Christine & Urs Breitenmoser recibieron sus respectivos doctorados en Zoología en la Universidad de Berna, Suiza. Desde finales de los años 80 han estado llevando a cabo proyectos de conservación de carnívoros en Suiza y en Europa. Con el fin de mejorar la coordinación de actividades de conservación en Suiza, fundaron la organización sin ánimo de lucro KORA. Desde 2001 ambos codirigen el Grupo Especialista de Felinos de la Unión Internacional para la Conservación de la Naturaleza (UICN/SSC). Durante los últimos años, han desarrollado diversas herramientas para mejorar la comunicación y la formación de especialistas en conservación de felinos, tales como la página web www.catsg.org, con información sobre las 37 especies de felinos; la biblioteca digital "Cat Library", con más de 6.000 documentos sobre conservación de felinos silvestres, y la revista "Cat News", con información sobre programas de conservación de felinos a nivel global. El Grupo Especialista de Felinos reúne a 210 especialistas de 57 países y es la única organización que trabaja globalmente, integrando conocimientos de científicos, investigadores, gestores y representantes de ONGs, con el fin de desarrollar directrices para la conservación de felinos de todo el mundo.

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We need another and a wiser and perhaps a more mystical concept of animals. Remote from universal nature, and living by complicated artifice, man in civilization surveys the creature through the glass of his knowledge and sees thereby a feather magnified and the whole image in distortion. We patronize them for their incompleteness, for their tragic fate of having taken form so far below ourselves. And therein we err, and greatly err. For the animal shall not be measured by man. In a world older and more complete than ours they move finished and complete, gifted with extensions of the senses we have lost or never attained, living by voices we shall never hear. They are not brethren, they are not underlings; they are other nations, caught with ourselves in the net of life and time, fellow prisoners of the splendour and travail of the earth.

Necesitamos otro concepto más sabio y quizás más místico sobre los animales. Alejado de la naturaleza universal y viviendo en un complejo artificio, el ser civilizado analiza a las criaturas a través de la lupa de su conocimiento, y es así como ve la pluma magnificada y toda la imagen en distorsión. Los tratamos con condescendencia por ser incompletos, por su trágico destino de haber adoptado una apariencia muy por debajo de la nuestra. Y es aquí donde erramos, erramos enormemente. Porque el animal no debe ser medido por el hombre. En un mundo más viejo y más completo que el nuestro se mueven plenos y consumados, dotados de extensiones de los sentidos que ya hemos perdido o que jamás llegamos a poseer, viviendo a merced de voces que jamás oiremos. No son hermanos, no son vasallos; son otros mundos, atrapados junto a nosotros en la red de la vida y del tiempo, compañeros prisioneros del esplendor y del arduo trabajo de la Tierra.

Henry Beston,
The Outermost House (1928)

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Conservación *Ex situ* del Lince Ibérico: Un Enfoque Multidisciplinar
Iberian Lynx *Ex situ* Conservation: An Interdisciplinary Approach

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Conservación *Ex situ* del Lince Ibérico: Un Enfoque Multidisciplinar
Iberian Lynx *Ex situ* Conservation: An Interdisciplinary Approach

Astrid Vargas

Christine Breitenmoser & Urs Breitenmoser

Publicado por/Published by:
Fundación Biodiversidad

Astrid Vargas ha dedicado su carrera profesional a la conservación de especies amenazadas, participando tanto en aspectos de investigación y gestión como en temas de sensibilización y educación. Licenciada en Medicina Veterinaria en 1988 (Universidad Complutense de Madrid), en 1994 se doctoró en Zoología y Fisiología por la Universidad de Wyoming, EEUU, donde realizó su tesis sobre etología y biología de la conservación del turón de patas negras (Mustela nigripes), uno de los mamíferos más amenazados del planeta. Entre 2004 y 2009 coordinó el Programa Nacional de Cría en Cautividad de este mustélido, participando activamente en la reintroducción de más de 900 ejemplares en 6 áreas diferentes del Altiplano norteamericano. Durante ese mismo periodo, Astrid colaboró con la ONG "Idea Wild" apoyando proyectos de conservación de fauna en Perú, Venezuela, Guatemala, Bolivia, México y Chile. Entre 1999 y 2002, Astrid participó en proyectos de conservación en Madagascar, realizando estudios de campo sobre la fosa y el sifaka de corona dorada, y coordinando el proyecto "Iniciativa de Conservación y Gestión de Recursos Naturales en la Región de Daraina, Noreste de Madagascar", proyecto que resultó en la creación de un área protegida en dicha región en el año 2005. Desde diciembre de 2003 Astrid dirige el Programa de Conservación Ex situ del Lince Ibérico y es responsable de la gestión del centro de cría de El Acebuche, en el Espacio Natural de Doñana.

Conservación *Ex situ* del Lince Ibérico: Un Enfoque Multidisciplinar
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Editor principal/Chief editor: Astrid Vargas
Editores asociados/Associated editors:
Christine Breitenmoser & Urs Breitenmoser

Publicado por/Published by: Fundación Biodiversidad
En colaboración con/in collaboration with: IUCN Cat Specialist Group

Conservación *Ex situ* del Lince Ibérico: Un Enfoque Multidisciplinar
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Editado por /Published by:
Fundación Biodiversidad en colaboración con/in collaboration with: International Union for Conservation of Nature, Species Survival Commission (IUCN/SSC) Cat Specialist Group

Editor principal/Chief editor: Astrid Vargas (Director, Iberian Lynx *Ex situ* Conservation Programme)
Editores asociados/Associated editors: Christine Breitenmoser, Urs Breitenmoser
(Co-chairs, IUCN/SSC Cat Specialist Group)

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Graphic design and layout /Diseño gráfico y maquetación: Juan Carlos Gauli (jcgauli@gmail.com)
Photographic art in book covers and section openings/
Arte fotográfico de las cubiertas del libro y apertura de secciones:
Dr. Joe Zammit-Lucia (www.jzlimages.com)
Chapter's opening photos/Fotos de apertura de capítulos:
Antonio Rivas (Programa de Conservación *Ex situ*); José María Pérez de Ayala (MARM);
Antonio Sabater (Enfoque-10); Héctor Garrido (EBD-CSIC); Alexander Sliwa (EAZA);
Luis D. Klink (Programa de Conservación *Ex situ*); Tanya Shenk (CDW);
Marianne Hartmann (Tierstation Bockengut); Urs Breitenmoser (IUCN CatSG);
Pete Oxford; Andoni Candela, y E. Abad.
Recommended citation/cita recomendada:
Vargas, A., Breitenmoser, Ch. & Breitenmoser, U., Eds. 2009.
Iberian Lynx *Ex situ* Conservation: An interdisciplinary approach. Fundación Biodiversidad, Madrid, Spain.

Printed on FSC certified paper. Impreso en papel certificado FSC
Printed in Madrid by/Impreso en Madrid por La Trébere: latrebere@telefonica.net

Fuentes Mixtas
Grupo de producto de bosques bien gestionados y otras fuentes controladas.
FSC www.fsc.org Cert no. SW-COC-003552
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Libro Amigo de los Bosques GREENPEACE

Depósito legal: M-35518-2009

Programa de Conservación *Ex situ* del Lince Ibérico
Iberian Lynx *Ex situ* Conservation Programme

PROGRAMA DE CONSERVACIÓN EX-SITU
LINCE IBÉRICO

Coordinación Nacional / National Coordination

GOBIERNO DE ESPAÑA
MINISTERIO DE MEDIO AMBIENTE Y MEDIO RURAL Y MARINO

Ejecución del Programa (2003-2008)
Programme Implementation (2003-2008)

GOBIERNO DE ESPAÑA
MINISTERIO DE MEDIO AMBIENTE Y MEDIO RURAL Y MARINO

JUNTA DE ANDALUCIA
CONSEJERÍA DE MEDIO AMBIENTE

Comisión Multilateral / Mulitlateral Commission

JUNTA DE ANDALUCIA
CONSEJERÍA DE MEDIO AMBIENTE

JUNTA DE EXTREMADURA
Consejería de Industria, Energía y Medio Ambiente

Castilla-La Mancha

ICNB
Instituto da Conservação da Natureza e da Biodiversidade, I.P.

GOBIERNO DE ESPAÑA
MINISTERIO DE MEDIO AMBIENTE Y MEDIO RURAL Y MARINO

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Prólogo

El lince ibérico, símbolo de la fauna peninsular, es hoy la especie de felino más amenazada del mundo y su futuro es tan incierto que, si se cumpliesen los peores pronósticos, podría desaparecer en el plazo de diez años.

La gravedad de su situación ha hecho saltar las alarmas en todos los ámbitos, tanto nacionales como internacionales y ha conducido a incrementar los esfuerzos para tratar de evitar su extinción.

Así, en los últimos años, se han puesto en marcha nuevas medidas y proyectos, y se han reorientado iniciativas y planes ya existentes para intentar salvar al lince ibérico de su extinción.

Es el caso de la Estrategia para la Conservación del Lince Ibérico, aprobada en España en 1999, recientemente actualizada, que tiene como misión aunar la acción de todas las partes competentes en la conservación de esta especie endémica de la Península.

Sin embargo, la recuperación del lince ibérico es a la vez fin y medio, ya que su supervivencia implica intrínsecamente la del monte mediterráneo, donde el lince tiene su hábitat natural y donde ha ido perdiendo progresivamente territorio, quedándose desplazado en áreas cada vez más restringidas y aisladas.

La meta final de la Estrategia para la Conservación del Lince Ibérico es contribuir a que el lince vuelva ser una pieza funcional del ecosistema donde habita, el monte mediterráneo, para lo que se incide en el impulso de la investigación aplicada a la conservación del lince.

Esta novedosa Estrategia establece metas cuantitativas concretas y fija un calendario para conseguir que en el año 2011 el lince ibérico quede fuera de la categoría de especie “en peligro crítico”, la más grave de la escala de la lista roja de la Unión Internacional para la Conservación de la Naturaleza y pasar a su catalogación como especie “en peligro”. En una segunda fase, la Estrategia Nacional determina una “hoja de ruta” con el objetivo de que en el año 2020, el lince ibérico pueda pasar a ser catalogado como especie “vulnerable”. El desarrollo a medio y largo plazo de nuevas poblaciones de lince ibérico es parte integrante de este proceso previsto por la Estrategia y se apoya significativamente en la cría en cautividad. A la vista de sus resultados, la puesta en marcha del Programa de Conservación *Ex situ* del Lince Ibérico en España es ya un éxito palpable, tras cinco temporadas consecutivas de reproducción, en las que se ha logrado un crecimiento exponencial de la población cautiva.

El Programa de Conservación *Ex situ* del Lince Ibérico representa de esta forma un “seguro de vida” frente a la posible extinción de la especie, preparando animales para su futura reintroducción en la naturaleza (prevista para el año 2010) y asegurando la conservación de la diversidad genética de esta especie.

Estas y otras claves para la conservación del lince ibérico fueron analizadas durante el curso que la Fundación Biodiversidad organizó en 2006 en su sede sevillana, en el que se consiguió reunir a un selecto y amplio grupo de expertos nacionales e internacionales en la materia.

Durante dos meses, científicos y técnicos de reconocido prestigio han pasado por nuestras aulas para hacernos partícipes de sus conocimientos sobre la recuperación de éste y otros felinos y trasladarnos su experiencia sobre posibles vías para sacar al lince ibérico de su gravísima situación actual.

Fruto de aquellas jornadas, y del esfuerzo de cinco años de cría para la conservación del lince ibérico, es el libro que ahora presentamos y que ha logrado recopilar entre sus páginas, de forma singular, las valiosas aportaciones de aquellos expertos que se dieron cita en Sevilla, a través de una publicación que deseamos contribuya a incrementar el conocimiento global de la comunidad científica, además de elevar la conciencia del público en general, sobre la recuperación de esta especie, tan bella como amenazada.

Josep Puxeu Rocamora
Presidente del Patronato de la Fundación Biodiversidad

Foreword

The Iberian lynx, a symbol of the fauna of the Iberian Peninsula, is today the most endangered cat species in the world. Its future is so uncertain that it may disappear in the next ten years if the worst forecasts prove right.

The gravity of the situation has raised widespread alarm on a national and international level and led to increasing efforts to try to avoid the extinction of the species.

In recent years, new measures and projects have been established, and already existing initiatives and plans aimed at preventing the extinction of the Iberian lynx have been reoriented.

The Strategy for the Conservation of the Iberian Lynx was adopted in Spain in 1999 and has recently been updated. Its mission is to bring together the actions of all the relevant players in the conservation of this species, which is endemic to the Iberian Peninsula.

However, the recovery of the Iberian lynx is both a means and an end. The survival of the species intrinsically implies the survival of Mediterranean forest and scrubland, the natural habitat of the lynx, where the species has progressively lost its territory and is being displaced to increasingly restricted and remote areas.

The final goal of the Strategy for the Conservation of the Iberian Lynx is to devote efforts so that the lynx can be a functional part of its ecosystem once again. This is carried out, among other things, by encouraging research applied to the conservation of the species.

This innovative Strategy includes specific quantitative targets and a timetable. First, the aim is to ensure that by 2011 the Iberian lynx is no longer Critically Endangered, the most serious category of threat of the IUCN Red List, and can be transferred to the category of Endangered. In a second stage, the National Strategy has established a roadmap with the objective of ensuring that the species qualifies for the category Vulnerable by the year 2020. The medium- and long-term development of new populations of Iberian lynx is an important part of the process established in the Strategy, which is significantly based on captive breeding. The Iberian Lynx *Ex situ* Conservation Programme in Spain has already proven to be successful after five consecutive breeding seasons that have led to an exponential growth of the captive population.

This Conservation Programme represents a “life insurance” against the possible extinction of the species, as it prepares animals for future reintroduction into the wild – planned for 2010 – and ensures the preservation of the genetic diversity of the species.

These key elements for the conservation of the Iberian lynx and others were analysed during the course organised in 2006 by Fundación Biodiversidad in its headquarters in Seville, which brought together a broad group of the best national and international experts on various subjects. For two months, renowned scientists and other experts visited our classrooms to share their knowledge on the recovery of the Iberian lynx and other felid species as well as their experience on possible ways to overcome the extremely serious plight of the Iberian lynx.

The book we are presenting today is a result of the course and of the effort of five years of captive breeding for the conservation of the Iberian lynx. This publication compiles the valuable contributions of the experts who met in Seville and is intended to increase the global knowledge of the scientific community and raise awareness in the general public about the recovery of this beautiful and highly endangered species.

Josep Puxeu Rocamora
President of Board of Trustees of Fundación Biodiversidad

Prefacio

Cierra los ojos durante unos minutos e imagina qué posibles problemas podría encontrar un felino de tamaño medio en un paisaje cultivado, dominado por los seres humanos: pérdida de hábitat, disminución de presas, sequías, fuegos, persecución directa, accidentes de tráfico, fragmentación de la población, deriva genética, enfermedades, y falta de acuerdo respecto a las medidas de conservación y reparto de responsabilidades. Así era la situación del lince ibérico en 2002, cuando fue catalogado en la Lista Roja de la UICN como Críticamente Amenazado; la primera especie de felino en esa categoría. Quedaban menos de 150 linces en dos poblaciones en declive, ambas en Andalucía, una en Andújar-Cardeña, en Sierra Morena, y la otra en la región de Doñana. Desde entonces, hemos progresado, y este libro es otro paso hacia delante. No el único, pero sí una de las mejoras más obvias en los últimos años ha sido el establecimiento de un programa de cría para la conservación de esta especie.

El primer centro de cría para el lince ibérico se construyó en El Acebuche, Doñana, en 1991, pero los linces nacieron allí por primera vez en 2005. Había falta de experiencia –nunca antes había nacido un lince ibérico en cautividad—pero la gran demora en conseguirlo fue principalmente consecuencia de las profundas dudas en relación a la importancia de la cría en cautividad y de su papel en la conservación del lince ibérico. Existe un gran excepticismo entre los biólogos de campo y los conservacionistas respecto a la cría en cautividad y la reintroducción. Parte del miedo consiste en que la cría en cautividad pueda “sobreexplotar” las poblaciones silvestres, y que las reintroducciones son difíciles de llevar a cabo y conllevan una alta probabilidad de fracaso. De hecho, las experiencias de reintroducciones realizadas con linces euroasiáticos y linces canadienses extraídos de poblaciones silvestres han sido ambivalentes, y los proyectos de reintroducción de felinos nacidos en cautividad tampoco son concluyentes. Sin embargo, podemos aprender de estas experiencias, tanto de sus éxitos como de sus fracasos, y lo cierto es que la situación del lince es tan crítica que necesitamos un enfoque que abarque el máximo rango de posibilidades. Ya no es “esto o lo otro”, sino “esto, y además, lo otro”.

Hoy, el Programa de Cría para la Conservación no solo proporciona una “copia de seguridad” para preservar la diversidad genética de esta especie, es también una parte integral de la estrategia global para la conservación del lince ibérico. Las metas principales del Programa *Ex situ* incluyen el mantener una población cautiva bien manejada desde el punto de vista genético y proporcionar linces para programas de reintroducción. Nuevos conocimientos obtenidos gracias al trabajo con la población cautiva, tales como el estudio de patógenos y la observación y estudio de episodios agresivos entre hermanos de camada, tienen una consecuencia directa sobre la gestión de las poblaciones cautiva y silvestre. Y, por último, pero no por ello menos importante, el programa de cría ayuda a concienciar nacional e internacionalmente acerca de la situación del lince ibérico y sus necesidades de conservación.

El proceso de conservación del lince y su programa de cría siguen un enfoque multidisciplinar, que se resalta en la gran diversidad de temas cubiertos en este libro. Gran parte de esta obra es producto de proyectos colaborativos de investigación y, como libro propiamente dicho, comenzó a tomar forma tras una serie de seminarios organizados en Andalucía en 2006 por la Fundación Biodiversidad, en colaboración con el Programa de Conservación *Ex situ*. La meta de esta serie de seminarios consistió en revisar el conocimiento que existía sobre la biología y ecología del lince, haciendo énfasis especial en su relación con el programa de cría y, a su vez, en compartir conocimientos con expertos en las disciplinas de ecología, genética, comportamiento animal, veterinaria y sanidad animal, reproducción y reintroducción. Este libro recopila gran parte de las charlas presentadas durante los seminarios de 2006 y actualiza los datos para presentar de modo integrado el trabajo de colaboración llevado a cabo en el Programa de Cría para la Conservación del lince ibérico entre 2004 y 2008.

La obra está organizada en cinco partes. La primera parte presenta una revisión sobre la situación del lince en la naturaleza y las actividades que se están llevando a cabo *in situ*. La segunda parte trata sobre aspectos genéticos, comportamiento y manejo de linces y otras especies de felinos en cautividad. La tercera parte recopila diversos trabajos sobre aspectos veterinarios, tratando temas que han ido tomando cada vez mayor importancia en la conservación de especies. La cuarta parte versa sobre la fisiología reproductiva de diversas especies de felinos, poniendo especial énfasis en los hallazgos más recientes sobre la reproducción de lince ibérico y de otras especies de linces. Por último, la quinta parte presenta una revisión sobre técnicas de reintroducción, así como casos de estudio sobre proyectos de reintroducción de felinos.

Los capítulos recopilados en esta publicación representan el trabajo de colaboración internacional que se ha llevado a cabo en pro de la conservación del lince ibérico durante los últimos cinco años, pero también presenta estudios y trabajos que se están desarrollando con otros felinos en distintas partes del mundo. Cada capítulo ha sido tratado como una publicación científica, siendo revisado por tres expertos en cada tema concreto. Aunque el idioma principal de conjunto de la obra es el inglés, los resúmenes y textos de las ilustraciones, así como el prólogo, el prefacio y el epílogo, se presentan en ambos idiomas. En conjunto, un total de 124 coautores de 10 países han participado en los 43 capítulos que contiene el libro, que incluye un epílogo de Miguel Delibes, uno de los científicos más prominentes en España, quien cierra el libro presentando su visión sobre el presente y el futuro de esta especie.

A la vez que se presenta trabajos de investigación científica en diversas disciplinas, el libro también desea llegar al público a través de su estética y ofrece más de 200 fotografías cedidas por fotógrafos nacionales e internacionales. El artista de reconocido prestigio Joe Zammit-Lucia ha diseñado específicamente para esta publicación las fotografías que aparecen en la portada y la contraportada, así como las fotografías que sirven como apertura de cada una de las secciones del libro. Los versos y citas que aparecen acompañando a cada capítulo han sido en su mayoría seleccionados por los propios autores. La diversidad de temas, junto la selección de expertos y autores de diversas partes del planeta, demuestran que el lince ibérico necesita apoyo internacional para su supervivencia, y asimismo revela cómo la sociedad global de conservacionistas está interesada e involucrada en la recuperación de esta especie, resaltando cuán necesario es el trabajar juntos para prevenir la extinción de este magnífico felino.

Urs Breitenmoser, Christine Breitenmoser y Astrid Vargas

Preface

Close your eyes and think for a few minutes about what potential threats and problems a medium-sized cat species could possibly encounter in a cultivated, human-dominated landscape: Loss of habitat, prey depletion, droughts and fire, direct persecution, traffic accidents, population fragmentation, genetic drift and inbreeding, diseases, and strong dissension regarding conservation measures and responsibilities. And no captive population. That was the Iberian lynx in 2002, when it was listed as Critically Endangered in the IUCN Red List as the first cat species ever. Less than 150 lynx remained in two declining populations in Andalucía, near Andújar-Cardeña in Sierra Morena and in the Doñana region.

Since then, we have made progress, and this book is another step forward. Not the sole, but one of the most obvious improvements in the past years has been the establishment of a conservation breeding programme. The first breeding centre for the Iberian lynx was built in El Acebuche, Doñana, in 1991, but lynx were born there only in 2005. There was a lack of experience –no Iberian lynx was ever born in captivity before– but the long delay was mainly a consequence of profound doubts regarding the importance of the conservation breeding programme and its role in the conservation of the Iberian lynx. There is a widespread scepticism among field biologists and conservationists about captive breeding and reintroduction. They fear that captive breeding may "over-exploit" the remnant populations, and that reintroductions are difficult to do and bear a high risk of failure. Indeed, the experiences with wild-to-wild reintroduction of Eurasian lynx and Canada lynx are ambivalent, and projects using captive-born cats of any species so far are not conclusive. Nevertheless, we can learn from all these earlier projects, both from their successes and failures, and the situation of the Iberian lynx is so critical that we need a comprehensive approach. It is no longer "either – or", it is "as well as".

Today, the conservation breeding programme does not only provide a safety backup for the lynx´s genetic diversity, it is an integral part of the overall conservation strategy for the species. The main goals of the *ex situ* conservation programme are to maintain a genetically well-managed captive population and to produce lynx for reintroductions. Beyond this, the conservation breeding programme provides insight into particular questions such as reproductive biology or health problems and diseases that could affect the species. Findings as a result of captive breeding, such as the study of pathogens and the observation of sibling aggression among lynx cubs have immediate consequences for the management of both the captive- and the free-ranging populations, and last but not least, the captive breeding programme helps to raise worldwide awareness for the fate of the Iberian lynx and its conservation needs.

The Iberian lynx conservation process and its captive breeding programme follow an interdisciplinary approach, emphasised by the wide range of topics covered in this

book. Largely, the book is a product of five years of collaborative research and, as a book, it began to take shape during a series of seminars on Iberian Lynx *Ex situ* Conservation, organized by the Biodiversity Foundation together with the Conservation Breeding Programme, which took place in Andalusia in autumn 2006. The goal of the seminar series was to review current knowledge on Iberian lynx biology and ecology as it relates to conservation breeding efforts and to share knowledge and experiences with experts on related fields of wild felid ecology, behaviour, genetics, health and veterinary aspects, reproduction, and reintroduction, in order to further advance the various aspects of the conservation programme. The seminar series was structured in four sessions, each lasting two days. The present book summarises the experiences presented in the talks, all of which have been updated to cover the work that has taken place in the conservation breeding programme between 2004 and 2008.
The organization of this book includes five sections. The first section presents a review of the *in situ* situation and field conservation activities. The second section deals with genetic aspects and the behaviour and husbandry of Iberian lynx in captivity. The third section compiles papers on veterinary aspects and health issues, which became increasingly important in recent years. The fourth section looks at reproductive physiology of a variety of felid species, placing special emphasis on the latest findings on lynx reproduction. Lastly, the fifth section provides an overview of reintroduction techniques and case studies of felid reintroduction projects.
The chapters compiled in this publication represent the collaborative international work carried out towards the conservation of the Iberian lynx during the past five years, but also portrays similar studies and approaches from other felid species from various areas of the world. Each chapter has been treated as a peer-reviewed paper for a scientific journal, and has been revised by three different experts in the specific subject. Although the book´s main language is English, abstracts and illustration captions from each chapter are presented in both English and Spanish; the prologue, preface and epilogue are also offered in both languages. Altogether, 124 contributors from 10 different countries have taken part in the 43 chapters, which include an epilogue by Miguel Delibes, one of Spain´s most prominent scientist, who wraps up the book presenting his vision about the present and future of the critically endangered lynx.
While presenting scientific research in a wide variety of disciplines, the book also hopes to appeal to the eye of the viewer by including more than 200 photographs contributed by national and international photographers. World renowned photographic artist Joe Zammit-Lucia, has specifically designed the photographs that appear in the front and back covers, as well as the artwork in the opening page of each of the sections. The verses and quotes that appear throughout the book have been largely selected by the authors. The variety of themes together with the selection of experts and authors from across the world demonstrates that the Iberian lynx needs international support for its survival, and also reveals how the global conservation society is interested and involved in the recovery of this species, emphasizing how much we all need to work together to prevent the extinction of this magnificent cat.

Urs Breitenmoser, Christine Breitenmoser and Astrid Vargas

Caminante, son tus huellas
el camino y nada más;
caminante, no hay camino,
se hace camino al andar.

Al andar se hace camino
y al volver la vista atrás
se ve la senda que nunca
se ha de volver a pisar.

Caminante no hay camino
sino estelas en la mar...

Antonio Machado
(1875-1939)

Biology and current status

BIOLOGÍA Y SITUACIÓN ACTUAL

If a man walks in the woods for love of them half of each day, he is in danger of being regarded as a loafer. But if he spends his days as a speculator, shearing off those woods and making the earth bald before her time, he is deemed an industrious and enterprising citizen.

Henry David Thoreau
(1817-1862)

Photo: Antonio Sabater

Life history and ecology of the Iberian lynx

Biología y ecología del lince ibérico

Francisco Palomares

Resumen
El lince ibérico, actualmente la especie de felino más amenazada en el mundo, es un animal de tamaño medio, solitario y territorial, cuyas únicas poblaciones se encuentran en la mitad sur de la Península Ibérica. Esta especie de lince presenta una alta especialización en cuanto a sus necesidades alimenticias y de hábitat: consume casi exclusivamente conejos europeos y habita en zonas mediterráneas de monte bajo. Las hembras normalmente se reproducen una vez al año entre los 3 y 9 años de edad, y suelen sobrevivir sólo 2 de las 3-4 crías que nacen en cada camada. Los linces jóvenes abandonan el territorio materno cuando tienen entre 1 y 2 años de edad, momento en el que se dispersan en búsqueda de nuevos territorios donde asentarse como residentes. El área de campeo de los linces adultos ocupa entre 4 y 30 km², dependiendo de factores como el sexo y la densidad de conejos. Los machos tienden a tener mayores áreas de campeo que las hembras. Los territorios de los machos y hembras adultos pueden coincidir en gran parte, pero esto no suele ocurrir entre ejemplares adultos del mismo sexo. En la naturaleza, el lince ibérico tiene una mortalidad elevada por causas no naturales, especialmente fuera de las áreas protegidas. Existe una fuerte interferencia entre el lince y otras especies de carnívoros; los linces matan a especies de tamaño más pequeño, como los zorros, meloncillos, ginetas y gatos domésticos, lo que produce cambios en el uso del espacio y densidades más bajas de algunas especies de carnívoros más pequeños.

Palabras clave
Lince ibérico, *Lynx pardinus,* ecología espacial, reproducción, uso del hábitat, organización social

Abstract

The Iberian lynx, currently the world's most endangered felid species, is a medium-sized solitary and territorial felid whose only populations occur in the southern half of the Iberian Peninsula. This lynx species is highly specialized in its diet and habitat requirements, consuming almost exclusively European rabbits and inhabiting Mediterranean scrubland. Females normally breed once a year between 3 and 9 years of age, and only 2 of the 3 kittens of the litter usually survive. Between 1 and 2 years of age juvenile lynx abandon their mother's territory and disperse in search of new territories to settle as residents. The home ranges of adult lynx measure between 4 and 30 km^2, depending on factors such as sex and rabbit density. Males tend to have larger home ranges than females. Core areas greatly overlap between adult males and females, but there is no overlap between adult individuals of the same sex. In the wild, the Iberian lynx experiences a high mortality from non-natural causes, particularly outside protected areas. There is strong interference between lynx and other carnivore species, with lynx killing species of smaller size such as red foxes, Egyptian mongooses, common genets, and domestic cats, which results in changes in the space use and lower densities of some of the smaller carnivores.

Keywords

Iberian lynx, *Lynx pardinus,* spatial ecology, breeding, habitat use, social organization

Photo: Antonio Sabater

Figure 1. Iberian lynx breeding female emerging from a hollow tree den in Doñana.

Figura 1. Hembra reproductora de lince ibérico saliendo de su madriguera, ubicada en un árbol hueco de Doñana.

Life history and ecology of the Iberian lynx

Francisco Palomares

Introduction

The Iberian lynx *(Lynx pardinus)* is one of the 36 existing species of felids, and one of the four species of the genus Lynx in the world. Two lynx species –the Iberian lynx and the Eurasian lynx *(Lynx lynx)*– occur in Europe and Asia, and two other species –the bobcat *(Lynx rufus)* and the Canada lynx *(Lynx canadensis)*– occur in North America. The direct ancestor of the Iberian lynx was the Cave lynx *(L. pardinus spelaeus)*, which was larger than the current species and widespread in most of Europe. About one million years ago, a population of Cave lynx became isolated in south-west Europe and gave rise to the Iberian lynx as we know it today.

The coat of the Iberian lynx is variable. Whereas individuals in the Doñana area have a pattern of large dark spots and stripes on a tawny background, those in Sierra Morena have small, less distinct spots on a more or less grayish background. Males are somewhat larger than females, and have been recorded to weigh 12-14 kg, and 9-10 kg, respectively in Doñana (Beltrán and Delibes, 1993).

The Iberian lynx is currently the world's most endangered felid species (Novell and Jackson, 1996). Unlike the other lynx species, the Iberian lynx already had a small natural range which was limited to the Iberian Peninsula. After a very dramatic decrease in the size of its range in recent decades, only two populations remain in southern Spain –Doñana and Eastern Sierra Morena. The most significant factors in the population decline of the species are habitat loss and fragmentation, direct persecution, and the decline of its basic prey, the European rabbit *(Oryctolagus cuniculus)* (Rodríguez and Delibes, 1992; Palomares et al., 2002; Guzmán et al., 2004).

Feeding ecology

The Iberian lynx feeds almost exclusively on rabbits (Delibes, 1980). In several areas of the centre and south of the Iberian Peninsula where its diet has been studied, remains of rabbits have been found in 85 to 99% of the scats analyzed (Delibes, 1980; Aymerich, 1982; Beltrán and Delibes, 1991; Gil-Sánchez et al., 1997; Palomares et al., 2001). The size of the rabbits consumed changes throughout the year depending on their abundance; very young and juvenile rabbits have been found to be more present in the diet in spring and summer than in the rest of the year (Calzada, 2000; Calzada et al., 2003). Sometimes lynx also prey on geese, ducks, rats, hares, partridges, magpies, pigeons and even juvenile red and fallow deer.

To date, there is no evidence that Iberian lynx can survive and breed feeding exclusively on prey other than rabbits. Therefore, the species cannot live in areas in which rabbits are absent or very scarce.

Habitat requirements

The Iberian lynx needs the Mediterranean scrubland to live (Palomares et al., 2000); if it is not available, rocky areas with some scrubland can also be suitable (Fernández et al., 2006). The Mediterranean scrubland is

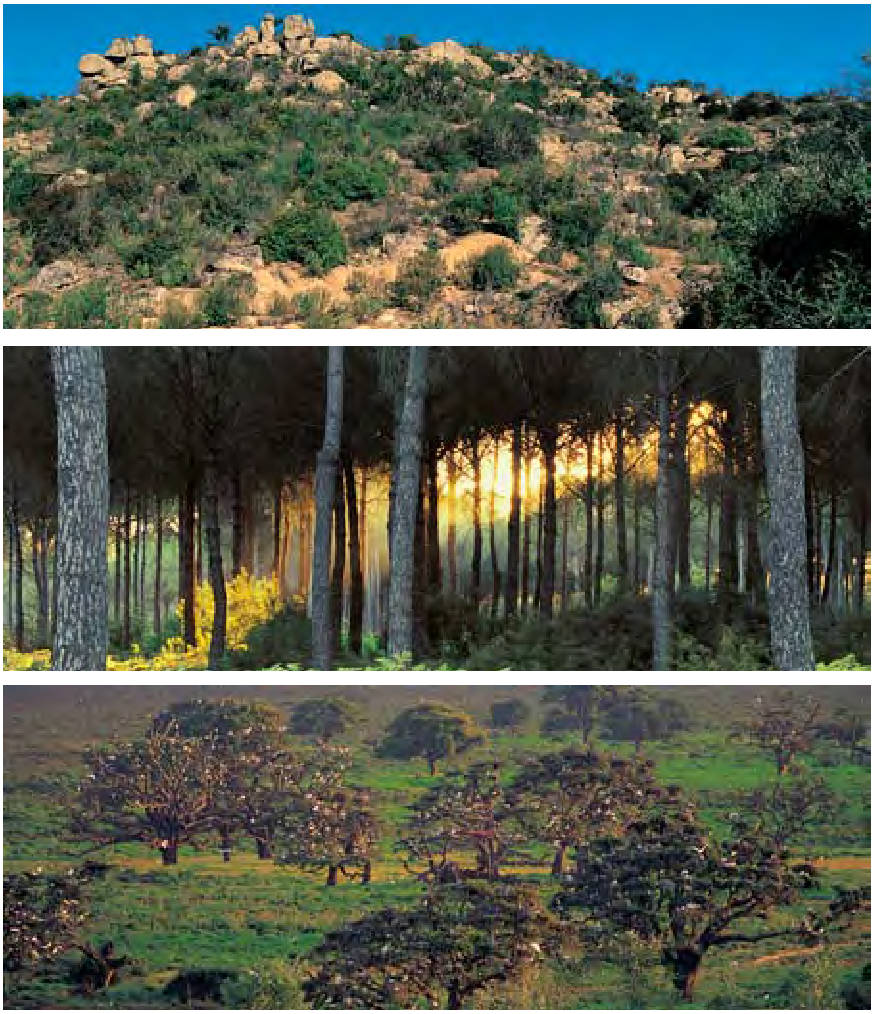

Photo: Antonio Sabater

Photo: Antonio Sabater

Photo: Antonio Sabater

Figure 2. Mediterranean forest and scrubland in Sierra Morena (a) and Doñana (b, c).

Figura 2. Bosque y matorral mediterráneo en Sierra Morena (a) y Doñana (b, c).

dominant in at least 75% of the areas where the Iberian lynx occurs and breeds regularly. In these areas, the average scrub cover is 55%. Remaining areas are usually strips of land between the scrubland and open habitats such as the edges of marshes or *dehesas* (Mediterranean savannah-like formations) (Fernández et al., 2007). Rabbit abundance must be at least between 1 and 5 rabbits/ha –in the least and most abundant seasons of the year, respectively– for breeding to take place (Palomares et al., 2001).

Dispersing individuals are not as demanding as residents in terms of habitat requirements. At that stage of their lives, they can use any kind of natural habitat as long as it is associated to some type of scrubland (Palomares et al., 2000). They are also less demanding with regard to rabbit abundance and can use areas with lower densities than those where they settle down (Palomares, 2001).

Availability of suitable breeding dens and the presence of water are also important components in lynx habitat. In Doñana, females always choose old, large hollow trees as breeding dens (at least 90 cm diameter at breast height; Fernández and Palomares, 2000) (Figure 1). In Sierra Morena, females probably breed in rock cavities and small caves. In summer, the daily movements of lynx depend on the location of water sources, where they are often sighted drinking (Palomares et al., 2001).

Therefore, the Iberian lynx needs a habitat where the dominant vegetation is the Mediterranean scrubland (Figure 2), with relatively good abundance of rabbits. Areas of Mediterranean scrubland without rabbits or areas with plenty of rabbits but without plant cover provided by scrubland or shrubland are not suitable for the


Photo: Antonio Sabater

Figure 3. Iberian lynx wild born cubs.

Figura 3. Cachorros de lince ibérico nacidos en la naturaleza.


Photo: Antonio Sabater

Figure 4. Interspecific interaction between an Iberian lynx and a red fox.

Figura 4. Interacción interespecífica entre un lince ibérico y un zorro rojo.

species. Most likely, the specie's habitat must also have places that can make good breeding dens, such as old hollow trees or rock cavities, and permanent water sources during the dry season.

Social and spatial organization

Lynx are solitary felids (Ferreras et al., 1997). It is unusual to see two individuals together apart from the female with her young during the period of dependence on the mother, which usually ends when the cubs are 7-8 months old. Males do not help to raise the kittens. Juvenile lynx abandon their mother's territory and disperse in search of new territories to settle as residents when there are between 1 and 2 years of age (Ferreras et al., 2004; Palomares et al., 1999).

The home ranges of adult lynx measure between 4 and 30 km^2, depending on factors such as sex and rabbit density (Ferreras et al., 1997; Palomares et al., 2001; Palomares, 2001). Males tend to have larger home ranges than females. Home ranges are smaller in areas where rabbits are abundant than in those where rabbits are scarcer. The areas of greatest use of resident adult lynx home ranges strongly overlap with those of individuals of the other sex, but not with territories of individuals of the same sex. Although lynx tend to be monogamous, the territories of some males may encompass those of more than one female.

Reproduction

In the wild, female lynx normally breed only between 3 and 9 years of age (Palomares et al., 2005). Breeding has been recorded in 2-year-old captive females, but the cubs seem to have lower chances of survival. Although lynx usually breed once a year, sometimes they do not breed for unknown reasons. Estrus peaks in December-January and litters are born between March and April. However, late estrus and births may occur (Fernández et al., 2002; Palomares et al., 2005). The most frequent litter size is 3 kittens, but sometimes 2 or 4 kittens are born (Figure 3). In most cases only two offspring of the litter survive until the age of dispersal.

Movements during dispersal

Lynx have dispersal limitations that make them very sensitive to barriers imposed by the construction of large infrastructures or the alteration of natural habitats (Ferreras, 2001; Ferreras et al., 2004; Revilla et al., 2004). In the Doñana area, a dispersing lynx travels an average distance of 172 km over a surface of 231 km^2 and moves a straight-line distance of 23 km away from the point of capture (Palomares et al., 1999). Lynx movements are determined by the structure and composition of habitats around breeding areas. Dispersing individuals have difficulties crossing open areas over 5 km wide. They are therefore very sensitive to fragmentation or destruction of the natural scrubland habitats they need to move about without major problems.

Mortality

Most lynx deaths in the Doñana area –at least 62%– are caused by human actions (Ferreras et al., 1992). In a radio-tracking study of more than 50 individuals, the causes of mortality recorded were the following: direct shooting (21% of deaths), illegal trapping with leg-hold traps and snares and hunting with dogs (21%), road accidents (17%) and drowning in water wells (4%). Humans appear to have been and continue to be responsible for a high proportion of deaths in Doñana as well as other areas of the specie's range.

Interactions with other carnivores and effects on prey

In Doñana, lynx often kill but do not consume other carnivore species of smaller size, including red fox *(Vulpes vulpes)*, domestic cat *(Felis catus)*, Egyptian mongoose *(Herpestes ichneumon)* and common genet *(Genetta genetta)* (Figure 4) (Palomares et al., 1996; Palomares and Caro, 1999). At least in the smaller carnivores –Egyptian mongooses and genets –, these interactions lead to densities between 10 and 20 times lower in areas inhabited by Iberian lynx than areas with similar vegetation but no lynx (Palomares et al., 1996). As for interactions between Iberian lynx and Egyptian mongoose, the abundance of mongoose has proven to be directly related to the abundance of lynx. The two species only coexist in areas with low lynx density (Palomares et al., 1998). This exclusion relation is not as clear with red foxes as it is with Egyptian mongooses and genets. However, red foxes have shown to be able to use areas with higher lynx densities mainly during the resting period of lynx, thus reducing the chances of encountering them (Fedriani et al., 1999). The presence of Iberian lynx benefits rabbit populations. In spite of being predated by lynx, rabbits reach higher densities in areas with an abundance of lynx due to the control exerted by lynx on other carnivores that also consume rabbits (Palomares et al., 1995).

Final remark

Initial research studies briefly outlined here on life history and ecology of lynx, including social and spatial organization, reproduction, activity patterns or habitat use, have been basic to understanding Iberian lynx requirements and, hence, have helped develop sound conservation plans for this species. Now, when the species is critically endangered and its survival in nature depend on the performance of these plans, systematic applied research is even more important to help managers understand the response of individual free-ranging lynxes and populations to the conservation measures implemented. Adaptive management based on sound research will allow for founded modifications of conservation plans if needed.

Acknowledgements

The information used in this review has been collected as result of several research projects, funded mostly with public funds from Spanish regional and national administrations, as well as several EU LIFE projects. M. Delibes, A. Rodríguez, P. Ferreras, E. Revilla, J. Calzada, J.A. Godoy, A. Vargas, J. Román, G. Ruiz, J.J. Aldama, N. Fernández, J.V. López-Bao, J.C. Rivilla, A. Píriz, S. Conradi and numerous students and collaborators have contributed to the current knowledge on the Iberian lynx reflected in these pages.

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I heard a thousand blended notes / While in a grove I sate reclined, In that sweet mood when pleasant thoughts / Bring sad thoughts to the mind.
To her fair works did Nature link / The human soul that through me ran; And much it grieved my heart to / think What Man has made of Man.

William Wordsworth
(1770-1850)

Status and trends of rabbit populations in the Iberian Peninsula

Estado actual y tendencias poblacionales del conejo en la Península Ibérica

Carlos Calvete

Resumen

El conejo silvestre *(Oryctolagus cuniculus L.)* es una especie clave en el ecosistema mediterráneo de la Península Ibérica. En las últimas décadas, no obstante, esta especie ha sufrido un dramático declive en su abundancia poblacional debido principalmente a la pérdida de hábitat y, en especial, a la incidencia de la mixomatosis y la enfermedad vírica hemorrágica (RHD). En la actualidad, el manejo y recuperación de las poblaciones de conejo silvestre es uno de los principales hitos en el ámbito de la conservación. Sin embargo, el impacto de la RHD en la dinámica de las poblaciones de esta especie parece ser uno de los principales obstáculos para su recuperación. En el presente capítulo, y bajo las premisas establecidas por un modelo matemático previamente desarrollado para explorar la epidemiología de la RHD, se evalúan de forma teórica los posibles resultados de la aplicación de diferentes estrategias de manejo de las poblaciones de conejo silvestre. Siguiendo los supuestos de dicho modelo, la mejora del hábitat sería el mejor modo –solo o en combinación con otras estrategias de manejo– para incrementar la densidad de conejos de forma permanente en aquellas poblaciones que ya están en equilibrio con la RHD. La mejora de aquellas poblaciones que todavía no hubieran alcanzado este equilibrio, sin embargo, parece ser compleja debido, probablemente, a las posibles interacciones entre la RHD y otros factores como la depredación. Por otro lado, el uso indebido de las translocaciones surge como un obstáculo añadido a la mejora de las poblaciones, debido a diversos mecanismos –tales como la competencia mediada por la enfermedad– que podrían perjudicar la viabilidad de las poblaciones nativas. Finalmente, se concluye que es necesario promover investigaciones futuras, tanto para evaluar las implicaciones de la RHD en la biología del conejo silvestre como para definir y desarrollar nuevos modos de gestión de la especie.

Palabras clave

Control de enfermedades, epidemiología, neumonía hemorrágico-vírica (RHD), manejo de hábitat, *Oryctolagus cuniculus*

Abstract

The European wild rabbit *(Oryctolagus cuniculus L.)* is a keystone species in Mediterranean ecosystems of the Iberian Peninsula. During the last decades wild rabbit populations have suffered a dramatic decline because of habitat loss and especially due to the incidence of myxomatosis and Rabbit Haemorrhagic Disease (RHD). Currently, enhancement of rabbit populations is a primary concern in conservation; however, the impact of RHD in rabbit population dynamics seems to be one of the major challenges to rabbit recovery. Under the theoretical insights obtained in a previous RHD epidemiology modelling approach, we evaluated the possible outcomes of several rabbit management strategies. Following model assumptions, habitat improvement was the best way –alone or in combination with other management strategies– to permanently increase rabbit densities in populations at equilibrium with RHD. The enhancement of rabbit populations in areas that had not yet reached equilibrium with RHD seemed to be more complex, likely due to possible interactions of disease with other factors like predation. The misuse of translocations arose as an added obstacle to rabbit enhancement because of underlying mechanisms, such as apparent disease-mediated competition, that could yield harmful effects on native populations. More research is needed in order to evaluate the implications of RHD on rabbit biology and to provide novel approaches to rabbit management.

Keywords

Wildlife disease control, epidemiology, Rabbit Haemorrhagic Disease (RHD), habitat management, *Oryctolagus cuniculus*

Photo: Antonio Rivas

El conejo silvestre en la Península Ibérica es un claro ejemplo de cómo una especie tan importante para el ecosistema ha sido tradicionalmente considerada una especie humilde o de carácter menor simplemente por ser común, y de cómo, esta aparente humildad, le ha hecho históricamente víctima de una gestión basada en el acerbo popular en vez de en el conocimiento científico.

Carlos Calvete

Status and trends of rabbit populations in the Iberian Peninsula

Carlos Calvete

Introduction

The European wild rabbit *(O. cuniculus L.)* is one of most important vertebrate species in Iberian Mediterranean ecosystems, where this species is native. The rabbit has a deep impact on microclimates, floral composition and invertebrate biodiversity of these ecosystems, largely contributing to their maintenance. Moreover, rabbits contribute significantly to the food supply of more than 30 predator species in the Iberian Peninsula (Delibes and Hiraldo, 1981), including highly endangered native predator species that depend on rabbit abundance. For these reasons, the wild rabbit is a primary conservation concern in the Iberian Peninsula.

Rabbits have been historically abundant in many areas of the Iberian Peninsula. Their populations, however, have been declining since the mid XX century, in part due to changes in agricultural practices and the subsequent habitat loss, yet particularly due to the impact of two viral diseases: myxomatosis in the 1950s and Rabbit Hemorrhagic Disease (RHD) at the end of the 1980s, causing two consecutive abrupt declines in wild rabbit numbers. The magnitude of rabbit populations collapse folations seems to be associated with bioclimatic factors traditionally related to habitat suitability and rabbit abundance. However, the high variability of current rabbit occurrence between and within habitats makes the relationships between mean rabbit abundance and these bioclimatic factors not so clear. This irregular distribution pattern suggests that unknown factors sometimes could be hindering and others promoting rabbit recovery at local level. Factors that could be invoked like putative and not mutually exclusive causes of this distribution pattern are: 1) variation of the impact of mortality factors (e.g. predation or hunting) at a local scale, 2) stochastic events (e.g., flooding or drought) and, especially, 3) variations in the dynamics of the wild rabbit-RHD virus system.

RHD is an infectious disease, primarily transmitted by direct contact. The etiological agent of RHD is the Rabbit Haemorrhagic Disease Virus (RHDV), a member of the family *Caliciviridae*. The main epidemiological feature of this disease is that lethality is frequently very high (about 80-90%) in rabbits older than 8 weeks of age, but less so in younger rabbits (see review of Cooke, 2002). The existence of non-pathogenic RHD-like viruses or variation in the genetic resistance to RHD between rabbit populations have been posed as possible factors that could cause local variations in the dynamics of the wild rabbit-RHD virus system in the Iberian Peninsula. Nevertheless, to date, there is no evidence regarding the presence of protective, non-pathogenic RHD-like viruses in southern European rabbit populations or evidence of differences in the genetic resistance to the disease in Iberian populations (Alda et al., 2006). Another possibility, however, has been described by a mathematical model representing the epidemiology of RHD (Calvete, 2006a). This model suggested that the impact of RHD could be highly dependent on rabbit population dynamics. The outcome of this model were largely compatible with observed patterns of rabbit distribution in the Iberian Peninsula and showed that, the presence of a unique, highly pathogenic RHD virus can be compatible with the existence of high-density populations at equilibrium with the disease.

On the other hand, even though wild rabbits are not currently at risk of extinction, considerable efforts have been made to enhance rabbit populations for hunting and conservation purposes in the Iberian Peninsula since the arrival of RHD. These efforts have borne a considerable increase of scientific research on rabbit ecology, management and conservation, but, mainly, research has been aimed towards enhancing management of rabbit populations.

Despite these efforts, the scientific knowledge generated to date still seems to be insufficient to manage rabbit populations successfully. Indeed, the results obtained in many management experiences are negligible, and this failure is usually attributed to causes such as low habitat suitability, mortality by predation and the impact of viral diseases. Among those, mortality caused by RHD during or just after management programmes seems to be one of the more frequently events pointed as the cause of failure. Given the above mentioned reasons, although the field validation of the RHD model (Calvete, 2006a) has not been performed yet, the analysis of the possible effects of RHD on rabbit ecology and management can provide new insights into lines of future research and management efforts.

Theoretical insights into RHD and rabbit ecology

The theoretical relationships between rabbit density and habitat carrying capacity (K) are shown in Figure 1. Carrying capacity is defined as the maximum density of reproductive individuals in an area in the absence of RHD. Carrying capacity is dependent as much on intrinsic habitat features that affect rabbit productivity and survival as on extrinsic mortality factors that are unique to RHD. For simplicity, the analysis assumed a linear relationship (continuous line) between rabbit density before RHD arrival and K, with rabbit density being low at values around K_0, medium at values around K_1, and high at values around K_2.

RHD had a differential short-term initial impact on naïve populations. In Australasia, a higher initial impact of RHD was associated with higher rabbit population densities, since high densities of susceptible rabbits favoured the initial transmission of the virus. In the Iberian Peninsula, retrospective analyses resulted in similar patterns, suggesting that the short-term initial impact of the disease was higher in populations located in more suitable habitats, whereas, in populations located in medium-low suitability habitats, the disease needed several additional years to attain its highest impact (Cooke, 2002; Calvete et al., 2006). Lacking more precise knowledge about the short-term initial impact of RHD, its relationship with K was assumed to be that shown in Figure 1 (dotted line). RHD affected rabbit populations at densities higher than a threshold density value (D_{th}), a threshold that was necessary for effective virus transmission and continued persistence. The short-term initial impact of RHD was higher in denser populations (around K_2 values) and lower in populations around K_1 values.

Following the initial impact of RHD, rabbit populations tended to reach a long-term equilibrium with the disease, as predicted by the model (dashed line). In agreement with the outcomes of this model, in the range from K_0 to K_1 there was little variation in rabbit density. Rather, the highest long-term impact of RHD was reached in populations with medium-low pre-RHD density levels (around K_1 values). In contrast, disease impact was lower around K_0, due to the reduced transmission rates of the virus, and in high-density populations, around K_2 values, due to higher viral transmission rates and the consequent lower mean age of rabbit infection. When the mean age of infection is lower, a greater proportion of rabbits are infected at ages at which the RHD virus lethality is reduced, either by age resilience or the presence of maternal antibodies, resulting in a lower mortality from RHD at the population level.

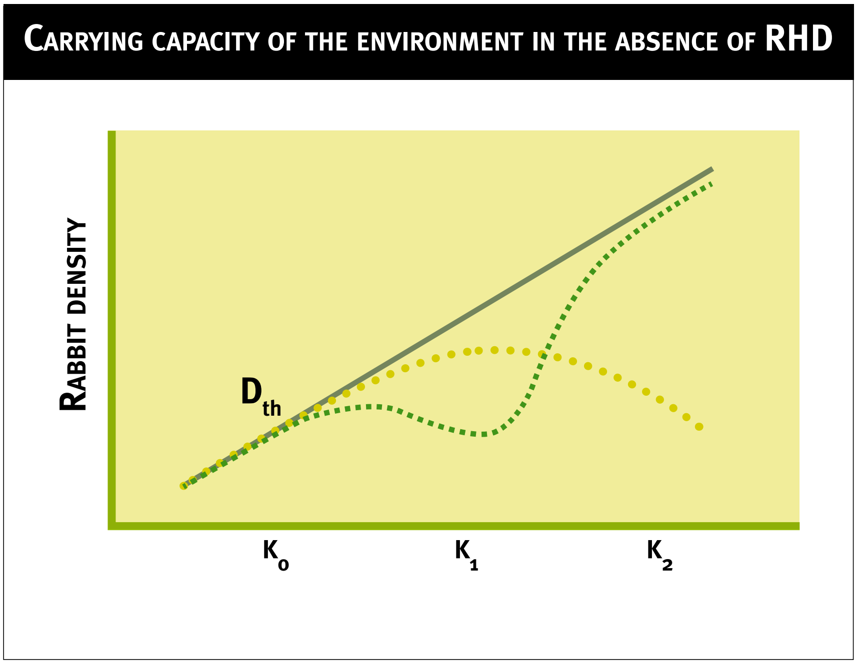

Figure 1. Theoretical relationship between rabbit density and habitat carrying capacity (K) before the arrival of RHD.
Continuous line: rabbit density before the arrival of RHD.
Dotted line: rabbit density after the short-term impact of RHD.
Dashed line: rabbit density at long- term equilibrium with RHD following the model of Calvete (2006a).
Dth: Threshold rabbit density for effective RHD-virus transmission.

Figura 1. Relación teórica entre la densidad de conejos y la capacidad de carga (K) del hábitat antes de la llegada de la enfermedad vírica hemorrágica (RHD).
Línea continua: densidad de conejos antes de la llegada de la RHD.
Línea punteada: densidad de conejos tras un corto periodo de impacto de la RHD.
Línea rayada: densidad de conejos bajo un equilibrio a largo plazo con la RHD, siguiendo el modelo de Calvete (2006a).
Dth: Umbral de densidad de conejos para un transmisión efectiva del virus de la RHD.

The dashed line in Figure 1 is actually an oversimplified way to represent the long-term impact of RHD, as it should be a cloud of points with a higher dispersion in relation to the vertical axis at values around K_1. This dispersion is determined by equilibrium between rabbit population productivity and mortality rates due to factors other than RHD. The transition of populations from short-term initial RHD impact (dotted line) to long-term equilibrium with the disease (dashed line) was likely to be highly dependent on both population dynamics and the life-history of each population. Differential transition times resulting from different population dynamics or the concurrence of factors limiting population growth at local scale may explain the currently highly variable pattern of rabbit distribution and population trends.

A extension of the theoretical predation model described by Pech et al. (1995) to incorporate the theoretical impact of RHD suggested that a gradient of possible interactions between RHD and predation impact could arise during this transition process (Calvete and Estrada, 2000). This way, the combined impacts of RHD and predation could reduce rabbit populations to lower densities than each one could do so by itself, which confirms the empirical evidence reported by Reddiex et al. (2002). In addition, the extended model suggested that the probability that rabbit populations can be maintained at low densities by predators due to a "predator-pit" phenomena could also increase in presence of RHD.

Theoretical insights into RHD and rabbit management strategies

Habitat management

The most frequent applied habitat management strategies have included scrub management to create natural pastures, construction of artificial refuges and creation of artificial pastures. However, due to the generally limited funding and logistic resources, habitat management strategies have been hardly maintained throughout time at local scale, and for example, many times artificial pastures are sowed only once at the start of management programmes.

Habitat management, is aimed to increase carrying capacity by mainly enhancing rabbit population productivity, therefore, habitat management would be the best way to promote rabbit populations in presence of RHD (Calvete, 2006a). However, following Figure 1, habitat improvement would not always yield a clear positive growth in populations. For example, we would consider a rabbit population at equilibrium with RHD located in a habitat at carrying capacity around K_0. In an attempt to enhance the rabbit population we would perform a habitat management programme that only increased habitat carrying capacity until values around K_1. It is obvious that the results would be fairly disappointing, as no positive change in rabbit density would take place, although epidemiology of RHD would have changed dramatically. This scenario could take place under poorly funded management programmes in which long-term habitat improvement was low or, also, under poorly designed programmes in which habitat improvement was high but only during a short time. Another example would be if habitat improvement was depending on the temporary (discontinuous) availability of funding and no habitat maintenance effort was performed when funding was unavailable. In this case, rabbit population would be subjected to recurrent perturbations from its equilibrium with RHD by repeatedly increasing the impact of the disease.

Control of mortality factors and harvesting of populations

In equilibrium state with RHD, reduction of mortality alone could be useful in only some populations. However, In situations in which a previous improvement of habitat has been performed, a temporary reduction of mortality would help to achieve a quicker increase in rabbit populations. The same suitability of mortality reduction, for example predation impact reduction, could arise in rabbit populations that are not in equilibrium with the disease, since in this case predation control could help rabbits escape from predator regulation and reach the equilibrium with RHD at higher densities.

Effective harvesting reduction should yield similar results on rabbit recovery as predation impact control. Moreover, outcomes of the RHD-model suggested that a decrease in rabbit density caused by excessive harvesting pressure (hunting or capturing for translocation) may increase the impact of RHD. Thus, a sustainable harvesting is essential to rabbit maintenance. Several theoretical approaches have been carried out to estimate the impact of harvesting on rabbit populations in Iberian Peninsula in absence of RHD (Angulo and Villafuerte, 2003; Calvete at al., 2005a). The discrepancies in results of both works are, however, clear evidence that, to date, we are still far from designing sustainable harvesting plans.

Vaccination campaigns

The use of vaccination as a disease prevention method in wild rabbits has increased greatly in the past several years. Currently, wild rabbits are vaccinated against myxomatosis and/or RHD with commercial vaccines developed for use in domestic rabbits. While, in general, the efficacy of these vaccines in eliciting immune responses in wild rabbits is relatively high (at least in the case of vaccines against RHD), there is some degree of variability (Calvete et al. 2005b; Cabezas et al., 2006). Wild rabbits must be vaccinated individually; in these vaccination campaigns, rabbits are captured by trapping or ferreting, vaccinated, and, ideally, released at the site of capture. In general, the success of vaccination campaigns has been negligible, although their effectiveness has been tested in very limited short-term field experiments, and only at the individual level (Calvete et al. 2004a; Calvete et al., 2004b). More recently, a transmissible recombinant vaccine has been developed to enhance the theoretical effectiveness of future vaccination campaigns (Torres et., al 2001).

The single evaluation of the effectiveness of vaccination campaigns at the population level has been only performed by modelling and only for the case of RHD (Calvete, 2006b). This theoretical approach showed that vaccination campaigns in populations at equilibrium with the disease could yield positive or negative population growth rates, depending on rabbit population dynamics and subsequent RHD dynamics. Negative growth rates were observed in simulated populations located in habitats with carrying capacity around or under K_1 (Fig. 1). Since low density populations are the main targets of vaccination campaigns, this model suggested that current immunisation programmes may have harmful effects on many managed rabbit populations. In populations subject to immunisation, prior knowledge of RHD dynamics should be therefore essential to minimise the risk of harmful effects.

Other different scenarios would arise if vaccination campaigns were carried out in populations that had not yet reached equilibrium with the disease. In this situation, vaccination, alone or in combination with other management tools, may facilitate a quicker recovery of populations. However, many doubts arise regarding the interference of vaccination on RHD epidemiology and how rabbit population would reach equilibrium with the disease just after vaccination cessation. It is important therefore to evaluate the outcomes of vaccination campaigns performed under these scenarios.

Translocations

Rabbit translocations are frequently performed for hunting purposes, with thousands of wild or captive-born individuals being translocated every year. In addition, rabbit translocations have dramatically increased in the last years due to conservation programmes that not only work towards recuperating rabbit populations, but also use rabbits as a means to provide temporary prey to predators. It has been largely shown that short-term rabbit mortality is a critical issue in translocations, however, the few surveys carried out to evaluate the medium- to long-term success of rabbit translocations have shown that survival is generally low and that some of the main mechanisms underlying this management strategy remain unknown (Moreno et al., 2004; Cabezas and Moreno, 2007).

Following model assumptions, the effects of these translocations on restocked populations should be similar to that described for vaccination campaigns against RHD (Calvete, 2006b). In some populations at equilibrium with the disease, therefore, restockings could yield negative population growth rates due to the increase of short- medium-term RHD-mortality. Taking into account that most of translocated rabbits are temporally immunized against RHD via vaccination before their release, successive translocation trials in populations in which restocking yielded negative growth rate would derive in a process of apparent competition mediated by disease. In this scenario, restocking would increase RHD mortality among native rabbits whereas vaccinated translocated rabbits (probably not as well adapted to the new environment) survive to disease and predominate over native rabbits, deteriorating population´s long-term fitness.

Another interesting scenario would arise when the restocking was carried out in an area where RHD-virus was absent or its transmission was severely reduced because the native population was at low density. In this case, when the new restocked population increased in density after one or several breeding seasons in absence of RHD-virus transmission, the casual reintroduction of the virus or the increase of its transmission rate subsequently to rabbit density increase would cause a RHD outbreak that would dramatically lessen population density again.

Conclusion

We are currently witnessing a process of re-distribution of wild rabbit populations in the Iberian Peninsula, mainly driven by habitat loss and introduced viral diseases. Many efforts are being carried out to enhance rabbit populations, trying to integrate hunting and conservation goals. Under the assumption of the theoretical approach to the rabbit-RHD system dynamics delineated here, however, it seems that the effects of applied management strategies would be uncertain. To date, there is still a considerable lack of knowledge about actual implications of RHD on rabbit ecology, and future research efforts should be devoted to evaluating which strategy or combination would yield the best results in terms of population improvements. However, until all these matters are assessed by future research, in the light of present assumptions on RHD epidemiology the next points could contribute to optimize wild rabbit recovery programmes.

Habitat managing aimed to increase habitat carrying capacity by mainly enhancing population productivity, either alone or in combination with mortality reduction, should be the main goal of rabbit recovery strategies. The primary mechanism by which habitat carrying capacity and rabbit productivity can be increased is managing habitat to increase refuge (mainly warrens) and the quantity and the quality of available food during breeding seasons.

Following the spread of RHD, many of the highest rabbit densities are currently found to be located primarily in agricultural landscapes mainly devoted to farming annual species on what rabbits preferentially feed during the breeding season. Therefore, replicating the landscape structure of traditional agricultural systems could help rabbit recovery. On the other hand, rabbit management programmes primarily based on scrub management to create natural pasture areas or the planting of crops that are cultivated only once, are probably not sufficient for long-term increases in population productivity. In consequence, they are not sufficient for reaching a population density at which RHD impact decreases, excepting in those cases in which mortality rates are simultaneously reduced.

The main non-stochastic mortality factors that affect rabbits (excluding RHD) are myxomatosis, predation and hunting. Myxomatosis is a viral disease that was introduced into Europe in the 1950s, and remains a major cause of mortality in wild populations. To date, however, there is no effective method to control mortality from this disease. On the other hand, predation impact reduction performed by means of predator control (e.g. fox removal) yields unclear results and has harmful effects on other predator species. Nevertheless, strategies aimed to facilitate predator avoidance, such as increasing warren density, changing landscape structure to minimize predation risk or building predator exclusion fences are measures that can be implemented for long-term reduction of predation impact in areas of concern in lynx conservation. Regarding hunting impact, effective reduction in hunting should have similar effects on rabbit recovery as predation control, with the advantage that hunting is a man-made activity that can be, or at least should be, easily reduced or even halted at the local or regional level.

For those populations that are currently at equilibrium with RHD, the long-term increase of habitat carrying capacity is the only method, either alone or in combination with other management strategies such as vaccination and/or restocking, for most negatively affected populations to reach stable densities similar to those observed prior to the introduction of RHD. In the absence of this long-term increase, the application of these other management strategies would be expected to yield only temporary positive or negative population growth rates, depending on the subsequent RHD dynamics. In contrast, in populations at densities lower than those obtained at equilibrium with the disease, the application of these management strategies for reaching equilibrium with the disease seemed to be more complex, due to possible interactions of disease with other factors, including predation, increasing, therefore, the uncertainty of results.

Until the managed rabbit population reached its final equilibrium with RHD at higher rabbit density, however, in many cases the population probably should experience a transitional process, with few changes in rabbit density but increased incidence of RHD. The duration of this hypothetical transitional process is not known, but it is probably highly dependent on population dynamics. Thus, a well-designed management programme should acquire the funding necessary for the long-term maintenance of the increased habitat carrying capacity (perhaps during 3-5 years minimum), independent of the short-term results on rabbit abundance.

Finally, for native populations that have not reached yet equilibrium with RHD, restocking may be an effective tool for recovering populations more quickly, especially in low-density populations regulated by predators. In these cases, however, apparent competition mediated by disease would have dramatic effects if population reinforcement was not sufficient to allow rabbits to escape predator regulation. Under this scenario, the combined effects of predation, apparent competition and the subsequent loss of population fitness could result in the extinction of rabbit populations. In addition, translocations carry an inherent risk of the possible transmission of new disease agents into release areas. Since their effectiveness is highly uncertain, due to the number of possible mechanisms that can cause failure or result in harmful effects on restocked populations, their application should be supervised and highly restricted in current rabbit promotion programmes in order to avoid abuse in their use as a rabbit management tool.

On the other hand, once the newly restocked population increased in density, the accidental introduction of the RHD virus would cause an outbreak of the disease, which would again dramatically lessen population density. To prevent this and to assure that the newly introduced rabbit population grows in the presence of the disease, it is necessary that rabbits and RHD-virus be translocated simultaneously. Since reservoirs and chronically infected rabbits may eliminate RHD for long periods of time, the joint translocation of rabbits and virus should be performed by translocating a relatively high number of rabbits from populations that already have reached equilibrium with RHD at high population density, and in which a high proportion of rabbits have already been infected by the virus. Conversely, the translocation of captive-born rabbits without previous contact with the virus, or the translocation of rabbits from populations in which virus transmission is reduced (low density populations), may be the worst option to attain this goal.

Nevertheless, RHD virus transmission in the wild probably depends not only on rabbit population dynamics and density but also on the density of infective viral particles in the environment. The latter, in turn, may depend on rabbit density and RHD dynamics at an unknown lag time; e.g. virus persistence in carcasses of RHD-killed rabbits in warrens. Thus, even when translocating a large number of rabbits from high density populations, it is unlikely that the newly transplanted population will immediately exhibit an equilibrium with RHD similar to that of the source population, even when the habitat and density of both populations are nearly identical. It is more likely that the new population would require several yearly cycles to reach this equilibrium, otherwise translocation would fail in the medium-term. One exciting option would be the controlled release of RHD virus during translocation and during the growth process of the new population, until the population and the virus reached equilibrium. This management practice could be applied independent of the origin of the translocated rabbits and may reduce the uncertainty of success of translocations and their dependence on initial RHD dynamics.

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If this is not done, future ages will certainly look back upon us as a people so immersed in the pursuit of wealth as to be blind to higher considerations. They will charge us with having culpably allowed the destruction of some of those records of Creation which we had it in our power to preserve; and while professing to regard every living thing as the direct handywork and best evidence of a Creator, yet, with a strange inconsistency, seeing many of them perish irrecoverably from the face of the earth, uncared for and unknown.

Alfred Russell Wallace
(1823-1913)


Photo: Antonio Sabater

A new Strategy for the Conservation of the Iberian Lynx

Una nueva Estrategia para la Conservación del Lince Ibérico

Javier Calzada, Luis Mariano González, J. Nicolás Guzmán and Borja Heredia

Resumen

Recientemente, el Grupo de Trabajo del Lince Ibérico aprobó una nueva Estrategia para la Conservación del Lince Ibérico. Esta Estrategia surge en un marco de trabajo diferente al que existía cuando se aprobó la primera Estrategia para el Lince Ibérico, en 1999. El lince ibérico está en la peor situación demográfica en la que ha estado a lo largo de su historia pero, por otra parte, nunca antes se había contado con tantos recursos humanos y económicos, ni con tanta atención política y preocupación social por la especie. La meta final de la Estrategia es que el lince ibérico sea una pieza funcional del monte mediterráneo. Para ello, la recuperación de la especie pasa tanto por gestionar con éxito las poblaciones que quedan, como por la elección y adecuación de áreas donde desarrollar proyectos de reintroducción que conduzcan al establecimiento de nuevas poblaciones silvestres. La nueva Estrategia marca un camino a seguir en el proceso de conservación y recuperación del lince ibérico, estableciendo metas numéricas concretas a lograr en un plazo determinado. Estas incluyen: 1) Estabilizar las poblaciones existentes luchando contra las amenaza para la especie. 2) Aumentar el número de linces que viven en las poblaciones silvestres. Se pretende conseguir que, para el año 2011, el lince pase de estar catalogado como "en peligro crítico de extinción, CR" a "en peligro, EN". 3) Aumentar el número de poblaciones silvestres, y lograr que para el año 2020 la especie deje de "estar en peligro, EN", para pasar a ser considerada "vulnerable, VU". Según el contexto español, la primera meta se debería conseguir a través de los Planes de Recuperación Autonómicos, que deben adoptar las líneas marcadas en la Estrategia y desarrollarlas completa y competentemente. La segunda meta es hacer crecer las poblaciones de linces hasta que, al menos una de ellas, supere los 50 individuos maduros (sin que éstos supongan más del 90% de todos los linces maduros silvestres). Si se considerase necesario, se recomienda desarrollar "Proyectos de Refuerzo e Intercambio Poblacional" para contribuir a aumentar la abundancia de linces en las poblaciones existentes. La tercera meta es conseguir que el número total de linces maduros presentes en la naturaleza sea superior a los 250 individuos maduros y que las poblaciones no muestren signos de declive. La única manera de lograrlo es mediante "Proyectos de Restauración del Hábitat y Proyectos de Reintroducción" en todas las Comunidades Autónomas de España donde el lince ibérico está presente o estuvo presente hasta hace poco.

Palabras clave

Planes de conservación, Plan de recuperación de especies, *Lynx pardinus*

Abstract

A new Strategy for the Conservation of the Iberian Lynx *(Lynx pardinus)* has recently been approved by the Spain's maximum authorities in Environmental Policy at the Sectorial Conference for the Environment. The new Strategy has been developed in a different working framework from the one that led to the first Strategy for the Conservation of the Iberian Lynx in 1999. The demographic situation of the Iberian lynx has never been worse. However, there have never been so many human and financial resources available, and the species has never been the focus of so much public attention and concern. The ultimate goal of the Strategy is to ensure that the Iberian lynx becomes a functional part of the Mediterranean scrubland habitat again. To this end, the recovery of the species involves both successfully managing the remaining populations and choosing and restoring areas to carry out reintroduction projects that will lead to the establishment of new wild populations. The new Strategy has set a roadmap for the conservation and recovery of the Iberian lynx, as well as specific numerical targets that must be met in a given period of time. These targets include: 1) Stabilize the populations by combating the causes of threat to the species; 2) Increase the number of individuals in the wild populations so that the Iberian lynx can be downlisted from Critically Endangered (CR) to Endangered (EN) by 2011; and 3) Increase the number of wild populations, so that the species can be downlisted from Endangered (EN) to Vulnerable (VU) by 2020. According to the Spanish system, the first target should be achieved through Regional Recovery Plans, which must adopt the guidelines established in the National Strategy and develop them fully and efficiently. Achieving the second goal requires increasing the number of individuals in the lynx populations until at least one of them has more than 50 mature individuals, which must not amount to more than 90% of all the wild mature individuals. If necessary, "Restocking" and "Population Exchange Projects" are recommended to help increase the abundance of lynxes in the existing populations. To achieve the third target, the combined wild populations must comprise at least 250 mature individuals and not show signs of decline. This could only be attained through "Habitat Restoration and Reintroduction Projects" carried out in all the Autonomous Communities of Spain where the Iberian lynx occurs or occurred until recent times.

Keywords

Conservation planning, Species Recovery Plan, *Lynx pardinus*

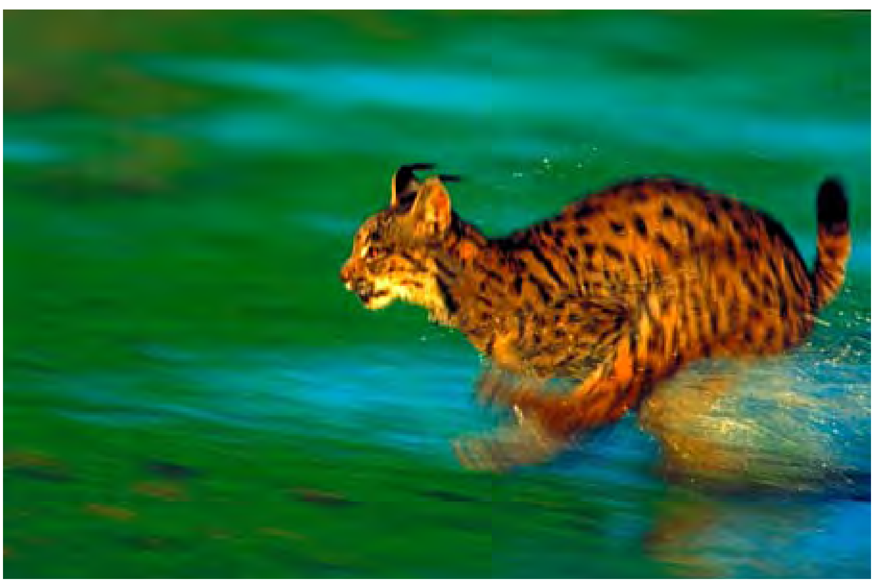

Photo: Antonio Sabater

A new Strategy for the Conservation of the Iberian Lynx

Javier Calzada, Luis Mariano González, J. Nicolás Guzmán and Borja Heredia

Introduction

Spain is a unitary state which, in fact, functions almost like a decentralized federation of regions, called "Autonomous Communities". Each of the 17 Autonomous Communities has powers to manage its own interests with a great deal of independence. The Environment is one of the areas over which Autonomous Communities have full powers. Therefore, every region is in charge of nature conservation and protects its resources with total independence. When an Action Plan is needed for the conservation of an endangered species, each Autonomous Community must draw up, adopt and implement its own Plan, which must include the necessary measures for the protection of the species in its territory (Article 31.6 of Act 4/89). In this case, the Iberian lynx *(L. pardinus)* has been listed as Endangered on a national level since 1990 (Royal Decree No. 439/90). Five Autonomous Communities in Spain where the Iberian lynx occurs or occurred until recently –Andalusia, Castille-La Mancha, Castille y Leon, Extremadura and Madrid– must therefore draw up and implement their own Species Recovery Plan to eliminate the risk of extinction of the species in their territory. However, for the species to recover successfully, Regional Plans must be designed and implemented in a coordinated and consistent way. This is done by means of National Conservation Strategies, whose purpose is to coordinate and combine the efforts of all the relevant departments and levels of government to achieve a recovery of the species.

The 2008 Strategy for the Conservation of the Iberian Lynx

The first National Conservation Strategy for the conservation of the Iberian lynx was adopted in 1999 (Dirección General de Conservación de la Naturaleza, 1999). Although the Strategy was planned to be valid for an indefinite period of time, it was decided that it should be reviewed annually and updated every four years. In 2003 no updates to the Strategy were considered necessary. However, new data available on the species –especially on its abundance, distribution and annual productivity– and the new circumstances of its management –the launch of new LIFE projects, and the Iberian Lynx *Ex situ* Conservation Programme– led the Iberian Lynx Working Group (a group of Iberian lynx experts of the national and regional environmental departments) to review and agree on a new

	1999	2007
Annual surveys were carried out	No	Yes
Estimated population size (individuals over 1 year old)	* 1,136	*** 107
No. of breeding females	* 350	*** 32
Area of occupancy	* 14,569 km²	** 2,200 km²
Area of occupancy where breeding occurs	* 10,669 km²	** 925 km²
No. of breeding populations	* 9	** 2
Range States of the Iberian lynx	* Spain and Portugal	** Spain
Spanish Autonomous Communities (administrative regions) with breeding populations	* 5	** 1
Conservation status (IUCN)	Endangered	Critically Endangered
Prior National Strategy	No	Yes
Recovery Plans approved	0	2 (Regions of Extremadura and Castille-La Mancha)
Action Plan for the Iberian Lynx in Europe	No	Yes
Iberian Lynx *Ex situ* Conservation Programme	No	Yes
LIFE Projects devoted, at least partly, to the species	2	6
Management teams exclusively devoted to the species	No	Yes
Knowledge of the specie's ecology and biology	Yes	Yes
Management Plan for the Iberian Lynx in Doñana National Park	Yes	Yes
SACs for the Iberian lynx (Natura 2000 Network)	No	SCIs approved

Table 1. Differences in conservation status, knowledge and management of the species between 1999, when the first Strategy for the Conservation of the Iberian Lynx was adopted, and 2007, when the second Strategy was drawn up. Demographic data are taken from the following studies: *Rodríguez and Delibes, 1990; ** Guzmán et al., 2005; * CMA-Junta de Andalucía (Regional Ministry for the Environment, Andalusian Regional Government), 2006.**

Tabla 1. Diferencias en el estado de la conservación, conocimientos y manejo de la especie entre 1999, año en el que fue adoptada la primera Estrategia para la Conservación del Lince Ibérico, y el año 2007, cuando se redactó la segunda Estrategia. Los datos demográficos proceden de los siguientes estudios: *Rodríguez y Delibes, 1990; ** Guzmán et al., 2005; * CMA-Junta de Andalucía 2006.**

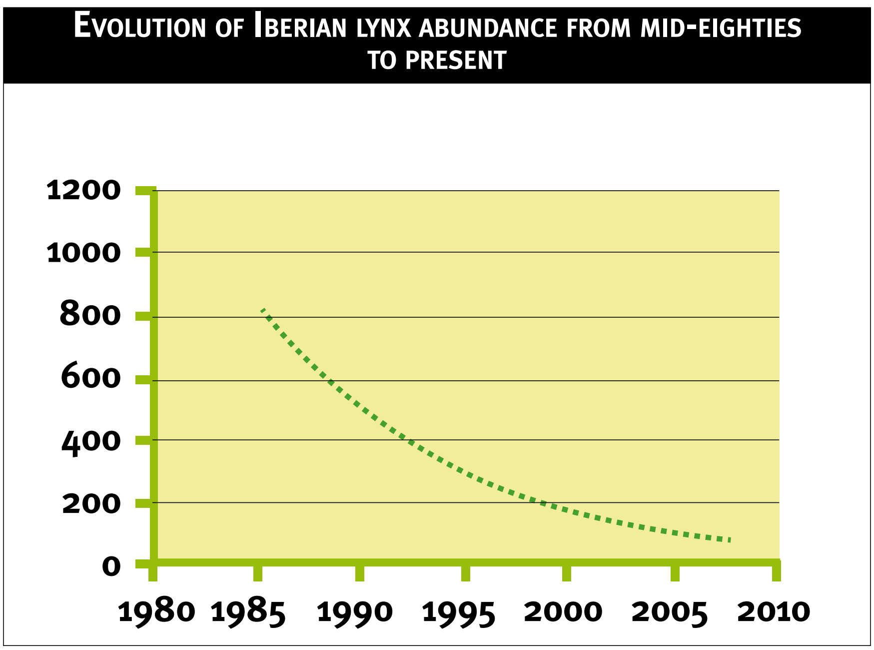

Figure 1. Evolution of Iberian lynx abundance from mid-eighties to present. The graph only considers abundance of individuals over 1-year-of-age (i.e., it does not take into account young-of-the-year). Estimates after 2001 are related exclusively to Andalusia. Data based on Rodríguez and Delibes, 1990; Castro and Palma, 1996; Guzmán et al., 2005; ; and Simon et al this book.

Figura 1. Evolución de la abundancia del lince ibérico desde mediados de los años 80 hasta el presente. El gráfico sólo tiene en cuenta la abundancia de ejemplares mayores de 1 año de edad (es decir, no incluye los cachorros ni juveniles del año). Las estimaciones posteriores al 2001 se refieren exclusivamente a Andalucía. Los datos están basados en los estudios de: Rodríguez y Delibes, 1990; Castro y Palma, 1996; Guzmán et al., 2005; ; y Simón et al, este libro.

document which has been adopted by the Iberian Lynx Working Group in 2007, and, in 2008, has been approved by the Spain's maximum authorities in Environmental Policy in the Sectorial Conference of the Environment.

A different state of affairs

The Iberian lynx is facing its worst demographic situation ever (Delibes and Calzada, 2005). In just a few years the population has declined from over a thousand individuals over 1-year-old distributed in about 10 subpopulations in different regions of Spain and Portugal (Rodríguez and Delibes, 1990; Castro and Palma, 1996) to less than two hundred individuals, most of which are grouped into two subpopulations in the region of Andalusia (Guzmán et al., 2005. See Table 1 and Figure 1). The Atlas and Red Book of Mammals of Spain, *Atlas y Libro Rojo de los Mamíferos de España* (Calzada et al., 2007) shows that, in only 16 years –between 1985 and 2001– the area of occupancy of the species has decreased by 87%, its breeding area has shrunk by 93%, the number of breeding females has declined by more than 90%, and the number of individuals over 1-year of age has fallen under 86%. These data led the IUCN to list the Iberian lynx as Critically Endangered, the highest category of threat for a species (IUCN, 2002). More recent population data obtained between 2001 and 2006 indicates some improvements (CMA-Junta de Andalucía, 2006). For instance, the Sierra Morena population is beginning to increase, while Doñana remains stable (Simón et al., this book).

Different players

In recent years, the political, social and economic attention given to the species has improved. In 1999, when the first Strategy for the Conservation of the Iberian Lynx *(L. pardinus)* in Spain was adopted, it was the first document drawn up to plan the management of the species. There was only one honorable exception, however: the Management Plan for the Iberian Lynx in Doñana National Park, drawn up many years before (Delibes et al., 1986). To date, two of the five Regional Recovery Plans have been approved, as well as an Action Plan for the species adopted by the Council of Europe, and a National Iberian Lynx *Ex situ* Conservation Programme (Delibes and Calzada, 2005). In 1999, the Sites of Community Importance (SCIs) for the Iberian lynx of the Natura 2000 Network had not been proposed yet. Today, the list has already been approved. In 1999 no attempts to breed the species in captivity had succeeded, whereas the species now breeds regularly every year in specialized centers. LIFE Projects involving the species had only been granted on two occasions, whereas the sixth one is currently under way. As a matter of fact, the latest LIFE Nature Project (see Simón et al., this book) is the most generous one ever granted so far in Europe. Besides, today there are several professional teams exclusively devoted to the management of the Iberian lynx, which was not the case in 1999 (Table 1).

In short, the current framework is totally different to the context in which the first Strategy was adopted. We know that the status of the Iberian lynx is the worst ever, but never before had so many resources been available for its management and conservation; the species has never received as much political attention, the public has never been so aware of and sensitive about its difficult plight, and never before have so many people been involved in the study, management and conservation of the Iberian lynx.

A new global commitment

The ultimate goal of the Strategy for the Conservation of the Iberian Lynx is "**to make the Iberian lynx a functional part of the Mediterranean scrubland habitat again**". The intention is not to maintain the lynx in captive populations, or "unnatural" wild populations where the resources of the species need to be supplemented forever.

The new Strategy for the Conservation of the Iberian Lynx *(L. pardinus)* acknowledges the undeniable fact that the recovery of the lynx cannot be achieved in the territory of just one Autonomous Community. This is due to the biological and ecological characteristics of the species and its habitat requirements (Delibes, 1980; Palomares, 2001; Palomares et al., 2000, 2001). This, added to the certainty that only two breeding populations of the species remain –both in Andalusia–, means that a solid, close and honest relationship is necessary between all the different departments and levels of government to achieve a recovery of the species.

The recovery of the species clearly involves both successfully managing the remaining populations and choosing and restoring areas to carry out reintroduction projects that will lead to the establishment of new populations of Iberian lynx.

Overview of the Iberian lynx conservation and recovery process in the National Strategy

According to the Strategy, the conservation and recovery process of the Iberian lynx must absolutely involve the following steps:

Stabilize the existing populations. If the declining trend continues, the species will become extinct in the wild. To avoid this, it is urgent to stabilize the remaining wild populations. This implies eliminating the causes of threat that have been described (Dirección General de Medio Natural y Política Forestal, 2009). It must be highlighted that an increase in the abundance or range of a species in itself does not imply that it is sustainable (Clark et al., 2002). The recovery of the species can only be achieved by eliminating actual threats. However, even if all the threats affecting the wild populations disappeared and the populations remained stable, they might still become extinct because of their very small size. This could simply be caused by demographic stochasticity, an environmental disaster and/or due to problems derived from poor genetic diversity. It is therefore vital to increase the number of individuals in the remaining populations, to create new populations and to promote genetic exchange between all of them.
To meet these commitments, the Strategy has drawn up a clear roadmap with specific numerical targets that are to be achieved in specific timeframes. This is an innovative feature of the National Strategy, which also shows an attitude of political commitment. It is the first time that a strategic plan for the conservation of a species in Spain has taken on such clear, specific and measurable commitments.

Increase the number of individuals in the wild populations. The IUCN requirements for the Iberian lynx to be downlisted from Critically Endangered, CR C2a(i), to Endangered, EN, are as follows: until the population is stabilized, at least one of the two subpopulations must contain more than 50 mature individuals –adults capable of breeding–, none of the subpopulations must contain more than 90% of all mature individuals, and, in any case, there must not be extreme numerical fluctuations. Therefore, the first numerical target of the recovery process must be to increase the number of individuals in the lynx populations until at least one of them has more than 50 mature individuals*.These 50 individuals must not amount to more than 90% of all the wild mature individuals. To attain this goal, the source patches in each population should be enhanced and allowed to expand. The year 2011 was set as a deadline to meet this target at the meeting of the Iberian Lynx Working Group held on 27 March 2007, where the Strategy was discussed and adopted**.

Increase the number of wild populations. The next step will be to get the Iberian lynx downlisted from Endangered, EN, to Vulnerable, VU. For this to happen, the wild population must contain more than 250 mature individuals* and not show signs of decline. To achieve this, the habitat must be restored so that it can be used by the species and new lynx populations must be created through reintroduction projects. The National Strategy has set the year 2020 as the deadline to meet this specific target**.

In order to reach the specified goals, it is obvious that a stable captive population must be maintained to guarantee that the species will not completely disappear if the efforts to conserve the wild populations fail. In addition to providing a safety net for the species, the captive population should be able to provide individuals for Reintroduction and Restocking Projects.

**: It is difficult to calculate the number of mature individuals of wild Iberian lynx according to the definition used by the IUCN to set its categories of threat. However, a survey of the number of females holding a territory is made every year. In this species, the territory of a female is not equivalent to two mature individuals using IUCN criteria. This is because the social organization of the lynx is not always structured into monogamous pairs, and not all individuals with a territory actually breed. From a conservative approach, the target of reaching 50 mature individuals could be considered met if there were 25 breeding females; the target of reaching 250 mature individuals could be considered met if there were 125 breeding females.*

*** : Once the target is met, a taxon may be moved from a category of higher threat to a category of lower threat if none of the criteria of the higher category has been met for five years or more (IUCN 2001).*

How to meet the commitments

The first priority is to minimize the threats to the species. Unless this condition is met, the Strategy will never succeed. The Strategy contains a summary of the threats leading the species to extinction and the basic actions proposed to combat them. Yet, it is only an outline of the actual task of planning the management and conservation of the species. It is through **Regional Recovery Plans** that Autonomous Governments must adopt the guidelines set in the Strategy and develop them fully and efficiently. Each objective included in the Strategy must be broken down into more specific, realistic and measurable goals and actions related to it. These goals and actions should be adopted in the Plan with a deadline and information on who will be in charge and what budget has been planned for their implementation. More importantly, provision must be made for monitoring the actions and reviewing the Plan. A monitoring procedure is particularly necessary to check the degree of compliance with the goals; it should include a timeframe of reviews with the possibility of adjusting the actions –and even the Plan itself– to ensure that the goals are reached and thus that the species is on its way to recovery–The monitoring procedure must be designed so that it is possible to differentiate between a defect of the Plan –if the actions are implemented but do not lead to meeting the goals as expected– and a lack of compliance with the Plan– if the actions are not implemented. Regional Recovery Plans must be developed as the main tool to fight against the threats to the species and achieve the specie's recovery. Recovery Plans, however, are not the only executive plans for species conservation in Spain. There are many other local and regional action plans that are mainly targeted to reducing threats to the species, ranging from the Management Plan for the Iberian Lynx in Doñana National Park to the LIFE Project currently under way. However, it is through Recovery Plans that regional governments must plan and design the general implementation of the recovery process in each Autonomous Community. They provide an axis for all the other Plans to join in.

After minimizing the direct threats to the Iberian lynx, the most important action for the conservation of the species in the wild is to increase the number of individuals in the existing wild populations. This is aimed at protecting the species from a direct threat of extinction due to stochastic, demographic and/or environmental factors. The chances that a lynx population will disappear are inversely related to its size. In fact, over the last 30 years, all the populations of Iberian lynx with less than 50 individuals have disappeared, except for the one in Doñana (Rodríguez and Delibes, 2002, 2003). The remaining populations must grow and expand. Sometimes it may be necessary to increase the carrying capacity of source patches by "compacting" territories or by implementing **Restocking and Population Exchange Projects** (Simón et al., this book). With such few populations of Iberian lynx, the conservation of the species is unlikely to be guaranteed even if we increase the number of individuals they contain. The reason for this is that these populations might disappear simply because of environmental stochasticity –e.g., a fire, a flood or the outbreak of a new disease in the population. This risk, as well as others, would decrease with the creation of new populations through **Reintroduction Projects**. In fact, representatives of the five Spanish Autonomous Communities have agreed on the goal of achieving a new breeding population of lynx with long-term viability in each of these regions; another one will is planned to be established in Portugal (Vargas et al., this book). The commitment acquired through the National Strategy establishes that each Autonomous Community must have a Reintroduction Project under way by 2012. This implies, at least, finding and selecting a number of potential areas that can be made suitable if necessary via **Habitat Restoration Projects** so that Reintroduction Projects can be implemented with high chances of success.

One of the main tools to achieve reintroduction goals is through the **Iberian Lynx *Ex situ* Conservation Programme**, which is included in the National Strategy. The main goal of the Programme is to contribute to the specie's recovery by providing specimens born and raised in captivity fit for reintroduction into the wild (Vargas et al., 2008; Vargas et al., this book). Besides, it also offers protection in the event of a catastrophic extinction. In Andalusia there are already two dedicated Breeding Centers and a partner centre for breeding Iberian lynx in captivity. The Ministry of the Natural, Rural and Marine Environment has agreed to fund one new breeding center per Autonomous Community that has expressed and open commitment to recover the Iberian lynx in areas of historical occupancy. *In situ* and *ex situ* efforts are thus linked by MOUs that tie the building of breeding centers to preparation of habitat for lynx reintroductions. Besides the two dedicated centers that are already established in Andalusia, new centers are scheduled to be opened in Extremadura and Castille-

Steps	Goals	Main management tool
1) Stabilize current populations	Eliminate threats	Recovery Plans
2) Increase number of individuals in populations	Strengthen populations	Restocking and population exchange projects *Ex situ* Conservation Programme
3) Create new populations	Restore habitat Reintroduce lynx	Habitat restoration projects Reintroduction projects *Ex situ* Conservation Programme

Conditions for success:

- All departments in charge of environmental issues at any level of government must believe in the recovery process and participate in an active, coordinated and efficient way.
- Research applied to the conservation of the species must be promoted – especially studies aimed at assessing the outcome and efficacy of the various Plans and Projects that must be carried out.
- Society needs to be convinced of the need to conserve the Iberian lynx. The recovery process of the Iberian lynx will be difficult, long and costly, and unless there is enough support from society it will be impossible to implement it successfully.

Table 2. Steps established for the Recovery Process in the Strategy for the Conservation of the Iberian Lynx *(Lynx pardinus)*, main goals and management tools planned for the recovery of the species; conditions for success.

Tabla 2. Pasos establecidos para el Proceso de Recuperación dentro de la Estrategia para la Conservación del Lince Ibérico *(Lynx pardinus)*, objetivos principales y herramientas de manejo previstos para la recuperación de la especie; condiciones necesarias para el éxito.

La Mancha (Vargas et al., this book). These centers will each maintain approximately 8 Iberian lynx breeding pairs, that will be exchanged between facilities according to genetic criteria and that will also provide lynxes for reintroduction projects. Portugal has also joined in by building a breeding center in the Algarve region (Vargas et al., this book) while committing to sign up agreements to prepare areas for future reintroduction efforts. Table 2 shows an outline of the steps, main goals and management tools planned for the recovery of the species. This framework was established through the overview of the Recovery Process of the Strategy for the Conservation of the Iberian Lynx.

For this Strategy to be successful, all official departments in charge of environmental issues at any governmental level must believe in the recovery process and participate in an active, coordinated and efficient way. It will be necessary to promote research applied to the conservation of the species (Palomares, this book) –especially studies aimed at assessing the outcome and efficacy of the various Plans and Projects that must be carried out. However, the most important thing to do is to convince society of the need to conserve the Iberian lynx. The recovery process of the Iberian lynx will be difficult, long and costly, and unless there is enough support from society it will be impossible to implement it successfully (Jiménez, this book).

Acknowledgements

The Strategy for the Conservation of the Iberian Lynx was drawn up thanks to the valuable contributions of the representatives of the regions of Andalusia, Castille-La Mancha, Castille y Leon, Extremadura and Madrid, the Spanish Ministry of the Natural, Rural and Marine Environment, the Iberian Lynx *Ex situ* Conservation Programme, Doñana Biological Station-CSIC (Spanish Council for Scientific Research), and the following NGOs: Ecologistas en Acción, Fundación CBD-Hábitat, SECEM and WWF/Adena. We wish to express our gratitude to all of them.

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We will either find a way, or make one.

Hanibal
(247-183 aC)

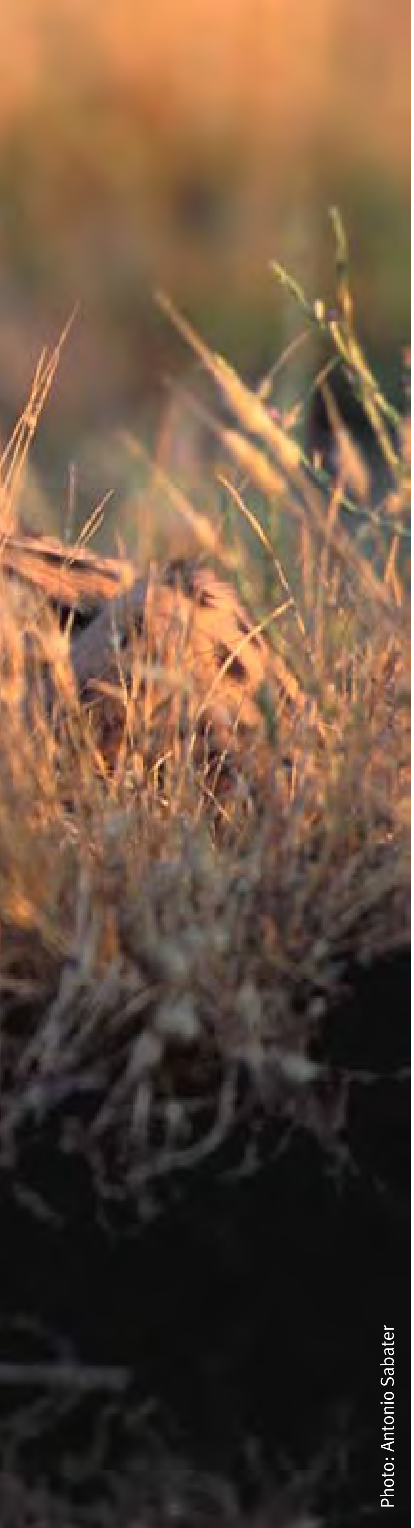

Photo: Antonio Sabater

Conservation status and Action Plan for the recovery of Iberian lynx populations in Portugal

Estado de conservación y Plan de Acción para las poblaciones de lince ibérico en Portugal

Pedro Sarmento, Joana Cruz, Catarina Ferreira, Pedro Monterroso, Rodrigo Serra, Pedro tarroso and Nuno Negrões

Resumen
Durante la segunda mitad del siglo XX, la población portuguesa del lince ibérico se distribuía en tres núcleos principales: el Valle de Sado, Malcata y Contenda-Barrancos. A lo largo de las décadas siguientes, las poblaciones de lince sufrieron una regresión significativa en estas áreas, probablemente debido a las prácticas silvícolas en gran parte del hábitat disponible, así como a la escasez de conejos a consecuencia de la introducción de enfermedades víricas. Según el último censo de linces realizado entre los años 2002 y 2004, el lince actualmente se encuentra al borde de la extinción en Portugal. El Instituto para la Conservación de la Naturaleza y Biodiversidad, consciente de la situación crítica de esta especie en Portugal, adoptó un Plan de Acción para la Conservación del Lince Ibérico con el fin de disponer de un método coherente y eficaz para la conservación del lince en territorio portugués. Este Plan de Acción se está aplicando en todos los espacios de la Red Natura 2000 situados en el área de distribución histórica del lince y que, a su vez, ofrecen condiciones apropiadas para la presencia potencial de la especie. También se están teniendo en cuenta aquellos espacios con características que podrían ser optimizadas para la supervivencia del lince. El Plan tiene como objetivo realizar actividades estratégicas de preparación para la reintroducción, con el fin de posibilitar, a largo plazo, la reintroducción del lince ibérico en áreas de distribución histórica. El Plan integra varios proyectos de conservación que están actualmente en curso y que incluyen la regeneración del hábitat y la recuperación de las especies de presa, así como la construcción de un centro de cría que se integraría en el programa global de Conservación *Ex situ* del Lince Ibérico.

Palabras clave
Restauración de hábitat, conservación *in situ*, recuperación de conejos, reintroducción, cría en cautividad

Abstract
From the first half of the 20th century onwards, the Portuguese Iberian lynx population was distributed in three major nuclei: Sado Valley, Malcata and Contenda-Barrancos. In the following decades these areas were subjected to a process that culminated in the specie's considerable regression, probably as consequence of a major allocation of potential habitat to forestry and of prey scarcity as a result of viral diseases. The most recent survey, conducted from 2002 till 2004, revealed that the species is presently on the verge of extinction. Aware of the critical situation of the Iberian lynx in Portugal, the Institute of Nature Conservation and Biodiversity developed a Conservation Action Plan for the Iberian lynx in order to provide a consistent and effective approach to conserve the species in Portuguese territory. The on-going Action Plan is being applied in all Natura 2000 Sites, located in the lynx historical distribution that present suitable characteristics for the species potential presence or landscape features that can be optimised for lynx survival and that can be relevant for the species life-cycle. The goal of this Plan is to apply pre-release strategic reintroduction activities in order to make it possible, in the long-term, the reintroduction of Iberian lynx. Integrated in the plan, there are several ongoing conservation projects, which include habitat and prey restoration and the construction of a breeding centre that will be integrated within the overall Iberian Lynx *Ex situ* Conservation Programme.

Keywords
Habitat restoration, *in situ* conservation, rabbit recovery, reintroduction, captive breeding

Photo: José María Pérez de Ayala

Conservation status and Action Plan for the recovery of Iberian lynx populations in Portugal

Pedro Sarmento, Joana Cruz, Catarina Ferreira, Pedro Monterroso, Rodrigo Serra, Pedro Tarroso and Nuno Negrões

Introduction

A recent national survey has brought to light the critical status of the Iberian lynx (*Lynx pardinus),* in Portugal (Sarmento et al., 2009). Field evidence showed that the species is currently in a pre-extinction stage and this scenario was re-enforced by a similar operation conducted in Spain (Guzmán et al., 2005). Consequently, the Iberian lynx was classified as a Critically Endangered species under IUCN criteria in the updated version of the Portuguese Red Book of Vertebrates and in the IUCN Red List of Threatened Species (Cabral et al., 2005; IUCN, 2007). The Iberian Lynx is a vulnerable species because of its dependence on only one prey, the rabbit *Oryctolagus cuniculus*, and its narrow habitat spectrum (Delibes et al., 2000; Calvete, this book; Calzada et al., this book). The decline in rabbit populations, caused by habitat changes and viral diseases, has had a direct impact on lynx numbers as well as high rates of non-natural mortality and habitat destruction (Ferreras et al., 1992; Guzmán et al., 2005). As a result of this process, currently lynxes are essentially constrained to two isolated populations (Doñana National Park and Cardeña-Andujár), both located in the Spanish Autonomic region of Andalusia (Guzmán et al., 2005; Calzada, this book). Potentially, dispersing animals could occasionally appear in Toledo, Guadalmez river, Central Sierra Morena and Sierra del Relumbrar y Alcaraz (Alda et al., 2008).

The current dramatic status of Iberian lynx populations, associated to its ecological importance were the basis for its classification as a top priority species for conservation in Portugal. This situation was the catalyzer for the establishment of a National Conservation Action Plan (Sarmento et al., 2005), which was legally approved in 2008 (Portuguese Government Issue 12697/2008, May 6th) and whose ultimate goal is to promote the specie's recovery and conservation in national territory through the restoration of historic population nuclei through reintroduction. In this paper, we discuss the recent evolution of lynx populations in Portugal and describe the on-going and proposed conservation measures conceived to reverse the specie's decline and assure its long-term conservation in the country.

Recent history – past and present situation

A recent geographic analysis of the distribution of museum specimens, "naturalised" lynxes, skins and photographs of hunted animals, using data from 1950 to the early 1990s, put into evidence the existence of three potentially major historical nuclei: Sado Valley (currently Cabrela-Monfurado Natura 2000 Sites), Malcata and Moura-Barrancos (Sarmento et al., 2009; see Figure 1 for geographic locations). These nuclei probably constituted the Portuguese Iberian lynx core areas in the mid-20th century. It seems that these areas were subjected to a degradation process, as a consequence of a major allocation of potential habitat to forestry and of prey scarcity as a result of viral diseases that has led the Iberian lynx towards an extinction vortex since the 1960s. By the mid 1970s, Palma (unpublished data) performed the first Portuguese scientific study on the species ecology in Serra da Malcata and attempted to describe the lynx distribution range and density in the country. A national total population of 50 individuals was estimated, which, according to the author, were distributed through Serra da Malcata, Sado Valley and Algarve.

More recently, a national lynx status survey was undertaken from 1994 to 1997 (Ceia et al., unpublished data), under the coordination of the Institute of Nature Conservation (ICN, re-designated in 2007 as the Institute of Nature Conservation and Biodiversity, ICNB), following the same criteria and methodology of the 1988 lynx Spanish survey (Rodríguez and Delibes, 1990), which had been based on personal interviews and questionnaires. Five lynx populations were estimated, distributed throughout 2,400 km^2 and harbouring a total population of 40-53 individuals. Three out of the five identified areas (Serra da Malcata, Serra de São Mamede and Guadiana) corresponded to the western extensions of the Sierra de Gata, Sierra de San Pedro, and Western Sierra Morena Spanish populations, respectively (see Figure 1).

In the beginning of this decade a new census was conducted in an Iberian cale (Guzmán et al., 2005; Sarmento et al., 2009), using only reliable methods that could undoubtedly confirm the species occurrence. For Portugal, no evidences of the species presence were obtained and the study revealed that the Iberian lynx is presently in the verge of extinction. In recent years, reliable information is becoming rarer. The last scat confirmed by DNA analysis as being from lynx origin was collected in Moura-Barrancos in late 2001 (Santos-Reis, unpublished data), and from this date forward, no other reliable information was obtained regarding the occurrence of the Iberian lynx in Portugal. This scarcity of evidence together with low habitat suitability in most of the lynx's historical range, points to a catastrophic situation for the species in Portugal (Sarmento et al., 2009). Although we cannot confirm extinction, the scenario is highly pessimistic.

On-going and future conservation actions

Aware of the considerable difficulties pointed above, the ICNB, developed a Conservation Action Plan for the Iberian lynx (CAPIL) in order to provide a consistent and effective approach to conserve the species in Portuguese territory (Portuguese Government Issue nb. 12697/2008, May 6th). The goal of this Plan is to apply pre-release strategic reintroduction activities (IUCN/SSC, 1998) in order to make possible, in the long-term, the reintroduction of the species in Portugal, and thus, to assure its viability as a fundamental element of Mediterranean ecosystems. For achieving this goal it is necessary to establish a suitable connection between *ex situ* and *in situ* actions (Vargas et al., this book). The Action Plan is being applied in all the areas of Natura 2000 Network, located in the lynx historical range, which include suitable characteristics for the presence of the species or landscape features that can be optimised for lynx survival and that can be relevant for the species life-cycle (Figure 1). In this framework we include residence, dispersal and reproduction habitats as defined by Palomares, 2001.

In situ actions – the application of the Action Plan

Conservation measures will likely be implemented at three scales of decision-making: home-range level (micro-scale), population level (macro-scale) and ecological corridors, providing a broad direction for management activities by establishing objectives and guidelines. The following managements units will be designed in order to achieve conservation (Ruediger et al., 2000; Figure 1):

- **Micro-units for lynx management (MULs):** These micro-units are intended to provide the fundamental or smallest scale for evaluation and monitoring of the effects of management actions on lynx habitat and prey. The MULs should be considered as theoretical home-ranges that should incorporate all the habitat requirements for the completion of the Iberian lynx life cycle and should be managed as if the species was present, even in case of no detection.

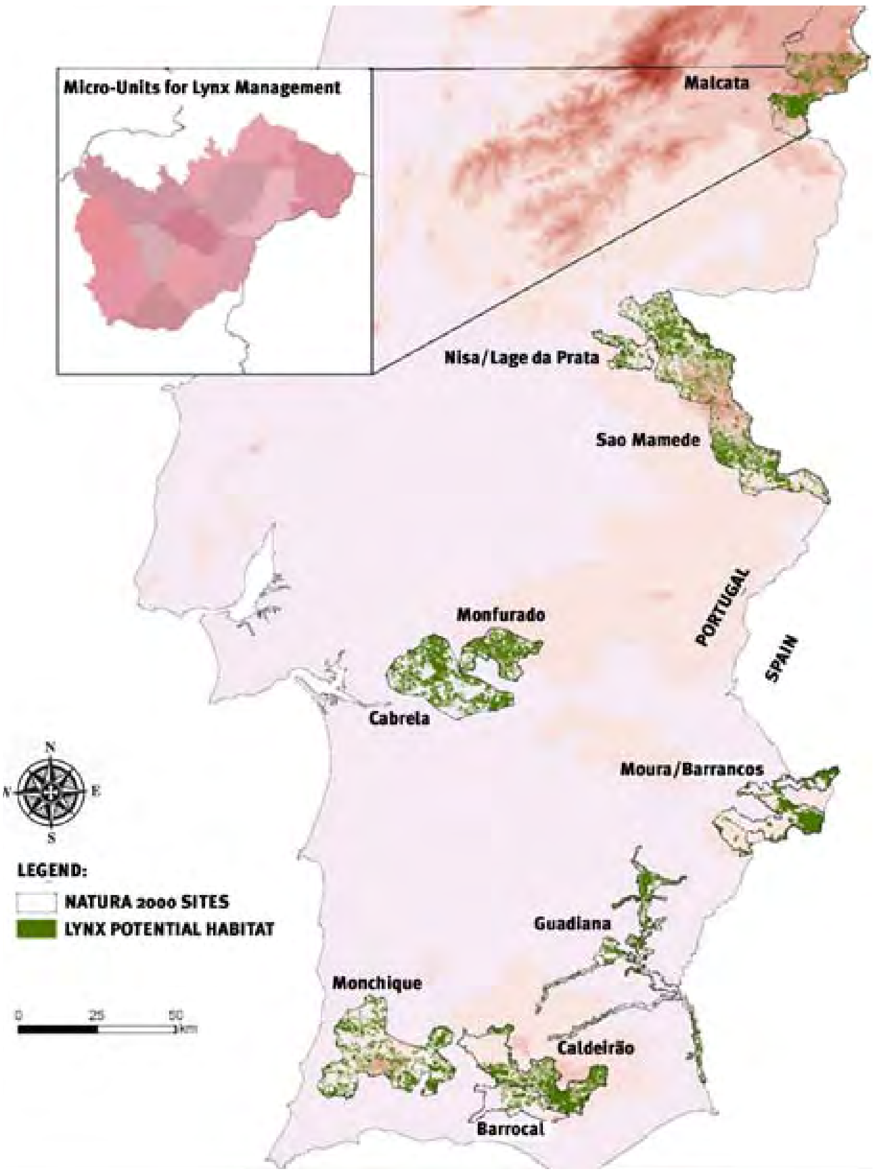

Figure 1 – Natura 2000 Sites selected for the application of the Portuguese Iberian Lynx Conservation Action Plan and examples of lynx management units for the Malcata area.

Figura 1 – Espacios Natura 2000 seleccionados para la aplicación del Plan de Acción para la Conservación del Lince Ibérico en Portugal y ejemplos de unidades de manejo del lince en el área de Malcata.

• **Macro-units for lynx management (MALs):** Action and programme planning should not be only focused at the home-range level (MULs). The landscape patterns of significant areas that correspond to potential populations should be taken into account. In this context, several MULs corresponding to theoretical populations will constitute a Macro-unit for lynx management (MALs).

• **Ecological corridors:** Dispersal is a key issue for lynx survival, since the meta-population equilibrium could only be achieved when the genetic flow between populations is maintained. Thus, the presence of linear landscape elements that warrants survivorship and movement is critical in terms of conservation.

The CAPIL actions were integrated in the Natura 2000 Network Management Plan, which will be the basic tool for conserving the habitats and species of European importance in Portugal. For the establishment of

A HABITAT TYPE	MANAGEMENT GUIDELINES
Denning habitat	Identify priority areas for land acquisition or warrant
	Recover potential areas of Mediterranean scrubland
	Promote programmes for controlling feral cats and dogs
	Promote forestry practices compatible with lynx conservation
	Establish economic incentives
	Promote sustainable rabbit hunting
	Enforce the vigilance towards illegal hunting
	Co-ordinate the decision-making process for infrastructure edification with lynx conservation
Foraging habitat	Identify priority areas for land acquisition or warrant
	Promote crop fields for rabbits
	Conduct rabbit restocking operations
	Evaluate the impact of rabbit diseases
	Promote sustainable rabbit hunting
	Enforce the vigilance towards illegal hunting
	Co-ordinate the decision-making process for infrastructure edification with lynx conservation
Corridors	Identify priority areas for land acquisition or warrant
	Stop the physical remove of scrubland vegetation and riparian habitats
	Promote sustainable rabbit hunting
	Enforce the vigilance towards illegal hunting
	Co-ordinate the decision-making process for infrastructure edification with lynx conservation

Table 1 – Natura 2000 major management guidelines for Iberian lynx conservation according to the different types of habitats.

Tabla 1 – Principales directrices de manejo de la Red Natura 2000 para la conservación del lince ibérico según el tipo de hábitat.

these measures, we mapped lynx potential habitat for all Natura 2000 Sites that had been classified with lynx presence (Figure 1). Using this data, management guidelines were defined for each habitat category according to its importance for lynx conservation (Portuguese Ministerial Council Resolution nb. 115-A/2008, July 21st; Table 1). These guidelines are being applied since 2005 for evaluating human activities that can directly or indirectly influence future lynx conservation. Currently is on-going a national evaluation on the effects of management guidelines on the conservation of natural habitats and species of European Community interest within the Natura 2000 Network areas.

On-going *in situ* conservation actions

Three major conservation projects are currently on-going:

• Habitat recovery in Serra da Malcata. Until 2007, most of the *in situ* conservation actions carried out in Portugal have concentrated in Serra da Malcata Nature Reserve where, since 1997, a continuous programme, financed by the LIFE Programme (LIFEB4-3200/99/006423) and by FEDER, for habitat and prey restoring is on-going. The main actions of this project include preventing the degradation of the Mediterranean forest, restoring land-use practices that favour rabbit presence (creation of pastures and shelters) and rabbit restocking. Several years after the systematic application of these measures it was possible to increase lynx carrying capacity at the Reserve and, as a consequence, to establish a reintroduction plan following IUCN guidelines (IUCN/SSC, 1998).

• LPN/FFI Life Project. In 2006, the League for Nature Protection (LPN), a Portuguese Non-Governmental Agency, in association with Flora and Fauna International (FFI), engaged in an EU LIFE project for the conservation of Iberian lynx in the area of Moura-Barrancos Natura 2000 Site (PTCON0053), called "Habitat recovery for the Iberian lynx in the Moura-Barrancos Natura 2000 Site" (LIFE06/NAT/P/000191). The main purpose of this project is to increase habitat suitability for lynx through the establishment of medium to long-term management agreements with landowners and hunting reserves in order to make a rational use of natural resources and to explore game species in a sustainable way compatible with lynx conservation (for further details please go to http://www.lpn.pt).

• Odelouca dam mitigation actions. Following the assessment of water resources in the Algarve (South Portugal), the Portuguese Government has applied for financial support to the European Union for the building of a dam in the Odelouca region, a Natura 2000 Site (PTCON00037) and part of the historical range of the Iberian lynx. The package of environmental compensatory measures presented to the EU includes several actions, being habitat recovery, rehabilitation and viability analysis activities for Iberian lynx and rabbit, conversion of monocultures into Mediterranean bush, valorisation of ecological corridors between habitat patches, and the construction of a captive breeding centre for the Iberian lynx as the most relevant.

Future actions

Future *in situ* conservation actions will include a feasibility analysis for reintroduction in potentially areas, located in the south-east of Portugal, several regional projects, financed by EU funds, for habitat management and prey increasing and the establishment of a national systematic monitoring system for rabbit populations. The first measure is crucial for the success of the Action Plan since it is of critical importance that the best possible sites and release methods are utilized. The study will be conducted in three phases: 1) construction of a landscape-scale statistical model; 2) construction of a large-scale statistic model, and 3) development of a metapopulation model. Following this action, several regional projects for habitat and prey conservation will be applied in order to maintain or augment potential sites for lynx colonization.

Ex situ conservation

Portugal is represented in the Iberian Lynx Captive Breeding Team (CCCLI) through the ICNB since 2001. Based on the Spanish *Ex situ* Conservation Action Plan (Vargas et al., 2005, 2008), the ICNB has applied its global aims and objectives and developed its own *Ex situ* Conservation Plan (Serra et al., 2005), which, among other measures, foresees the construction of a captive breeding facility within the Iberian network of breeding centres and under the technical and scientific guidance of the CCCLI (Vargas et al., this book). The construction of the breeding centre in Silves (Algarve, Southern Portugal) is currently taking place and will be able to maintain up to 16 breeders coming from the captive breeding programme as of mid-2009.

Conclusions

The dramatic decline of the Iberian lynx and the critical status of its free-ranging populations in Portugal are now acknowledged by both scientific and political groups nationwide. Nevertheless, it is assured that the recovery of the Iberian lynx populations will be a considerable challenge with a high degree of uncertainty in both Iberian countries. In order to achieve the goal of restoring the species in Portugal and Spain it is necessary to apply a daring plan that will involve inter-agency and international collaboration; therefore, on-going actions involve several teams that are co-ordinately conducting a multidisciplinary approach towards Iberian lynx recovery. All the same, all agents involved in this laborious task need to be aware that, even with unlimited resources and in ideal conditions, the future of the Iberian lynx in the Iberian Peninsula is still uncertain, and only a professional attitude, coupled with social and political support, can prevent the vanishing of this majestic cat species.

Acknowledgments

Authors which to thank all field collaborators during Iberian lynx surveys in 2002, which provided most of field data available, and to Catarina Eira for the revision of the manuscript.

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Photo: Antonio Rivas

Imagine there's no heaven / It's easy if you try / No hell below us / Above us only sky / Imagine all the people / Living for today / Imagine there's no countries / It isn't hard to do / Nothing to kill or die for / And no religion too / Imagine all the people / Living life in peace / You may say I'm a dreamer / But I'm not the only one / I hope some day you'll join us / And the world will be one / Imagine no possesions / I wonder if you can / No need for greed or hunger / A brotherhood of man / Imagine all the people / Sharing all the world / You may say I'm a dreamer / But I'm not the only one / I hope some day you'll join us /And the world will live as one.

John Lennon
(1940-1980)


Photo: Andoni Candela

Conservation of free-ranging Iberian lynx *(Lynx pardinus)* populations in Andalusia

Conservación de las poblaciones silvestres de lince ibérico *(Lynx pardinus)* en Andalucía

Miguel A. Simón, Rafael Cadenas, José María Gil–Sánchez, Marcos López-Parra, José García, Leonardo Fernández, Gema Ruiz and Guillermo López

Resumen

A finales del siglo XX se comprobó que el estado de conservación del lince ibérico era extremadamente crítico, cuando solo permanecían en la naturaleza alrededor de 150 individuos en dos poblaciones aisladas, Doñana y Sierra Morena oriental, ambas en Andalucía. La Junta de Andalucía, en colaboración con algunos socios, adoptó diferentes medidas de conservación que recibieron apoyo de la Unión Europea en forma de dos proyectos LIFE de conservación. Estos proyectos han sido esenciales para evitar la posible extinción de la especie en la naturaleza y para estabilizar ambas poblaciones silvestres. En la actualidad, los esfuerzos de la conservación *in situ* se están centrando en los siguientes objetivos: 1) Mantener y expandir las dos poblaciones existentes; 2) Crear nuevos núcleos poblacionales –siguiendo los criterios de la IUCN para las reintroducciones; 3) Maximizar la variabilidad genética mediante la "conexión genética" de ambas poblaciones; 4) Continuar promoviendo el apoyo local, nacional e internacional para garantizar la recuperación de esta especie gravemente amenazada. Hoy, la población de Sierra Morena continúa creciendo anualmente (tanto numéricamente como en superficie). De hecho, el número de individuos adultos (más de un año de edad) se ha incrementado desde los 38 registrados en 2001 hasta los 95 en 2008. La población de Doñana permanece estable, y se está llevando a cabo un programa de reforzamiento (principalmente genético, pero también demográfico). Los esfuerzos en la conservación *in situ* se están centrando también en la recuperación de nuevos núcleos poblacionales mediante programas de reintroducción. Los trabajos de preparación se están llevando a cabo desde 2005 y las primeras sueltas están programadas para el 2009.

Palabras clave

Recuperación, diversidad genética, refuerzo demográfico, reintroducción

Abstract

The conservation status of the Iberian lynx was found to be extremely critical by the end of the 20th Century, when only 150 individuals remained in the wild secluded into two isolated populations, Doñana and eastern Sierra Morena, both located in Andalusia. The Andalusian Government, together with a number of partners, adopted different conservation measures that received support from the European Union in the form of two LIFE–Nature conservation projects. These projects have proved essential to avoid the potential extinction of the Iberian lynx in the wild and to stabilize both free–ranging populations. Presently, *in situ* conservation efforts are focused on the following objectives: 1) Maintaining and expanding the two existing populations; 2) Recovering extinct population nuclei –following IUCN reintroduction criteria; 3) Maximizing genetic diversity by "genetically connecting" the two existing populations; 4) Continuing to promote local, national and international support to ensure the recovery of this highly endangered species. Nowadays, the Sierra Morena population continues to grow –both numerically and in surface area– at an annual basis. In fact, the number of lynxes has increased from 38 adults (individuals of more than one–year–of–age) registered in 2001 to 95 in 2008. The Doñana population remains stable and a translocation programme, with the ultimate goal of genetic and demographic reinforcement, is currently taking place. Also, *in situ* efforts are presently focused on the recovery of historical population nuclei through reintroduction programmes. Preparation works are being carried out since 2005 and the first releases are scheduled to begin in 2009.

Keywords

Recovery, genetic diversity, demographic reinforcement, reintroduction

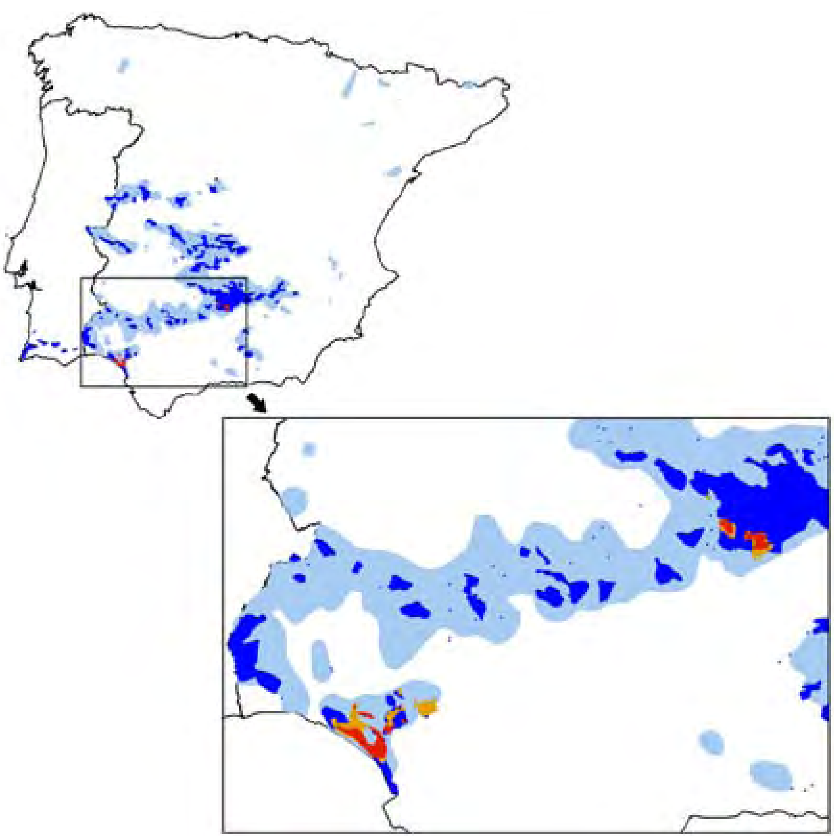

Figure 1. Iberian lynx distribution range in the 60's (only Spain; pale blue) (Rodríguez & Delibes, 1990), in the early 90's (dark blue) (Rodríguez & Delibes, 1990; Castro & Palma, 1996), in 2002 (red)(Guzmán et al., 2004), and in 2008 (orange) (LIFE project unpublished data).

Figura 1. Área de distribución del lince en los años 60 (sólo en España; azul pálido) (Rodríguez y Delibes, 1990), a principios de los 90 (Azul oscuro) (Rodríguez & Delibes, 1990; Castro & Palma, 1996) y en 2002 (rojo) (Guzmán et al., 2004).

Conservation of free-ranging Iberian lynx *(Lynx pardinus)* populations in Andalusia

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Introduction

The Iberian lynx *(Lynx pardinus)*, a species endemic to the Iberian Peninsula (Ferrer & Negro, 2004), is currently endangered in Spain and virtually extinct in Portugal (Sarmento et al., this book). It is considered the most endangered felid in the world, as it has been catalogued as "critically endangered" by the IUCN (2003). Legal protection for the Iberian lynx is granted by the Bern Convention, and the protection of its habitat, the Mediterranean scrubland, is a considered a priority by the European Commission (Habitat Directive 92/43). Although it is known that the Iberian lynx inhabited throughout the Iberian Peninsula, its distribution area suffered a 98% decline during the second half of the last century (Rodríguez et al., 1992; Guzmán et al., 2004; Calzada et al., this book) and, by 2002, about 170 individuals remained, distributed into two isolated breeding populations: the Doñana Natural Space and its surrounding biosphere reserve, and the Andújar–Cardeña mountains in Eastern Sierra Morena, both in Andalusia (Guzmán et al., 2004; see Figure 1). The Iberian lynx is a specialized predator, with more than 90% of its diet comprised of wild rabbits (Gil–Sánchez et al., 2006; Palomares, this book "a"; Calvete, this book). Mediterranean scrubland areas represent the optimal habitat for this species (Palomares, 2001; Palomares, this book "a"). Current threats to the species include 1) scarcity of rabbits due to mixomatosis and rabbit haemorrhagic disease (RHD) (Calvete, this book); 2) habitat destruction, and 3) human–caused mortality (road kills, leghold traps, poaching, etc.) (Guzmán et al., 2004).

Given this critical scenario, in early 2000 the Andalusian Regional Ministry of Environment began working on measures to avoid the Iberian lynx extinction. These included: 1) to attain an accurate knowledge of the actual distribution range of the species; 2) to carry out a monitoring programme of both rabbit and lynx populations; 3) to sign collaborative agreements with land owners; 4) to recover rabbit populations; 5) to control non–natural causes of mortality; 6) to establish a captive population to help preserve current genetic diversity, and 7) to ensure support from the local people that inhabit near both wild populations. Initial results from this Andalusian programme allowed to determine what was the actual distribution range of the Iberian lynx and helped develop accurate population monitoring methods. By 2002, a more ambitious and participative LIFE–Nature conservation project was granted. The project, entitled "Recovery of the Iberian lynx populations in Andalusia" (LIFENAT 02/E/8609) lasted four years (2002–2006) and involved the participation of the Andalusian Regional Ministry of the Environment, the three main Andalusian hunter associations (*Asociación de Titulares de Cotos de Andalucía –ATECA–, Federación Andaluza de Caza –FAC–* and *Asociación de Productores de Caza –APROCA–*) and two conservation organizations (*Ecologistas en Acción Andalucía –EEA–* and *–CBD–Hábitat Foundation, belonging to the Spanish Environment Ministry*).

The main objectives of this project (which included both short– and medium–term conservation measures) were 1) to augment lynx population size; 2) to maximize the connection between isolated populations; 3) to promote habitat conservation and resources availability; 4) to improve public perception of the species; 5) to contribute to the maintenance of a genetic "pull" representative of the species; 6) to create an effective lynx population monitoring system, and 7) to decrease threats for the lynx. Scientific oversight was provided by the Superior Council for Scientific Research (CSIC), Doñana's Biological Station. The main goal of this LIFE project was to stop and revert the rate of decline that had been observed during the previous decades. This included avoiding potential losses in both populations (through controlling threats and stabilizing resources availability), while increasing their carrying capacity to allow an increase of the population size. These specific goals were successfully attained, since both population nuclei could be stabilized and Sierra Morena one began increasing (LIFE project, unpublished data). Such results helped establish the basis for requesting a second LIFE–Nature project. This project, entitled "Conservation and Reintroduction of the Iberian Lynx in Andalusia" (LIFENAT 06/E/209), was granted in 2006 and will be ongoing until 2011. This new LIFE project focuses on the following objectives: 1) Maintaining and expanding the two existing populations; 2) Recovering extinct population nuclei –following IUCN reintroduction criteria–; 3) Maximizing genetic diversity by "genetically connecting" the two existing populations; 4) Continuing to promote local, national and international support to ensure the recovery of this highly endangered species. The project counts with the participation of the Andalusian Regional Ministries of Environment, Public Works and Agriculture, the Extremadura Regional Ministry of Environment, the three main Andalusian hunter associations (ATECA, FAC and APROCA) and four conservation organizations (EEA, CBD–Hábitat Foundation, the Spanish Society for the Conservation and Study of Mammals (SECEM), and World Wildlife Fund (WWF)/Spain). The project was structured based on the "Recovery Strategy for the Iberian Lynx", presented in the II International Seminar on the Conservation of the Iberian Lynx, which was held in Córdoba in December 2004 (Olszanska & Breitenmoser, 2004). Presently, we are into the third year of this LIFE project and preliminary results indicate that the Sierra Morena population continues to increase, and also Doñana is beginning to do it, as it is currently being genetically and demographically enhanced via translocations of selected Sierra Morena individuals. In addition, firsts releases of the reintroduction of the species into areas of historical occupancy is scheduled to take place at the end of 2009.

Materials and methods

Starting point

During 2001–2002, the actual distribution range of the Iberian lynx was studied in Andalusia. Transects searching for lynx signs (mainly scats and tracks) were performed in 366 5x5 km UTM squares, covering the known distribution area in 1988 (Rodríguez & Delibes, 1990). Lynx scats were confirmed by molecular methods (J.A. Godoy, unpublished data). Only 55 out of the 366 squares were positive, corresponding with the two previously identified nuclei: Doñana and the Andújar–Cardeña mountains, in the Sierra Morena range. About 80% of the distribution area was composed of private hunting lands. Afterwards, a quantification of rabbit abundance was performed through the lynx distribution area by means of transects. Rabbit abundance ranged between 0,6 and 4 rabbits/ha.

Figure 2. (A). Fauna underpass placed in a road of Doñana area. (B). Construction of speed bumps in a road of Doñana area.

Figura 2. (A). Paso de fauna en una carretera de Doñana. (B). Construcción de resaltos en una carretera de Doñana.

Agreements with landowners

The establishment of agreements with land owners and with hunter societies allowed the conservation projects to perform management measures throughout the lynx distribution. A total of 125,088 Has have currently being protected via collaborative agreements with landowners: 106,359 Has signed with the Andalusian Regional Ministry of the Environment, 13,729 Has with CBD–Habitat Foundation, and 5,000 Has with WWF–Spain. In addition, the Iberian lynx presently occupies 60,000 Has of public lands.

Figure 3. Well covered with a metallic net protected from the entrance of Iberian lynxes. Drowning in wells was detected as a relevant cause of mortality during the 90's.

Figura 3. Pozo cubierto con una malla metálica para evitar la caída de linces. El ahogamiento en pozos se detectó como una causa relevante de mortalidad durante los años 90.

Conservation strategy

Both Iberian lynx LIFE conservation projects have been based on a general strategy focused on:

• **Stabilization of existing territories:** This has been carried out focusing on the following points: 1) stabilising and increasing availability of resources through habitat improvement, and 2) decreasing actual threats for the lynx. Habitat improvement management is focused on getting optimal vegetation structure both for rabbits and lynxes (Palomares, 2001). It is mainly based on the improvement of meadows (small scrub clearances and cereal plantations) and increasing shelters for rabbits (artificial burrows, hiding areas, etc). Also, reduction of hunting pressure on rabbits is being carried out in critical areas. Rabbit releases are being performed in areas where rabbit abundance is under 1 rabbit/Ha in autumn, which is considered the minimum necessary to sustain Iberian lynx territories (Palomares, 2001). Rabbit releases are mainly being conducted using enclosures of about 5 Has with a predator exclusion fence (that is permeable to lynxes but not to other carnivores), although soft releases in the field without enclosure have also been performed. The last tool to increase rabbit availability is the establishment of supplementary feeding stations, aiming at maintaining specific lynxes in a determined area. These stations are baited with domestic rabbits and allow managing the population in case of potential rabbit populations crashes.

• **Decreasing causes of mortality.** Threats affecting Iberian lynx are mostly human–caused, although other causes can also affect the specie'spopulation dynamic. The main human.related threats affecting Iberian lynx include poaching, road kills and drowning in wells (Rodríguez & Delibes 1992; Guzmán et al., 2004), whereas the main natural threat includes mortalilty due infectious diseases (Meli et al., 2009; López et al., this book; Meli et al., this book). Several measures have been implemented to control these threats. Specific surveillance against illegal hunting methods is being carried out by LIFE project staff in areas of lynx occurrence. Also, control of road kills, mainly in the Doñana population, is one of the most important issues being addressed through the current LIFE project. Part of this control is being addresed by the construction of wildlife underpasses and

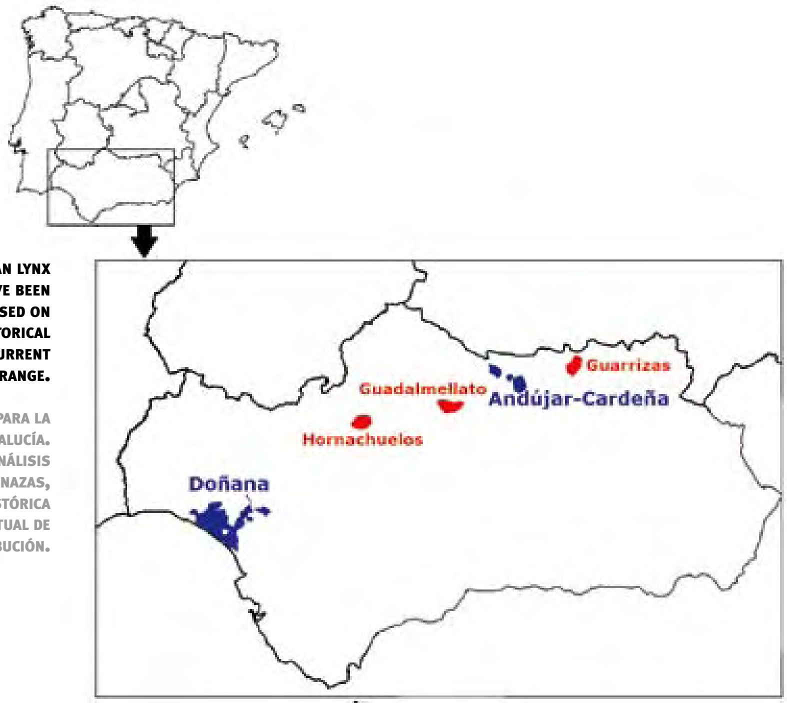

Figure 4. Optimal areas (in red) for Iberian lynx reintroduction in Andalusia. Areas have been selected by the multi–criteria analysis based on habitat, threats, resources availability, historical distribution and protection degree. In blue, current distribution range.

Figura 4. Áreas óptimas (en rojo) para la reintroducción del lince ibérico en Andalucía. Las áreas se han seleccionado por un análisis multi–criterio en función del hábitat, amenazas, disponibilidad de recursos, distribución histórica y grado de protección. En azul, área actual de distribución.

ecoducts (Figure 2), placing wildlife exclusion fences on problem roads, installing dissuasive road night lights (catadioptrics), advertising road signals and speed bumps for traffic, etc. Moreover, specific brochures and booklets have been developed to increase awareness among drivers in the Doñana area. In addition, to address the problem of lynxes drowning in wells, all known wells in areas of lynx occupancy have been covered with metallic nets (Figure 3). Finally, to control the risk of infectious diseases (López et al., this book; Meli et al., this book) a surveillance programme is being carried out throughout both Iberian lynx populations. This programme includes thorough health screenings of all handled animals (Martínez et al., this book), and also an indirect surveillance through scat analysis.

• **Settlement of new territories:** This is performed by increasing carrying capacity and decreasing threats for the lynx. The recovery of new territories has been made using territory recovery units (TRUs) as the main methodology. TRUs are composed of different habitat improvement measures performed in about 500 Ha (mean surface area of an Iberian lynx home range). Wild rabbit releases were included in TRUs only when rabbit abundance is under 1 rabbit/Ha.

• **"Compression" of occupied areas:** This strategy works under the same premises of the abovementioned ones; i.e., by increasing carrying capacity it is possible to reduce home range surfaces, which in turn will allow a higher number of individuals to inhabit a specific area.

• **Public awareness campaigns:** These campaigns have been taking place at a regular basis since 2002, and they are developed by all partners that participate in the conservation of this species. The incorporation of the hunter societies to the communication group has been one of the most effective ways to gain access to the hunter's opinion and support. Conservation associations have also been important to bring national and local awareness regarding the situation of the species.

• **Reintroduction:** This entails a medium– to long–term aspect of the conservation strategy. The first macro–habitat analysis for the suitability of areas for potential Iberian lynx reintroduction in Andalusia was performed in 1999 (F. Palomares, unpublished data). This habitat model become the basis for further work, although resource availability was not considered in it. Given that this factor is thought to be the most important one to take into account when developing a reintroduction strategy, a more exhaustive work was needed. Initial work towards Iberian lynx reintroduction began under the first LIFE conservation project, in 2002, and it has always being based on the IUCN guidelines for reintroduction (IUCN, 1998). The detection of potential reintroduction areas in Andalusia was performed by a multi–criteria analysis (MCA). The MCA is a decision–making tool developed for complex problems that follows a logical, well–structured, decision–making process where multiple criteria are involved. The steps followed in this analysis were: 1) the identification of factors determining the suitability of areas that could host Iberian lynx; 2) compilation of information on everyone of the identified factors; 3) integration of all information in a Geographical Information System (GIS), and 4) evaluation of the resulting models. The identified factors that were important in determining lynx presence included habitat features (vegetation structure, altitude and slope), resource availability: (rabbit abundance and drinking points availability) and threats (proximity to urban nuclei and to communication routes). Afterwards, a Weighted Linear Combination (WLC) analysis was performed (Barredo, 1996). With this method, the suitability of a pixel is obtained as the sum of partial suitability values of every factor in that area. The final potentiality map for Andalusia (Figure 4) took also into account the historical distribution of the species and the degree of protection degree of each area. The result was the identification of three optimal nuclei where to carry out initial Iberian lynx reintroductions in Andalusia: Guarrizas Valley, Guadalmellato Valley and Hornachuelos Natural Park. Once this map was generated, all three areas were analysed in a fine–scale comparison through hierarchical analysis of 35 variables grouped into seven groups: 1) Social attitude; 2) Possibility of integration in the current metapopulation; 3) Carrying capacity; 4) Habitat structure; 5) Possibilities of expansion to other a–priori suitable areas; 6) Illegal persecution pressure and 7) other threats. Among the three selected areas, the Guadalmellato Valley area was selected as the best one and, thus, the first reintroduction –scheduled for late 2009– will take place at this specific site. Ever since after signing agreements with the owners, habitat management measures are already being made in the area.

Lynx and rabbit monitoring programmes

A monitoring programme of both rabbits and lynxes is being carried out by the Andalusian Regional Ministry of the Environment since 2001. Rabbit abundance is continuously monitored – via transects and latrine censuses– in areas with lynx presence. A disease surveillance programme (focusing mostly on RHD) is also carried out every year during critical time–periods. Iberian lynx monitoring is carried out by means of photo–trapping, radiotelemetry, direct observations and search for lynx signs (tracks, scats, etc.). Evaluation of actions undertaken at the population scale has been performed using information on inter–annual rabbit latrine survey in 2.5 km UTM squares, since its surface (625 Ha) is about the size of a breeding female home range (Ferreras et al., 1997; Palomares, 2001). In Sierra Morena, treated squares have been compared to control ones (without management actions).

Genetic and demographic reinforcement via translocation of selected individuals

The low effective size has, in fact, reduced the genetic diversity of both Iberian lynx wild populations and, data shows that genetic drift has taken place in Doñana and Sierra Morena (Godoy et al., this book). In addition, Doñana's genetic diversity is 30% lower than that of Sierra Morena (Godoy et al., this book). The most effective way to increase genetic diversity in the Doñana Iberian lynx population is via translocations of individuals from Sierra Morena (Palomares, this book "b"; Godoy et al., this book). To date, two individuals from Sierra Morena have been released in Doñana, one in 2008 and the second one in 2009. Both were soft–released, being kept between 2–4 weeks in large (3–8 Has) acclimatization pens placed within the most protected environs of the Doñana area. Translocations will continue to take place until at least four individuals from Sierra Morena will settle in Doñana.

Results

Conservation strategy

• **Stabilization and compression of territories, and settlement of new ones:** When comparing treated squares (squares where TRUs have been performed into) with control ones, the former increase overall rabbit abundance in 6 years, has been about 100%, whereas untreated squares have not shown significant differences in rabbit abundance during the same period of time. The most effective conservation measures have been the supplementary feeding stations and the rabbit release enclosures. Yet, the most important aspect that affects rabbit population dynamics in our study area has been the RHD (Figure 5; see Calvete, this book). In Doñana, rabbit abundance has slightly increased since 2002, although the effect of the carried out actions is not clearly related to this increase. Due to landscape features, the evaluation of actions over the rabbit population, however, is much more difficult to be performed in Doñana than in Sierra Morena. A TRU has been considered successful when the rabbit increase has allowed the settlement and reproduction of an adult female, while we have considered them partially successful if settlement has been recorded but without reproduction. Most TRUs both in Sierra Morena

Individual	Year	Age	Population	Individual	Year	Age	Population
Aura	2002	<1	DON	Candiles *	2006	<1	SMO
Saliega	2002	<1	SMO	Caña	2006	<1	SMO
Garfio	2003	3	SMO	Cardo	2006	<1	SMO
Cromo *	2003	<1	SMO	Ceniza	2006	<1	SMO
Fran *	2003	1	SMO	Córdoba	2006	<1	SMO
Jub	2003	3	SMO	Coscoja	2006	<1	SMO
Adelfa	2004	<1	SMO	Cuco	2006	<1	SMO
Alhucemas	2004	<1	SMO	Al–Andalus	2007	3	SMO
Aliaga	2004	<1	SMO	Alfonso *	2008	10>	SMO
Almoradux	2004	<1	DON	Azuel *	2008	Unkn.	SMO
Artemisa *	2004	<1	SMO	Calabacín *	2008	2	SMO
Arcex	2005	1	SMO	Cascabel	2008	2	SMO
Beta	2005	<1	SMO	Charqueña	2008	2	SMO
Biznaga	2005	<1	SMO	Era	2008	<1	SMO
Boj	2005	<1	DON	Esparto	2008	<1	SMO
Azahar *	2006	2	SMO	Espina	2008	<1	DON
Bandolero	2006	1	SMO	Estela II	2008	<1	DON
Barraca *	2006	1	SMO	Damán II	2009	2	SMO

Table 1. Iberian lynx individuals extracted from the field by the LIFE conservation project to be incorporated as founders of the *Ex situ* Conservation Programme (* = individuals considered to have limited potential for survival in the wild at the time of capture).

Tabla 1. Ejemplares de lince ibérico extraídos de la naturaleza por el Proyecto LIFE para ser incorporados al Programa de Conservación *ex situ* del Lince Ibérico (*=individuos extraídos por tener un bajo potencial de supervivencia en la naturaleza en el momento de captura).

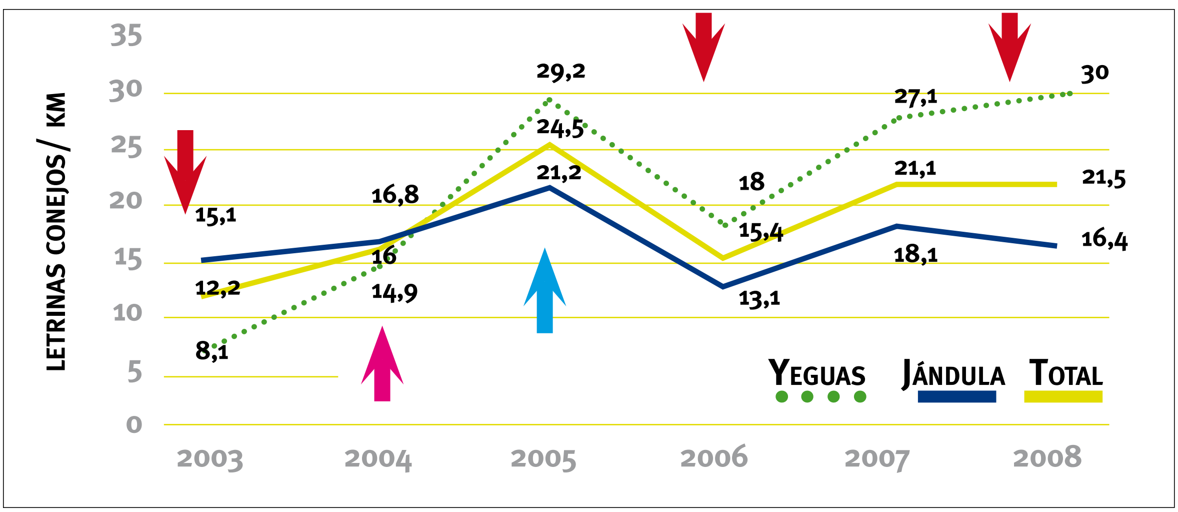

Figure 5. Evolution of the rabbit relative abundance in 2.5 Km UTM squares in the Iberian lynx occupancy area (n = 28squares). The pink arrow marks the beginning of the supplementary feeding programme in the three corresponding squares. The blue arrow marks the beginning of the LIFE project activities. Red arrows mark Rabbit haemorrhagic disease outbreaks.

Figura 5. Evolución de la abundancia relativa de conejos en unidades UTM en cuadrados de 2,5km en el área de distribución del lince (n = 28 cuadrados). La flecha rosa marca el inicio del programa de alimentación suplementaria en los tres cuadrados correspondientes. La flecha azul marca el inicio de las actuaciones del LIFE. Las flechas rojas marcan las epizootias de la Enfermedad Hemorrágica del conejo.

and in Doñana have been considered successful. In Sierra Morena, eight out of the 13 established TRUs can be considered successful and two partially successful. A total of 12 new Iberian lynx territories have been established in Sierra Morena since 2001, where the population has increased from 38 adults (considering animals over 1 year–of–age; n=60 individuals including young–of–the year) in 2002 to 95 adults (n=150 individuals including young–of the year) in 2008 (Figure 6). In Doñana, nine new female Iberian lynx territories have been established since 2002, although the effect of the habitat management units is difficult to determine. The number of individuals in the Doñana population has remained stable at approximately 31–32 adults, yet the number of territorial females has

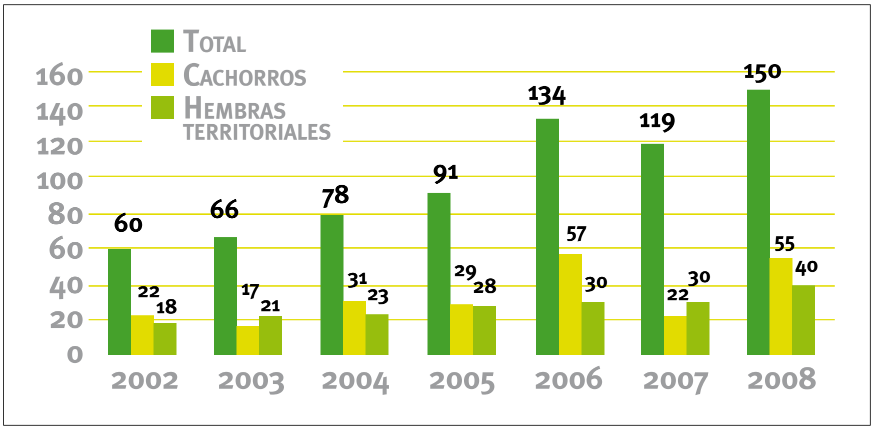

Figure 6. Evolution of the Iberian lynx population in Sierra Morena between 2002 and 2008: estimated total number of individuals, cubs and territorial females.

Figure 6. Evolución de la población de Sierra Morena entre 2002 y 2008: número total de individuos estimados, cachorros y hembras territoriales.

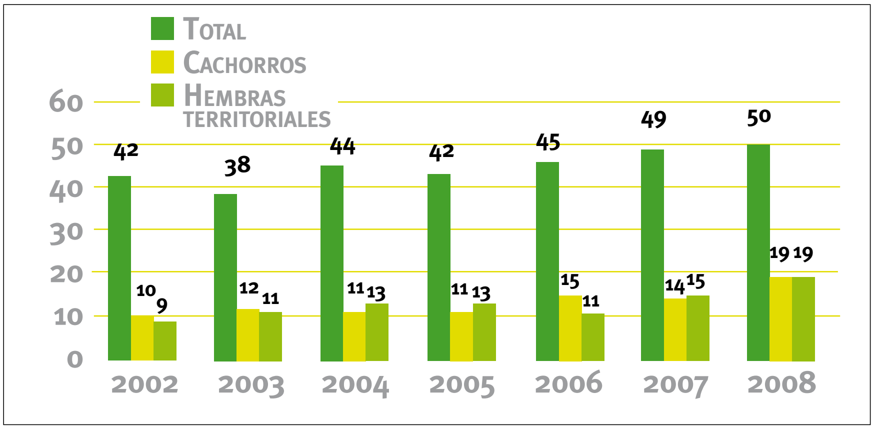

Figure 7. Evolution of the Iberian lynx population in Doñana between 2002 and 2008: estimated total number of individuals, cubs, and territorial females.

Figura 7. Evolución de la población de Doñana entre 2002 y 2008: número total de individuos estimados, cachorros y hembras territoriales.

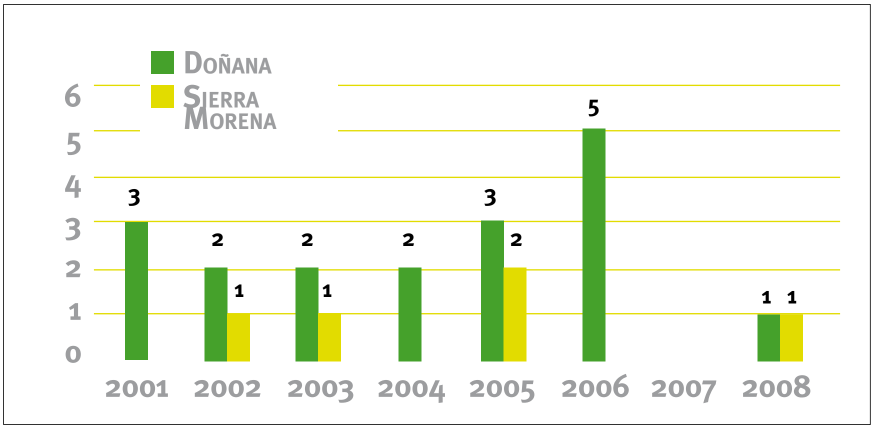

Figure 8. Road mortality in the Iberian lynx populations between 2001 and 2008. Dark green bars represents the Doñana population and light green bars the Sierra Morena population.

Figura 8. Mortalidad en carretera en las poblaciones de lince ibérico entre 2001 y 2008. Las barras verdes oscuras representan a la población de Doñana y las verdes claras a la población de Sierra Morena.

increased from 10 to 19 between 2002 and 2008 (Figure 7), while the total number of males has decreased. This is largely due to the loss of males after the 2007 Feline leukaemia (FeLV) outbreak in the Coto del Rey nuclei (López et al., 2009; Meli et al., 2009; López et al., this book; Meli et al., this book).

• **Decreasing causes of mortality:** Measures to control road mortality seem to be working efficiently up to now. Although road kills increased during 2003–2006, they have decreased during 2006–2009 (Figure 8; Martínez et al., this book). Control of illegal hunting methods has helped decrease the number of leghold traps and snares to almost cero. In 2008, the epidemiological surveillance programme allowed to detect the first case of poisoning in an Iberian lynx. Fortunately, the perpetrator could be identified and he is currently down by law. The epidemiological surveillance programme is also working effectively. Thanks to this programme, which is led by staff of the Andalusian Government, the *Ex situ* Conservation Programme and the University Zürich, an outbreak of feline leukemia virus that occurred in Doñana population in 2007 could be rapidly detected and controlled (López et al., 2009; Meli et al., 2009; López et al., this book; Meli et al., this book).

• **Public awareness campaigns:** The outreach campaigns have made the population aware of the situation of the Iberian lynx and supportive of the proposed reintroduction in this area. Altogether, outreach efforts have largely focused on the most critical groups: hunters, farmers, local populations of Doñana, Sierra Morena and the proposed release site, and children. As part of these communication efforts, the LIFE conservation project publishes a monthly bulletin and maintains an updated webpage (www.lifelince.org). Public information is proving to be a very effective tool for local awareness and support.

• **Reintroduction:** Habitat management measures are presently being carried out in the Guadalmellato Valley, where the first Iberian lynx reintroduction is scheduled to take place. Six wild–born individuals will be released during the second half of 2009. The selected method includes a soft–release technique in on–site acclimatization enclosures of approximately 4 Ha each. Most landowners in the area have already signed collaborative agreements with the Andalusian Regional Ministry of the Environment within the frame of the Iberian lynx LIFE–Nature conservation project.

Genetic and demographic reinforcement

The genetic and demographic reinforcement performed in Doñana via translocations of Sierra Morena males is still incipient. The first translocated individual was released in Doñana National Park in January 2008. It was a three–year–old male named *"Baya"* that belonged to the Cardeña subpopulation (Ruiz et al., this book). It was released in Coto del Rey subpopulation, (a high rabbit density place) where there were three adult females and no adult males due to the FeLV outbreak that took place in 2007 (see López et al., 2009). *Baya* immediately settled in the area and copulated with the three territorial females. All three became pregnant and gave birth to a total of eight young, of which three were still alive by the end of 2008 (for details see text box by Ruiz et al., in Palomares, this book). The introduction of Sierra Morena genes into the Doñana population is considered essential for the long–term genetic health of this wild population (Godoy et al., this book). In January 2009, *"Caribú"*, a three–year–old male that originated from the Andújar subpopulation, was soft–released in Doñana's Biological Reserve, one of the best protected areas within the National Park. This place is a low–density rabbit area where one adult female and one adult male inhabit. In contrast with the first experience, this male has not shown any interest on attempting to settle within the vicinity of the release site, and it is currently dispersing northwards out of the Doñana population in a moving considered as "homing behaviour". Experiences with other lynx species (unpublished data) point out the possibility that this individual returns to the Doñana population by itself after several months.

Safeguarding genetic diversity in captivity

Both LIFE conservation projects have helped provide all the Iberian lynx founders that presently conform the Iberian Lynx Conservation Breeding Programme (Vargas et al., this book). Since 2002, 36 individuals have been brought into captivity (6 from Doñana and 30 from Sierra Morena; Table 1). From these, 24 lynxes were specifically extracted

Photo: Andoni Candela

for the captive breeding programme, whereas the other 10 lynxes were brought into captivity because they had problems that compromised their survival in the wild. The extraction rate was based on a model developed in order to avoid potential impacts on the population dynamics of this species (Palomares et al., 2002).

Discussion

The conservation strategy developed for the Iberian lynx in Andalusia seems to be advancing in the right way. The Sierra Morena population has experienced a substantial increase since 2001, when active conservation measures began to be implemented. The number of lynxes in this population has increased at a much higher rate than the surface area they are occupying; i.e., territories are being "compacted" and there seems to be current evidence of population saturation in Sierra Morena (LIFE project, unpub. data). The productivity is following an every–other–year cycle, probably due to the stratification of one–year–old individuals inside the population that hinder the survival of cubs born the following year. Moreover, about 70% of the juveniles are lost within their first year of life, and there have been several recorded cases of intraspecific aggression, which seems to be one of the major causes of this loss. Moreover, juveniles are currently settling in areas with very low rabbit densities or with very poor habitat structure. Population expansion seems difficult due to lack of resources in all areas surrounding the current distribution area. Nevertheless, there are suitable areas for the establishment of Iberian lynx within 15–45 km from the current Andújar–Cardeña population. In fact, all available information points towards asserting that the rapidly growing Yeguas River subpopulation has motivated the foundation of a new population nucleus in Southern Castille–La Mancha Community, 15 km away from the Andalusian Sierra Morena population (Castille–La Mancha Government, unpublished data). Iberian lynx conservation efforts in Doñana have promoted the establishment of an important number of new territories outside the National Park during the period 2001–2009, which have made up for the dramatic loss of territories suffered inside the National Park between 2001–2003. The key to this change of trend outside the National Park has been the awareness of the local population and the decrease in road mortality. The number of territorial females 19) and wildborn cubs (24) recorded in Doñana in 2008 have been the maximum ever recorded in this population. We believe that all

Photo: Miguel Ángel Simón

the abovementioned measures have prevented the extinction of this small population. However, more active management measures are needed to ensure the long–term conservation of this population.

Reintroductions are considered to be key in moving forward towards the recovery of the endangered Iberian lynx. Once the protection and effective conservation of the currently existing populations are granted, the next step is the establishment of new free–ranging lynx populations in areas of historical occupancy (see Calzada et al., this book). The Iberian lynx is critically endangered, and can only be recovered through hard work and solid partnerships between national and international stakeholders. The effort of politicians, biologists, scientists, veterinarians, hunters, and the whole society is sorely needed to help recover the lynx populations that roamed in the past throughout the Mediterranean forests and scrublands of the Iberian Peninsula.

Acknowledgements

F. Coves, C. Castillo, J. Guirado, M. Martín, C. Gañán, M. Delibes, F. Palomares, N. Fernández, A. Vargas, F. Martínez, M. Navarro, M. Briones, and N. Guzmán give support to the all the Iberian lynx conservation programmes carried out in Andalusia. The other members of the LIFE project field team (G. Valenzuela, G. Garrote, M.A. Díaz–Portero, A. Leiva, E. Rojas, R. Arenas, J. Rodríguez, J. Bueno, S. Lillo, J. Pérez, M.I. García, M. Moral, J.A. Báñez, R. Sanabria, R.B. Millán, D. Palacios, CBD Hábitat Foundation team and WWF/Adena team), together with the rest of the LIFE project team (S. Saldaña, M.P. Delgado, R. García, I. Tenorio, M. Vara, Ecologistas en Acción, SECEM, ATECA, APROCA and FAC) are making all this work possible.

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When despair grows in me
and I wake in the middle of the night at the least sound
in fear of what my life and my children's lives may be,
I go and lie down where the wood drake
rests in his beauty on the water,
and the great heron feeds.
I come into the peace of wild things
who do not tax their lives with forethought of grief.
I come into the presence of still water.
And I feel above me the day-blind stars
waiting for their light. For a time
I rest in the grace of the world, and am free.

The Peace of Wild Things, Wendell Berry


Photo: Héctor Garrido

Interdisciplinary methods in the Iberian Lynx *(Lynx pardinus)* Conservation Breeding Programme

Métodos multidisciplinares en el Programa de Cría para la Conservación del Lince Ibérico

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Resumen

El Programa de Cría para la Conservación del Lince Ibérico se plantea bajo un enfoque multidisciplinar, integrado en la Estrategia Nacional para la Conservación del Lince Ibérico, y desarrollado en colaboración con instituciones regionales, nacionales e internacionales. Las metas principales del Programa de Conservación *Ex situ* son: 1) mantener una población cautiva sana y gestionada genética y demográficamente; 2) crear nuevas poblaciones naturales de lince ibérico *(Lynx pardinus)* mediante la reintroducción de la especie. Con el fin de lograr el primer objetivo, el Programa tiene como cometido mantener el 85% de la diversidad genética actualmente existente en la naturaleza durante los próximos 30 años, lo cual requiere el manejo de 60 ejemplares reproductores (30 machos, 30 hembras). Las proyecciones de crecimiento indican que el Programa *Ex situ* debería alcanzar su meta poblacional para el año 2010 y que una vez logrado este primer paso, podrá dar comienzo la reintroducción de ejemplares nacidos en cautividad. El Programa se encuentra actualmente por delante de las proyecciones, habiendo alcanzado su objetivo poblacional en el año 2009, aunque la primera reintroducción de ejemplares de lince nacidos en cautividad sigue programada para el año 2010. Hasta la fecha, el trabajo actual del Programa *Ex situ* se ha centrado en la cría y mantenimiento de ejemplares sanos, tanto desde un punto de vista fisiológico como etológico, para lo cual se han utilizado técnicas de manejo y de investigación siguiendo un enfoque multidisciplinar, es decir, integrando conocimientos generados a partir de los campos de la biología, etología, nutrición, veterinaria, genética, fisiología, endocrinología y ecología de la especie. Hemos considerado particularmente importante el adaptar nuestros métodos de manejo para ayudar a fomentar los comportamientos naturales del lince (caza, territorialidad, interacciones sociales) y ofrecer así un entorno cautivo que minimice el estrés y favorezca la reproducción natural. El presente capítulo tiene como objetivo ofrecer una visión global sobre cómo los conocimientos científicos aportados a través diversas disciplinas han sido esenciales para el diseño y desarrollo del Programa de Cría del Lince Ibérico. El capítulo se divide en 6 secciones (Manejo genético y demográfico, Etología y Manejo de la población cautiva, Aspectos Sanitarios, Fisiología Reproductiva, Reintroducción, Divulgación/Capacitación) y presenta, a modo de resumen, la información y los resultados contenidos en aquellos capítulos de este libro que tratan sobre la conservación del lince ibérico.

Palabras clave
Genética, manejo, etología, fisiología reproductiva, medicina veterinaria, reintroducción

Abstract
The Iberian Lynx Conservation Breeding Programme follows a multidisciplinary approach, integrated within the National Strategy for the Conservation of the Iberian Lynx, which is carried out in cooperation with national, regional, and international institutions. The main goals of the *Ex situ* Conservation Programme are to: 1) maintain a genetically and demographically-managed captive population; 2) create new Iberian Lynx *(Lynx pardinus)* free-ranging populations through reintroduction. To achieve the first goal, the Programme aims at maintaining 85% of the genetic diversity presently found in the wild for the next 30 years. This requires managing 60 (30 males, 30 females) Iberian lynx as breeding stock. Growth projections indicate that the *Ex situ* Programme should achieve such population target by the year 2010 and, once this goal is reached, reintroduction efforts could begin. The Programme actually surpassed this population target in 2009, although the first reintroduction of captive-born Iberian lynxes is still scheduled for 2010. To date, *ex situ* efforts have focused on producing physiologically and behaviourally sound captive-born individuals. To achieve this goal, we have used management and research techniques that rely on multidisciplinary input and knowledge generated on the specie's life history, behaviour, nutrition, husbandry, genetics, veterinary science, physiology, endocrinology and ecology. Particularly important has been adapting our husbandry schemes based on research data to promote natural behaviours in captivity (hunting, territoriality, social interactions) and a stress-free environment that is conducive to natural reproduction. The aim of this chapter is to provide an overview of how scientific knowledge from various disciplines is proving essential to shape management efforts within the Iberian Lynx Breeding Programme. The chapter is divided into 6 sections: Genetic and Demographic Management, Captive Husbandry and Behavior, Health and Veterinary Aspects, Reproductive Physiology, Reintroduction, & Outreach/Capacity Building, and it summarizes information and results contained in various chapters of this book as they relate to Iberian lynx conservation efforts.

Keywords
Genetics, husbandry, behaviour, reproductive physiology, veterinary medicine, reintroduction

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Introduction

Owing to the precarious situation of the Iberian lynx *(Lynx pardinus)*, in the wild (Calzada et al., this book; Simón et al., this book), conservation measures need to be implemented effectively and efficiently, integrating efforts and working tools. Iberian lynx conservation could be conceived as a puzzle whose pieces should fit together adequately. One of such pieces involves *ex situ* conservation, which includes –among other activities– captive breeding, genetic and demographic management of the captive population, management of a Biological Resources Bank (BRB), preparing captive-born animals for release, as well as capacity building, education, and outreach efforts (Vargas et al., 2005b).

Current Iberian lynx conservation breeding efforts focus on producing physiologically and behaviourally sound captive-born individuals (Figure 1) that are suitable for future reintroduction efforts. For this purpose, we use management and research techniques that rely on multidisciplinary input generated on the specie's life history, behaviour, nutrition, veterinary and health aspects, genetics, reproductive physiology, endocrinology and ecology. The Programme stresses the importance of adapting our husbandry schemes based on research data to promote natural behaviours in captivity (hunting, territoriality, social interactions) and a stress-free environment that is conducive to natural reproduction. Some relevant research areas include: determining fecal hormone profiles for adult and subadult lynx (Pelican et al., this book; Dehnhardt et al., this book) studying reproductive behaviour and cub development (Antonevich et al., this book), determining reproductive health of male and female breeders (Roldan et al., this book; Göritz et al., this book), developing a non-invasive pregnancy test (Jewgenow et al., this book), establishing sound bio-security and biomedical protocols (Martínez et al., this book), establishing reference values for blood parameters (Pastor et al., this book; García et al., this book), genotyping all founders and making paring recommendations based on genetic distance between breeders (Godoy et al., this book; Leus and Lacy, this book). Within this scheme, one of our goals is to minimize the use of potentially invasive methods while simultaneously enhancing the trust between the animals and their keepers to assist in securing information on animal weight and gestational status. Between 2005 and 2008, a total of fifteen pregnancies resulted in the birth of 31 live offspring, of which 24 survive to date (Vargas et al., 2008 a; Figure 2). While describing various organizational aspects of the Iberian Lynx Conservation Breeding Programme, this chapter will present an overview of the information contained in various chapters of this book while emphasizing how results from multidisciplinary life science research can be integrated into an adaptive management approach to help recover the world's most endangered felid species.

The Iberian Lynx *Ex situ* Conservation Programme

The Iberian Lynx *Ex situ* Conservation Programme is integrated within the National Strategy for the Conservation of the Iberian Lynx, officially endorsed by the Spanish National Commission for the Protection of Nature (MARM, 2008; Calzada et al., this book). National, regional and international institutions collaborate with the Programme, which is currently implemented through a "multilateral commission" that includes the central governments of Spain and Portugal, together with the autonomous governments of Andalusia, Extremadura and Castille-La Mancha, Spain. Portugal, where no Iberian lynx populations were detected during the last 2002–2003 census, has developed its own *ex situ* conservation action plan in coordination with the Spanish Programme (Serra et al., 2005) and it is presently building a captive breeding facility while beginning to work on improving habitat for future re-establishment of lynx populations (Sarmento et al., this book).

Photo: Antonio Rivas

Figure 1. Iberian lynx female with her three-week-old captive-born cubs. Mothers tend to maintain their litter in the den for approximately one month and, afterwards, they frequently move their cubs to a different den. Generally, cubs begin to explore their immediate environment during their fifth week of life.

Figure 1. Hembra de lince ibérico con sus cachorros de tres semanas nacidos en cautividad. Las madres tienden a mantener la camada en su paridera natal durante el primer mes de vida y después suelen mover los cachorros a otra paridera. Generalmente, los cachorros comienzan a explorar su entorno más inmediato durante su quinta semana de vida.

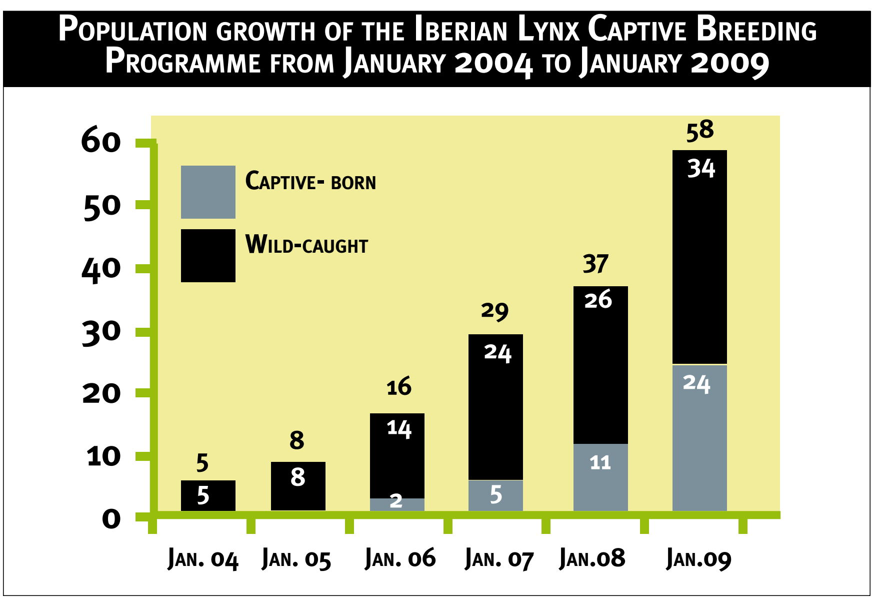

Figure 2. Population growth of the Iberian Lynx Captive Breeding Programme from January 2004 to January 2009 (Grey bars = captive-born cubs; black bars = wild-caught individuals).
Note: Between March and April, 2009, 18 new cubs have been born in the Breeding Programme, bringing up to 42 the number of Iberian lynx cubs raised in captivity to date.

Figura 2. Crecimiento poblacional del Programa de Cría del Lince Ibérico entre enero de 2004 y enero de 2009 (barras grises = cachorros nacidos en cautividad; barras negras = ejemplares provenientes de la naturaleza). Nota: Entre marzo y abril de 2009, han nacido 18 nuevos cachorros en el Programa de Cría, elevando a 42 el total de cachorros de lince ibérico criados en cautividad.

The route map of the *Ex situ* Programme is the Iberian Lynx Captive Breeding Action Plan, an adaptive management document reviewed annually by an advisory captive breeding committee. The Plan's most recent update, which emphasizes habitat conservation using *ex situ* efforts as a working tool, has been recently endorsed by the highest Spanish authorities at the Sectorial Conference, in May 2008 (Vargas et al., 2008b). The main *Ex situ* Programme goals are twofold: 1) To maintain a genetically and demographically managed captive population that serves as a "safety net" for the species and 2) To help establish new Iberian lynx free-ranging populations through reintroduction programmes. To accomplish these goals, the Iberian Lynx Conservation Breeding Programme encompasses management and applied research strategies in the following six areas.

Genetic and demographic management of the captive population

Iberian lynx genetic goals for the *Ex situ* Programme were established based on recommendations provided by the IUCN/SSC Conservation Breeding Specialist Group in collaboration with the Iberian lynx *in situ* conservation managers. Population modelling using the Programme PM2000 (2004) revealed that it would be impossible to maintain 90% of existing gene diversity for 100 years because the wild population could not withstand extraction of the required 12 founders per year for 5 years (Lacy and Vargas, 2004; Leus and Lacy, this book). At the time of this first analysis in Spring 2004 there were six wild-caught lynxes already at El Acebuche captive breeding center, a facility that was constructed in 1991 in Doñana National Park, Southeastern Spain. At the same time, modelling suggested the feasibility of maintaining 85% of the existing genetic diversity for the next 30 years. The outcome eventually would be the *ex situ* management of 60 individuals (30 males, 30 females) as breeding stock (Lacy and Vargas, 2004; Godoy et al., this book; Leus and Lacy, this book). This goal could be attained by adding four founders (mostly cubs or juveniles) per year for 5 years as well as one extra founder every 2 years (from the handicapped lynxes that normally enter rescue centres) for the entire duration of the Programme. This level of extraction rate would have a minor impact on the viability of the wild populations according to the model designed by Palomares et al.,(2002). Following this scheme, the Programme could achieve its population target of 60 individuals by 2009 and reintroduction of captive-raised lynxes could begin in 2010, provided that adequate habitat was prepared. Originally, modelling predicted the availability of 12 to 13 captive-born lynxes annually from 2011 through 2019 (Lacy and Vargas, 2004). A recent update of the projections, using data from actual captive reproduction over the past 5 years, indicates that the annual number of animals available for release every year will oscillate between 20 and 40 Iberian lynxes (Godoy et al., this book).

During the past seven years (from Spring 2002 to 2008), the Andalusian government has extracted 26 wild-born Iberian lynxes for the Captive Breeding Programme; in addition, 10 more lynxes were brought into captivity because they had problems that compromised their survival in the wild (Simón et al., this book), although not all of these lynxes can be considered as potential founders of the captive population. The year 2008 marked the end of the planned extractions of wild lynxes for incorporation into the breeding population; still, those found injured or handicapped –with compromised probabilities of survival in the wild– will continue to trickle into the Breeding Programme. At the time of writing this chapter, there are 58 lynxes in the Programme (Figure 2), of which 42 (28 founders and 14 captive-born) and 6 (all founders) have pure Sierra Morena and Doñana ancestry, respectively. Ten of the captive-born cubs in captivity descend from crossings between Doñana and Sierra Morena founders (Figure 3). Thus, the Programme is presently ahead of growth projections (Godoy et al., this book) and could already begin to provide captive-born individuals for reintroduction efforts.

To provide the captive space needed to achieve the Programme's genetic goals, construction of two new Iberian lynx breeding centers –Granadilla (Extremadura) y Odelouca (Silves, Portugal)– is underway. An additional center in Cabañeros (Castille-La Mancha) is also scheduled for future construction. All breeding centers are strategically placed so they can be co-managed by administrations that commit to habitat preparation for future reintroductions. Breeding centers will function as a network, following EEP standards, each managing approximately eight Iberian lynx breeding pairs. All centers considered, the captive population will be managed as a metapopulation, a genetic counterpart to the Sierra Morena and Doñana free-ranging populations (Godoy et al., this book). Construction of breeding centers is tightly linked –via Memorandums of Understanding– to a regional compromise to prepare habitat for future reintroduction efforts. In this fashion, *in situ* and *ex situ* efforts work together towards a common goal.

Photo: Antonio Rivas

FIGURE 3. MATING BETWEEN A MALE FROM SIERRA MORENA AND A FEMALE FROM DOÑANA. BETWEEN 2005 AND 2008, OUT OF THE 24 CAPTIVE-RAISED CUBS, TEN WERE OF MIXED-ORIGIN "DOÑANA X SIERRA MORENA".

FIGURA 3. APAREAMIENTO ENTRE UN MACHO DE SIERRA MORENA Y UNA HEMBRA DE DOÑANA. DE LOS 24 CACHORROS CRIADOS EN CAUTIVIDAD ENTRE 2005 Y 2008, 10 HAN SIDO DE ORIGEN MIXTO "DOÑANA X SIERRA MORENA"

Photo: Héctor Garrido

FIGURE 4. ROUND-THE-CLOCK VIDEO-SURVEILLANCE SYSTEM. THIS SYSTEM ALLOWS FOR EARLY DETECTION OF POTENTIAL PROBLEMS WHILE IT PERMITS STUDYING THE LYNXE'S BEHAVIOUR WITHOUT DISTURBING THE ANIMALS. THANKS TO THIS SYSTEM, WE HAVE BEEN ABLE TO DETECT SEVEN ABANDONED CUBS (ALL BY PRIMIPAROUS MOTHERS) AND 18 SIBLING FIGHTS, THUS ALLOWING THE TEAM TO REACT IN TIME AND SAVE SEVERAL IBERIAN LYNX LIVES.

FIGURA 4. SISTEMA DE VIDEOVIGILANCIA (24-HORAS AL DÍA). ESTE SISTEMA PERMITE LA DETECCIÓN TEMPRANA DE CUALQUIER PROBLEMA POTENCIAL Y FAVORECE EL ESTUDIO DEL COMPORTAMIENTO DE LOS LINCES SIN CAUSARLE MOLESTIAS DE NINGÚN TIPO. GRACIAS A ESTE SISTEMA SE HA PODIDO DETECTAR EL ABANDONO DE VARIOS CACHORROS (TODOS POR PARTE DE MADRES PRIMERIZAS) Y DIVERSAS PELEAS ENTRE HERMANOS N=18, PERMITIENDO REACCIONAR A TIEMPO Y SALVAR LA VIDA DE VARIOS EJEMPLARES DE ESTA ESPECIE.

TABLE 1. MATING, GESTATION, PARTURITION AND CUBS WEANED (3-MONTHS-OF AGE) OF CAPTIVE IBERIAN LYNX BETWEEN 2005 AND 2008. THE GESTATION PERIOD IS MEASURED AS THE TIME ELAPSED BETWEEN THE FIRST OBSERVED MATING AND THE DELIVERY OF THE FIRST OFFSPRING, WHILE "LABOUR" IS MEASURED FROM THE FIRST OBSERVED CONTRACTION TO THE DELIVERY OF THE LAST YOUNG. ("M.F.UNK"= MALE, FEMALE, UNKNOWN; NP= NON PREGNANT; N/A = NOT APPLICABLE; *= WHELPING BEFORE DUE TIME; **=ONE HAND-RAISED CUB; *=ALL CUBS HAND-RAISED).**

TABLA 1. APAREAMIENTOS, GESTACIONES, PARTOS Y CACHORROS DESTETADOS (3 MESES DE EDAD) EN EL PROGRAMA DE CONSERVACIÓN *EX SITU* DEL LINCE IBÉRICO ENTRE 2005 Y 2008. EL PERIODO DE GESTACIÓN SE MIDE COMO EL TIEMPO TRANSCURRIDO DESDE LA PRIMERA CÓPULA OBSERVADA HASTA EL NACIMIENTO DEL PRIMER CACHORRO, MIENTRAS QUE EL TRABAJO DEL PARTO ("LABOUR") SE MIDE COMO EL TIEMPO TRANSCURRIDO DESDE LA PRIMERA CONTRACCIÓN OBSERVADA HASTA EL NACIMIENTO DEL ÚLTIMO CACHORRO. ("M.F.UNK"= MACHO, HEMBRA, DESCONOCIDO; NP = NO GESTANTE; N/A=NO APLICABLE; * NACIMIENTO ANTES DE TÉRMINO; **=UN CACHORRO CRIADO A MANO; ***TODOS LOS CACHORROS CRIADOS A MANO)

TABLE 1.

Year	Female	Male	Date 1st mating	Number of matings	Gestation (days)	Labour	Cubs Born Total(m.f.unk)	Cubs Weaned Total(m.f.unk)
2005	*Saliega*	*Garfio*	23/01/2005	>5 (unk)	64	?	3(1.2)	2 (1.1)
2006		*Garfio*	19/01/2006	43	63	?	2(0.2)	2 (0.2)
2007		*Jub*	18/01/2007	43	64	1 h	2(1.1)	2(1.1)
2008		*Jub*	18/01/2008	65	64	32'	3(1.2)	2(1.1)
2005	*Aura*	*Jub*	28/01/2005	25	NP	N/A	N/A	N/A
2006		*Garfio*	30/01/2006	26	NP	N/A	N/A	N/A
2007		*Garfio*	15/01/2007	21	65	6 h	3(2.1)	3(2.1)
2008		*Garfio*	07/02/2008	21	65	5h 55'	2(1.1)	2(1.1)
2005	*Esperanza*	*Garfio*	11/02/2005	20	NP	N/A	N/A	N/A
2006		*Jub*	09/02/2006	25	65	>6h	2(1.1)	1(0.1)**
2007		*Jub*	03/01/2007	24	66	4h 40'	2(1.1)	1(1.0)
2008		*Jub*	06/02/2008	33	66	2h 43'	1(0.1)	1(0.1)
2006	*Aliaga*	*Cromo*	15/02/2006	13	56 *	50´	2(2.0)	0
2007		*Cromo*	22/01/2007	23	NP	N/A	N/A	N/A
2008		*Cromo*	07/02/2008	33	64	5h 22'	3(0.3)	3(0.3)***
2006	*Adelfa*	*Cromo*	19/01/2006	19	NP	N/A	N/A	N/A
2007		*Cromo*	27/01/2007	33	61 *	1 h	3(2.0.1)	0
2008		*Garfio*	01/02/2008	32	63	3h 25'	2(2.0)	2(2.0)
2007	*Artemisa*	*Almoradux*	02/01/2007	23	42 *	N/A	2(2.0)	0
2007	*Brisa*	*Arcex*	20/01/2007	15	NP	N/A	N/A	N/A
2008	*Brisa*	*Arcex*	19/01/2008	25	59 *	9h	2(0.1.1)	1(0.1)***
2008	*Boj*	*Arcex*	02/02/2008	31	65	6h	3(2.1)	2(0.2)***

Captive husbandry and behaviour

Captive husbandry is based on interdisciplinary input from a variety of animal-care fields, such as nutrition, behaviour, genetics, physiology and veterinary medicine, together with the systematic use of the scientific method. One of the Programme's key husbandry challenges is to strike a balance between fostering natural behaviours in captivity (hunting, territoriality, social interactions, etc.) and creating a stress-free environment where animals are more prone to mate. For this reason, the Breeding Programme favours naturalistic enclosures and promotes natural behaviours via environmental enrichment (Manteca, this book; Martos, this book). In order to get important information about the animals (such as their body mass or determining whether or not the females are pregnant), certain training techniques are being used. Some of these include obtaining regular weights by having the lynx step on a measuring scale. Such techniques are designed to avoid using invasive methods, which would stress the animals, and they also serve as a way to strengthen the trust between the animals and their keepers. Special care is taken to avoid domestication of captive lynxes, although this becomes a greater challenge in the case of hand-reared, abandoned cubs (Rivas et al., this book).

Lynx behaviour and activity patterns are also being carefully observed by a round-the-clock video surveillance system (see description in Vargas, 2005a) (Figure 4), which provides a great deal of information on conducts that could not easily be learned through observations in the wild, such as the ontogeny of predatory behaviour or the dynamics of the weaning process (Figure 5). Among other things, we have learned that mating behaviour in the Iberian lynx follows a similar pattern as that of other felids (Figure 3) (Lanier and Dewsbury, 1976; Rodríguez-Llanes, 2006). Breeding season in captivity mostly takes place throughout January and February, with most births occurring in March and April, as it is the case in the wild (Palomares et al., 2005; Palomares et al., this book "a"). The actual mating period lasts between two and three days during which lynxes copulate an average of 28 times (range: 13-65; n=460 copulatory bouts in 21 pairs; Table 1). Gestation period, counted from the time of the first copulation, ranges from 63-66 days (n=12). All parturitions that have occurred before 61 gestational days (n=4) have resulted in the birth of either dead or weak and not completely developed young, which were considered premature. Although variation between individuals is very high, most females are very consistent regarding their own timing to enter estrous and regarding the number of days they are gravid (Table 1).

Labour during whelping (regarded as the time lapsed between the first visible contractions until the delivery of the last offspring) varies widely between females, with some of them delivering each young within 10-15 min intervals while others taking up to 9 hours between the delivery of each offspring (Table 1). Primiparous females have a higher rate of failure to raise their young than multiparous ones. Out of the eight females that have whelped at El Acebuche center, only two first-time mothers –*Saliega* and *Aura*– managed to nurse all their young until weaning age. Two other dams –*Esperanza* and *Boj*– kept only one of their cubs after whelping and abandoned the rest of the offspring in their first litter, while three other females –*Adelfa*, *Aliaga* and *Brisa*– miscarried one of their offspring and delivered the rest of the cubs prematurely during their first gestation. One female, *Artemisa*, miscarried two undeveloped fetuses at 42 gestational days. Our video-surveillance system permits early detection of problems, which has resulted in the rescue of 7 Iberian lynx cubs that were hand-raised after being abandoned by their first-time mothers (Figure 5) (Rivas et al., this book).

This non-intrusive surveillance system has also allowed us to identify the existence of a sensitive period when Iberian lynx cubs become highly competitive to the point of siblicide (Vargas et al., 2005 b; Antonevich et al., this book). Spontaneous aggression erupted at 44 days of age in the first Iberian lynx litter born in captivity. The largest cub (a female) in a litter of three was killed by a brother who delivered lethal bites to the larynx and skull. Agonistic behaviour has been observed in nine of eleven subsequent Iberian lynx litters of two or more cubs, with the most intensive fighting occurring around the end of the sixth and seventh post-natal weeks, respectively (Antonevich et al., this book). This same phenomenon has been observed in the Eurasian lynx by Russian scientists at the Tcherngolovka facility who recorded aggressive behaviour in 16 of 31 litters, with deaths occurring in four cases (Naidenko and Antonevich, this book). The authors indicate that the highest prevalence of agonistic behaviour in Eurasian lynx cubs occurred at 36 to 64 days of age, with the greatest frequency during the seventh post-natal week. Although siblicide in Iberian lynx has not been directly observed in nature, a 1-month-old cub was found in the wild in 2003 with severe injuries compatible with bites from another cub. It may also be relevant that free-ranging Iberian lynxes generally give birth to three cubs, but 70% of the females

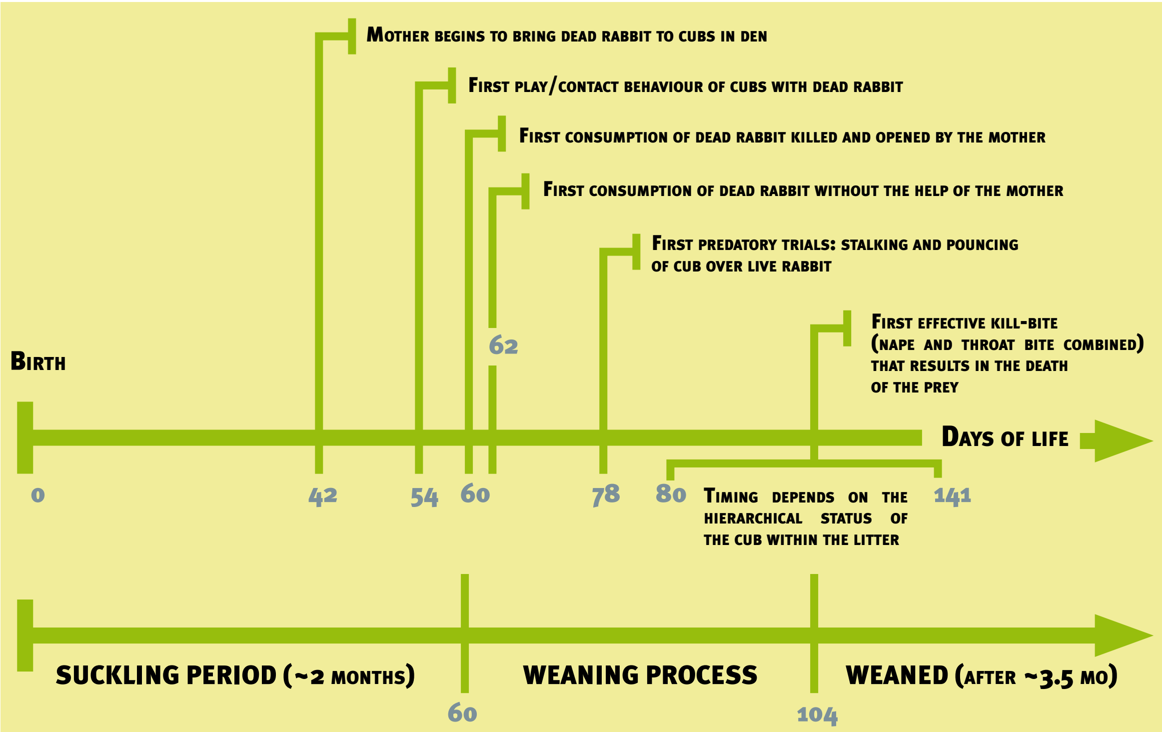

Figure 5. Developmental landmarks of predatory behaviour in mother-raised Iberian lynx cubs, coupled with timing of the lactation and weaning processes (n=9; adapted from Vázquez et al., 2007). During and after the weaning period, mothers play an active role in teaching their offspring how to hunt.

Figura 5. Hitos del desarrollo predatorio en cachorros de lince ibérico criados por sus madres y comparativa con la duración de los procesos de lactancia y destete (n=9; adaptado a partir de Vázquez et al., 2007). Durante y tras el proceso de destete, las madres juegan un papel activo en enseñar a cazar a sus crías.

are usually observed with only two young after approximately three months post-parturition (Palomares et al., 2005). Sibling aggression in Iberian lynx has influenced programme husbandry as staff must be vigilant and prepared to break up aggressive bouts during the sensitive period.

Health and veterinary aspects

The health considerations involved in captive breeding, reintroduction and translocation programmes are a source of great concern to conservation biologists, since captive-bred animals could potentially transmit infectious diseases to wild populations (e.g., tuberculosis in reintroduced Arabian oryx, Oryx leucoryx; Viggers et al., 1993) and vice-versa (e.g., wild-caught black-footed ferrets transmitted canine distemper to potentially uninfected, captive individuals; Williams et al., 1988). It is generally felt that most of these conservation programmes were lacking: 1) sufficient information on disease distribution and risk in captive populations; 2) sufficient information on disease incidence, distribution and risk in wild populations; 3) quarantine systems to prevent disease transmission; 4) a system to adequately track and detect pathogens.

Because relatively little was known about the diseases affecting the Iberian lynx, actions to improve our knowledge of the main diseases affecting the species was imperative. The Iberian Lynx Conservation Breeding Programme established a Veterinary Advisory Team (GAAS) dedicated to address diverse aspects of veterinary management and research, as well as protocol development (Martínez et al., 2006). To improve the understanding of the various diseases that could potentially affect the species, the Programme's main lines of action involve the establishment of preventive disease protocols for the captive population, capacity building of veterinary staff working with *in situ* and *ex situ* populations, and conducting research on general veterinary science (Martínez et al., this book).

Photo: José María Pérez de Ayala

Figure 6. Sometimes it is not easy to differentiate between play and fighting behaviours, especially because play-fighting is common in Iberian lynx cubs. In general, sibling fights do not occur during playful bouts.

Figura 6. A veces no es fácil diferenciar entre el comportamiento lúdico y el comportamiento agonístico, especialmente porque "jugar a pelearse" es frecuente en los cachorros de lince ibérico. Generalmente, las peleas entre hermanos de camadas no surgen durante el juego.

Research projects have helped determine the incidence and prevalence of infectious pathogens in captive and wild lynx populations (Millán, 2006; Meli et al., this book; López et al., this book), determination of normal vs pathological blood values (Pastor et al., this book; García et al., this book), and establishing parameters that point towards a potential renal disfunction in wild and captive lynxes (Jiménez et al., 2008; Jiménez et al., this book). The results of research, protocol development, and standardization efforts, coupled with dissemination and sharing of knowledge and experience among veterinarians working in the Programme are all contributing to more consistent diagnosis and treatment.

Reproductive physiology

Reproductive physiology studies and associated technologies increase the success rate of any captive-breeding programme and are important in helping with the conservation of wild felids in captivity (Wildt et al., 2009; Wildt et al., this book). Reproductive technologies are available for three major purposes: 1) assessing fertility and monitoring reproductive status; 2) assisting in breeding and maintenance of gene diversity; and 3) learning more about reproductive mechanisms of the endangered Iberian lynx.

Consistent with the need for more basic species information, our husbandry and breeding efforts have established/reconfirmed normative data on sexual maturity (males, 2-3 years; females, 2-3 years; Roldan et al., this book; Göritz et al., this book, respectively), oestrus length (3-7 days), copulations per pairing (28) and gestation interval (63-66 days) (Table 1). Faecal hormone monitoring has demonstrated that females experience ovarian cycles from January through May whereas males maintain testosterone year-round (Pelican et al., this book; Dehnhardt et al., this book). But species peculiarities also have been revealed, for example, oestrogen metabolite concentrations during pregnancy are significantly greater than in other felid species (Pelican et al., this book; Brown, this book). Additionally, progestin excretion profiles are unusually lengthy, largely because ovarian corpora lutea (sites of earlier ovulations) remain active much longer than in most other felids (Göritz et al., this book). Prolonged non-pregnant luteal activity has also been described in the closely related Eurasian lynx (*Lynx lynx*; Jewgenow et al., 2006; Dehnhardt, this book) and in the Canada lynx (Fanson et al., this book) suggesting an idiosyncratically-conserved mechanism for lynxes in general.

Regarding male reproductive physiology, sperm traits seen in the Iberian lynx are lower than those reported for some other felid species, yet higher than those reported for the Eurasian lynx and the bobcat (Gañán et al., in press; Roldan, et al., this book). It has also been found that thawed-out Iberian lynx spermatozoa are capable of

Photo: Antonio Rivas

Photo: Antonio Rivas

Figure 7. Five-week-old Iberian lynx hand-raised cubs. Whenever possible, it is important to maintain hand-raised cubs in pairs/triplets. If this were not possible, it is recommendable not to maintain the cub in isolation but to provide a similar-size cub from a closely related species in order to promote adequate socialization and behavioural development.

Figura 7. Cachorros de lince ibérico de cinco semanas criados a biberón. Siempre que se pueda, es importante mantener a los cachorros criados a mano en parejas o en grupos de tres. Si esto no fuese posible, se recomienda no mantener al cachorro en aislamiento e intentar proporcionarle la compañía de algún cachorro de otra especie de felino de tamaño similar con el fin de promover una buena sociabilización y un desarrollo adecuado del comportamiento.

fertilising viable in vitro matured domestic cat oocytes, thus opening up the possibility of examining functional capacity of spermatozoa from this species under laboratory conditions (Gañán et al., in press).

The *ex situ* population is also being used to explore a novel means of diagnosing pregnancy. Because all ovulating lynxes produce rising progesterone (regardless of conception), conventional hormone monitoring is not useful for identifying a gestating female (Pelican et al., this book). However, increasing concentrations of the hormone relaxin (in blood or urine) are indicative of pregnancy in felids (Van Dorsser et al., 2007). A unique non-invasive means of collecting blood has been developed using blood-sucking Triatomine bugs (*Rhodnius prolixus* or *Dipetalogaster maxima*) placed in specially-drilled hiding holes in the lynx's cork nestbox (Voigt et al., 2007; Jewgenow et al., this book). As much as 2 ml of blood can be extracted per bug, more than adequate for the assay. Urine is captured using special collection devices distributed throughout the animal's enclosure. Pregnant females express a positive relaxin signal from 32 to 56 days post-copulation of a 65-day gestation (Braun et al., 2009; Jewgenow, this book), thereby allowing managers to prepare for an impending parturition.

In order to assist breeding and maintaining genetic diversity, the Iberian Lynx *Ex situ* Programme collaborates with the maintenance of Biological Resources Banks (BRBs) for conservation of biomaterial gathered from wild and captive Iberian Lynx populations. Biomaterials are presently being safeguarded at two locations: the National Museum of

Natural Sciences in Madrid (Roldan et al., this book) and the Miguel Hernández University in Elche, Alicante (León et al., this book). Although the Museum of Natural Sciences places special emphasis on reproductive samples and the MH University focuses on multipotential somatic cells, both banks preserve tissue, blood, serum, and other biological materials. The conservation of gametes will allow the Breeding Programme to extend future options without the limitation of space or the risk of disease transmission, while opening the opportunity of prolonging the possibilities of reproduction for individual animals after their death (Roldan et al., this book). The storage of samples at both BRBs provides a safety net for these valuable materials and ensures that biosamples will be available for additional analysis whenever needed, which is a crucial resource for potential retrospective studies.

Reintroduction

The small size of current Iberian lynx free-ranging populations renders them highly vulnerable to stochastic events. Thus, it is imperative to create, as soon as possible, new wild populations, while simultaneously increasing numbers in the existing ones. Prior to any reintroduction/translocation a detailed viability study is required (IUCN Guidelines for reintroductions: IUCN, 1998). It is important to determine if the cause or causes that brought the species to extinction in the specific area have been eradicated and, if so, if there is administrative and local population support for the programme and if the habitat is prepared to support a viable population of the species (Simón et al., this book). All reintroductions and translocations must be performed using scientific support and the Iberian lynx should be no exception (Calzada et al., this book; Palomares et al., this book “b”). Such conservation techniques require an interdisciplinary approach, with input from experts in ecology, veterinary medicine, genetics, physiology and behavioural sciences, as well as support from socio-political and information sciences. All stages of programme development and implementation must have well-defined protocols that document objectives, methodology, responsibilities, as well as the accountability of the organizations and individuals involved (MARM, 2008).

The Andalusian government, by means of the current LIFE-Nature Project for the Reintroduction of the Iberian lynx in Andalusia, has evaluated different potential areas for lynx reintroduction in this Spanish region and has selected two areas that comply with IUCN criteria (Simón et al., this book). The first reintroduction is scheduled to take place in 2009 using wild-born individuals from the Sierra Morena population (Simón et al., this book). Reintroduction of captive-born lynxes is scheduled for 2010. Captive candidates will be chosen based on genetic and behavioural criteria. Preparation for release will include maintaining animals in large preconditioning enclosures with minimal human contact and exposure to natural stimuli, including live prey. Lessons from other reintroduction programmes will be essential for the planning and implementation of the first Iberian lynx reintroduction.

Capacity building and outreach efforts

Awareness, education, and scientific training are essential to all conservation breeding programmes. Education and awareness efforts should be focused on changing prevalent attitudes that contribute to habitat destruction and species extinction. One advantage enjoyed by conservation breeding programmes is their ability to gain public attention, particularly if the animal in question is charismatic and attractive to the broader public. The Iberian lynx is one such case, and raising public awareness for the need for habitat conservation to guarantee survival of the species in the wild is one of the Programme’s objectives. It is important to emphasize that breeding and keeping lynxes in captivity, with no hope of ever returning them to the wild or of recovering the natural populations, would be a pointless exercise. The Breeding Programme encourages, cooperates with, and supports media interest in the Iberian lynx, while taking every opportunity to remind the public of the primary importance of habitat conservation.

One way of providing an open window to the Breeding Programme is by sharing on-line information via a frequently updated Iberian lynx web page *(http://www.lynxexsitu.es)*, featuring monthly newsletters, pictures and videos of all captive lynxes, along with general interest and scientific articles, plus information on the Programme’s protocols and working methods. An English language version is currently in production to further expand the scope of communication and awareness efforts. As part of its training efforts, El Acebuche Center organizes on-site internships for recent college graduates interested in acquiring first–hand knowledge on the Iberian Lynx Conservation Programme.

Conclusions

To date, the Iberian Lynx Conservation Breeding Programme includes 58 (30, 28) lynxes and is ahead of Action Plan forecasts (Lacy and Vargas, 2004; Leus et al., this book; Godoy et al., this book). Between 2005 and 2008, 15 litters have been produced (three prematurely) with 24 surviving young (Figure 2). The Programme's management team works closely with *in situ* conservation authorities, managers and researchers. For example, skills and resources are being shared to address the increase in disease concern for lynxes and prey, and projects are coordinated to monitor genetic status of the wild and captive populations. Most importantly, our multidisciplinary research is generating new insights into the unique biology of this species while our intensive management is on course to meet the overall recovery goal of attempting reintroduction of captive-raised lynxes by 2010. Finally, the Programme carries out a capacity-building plan aimed at preparing professionals for working on endangered species conservation as well as sensitizing the general public and decision-makers about the importance of conserving habitat for the recovery of this charismatic felid. In this fashion, the guidelines and experiences offered in this book illustrate how an *ex situ* breeding programme can be integrated to compliment the challenge of recovering wild Iberian lynxes in nature, which is always the highest priority.

Acknowledgements

The authors would like to thank all the institutions –and associated staff– that collaborate with the Iberian Lynx Conservation Breeding Programme, including the Spanish Ministry of the Natural, Rural and Marine Environment, the governments of Andalusia, Extremadura, Castille-La Mancha and Portugal, the Life-Nature Project for Iberian Lynx Conservation in Andalusia, the Species Survival Commission of the International Union for the Conservation of Nature (Cat Specialist Group and Conservation Breeding Specialist Group), the National Scientific Research Council (CSIC: Doñana Biological Station in Seville, the National Museum of Natural Sciences in Madrid, and Almeria's Arid Zones Station), Doñana's National and Natural Park, TRAGSA/TRAGSEGA, EGMASA, the Jerez Zoo, the Diagnostic Center for Analysis (CAD) of Málaga, the Miguel Hernández University in Elche, the Autonomous University of Barcelona, the Institute of Zoo and Wildlife Research (Berlin, Germany), the University of Zurich–Vet Clinical Laboratory (Switzerland), the Complutense University of Madrid, the University of Huelva, the University of Cordoba, KORA (Switzerland), the Smithsonian Insitution Conservation and Research Center (USA), the Biodiversity Foundation, the Terra Natura Foundation (Spain), the Rainforest Foundation (Spain), The Gypaetus Foundation (Spain), the BBVA Foundation (Spain), the Dierenrijk Zoo Foundation (Holland), ConZOOlting, the Fuengirola Zoo (Spain), the Barcelona Zoo (Spain), Parques Reunidos (Spain), the European Association of Zoos and Aquariums, the Iberian Association of Zoos and Aquariums, the International Society for Endangered Cats (ISEC), Pole-Position, CatsCraze, Coronel Tapiocca, Pfizer, Merial, Fort Dodge, Farmadiet, Disvisión, Guadiamar Reference Veterinary Services, Avila Veterinary Hospital, Condado de Huelva Veterinary Clinic, Masnou/Rocaberti Veterinary Clinics, Noray, the Andalusian School of Biologists, the Spanish Society for the Study and Conservation of Mammals (SECEM), WWF-Spain, Ecologists in Action (Spain), the Ornithological Society of Spain (SEO/Birdlife), and the National Park System volunteer programme. It is thanks to the combined efforts and wholehearted support from many different individuals, teams and organizations that the Iberian Lynx Conservation Programme moves forward towards the recovery of one of the world's most endangered mammals.

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Photo: José María Pérez de Ayala

Genetics, behavior and husbandry

GENÉTICA, COMPORTAMIENTO Y MANEJO

Managing conservation breeding programmes oriented towards reintroduction is a case of artfully combining the following advice:

Before beginning, plan carefully
Marcus Tullius Cicero

You'll never do a whole lot unless you're brave enough to try
Dolly Parton

Photo: Luis D. Klink

Genetic and demographic management of conservation breeding programmes oriented towards reintroduction

Gestión genética y demográfica de los programas de cría en cautividad con fines de reintroducción

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Resumen

En el presente capítulo se resumen los principios generales de la gestión genética de los programas de cría en cautividad con fines de reintroducción, uno de cuyos objetivos más importantes es mantener tanta diversidad genética como sea posible. Esta diversidad genética representa el potencial evolutivo de una población y está correlacionada con su aptitud biológica. Las poblaciones en cautividad suelen ser pequeñas, carecen de flujo génico sin intervención humana y no viven en condiciones naturales. Esto las hace vulnerables a cambios genéticos que pueden afectar al éxito de la reintroducción, tales como la pérdida de diversidad genética debido a la deriva genética, la endogamia, la depresión por endogamia y la adaptación genética a la vida en cautividad. La mejor forma de mantener la diversidad genética en poblaciones cautivas implica: 1) maximizar el número de ejemplares fundadores (sin poner en peligro la población silvestre de donde se extraen los ejemplares), 2) maximizar la tasa de crecimiento durante la etapa de crecimiento de la población (lo que implica un buen conocimiento de la historia natural y los cuidados que requiere la especie en cautividad), 3) maximizar la capacidad de carga y 4) realizar los emparejamientos de los ejemplares en función de su índice medio de parentesco o mean kinship (mk), sobre todo durante la "etapa de capacidad" del programa, y además, 5) minimizar la endogamia. El valor mk de un individuo es una medida con la que se establece su parentesco con toda la población. Los ejemplares con valores de mk bajos tienen pocos parientes en la población. Si uno de estos individuos muere, es muy probable que se pierda para siempre esa diversidad genética única. En cambio, es probable que la mayor parte del material genético de un individuo con muchos parientes ya esté representado en ellos. Por tanto, la diversidad genética de una población cautiva se puede maximizar dando prioridad a los individuos con valores de mk bajos, emparejando individuos con valores de mk similares para la reproducción, y minimizando la endogamia. Se necesita un buen grado de conocimiento para tener en cuenta las características biológicas y sociales de la especie, las peculiaridades no genéticas de los individuos en cuestión y las circunstancias prácticas. Por último, los individuos criados en cautividad más idóneos para la reintroducción deben estar bien representados en la población cautiva y, asimismo, deben ser cuidadosamente seleccionados, ya que ellos mejorarán la diversidad genética de la población silvestre. Todos estos principios se explican en este capítulo, prestando particular atención al caso específico del lince ibérico.

Palabras clave
Reproducción en cautividad, diversidad genética, fundadores, metas poblacionales, selección de parejas, índice medio de parentesco

Abstract

This paper aims to summarise the general principles of the genetic management of conservation breeding programmes with the aim of reintroduction. One of the most important aims for such programmes is to retain as much gene diversity as possible. Gene diversity represents the evolutionary potential captured within the population and is correlated with population fitness. Populations in captivity are often small, lack gene flow between subpopulations without human intervention, and live under unnatural conditions. This makes captive populations vulnerable to genetic changes that may affect reintroduction success, such as loss of genetic variation through genetic drift and inbreeding, inbreeding depression, and genetic adaptation to captivity. Gene diversity can best be maintained in captive populations by 1) maximising the number of founders (without compromising the wild source population); 2) maximising the growth rate in the growth stage of the population (implying good knowledge of natural history and captive husbandry); 3) maximising carrying capacity, and 4) basing the pairings of individuals, especially during the capacity stage of the Programme, on their mean kinship (mk) values, while 5) minimising inbreeding. The mk value of an individual is a measure for the relatedness of this individual to the entire population. Animals with low mk values have few relatives in the population and vice versa. If an individual with few relatives dies, chances are high that unique genetic variation is lost forever. In contrast, most of the genetic material of an individual with many family members (i.e., high mk) is likely also present in its relatives. By giving breeding priority to low mk individuals, combining individuals with similar mk values for mating, and minimising inbreeding, the amount of gene diversity retained can be maximised. A degree of compromise will be necessary to take into account the biological and social characteristics of the species, non-genetic peculiarities of the individuals involved, as well as practical circumstances. Finally, the captive born individuals best chosen for reintroduction are those that benefit the gene diversity of the wild population, but are genetically well represented in the captive population. The above principles are explained while paying particular attention to the specific case of the Iberian lynx.

Keywords
Captive propagation, gene diversity, founders, population targets, pair selection, mean kinship

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Introduction: Why is genetic management so important?

One of the most important aims for the management of any captive population of endangered species, and especially one that is to function as a source for individuals to be reintroduced into the wild, is to retain as much gene diversity as possible. This is because gene diversity represents the evolutionary potential captured within the population and because there is a correlation between gene diversity (heterozygosity) and population fitness (Reed and Frankham, 2003). The more variation in genetic traits present in the population and in the individuals within it, the higher the chance that at least a proportion of the individuals is able to survive the challenges offered by the variable wild environment. Populations in captivity are often small, lack gene flow between subpopulations (different enclosures or captive colonies) without human intervention and live under unnatural conditions (de Boer, 1989). These characteristics make captive populations vulnerable to a number of genetic changes that affect reintroduction success, among which loss of genetic variation (and therefore of evolutionary potential) through genetic drift and inbreeding, inbreeding depression, and genetic adaptation to captivity. Gene diversity can be measured by employing various molecular techniques to study the nuclear and/or mitochondrial DNA of a sufficiently large sample of individuals in the population, or can be inferred from detailed pedigree records with the aid of computer modelling.

If captive propagation is expensive and prone to losing gene diversity, why do it?

The maintenance of a captive population of an endangered species is a very costly affair. If captive populations are vulnerable to rapid loss of evolutionary potential, and at the same time cost a lot of time and resources, why do it? The same artificial conditions that cause the loss of gene diversity in captivity also provide the captive population with a relatively safe environment (no predators, sufficient food and shelter, medical treatment etc.). Assuming one has sufficient knowledge of the basic biology and therefore husbandry needs of the species, a much faster population growth can be obtained in captivity. Mortality rates may be lower in captivity than in the wild and for some species the reproductive output per female per year can be increased (e.g., double clutching in birds). The assumption of sufficient knowledge of the biology and husbandry of the species is not one to be taken lightly. If reproduction is lower and/or mortality is higher in captivity than in the wild, extracting animals from the wild to establish and support the captive population will act as a drain on the already endangered wild population. Sometimes closely related species for which there is more knowledge on the natural history in the wild and/or that have already been kept successfully in captivity can act as a model for the development of suitable husbandry and breeding techniques. In the case of the Iberian lynx for example, experience with the Eurasian lynx *(Lynx lynx)* taught that in captivity there is regular mortality of cubs due to one cub attacking and fatally injuring another cub of the same litter. This knowledge allowed housing and breeding management in the Iberian lynx *ex situ* breeding programme to be adapted such that this cause of cub mortality could be minimised without compromising the family unit and normal lynx rearing behaviour (Vargas et al., 2005).

Apart from providing a safe environment, the more controlled conditions in captivity allow for pro-active genetic and demographic management in order to minimise and slow the rate of loss of gene diversity, even when the population is relatively small. The relevant techniques employed will be discussed below.

Starting the captive population – how many founders?

The wild caught individuals at the basis of a captive population are known as founders. The founders are a sample of the wild population and therefore the statistical laws of sampling apply (Lacy, 1994). The larger the number of founders taken from the wild population, the higher the proportion of the wild population's gene diversity that will be present in the source population. In the case of critically endangered species, this creates an interesting dilemma. Catching many wild individuals as founders for the captive population may further endanger the wild source population(s). Also, the smaller and more fragmented the wild population becomes, the less gene diversity is available for the start of a captive population. For this reason IUCN recommends in its guidelines for the *ex situ* management of endangered species, to start a captive breeding programme when a species still numbers in the thousands in the wild (IUCN, 1995). Considering the very low numbers of Iberian lynx in the wild, there was no time to lose and the decision to start an *ex situ* breeding population was entirely warranted (Delibes et al., 2000).

If catching more founders is better for the captive population but less good for the remnant wild population, then how many founders are enough? Luckily, sampling and genetic theory indicates that only modest numbers of founders, namely at least 20 unrelated wild individuals, are sufficient to capture 97.5% of the gene diversity of the wild population within the founder population. Even a modest further increase in amount of gene diversity captured would require many more founders (Crow and Kimura, 1970; de Boer, 1989; Lacy, 1994; Frankham et al., 2002). About 20 unrelated founders are therefore considered an acceptable compromise between gene diversity captured and number of individuals needing to be extracted from the endangered wild population.

At this stage it is worth pointing out a few caveats: 1) A wild caught-individual can only be truly considered a founder once it has surviving descendants in the captive population. Therefore, likely more than 20 individuals will have to be caught as not all individuals may breed. 2) The founder generation is only the start of the captive breeding programme. How breeding progresses from the founder generation onwards (determined by management and the natural history characteristics of the species which influence growth rate, population size, founder representation, inbreeding level, etc.) will determine the rate of loss of the gene diversity present in the founder population. Twenty wild caught individuals should therefore be considered a theoretical minimum (see further in this paper for what is appropriate specifically for the Iberian Lynx population).

Breeding from the founders – more is better!

Each generation of individuals born in captivity is in a genetic sense a sample of the previous generation (Lacy, 1994). This can be understood as follows (de Boer, 1993): Every time a parent has an offspring, 50% of its genetic material is passed on to that offspring. If a parent has a second offspring and if the transmittance of the genetic material to a second offspring is independent of that to the first one, it is highly unlikely that they will each receive either exactly the same 50%, or the complementary 50%. On average, 50% of the genetic material will only be present in one offspring and not in the other, 25% will be present in both and 25% in neither. Statistically speaking, the genetic material of the two offspring combined represents 75% of the genetic material of the parents. If the parents have three offspring, this becomes 87.5%, four 93.75% etc. Conversely, should the parents die after having had three offspring, on average 12.5% of their genetic material would not be represented in those offspring – therefore 12.5% of their genetic variation would be lost. All of this holds a number of implications:

1. The more offspring a founder produces, the more of its genetic variation will have been passed on to the

next generation. In real life, the above theory holds true for each founder allele separately and some loci will be linked on the same chromosomes and are therefore not transmitted independently. Things are therefore not quite that simple, but it has nevertheless been estimated that 12 offspring per founder are sufficient to provide 99% probability that all alleles of a founder are transmitted to at least one offspring (Thompson, 1994).

2. The offspring will only be able to retain and preserve the genetic variation of the founders if they themselves survive and reproduce before they die. Genetically speaking, a founder that has had 12 offspring, but of which 9 never bred, might from a genetic point of view as well only have had three offspring. Therefore, it is likely that more offspring need to be produced than sampling theory indicates (to compensate for stochasticity in survival and reproduction of the offspring).

3. As each founder needs to produce many offspring, a steep growth rate is an important goal in the foundation phase of a captive breeding programme.

4. In captive populations that are small and remain small, a lot of gene diversity will be lost quickly due to the above process. Which alleles are lost is largely due to chance (a process called genetic drift), especially if there is no proactive management of who will breed with whom in the capacity phase of the population (see below).

5. Gene diversity is lost per generation. Therefore the fewer generations a population passes through in a given number of years, the slower gene diversity will be lost. This is partly determined by the natural history of a species, but can also be influenced to some degree by breeding management.

6. The growth rate during the foundation phase strongly influences the rate at which gene diversity is lost in the future of the captive breeding programme. In addition, when a founder dies, any of its genetic variation that was not yet passed on to the next generation will have been irretrievably lost from the captive population. There is also always a chance that mortality may occur among the offspring of a founder before they themselves have had the chance to reproduce and pass on the retained gene diversity. For these reasons, as long as carrying capacity in captivity has not yet been reached, founders should not be prevented from breeding, even those founders that appear to be much more prolific than the others. It is preferable to try to correct unevenness in founder representation during the capacity stage of the programme, rather than to risk losing alleles during the founding stage (Lacy, 1994). As long as all founder alleles are passed on with an acceptable probability, the fact that some have a higher frequency than others can be addressed later.

Capacity stage – how many Iberian lynx are enough?

From a genetic point of view, the smaller a population at the carrying capacity stage of a breeding programme (the stage at which the population will be kept stable at that size), the more gene diversity is lost. Although bigger is better, both space and financial and human resources are always limited. This begs the question, how big is big enough? What should be the minimum population of Iberian lynx kept in captivity in order to be able to retain a sufficient amount of gene diversity? And how much gene diversity is enough? This leads to the search for an acceptable compromise between the theoretic ideal and what is possible in practice.

First, it is necessary to introduce the concept of Effective Population Size (Ne). This theoretical concept can be intuitively understood by taking into consideration that a population with 500 males and 20 females is not as "effective" as one with 260 males and 260 females, or that having only 10% of the males doing all the breeding is not genetically the same as having all reproductive aged males contributing equally to the next generation. Or that a population with 500 individuals that crashes to 50 and then grows back to 500, is not as "effective" as one that had a stable population size of 500.

The effective population size is defined as the size of an ideal population that would have the same rate of genetic drift and of inbreeding as is observed in the real population with N individuals (Lacy, 1994; Frankham et al., 2002). In an ideal population there is random breeding, constant population size, equal sex ratio and non-overlapping generations. Needless to say, real life populations are far from "ideal". The ratio of Ne to N is influenced by the number of breeding animals in the population (some are pre- or post-reproductive and some animals at reproductive age may not breed for other reasons), variation in family size (not all individuals produce the same number of offspring), unequal sex ratio (leaving some animals of the more abundant sex with fewer breeding opportunities), and fluctuations in population size. There are different methods of calculating Ne that each makes adjustments for these different parameters influencing Ne (Frankham et al., 2002).

There is a relationship between the effective population size and the gene diversity retained: $Gt=G0\ (1-1/(2Ne))^t$, whereby Gt is gene diversity retained at generation t, G0 is gene diversity present in generation 0 (Frankham et al., 2002). The higher the effective population size, the more gene diversity will be retained. This implies that, apart from the number of founders, the growth rate and the true size of a population, the amount of gene diversity that can be retained in a captive population also depends on how closely the true population behaves to the ideal population. No real population will achieve the theoretical 'ideal', but a technique for management of breeding in captive populations has been developed to help maximise the ratio of Ne/N in captive populations (see below).

Year	N	Capture of founders	Releases	Cumulative releases
2004	6	0	0	0
2005	11	4 + 1	0	0
2006	18	4	0	0
2007	25	4 + 1	0	0
2008	35	4	0	0
2009	46	4 + 1	0	0
2010	56		5	5
2011	67	1	5	10
2012	73		8	18
2013	72	1	13	31
2014	73		12	43
2015	73	1	13	56
2016	72		12	68
2017	73	1	13	81
2018	73		12	93
2019	72	1	13	106

Figure 1. Growth projections for the Iberian lynx Conservation Breeding Programme (from Lacy and Vargas, 2004).

Figura 1. Proyecciones de crecimiento del Programa de Cría para la Conservación del Lince Ibérico (Lacy y Vargas, 2004).

Current genetic theory indicates that the minimum viable population size needed to balance the loss of gene diversity due to drift with the generation of new diversity through mutations is an effective population (Ne) of 500 - 5.000, which for wild populations often corresponds to a true population size (N) of about 5.000 – 50.000 individuals (Thomas, 1990; Nunney & Campbell, 1993; Frankham et al., 2002). Even when taking into consideration that the Ne/N ratio for captive breeding programmes under proper genetic and demographic management is often close to 0.3 (Frankham et al., 2002; Mace, 1986), this still implies a required true population size of about 1.700 – 17.000 which is a practical impossibility in terms of space, finances and resources for the vast majority of programmes. Even when space for a captive breeding programme is shared between a large number of zoos and other holding collections, the number of species needing captive breeding is so high that the demand for space usually far exceeds what is available. However, if a modest amount of loss of gene diversity is accepted, a smaller population is required to achieve this goal. Currently, the world zoo and aquarium community generally considers a goal of retaining 90% of gene diversity present in the source population after 100 years of breeding in captivity to be an acceptable compromise between a modest loss of gene diversity and accommodating more breeding programmes (because they are of smaller size). This goal can generally be achieved with a few hundred, rather than a few thousand individuals. Ninety percent of gene diversity retained after 100 years corresponds to an average level of inbreeding of 10%, meaning that on average the individuals would be related to the equivalent level of just below that of half-siblings or that of aunt and nephew (F = 0.125).

How has all this been applied to the particular situation of the Iberian lynx? The software programme PM2000 was designed to establish the genetic and demographic goals for pedigree managed captive breeding programmes (http://www.vortex9.org/pm2000.html) (Pollak et al., 2007). Based on the generation length, the population growth rate, the current population size, the current effective population size, the ratio of Ne/N, and the current gene diversity, me calculates the population size needed to reach a particular genetic goal (e.g., retention of 90% of gene diversity at the end of 100 years). These population parameters can either be calculated from pedigree data, or can be entered by the user. Lacy and Vargas (2004) employed PM2000 in order to determine the goals for the captive Iberian lynx population. At the time of the analyses, the *ex situ* Iberian lynx population consisted of a total of six animals, all wild caught animals. As no studbook data were available that could be used to calculate the other parameters, a number of assumptions were made, based on experience with similar species: maximum annual population growth rate 21.5%, generation time 5.25 years, Ne/N ratio 0.3, and current gene diversity 90%. The analyses indicated that the goal of maintaining 90% of gene diversity for 100 years is not obtainable for the Iberian lynx because the number of extra founders needed to achieve this (12 extra founders per year for the next 5 years) is more than the wild populations can sustain (Palomares et al., 2002), and the number of individuals required at carrying capacity (500) is exceeding the availability of space and resources for the programme. Furthermore, the primary goal of this captive population is not to provide a long term (e.g. 100 years), large, back up population for the wild population, but to provide a genetically healthy, yet relatively small, short term population that can relatively quickly supply individuals for reintroduction. Further modelling indicated that it will be possible to maintain 85% of gene diversity for 30 years (a more realistic time span) with a nucleus population of 60 breeders (feasible in terms of space and resources), if 4 wild cubs can be added to the programme each year, for the next five years (spread over Doñana and Sierra de Morena – a rate deemed viable according to the study by Palomares et al., 2002), as well as one extra founder every two years for the whole duration of the programme in the form of animals entering rescue centres (Figure 1).

Is 85% gene diversity retained (and therefore an average level of inbreeding after 30 years of 15%) much worse than 90% (and 10% inbreeding) and will this compromise the success of reintroduction? This is again a matter of probabilities. There is no guarantee that a captive population, or a reintroduced population derived there from, with less gene diversity and higher inbreeding levels will go extinct or suffer in other ways, but the chance that it does increases. It is therefore always a case of trying to achieve the highest possible retention of gene diversity within the restraints of the particular situation of the species and the space and resources available.

Who breeds with whom?

From a genetic point of view, what you would ideally like to do in an *ex situ* programme is magically freeze the founders and thaw them out again at some point in the future so they can be available for breeding. In that way, all gene diversity would have been retained and all allele frequencies would remain exactly the same. Although gene banking and reproductive technologies allow us to take important steps in this direction (Ballou, 1984), generally the gene diversity of the founders is preserved for the future by having them breed by natural means and pass on their genes to the next generation. Ideally one would like each founder to have a very large and equal number of offspring. This not only maximises retention of gene diversity but would also preserves the existing allele frequencies. In other words, one would ideally like to “stop” selection such that the gene diversity that is available for reintroduction is the same as that collected from the wild generations earlier.

Real life is of course a different matter. Some founders will be more prolific than others. Allele frequencies will change (and some alleles may be lost) due to a combination of genetic drift (i.e., chance) and some individuals having better adapted to life in captivity. Whereas the emphasis is very much on maximising growth rate during the growth phase of the population, during the carrying capacity stage attention should be paid to correcting inequalities that have developed in the founder representation (Lacy, 1994).

In order to achieve this, the technique that is currently employed by the zoo and aquarium community is to base the pairings of the animals (i.e., who breeds with whom) on their mean kinship value. Mean kinship is a measure of the relatedness of an individual to every living individual in the population (Ballou, 1991; Ballou & Lacy, 1995). It is calculated as the average of the coefficients of kinship of an animal with every animal in the population. The coefficient of kinship between a pair of animals in turn is the inbreeding coefficient of any offspring produced by that pair of animals. Priority for breeding is given to individuals with low mean kinship values (and few relatives). After all, if such an individual should die before it gets a chance to breed, there are very few other individuals in the population that share some of its genetic variation and can help pass this on to the next generation. Chances are high that alleles only present in this individual will be lost from the population. For an individual with a high mean kinship value, chances are high that the majority of its gene diversity is also present in its many relatives. Individuals with high mean kinship values are therefore given lower breeding priority. Furthermore, efforts should be made to combine individuals with similar mean kinship values. If an animal with low mean kinship would be combined with one with high mean kinship, the resulting offspring would be half important and half not important. Every time this offspring is bred, not only the rare alleles are spread, but also the already common alleles of the individual with high mean kinship value (Wilcken & Lees, 1998). The offspring is also going to be related to many individuals in the population which will make it harder to find breeding opportunities for it that do not result in inbreeding.

Apart from basing breeding priority and pair combinations on mean kinship, it is also important to minimise the level of inbreeding in a population. Inbreeding not only increases the level of homozygosity and reduces the amount of gene diversity retained, it often also causes inbreeding depression (a reduction in fitness of the inbred individual), partly due to an increased probability for homozygosity of recessive deleterious or lethal alleles (Frankham et al., 2002). Inbreeding depression may express itself in many forms, some of which may not be immediately obvious unless one consciously sets out to investigate them, e.g. reduced juvenile survival, reduced adult survival, less successful mate acquisition, lower social dominance ranking of inbred individuals, increased sensitivity to infections, reduced fertility, increased bilateral asymmetry, lower resistance to environmental stresses, etc. Despite earlier skepticism about the importance of inbreeding depression in wildlife populations, numerous wild populations have now been shown to suffer from inbreeding depression (Roelke et al., 1993; Crnokrak and Roff, 1999; Dietz et al., 2000; Sunquist and Sunquist, 2001; Frankham et al., 2002; Keller and Waller, 2002; Pimm et al., 2006). Captive populations too have been shown to suffer from inbreeding depression (Ralls and Ballou, 1986; Ralls et al., 1988; Boakes et al., 2007).

The level of inbreeding depression significantly influences the extinction risk of a population (O'Grady et al., 2006), and it would be dangerous and unwise to presume any population will be safe from inbreeding depression. In addition, populations with a high level of inbreeding that appear to be coping well enough in captivity may have significantly lower success rates upon reintroduction (i.e., in a more challenging environment) compared to non-inbred released individuals. For example, inbred white-footed mice *(Peromyscus leucopus)* showed significantly lower survival upon reintroduction than non-bred individuals (Jiménez et al., 1994).

Inbreeding is 'reversible', meaning that when an inbred individual is mated with an unrelated animal, the resulting offspring are no longer inbred. For this reason, it may sometimes be preferable to allow a modest level of inbreeding among low mean kinship animals, rather than pairing unrelated individuals with very different mean kinship levels. Naturally, these theoretically ideal breeding strategies for captive management need to be 'married' with the specific social and life history strategies of the species.

Choosing individuals to reintroduce

The modelling by Lacy and Vargas (2004) showed that the Iberian lynx *ex situ* population may be able to supply modest numbers of individuals for reintroduction after 8 years, and after 12 years, 12 to 13 cubs could be supplied for reintroduction per year, while still maintaining the required (for maintenance of gene diversity) nucleus population of 60 breeders (Figure 1). Especially for species as critically endangered as the Iberian lynx, the temptation is large to start reintroducing as soon as possible. Reintroductions are risky and the probability for whole or partial failure of particularly the first release attempts is high. The urge to reintroduce is therefore best contained until the required, secure, reliable nucleus breeding population has been obtained (Lacy, 1994). Rather than limiting breeding to keep the population at carrying capacity, growth can at this stage be maintained and the "surplus" animals used for reintroduction.

This automatically leads to the question, which individuals are best chosen for reintroduction? What are the best genetic criteria upon which to base this choice?

It is obvious that reintroduced individuals have to help improve the genetic and demographic health of the wild population. From this point of view, the reintroduced individuals need to be physically healthy, have a high reproductive fitness, a high gene diversity, and as low an inbreeding level as possible. What is often forgotten however is that the removal of the animals destined for reintroduction should also not compromise the genetic and demographic health of the nucleus *ex situ* breeding population. For that reason, individuals for reintroduction are preferably those that benefit the gene diversity of the wild population (i.e., have few relatives in the reintroduced population), but are genetically overrepresented in the captive population (Frankham et al., 2002). As reintroductions are risky however, first attempts are best tested with animals that are overrepresented in both the wild and captive populations. Once release methods have been tested and fine tuned and once survival and reproduction in the reintroduced population have improved, animals more valuable to the wild population can be added (Frankham et al., 2002).

Acknowledgements

This paper draws largely on the published work of, and personal communication with, Jonathan Ballou, Astrid Vargas and their colleagues, who deserve special thanks. Laurie Bingaman Lackey of ISIS (International Species Information System) provided helpful comments to the manuscript. K. Leus is also affiliated with the European Association of Zoos and Aquaria, and wishes to thank the Flemish Government for structural support to the Centre for Research and Conservation of the Royal Zoological Society of Antwerp.

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Genetic issues in the implementation of the Iberian Lynx *Ex situ* Conservation Programme

Aspectos genéticos en el Programa de Conservación *Ex situ* del Lince Ibérico

José A. Godoy, Mireia Casas–Marce and Jesús Fernández

La vitalidad se revela no solamente en la capacidad de persistir sino en la de volver a empezar.

Francis Scott Fitzgerald (1896-1940)

Resumen

Una de las principales utilidades de la genética de la conservación es la de apoyar a la práctica de la conservación aportando recomendaciones y procedimientos específicos para la gestión de los programas *ex situ* y para otras medidas de conservación. Estas recomendaciones se deben ajustar a las particularidades de la historia de vida, demográfica y evolutiva de la especie en cuestión. Para el establecimiento del Programa de Conservación *Ex situ* del Lince Ibérico la primera decisión que hubo que tomar fue si había que mezclar las dos poblaciones silvestres (Sierra Morena y Doñana) o tratarlas como dos unidades separadas. Aunque la diferenciación para marcadores moleculares entre estos grupos es grande, esto se puede explicar por la acción predominante de la deriva genética en tiempos recientes. Además, hasta la fecha no se han encontrado diferencias en características potencialmente adaptativas entre estas poblaciones y éstas son poco probables considerando la historia demográfica y la capacidad de dispersión que tiene esta especie. Por lo tanto, ambas poblaciones se trataron como una única unidad y se recomendó la translocación de animales desde Sierra Morena a Doñana. El siguiente paso fue determinar la proporción de individuos que había que capturar de cada población para maximizar la diversidad genética de partida. Teniendo en cuenta las diferencias en la composición genética de las dos poblaciones, la contribución óptima de cada una de ellas a la población cautiva se estimó en un 64% de individuos de Sierra Morena y un 36% de individuos de Doñana. A mediados del 2008 la población cautiva incluía cuatro fundadores de Doñana y 24 de Sierra Morena. El análisis empírico de la población cautiva reveló una heterocigosidad esperada algo por debajo del máximo potencial, debido principalmente a una representación insuficiente de la población de Doñana. La gestión de la población cautiva ha sido guiada por dos criterios principales: asegurar la reproducción de todos los fundadores (para que su información genética no se pierda) y la aplicación de un diseño de cruzamientos basado en la estrategia de mínima coancestría. Siguiendo este principio, se han favorecido los cruzamientos entre individuos procedentes de distintas poblaciones, que podemos estar seguros que están menos relacionados que las parejas procedentes de una sola población. El Programa *Ex situ* ha tenido tanto éxito que se han superado las proyecciones de crecimiento

Photo: José María Pérez de Ayala

iniciales y se ha alcanzado la capacidad de carga original dos años antes de lo previsto. Se necesita urgentemente, por tanto, continuar con la expansión del Programa a nuevos centros para acomodar este crecimiento poblacional. Al mismo tiempo, se podría disponer de individuos nacidos en cautividad para reintroducciones ya en el año 2009. Una vez que todos los centros proyectados estén funcionando a capacidad de carga, se deberían manejar conjuntamente las poblaciones cautivas y las silvestres para maximizar la diversidad genética global, optimizándose al mismo tiempo el intercambio de individuos entre ellas.

Palabras clave
Unidades de conservación, diversidad genética, fundadores, translocaciones, reintroducciones, emparejamientos de mínima coancestría, marcadores moleculares

Abstract
One of the main roles of conservation genetics is to support conservation practice by providing specific recommendations and procedures for the management of *ex situ* programmes and other conservation actions. However, these recommendations must be tailored to the particularities of the life, demographic and evolutionary histories of the species and populations we deal with. In the establishment of the Iberian Lynx *Ex situ* Conservation Programme the first decision that had to be taken was whether to mix the two wild populations (Sierra Morena and Doñana) or to treat them as two different units. Although differentiation for neutral molecular markers was large between groups, this might be explained by the predominant action of genetic drift in recent times. Moreover, no differences on potentially adaptive features have been reported between both populations and these are unlikely given the known demograhpic history of the species and its dispersal capacity. Consequently, they were treated as a single unit, and translocations of animals from Sierra Morena to Doñana were advised. Next step was determining the proportion of individuals to be captured from each of the wild populations to maximize levels of starting genetic diversity. Based on the genetic variation within and between both populations, the optimal contribution of each to the captive stock was estimated in 64% of individuals from Sierra Morena and 36% from Doñana. By mid-2008 the captive population included four founders from Doñana and 24 from Sierra Morena. Empirical analysis of the captive population revealed an expected heterozygosity a little below the potential maximum, mostly due to insufficient representation of Doñana population. Management of the captive populations has been driven by two main criteria: using all the available founders as breeders (to allow the maintenance of their genetic information) and the application of a minimum coancestry mating scheme. According to the latter principle, matings between individuals coming from different wild populations were favoured, as one can be sure that they are less related than couples formed within populations. The *Ex situ* Programme has been so successful that initial growth projections have been surpassed, and the original carrying capacity of the Programme has been reached two years in advance of original predictions. Therefore, continuing the expansion of the Programme to new centers is urgently needed to cope with population increase. In parallel, captive individuals for reintroduction to the wild might become avaliable already by 2009. Once all the planned centers are running at carrying capacity, both wild and captive populations should be managed jointly to keep the highest levels of global genetic diversity, while optimising the exchange of individuals between them.

Keywords
Conservation units, genetic diversity, founders, translocation, reintroductions, minimum coancestry mating, molecular markers

Genetic issues in the implementation of the Iberian Lynx *Ex situ* Conservation Programme

José A. Godoy, Mireia Casas–Marce and Jesús Fernández

Introduction

E*x situ* conservation programmes are important conservation tools, especially when they complement rather than substitute *in situ* programmes. Accordingly, the ultimate goal of such programmes should be to serve as a backup from an eventual extinction in the wild and to support wild populations by providing healthy and fit individuals for reinforcement or reintroductions. This goal can rarely be achieved if genetic issues are ignored. In particular, global objectives of any *ex situ* conservation programme must include the preservation of the adaptive potential of the species, the minimization of the risks of inbreeding depression and the prevention of adaptation to the captive environment. In order to achieve these aims, captive breeding programmes must be scientifically managed based on all available genetic and demographic information, with the application of most effective managing strategies.

A primary task of any *ex situ* programme is to define the targeted units of conservation. For a species with some degree of intraspecific structure, it has first to be decided whether perceived genetic units should be mixed in a single captive population or bred separately. Secondly, it has to be decided the number and origin of the founders, the objective being to capture the highest possible proportion of the genetic diversity still present in the wild, while minimizing the impact of extractions on the wild populations and considering the intrinsic capacity limitations of the Programme. Finally, management strategies need to be applied to minimize the losses of the captured genetic diversity and the accumulation of inbreeding over successive generations.

The generalities of genetic issues in *ex situ* programmes have been thoroughly treated in a separate chapter of this volume (Leus and Lacy, this book). In this chapter we will elaborate on these issues for the particular case of the Iberian lynx. Firstly, we will discuss whether lynx populations should be considered as a single management unit based on the existing knowledge on the genetic and demographic history of the species. Secondly, we will evaluate the genetic status of the captive Iberian lynx population as of mid-2008. Finally, we will explore alternative strategies and discuss the role that genetic markers may play in the genetic management of this captive population.

Identification of management units: to mix or not to mix

In structured populations, a first important decision is whether the different population segments should be combined and managed as a single unit or managed separately. Mixing is the best option when genetic differentiation arises as a consequence of genetic drift acting independently in recently isolated and declining populations. In this situation mixing would revert the accumulated inbreeding and maximize the potential genetic diversity of the captive stock. However, if the genetic differentiation is due to long periods of independent evolution in isolation, mixing might negatively affect individual fitness and population viability, an effect known as outbreeding depression (Edmands, 2007). The causes of outbreeding depression might be extrinsic, when local adaptation to particular environments is lost in the hybrids, or intrinsic, when due to genetic incompatibles, either chromosomal or

Species or population	N samples	N loci	He (SE) ±	AR (SE)	Reference
Iberian lynx *(Lynx pardinus)*	**150**	**36**	**0.50 (0.02)**	**3.94 (1.87)**	**Godoy , unpublished data**
Iberian lynx, Doñana	**93**	**36**	**0.31 (0.05)**	**2.39 (1.02)**	**id.**
Iberian lynx, S. Morena	**57**	**36**	**0.47 (0.03)**	**3.58 (1.48)**	**id.**
European lynx *(L. lynx)*	401	11	0.61 (0.06)	7.30 (3.53)	(Rueness et al., 2003)
L. lynx Norway	210	11	0.52 (0.07)	4.40 (2.46)	id.
L. lynx Sweden	93	11	0.51 (0.07)	4.10 (1.85)	id.
L. lynx Finland	48	11	0.63 (0.06)	5.40 (2.32)	id.
L. lynx Baltic	32	11	0.61 (0.08)	4.70 (2.34)	id.
L. lynx Russia	11	11	0.70 (0.04)	5.00 (1.70)	id.
Cheetah *(Acinonyx jubatus)*	89	38	n.d.	6.1 (1.8)	(Marker et al., 2008)
A.j., Gobabis	11	38	0.70 (0.13)	4.5 (1.3)	id.
A.j., Grootfontein	11	38	0.70 (0.12)	4.3 (1.3)	id.
A.j., Okahandja	18	38	0.66 (0.12)	4.4 (1.3)	id.
A.j., Omaruru	15	38	0.67 (0.14)	4.5 (1.3)	id.
A.j., Otjiwarongo	21	38	0.64 (0.14)	4.6 (1.5)	id.
A.j., Outjo	7	38	0.64 (0.14)	3.7 (1.0)	id.
Lion *(Panthera leo)*	60	51	0.56 (0.04)	5.29 (2.66)	(Driscoll et al., 2002)
P. l., Gir Forest	10	51	0.10 (0.03)	1.31 (0.65)	id.
P. l., Ngorongoro Crater	10	51	0.44 (0.04)	2.84 (1.24)	id.
P. l., Serengeti NP	10	51	0.52 (0.04)	3.61 (1.7)	id.
P. l., Kalahari Gemsbok NP	10	44	0.40 (0.04)	2.68 (1.38)	id.
P. l., Etosha Park	10	44	0.49 (0.04)	3.05 (1.43)	id.
P. l., Kruger National P	10	44	0.48 (0.04)	3.32 (1.7)	id.
Puma *(Puma concolor)*	30	84	0.56 (0.67)	6.64 (0.42)	(Driscoll et al., 2002)
P. c. coryi BCS	10	84	0.15 (0.17)	1.51 (0.40)	id.
P. c. hippolestes IDO	10	84	0.35 (0.41)	2.51 (0.51)	id.
P. c. subsp. SA	10	84	0.68 (0.81)	5.99 (0.54)	id.
Cat *(Felis catus)*	15	51	0.70 (0.02)	6.18 (2.46)	id.

Table 1. Genetic diversity parameters estimated with microsatellite markers for Iberian lynx and several other felid species. He: expected heterozygosity; AR: allelic richness. The few examples of populations with levels of genetic diversity similar to or lower than Iberian lynx are highlighted.

Tabla 1. Diversidad genética estimada con marcadores microsatélite para el lince ibérico y otros felinos. He: heterocigosidad esperada; AR: riqueza alélica. Se destacan los escasos ejemplos de poblaciones con niveles de diversidad similares o menores que los del lince ibérico.

genic. The decision to mix or not to mix is, therefore, largely based on the direct evaluation of the existence of adaptive divergences or on the evaluation of the opportunities for such divergences to have arisen in view of the evolutionary and demographic history of the species. For this purpose, the analysis of genetic patterns for molecular makers might be extremely useful. Let us, thus, review what we know about the demographic history of the Iberian lynx, current genetic patterns in the species, and the possibilities of adaptive divergences between populations.

Demographic history

The Iberian lynx has gone through a well documented steep decline and fragmentation process during the last century which has restricted its current distribution to two isolated populations: Doñana and Sierra Morena (Castro and Palma, 1996; Rodríguez and Delibes, 1992, 2002, 2003; Calzada et al., this book; Simón et al., this book). Although both populations are the consequence of a common process of decline and fragmentation, they have slightly different recent demographic histories. Doñana is a peripheral population that became effectively isolated from the rest of the species distribution probably more than five decades ago, and has remained isolated and at a census size of around fifty since then. Conversely, the Sierra Morena population has remained connected to surrounding populations until recently, and has been continuously shrinking in size until recent active conservation measures have stabilized its census size at around 180 (Simón et al., this book). Under this demographic history, the action of genetic drift may have resulted in a pattern of low genetic diversity and high inbreeding within populations and high genetic differentiation between them, although the magnitude of these changes is difficult to predict without precise estimates of demographic parameters over time.

Contemporary genetic patterns

Contemporary genetic patterns in Iberian lynx have been analysed using both mitochondrial and microsatellite markers (Johnson et al., 2004; Godoy, unpublished data). The two main findings are that genetic diversity is globally low, but lower in Doñana than in Sierra Morena, and that these two populations show a high level of genetic differentiation.

Mitochondrial diversity is extremely low in the Iberian lynx, with only two haplotypes observed which differ in one single position. These two haplotypes seem to be alternatively fixed in each population at present. However, our previous study detected the presence of the Doñana haplotype in a museum specimen from Sierra Morena, and also showed the occurrence of a third now extinct haplotype in Western Sierra Morena (province of Huelva) (Johnson et al., 2004). These results with mitochondrial DNA provide direct evidence for diversity losses in recent times due to drift in the small remnant populations and to the extinction of differentiated populations.

Iberian lynx nuclear microsatellite diversity levels are also globally low, both in terms of heterozygosity and allelic diversity. Most felid species analysed to date have higher microsatellite diversity than the Iberian lynx, including its sister species, the Eurasian lynx, as well as some of the species that, like the cheetah, once became paradigmatic examples of species with low genetic diversity. On the contrary, some extreme examples of small isolated populations like the Gir forest lions and the Florida panther (before the successful translocation of pumas from Texas) do seem to harbour lower diversity than the Iberian lynx (Table 1). Interspecific microsatellite diversity comparisons must be interpreted with care because of the possible ascertainment bias, but they actually suggest lower genetic diversity in the Iberian lynx than in closely related populations/species with similar life history. Moreover, microsatellite heterozygosity and allelic diversity are 33% lower in Doñana than in Sierra Morena.

A high level of genetic differentiation is observed between Doñana and Sierra Morena populations, i.e., allele frequencies are currently different in both populations, so that a significant fraction of the total genetic variation is distributed between populations. Furthermore, private alleles (i.e., alleles that are found in one population but not in the other) are up to four times more abundant in Sierra Morena than in Doñana, indicating that the genetic variation found in Doñana may be mostly, but not totally, a subset of that found in Sierra Morena.

As expected from the known demographic history of the species, genetic patterns found in the Iberian lynx population could have been shaped by the predominant action of genetic drift in recent times, according to size and time since isolation of each population. Accordingly, the strong differentiation found between Doñana and Sierra Morena populations should be mainly due to random allele frequency fluctuations occurring in each

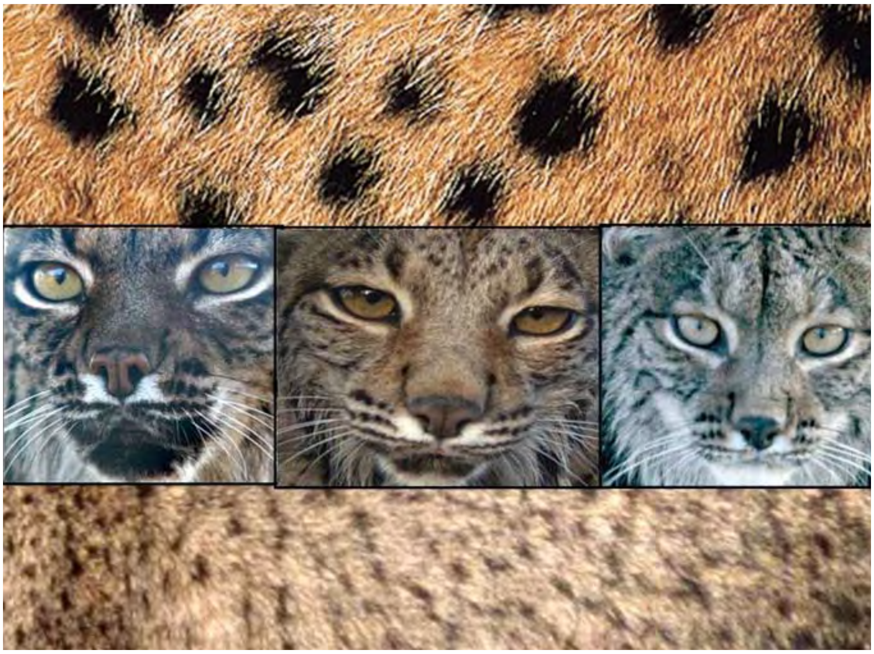

Figure 1. Phenotypic diversity for morphological traits in Iberian lynx. The two extreme coat patterns –small and large spots– still coexist in Sierra Morena, but the small spot pattern was lost in Doñana during the 1960s.

Figura 1. Diversidad fenotípica para rasgos morfológicos en lince ibérico. Los dos pelajes extremos –mota pequeña y mota grande– todavía coexisten en Sierra Morena, pero el de mota pequeña desapareció de Doñana en la década de 1960.

population since their isolation from each other, and must not be interpreted as the consequence of differential adaptation pressures/responses or a long history of independent evolution. Moreover, low levels of current genetic diversity are probably the consequence of a steep and rapid loss in recent times, especially in Doñana, where the loss might have reached over 33% in magnitude. Diversity projections using simple genetic drift models and genetic estimates of effective population sizes predict a further reduction of heterozygosity to 50% from current levels in only 12,5 (60 years, assuming a generation length of five years) and 16,6 generations (83 years) for Doñana and Sierra Morena, respectively. Furthermore, an effective size like that estimated for Doñana could retrospectively explain the loss of 33% heterozygosity from an ancestral diversity similar to that currently present in Sierra Morena in less than ten generations (50 years). Under such scenario, diversity losses may be equated to inbreeding accumulation, so that a 33% diversity reduction in Doñana would reflect a population inbreeding coefficient for Doñana well over that expected for full-sibling matings. Such high inbreeding values are often associated to a high frequency of genetic disorders, low fertility and other negative effects, as illustrated by the notorious case of the Florida panther (Roelke et al., 1993). A high incidence of glomerulonefritis and lymphoid depletion has been reported for Iberian lynx (Jiménez et al., this book; Jiménez et al., 2008; Peña et al., 2006). Establishing that these and any other observed deficiencies and abnormalities in Iberian lynx are due to inbreeding depression or genetic depauperation will not be easy to proof, but this possibility must be seriously considered in view of the observed genetic patterns and the recent demographic history of the species.

Evidences and chances for adaptive divergences

Direct evidence for adaptive divergences between Sierra Morena and Doñana populations are lacking: no differences in life history traits or behaviour have been reported to date, at least none that may not be attributed to a plastic response to a slightly different environment (e.g., dens in caves vs. shallow trunks, in Sierra Morena and Doñana, respectively).

A recent study reported morphometric differentiation between Iberian lynx populations, but also demonstrated temporal morphological changes within Doñana. Consequently, morphometric differentiation was attributed to environmental stress and genetic drift acting on small and isolated populations (Pertoldi et al., 2006). The two remnant populations also differ currently in pelage pattern frequencies: the small-spot pattern that is predominant in Sierra Morena is currently absent from Doñana population; however, both patterns coexisted in Doñana until the 1960s (Beltrán and Delibes, 1993) (Figure 1).

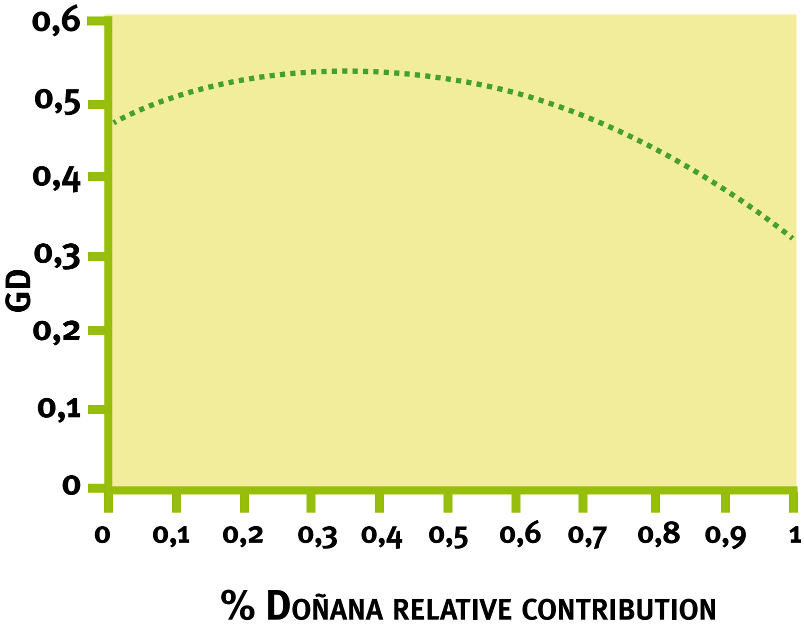

Figure 2. Doñana relative contribution. Gene diveristy (GD; or expected heterozygosity) of the captive population with varying contributions of each population, based on empirical estimates of microsatellite diversity and differentiation and following Toro and Caballero (2005). Gene diversity peaks at 0,543 when 36 % Doñana and 64 % Sierra Morena is considered.

Figura 2. Diversidad génica (GD; o heterocigosidad esperada) de la población cautiva en función de la contribución relativa de cada población, en base a las estimas empíricas de diversidad y diferenciación genética para marcadores de microsatélites y siguiendo a Toro y Caballero (2005). La diversidad génica alcanza un máximo en 0,543 cuando la contribución es de un 36 % de Doñana y un 64 % de Sierra Morena.

Notwithstanding, these two remnant populations were probably interconnected by gene flow prior to their recent isolation. The potential for dispersal and gene flow in the species is probably high, as in other lynx species, although it has been shown to be strongly affected by habitat matrix (Revilla et al., 2004). The anecdotal case of an animal from Doñana that made its way back after being liberated in Andújar (Eastern Sierra Morena) illustrates the potential for long distance movements in the species. Such high dispersal rates in the past would have hindered the development of strong adaptive divergences between these two populations.

When the points discussed above are considered together, the management of the species as a single unit arises as the most sensible management option. In other words, in view of current genetic patterns and the demographic history of the species, the risks of inbreeding depression largely exceed risks of outbreeding depression in the Iberian lynx. The Iberian Lynx *Ex situ* Programme is consequently managed as a single mixed population and already harbours several captive-born interpopulational litters (Vargas et al., this book). Moreover, the first wild progeny of interpopulational crosses were born in Doñana in spring 2008 following the release of a single Sierra Morena male, which becomes the first example of successful translocations of Iberian lynxes.

Genetic objectives and current status of the *Ex situ* Programme

Ex situ conservation programmes must aim at capturing as high a proportion as possible of the genetic diversity still present in the wild. This can only be achieved by maximizing the number of individuals brought into captivity, although in most real-world scenarios this has to be tampered by the need to minimize the impact of extractions from the wild populations. Twenty to 30 randomly selected founders would represent 97.5-98.3% of the sampled diversity, so this number is usually recommended as a reasonable compromise in most real-life situations (Frankham et al., 2002). Furthermore, as long as there is some genetic structure –as is the case for the Iberian lynx– global diversity will be maximized by the mixing of the two genetic stocks in appropriate proportions. This proportion depends on diversity levels within populations and on the degree of differentiation among populations (Caballero and Toro, 2002). From our empirical estimates of each population's diversity and their degree of differentiation, optimal contribution of each population to the mixed captive stock is determined as 36% and 64% for Doñana and Sierra Morena, respectively (Figure 2). When these proportions are met, the heterozygosity of the *Ex situ* Programme can reach 0.54.

As expected, the genetic diversity represented in the captive population has progressively increased as

He and N of captive population through time

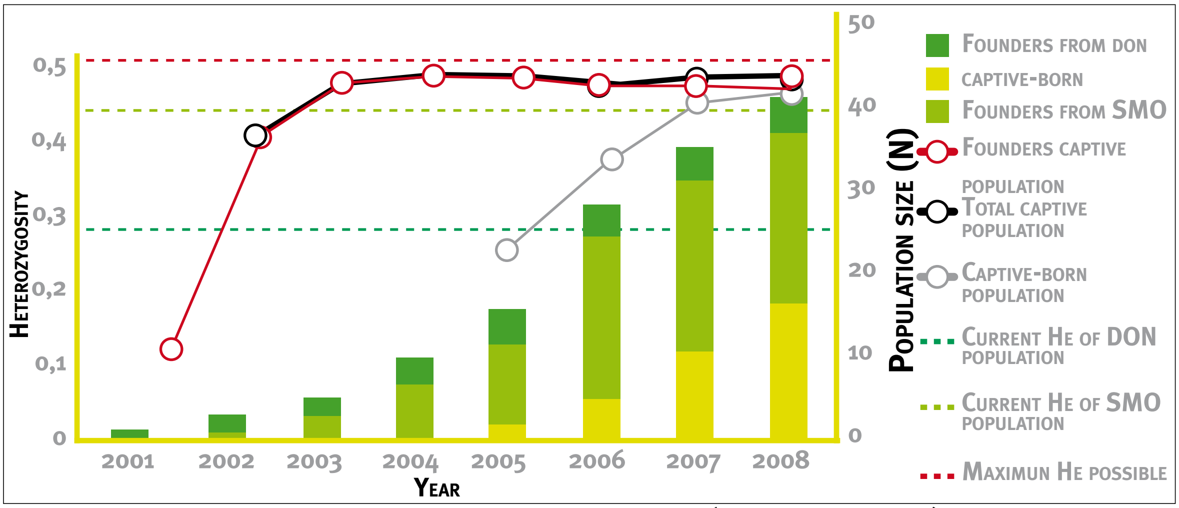

Figure 3. Changes in captive population size and composition and in microsatellite diversity (expected heterozygosity, He) from 2001 to 2008. Horizontal lines indicate the expected heterozygosity in Doñana, Sierra Morena and the theoretical maximum He of the pool (see figure 2). Only 17 of the 24 captive-born cubs surviving by mid-2008 were included in the genetic diversity analysis.

Figura 3. Cambios en el tamaño poblacional y la diversidad genética para marcadores microsatélites (heterocigosidad esperada, He) de la población cautiva entre 2001 y 2008. Las líneas horizontales muestran la heterocigosidad esperada en la población de Doñana, Sierra Morena y la máxima teóricamente posible para la población cautiva (ver figura 2). En el análisis de diversidad sólo se incluyen 17 de los 24 cahorros nacidos en cautividad que sobreviven a mediados de 2008.

new founders are incorporated to the Programme (Figure 3). By mid-2008, the number of potential founders (individuals that have been captured but which have not necessarily reproduced) in the Iberian lynx *ex situ* population was four from Doñana and 24 from Sierra Morena. Microsatellite analysis of these founders shows that they globally harbour an expected heterozygosity of 0.50, and that they represent 96% of Sierra Morena's heterozygosity, but only 77% of Doñana's. The inability to reach the theoretical maximum heterozygosity of 0.54 is due both to an insufficient representation of Doñana's genetic diversity and to a biased composition towards Sierra Morena, what calls for the increase of Doñana´s representation in the captive stock. Based on these recommendations, two new juvenile females from Doñana were recently added to the captive stock (Simón et al., this book).

Management of the captive population: the early times

Once the potential founders to be taken from the wild have been chosen and brought to captivity, all the management strategies must be directed (from the genetic point of view) to maintain the highest possible level of the genetic diversity present in the moment of the establishment (Frankham et al., 2002). It must be noticed that all the genetic information available to be preserved is the one present in the *founders* (i.e., the individuals captured in the wild without previous knowledge of their ancestors). Therefore, the correct use of these animals is the key point in a conservation breeding programme.

Selecting the breeders

Following this philosophy, it seems that the sensible way to act is to guarantee that all the founders have an equal opportunity/probability of transmitting their genetic material (Leus and Lacy, this book). First of all, this implies using all the founders as actual breeders. In the initial stages of an *ex situ* conservation programme the resources are usually scarce, and space, number of adult animals, and personnel limitations reduce the number of couples that can be mated in each reproductive season. In the case of the Iberian lynx, only three couples could be used as breeders in the early years (when only one center, El Acebuche, had lynxes of breeding age). This fact led to a scenario where some founders have been "overused" (e.g., a single male has contributed up to 11 offspring) while others remained unused because they were too young to breed. The incorporation of new facilities into the conservation programme has helped to increase the number of founders reproducing, increase the programme census size and reduce its probability of extinction due to demographic stochasticity. Besides this, the spreading of the population into different locations would prevent the complete loss of the captive population caused by punctual catastrophes like fires or disease breakouts.

The usual management strategy to assure the proper representation of all founders in any generation is the minimisation of the global coancestry, as explained in a previous chapter (Leus and Lacy, this book). This feature is due to the property of the minimum coancestry method of minimising the variance of the contributions of any individual in the genealogy to a particular generation and, therefore, equalising the contributions of all the ancestors, including the founders (Caballero and Toro, 2000). Moreover, several authors (Ballou and Lacy, 1995; Caballero and Toro, 2000) have pointed out that the global coancestry of a population is inversely related to the number of founders genome equivalents and, so, minimising the coancestry would maximise this latter parameter, that include both the effective number of founders and the effective number of non-founders (related with the occurrence of bottlenecks within the *Ex situ* Programme).

In most conservation programmes, founders are assumed to be unrelated and, thus, their genetic information is considered exclusive and worthy, what gives support to the idea of the equalisation of the founder contributions. But in the case of the Iberian lynx the wild populations have been small during a large period of time, making much likely that animals starting the captive population are actually related and, thus, genetically redundant to some extent. Conversely, the long period of isolation between the populations of Doñana and Sierra Morena implies that any eventual relationship between animals coming from different locations might be very small. Consequently, founders from different origins can be assumed unrelated and only relationships within subpopulations need to be accounted for. Some authors think that the denial of the coancestry between founders could result in a rather moderate loss in genetic diversity, depending on the population structure (Rudnick and Lacy, 2008), or even be advantageous for the maintenance of adaptations for survival in the wild (Saura et al., 2009). Notwithstanding, in highly endangered population starting from an already low genetic diversity, no extra losses can be afforded and the incorrect assumption of unrelated founders may lead to further increases of inbreeding due to a wrong planning of matings. Therefore, we encourage the relationship between founders to be estimated and accounted for when planning the matings in the Iberian Lynx *Ex situ* Conservation Programme, at least when it reaches the steady state and the centers are at their carrying capacity.

The relationship between wild individuals (or the amount of genetic information they share) is usually unknown, but it can be estimated from molecular data. Several estimators have been developed in the last decades (see Fernández and Toro, 2006 and Oliehoek et al., 2006 for a revision). In general, the methods can be divided into two categories: those estimating the relationship between single pairs of individuals (i.e, pairwise methods) and those calculating the relationships of all individuals at a time (the so called genealogy reconstruction methods). Advantages and shortcomings of each method can be found in the cited literature. Data from 36 microsatellites genotyped in the founders of the Iberian Lynx *Ex situ* Conservation Programme were processed with a couple of methods (those developed in Fernández and Toro, 2006 and Oliehoek et al., 2006). Although some differences were found between the results obtained with each method, both detected a couple of individuals from Doñana (*Boj* and *Esperanza*) and another from Sierra Morena (*Candiles* and *Garfio*) that appeared to be significantly related to a level similar to that of half-sibs.

Molecular tools are also useful once the Programme is running. On one hand, they can be used to complete/correct the pedigree if some doubts about any of the relationships arise. For example, *Adelfa*'s 2008 litter could be anambiguously asigned to *Garfio* after molecular analysis, while *Cromo*, who also mated with her, was discarded as parent. On the other hand, molecular analysis are also required to determine the relationships between captive individuals and wild populations and can thus help in the design of reintroductions of captive stock back into nature.

Selecting breeding pairs

The second step where the manager can act within a conservation breeding programme is in the design of the mating plan. In the short-term, the effect of the mating strategy is more evident on the levels of inbreeding of the animals kept. Highly endangered populations, like the Iberian lynx, should be prevented of further increases of inbreeding to avoid the deleterious consequences of inbreeding depression. Consequently, the most sensible strategy should be implementing a minimum coancestry mating design, as the inbreeding of the offspring will be the coancestry between the individuals acting as parents (Caballero et al., 1996). The general idea is to avoid as much as possible the mating of relatives. In the Iberian Lynx *Ex situ* Conservation Programme, as explained before, the most unrelated couples that can be paired are those comprising one parent from Doñana and the other from the Sierra Morena. Once the management of both subpopulations as a single unit has been decided, the generation of 'mixed' couples should thus be promoted.

Regarding the genetic long-term consequences, it is well known from the classical theory that there are other mating schemes that assure lower levels of inbreeding in a distant future (as well as higher levels of genetic diversity maintained). This is the case of circular mating (Caballero et al., 1996; Kimura and Crow, 1963), which provide lower inbreeding rates (ΔF) in the long-term due to the subdivision of the population inherent to this management strategy. In fact, as pointed out by Woolliams and Bijma (Woolliams and Bijma, 2000), the lowest overall long-term ΔF can be obtained by mating related individuals (as it occurs in subdivided populations) but the inbreeding generated in the short-term cannot be assumed in conservation breeding programmes of large mammals, as the one we are dealing with.

When theory and real life collide

From a theoretical point of view, the two steps of selecting the parents (and/or their contributions) and deciding the way they are to be mated could be performed separately. However, reproductive limitations of the species or logistic reasons may show that some configurations of the first step (i.e., scheme of contributions) are impossible to implement in a sensible mating design. For example, the optimal result could imply the generation of many offspring from a single female or the mating of one female to several males, which could be not feasible in a single breeding season. Moreover, behavioural matters are also very important in the designing of the mating plan, as particular couples may be incompatible even when they would be recommended from a genetic point of view. Reproductive technologies, like artificial insemination and embryo transfer, could partially overcome these limitations; these are being developed for the Iberian lynx (Roldan et al., this book), but they cannot yet be used as routine in the *Ex situ* Conservation Programme. In these scenarios, management decisions should be taken in a single step, in the process that has been called mating selection (Fernández et al., 2001). Mating selection allows fitting any mating/logistic limitation required and, at the same time, optimising the contributions of the available parents and implementing minimum coancestry mating schemes, if desired.

Management of the captive population: expanding to other centers

When a center reaches its carrying capacity, the need for genetic management still applies as stated above, but the surplus animals must be transferred to another center. Contrary to what happened in the early stages when the use of all founders had to be guaranteed, adult animals that are already adapted to the center should not be moved but young individuals that have not yet reproduced are better candidates. This is a way of avoiding mating failures of those individuals that were successful in previous rounds, due to the stress induced by the change of center.

But, which particular animals should be moved from all the available? The general idea is that individuals belonging to overrepresented lineages should be the ones transferred, i.e., we leave in the original center a set of individuals with the minimum global coancestry. This procedure has a twofold justification: i) if the animal dies or it is unable to reproduce in its new location, the genetic information it carried will not be lost, as it is present in its relatives left in the origin; ii) following this strategy, all centers will harbour quite a similar genetic composition becoming copies of each other. This way, the security of the whole programme will improve because any problem within a particular center is less important as the genetic information of that subpopulation can be replaced by the one present in any other subpopulation.

When all the centers are at carrying capacity and no more centers are planned, the movement of animals between centers should not stop. However, the migration scheme must be planned carefully to avoid unnecessary translocations with the problems and risks they imply. At that stage we must consider all the centers as a metapopulation and design their dynamic as a whole. Recently, Fernández et al., (Fernández et al., 2008) have presented a management system which allows for the joint control of the contributions and matings within each subpopulation and the migration scheme among them. Therefore, this strategy provides the configuration that keeps the highest possible levels of genetic variability in the metapopulation while avoiding a large increase of inbreeding in any subpopulations via a proper design of migrations. The system is dynamic and can detect punctual or structural disequilibria and act to correct them.

Prospects for an integrated management of wild and captive populations

The concept of metapopulation can be applied to the movement of individuals between different captive populations but also to the possible migration between the different populations in the wild. Translocations of individuals can be viewed as migrations performed to increase the census size of more endangered populations and to avoid the rise of inbreeding. The Iberian Lynx *In situ* Conservation Programme has transferred a couple of males from the Sierra Morena population to the Doñana population (Simón et al., this book), which is smaller, bears higher inbreeding levels and, therefore, is at higher risk. The first male to be translocated managed to breed and some of his offspring are still alive and growing.

In later stages of the Conservation Breeding Programme, movements of individuals should also include migrations between both types of populations: wild and captive. Obviously, *ex situ* animals are to be released into nature, as this is the final objective of the captive programme. This includes population reinforcement in areas where animals are still present and reintroduction in areas where they are absent. Captive-born Iberian lynxes were scheduled to become available for reintroductions/reinforcements in 2010, based on demographic projections using demographic and life history parameters estimated for the wild, on an anticipated steady rate of incorporations of four animals per year between 2004 and 2008 and one more animal every two years, and taking into account an estimated carrying capacity of 60 breeders (Lacy and Vargas 2004; Vargas, this book). Fortunately, these early projections have been surpassed as captive population size reached 52 already by mid-2008, in good part due to intensive husbandry aimed at maximizing newborn surviv al., New demographic simulations with updated demographic data and parameters estimated for the captive stock predict the production of 20-40 cubs per year starting in 2010. This updated estimate of population growth underscores the urgent need of additional breeding/housing spaces (Vargas et al., this book) and allows the consideration of reintroductions of captive-born animals already for 2009.

But if only the founders´ genetic variability is continuously distributed, this may have a counteracting effect diminishing the global variability in the wild (Wang and Ryman, 2001) for the problems incurred with a wrong implementation of supportive breeding. Therefore, animals from the natural populations should enter periodically the captive programme to avoid the overrepresentation of particular lineages and to capture the genetic information not included in the original establishment of the *Ex situ* Programme. In the end, the metapopulation to be managed will include all natural populations and all breeding centers. The general aim will be to maintain the highest levels of genetic diversity in the global metapopulation while avoiding inbreeding in any of the subpopulations through a wise planning of captures, reintroductions and translocations.

Hopefully, the *Ex situ* Programme and intensive management will eventually become unnecessary if we manage to achieve the ultimate goal of establishing a demographically stable and genetically healthy network of Iberian lynx wild populations interconnected by natural dispersal.

Acknowlegements

The molecular genetic analysis of the Iberian lynx populations was performed with the invaluable technical assistance of Laura Soriano and as part of a project funded by the Ministerio de Educación y Ciencia (Ref: CGL2006-10853/BOS). The Life-Nature Project for Iberian Lynx Conservation in Andalusia, Consejería de Medio Ambiente, the team of the Iberian Lynx *Ex situ* Programme at El Acebuche, and the Biological Resource Banks at the National Museum of Natural History (CSIC) and the University Miguel Hernández, kindly contributed samples to this study.

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Photo: J.M. Pérez de Ayala

We can't solve problems by using the same kind of thinking we used when we created them.

Albert Einstein
(1879-1955)


Photo: Anastasia Antonevich

Studbook management of captive Eurasian lynx *(Lynx lynx)*

Gestión del libro genealógico *(studbook)* del lince euroasiático *(Lynx lynx)* en cautividad

Lars Versteege

Resumen

El libro genealógico o “studbook” del lince euroasiático (ESB) se estableció en 2002 siguiendo las recomendaciones del TAG (Grupo Asesor del Taxón) de Felinos de la EAZA. Entre los numerosos motivos que dieron lugar a la creación de este libro genealógico cabe resaltar que casi el 50% de los individuos en cautividad eran de origen desconocido, que habían tenido lugar muchas reintroducciones a partir de animales cautivos, que se estaban obteniendo muchos individuos mediante la cría en cautividad y que los linces cautivos estaban siendo objeto de una gran diversidad de estudios. Con el fin de organizar la información existente, se pidió a todos los zoológicos que enviaran sus registros históricos sobre los linces cautivos y se compararon los datos con la información proporcionada por el ISIS (Sistema Internacional de Información de Especies). El estudio del libro genealógico reveló un gran número de problemas y discrepancias, entre los que figuraban los siguientes puntos: alto nivel de endogamia, gran número de individuos de origen desconocido y numerosos individuos de origen mixto. Además, se pudo comprobar que muchos individuos se encontraban en condiciones de cautividad subóptimas. Se enviaron recomendaciones a los zoológicos instándoles a cooperar para evitar una mayor endogamia en la población cautiva, a mejorar el diseño de sus instalaciones para la cría en cautividad de la especie y a convencer a las instituciones implicadas para que participasen en estudios genéticos que ayudasen determinar las distintas subespecies. El problema de las subespecies es importante para el programa de reproducción, debido a la gran dificultad que existe para gestionar cruces sin tener la certeza de cuántas subpoblaciones contiene la población cautiva. Por otra parte, dado que sigue habiendo zoológicos que participan en los proyectos de reintroducción, es imprescindible conocer el origen de los individuos destinados a ser reintroducidos. Un grupo de investigadores suizos participa en el muestreo genético de linces y ya ha establecido un banco de datos para algunas de las subespecies. Mediante este programa se solicita a los zoológicos que albergan esta especie a que colaboren todo lo posible proporcionando muestras de subespecies conocidas. De esta forma, el ESB sirve de catalizador para las instituciones de la EAZA que proporcionen muestras genéticas de sus linces euroasiáticos.

Palabras clave

Lince euroasiático, subespecie, endogamia, muestreo genético

Abstract
The Eurasian lynx studbook (ESB) was established in 2002 after recommendations from the EAZA Felid TAG (Taxon Advisory Group). The reasons to establish this studbook were manifold. Almost 50% of the lynxes in captivity were of unknown origin, many reintroductions had been taking place, many lynxes were bred and many different studies were being conducted with the captive specimens. In an attempt to address these problems in an organized manner, all zoos were asked to send their historical registration on lynxes, and this data was compared with the information received from ISIS (International Species Information System). Through careful investigation of the studbook many discrepancies and problems were identified. Specifically, it was found out that the level of inbreeding in the captive population was very high. Secondly, the number of lynxes of unknown origin was also high. In addition, there were a large number of identified lynxes of mixed genetic origin. Finally, many lynxes were kept in suboptimal captive conditions. Recommendations were sent out to zoos urging cooperation to avoid further inbreeding of the population, to improve enclosure design and husbandry procedures for this species and to convince involved institutions to participate in the genetic studies aimed at determination of the various subspecies. The subspecies problem is an important one for the programme, since it is very difficult to manage the captive population if it is unclear how many subpopulations it consists of. Furthermore, as there are still zoos involved in reintroduction projects, it is imperative to find out the origin of lynxes that are targeted for reintroduction. A Swiss research group is currently involved in genetic sampling of lynxes and has already set up a databank for some subspecies. Zoos keeping lynxes are encouraged to cooperate as much as possible by providing samples of known subspecies. In this way, the ESB serves as a catalyst for the EAZA institutions that provide genetic samples from their lynxes.

Keywords
Eurasian lynx, subspecies, inbreeding, genetic sampling

Studbook management of captive Eurasian lynx *(Lynx lynx)*

Lars Versteege

Status of the Eurasian lynx in the wild

Until the mid-1900´s geographic distribution of the Eurasian lynx *(Lynx lynx)* spread throughout many countries of Europe and Asia, including Russia. Afterwards, population numbers were drastically reduced as a result of habitat destruction, as well as due to hunting and trapping for their fur (Breitenmoser and Breitenmoser-Würsten, 2008). In the second half of the twentieth century, legal protection helped the Eurasian lynx recover in the Northern countries. Also reintroduction programmes were established in certain areas of Central and Western Europe (von Arx et al., this book). Through these programmes many lynx have been reintroduced back into forested areas of Switzerland, Slovenia, Austria, France, the Czech Republic, Germany and Italy, where they had become extinct at the end of the 19th century (Breitenmoser and Breitenmoser-Würsten, 2008).

Geographic distribution

The Eurasian lynx is widespread throughout large forest tracks of northern Europe and Asia. Russia is the heartland of its range, which extends Eastwards into China and Southwards into the Northern flank of the Himalayas. In earlier times, this species was distributed across Europe, Asia and Russia. Presently, its range is limited to the areas represented in Figure 1 (adapted from Breitenmoser and Breitenmoser-Würsten, 2008).

Throughout this distribution range, different subpopulations have been described, although not all of them have been formally recognised. The Northern lynx *(L. l. lynx)* distribution range spreads from North-West Europe to Western Russia. In the Carpathian Mountains and Greece, roams the Carpathian lynx *(L. l. carpathicus*; Figure 2) while the Caucasian lynx *(L. l. dinniki)* lives in the Caucasian mountains, Iran and Turkey. The Turkestan lynx *(L. l. isabellinus)* can be found from Kashmir to Mongolia, the Irkutsk lynx *(L. l. kozlovi)* has its range in Central Siberia, while the Siberian lynx *(L. l. wrangeli)* inhabits Eastern Siberia. The existence of another Siberian lynx subspecies *(L. l. wardi)* is currently under debate. For the distribution of the different subspecies see Figure 1 (Breitenmoser and Breitenmoser-Würsten, 2008).

Justification for a European Studbook (ESB)

Why should a studbook be established for such a common species? The Eurasian lynx is not considered as a high priority species for conservation action by the IUCN, as it is the case with its sister taxa, the Iberian lynx (Calzada et al., this book). The species is frequently displayed in zoos, being present in almost 50% of the zoos that are EAZA (European Association of Zoos and Aquaria) members. Yet, the establishment of a studbook was considered a priority by the EAZA Felid TAG (Taxon Advisory Group) in order to attain the following goals:

Figure 1. Current distribution of the Eurasian lynx (adapted from Breitenmoser and Breitenmoser-Würsten, 2008).

Figura 1. Distribución actual del lince euroasiático (adaptado de Breitenmoser y Breitenmoser-Würsten, 2008).

Figure 2. *Lynx lynx.*

Figura 2. *Lynx lynx.*

	EAZA TAG Survey 2002		After studbook investigation	
	Individuals	Institutions	Individuals	Institutions
L. l. lynx	104	35	89	31
L. l. kozlovi	1	1	2	2
L. l. wrangeli	60	21	50	20
L. l. carpathicus	7	2	31	13
L. l. wardi	0	0	3	1
L. lynx ssp.	138	46	116	49
Mixed origin	0	0	27	12
Total	310	105	318	128

Table 1.
Comparison between the results from the EAZA TAG survey and studbook analyses. The last two columns depict the most probable number of individuals of each lynx subspecies held in EAZA institutions in 2002.

Tabla 1. Comparación entre los resultados de la encuesta del EAZA TAG y los análisis del studbook. Las dos últimas columnas muestran el número más probable de individuos de cada subespecie mantenido en instituciones de la EAZA en el 2002.

1. Defining Subspecies: This was deemed necessary because almost 50% of the total captive population was of unknown origin or unspecific status.
2. Various reintroductions were taking place and the genetic origin of the released animals was unclear.
3. Genetic management was needed to make sure that inbreeding was avoided so that a genetically healthy captive population could be established within the European Species Program (EEP).
4. Many different organisations were conducting research on lynx genetics, physiology, reintroduction success, etc, but there was not a mechanism to compile and make such information available to others.

The establishment of the Eurasian lynx European Studbook (ESB)

The first step in trying to address the abovementioned problems was to identify all the zoos that kept Eurasian lynxes in their collection. The EAZA Felid TAG survey, carried out in 2002 (Versteege et al., 2002), included a section on Eurasian lynx that helped provide the information on lynx holding institutions. Subsequently, all lynx holding institutions were contacted and asked to send their historical reports for this species. All EAZA institutions that were members of ISIS (International Species Information System) were urged to have their data updated in the ISIS database, which made information easily accessible. Historical information received from all the zoos was double-checked with the information received from ISIS; in addition, information from the EAZA members that were not ISIS members was gathered and included in the database. Through intensive searching and questioning many discrepancies were resolved, yet a lot of information remained unknown.

Analysis of the European Studbook (ESB)

After a year of data gathering, comparisons were made between data received from the EAZA Felid TAG Survey and data received through the studbook research. The discrepancies between both results were notable, as shown in Table 1. The most important conclusion from this analysis was that the registered records of Eurasian lynxes in many institutions were very poor. Many institutions registered their lynxes as *"Lynx"* without subspecific identification. Also, many institutions automatically registered them as the nominate subspecies *"Lynx lynx lynx"*. An interesting finding was that the number of Carpathian lynxes that were identified after studbook investigation changed from seven listed in the EAZA TAG Survey to 31 after the studbook investigation. Also, the increase in number of lynxes that were identified as "mixed origin" was also remarkable (from 0 to 27, according to survey comparisons).

Specific problems of the European Studbook (ESB)

Results from this research helped identify the following problems:

- A high level of inbreeding within the Eurasian lynx captive population.
- A high number of lynxes of unknown origin.
- A high number of lynxes of mixed origin.
- Many lynxes were kept in suboptimal captive conditions.

Given that the determination of different subspecies is still unclear and that many assumptions have been made without proper scientific studies (e.g., genetic evidence of subspecies by DNA testing), it is not possible to identify and manage "pure" breeding populations. This means that animals that appear in the studbook listed as pure subspecies, may not be pure at all, or might belong to another subspecies (e.g., the uncertainty about the *"wardi"* subspecies).

As lynxes are easily available to zoos, there had never been standardized husbandry recommendations for this species. Besides from following animal welfare standards, there were no reasons for zoos to invest in good husbandry procedures. In general, lynxes were kept in small cages and, even to date, a large number of zoos have not invested in large, naturalistic enclosures for maintaining lynxes. One of the tasks of a breeding programme is to provide husbandry guidelines for the species to upgrade the husbandry and welfare standards for the species in captivity. But because there is less attention for lynx than there is for larger, charismatic mammals, not much pressure was put on compiling husbandry guidelines for lynxes. With the help of students, basic guidelines were developed but not formally published. Slowly, zoos are improving their facilities and presently asking for advice on how to design new lynx enclosures and how to take care of their captive animals.

Recommendations that emerged from the Eurasian lynx ESB analyses

Due to the problems faced by Eurasian lynxes maintained in captivity, several recommendations were made to improve the husbandry and management of this species in zoos. These included:

- Avoid inbreeding. All institutions that have related breeding pairs should cease breeding those animals and consult the studbook keeper for advice.
- Improve husbandry and enclosure design. All zoos are urged to improve their facilities for lynxes. Since there is a "demand" for pure Carpathian lynxes, zoos wanting to display this subspecies are encouraged to design and build large, naturalistic enclosures and provide enrichment opportunities.
- Further research is needed to identify the different subspecies. From the studbook management point of view, all lynxes from different origin are treated as subspecies until further scientific research determines the validity of the subspecies or provides alternative conclusions. For instance, all lynxes originating from Slovakia are Carpathian lynxes, and all lynxes from Sweden are Northern lynxes (lynx subspecies). By using this strategy, the possible loss of important bloodlines might be prevented. All mixed progeny between lynxes from different origins is considered as "hybrids", and a specific "non-breeding" recommendation is forwarded in such cases.

The importance of determining subspecies

The outcome of the genetic research carried out on lynxes of different origin is of extreme importance to the studbook management for the following reasons:

Genetic management

In 2002, a total of 318 individuals in 129 zoos were identified as belonging to seven different populations (Table 1). If genetic research eventually shows that within the Eurasian lynx there are this many subspecies, it would mean that zoos would have to manage seven different populations. If genetic research shows that only two clear subspecies can be recognised, then only two separate populations would need to be managed.

Reintroductions

The IUCN/SSC Guidelines for Reintroductions state that "an assessment should be made of the taxonomic status of individuals to be reintroduced. They should preferably be of the same subspecies, or race, as those which were extirpated, unless adequate numbers are not available". This statement indicates that, prior to any Eurasian lynx reintroduction project there has to be a clear understanding of the genetic origin of the selected candidates for release.

Management recommendations

To address the Eurasian lynx captive population problem the proposed measure includes performing the appropriate analyses to identify each breeding individual via genetic analyses. Since this would entail a significant amount of work, it would be important to look for collaborations with organizations experienced in this field. An important contact is the research group in Switzerland, which has already been involved with scientific studies regarding lynx genetics (Breitenmoser-Würsten et al., 2003; Breitenmoser-Würsten and Obexer-Ruff, 2007). They have already set up a genetic databank for some of the possible subspecies. The ESB could function as source to define which lynxes should be prioritized for research and provide more samples from animals from known origin to establish a genetic "footprint" for the database. These genetic footprints will serve as base from which the entire ESB population could be identified. In addition, the ESB would serve as a central point for managing and distributing the genetic samples provided by the various EAZA institutions. This approach will be important to help manage both captive and reintroduced populations.

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No sabemos lo que estamos haciendo en estos momentos o cómo afectarán al futuro nuestras acciones actuales. Lo que sí sabemos es que sólo hay un planeta para seguir haciéndolo y sólo una especie capaz de cambiar las cosas de una forma considerable.

Bill Bryson

Photo: Antonio Rivas

Hand-rearing of Iberian lynx cubs

Crianza artificial de cachorros de lince ibérico

Antonio Rivas, Fernando Martínez, Iñigo Sánchez,
José María Aguilar, Miguel Ángel Quevedo, Juana Bergara,
Eva Vázquez, Mariano Cuadrado and Astrid Vargas

Resumen

La crianza artificial está considerada como una actuación de emergencia que permite sacar adelante con éxito cachorros que no han podido ser criados por sus madres y que tienen pocas posibilidades de supervivencia. En el Programa de Conservación *Ex situ* del Lince Ibérico se han criado a mano satisfactoriamente ocho cachorros desde el año 2001. La crianza artificial es un proceso delicado que se inicia con la recepción del cachorro y finaliza cuando éste es capaz de valerse por sí mismo, en torno a los cuatro meses de edad. Durante el proceso de crianza, las variables a controlar y las técnicas a emplear irán adaptándose a la edad del cachorro, siendo los primeros días de vida los más críticos para garantizar su supervivencia. El cachorro recién ingresado debe acostumbrarse a las condiciones del nuevo alojamiento (la temperatura ambiental debe estar en torno a los 30 ºC y la humedad mantenerse al 50% durante la primera semana de vida), así como habituarse a la leche de reemplazo (se utilizan marcas con presencia del aminoácido taurina en su composición); al ritmo de lactancia (ocho tomas diarias de alimento cada tres horas durante los seis primeros días), y si no ha recibido calostro natural, se le deberán suplementar los anticuerpos necesarios para reforzar su sistema inmune (aporte vía oral de suero sanguíneo de un adulto sano a razón de 2 ml/100 g cada 12 horas durante dos días). Según los datos obtenidos a partir de cachorros criados a mano en el Programa de Conservación *Ex situ* –todos ellos abandonados por sus madres– un neonato de lince ibérico pesa aproximadamente 175±18 g y tiene una temperatura corporal de 34,9±0.7 ºC. Entre el primer y tercer día de vida, los cachorros criados a biberón incrementan su peso en 17±3 g al día y rápidamente pasan a una tasa de crecimiento diario de 35±6 g entre los cuatro y 40 días de vida. A partir de la quinta semana, debe comenzarse el periodo de destete, que se completará en torno a los 100 días de vida. Para asegurar que los linces criados a mano presenten unas conductas afines a los objetivos del Programa de Conservación *Ex situ* es preciso llevar a cabo, de forma paralela a la alimentación y cuidados, un correcto programa de socialización a partir de la segunda semana de vida de los cachorros.

Palabras clave

Crianza artificial, calostro natural, ritmo de lactancia, tasa de crecimiento, neonatos, socialización

Abstract

Hand-rearing of cubs is an emergency measure that makes it possible to successfully raise cubs whose mothers cannot take care of them and that consequently have low chances of survival. In the Iberian Lynx *Ex situ* Conservation Programme, eight cubs have been successfully hand-reared since 2001. Hand-rearing is a delicate process that starts when the cub is taken and finishes when the individual is able to take care of itself, around the age of four months. During the hand-rearing process, the variables to control and techniques to use should be adapted to the age of the cub. Indeed, the first days of a cub's life are most critical in guaranteeing its survival. Upon arrival, cubs must adjust to the conditions of their new environment (ambient temperature should be kept at about 30 °C and moisture should be 50% in the first week of life) and get used to the milk replacer (brands containing the aminoacid taurine are recommended) and the feeding schedule (eight daily feedings, that is, one feeding every three hours in the first six days); cubs that did not receive colostrum from their mothers need to be given the necessary antibodies to strengthen their immune system (via oral administration of blood serum from a healthy adult, 2 ml/100 g every 12 hours for two days). According to the data obtained from hand-reared cubs in the *Ex situ* Conservation Programme –all cubs had been abandoned by their mothers– a neonate Iberian lynx weighs about 175±18 g and has a body temperature of 34.9±0.7 °C. Between the first and the third day of life, the daily weight gain of hand-reared cubs is 17±3 g; between the 4^{th} and 40^{th} day of life, their mean daily growth rate is 35±6 g. Weaning should start around the fifth week of life and be completed around the age of 100 days. To ensure the behavior of hand-reared individuals matches the goals of the *Ex situ* Conservation Programme, an appropriate socialization programme should begin on the cub's second week of life, in parallel to the feeding and care of the cubs.

Keywords

Hand-rearing, natural colostrum, lactation, growth rate, neonates, socialization

Photo: Antonio Rivas

Figure 1.
Iberian lynx newborn cub (abandoned by its primiparous mother).

Figura 1.
Cachorro de lince ibérico recién nacido (abandonado por su madre primípara).

Hand-rearing of Iberian lynx cubs

Antonio Rivas, Fernando Martínez, Iñigo Sánchez, José María Aguilar, Miguel Ángel Quevedo, Juana Bergara, Eva Vázquez, Mariano Cuadrado and Astrid Vargas

Introduction

The best option for the proper development of an Iberian lynx *(Lynx pardinus)* captive-born cub is to be reared by its mother. Hand-rearing should only be considered when all factors and circumstances point to a high risk of disease and/or death of the cub, the mother or the cub's siblings. The few remaining populations of Iberian lynx are in such an extreme situation that each individual is valuable for the conservation programme. Thus, all efforts aimed at avoiding the loss of a cub, even if premature, are justified.

Data obtained from the 15 litters born at El Acebuche Breeding Center, between 2005 and 2008, via our camera-surveillance system indicate that, in the first 20 days of life, mothers spend between 67 and 79% of the day –16 and 19 hours, respectively– with their young, providing the care and attention they need. These figures substantiate the effort involved in trying to substitute for maternal care and the importance of knowledge and experience in successful hand-rearing.

This chapter is a compilation of the most important sections of the "Guidelines for Hand Rearing Iberian Lynx Cubs" (Rivas et al., 2008). It has been drawn up thanks to the experience acquired by the staff involved in the breeding of lynx at El Acebuche Breeding Center and at the the Jerez Zoo, as well as the advice and experience of experts on the subject, and a review of literature on the topic (Andrews, 2008; Hedberg, 2002; Meier, 1986; Gunn-Moore, 2006a).

What is a lynx cub like?

The gestation period for an Iberian lynx is 63-66 days (mode = 64; Vargas et al., this book). Cubs that are born to term weigh between 157-193 g (n=5). Iberian lynx neonates are altricial, ie., they are born helpless, blind and deaf, and completely dependent upon their mothers (Figure 1). Neonates are not capable of regulating their own body temperature, so if they do not have their mother´s warmth, they quickly become hypothermic. In addition, neonates depend on their mother's stimulation of the perianal area in order to defecate and urinate. Neonates have no teeth and, during their first two weeks of life, they spend most of their time nursing and sleeping.

Cubs can be sexed by measuring the distance between the genital and anal sphincters. At 3-4 weeks of age the distance is ≤16 mm in females and >16 mm in males (Palomares, pers. comm.; *Ex situ* Programme, unpub. data). In felids, as in other carnivores, immunity of cubs is essentially passive at first. Cubs receive most of

<table>
<tr><th>Days</th><th>00</th><th>01</th><th>02</th><th>03</th><th>04</th><th>05</th><th>06</th><th>07</th><th>08</th><th>09</th><th>10</th><th>11</th><th>12</th><th>13</th><th>14</th><th>15</th><th>16</th><th>17</th><th>18</th><th>19</th><th>20</th><th>21</th><th>22</th><th>23</th><th>24</th><th>25</th><th>26</th><th>27</th><th>28</th><th>29</th><th>30</th><th>31</th><th>32</th><th>33</th><th>34</th><th>35</th><th>36</th><th>37</th><th>38</th><th>39</th><th>40</th><th>41</th><th>42</th><th>43</th><th>>44</th></tr>
<tr><td>Eyes</td><td colspan="12">Closed</td><td colspan="4">Half way opened</td><td colspan="29">Opened</td></tr>
<tr><td>Dentition</td><td colspan="18">No teeth</td><td colspan="4">Canines</td><td colspan="22">Incisors and Canines</td><td colspan="1">Premolars</td></tr>
<tr><td>Ears</td><td colspan="34">Halfway folded</td><td colspan="11">Upright</td></tr>
<tr><td>Claws</td><td colspan="25">Non-retractile</td><td colspan="20">Retractile</td></tr>
<tr><td>Locomotion</td><td colspan="16">Cannot stand steedly on the four limbs</td><td colspan="18">Can stand on four limbs: unestable movements</td><td colspan="11">Good movement capacity</td></tr>
<tr><td>Downy fur</td><td colspan="14">Complete</td><td colspan="12">Missing on head</td><td colspan="19">Missing on limbs</td></tr>
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Figure 2. Developmental landmarks of Iberian lynx cubs (Rivas et al., 2008).

Figura 2. Hitos del desarrollo en cachorros de lince ibérico (Rivas et al., 2008).

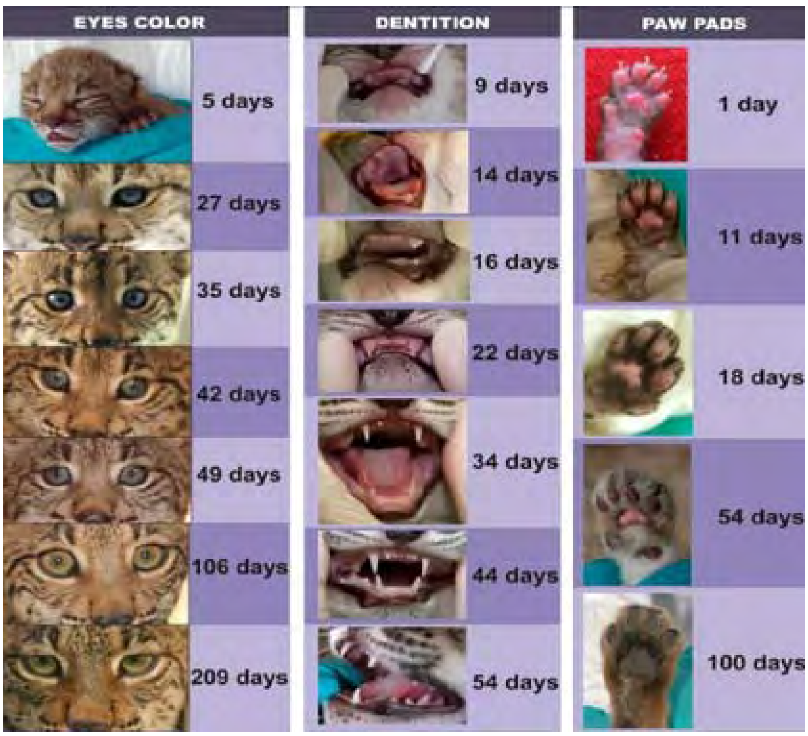

Figure 3. Changes in eye color, dentition, and darkening of paws in iberian lynx cubs.

Figure 3. Cambios en el color de ojos, dentición y oscurecimiento de almohadillas plantares en cachorros de lince ibérico.

their antibodies (IgG) from their mother´s colostrum, the first milk which will better prepare the immune system to respond to potentially infectious agents present in the environment. A colostrum-deprived cub will be more prone to disease. It is vital to maintain strict hygiene for all cubs, but especially for the colostrum-deprived ones.

Development of an Iberian lynx cub

Figure 2 shows distinct developmental landmarks of Iberian lynx hand-raised cubs. Following are specific features of each of these landmarks:

Eyes: The eyes start to open in the second week of life (8-14 days) and are fully open in the 3rd week (around 14-18 days of age). At first the iris is light gray. In just a few days it becomes deep blue, and then gradually it starts turning amber brown or emerald green, which will end up being the adult eye color (Figure 3).

Dentition: The dental formula of an adult Iberian lynx is I3/3, C1/1, P2/2, M1/1. The deciduous dentition set has the following formula: dI3/3, dC1/1, dP2/2. In the domestic cat, deciduous dentition starts to erupt in the 2nd week of life, with the incisors emerging on the first place, immediately followed by canines, and finally premolars at five or six weeks of age (Murtaugh, 1994). In contrast, in Iberian lynx cubs the first teeth to erupt are the upper canines, between 15 and 20 days of life. Lower canines immediately follow. Between 22-26 post-natal days we observe the eruption of the incisors and, finally, premolars emerge staring at week six after birth (Figure 2).

The order of eruption of permanent teeth replacing deciduous ones is incisors, molars, canines and finally premolars (García-Perea, 1996). Deciduous canines begin to emerge during the fifth post-natal month, and are completely replaced by permanent ones by the sixth month of life.

Coat: The first coat of cubs is formed by a mantle of fine, downy and compact hair that provides the necessary cover, protection and camouflage in their first weeks of life. The down keeps cubs warm while they are unable to thermoregulate. It gradually starts to disappear from the forehead and distal part of the limbs at around 11 post-natal days, follows a cranial-caudal pattern of thinning until it eventually disappears completely at around 70 days of age.

Ears: Lynx cubs are born with their ears folded. Cubs born after a full-term gestation (63-66 days) also present little hair tufts on the tips of their ears, a hallmark characteristic of the genus Lynx. The ears start to unfold around the tenth day of life and remain half-folded until the fifth week (31-36 days), when they become fully erect.

Claws: Cubs are born with their claws fully extended and covered from the base with a sheath of connective tissue. The claws only become completely retractable –another trait of felid species – between the third and fouth post-natal week.

Paw pads: Cubs are born with light-colored paw pads that gradually become darker until they are about three months old (Figure 3).

Motor skills: Although cubs are able to move from the very beginning, their movements are clumsy and shaky. They begin to crawl and are strong enough to stand on their four limbs between the second and third week of life. They only acquire enough motor skills to start to walk and explore their surroundings at four weeks of age.

Situations that may require hand-rearing of cubs

- Cubs born in the captive breeding center that are at risk of death due to any one of the following circumstances:
    - Lack of maternal instinct towards the litter or a specific cub.
    - A disease of the mother that poses a risk for the normal care of the cubs.
    - Cubs showing signs of weakness or disease that are starting to be neglected by their mother. This is a sign that the cub might have an infectious disease or a developmental disorder.
- Cubs taken from the wild to be included in the captive breeding programme.
- Cubs taken from the wild because their life is considered to be in danger – e.g., signs of generalized weakness or disease, risks posed to litter viability by external situations, etc.

General considerations

The mother should always be the one to take care of the cubs if possible. Once the decision has been made to hand-rear an Iberian lynx cub, the following three aspects must be controlled and considered at each step of the way:

- Keep the cub WARM (cubs are not capable of thermoregulating)
- Keep the cub HYDRATED (dehydratation can happen very fast)
- Keep the cub WELL NOURISHED

In addition, it is considered important to provide passive immunity to the neonate, as far as it is possible to do so. It is also important to make every effort not to rear a cub in isolation. One or several Iberian lynx cubs can be hand-reared with those of other *Lynx* species (e.g., bobcat *L. rufus*) or small felids (e.g., domestic cat *F. silvestris catus*, wildcat *F. silvestris silvestris*) as long as they have previously undergone a medical examination and do not have or carry contagious infectious diseases. Four weeks is a good age to introduce a domestic kitten or a cub of another species of small felid (Mellen, 1998). A high standard of hygiene must be followed when handling the animal, preparing its food, and cleaning feeding bottles and other utensils. This is particularly important for those cubs that have not had mother's colostrum. Changes in handling and feeding must be gradual.

Hand-raised neonates should be taken care of by no more than 2-3 people in order to ensure consistency in the handling and feeding of the animals as well as maintaining a good record-keeping.

Necessary equipment for hand-rearing of cubs

Housing

During the first three weeks, the cub must be kept in an incubator, in a box that is easy to clean or in a pet carrier box where it can be comfortably housed. It must be possible to control the inside temperature of the box. Bedding should be soft (i.e., fleece pads or flannel blankets) and it is important that it sheds no "fur" that the cub could lick and ingest. Housing must progressively change as the cub grows. At around 15-20 post-natal days, cubs are fairly mobile and need more space, it is advisable to provide them at his stage with an area of approximately 1x1 m to move around. At around five weeks, mother-raised cubs begin moving all over their enclosures, at this age, it is important to provide hand-raised cubs with a larger area where to exercise and explore.

Housing temperature

The temperature of the incubator and the room should be continuously monitored with appropriate thermometers. The incubator or denning box, can also be provided with additional hot water bottles or electric heating pads. The sources of heat should be placed so that there is always a temperature gradient and some areas are warmer than others, enabling neonates to find the most comfortable spot for them. The sources of heat should not come into direct contact with the animal, as they may cause burns. Socks or pads can be used to cover bottles.

It is vital that the inside of the incubator and the room where it is placed have a similar temperature, so that the neonate does not suffer a sudden change in temperature when being fed and cared for outside of the incubator. The room and incubator temperature should be regulated depending on the age of the cub. The recommended temperature gradient is as follows (Prats, 2004; Gunn-Moore, 2006b; Murtaugh, 1994):

- 1^{st} week: 30-32 ºC
- 2^{nd} week: 27-29 ºC
- 3^{rd} and 4th week: 27 ºC
- 5^{th} week: 24 ºC
- After the 6^{th} week: 21 ºC

Observation of the cub between two feeds will tell whether the animal is too cold or too warm. If the cub is agitated, restless and/or making whining sounds it may be a sign that the temperature is not well regulated.

Housing humidity

Housing must be maintained at around 50% humidity. For humidity control, one can use humidifiers or maintain containers full of water near heat sources. Neonates are under a high risk of dehydration, and thus it is important to maintain adequate levels of humidity in the room. Levels that are too high (85-90%) or too low might compromise the health of the cub.

Bottles and nipples

When selecting a nipple, try to match it with the teat size of the mother. For Iberian lynx neonates, the ideal ones are PetAg® small nipples for 60 cc feeding bottles. Place a hole directly in the center of the nipple with a heated needle. The size of the hole should allow suckling at a slow, steady rate. To test size, fill the bottle with hot milk and hold it upside down. Only a small drop should come out each time the bottle is put upside down. Constant dripping means the hole is too large, which can cause milk to be ingested too rapidly and increase the chances of bloating and of aspiration into the lungs (see section "Aspitarion").

Once a nipple works with a cub it should always be used exclusively for the same cub, until it needs to be replaced by another one because it is too small or too worn. Disinfectants and wet heat sterilization gradually damage the nipples, which eventually have to be replaced. The size and volume of the bottle will increase as the cub grows and needs more food.

Several nipples, bottles and bottle brushes should be available while hand-rearing the young. They should be cleaned thoroughly and handled with great hygiene. After each feeding, they must be washed with soap, very well rinsed and sterilized with steam or a bottle sterilizer.

Milk replacers

There are many brands available on the market, in both powdered and liquid forms. The most popular ones for cats are KMR®, Esbilac® and Lactadiet® Although the milk composition of the Iberian lynx is not known, it seems to be similar to that of other *Lynx* species, such as the Eurasian lynx (21.7% solids, 28.6% fat, 47% protein and 20.7% carbohydrates) (Jenness and Sloan, 1970).

The milk replacer for domestic kittens with the closest composition to that of the lynx is PetAg's KMR® (27% crude fat, 40% protein, 7% ash, 5% moisture). It also provides an appropriate amount of taurine, an essential amino acid for cats. Esbilac® and Lactadiet® have also being used with Iberian lynx and they are considered appropriate for this species.

Most mammals synthesize taurine from other amino acids. However, cats cannot synthesize a sufficient amount and, therefore, must acquire the rest through diet. Taurine deficiency can lead to impaired vision (feline central retinal degeneration), heart disease (dilated cardiomyopathy) or a decreased reproductive performance and growth.

Electrolytes

Electrolytes are solutions that are easily digestible at any body temperature. They are used as a source of energy and hydration in the first few feedings of cubs that are going to be bottle-fed (see "Feeding"), as a gut stabilizer during episodes of diarrhea (see "Diarrhea") and to treat cubs with hypothermia (see "Treating hypothermisc cubs"). In general, electrolytes –such as Pedialyte® and Glucolyte®– provide a good energy supplement to neonates, particularly those that are cold and/or weak.

Artificial colostrum

Colostrum is the first milk produced by the mother in the first hours after parturition. It is very rich in vitamins, proteins and fat and has a slightly laxative effect. It is especially valuable because it contains the antibodies that neonates need to start to develop there non-existent immune system. The placenta only allows for absorpotion of 20% of the passive immunity (Prats, 2004), the rest is acquired from the dam´s milk. In the domestic cat, immunoglobuline levels are constant throughout lactation, as opposed to dogs where immunoglobuline concentration is higher in the first few post-natal days (Prats, 2004). Also, in domestic kittens the gut is permeable to the large molecules of IgG in colostrum up to 72 hours after birth, and the greatest concentration is absorbed in the first 24 hours (Murtaugh, 1994; Romagnoli, 2001). For this reason it is important that abandoned neonates take colostrum during the first two days of life, when their intestine is permeable to the large IgG molecules. Nevertheless, kittens can still receive a good immunological support at later stages, since the dam's milk contains good concentrations of antibodies.

For the Iberian lynx, we recommend to provide antibodies to neonates that have not received colostrum from their mother. There are various alternatives, such as formulas available in the market (e.g., Lactadiet´s Artifical Colostrum for Kittens), or adding Iberian lynx blood serum, with an optimal concentration of antibodies, to the formula used to feed them (Bush et al., 1994; Bush et al., 1998; Prats, 2004 see "Provision of antibodies" via blood serum).

Measures of hygiene/Biosecurity

Neonates have a poorly developed immune system, particularly if they have not received colostrum. It is therefore essential to maintain strict hygiene to prevent the spread of germs to the cub during feeding and handling. The cub rearing area must be perfectly clean and separated from other areas with animals. The staff that is present in the rearing area must wear specific clothing for handling the cub –e.g., disposable coveralls, shoe covers, talcum-free gloves, and masks–. Any gear used with the cub –whether it is to prepare and supply food or provide regular care– must be disinfected and shouldn't be used with other animals and vice-versa.

Scale

It is necessary to have an accurate scale with a basket to weigh the cub daily in the first few weeks of life. An infant care spredsheet must be kept so that the evolution of the cub can be checked anytime. It should include the cub's daily weight and other data of interest such as amount eaten per feeding, temperature and so on (see "Template" in "Guidelines for Hand Rearing Iberian Lynx Cubs"; Rivas et al., 2008).

Important aspects and stages in hand-rearing

Receiving the cub

The cub must be received in a calm, quiet environment where only the necessary staff is present. The animal is usually stressed, so it is important to try to comfort it. Assess the cub's general condition: Are the airways open? Is the

	rectal temperature
1st day	34,5-36 °C
1st-2nd week	36-37 °C
3rd-4th week	37,8 °C
After week 4	38,5 °C

Table 1. Average temperature values for domestic kittens (Prats, 2004).

Tabla 1. Valores medios de temperatura en gatos domésticos (Prats, 2004).

	rectal temperature
0-2 days	34,2-35,6 °C
3-6 days	35,8-37 °C
7-14 days	36,6-37,6 °C

Table 2. Temperature ranges from six hand-raised Iberian lynx cubs at El Acebuche Breeding Center (Rivas et al., 2008).

Tabla 2. Rangos de temperatura en seis cachorros de lince ibérico criados a mano en el Centro de Cría de Lince Ibérico El Acebuche (Rivas et al., 2008).

Photo: Antonio Rivas

Figure 4. Weighing an Iberian lynx cub.

Figura 4. Pesaje de un cachorro de lince ibérico.

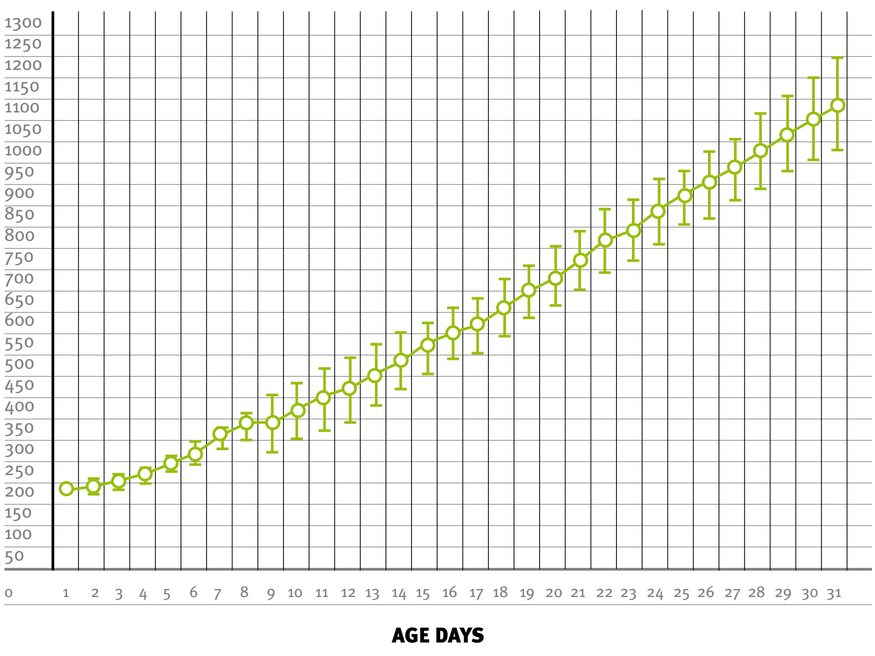

Figure 5. Changes in weight (avg + SD) during the first month of life in hand-reared Iberian lynx cubs (n=7) (Rivas et al., 2008).

Figura 5. Cambios en el peso (media + DE) durante el primer mes de vida en siete cachorros de lince ibérico.

cub breathing? Does it have a heartbeat? As a precaution against possible problems, the staff must be trained in cardiopulmonary resuscitation (CPR) techniques (Read and Meier, 1996) and have emergency equipment at hand.

The first priority is to keep the cub at the right temperature. Use a pediatric thermometer to measure rectal temperature. When hypothermia is severe (temperature <34 ºC) follow the steps shown in section "Treating hypothermic cubs" on treating hypothermic cubs to try to stabilize the animal. The room and incubator should be kept at the temperature shown in section "Housing temperature".

Stimulate urination and defecation as explained in section "Stimulation of urination and defecation", as the cub may have been abandoned by/pulled from its mother for quite a few hours.

Comfort the animal by massaging its whole body with a gauze and/or toothbrush dampened with warm water to mimic the mother's licking. Assess the sucking reflex by placing the tip of your little finger on the cub's mouth (make sure that your hands are throughly washed or that you are wearing powder-free gloves. If suckling reflex is absent, it may be because the animal is still nervous. If so, continue to comfort it by massaging it as described above. Once the cub is stable, a thorough medical examination should be performed.

Siblings are best left together, for warmth and comfort, unless a distinct need for separation occurs due to disease or other reasons. If there is only a single cub, provide a small stuffed toy –caution: the toy should not contain any plush fabric that may come off and be swallowed by the cub– or a rolled up towel to lie against for warmth and comfort.

Body temperature

In the first few weeks, neonates are unable to regulate their own temperature; therefore, caregivers must carefully control the cub's temperature environment. Avoid tendency to keep too warm. A few signs that the temperature is too warm are the following: the insides of the ears feel hot, the neonate is urinating on itself, or the paw pads are very red. The cub's body temperature will vary depending on its age (Gunn-Moore, 2006b; Prats, 2004; Tables 1 and 2). It is important to keep the cub's temperature stable; it must therefore be continuously monitored. A rectal temperature <34 ºC may lead to the cub's death (Gunn-Moore, 2006b).

Weight

Iberian lynx cubs weigh about 157-193 grams at birth. It is important to weigh the cub every day before its first feeding and record weight values in the infant care sheet (Figure 4, weighing a cub). A loss of 10% in the first 24 hours may be normal; however, after that, the cub should gain weight every day. Any weight loss –or lack of weight gain– must be looked into, as it is a sign that there is a problem. A weight loss exceeding 10% poses a risk to the cub's survival (Gunn-Moore, 2006b). Steady weight gain is the sign of a cub developing normally.

Daily growth rates vary depending on the age of the cub, and it should be calculated and monitored every day. In the Eurasian lynx *(Lynx lynx)*, different weight gain periods have been established (Naidenko, 2006): <20 to 41 days: 30-40 g; 41-60 days: 20-30 g; 60-80 days: 40-50 g. Hand-raised Iberian lynx cubs (n=7 between 2006-2008) showed daily increases in body weight of 16,6±2.5 g during the first three post-natal days and of 34,5±6 g between days 3-40 after birth (Figure 5).

Another aspect we consider important to register is the ratio between the total volume of food ingested and the cub´s body weight (V/BWx100). As a general rule, cubs should ingest approximately 20-30% of their body weight daily (Ward, 2002). Data on hand-raised Iberian lynx cubs show that, during their first week of life, they ingest a volume of milk that is equivalent to 44±5% of their body weight. This proportion gets smaller as the cubs grows, turning into values of 30±3% between 8 y 19 post-natal days; 19±2% at 20 y 34 post-natal days, and 11±1% at 35-40 days of life.

Stimulation of urination and defecation

Cubs usually defecate before they are fed. Stimulate the perineal region with a warm, wet cotton ball or gauze in circular and upward movements, first, softly around the external genitals and finally around the anus (Figure 6). It is normal for cubs to urinate first and then defecate. Always keep the anal and genital area clean. Do not force the cub by over stimulation if it does not defecate or urinate, as it will always make an effort to try to empty his system. Too much effort can lead to gastrointestinal problems. Neonates that are just a few hours old may pass meconium stools, which are thick, sticky, dark and foul-smelling. Stool color is a good indicator of digestion: yellow-brown is normal, greenish indicates too much food and white indicates that the cub is not digesting milk properly.

Photo: Antonio Rivas

Figure 6. Stimulation of urination and defecation.

Figura 6. Estimulación de la micción y defecación.

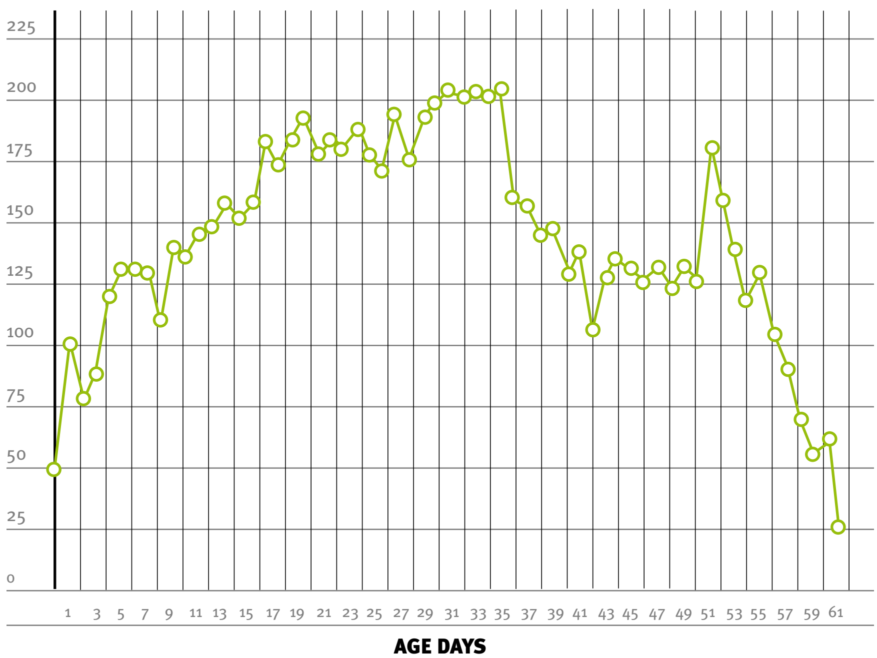

Figure 7. Average daily milk volume taken by seven Iberian lynx hand-raised cubs.

Figura 7. Volumen medio de leche ingerido por siete cachorros de lince ibérico criados a biberón.

Feeding

Feeding the cub

A cub's intestine is usually filled at birth, so feeding is not necessary in the first few hours after separation from the mother. Only stable cubs without hypothermia and responding to stimuli should be fed. If rectal temperature is below 34 ºC, digestion of food will not be possible.

There should always be more formula in the bottle than needed, allowing for better flow and preventing that the cub swallows air which would lead to bloating. The bottle with the formula should be maintained at

38 °C during the course of the feeding. This is done by placing the bottom half of the bottle in a container with warm water so that it keeps its temperature while other tasks are performed– weighing the cub, stimulating urination, and so on.

The average volume of milk ingested by Iberian lynx cubs in their first 20 days of life is 21±7 ml; increasing to 42±6 ml between 21 and 50 post-natal days. Total milk volume also varies according to age, reaching its peak at around one month of age (Figure 7). Afterwards, meat is provided to the cub and the daily amount of milk ingested starts to decline.

Do not overfeed as it may lead to diarrhea. Neonates suckle until they are tired, rather than full; they should therefore be given time to rest during the feeding in the first few days. Any food changes must be made gradually, giving the digestive system time to adjust. After feeding, try to favor elimination of any potentially ingested bubbles by giving a soft massage with the palm of our hand on the cub's belly and gently tapping its back, always maintain a slightly inclined position so the cub´s head is above the rest of its body.

Formula preparation

Formula refers to milk diluted with mineral water or electrolytes. It is vital to maintain high standards of hygiene and cleanliness when preparing the formula. This is particularly important with cubs that have not received colostrum from their mothers and therefore have not had acquired immunity. Thoroughly clean all the utensils used and the area where the food is prepared. Only use material –bottles, nipples, teaspoons, etc.– that has previously been sterilized. If the cub has not received colostrum, provide artificial colostrum for cubs during the first two days, or provide Iberian lynx serum with optimal concentrations of antibodies as explained in section "Provision of antibodies via blood serum".

It is important to note that the composition of mother's milk changes during the course of lactation (Oftedal, 1984) and is more diluted in the first 2-3 days.

The formula can be prepared in advance for a 24-hour period as long as it is kept refrigerated and the bottles are filled specifically for each feeding. Mix formula thoroughly and allow any air bubbles to settle and disappear prior to feeding. Gently transfer the necessary amount of formula to the bottle to prevent new bubbles from forming. The formula may be warmed in a double boiler (bain-marie) or in a bottle warmer. Use of microwaves is not recommended, as they do not warm food homogeneously and may burn the animal. The temperature of the formula must always be tested before feeding. This is done by pouring a few drops on the inside of your wrist. When the formula is warm but does not burn the skin it is at the right temperature.

Feeding position

Sit and place the cub on a towel on one of your legs. Keep the cub on its stomach with its head slightly elevated (Figure 8). Place your forearm, a rolled up towel or a pillow in front of the cub; the cub will push and knead with its front paws as it suckles, as it would do with its mother's teats to stimulate milk flow. Tilt the bottle so that the nipple is always full of milk to avoid air swallowing. Test milk temperature just before feeding the cub.

Common factors adversely affecting suction

Cubs are stressed by the new situation; it is therefore normal for them to lack the sucking reflex. Comfort the animal by massaging its whole body with a gauze and/or toothbrush dampened with warm water to mimic the mother's licking. This may calm it down and get it to suckle. Some cubs will not drink if milk is too cool. A cub may fuss and not eat well when fed by a new caregiver until it gets used to the new person.

Feeding schedule for Iberian lynx cubs

- 1st week: feed every 3 hours, 7-8 feeds/day
- 2nd-3rd week: feed every 3.5 hours, 5-6 feeds/day and 5-6 hour's sleep at night
- 4th week: feed every 4 hours, 4 feeds/day and 8 hours of sleep at night
- 5th week to weaning: feed every 4 hours, 4 feeds/day; introduce solid food very gradually.
- Weaning to 6 months: 3 feeds/day

Photo: Antonio Rivas

Figure 8. Feeding position.

Figura 8. Postura durante la alimentación del cachorro.

This feeding schedule is just a guideline; the cub often shows us the best regime to follow.

The first few –two or three– feedings of a neonate should be straight electrolytes, between 5 and 10 cc (see "Electrolytes"). This makes it possible to check the swallowing reflex is good and reduces the risks in case of aspiration into the lungs.

Weaning

Transition to eating solid food should begin at 5-6 weeks of age, when the upper and lower canines and incisors are present and the deciduous premolars start to erupt (see "Development of an Iberian lynx cub"). The cub will progressively refuse to be bottle-fed and will start to prefer solid food. Three feeding stages have been differentiated in Iberian lynx cubs raised by their mothers in captivity (Vázquez et al., 2007):

- From 0 to 60 days they only suckle
- From 61 to 104 days they suckle but also ingest solid food
- From 105 days they no longer suckle (weaning is at around 3.5 months)

Weaning must be progressive so that the animals can adapt to the change of food. At first, cubs should be fed very small pieces of lean meat –preferably rabbit or chicken– mixed with some milk. Meat must not contain bones, skin, gristle or entrails. At first, it is normal for cubs to reject solid food, but they gradually start to chew meat, accept it willingly, and finally eat it with eagerness. They should progressively be given meat attached to large pieces of bone that they can bite without risking to choke on them.

Always have a bowl of water available for the cub to drink once it is eating only solids. If more than one cub is being hand-reared, it is very important to feed each individual separately in different areas of the enclosures to avoid disputes or fights for food and to be able to control what each animal is eating.

Common problems and what to do about them

Diarrhea

Diarrhea must be treated as soon as it is detected, as severe diarrhea can dehydrate a neonate very rapidly and result in death. In most cases, diarrhea is related to overfeeding or a feeding formula that is too concentrated; sometimes it is caused by a bacterial or parasitical infection, usually due to a lack of hygiene. Mild diarrhea responds well to a more diluted formula with mineral water at a 1:1 ratio until diarrhea stops.

In more serious cases, take the cub off formula completely and give only electrolytes (a 5-10% glucose solution), using the same amount and feeding schedule as with formula until diarrhea stops (Andrews, 1998). If the neonate is cold don't give any oral medication or fluid. In these cases a veterinary intervention is needed to start an intravenous or subcutaneous treatment.

Gradually reintroduce formula, beginning with half the strength of what it was before withdrawing, then slowly work up to its original strength as stools firm. Consult a veterinarian if diarrhea persists. Bene-bac® by PetAg®, a gel that stabilizes the natural gut flora of kittens, is very effective (Andrews, 1998). Antibiotics are not recommended, particularly for treating diarrhea, as they often upset the kitten´s normal growth of bacterial gut flora. Therefore, they can lead to an even worse situation (Gunn-Moore, 2006a).

Constipation

A cub whose feces are very hard, who is having too much difficulty defecating or has not defecated in 36 hours is considered constipated (Andrews, 1998). A few doses of liquid paraffin (Hodernal®, Emuliquen®) in approximate doses of 0.5 ml per feeding for 2-3 days (Gunn-Moore, 2006a) usually solves the problem. Another possibility is to give the cub a few drops of corn syrup added to each bottle for 2-3 feedings (Andrews, 1998). If the cub does not defecate after the treatment explained above, a very mild warm soapy enema can be given.

Bloat

Bloat is swelling of the abdomen caused by gas in the intestines or the peritoneal cavity. Use electrolyte therapy as for diarrhea or use Nutrical® at a rate of 4 cc daily divided by the number of feedings (Andrews, 1998).

Aspiration

This happens when formula is not well sucked and enters the airways. It may be due to the cub not being used to the bottle or to the cub being given too much food. It can lead to asphyxia of the cub or to aspiration-pneumonia. If it happens, the cub will start to cough and milk will come out of its nose. Apply the following technique to clear the airways: hold the cub belly down on the palm of your right hand, placing its head between the index and middle fingers. Place the palm of your left hand on the cub's back, holding the upper part of its head with the fingers of this hand. Bend forward with your legs flexed and swing the cub between your legs gently but firmly so that it can expel the fluid from its airways. This technique must be used with caution, as it could cause a brain hemorrhage if it is done violently.

Treating hypothermic cubs

Cubs are unable to regulate their own temperature, which can drop from an optimal to a critically low or high temperature in just a few hours. Risk is highest in the first few post-natal weeks, when body temperature ranges between 35-37 ºC, the shivering reflex is not yet present and there is not much subcutaneous fat (Prats, 2008).

A cub with a temperature of 38 ºC has a blood pulse between 200 and 250 beats per minute (bpm). However, if its temperature drops to 30 ºC its heartbeat will drop to 40-50 bpm. This may cause a malfunction of the respiratory system and ultimately lead to cardiac arrest. Domestic kittens whose rectal temperature is <34 ºC are in serious danger (Gunn-Moore, 2006a).

It is vital to re-stabilize the cub's temperature gradually. The cub may be placed in a warmed isolette or incubator. It should be done in a timeframe lasting between 1 to 4 hours, depending on the severity of hypothermia. A rapid increase in temperature may cause cardiovascular collapse and death of the animal.

Raising the temperature too much can also cause dehydration of the cub and also lead to its death. Continuous and careful monitoring of the animal's temperature is therefore of capital importance. Peripheral circulation is poor in severely hypothermic animals, so heat is not dissipated from the skin surface; therefore burns and severe skin damage can result, even at temperatures that would not damage normal individuals (Meier, 1986, 1984). Hypothermia reduces the absorption capacity of the gut and thus leads to poor digestion, which easily leads to hypoglycemic episodes (see "Hypoglycemia"). Do no provide formula, only eletrolytes, until the cub recuperates its normal temperature.

Provision of antibodies via blood serum

Serum should be taken from the cub's mother if she is in perfect health. If the mother is not available, serum can be taken from another healthy individual that has spent over a year in the breeding programme and whose serology study proves that it has good antibody titers. Serum must be separated in 4-6 ml aliquots; it can be frozen and thawed out for its use, although it is always better to provide it fresh. Administer the serum at approximately 35-37 ºC. The recommended dosages are as follows (Prats, 2004):

Oral: 2 ml/100 g body weight every 12 hours. Oral route has the slight problem that the volume that can be given is limited by the cub's stomach size, and also that it competes with the administration of other nutrients.

Subcutaneous (SC), intravenous (IV), Intra peritoneal (IP) routes: 2-5 ml/100 g every 8 hours.

Provide lynx serum at this rate for the first two days of life.

Hypoglycemia

Hypoglycemia is the result of a poor diet. If a cub refuses several feedings or food is not being assimilated because of a problem –e.g., hypothermia– it should be treated immediately. Cubs have very limited energy reserves and may die in just a few hours.

Treatment involves giving the cub a few drops of corn syrup orally as a source of energy. If the cub is also hypothermic, no oral treatment should be given. In this case parenteral treatment and stabilization of the cub's temperature should begin at the same time (Gunn-Moore, 2006a) (see "Treating hypothermic cubs").

If the cub still does not accept food, surgery will be needed to insert feeding tubes into the cub's stomach and feed it artificially.

Socialization

Hand-rearing must be encompassed with a socialization programme, oriented to ensure the development of natural behaviors in Iberian lynx cubs. In felids, socialization is crucial during development (Caro, 1995; Bateson, 2000; Rochilitz, 2000; Casey et al., 2005), and, thus, it is recommended that, whenever possible, hand-reared cubs should be raised in groups of two or three young. The objectives of hand-rearing an animal must be clearly defined from the beginning. Will the animal be used in education programmes and therefore be in contact with humans? Or will it be used for breeding or for reintroduction purposes? It sometimes requires great effort and time to check whether the final objective has not been compromised or undermined by our intervention. Hand-rearing is not recommended for cubs that will be reintroduced into the wild.

Hand-rearing implies the risk of raising cubs that will exhibit an abnormal behavior for their species. Such imprinted behavior is difficult to modify.

The sensitive period for socialization is considered to take place between the 2nd and 20th week of a cub's life. Iberian lynx cubs open their eyes around their 14^{th} day of life. This is the beginning of a developmental period during which they rapidly learn from external stimuli. They exclusively depend on their mother's milk until they are about nine weeks old –although they may continue to suckle until they are three or four months old–. This period of dependence on the mother is considered to be vital for the development of different behavioral traits.

It is important that the animals have contact with other individuals of their species during the sensitive period for socialization (Read and Meier, 1996). If this is not possible, they should be in contact with other felid species, preferably of the genus Lynx. If this does not happen, their adult behavior might be altered and they may not show any interest in mating with individuals of their species upon reaching sexual maturity.

Another aspect to consider is contact with humans during the sensitive period for socialization. As long as contact with other lynx is ensured, contact with humans does not seem to alter the development of reproductive behavior. However, regular contact with humans in cubs between two and 20 weeks of age can lead to very tame animals that are not afraid of humans. This may pose problems for handling these individuals once they have reached maturity.

Conclusion

Hand-rearing is a delicate and meticulous process. On most occasions, it involves handling Iberian lynx cubs that are only a few days old and sometimes even prematurely born. These animals have a deficient immune system and their viability completely depends on very thorough care and an appropriate environment. Given how difficult it is not to alter natural behavior patterns, an important factor is the final use of the animals –restocking and/or reintroduction projects, captive breeding, public display, etc. The *Ex situ* Conservation Programme's main goal aims at reintroducing animals yet, the highly critical status of the populations of *L. pardinus* justifies taking every necessary effort –including hand-raising of cubs– to successfully raise any individual with low chances of survival. Extreme care should be devoted to promoting natural behaviors in the species, even if hand-raised animals will never be targeted for release.

As shown in this paper, successful hand-rearing of Iberian lynx cubs involves following specific guidelines and requirements. There are two factors that are highly important and should not be forgotten, even though they cannot be measured with any thermometer or scale. They are the dedication and confidence of the human team in charge of taking care of the cubs. A great deal of time must be devoted to the process, and there will be many occasions in which the cub does not evolve as we expect or wish. In such moments, the difference between success and failure depends on the team's energy, knowledge and confidence.

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Photo: Antonio Rivas

In the end, our society will be defined not only by what we create, but by what we refuse to destroy.

John C. Sawhill
(1936-2000)

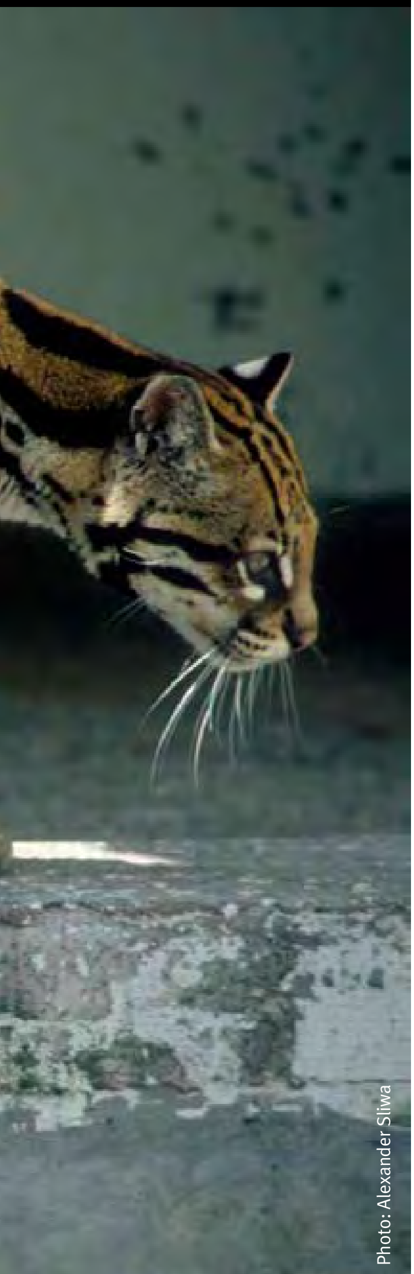
Photo: Alexander Sliwa

Behavioral problems of wild felids in captivity

Alteraciones del comportamiento en felinos salvajes en cautividad

Xavier Manteca

Resumen

Los felinos salvajes en cautividad muestran en ocasiones cambios de conducta que pueden ser indicativos de una falta de bienestar. Entre estos cambios destacan las estereotipias, la inhibición de la conducta maternal, el aumento de la conducta agresiva, y la reducción del consumo de alimento y de la conducta exploratoria. Las estereotipias son conductas repetitivas que resultan de una enfermedad o de los intentos repetidos por adaptarse a un ambiente difícil. Las estereotipias de desplazamiento son las más frecuentes en los felinos salvajes en cautividad y suelen aparecer cuando los animales se encuentran en situaciones que impiden o dificultan la expresión de su comportamiento normal, especialmente la conducta de alimentación, locomotora o de exploración. Varios estudios sugieren que los animales que durante las fases tempranas del desarrollo se mantuvieron en ambientes pobres en estímulos tienen más probabilidades de realizar estereotipias cuando son adultos. El estrés inhibe el comportamiento maternal y puede causar ocasionalmente canibalismo materno-filial. Así mismo, el estrés puede interrumpir o prolongar el parto y causar hipoxia cerebral en las crías. El estrés causado por un ambiente inadecuado o nuevo causa frecuentemente anorexia, que en algunos casos puede comprometer la salud e incluso la vida del animal. Algunas circunstancias relacionadas con el manejo y las instalaciones de los felinos salvajes en cautividad –tales como la introducción de nuevos animales, la existencia de recursos por los que los animales pueden competir y la alteración de la dinámica social típica de cada especie– pueden aumentar la frecuencia o intensidad de las interacciones agresivas. Las técnicas de enriquecimiento ambiental y, de forma ocasional, la utilización de psicofármacos y de feromonas constituyen las técnicas principales para prevenir y corregir las alteraciones de comportamiento que aparecen en los felinos salvajes en cautividad. El objetivo principal del enriquecimiento ambiental es facilitar la expresión del comportamiento normal de la especie, especialmente la conducta exploratoria y la interacción social. Las feromonas faciales y las feromonas apaciguadoras producidas por las hembras lactantes pueden ser especialmente útiles para prevenir o corregir los cambios de conducta causados por el estrés.

Palabras clave

Bienestar, estrés, estereotipias, enriquecimiento ambiental, psicofármacos, feromonas, socialización

Abstract
In captivity, wild felids sometimes exhibit behavioural changes that may be a sign of a lack of welfare. Such changes generally involve stereotypic behaviour (or stereotypies), the inhibition of maternal behaviour, increased aggressive behaviour, and decreased food consumption and exploratory behaviour. Stereotypies are repetitive behaviours resulting from a disease or repeated attempts to adapt to a difficult environment. Stereotypic pacing is the most frequent type of stereotyped behavior in wild felids in captivity. It usually appears when the animals are in a situation that prevents or hinders them from expressing their normal behaviour, particularly feeding, moving about, or exploring. A number of studies suggest that when animals are held in environments with few stimuli at an early stage of their development, they are more likely to perform stereotypies as adults. Stress inhibits maternal behaviour and may sometimes cause maternal cannibalism, or interrupt or delay delivery and cause brain hypoxia in the young. Stress caused by an inadequate or new environment often causes anorexia, which may in some cases compromise the animal's health or even its life. Some circumstances related to the housing and husbandry of wild felids in captivity, e.g. introducing new animals, competition for resources and altering the typical social dynamics of the species, may increase the frequency or intensity of aggressive interactions. Environmental enrichment techniques, and occasionally the use of psychotropic drugs and pheromones, are the main techniques to prevent and correct behaviour problems in captive wild felids. The main objective of environmental enrichment is to facilitate the expression of the normal behaviour of the species, especially exploratory behaviour and social interaction. Facial pheromones and appeasing pheromones produced by lactating females can be particularly useful to prevent or correct behavioural abnormalities caused by stress.

Keywords
Welfare, stress, stereotyped behaviour, environmental enrichment, psychoactive drugs, pheromones, socialization

Behavioral problems of wild felids in captivity

Xavier Manteca

Abnormal behaviour of wild cats in captivity

Stereotypies

Traditionally, stereotypies have been defined as behaviours that are repetitive, invariable, and without any apparent function (Fox, 1965). More recently, Rushen and Mason (2006) have described them as repetitive behaviours resulting from illness or repeated attempts at adapting to a difficult environment.

Stereotypic behaviour has seldom been described in animals in the wild (Carlstead, 1996). However, it is seen relatively frequently in wild animals in captivity, such as farm, companion and laboratory animals. It can occur as a response to an inadequate environment, following the administration of certain drugs, such as amphetamines or apo-morphine, or as a result of neurological disease or damage (Mason, 1993). Only stereotypies which occur in response to environmental conditions will be considered in this article.

The stereotypy most frequently exhibited by wild carnivores in captivity is known as pacing. It involves the animal making repetitive movements along an unchanging path, often incorporating a fixed sequence of movements in a specific place. This stereotypy makes up 97% of those described in captive carnivores (Clubb and Mason, 2003).

The causes of environmental stereotypies have been, and continue to be, the subject of many investigations, and a detailed revision of the neurophysiological mechanisms responsible for such behaviours is beyond the objectives of this article. Briefly, however, it appears that environmental stereotypies tend to be exhibited in situations where expression of normal behaviour is obstructed or made difficult. In particular, they can appear when the environment prevents the expression of feeding, locomotory or exploratory behaviours. So, for example, in many species the stereotypy is mostly seen just before feeding time, when the animals are motivated to perform exploratory behaviours in search of food (Carlstead, 1996). On other occasions, the inability to reach a certain place, or the company of another animal, can trigger a stereotypic behaviour (Carlstead, 1996). Likewise, some stereotypies seem to derive from an animal's attempts to escape the environment in which it is confined; in which case, the behaviour would be a consequence of the aversion caused by the environment (Rushen et al., 1993). However, the fact that, on occasions, stereotypic behaviour decreases in response to environmental enrichment techniques that do not seem to be related to food, exploration, locomotion or aversion, indicates that at least some stereotypies could be related to other behaviours or factors, such as sexual or nesting behaviour

(Rushen et al., 1993). Finally, stereotypies can also be triggered by the general activation of the central nervous system as a response to unspecific stimuli (Rushen et al., 1993).

It is often stated that stereotypies are a consequence of the animal being confined to a very limited space, and can be reduced or even eliminated by simply increasing the space available to the animal. Frequently however, the critical factor is not the amount, but the quality of space, and more precisely, the way in which the installation allows the animal to carry out its normal behaviour (Carlstead, 1996).

The tendency to perform stereotypies varies considerably between species, and between individuals of the same species. The differences between species can be at least partly related to some aspects of their natural history. Clubb and Mason (2003) have shown that, in captive wild carnivores, stereotypies are more frequent in species which use extensive areas in the wild. Interestingly, neonatal mortality in captivity also tends to be greater in these species than in those which use smaller areas. This would indicate differences between species in terms of the ease in which they are able to adapt to their captive conditions.

The differences between individuals within a species can have genetic and environmental origins. Thus, during early stages of development, a complex environment that is rich in stimuli may help prevent the development of stereotypies in later stages, due to greater behavioural flexibility and decreased sensitivity to stressful situations. This could explain the differences, described in some species, between individuals born in captivity and individuals captured in the wild in terms of their tendency to carry out stereotypical behaviours (Jones and Pillay, 2006).

Susceptibility to stressful environments, or those which restrict the expression of normal behaviours, also corresponds with genetic factors, so that some genotypes develop stereotypies in suboptimal environments, while others would seem to be more resistant (Cabib, 1993). Therefore, environmental stereotypies appear in animals with a certain individual or species predisposition, when they find themselves in environments that do not allow the expression of certain behaviours. Individual predisposition to perform stereotypies in suboptimal environments would result from a combination of genetic and environmental factors.

Stereotypies change over time, and stereotypies that have been performed over a long period, are often more difficult to stop and are also less responsive to management techniques such as environmental enrichment. Such techniques may be more useful to correct more recently acquired stereotypies (Mason, 1993). In general, stereotypies are considered to be indicators of a lack of welfare. This is due to both the circumstances that favour their development, such as restrictive environments that prevent the expression of normal species specific behaviour, and the fact that some stereotypies have negative consequences for the animal, causing injury or loss of body condition (Mason, 1993). However, it is important to bear in mind that stereotypies are not synonymous with a lack of welfare. Indeed, some animals can be found in a state of considerably bad welfare, yet still not develop stereotypic behaviours (Jones and Pillay, 2006). In fact, while environments that are conducive to the development of stereotypies are often inadequate from a welfare point of view, animals in such environments that do not perform stereotypies can have an inferior state of welfare to those who do develop them (Mason and Latham, 2004). Moreover, the importance of stereotypical behaviour from a welfare point of view will vary between long-standing and more recently acquired stereotypies. In summary, while stereotypies must be considered as potential indicators of a lack of welfare, it is important to consider the possible differences in their backgrounds and incentives, and in no case should the frequency of stereotypies be used as the sole indicator of welfare (Mason and Latham, 2004).

Inhibition of maternal behaviour

In natural conditions, maternal behaviour is essential for the survival of young. In captivity, although artificial rearing techniques can be called upon, it is more desirable that the breeding females express a sufficient degree of maternal behaviour to allow a natural upbringing, since it facilitates management and leads to the development of more appropriate offspring behaviour (Figure 1).

The proper expression of maternal behaviour results from an interaction between various factors, which are summarised below (Poindron, 2005):

- Hormonal changes preceding parturition, especially an increase in the plasma concentration of oestrogens, and in some species, prolactin (Jewgenow et al., this book; Pelican et al., this book).

Photo: José María Pérez de Ayala

Figure 1. Situations of stress may inhibit the expression of maternal behavior.

Figura 1. Las situaciones de estrés pueden inhibir la expresión del comportamiento maternal.

- Release of oxytocin following the dilation of the birth canal during the expulsion of the foetus.
- Stimuli from the young.
- Previous maternal experience (Vargas et al., this book).

Immediately after giving birth there is a sensitive period in which the female is especially receptive to any stimulus from the young. During this time, contact between the female and her young is crucial for maternal behaviour to become independent from hormonal stimuli and be maintained until the young are weaned and independent.

One of the most important causes of neonatal mortality in domestic cats is the inadequate expression of maternal behaviour, which can cause up to 19% of all kitten deaths (Young, 1973). A similar situation is highly likely in other felid species, such as the Iberian lynx, where maternal neglect occurs in more than half of first-time mothers (Vargas et al., this book). As has been previously commented, there are differences between species in terms of survival of young in captivity; species that use larger areas in the wild usually suffer a higher percentage of neonatal mortality in captivity (Clubb and Mason, 2003).

Stress also has a very important effect on maternal behaviour, sometimes inhibiting its expression, or even leading to filial cannibalism. In the domestic cat, filial cannibalism accounts for up to 12.5% of all offspring deaths up until weaning (Young, 1973). Furthermore, stress can interrupt or prolong parturition, which in turn can cause offspring cerebral hypoxia, reducing their strength and mobility. As the females maternal behaviour is stimulated by movements of the young, the hypoxia caused by an excessively long labour can inhibit maternal behaviour and lead to offspring abandonment.

Apathy and inhibition of food consumption

When animals find themselves in an environment lacking stimuli over a prolonged period of time, they respond either with apathetic behaviour (lack of movement and response to stimuli) or with 'out of context' behaviours intended to increase stimulation. The first of the two responses is relatively common in carnivores held in facilities that lack any form of enrichment and is characterised by the animal showing inactivity and lack of interest in anything that happens around it. On the other hand, these animals may have an abnormally intense response to a sudden stimulus. For example, Christian and Radcliffe (1952) describe the case of various captive wild animals, after being kept in extremely small cages, which died suddenly on being moved to a new installation. In all of the cases, there were signs of adrenal cortex atrophy, which indicates an inability to adapt to a new and potentially stressful situation.

Stress inhibits food consumption, and, at least in the domestic cat and most probably in other felids too, stress from an inadequate, or simply new environment, frequently causes anorexia. This can compromise the health, and even the life of the animal. It has been found in the domestic cat that a change in behaviour caused by a situation of stress following a change in the animal's environment, can lead to anorexia, immobility and general inhibition of behaviour. In some cases it can lead to death from liver failure due to the process of lipidosis (Fatjó et al., 2000).

Aggression

Different behaviours included within the term 'aggression' can vary considerably, in terms of the context in which they appear and the factors responsible for their control. Therefore, an attempt must be made to classify the types of aggressive animal behaviour.

The most objective system of classification is probably one based on the nervous structures involved in the control of aggressive behaviour. This classification distinguishes only three types of aggression: offensive, defensive and predatory. They are distinguished not only by the nervous structures that control them, but also by the context in which they appear, and by the relatively invariable motor sequences that characterize them (Moyer, 1968). Such sequences have been described in great detail in the domestic cat (Leyhausen, 1979).

Although aggression is a natural behaviour (Naidenko et al., this book; Antonevich et al., this book), some circumstances related to housing and husbandry of wild felids in captivity can increase the frequency and intensity of aggressive interactions. So, for example, the introduction of new animals, competition for resources and the alteration of typical social dynamics for each species (especially the normal age of dispersion of juveniles), can lead to aggressive behaviour. This, in turn, can have especially severe consequences in captivity, due to the subordinate animal being unable to escape (Koontz and Roush, 1996).

Techniques to prevent and correct behavioural abnormalities

Environmental Enrichment

Environmental enrichment includes a series of techniques whose objectives are to 1) increase the animal's control over its environment; 2) facilitate the expression of normal behaviour, especially exploratory behaviour and social interaction, or 3) provide the animal with cognitive challenges (Shepherdson, 1998).

Some of the enrichment techniques used for wild felids in captivity include hiding food so that the animal must dedicate time to searching for it; giving a supply of toys and installing platforms that provide the animals with a three dimensional space (Martos, this book).

The effects of environmental enrichment are variable and depend on the technique used and the characteristics of every species and individual. However, the effects of enrichment on behaviour and welfare of wild felids in captivity are generally positive, and may include the following (Carlstead and Shepherdson, 2000):

- A decrease in the proportion of time spent exhibiting stereotypic behaviour
- An increase in activity and exploratory behaviour
- A decrease in chronic stress occasionally associated with captive conditions

In other species it has also been found that environmental enrichment can decrease the response of acute stress when faced with sporadic aversive situations (Carlstead and Shepherdson, 2000). It is important to take into account that, occasionally, environmental enrichment programmes can have negative effects on the animal's welfare. For example, in animals housed in groups enrichment can cause an increase in aggression by stimulating competition for resources that did not previously exist. However, this problem usually disappears following an increase in the number of objects or feeding points used for the enrichment, so that the animals have no need to compete with one another (Bloomsmith et al., 1988).

In order to verify the effectiveness of an environmental enrichment programme, it is necessary to gather information about the animal's behaviour before implementing the Programme, so that possible changes made by the enrichment can be assessed (Martos et al., this book). Furthermore, in order to assess the effect of enrichment upon physiological parameters indicative of chronic stress, it can be useful to analyse the concentration of metabolites of cortisol in the faeces before and after carrying out the programme.

Psychoactive drugs

Psychoactive drugs can be useful to alleviate specific situations of stress. It is important to take into account, however, that under no circumstance should they be used over a prolonged period of time in order to mask the effects of inadequate housing or husbandry. Among those most commonly used in wild felids in captivity are the following:

-Long-acting Neuroleptics

A group of potentially useful psychoactive drugs are the so-called long acting neuroleptics (LANs), which are tranquilisers that provide effective therapeutic levels for at least a week, after only one application (Lingjaerde, 1973). LANs available on the market are obtained by creating a fatty acid ester of the active product and dissolving this in vegetable or medicinal oil. After administration, the slow hydrolysis of the solvent oil releases the ester which diffuses into the extracellular fluid, and is subsequently absorbed into the bloodstream, where it is hydrolysed into the active form (Ebedes, 1993).

LANs were used for the first time on wild animals in South Africa to alleviate the possible adverse effects of the stress of capture, handling and transport of wild animals (Ebedes, 1993). Following administration of a LAN, the following signs are typically observed (Ebedes, 1993):

- An indifference to unfamiliar surroundings
- An increase in appetite, presumably due to decreased fear
- A greater tolerance towards people and other animals

For these reasons, LANs can be especially useful in helping the animal to adapt to a new environment or the presence of unfamiliar conspecifics.

LANs have been used on a variety of felid species, including cheetahs, lions, jaguars, tigers, ocelots and leopards (Huber et al., 2001; Winterer and Wiesner, 1998). It is important to note, however, that there is very little information about the use of such pharmaceuticals in non-ungulate mammals, so they must be used with caution. Some species of artiodactyls have been known to perform aggressive behaviours towards people following the administration of long-acting neuroleptics (Ebedes, 1993).

-Other psychoactive drugs

Various psychoactive drugs have frequently been used to correct behaviour problems in the domestic cat, some of which, such as buspirone and fluoxetine, can be used in wild felids in captivity. Buspirone is an anxiolytic that does not belong to the benzodiazepines, and acts as a partial agonist to the 5-HT1 receptor for serotonin. Serotinin is a neurotransmitter that has an important regulating effect on the anxiety response. Buspirone has very few side effects and does not appear to create dependency. Furthermore, unlike benzodiazepines, it does not interfere with learning and memory. Fluoxetine is a selective inhibitor of serotonin reuptake, and is very useful against aggression problems between cats. The effects of the selective inhibitors of serotonin reuptake may not be immediate and can even take weeks to appear (Overall, 1997). Both drugs have occasionally been used on wild cats in captivity to control fear and aggression. However, they must be used with caution, since there is little information about their possible effects on wild animals.

Pheromones

Felids possess pheromone-producing glands on the sides of the head (temporal glands) around the mouth (perioral gland) and tail (caudal glands). Temporal and perioral glandular secretions are deposited as the animal rubs its head against an object, or sometimes another animal (Bradshaw and Cameron-Beaumont, 2000). The facial pheromones of the cat are of interest in clinical ethology because they can reduce the stress response and aggressive behaviour towards other animals of the same species (Pageat and Gaultier, 2003). The domestic cat's facial pheromone has been shown to be effective in other species of cats such as tigers. Therefore, it may be possible to use this pheromone, which is available on the market, as a useful tool to reduce stressful situations of wild cats in captivity, although this is obviously a subject that must be studied more in depth.

Lactating females of several mammalian species produce a pheromone called "appeasing pheromone", which, as its name suggests, reduces the aggressive behaviour of young, and also has anxiolytic effects. It is produced by glands within the skin around the abdominal area (Gaultier et al., 2008). An appeasing pheromone has recently been isolated in the domestic cat, and work will be carried out promptly to determine whether or not it also has an effect in other cat species.

Conclusions

Observing the behaviour of wild cats in captivity is a useful method to assess their welfare and, if necessary, implement corrective measures to facilitate their adaptation to housing conditions and management. This is especially relevant in captive breeding centres, as lack of adaptation to the environment results in a decrease in reproductive efficiency. Environmental enrichment programmes should be part of the day to day husbandry routine for wild cats in captivity. Furthermore, the use of pheromones to prevent or remedy stressful situations is a promising technique that deserves thorough investigation. Finally, LANs can be helpful in decreasing an animal's stress response, but should never be used as an alternative to adequate housing and management.

Acknowledgements

I want to thank the staff of the Iberian Lynx *Ex situ* Conservation Programme for their collaborative work and to Josie Dade for translating this manuscript.

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Produce una inmensa tristeza pensar que la naturaleza habla mientras el género humano no escucha.

Víctor Hugo
(1802-1885)


Photo: José María Pérez de Ayala

Environmental enrichment for captive felids

Enriquecimiento ambiental para felinos en cautividad

Ana Martos

Resumen

El ambiente que rodea a un animal influye directamente en su estado físico y mental. Este está compuesto por una serie de estímulos ante los cuales el individuo debe reaccionar. Cada especie está adaptada a un tipo de ambiente determinado y posee un repertorio conductual especialmente desarrollado para sobrevivir en él. En cautividad, las condiciones cambian, el entorno pierde complejidad y se vuelve predecible. Este hecho puede traducirse en una pérdida en la riqueza de comportamientos presentados por el individuo que, al no poder realizar determinadas conductas, puede desarrollar otras "no naturales" que podrían llegar a considerarse patológicas, como las denominadas estereotipias.

Un aumento de la complejidad del entorno y la utilización del enriquecimiento ambiental pueden llegar a reducir notablemente el porcentaje de tiempo que los animales dedican a realizar conductas no deseadas y aumentar el tiempo que dedican a otras que si lo son, como la exploración, la interacción social, el juego y la manipulación de objetos (Lozano-Ortega, 1999). El objetivo de este capítulo es el de recopilar información sobre la utilización de las técnicas de enriquecimiento ambiental destinadas a mejorar el bienestar de los felinos. Para ello se hablará de los diferentes tipos de enriquecimiento que se utilizan (enriquecimiento físico, ocupacional, alimentario, sensitivo y social), y de cómo afecta el enriquecimiento a la conducta de los animales. Por último, se estudiará la forma de aplicar todo lo debatido anteriormente, para lo cual se utilizará como referente el modelo de planificación de un programa de enriquecimiento ambiental del Disney Animal Kingdom Theme Park®, que propone una aplicación de estas técnicas siguiendo una serie de fases que se denominan secuencia SPIDER: selección de metas, planificación, implementación, recogida de datos, evaluación y reajuste. Al final del capítulo se debatirán algunas aplicaciones del enriquecimiento ambiental en los centros de cría para la conservación del lince ibérico.

Palabras clave

Enriquecimiento ambiental, planificación, Felidae, bienestar, cautividad, secuencia SPIDER

Abstract
The environment that surrounds an animal has a direct influence on its physical and mental state. It is formed by a series of stimuli that the individual must react to. Each species is adapted to a given type of environment and has a behavioral repertoire that has been especially developed to survive in it. In captivity, conditions change and the environment becomes less complex and predictable. This may lead to a decrease in the range of behaviors of the individual. The fact of not being able to perform a given behavior may lead captive individuals to perform other "unnatural" behaviors that may in some cases even be pathological in the case of stereotyped behavior.
Increasing the complexity of the environment and using environmental enrichment techniques can significantly reduce the time animals devote to performing undesired behaviors and increase the time devoted to normal behavior, such as exploration, social interaction, play and object manipulation (Lozano-Ortega, 1999). The purpose of this chapter is to compile information about the use of environmental enrichment techniques aimed at improving the welfare of felids. This implies reviewing the different types of enrichment used (physical, occupational, food, sensory and social enrichment) and how enrichment affects animal behavior. Finally, we discuss how the above-mentioned issues can be implemented, taking the model of an environmental enrichment programme used at the Disney Animal Kingdom Theme Park® as a reference. The Programme proposes to apply these techniques following a set of phases called the SPIDER model: Setting goals, Planning, Implementing, Documenting, Evaluating and Readjusting. At the end of the chapter, we discuss the use of environmental enrichment in breeding centers for the conservation of the Iberian lynx.

Keywords
Environmental enrichment, planning, Felidae, welfare, captivity, SPIDER model

Photo: Antonio Rivas

Environmental enrichment for captive felids

Ana Martos

Introduction

Environmental enrichment has been defined in many different ways at different times. Nevertheless, until the 90's, it simply consisted of the introduction of devices inside the enclosures for the animals to play with or to hide in.

Nowadays the definition has been amplified and comprehends any physical or social action, design or handling which "improves the quality of life of these animals, by recognition and recreation of the environmental stimuli necessary for optimal physical and psychological welfare".

We can find two different approaches concerning environmental enrichment: the naturalistic approach, based on the modification of the physical and social environment in order to simulate a natural environment (Quick, 1984), and the behavioral engineering approach, proposed by Markowitz, in which animals have some control over their environment by using devices to reward them or to promote certain behaviors.

In any case, the main objectives of an environmental enrichment programme are the following (Young, 2003):

1) Increasing behavioral repertoire of captive animals.
2) Reducing abnormal or pathological behavior of wild animals.
3) Increasing functional space inside enclosures by using three-dimensional space.
4) Increasing the animal's capacity of adaptation to changes.
5) Preparing the animals for a potential reintroduction.

Environmental enrichment for felids

This taxonomic group includes many species whose environmental enrichment is somewhat complicated due to the great extensions that typify their territories and hunting needs (Mellen et al., 1998). These territories are impossible to recreate in captivity, especially taking into account ethical problems and public reaction towards supplying live prey for zoological specimens. On the other hand, felids habituate relatively rapidly to novel conditions for which, according to Mellen (1998), "enrichment should be dynamic and constantly modified to effectively induce the behaviors in captives that are more characteristic of their wild counterparts".

However, there are many studies and publications about the different possibilities of enrichment. The proposal we analyze now has been based on the classification model formulated by Segonds Pichon (1994) and adapted by Lozano-Ortega for rescue and rehabilitation centers (1999).

Physical enrichment

This comprises space and furnishing, whether permanent or not. The objective is to achieve a complex environment, with an optimum level and frequency of stress-stimuli to provide challenges to which animals should respond by exercising natural behavior.

Space enrichment: area and volume

Distribution and space availability should be adapted to the specie's physical characteristics, size and number of individuals, but also to social organization and possible hierarchy. For example: when designing enclosures, straight angles should be avoided, as they are inexistent in nature and may cause animals to feel trapped, enhancing stress and undesired behavior (Kleiman, 1996). For small felids Mellen et al. (1998) pointed out the importance of complexity in enclosures rather than size. Complexity is defined as the number of visual barriers added in an enclosure which allows animals to completely hide from view (Mellen, 1998). According to this same author, in enclosures where seven or more visual barriers were present, pacing (abnormal behavior consisting of patrolling the enclosures, always following the same trail (Manteca, this book) was reduced or non-existent. In this same way, Carlstead et al. (1993) found out that time spent pacing was reduced by 50% through increasing complexity in leopard cats *(Felis bengalensis)* enclosures. Another factor to keep in mind when discussing space enrichment is volume. An adequate use of volume is capable of increasing the delimited functional space of an enclosure. For instance, creating a series of "aerial trails" (hanging trails, trails fixed to the enclosure walls or supported by posts) for the animals to roam on or, placing platforms at different heights for a better exploitation of vertical and horizontal space (Knapik, 1995) for animals to use as resting places or to provide shade. According to Mallapur (2001) leopards with platforms in their enclosures, or with the possibility of climbing up branches devote 79,2% of their time to resting on them, while leopards without this possibility show a greater amount of time spent in activity, and also run a higher risk of showing stereotypic behavior.

Furnishing

This is defined as any device with which an animal is capable of interacting. The most frequently used are trees, logs, branches, ropes, etc. These can be moved within the enclosure, added or removed to provide novelty, the creation of new trails, or stimulation of explorative behavior (Lozano-Ortega, 1999).

Furnishing is often used to recreate a captive environment as naturalistic as possible. To accomplish this purpose, a large variety of substrate can be used on the enclosure floor, in addition to placing trees and bushes proper to the animal's wild habitat. The introduction of furniture in an animal's enclosure diminishes stress, increases desirable and decreases abnormal behavior (Healy et al., 2000). Furniture can be exchanged between various enclosures, or enclosures can be switched either within the same species, the same family, or between predator and prey (AAZK, 1998). A constant introduction of new stimuli is convenient to elicit exploratory behavior and marking, in this way avoiding habituation to the environment which could result in an increase of stereotypic behavior (Mellen et al., 1997).

Occupational enrichment

This consists of providing an animal with various types of manipulative devices to choose from. Replacing or alternating them at intervals will increase their use and their efficacy as novelties for the animal, eliciting explorative behavior (AAZK, 1998). It is important to remove devices when animals loose interest in order to repeat the experience later on.

Manipulative devices can stimulate both solitary and social play (Kleiman, 1996) but it is convenient to provide sufficient quantity of items in order to avoid dominant individuals monopolizing them (Lozano-Ortega, 1999).

In the case of felids, the most widely used objects are imitations of prey, or at least devices which induce hunting-related behavior, even if these are not associated with food. Items such as Boomer balls or other plastic toys elicit stalking and pouncing behavior (Mellen et al., 1998). The effectiveness of using other materials, like cardboard and paper (Pitsko, 2003), pumpkins (Lewis, 1996), snake skin (Acuña, 1995), hosepipe-made objects (Pappas, 2002), etc. has also been noted. The use of mechanical mobile prey can also be included in this type of environment enrichment, and it is used, for example, for captive cheetahs to stimulate their locomotive activity and their mental health (Lindburg, 1998). Attempts should be made to provide a variety of materials, shapes and textures in order to select the items with the most satisfactory responses.

Food enrichment

As previously mentioned, felids are predatory animals, for whom there is no better food-related environmental enrichment than providing live prey for them to prey on (Figure 1). Nevertheless, in many cases, for example involving small enclosures and/or accessibility to the public, this type of feeding is not feasible. If food enrichment is desired it is recommended to use novel food, environmental enrichment devices, or to vary food presentation.

Novel food

This consists of a sporadic use of food that is not included in the animal's usual diet but which will not cause alimentary disorders if it is not offered daily. For example, introduction of animal carcasses to animals which are usually fed on a commercial diet may enhance physical well-being as, in many cats, it stimulates the stalk-rush-kill sequence which they might execute in wild with live prey (Mellen 1998; Gilchrist, 2005) and does not show any negative effect on health (Pearson et al., 2005). However, it is recommendable to complement this activity with other enrichment methods for a greater expansion of the behavioural repertoire. For fishing felids, for example, another method which can be used is to provide insects as well as live fish.

Food enrichment devices and food diversity presentation

Food enrichment devices are intended to prevent animals from obtaining food too easily; this is because in the wild, felids devote lots of their energy and time to acquiring prey (AAZK, 1998).

For instance, by simply hiding food portions within the whole enclosure, Shepherdson et al., (1993), registered an increased amount of exploratory behavior in leopard cats, from 5.5% to 14%, and the diversity of behaviours increased as well. The final objective is to provide "an environment in which an animal will obtain food as a consequence of its hunting behavior and food search" which is important for improving animal welfare and approximating it as much as possible to what would be a wild environment (Kleiman D., 1996).

Photo: Antonio Rivas

Figure 1. Iberian lynx chasing a rabbit.

Figura 1. Lince ibérico cazando un conejo.

Feeding schedule is also important. Shepherdson (1993) observed that an increment in the number of feedings per day, and at unpredictable times, augments exploratory behavior and diminishes stereotype behaviour frequency and durability.

Sensory enrichment

Olfactory enrichment

Sensory enrichment consists of using the more developed senses of each species in order to carry out environmental enrichment. In the felids case, the olfactory sense is the most heavily used in enrichment, to elicit exploration, flehmen, tracking, grooming, urine and cheek-rub markings ...

Some of the most successful odors used are almond and mint extracts (Powell, 1995), herb extracts (rosemary, chive, valerian), animal faeces (Baker, 1997) and essential oils (Pearson, 2002). All of them reported an increase in animal activity during the enrichment period. However it is necessary to survey animal reactions at all times, as some studies have reported an increase in pacing associated with odor introduction (Clark et al., 2004). In any case exposures should be short and spaced in time to avoid habituation (Schuett et al., 2001).

Auditory enrichment

It consists of stimulating an animal by using recordings of a diversity of sounds, usually predator, prey or same species vocalizations.

Social enrichment

Conspecific interactions

Although felids (except for lions and cheetahs) are generally solitary animals, housing them in pairs or in reduced groups could be beneficial in encouraging the appearance of competitive or cooperative behavior (AAZK, 1998). On the other hand, when being introduced into the wild or maintained in breeding centers, it is necessary for the animals to interact with other individuals of the same species (Figure 2).

For gregarious animals, it is important that many individuals of the same species be housed in a single enclosure, providing them sufficient space and hiding places, in case of necessity, in order to avoid antagonistic interactions (Lozano-Ortega, 1999). As for solitary felids, the possibility of introducing two individuals in a same enclosure depends as much on the species as on the individual concerned. This is because in the same species some individuals will tolerate cohabitation with others, and others not (Mellen et al., 1998).

Interactions with human beings

Influence of felid-keeper interactions has been previously discussed by many authors. According to Mellen (1998) it does not consist of animals becoming pets, but rather, consists of a certain grade of interaction which, even through gates and fences, has been found to have a beneficial effect on animal welfare. Likewise, there is a positive relation between the interaction levels with keepers and reproduction success. This type of interaction also has an effect on reducing time animals devote to pacing. Another kind of interaction between animals and keepers is employing instrumental conditioning or positive reinforcement for training animals, easing veterinary procedures and handling, besides increasing animal cognitive abilities and benefiting its welfare (Basset et al., 2006).

When the final destiny of a captive animal is to be reintroduced in the wild, one of the main problematic matters is to avoid the animal's loss of fear of human beings. Also to be avoided are neotony and extended infantile behavior which appear in artificially bred animals kept in captivity (Lozano-Ortega, 1999).

Planning an environmental enrichment programme

When an institution plans to carry out an enrichment programme, it is not enough to take some of the examples described in available information. For the Programme to be successful, it is necessary to elaborate an action plan including quantifiable goals and results. A successful environmental enrichment plan should anticipate animal necessities and provide them with the opportunity of choice, within their environment, as well as the possibility of confronting the various stimuli presented. It should be based on the animal's naturalistic behaviors, on their

Figure 2. Iberian Lynx breeding pair from the Iberian Lynx Conservation Breeding Programme.

Figura 2. Pareja reproductora de linces ibéricos del programa de Conservación *Ex situ*.

biological and cognitive necessities and finally, it should involve all responsible staff, from veterinary officers to nutritionists, scientists, keepers, management, etc.

Subsequent to these outlines, when planning this programme six points should be respected. These six points generate the SPIDER model created by the Disney Animal Kingdom® Theme Park staff, and are the following:

Setting goals

This consists of establishing what the programme aims at. The first thing to undertake is to carry out an investigatory task to achieve a full knowledge of the specie's naturalistic behavior, biology, history and habitat. In the same way, individual characteristics of the animals involved, and the handling conditions which could benefit or prejudice the establishment of the programme's intended measures should be taken into account. Once all these aspects have been considered, a decision should be made concerning the desirable behaviors which it is wished to encourage through enrichment and the undesirable behaviors it is hoped to avoid. Desirable behaviors should be prioritized according to their importance and the ease with which they can be induced. The final goal is to obtain a list of enrichment proposals, for which every proposal will be associated with a desirable behavior.

Planning

The following step is to achieve the approval, by means of all the staff concerned, of the list of proposals obtained in the above point. To achieve this approval, all the project should be subjected to a strict analysis of possible risks in which the enrichment programme could affect animal security and enclosures. All the materials and methods used should be analyzed from all points of view to assure their safety. Weighing risk and benefit for each proposal could be very useful at this stage.

Implementing

This aims to resolve questions such as who will be the one responsible for the enrichment system implementation, or the frequency with which the devices will be used. Executing a schedule, as in Figure 1, is recommended for this matter.

Documenting

Data collection consists of a daily survey on the way animals interact with enrichment. It can be accomplished with recordings, direct observations or other methods. The greater the specificity of the observations, the greater

SUN	MON	TUE	WED	THU	FRI	SAT
		1 11:30 Boomer Balls c1,c2,c3,c4	2	3 11:30 Removing Boomer Ball	4	5
6	7	8 11:30 Boomer Balls c5,c6,c7,c8	9	10 11:30 Removing Boomer Balls	11	12 18:30 Quails c1,c2,c3,c4
13	14	15	16	17	18	19 18:30 Quails c5,c6,c7,c8
20	21 Changes in feeding schedule (all week)	22	23	24	25	26
27	28	29	30	31		

Table 1. Example of a monthly schedule for environmental enrichment.

Tabla 1. Ejemplo de horario mensual para enriquecimiento ambiental.

value it will have in time. As an example of data collection, Disney's Animal Kingdom® Theme Park developed three 1-5 scales in order to achieve better validity and consistence when observing and quantifying results.

1) Direct evidence scale: the observer attends to the animal's contact with the enrichment device and is able to calculate the interaction intensity.
2) Indirect evidence scale: the observer may not be present when interaction takes place, for which the observer will use indirect evidence to determine if interactions with the enrichment devices occur or not, for example, finding signs which indicate use of devices.
3) Achieved goal scale: the observer indicates if the interaction with the enrichment devices has caused the desired behavior.

For all three scales it is necessary for the observers to establish a common consensus, in order for all of them to evaluate all reactions with the same numerical value to assure valid study.

Evaluating

An enrichment programme evaluation consists of an analysis of the data collected and allows us to answer the questions arising during the whole process.

Readjusting

In this last point corrective measures will be analyzed in order for the programme to be more effective. To achieve this, all questions arising in the data collection and evaluation stages will be taken in account, and the necessary changes will be reviewed in order to improve each one of the previous stages.

Conclusions and further recommendations

The characteristics of the Iberian lynx breeding center condition, to a large extent, the possibilities of using the various environmental enrichment methods. The Iberian Lynx Conservation Breeding Programme is able to recreate a naturalistic environment resembling the habitat of their conspecifics in the wild. For this purpose, elements proper to Mediterranean forests can be used, placing them in a determined manner to increase functional three-dimensional space in enclosures. Also, the introduction in enclosures of enrichment devices which allow placement modification is recommended, in order to obtain a more dynamic space. On the other hand, food enrichment in breeding centers is achieved with live prey supply (farm rabbit, quail, and when possible, wild rabbit). It is suggested to sporadically introduce other types of prey which increases time devoted to hunting; an example could be periodically providing hare. The possibility of using odor stimuli, manipulative items or effecting small changes in handling routine could also be studied, always respecting animal welfare and dealing with an environment resembling the wild as much as possible, without disregarding the final aim of the Programme, Iberian lynx reintroduction into its natural habitat.

Acknowledgements

The author wants to thank Astrid Vargas and all staff from El Acebuche for giving her the possibility of carrying out this work, and to acknowledge the great task they are accomplishing. Also, many thanks to Félix Zaragoza from the Universidad Alfonso X, for his help for obtaining part of the bibliography and for awaking my interest in environmental enrichment. Special thanks go to Jessica Reeves for translating this manuscript.

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Even the smallest of cats is a work of art.

Leonardo da Vinci
(1452-1519)


Photo: Sergey Naidenko

Sibling aggression in Eurasian lynx *(Lynx lynx)*

Agresión entre hermanos de camada en el lince euroasiático *(Lynx lynx)*

Sergey Naidenko and Anastasia L. Antonevich

Resumen

La agresión entre hermanos ha sido estudiada principalmente en aves, pero sólo ha sido descrita en algunas especies de mamíferos. En el lince euroasiático, las peleas entre hermanos de camada son relativamente comunes y se producen a una edad determinada, principalmente en la 7ª semana de desarrollo postnatal (63%). Este fenómeno no se produce siempre, y se ha observado en aproximadamente la mitad de las camadas que han sido objeto de este estudio. Las peleas suelen iniciarse de forma espontánea, con una duración que varía desde pocos minutos hasta varias horas, y –en el caso de lince euroasiático– no se repiten durante la ontogénesis de una misma camada. Las peleas coinciden con un período crítico en el desarrollo del lince, en el que los cachorros pasan de alimentarse de leche materna a comer comida sólida. Durante este período, también se produce un cambio en la tasa de crecimiento de los cachorros; sus relaciones sociales con sus compañeros de camada se intensifican, y se establece una jerarquía entre ellos. Los ganadores de las peleas entre hermanos aumentan su tasa de crecimiento durante esta fase crítica, lo cual podría proporcionarles mayores posibilidades de supervivencia en el medio silvestre. Además, los cachorros ganadores también inician más contactos sociales en la camada. Aunque no se ha observado una relación clara entre la probabilidad de peleas y el estado hormonal de los cachorros, la estimulación de las glándulas suprarrenales mediante la inyección ACTH parece aumentar la probabilidad de las peleas. Son necesarias más investigaciones para comprender el mecanismo que desencadena el comportamiento agresivo en el lince.

Palabras clave

Agresión entre hermanos de camada, lince euroasiático, masa corporal, relaciones sociales, glándulas suprarrenales

Abstract
Sibling aggression has been studied mainly in birds, but it has also been described for a few mammalian species. In Eurasian lynx these fights occur at a specific age, mainly at the 7th week of postnatal development (63% of fights). This is not an obligate phenomenon and in a half of all litters these fights were not observed at all. Usually, fights start spontaneously, last from few minutes to a few hours and are not repeated during ontogeny in the same litter. These fights coincide with a critical period in lynx ontogeny: cubs switch to solid food, change their growth rate, intensify social relations and establish a hierarchy among littermates. Fight winners increase their growth rate during this critical period, which may provide them a better chance to survive in the wild. They also initiate more social contacts in the litter. Although there were no clear relations between probability of fights and cub's hormonal status, the stimulation of adrenal glands with ACTH injection seemed to increase the occurrence of fights.

Keywords
Sibling aggression, Eurasian lynx, body mass, social relations, adrenal glands

Photo: Sergey Naidenko

Sibling aggression in Eurasian lynx *(Lynx lynx)*

Sergey Naidenko and Anastasia L. Antonevich

Introduction

Sibling aggression has been described in a variety of avian species (e.g. American white pelican *Pelecanus erythrorhynchus*, eurasian kestrel *Falco tinnunculus*, laughing kookaburra *Dacelo novaeguineae*) (Mock and Parker, 1997; Nathan et al., 2001; Massemin et al., 2003). This phenomenon (resulting sometime in the death of nestlings) should bring some advantages for the winners. The degree of such advantages may change depending on food supply, and is thought to be a way of brood size modification according to available resources (Mock and Parker, 1997; Drummond, 2001).

In mammals, competitive behaviour among siblings has been described in few species. Serious aggression has been described among litter-mates in some canids (Bekoff, 1974; Wandrey, 1975; Ovsyannikov, 1993). In two mammalian species sibling aggression sometimes results in the death of one littermate ("siblicide"). These are the domestic pig *(Sus scrofa)* (Fraser, 1990) and the spotted hyaena *(Crocuta crocuta)* (Frank et al., 1991). In both cases, siblings compete for the mother's milk as the only food source for the young animals. The losers are likely to starve to death (Fraser, 1990; Smale et al., 1995; 1999; Golla et al., 1999). The probability of this phenomenon in hyenas depends on the availability of food for adult animals (Wachter et al., 2002).

Eurasian lynx *(Lynx lynx)* became the third mammalian species where the fatal sibling aggression was described (Sokolov et al., 1994). It was noted in captivity, in five litters out of 10 with two to three cubs, and in one case it resulted in the death of one cub. In the wild this phenomenon was never observed, although high postnatal mortality was described in some studies (Jedrzejewski et al., 1996) and some indirect findings may indicate sibling aggression (U. Breitenmoser, pers. comm.). The monitoring of an Iberian lynx *(L. pardinus)* population in nature provides some indirect data that sibling aggression may occur in the wild as well (Palomares et al., 2005). Sibling aggression has also been described for Iberian lynx in captivity (Vargas et al., 2005; Antonevich et al., this volume).

Eurasian lynxes have reproduced successfully in many zoos. The average litter size is around two cubs (for review see Naidenko and Erofeeva, 2004). Usually, about 75% of the litters have more than one cub. Physical development of cubs has been described by different authors (e.g., Stehlik, 1980; Naidenko, 2006), but the ontogeny of social behaviour is poorly studied. The long-term research of a captive lynx population in Tchernogolovka, Russia, allowed us to collect data on lynx behavioural development during their postnatal ontogeny. The aim of this study was to sum up the data on lynx sibling aggression and to look at the effect of this phenomenon in the development of cubs.

Material and methods

This study was conducted at the experimental station Tchernogolovka of the A. N. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences (56°00' N, 38°22' E) from 1989 to 2006. Average annual temperature varied from +3.5 °C to +4.3 °C; average temperature in July was +19 °C and in January -11 °C. The station keeps the Northern lynx *(L. l. lynx)* subspecies (Versteege, this book) Adult lynx lived separately in three types of outdoor enclosures: treeless small enclosures of 8 m^2 (n=26) and 74 m^2 (n=6); and a large enclosure of 7500 m^2, which is a fenced part of natural mixed forest *(Pinus silvestris, Betula pendula, Picea abies)* with the dense brush *(Sorbus oucuparia, Rubus idaeus)*. More details of lynx husbandry conditions have been described earlier (Sokolov et al., 1994; Naidenko, 2001).

Females with cubs until the age of three months (n=28) lived mainly in medium sized enclosures (74 m^2). We were not able to conduct statistical analyses effect of the husbandry conditions on the occurrence of sibling aggression because too few litters were kept in small cages (n=3) and in the large enclosure (n=2), but both "aggressive" and "non-aggressive" litters were found in all types of enclosures.

Lynx reproduced every year at the station. Males and females were placed together during mating season in March (Naidenko and Erofeeva, 2004). All litters were born between May 12 and June 19. Litter size varied from 1 to 4. There were 31 litters with two to four kittens at the age of 45 days (peak of fights, see below) (Table 1). These litters belonged to 12 different females and were sired by six different males. Cubs born at the station stayed with their mothers until they were 10 months old, when they would disperse in nature (Schmidt, 1998; Zimmermann et al., 2005).

Females with cubs were fed daily a beef or chicken diet. In addition, they also received live and dead rats and rabbits, eggs, fish, curd and vitamins. The size of the daily amount of food varied from year to year, but was always more than 1 kg per day (Sokolov et al., 1994; Naidenko, 2001). Usually all cubs were weighed once per week (starting at 3-5 days of age), although this frequency was not constant for different litters (Naidenko, 2006). The weight of cubs was measured to the nearest 5 g. To estimate cub's daily growth rate (in g) we used the results of two successive weightings divided by the number of days between weighing them. To estimate increase in weight we calculated the ratio of the daily growth rate (in g) to the number of the last weighing and expressed it in percent (Naidenko, 2006). We estimated average cub's body mass at the time of fighting (for litters where fights were not observed, body mass was estimated for cubs at 45 days of age), representing as well the standard error and standard deviation.

Regular observations were conducted on cub's ontogeny using the data continuous recording method (Martin and Bateson, 1993). The frequency of observation was usually one six-hour period per every three days (Naidenko, 1997) and one to two 24-hour observations per month for each litter. The observations were conducted from a special shelter. Lynx fights were observed only three times during these regular observations; fights were mainly noted by accidents. In 2003-2004 we conducted detailed observations of kitten's behavior in four litters from three females (three triplets and one twin) comprising four males and seven females. For each social interaction we noted the initiator and the object. For further analysis cubs were categorized according to the results of fights: winners, losers and neutral cubs (in triplets).

Table 1. Litter size and other parameters related to sibling fighting behaviour in Eurasian lynx litters.

Tabla 1. Tamaño de camada y otros parámetros realcionados con el comportamiento agonístico en el lince euroasiático.

Litter size	2	3	4	Total
Number of litters	12	17	2	31
Number of litters where the fights or their consequences were observed	5	11	0	16
Sex ratio (m/f)	11/13	26/24*	3/5	40/42

* - sex of one kitten was not detected before it disappeared.

* - el sexo de uno de los cachorros no fue detectado antes de desaparecer.

We divided the data into two-week intervals to establish the influence of sibling aggression on cub's behavior, taking the age at which fights occurred in each litter as the reference point. The frequency of each form of behavior was recalculated per one-hour unit of activity using the formula:

$$Nh=N*60/A$$

where Nh=number of behavioral acts per one hour of activity, N=number of behavioral acts per six hours of observation, A=activity time (in min) for six hours. To define the differences and individual partner preferences for social interactions we compared observed frequencies with frequencies expected by chance. Expected frequencies were calculated for each litter (total number of social contacts per litter divided by the number of cubs), and the degree of difference between the observed and expected frequencies estimated using the Chi-square test (Lehner, 1996). An analysis of variance was used to test factors possibly affecting kitten's play behavior. All data analyses were conducted with the software Statsoft Statistica 6.0.

Blood sampling was conducted for all kittens at the 7th week of their life at the same time (8.00-11.00 h) to exclude the effect of the diurnal cycles of hormone excretion. Enzyme Immuno Assay (EIA) was conducted to estimate the concentration of testosterone, dehydroepiandrosterone, cortisol (commercial kits of "Immunoteck", Russia) and androstenedione ("DRG", USA) (Naidenko and Erofeeva, 2005; Naidenko, 2005). The level of the hormones of the adrenal glands was also measured one hour after ACTH-injection ("Synacten Depot", Novartis, Switzerland, 2,5 IU/kg of body mass) (Naidenko et al., 2007).

Results

Description of fights

We observed fights in 16 out of 31 litters (52%) with two to four kittens. Fights were observed in five out of 12 (42%) litters with two kittens and in 11 out of 17 (65%, difference test (%), P=0.24) litters with three kittens. In two litters with four kittens we observed no fights. They occurred in different litters at the age of 36-64 days (6th -10th postnatal weeks), with a highest frequency during their 7th week of life (n=10, 63%). Fights started with the spontaneous attack of one of the sibs on another one. No aggressive interactions between sibs were ever observed before the attack. Both males and females were aggressors and they attacked kittens of the same or another sex. When the aggressor was identified, females were aggressors in eleven cases (total number of females in these litters was 24), males –in four cases (n=18; difference test (%) P=0.11). The aggressor tried to bite the victim on the back of the head or throat (Figure 1). The attacked sib rolled up to the back and tried to defend itself. The female tried to stop fights using her forelegs and mouth. When the mother separated her cubs, the aggressor tried to continue the attack. The motivation level was sometimes so high that the aggressor lost its fear of humans. The attempts to continue the fight lasted for some hours. Lynx mothers sometimes stayed near the injured/attacked kitten and tried to prevent the aggressor to come nearby. Probability of fights was approximately the same for primeparous (50%; n=10) and multiparous (52.3%; n=21) females as well as the probability of the lethal result (10% and 14%, respectively). The attacked kitten continued to show defensive behaviour for a few more hours. In four cases, fights resulted in the death of kittens. Altogether, fights resulted in the death of less than 10% (4 over 43) of cubs in aggressive litters.

Photo: Sergey Naidenko

Figure 1. Attacked cub presenting a severe throat bite.

Figura 1. Cachorro atacado que presenta un severo mordisco en la garganta.

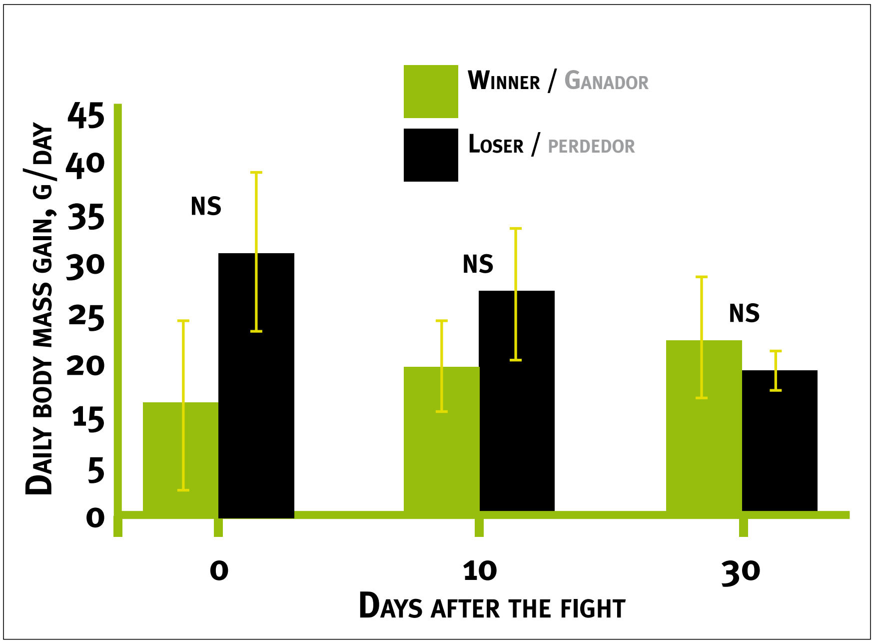

Figure 2. Increase in daily body mass in Eurasian lynx cubs after fights.

Figura 2. Aumento de masa corporal en cachorros de lince euroasiático tras las peleas.

Fight effect on kitten's physical development

Body mass of kittens was analysed very precisely for seven litters, where kittens survived after the fights and winners and losers were clearly detected. We did not find significant differences in body mass of kittens (winners and losers) and their growth rate after the fights (Figure 2). We tried to estimate the changes in growth rate of kittens at 10 and 30 days after the fight, in comparison to their growth rate at the day of fight. The results are significantly different 30 days after the fights, where the changes in the growth rate of winners are much higher than that of losers (Figure 3). These parameters varied a lot for different litters (-28 to 56 g/day for winners and -52 to 39 g/day for losers), but for all litters the winner got some advantage in changes of growth rate in comparison to the victim.

Kittens killing a sib should expect even more advantages after the death of a competitor. Their daily growth rate was significantly higher than in all other aggressors in the first ten days after the fight (47±8, n=3, and 20±16, n=7; U=1; Z=2.17; P< 0.05). So, the daily body mass gain of killers was almost 2.5 times greater than the weight gain of other aggressors. However, these differences disappeared in the following three weeks.

Fight effect on kitten's social behaviour

Fighting among cubs was observed during the developmental period for social play. The first elements of playing were observed at the age of one month, and by the end of the second month the frequency reached its maximum for the whole developmental period. However, frequency of play decreased significantly for some time after fights. For the first days after the fights the frequency of social play was significantly lower in comparison to other observations (ANOVA-test F=4.18; df=1; P<0.05).

The frequency of play also varied among littermates. Before fights, observed and expected play frequencies differed significantly (it was higher and lower, respectively, than the expected value) in two of the 11 cubs (18%) from four litters. In contrast, during the first two weeks after the fights, differences between expected and observed frequencies were registered for six cubs (55%; difference test (%) P<0.05). The most marked changes were seen on winners (in three of four cubs), which initiated play interactions in two cases significantly more often and in one case less often than expected. After another two weeks, differences between observed and expected frequencies of play were still seen in five cubs (45%; difference test (%) P=0.08). Thus, the occurrence of fights resulted in an increase in the asymmetry of play behavior. Play frequency for losers in the triplet litters never exceeded expected levels. In two cases there was no significant difference, and in one case the frequency was significantly lower. The frequency of loser's play was significantly higher only once: in twin litters during the third to fourth week after the fight.

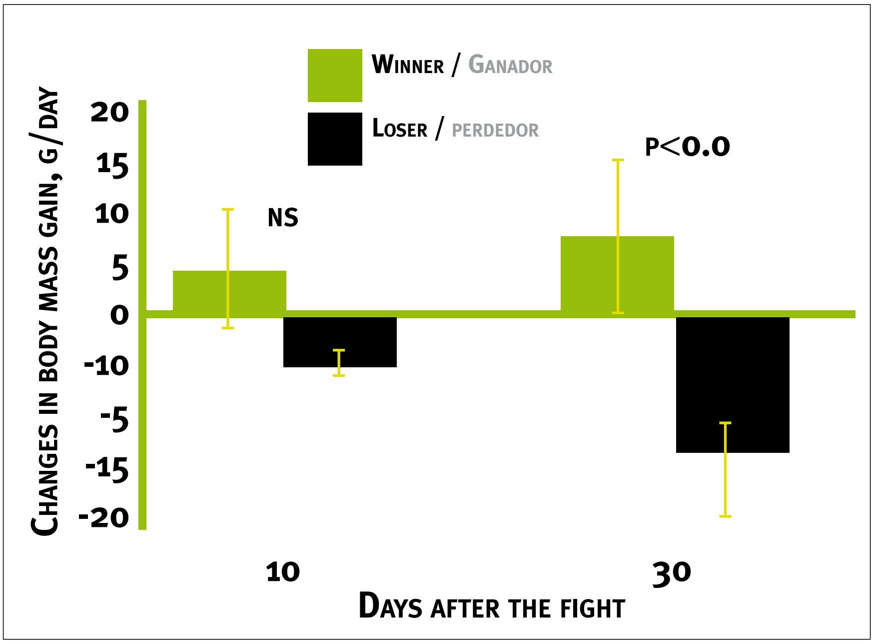

Figure 3. Changes in daily body mass gain in Eurasian lynx cubs after 10 and 30 days, respectively, from the fights.

Figura 3. Cambios en el aumento diario de masa corporal en cachorros de lince euraoasiático tras 10 y 30 días después de las peleas.

Hormonal status of the kittens

Hormonal status of winner and loser cubs during the fights, as well as that of neutral cubs, was compared. We did not find any significant difference in hormone concentration in the blood of winners and losers. The level of testosterone was similar for kittens that initiated fights and for the victims (Wilcoxon pair-matched test: T=5; Z=0.67; n=6; ns). Average levels of cortisol were slightly higher (158±33 ng/ml) in aggressive cubs compared to neutral kittens (147±41 ng/ml; ns) and victims (113±10 ng/ml; T=3; Z=1.21; n=5; ns). Also, cortisol levels did not differ in cubs from litters where no fights occurred. There were no significant differences in androstenedione during this period (T=7; Z=0.73; n=6; ns) for aggressors and losers.

A single injection of ACTH increased sharply the level of cortisol in the cub's blood plasma (Z=2.39; n=10, P<0.01). Sixty minutes after injection, the cortisol level increased 1.4-8.1 fold for different animals and more than three times on average (98±13 and 313±54 ng/ml, respectively). The level of androstenedione increased significantly as well (0.049±0.010 and 0.081±0.013 ng/ml; Z=2.60; n=10, P<0.01). The level of dehydroepiandrosterone and testosterone did not increase significantly. Two kittens attacked the sibs during the first hour after the ACTH injection. The probability of these fights was 18%, which was much higher than potentially expected values (0.02%). It seems that the adrenal glands play a crucial role in kitten's sibling aggression.

Discussion

Serious sibling aggression in lynx has been observed during the period of cub ontogenesis. Only 36-64 day-old cubs fought with each other. Usually, interactions of litter-mates in July (about 35-65 days of age) are mainly (more than 90%) playful contacts (Naidenko, 2001). Few aggressive interactions, which tend to happen around food, are much more ritualized than specific sibling fights (Sokolov et al., 1994). In contrast to spotted hyaenas, lynx fights usually happen only once. They may last for a few hours but when they stop the aggression is usually over.

Sibling aggression in lynx does not depend on the sex of either the aggressor or the attacked cubs. The behaviour of adult females was consistent in case of sibling fights: they always tried to stop the aggressive interactions. Although some cubs may get some advantage if the sibling dies, the mother will get a clear disadvantage with the decrease of it's reproductive success.

These fights rarely result in the death of one of the cubs; about 10% of them die. It does not look like the death of the competitor is a main result of sibling aggression in lynx. This phenomenon was never observed in the wild, but in captivity it was noted under different husbandry conditions (our station, St Petersburg and

Wuppertal zoos, Oslo University and El Acebuche Iberian lynx Breeding Centre (Vargas et al., 2005; Vargas et al., this book, Antonevich et al., this book). So, the effect of captivity on lynx sibling aggression could not be ruled out but needs more detailed research.

It has been suggested that fights result in the formation of a hierarchical structure in lynx litters (Sokolov et al., 1994). It may provide some advantages to the winner of the fight, especially if food is limited. The period of sibling aggression coincided with the development of social play (Naidenko, 1997). However, fights were followed by a decrease in frequency of play behavior, after which it increased again. In addition, we found an increase in the asymmetry of play interactions after the fights. During the first hours after a fight the injured kittens (losers) often showed defensive behavior towards the aggressors, the neutral sibs and their mother. This reaction disappeared a few hours later but was followed by asymmetry in the kitten's further play interactions (i.e., winners usually initiate playful contacts more often than we expected). For the first month after fights, a relatively high level of asymmetry in play interactions was seen. Such way, asymmetry in play behavior during this period allowed us to consider the possibility that hierarchical relationships were established in lynx litters during this period, even though the number of aggressive encounters was low.

The second month of cub's development in Eurasian lynx is very important. During this period, they start to take solid food at approximately 42-45 days of age (sometimes slightly earlier or later). The period after fights (after 60 days of age) –coinciding with when cubs start to take solid food– was characterised by an increase in the cub's growth rate (Naidenko, 2006). At 60 days, meat seems to become more important than milk (Naidenko, 1997). In the wild, we don't have any obvious evidence that females bring meat to the den.

In captivity, food was provided ad libitum for females with cubs (Sokolov et al., 1994; Naidenko, 2001). That might decrease the intra-litter competition and the differences in kitten's body mass dynamic. Regardless of this fact, fights seemed to always bring advantages to the winners. At least, changes in body mass gains were positive for the winners and negative for the losers. "Killers" had even a higher growth rate than non-killer winners. Probably, in case of food shortage, dominant cubs will get more chances to survive in nature.

The mechanisms of sibling aggression in lynx are not yet clear. For this species, the phenomenon does not correspond to high level of blood-circulating androgens, as it was assumed before (Naidenko and Erofeeva, 2005). Measurements of cortisol did not allow us to see clear differences between aggressors and other kittens. However, stimulation of cub's adrenal glands by an ACTH-injection increased both probability of fights and level of adrenal hormones (cortisol and androstenedione). Results indicate that sibling aggression in lynx might be related to the function of adrenal gland. Further research is needed to understand the trigger mechanism of aggressive behaviour in lynx.

Acknowledgements

This study was supported by RFBR 03-04-48763 and 07-04-00899, WTZ/RUS 02/035 and DAAD.

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Cats are intended to teach us that not everything in nature has a function.

Joseph Wood Krutch
(1893-1970)

Photo: Anastasia Antonevich

A comparative note on early sibling aggression in two related species: the Iberian and the Eurasian lynx

Nota comparativa sobre la agresión precoz entre hermanos de camada en dos especies afines: el lince ibérico y el lince euroasiático

Anastasia L. Antonevich, Sergey Naidenko, Juana Bergara, Eva Vázquez, Anastasio Vázquez, Javier López, Antonio Pardo, Antonio Rivas, Fernando Martínez and Astrid Vargas

Resumen

Las peleas precoces entre hermanos de camada en el lince euroasiático y el lince ibérico se distinguen de otros tipos de comportamiento agonístico por la ausencia de elementos rituales (amenazas) y por su alto nivel de motivación. En 2005, se observó una agresión súbita que acabó en fratricidio en la primera camada de lince ibérico nacida en cautividad. Este fenómeno se ha convertido en un desafío para este Programa y actualmente constituye uno de los mayores riesgos de mortalidad de cachorros nacidos en cautividad. Las agresiones se inician de forma espontánea, sin ninguna indicación de agresión anterior, con un ataque repentino y rápido por parte de uno de los cachorros de la camada. Las agresiones observadas nunca han tenido lugar mientras los cachorros se alimentaban y no parecen haber sido causadas por ningún tipo de competencia por el alimento. En ocasiones, las peleas tuvieron lugar tras la intensificación de otras interacciones sociales, pero en otras muchas se produjeron sin interacción previa (p.ej., cuando uno de los cachorros estaba durmiendo o simplemente sentado mirando hacia otro lado). El tamaño de la camada no influye sobre la probabilidad de peleas ni sobre la mortalidad de los cachorros en ninguna de las dos especies. La edad a la cual se produjeron las agresiones oscila entre 36 y 64 días en camadas de lince euroasiático. En el lince ibérico, dichas agresiones se observaron entre los 36 y 63 días de vida. En general las peleas entre hermanos tuvieron lugar entre la sexta y la octava semana de vida en cachorros de lince euroasiático (90% de los casos), aunque se dieron con mayor frecuencia durante la séptima semana de vida. En el lince ibérico, el 77% de las peleas tuvieron lugar entre la sexta y la octava semana de vida, siendo más frecuentes durante la sexta semana.

La duración del período agresivo posterior a una pelea varía entre camadas de lince ibérico (media=14; mín =1; máx=99 días). El periodo agresivo en el lince ibérico duró alrededor de 63 días (mín=45; máx=144) El número de ataques fue mayor en las peleas entre cachorros de lince euroasiático que en las de lince ibérico, pero el período agresivo posterior a las peleas fue mucho más corto en el lince euroasiático que en el ibérico. Ambas diferencias podrían deberse o bien al uso de distintas técnicas de manejo tras las peleas observadas en la especie amenazada, o bien a características específicas de cada especie. Aunque el sexo del cachorro no influyó sobre su papel en las peleas (agresor o víctima), el peso corporal sí resultó ser un factor importante. Las hembras fueron agresoras con mayor frecuencia que los machos en cachorros de lince euroasiático, mientras que en el lince ibérico se produjo el fenómeno contrario. La proporción de sexos de los agresores fue la misma que la proporción de sexos total en las camadas de linces. En general, antes de la pelea, el peso del agresor era mayor que el del cachorro atacado. Aunque existen algunas diferencias entre las peleas de lince euroasiático e ibérico, este fenómeno es similar en ambas especies y se diferencia claramente de la agresión entre hermanos de camada observada en otros grupos taxonómicos.

Palabras clave
Lince ibérico, lince euroasiático, fratricidio, comportamiento agresivo

Abstract
Early sibling fights in Eurasian and Iberian lynxes differ from other types of behavior in a lack of ritualized elements (threats) and a high motivation level. In 2005 sudden aggression, which ended up in siblicide, took place in the first Iberian lynx litter born in captivity. Fights became a problem, turning into one of the highest risks of mortality for captive born cubs. Fights started spontaneously, without any indication of previous aggression, with a very sudden and fast attack of one of the cubs in the litter. This aggression did not take place while the cubs were nursing or eating, and it did not appear to be caused by any kind of competition. Fights were not the result of an escalation of other social interactions, most times they occurred without any previous interaction (e.g., while one of the cubs was sleeping or just sitting, looking away). Litter size didn't influence fight probability or cub mortality in any of the two species. Age at which fights occurred varied from 36-64 day in Eurasian lynx litters. In naturally raised Iberian lynxes, the appearance of sibling fights was observed during 36-63 days of life. In general, fights occurred between the 6^{th} and 8^{th} week of the cub's life in 90% of the Eurasian cases (18/20), but mostly during the seventh week. In Iberian lynx litters, 77% (10/13) of fights also occurred between the 6^{th}-$8t^{h}$ postnatal weeks, being more frequent during the 6^{th} week.Duration of after-fight aggression varied across Iberian lynx litters (median=14; min=1; max=95 days). The aggressive period in Iberian lynx lasted around 63 days (min=45; max=144). The number of attacks was higher in Eurasian than in Iberian lynx fights, but the after-fight aggressive period in Eurasian lynx was much shorter than in the Iberian lynx. Both differences could be caused by either the after-fight husbandry procedures used in the endangered species or by species-specific differences. Although fights occurred between cubs of the same and different genders, body size made a difference. Female cubs were aggressors more often than males in Eurasian lynx and the opposite was true for Iberian lynx litters. Sex ratio of aggressors did not differ from overall sex ratio in lynx litters. In general, the aggressor was larger than the attacked cub. Although several characteristics differ between Eurasian and Iberian lynx fights, this phenomenon is similar in both species, yet it differs from sibling aggression in other taxa.

Keywords
Iberian lynx, Eurasian lynx, siblicide, aggressive behavior

A comparative note on early sibling aggression in two related species: the Iberian and the Eurasian lynx

Anastasia L. Antonevich, Sergey Naidenko, Juana Bergara, Eva Vázquez, Anastasio Vázquez, Javier López, Antonio Pardo, Antonio Rivas, Fernando Martínez and Astrid Vargas

Introduction

Early sibling aggression in Lynx *(Lynx lynx)* was described at Tchernogolovka biological station (Sokolov et al., 1994; Naidenko and Antonevich, this book). These fights differed from the other types of behavior in that cubs were focused on aggressive interactions that lacked ritualized elements such as threats. This kind of sibling interaction is known mostly from bird species, and it is rarely seeing in mammals (Drummond, 2006). Overwhelming advantages are expected for the winner in such a strongly exposed competition and studies about sibling fights in Eurasian lynx revealed some of them (Naidenko and Antonevich, this book). In 2005 sudden aggression appeared in the first Iberian lynx *(Lynx pardinus)* litter born in captivity (Vargas et al., 2005). This fight ended up in siblicide, with the death of the largest female in the litter. Ever since, fights have become a husbandry challenge, revealing a potentially high risk of mortality for captive-born Iberian lynx cubs. In 2007, a study of early sibling aggression on Iberian lynx was started with the purpose of developing husbandry strategies for countering cub fights while applying knowledge from siblicide in Eurasian lynx to its endangered sister taxa. The study aimed at comparing the main features of early sibling aggression in Eurasian and Iberian lynx.

Materials and methods

The study on Eurasian lynx was conducted at the "Tchernogolovka" biological station, Russia (see details in Naidenko and Antonevich, this book). The Iberian lynx study was held at El Acebuche Breeding Center, in Doñana, Huelva. In Iberian lynx litters and paired hand-raised cubs, 24-hour video-monitoring provided us with detailed information about cub development, including fighting behavior (Vargas et al., this book).

We used different approaches to manage Eurasian and Iberian lynx litters during fights. In the Eurasian lynx, managers usually did not intervene during fights nor did they separate cubs after fighting. After the first few fights, managers soon realized that those aggressive encounters were more of a rule rather than just pure accident. Since then, human intervention would only take place if Eurasian lynx cubs were severely injured during the fights.

As for Iberian lynx fights, given that the first fight that ever took place in the Breeding Programme resulted in the death of a cub, aggressive cubs were often separated from the others, in order to prevent the risk of severe injuries and/or death of the attacked cub. After-fight husbandry involved maintaining both cubs separated from each other and taking turns with their mother. Cubs were allowed to see and contact each other and their mom through the mesh, which impeded the potential for fighting, but kept the litter united. Switching cubs occurred between two and four times per day. Cubs were put together regularly to check if they had overcome the aggressive period. Once cubs had settled their differences, the family unit was brought back together.

Fight cases in Eurasian lynx litters were recorded from 1989 to 2007. In 2003-2007 a thorough study aimed at studying fights led to the observation of 15 Eurasian lynx litters. Iberian lynx litters included in this study were monitored between 2005 and 2008. Altogether, eight naturally-raised litters of 2-3 cubs and six hand-raised cubs paired in different ways were monitored. Duration of the aggressive period was estimated by calculating the time elapsed between the first fight and the first reunion where cubs did not fight. In hand-raised cubs, there was a pair of cubs that were not to put back together. In this case we used the last known attempt of reunion even unsuccessful as the last known day of aggression.

Results and Discussion

Context

In the Eurasian lynx, fights occurred in 53% (19/36) of litters of 2-4 cubs. The frequency of fights was higher in the years of precise cubs observations (67%, n=15). Litters with triplets had fights slightly more often than litters with twins (65% vs 39% of litters, respectively; P=0.08). However, litter size ratio is biased towards triplets (8/3-triplets/twins) in the years of precise observations. Such bias could cause underestimation of the probability of fights in twins. Mortality was not related to litter size (Naidenko and Antonevich, this book). Overall mortality rate was 5% of cubs. When taking into account all litters raised at the Tchernegolovka facility (n=36), siblicide occurred in 14% of all litters, which included 5% of all cubs born at the facility. When only taking into account cub mortality in litters where fights occurred (n= 19 litters), siblicide occurred in 20% of the fighting litters, i.e., in 9% of the cubs that fought.

Fights occurred in six of the seven naturally-raised Iberian lynx litters (86%). Among the six hand-raised male cubs, four different combinations of paired-cubs took place during the process of looking for compatible pairs. Also, a hand-raised female born in 2007 was paired with a 1-week younger bobcat female cub. Strong spontaneous aggression occurred in all paired hand-raised cubs, in some cases right after nursing from the bottle. Litter size ratio of aggressive naturally-raised litters was 5:1 (twins: triplets), and only one litter with triplets never had a fight. Cubs died from fighting-induced trauma in two litters. In the first case, one cub severed the others´ trachea and perforated its skull (Vargas et al., 2005) and in the other case, it was the mother who inflicted the lethal wounds on her cub while trying to separate her fighting offspring. Husbandry methods in all Iberian lynx litters were geared to prevent any potential deaths, so it is not possible to evaluate the actual mortality rate from these fights.

Age of fights

The age of fights varied from 36-64 day in Eurasian lynx litters. In Iberian lynx, the first fights were observed between 38-63 days of life. In hand-raised Iberian lynx cubs, fights occurred between 42-56 days of life, except for one cub that had been paired with a 1-week younger bobcat cub (for lack of another Iberian lynx cub) and fought at 74 days of age. Fights occurred between the 6th and 8th week of the cub's life in 90% of the Eurasian cases (18/20), but mostly during the seventh week (55% of cases; i.e., 11/20; chi-square=15.31; p<0.01). In Iberian lynx litters, 77% (10/13) of fights also occurred between the 6th-8th postnatal weeks.

Fight features

Every fight in both Eurasian and Iberian lynx litters consisted of several attacks. We considered one full attack to take place from the onset of fighting to the moment when the mother separated the cubs; if the cubs initiated another bout of fighting that would be considered another attack. We calculated the number of attacks for each fight in Eurasian lynx litters (median=5; min=2; max=18; n=6) and in Iberian lynx litters (median=3.5; min=2; max=7; n=6) in all the cases where it was possible to see. In Eurasian lynx litters, cubs remained aggressive

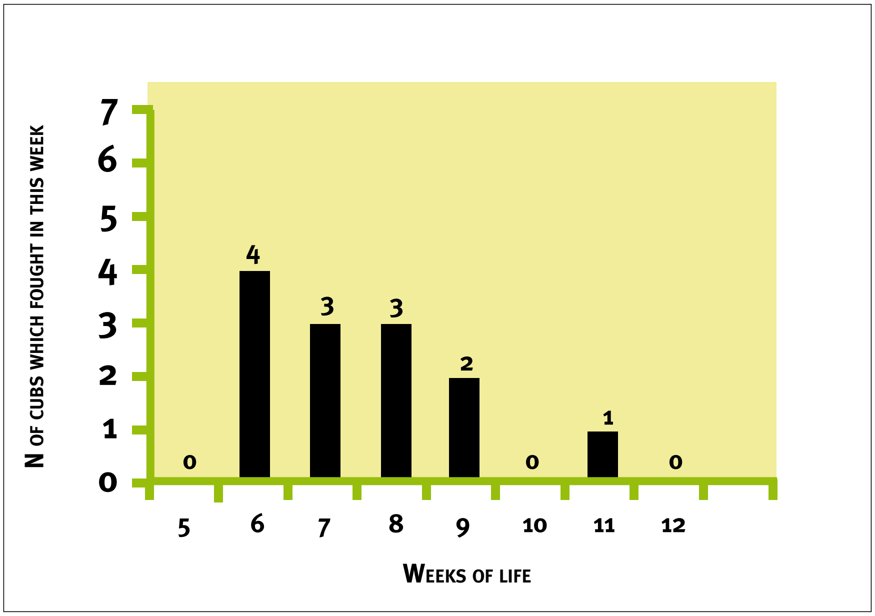

Figure 1.
Age of aggressor in Iberian lynx fight (mother-raised and hand-raised litters). In 2009, at the time of updating this data, seven new fights had taken place: three of them during the 6th week of life, one fight during the 7th week, two more during the 8th postnatal week, and one in the 9th week.

Figura 1.
Edad del cachorro agresor en camadas de lince ibérico. (Criados por sus madres y criados al biberón).
En 2009, durante la revisión de este capítulo, han tenido lugar siete peleas nuevas: tres de ellas durante la sexta semana de vida, una durante la séptima semana, dos durante la octava semana postnatal y una más durante la novena.

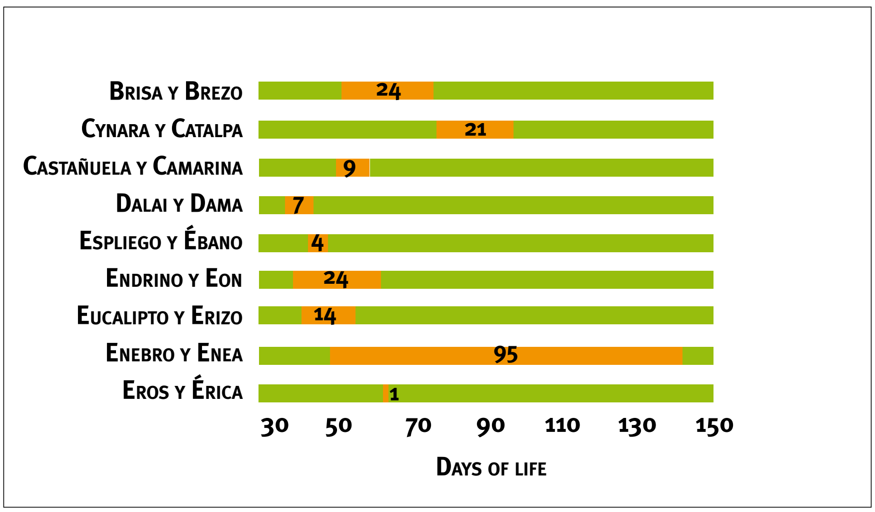

Figure 2.
Age of fight and duration of aggressive period in Iberian lynx litters, natural and hand-raised.
The end of the aggressive period has been calculated from the first peaceful reunion. (Orange: aggressive period; green: peaceful period).

Figura 2.
Edad en la que la pelea tuvo lugar y duración del periodo agresivo en camadas de lince ibérico, tanto de crianza natural como artificial. El final del periodo agresivo ha sido calculado a partir de la primera reunión pacífica. (Naranja: periodo agresivo; verde: periodo pacífico).

for only few hours. In one Eurasian lynx litter a new fight emerged nine days after the first one, but the cubs were not aggressive during the period between both fights. Eurasian lynx cubs were separated only on one occasion, after the first fight was observed, and the cubs were checked afterwards for aggressiveness toward littermates. Five days after the fight they were still aggressive, but aggressiveness was not present on the 9^{th} day after the first fight. Iberian lynx cubs were separated by keepers when fighting escalated to a point of concern, and they were later reunited at different time intervals after the fights (4-14 days), once the cubs seemed to have calmed down. Analysis did not reveal a husbandry influence on the duration of aggression between cubs. No correlation was found between time passed from the fight to the first attempt of reunion and the duration of the aggressive period (Spearman rank order correlation: R=0.52; t=1.2; n=6; ns), although this needs to be taken cautiously since sample size is rather small. Duration of after-fight aggression varied across Iberian lynx litters (median=14; min=1; max=95 days) (Figure 2). Thus, the aggressive period in Iberian lynx lasted, in average, until postnatal day 63 (min=45; max=144).

Photo: Anastasia Antonevich

Who are the aggressors and the victims?

Eurasian lynx cubs fought with littermates of both the same and opposite sexes. Although females were aggressors in 65% of the fights (m:f=6:11), the sex ratio of aggressors did not differ from the overall sex ratio of all Eurasian lynx litters (Naidenko and Antonevich, this book). In the mother-raised Iberian lynx litters, aggressors were males in 67% cases (m:f=6:2). Sex ratio was equal for mother-raised litters with several cubs (9:9). In hand-raised cubs sex ratio was 6:2 (m:f), taking the bobcat female into account, but all the hand-raised pairs were of the same sex (four male combinations in 2008 and one constant female pair in 2006). Although all pairs had fights, the small sample size does not allow us to analyze the sex ratio of aggressors. Nevertheless, we can state that fights occurred between cubs of the same and of opposite sexes; i.e., males and females were aggressors in different litters. The tendency was that, before the fight, the aggressor in Eurasian lynx litters was larger than the attacked cub, but in general, the neutral cub was usually the largest. Altogether, aggressors were heavier in 71% (10/14) of the cases. Similarly, in mother-raised Iberian litters the aggressor was larger than the victim in 86% of the fights (6/7 litters).

Response of mothers to fighting offspring

Females tried to stop cub fights as soon as they emerged. Eurasian lynx mothers used their forelegs and mouth to separate cubs in a very rough way. Iberian lynx dams seemed gentler and never used their paws to break a fight. One of the females, *Adelfa*, used her paws to prevent the aggressive cubs from contacting each other, but not to break up a fight between cubs. In both species, females would sometimes use their body to keep cubs apart, licking away the aggressor to move it further from the victim.

Conclussions

Early sibling aggression in Eurasian and Iberian lynxes occurred during a similar sensitive period and shared many common features, even though husbandry procedures to handle fights greatly differed between the two centers where these studies were carried out. Sibling aggression in other mammalian species tends to emerge during moments of highest competition between sibs, such as nursing, eating, etc. (Drummond, 2006). It has been argued that the lack of resources seems to be the underlying mechanism of litter size restrictions that cause aggression in larger litters (Hofer and East, 2008). Yet, in both lynx species early fights were spontaneous and did not appear to be the result of any direct competition. This characteristic distinguishes lynx fights from early aggression in other taxa (Fraser and Thompson, 1991; Drummond, 2006). The number of attacks per fight was slightly higher for Eurasian lynx encounters than for Iberian lynx ones, but the duration of the aggressive period for the Eurasian lynx was much shorter than for the Iberian species. These differences could be caused by the separation of viciously fighting cubs of the endangered species, so the fight was left unresolved and no behavioral asymmetry was established (Antonevich and Naidenko, 2008). We can also expect species-specific differences to exist in some characteristics of fights like the ways that mothers use to stop cubs from fighting. There is no evidence that sex influences the role of the cub in the fight, but data indicates that size does in both species. Although several characteristics differ between Eurasian and Iberian lynx fights, this phenomenon is similar in both species but differs from sibling aggression in other species of animals.

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Photo: José María Pérez de Ayala

Veterinary aspects

ASPECTOS VETERINARIOS

The earth has music for those who listen.

William Shakespeare
(1564-1616)

Integrating health issues into the conservation of the Iberian lynx *(Lynx pardinus)*

Integración de los aspectos sanitarios en la conservación del lince ibérico *(Lynx pardinus)*

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Resumen
Las acciones necesarias para la conservación del lince ibérico precisan, entre otras medidas, la integración de conocimientos y de personal veterinario dentro de un contexto multidisciplinar. Las acciones veterinarias son desarrolladas por el Grupo Asesor de Aspectos Sanitarios (GAAS) del lince ibérico e incluyen la elaboración e implementación de protocolos de trabajo (manuales), la realización de anestesias, exámenes y muestreos de los animales, necropsias, elaboración de informes técnicos y el mantenimiento de una base de datos biomédica. En el periodo transcurrido entre diciembre de 2003 y diciembre de 2008, se llevaron a cabo 318 inmovilizaciones en 126 individuos, 60 de ellos de vida libre. En la mayoría de los casos los animales fueron capturados con jaula trampa y para la anestesia se empleó la combinación de ketamina y medetomidina, que fue suplementada con isoflurano cuando fue necesario prolongar el procedimiento. En el mismo periodo se realizaron 52 necropsias, 38 en animales de vida libre, revelando que el atropello es la principal causa de muerte. El seguimiento intensivo de la población de vida libre ha puesto de manifiesto que las enfermedades infecciosas (leucemia felina, tuberculosis, moquillo canino, panleucopenia felina) suponen una seria amenaza para la supervivencia de la especie. Paralelamente, en la población cautiva se está desarrollando un programa de medicina preventiva que incluye cuarentenas, vacunaciones, análisis coprológicos, cultivos fecales y exámenes periódicos de los animales. En esta población se han descrito casos de clostridiosis, dermatitis, clamidiosis, enteritis con retención fecal, absceso dental, quilotórax idiopático, abortos y nacimientos prematuros, dermatomicosis, inflamación de glándulas perianales, hernias umbilicales y epilepsia idiopática. El conocimiento científico generado por los diferentes procedimientos clínicos, análisis y seguimientos se aplica en la gestión y en la conservación de la especie.

Palabras clave
Anestesia, necropsia, medicina preventiva, patologías en cautividad, base de datos biomédica

Abstract
Conservation actions for the endangered Iberian lynx require the integration of expertise and veterinary skills into an interdisciplinary effort. Veterinary actions are overseen by the Iberian Lynx Health Advisory Group (GAAS). These actions involve developing and implementing working protocols (manuals), anesthetizing animals, collecting samples, performing examinations and necropsies, preparing technical reports, and maintaining a biomedical database. Three hundred and eighteen anesthesias of 126 individuals were carried out during the period between December 2003 and December 2008, 60 of them belonged to the free-ranging population. In most cases, the animals were captured with a box-trap. A combination of ketamine and medetomidine was used for chemical immobilization. It was supplemented with isoflurane when necessary. Fifty two necropsies were performed in the same period, 38 of them on free-ranging animals, revealing road-kills as the main cause of mortality. Intensive monitoring of the free-ranging population has shown that infectious diseases (feline leukemia, tuberculosis, canine distemper, feline panleukopenia) can be a serious threat to the survival of the species. A preventive medicine programme is being implemented with the captive breeding population. It includes quarantines, vaccinations, fecal analyses, fecal cultures, and regular examinations of the animals. Several clinical cases in this population have been described: miscarriages and premature births, clostridiosis, dermatitis, chlamydiosis, enteritis with fecal retention, tooth abscess, idiopathic chylothorax, dermatomycosis, perianal gland inflammation, umbilical hernias and epilepsy. Scientific knowledge generated through the various clinical procedures, tests, and monitoring programs is applied to the management and conservation of this species.

Keywords
Anesthesia, necropsy, preventive medicine, diseases in captivity, biomedical database

Integrating health issues into the conservation of the Iberian lynx *(Lynx pardinus)*

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Introduction

In spite of its highly endangered status, relatively little was known about biomedical aspects of both free-ranging and captive Iberian lynx populations (Beltrán and Delibes, 1991; Ferreras et al., 1994; Torres et al., 1998; Briones et al., 2000; Pérez et al., 2001; Vicente et al., 2004). The development of a health programme that allows studying biomedical aspects and evaluating the incidence, as well as prevalence of infectious pathogens and diseases, is one of the most relevant aspects that need to be taken into account when designing a long-term conservation plan for any species including the Iberian lynx. Previous experiences in other species (Munson and Cook, 1993; Deem et al., 2001; Cleaveland et al., 2002; Deem and Karesh, 2002; Deem 2007; Furtado et al., 2008) clearly show that developing such a programme is fundamental and necessary. The low number of individuals in two isolated subpopulations (Simón et al., this book; Guzmán et al., 2004) make this species extremely vulnerable to stochastic factors, such as infectious diseases, as has already been observed in the Iberian lynx (Meli et al., 2008; Meli et al., 2009; López et al., 2009), and in other threatened terrestrial carnivores (Williams et al., 1988; Alexander and Appel, 1994; Roelke-Parker et al., 1996; Cunningham et al., 2008). In addition, low genetic variability (Godoy et al., this book) may result in a reduced immunological response to pathogens (Peña et al., 2006), or in the development of uncommon pathologies (e.g., facial neoplasms in the Tasmanian devil *(Sarcophilus harrisii),* McCallum, 2008).

In addition to health aspects, a multidisciplinary effort to integrate management, genetics and reproduction is crucial to obtain optimal individuals for reintroduction, always trying to avoid the effects of genetic adaptation to captivity (Frankham, 2007).

The Iberian Lynx Health Advisory Group

The Iberian Lynx Captive Breeding Action Plan determines the objectives and actions of the *Ex situ* Conservation Programme (Vargas et al., this book). The document is developed by a Breeding Committee, and is reviewed on an annual basis. The committee consists of several working groups specialized on different disciplines, including health aspects (Vargas et al., this book). Although the initial focus for the Health Advisory Group *(Grupo Asesor de Aspectos Sanitarios, GAAS)* was the *Ex situ* (captive breeding) Programme, the natural work

Fig 1. Material used for field anesthesia in Iberian lynx. Isoflurane vaporizer and oxygen tank are also included but not shown in this picture.

Fig 1. Equipo empleado para la anestesia de campo en los linces ibéricos. Aunque no aparece en la imagen, también se emplea un circuito con vaporizador de isofluorane y una bombona de oxígeno.

Fig. 2a. Anesthesia and sampling of an Iberian lynx in an enclosure at a captive breeding center. This animal had to be repeatedly immobilized due to an osteomielitis in a digit. According to protocol, a minimum of two veterinarians have to be involved in any Iberian lynx anesthesia.

Fig. 2a. Anestesia y muestreo de un lince ibérico en la misma instalación del animal, en un centro de cría. Este animal fue necesario anestesiarlo en repetidas ocasiones para el seguimiento y tratamiento de una osteomielitis en una falange.
Por protocolo, en toda anestesia de un lince ibérico, siempre hay un mínimo de dos veterinarios.

Fig. 2b. Organizing anesthesia and sampling of an Iberian lynx in the field.

Fig. 2b. Preparación en campo para la anestesia y muestreo de un lince ibérico.

Fig. 2c. Anesthesia on an Iberian lynx with a gastrointestional affection in a veterinary hospital. The animal has been radiographed before conducting a gastric fibroendoscopy.

Fig. 2c. Anestesia de un lince ibérico con una patología gastrointestinal en un hospital veterinario. Se han realizado radiografías del animal antes de proceder a una gastroscopia.

flow and the necessary collaboration between *in situ* (in its natural habitat) and *ex situ* programmes has led the GAAS to also encompass the health aspects of free-ranging animals. The interdisciplinary group consists of national and international veterinarians that commit themselves to collaborating with the Programme on a voluntary basis and work in such diverse, complementary and necessary aspects as research, pathology, clinical medicine, zoo medicine and wildlife rehabilitation. The group works in close collaboration with other groups of the Breeding Committee. Its main goals include the following:

Development and implementation of working protocols (Iberian lynx clinical manual, Iberian lynx hand-rearing manual, Iberian lynx necropsy manual)

These protocols are considered to be living documents that are periodically revised based on experience, scientific information and programme needs. The protocols standardize work processes, and aim at maximizing the information that can be obtained each time a lynx, either alive or dead, is handled. Of course, the priority in live animals is their health and well-being. The protocols are available on the *Ex situ* Conservation Programme's website (www.lynxexsitu.es).

Anesthesia, clinical examination, and sample collection

Procedures where anesthesia is needed include: quarantine examinations; health and reproductive examinations (Roldan et al., this book; Göritz et al., this book), both in animals included in the Breeding Programme and in free-ranging individuals; placement of radiotelemetry or GPS collars, and clinical or diagnostic procedures.

A complete and easily mobile equipment unit is available (Figure 1). In addition to the equipment necessary at each breeding center, specific equipment to anesthetize free-ranging individuals exists both in Sierra Morena and Doñana. The duplicate gear helps to avoid the risk of transmitting infectious agents between *in situ* and *ex situ* populations. Many anesthetic events have been carried out in the field, outside clinical facilities, but the procedure and equipment used have allowed the team to perform safe chemical immobilizations, monitoring, and sample collection (Figures 2a, 2b and 2c). At least two veterinarians participate in any Iberian lynx anesthesia: one of them exclusively monitors the animal, while the other is in charge of the clinical examination and sample collection. Independently of the reasons for the anesthesia, biological samples are systematically collected whenever possible according to protocol (Iberian lynx clinical manual). This sample collection has several goals: diagnosis, research (Pastor et al., this book; García et al., this book; Meli et al., this book; Göritz et al., this book), genotyping (Godoy et al., this book), and sample storage for the biological resource banks (BRBs) (Roldan et al., this book; León et al., this book). Several laboratories and institutions participate in sample analysis, backed up by the corresponding agreements: Unitat de Hematologia Clinica, Facultat de Veterinària de Bellaterra (Barcelona); Centro de Análisis y Diagnóstico (CAD) from Consejería de Medio Ambiente de la Junta

	Cub Young	Subadult	Adult	Unknown age	TOTAL
Infectious causes					
Bacterias	–	–	1	–	1
Virus	–	–	6 (6)	–	6 (6)
Non infectious causes					
Road-kill	4	6	4 (2)	–	14 (2)
Aggression	2	–	1 (1)	–	3 (1)
Illegal traping	–	–	1	–	1
Starvation	1	–	-	–	1
	–	–	1(1)	–	1 (1)
Unknown causes	6	–	2 (2)	3	11 (2)
	13	6	16 (12)	3	38 (12)

Table 1. Mortality causes of free-ranging Iberian lynx *(Lynx pardinus)* at different ages from December 2003-2008. Numbers in brackets indicate adult animals equipped with radiotelemetry or GPS collars.

Tabla 1. Causas de mortalidad en linces ibéricos *(Lynx pardinus)* de vida libre agrupados según edades en el periodo comprendido entre diciembre 2003-2008. Los números en paréntesis indican los animales que llevaban collares de radiotelemetría o de GPS.

	Cub Young	Subadult	Adult	TOTAL
Infectious causes	–	–	–	–
Non infectious causes				
Miscarriages & premature birthings	7	–	–	7
Fighting	3	–	–	3
Chylothorax	1	–	–	1
Electrocution	–	1	–	1
Enterotoxemia	–	1	–	1
Unknown causes	1	–	–	1
	12	**2**	**-**	**14**

Table 2. Mortality causes of Iberian lynx *(Lynx pardinus)* from the *Ex situ* Conservation Programme at different ages from December 2003-2008. The continuous video-surveillance, the hand rearing of ill and non attended cubs and the fast intervention during cub fighting have avoided the lost of more cubs.

Tabla 2. Causas de mortalidad de linces ibéricos *(Lynx pardinus)* del Programa de Conservación *Ex situ* agrupados en diferentes edades y en el periodo comprendido entre diciembre del 2003-2008. La videovigilancia continua, la cría artificial de cachorros no atendidos o enfermos y la rápida intervención en las peleas entre cachorros ha evitado la muerte de más animales.

de Andalucía (Málaga); Clinical Laboratory at the Vetsuisse Faculty, University of Zürich; Anatomía Patológica, Facultad de Veterinaria de Madrid; BanGes at the Museo Nacional de Ciencias Naturales, CSIC (Madrid); Laboratorio de Bioingeniería at Universidad Miguel Hernández (Alicante); Laboratorio de Biología Molecular, Estación Biológica de Doñana, CSIC (Sevilla); Laboratorio de la Estación Experimental de Zonas Áridas, CSIC (Almería), and the Department for Reproduction at the Institute for Zoo and Wildlife Research-IZW (Berlin).

Free-ranging lynx are usually captured by means of double entry box live-traps and then transferred to a squeeze cage where they are administered immobilizing drugs through direct intramuscular injection. To avoid stress and minimize trauma, captive animals are caught with squeeze-cage traps whenever possible (Figure 3a and 3b). On other occasions they are captured with nets or blowdarts. A total of 318 chemical immobilizations of 126 individuals, among them 60 free-ranging lynx, were performed between December 2003 and December 2008. The most commonly used method of chemical restraint consists of a mixture of 5 mg/kg ketamine hydrochloride (Imalgene1000®, Merial) and 50 µg/kg medetomidine (Domtor®, Pfizer). Induction is fast (lateral recumbence at 6.5 ± 4.2 minutes) and smooth, and the effect lasts about 40-50 minutes (Martínez, 2007). Anesthesia can be partially reverted with atipemazole (Antisedan®, Pfizer) if necessary. By the end of 2008, medetomidine was substituted by dexmedetomidine (Dexdomitor®, Pfizer) at a dose of 12.5 µg/kg, which has resulted in a reduction of cardiovascular depression, although more data is needed to confirm the benefits. Isoflurane or sevoflurane are used for longer procedures (Gómez-Villamandos et al., 2007), although on certain occasions (e.g., anesthesia for electroejaculations) ketamine has been used for supplementation to avoid sphincter

Fig. 3a. Squeeze-cage in a handling corridor in a breeding center.

Fig. 3a. Jaula de captura–compresión en un túnel de manejo en un centro de cría.

Fig 3b. Use of the squeeze-cage for direct intramuscular administration of anesthesia.

Fig 3b. Uso de la jaula de captura-compresión para la inyección intramuscular directa de anestésicos.

relaxation and to minimize the risk of contaminating semen with urine (which would kill the spermatozoa). No accidents have been registered during anesthesia, and all animals recovered without complications.

Necropsies and forensic examinations

For operational reasons and uniformity, the Centro de Análisis y Diagnóstico (first located in Sevilla, actually in Málaga, Spain) has been designated as the laboratory where all Iberian lynx necropsies shall be performed. This center is equipped with all the necessary materials and has the necessary trained staff to carry out necropsies even in emergency situations. Necropsies are performed following the established protocol (Iberian Lynx Necropsy Manual). In addition to determining the cause of death, post-mortem exams and sample collection provide biological material for epidemiological studies (Meli et al., this book), for genetic studies (Godoy et al., this book) and for the scientific collections of skeletons and skins. They also provide materials for the Biological Resource Banks (BRBs). Communication and coordination with the BRBs is key to collect, transfer, and process viable reproductive tissues (testes and ovaries) as fast as possible to avoid deterioration of these valuable samples (González et al., 2007).

A total of 52 necropsies, 38 of them on individuals from the free-ranging populations and the rest mostly from captive-born prematures and neonates, have been performed between December 2003 and December 2008. The causes of death in the free ranging population (Table 1) were as follows:

- Seven animals died due to infectious causes. Four of them died of diseases associated with FeLV infection (Meli et al., 2008; Meli et al., this book; López et al., 2009; López et al., this book) and one of infection with canine distemper virus (CDV) (Meli et al., 2008). The losses to the wild population caused by capture and transfer of animals with FeLV to a rehabilitation center are not included in this chapter (López et al., this book). One individual was infected with feline parvovirus (FPV) and had spleen rupture, probably caused by trauma (Guillermo López, pers. comm.). One lynx was infected with *Mycobacterium bovis*, although more cases were already known prior to this one (Pérez et al., 2001; Aranaz et al., 2004), this additional case underlines the susceptibility of the Iberian lynx for bovine tuberculosis. Six of the seven animals that died of infectious diseases were equipped with radiotelemetry or GPS collars, which reaffirms that tracking allows for the detection of deaths that, otherwise, would likely remain unnoticed. Similar results have been observed in other free ranging felids (Schmidt-Posthaus et al., 2002; Haines et al., 2005).
- Twenty animals died of non-infectious causes. The most frequent cause of death was road-kill (14 animals). Three animals died of aggression: two of them were cubs aged less than one month. The species that attacked them could not be determined. One adult lynx had lesions in the hindquarters that had probably been caused by hunting dogs. Another adult animal died after ingesting bait that had been deliberately impregnated with aldicarb. One animal died of lesions, dehydration, and cachexia following the amputation of a limb by a snare trap. The number of deaths that were detected and attributed to illegal poaching was considerably lower during the study period than in a previous study (García-Perea, 2000). Multiple pellets from two previous buckshot wounds were found during the necropsy of an animal that had died following a collision with a vehicle. In contrast to this, no pellets were found in any of the 126 live animals that had been examined and radiographed. The death of one dispersing juvenile was attributed to starvation based on the observed cachexia and the absence of infectious or toxic agents.
- The cause of death could not be determined in 11 cases. Only skeletal and dry tissues remains were available for examination in these animals, which did not allow clarifying the actual cause of death.

During the same period, mortality within the *ex situ* population (Table 2) was attributed to the following causes:

- Seven premature births and miscarriages were registered. Four offspring were stillborn, while two premature young died of septicemia caused by *Escherichia coli* (see *Discussion of clinical cases experienced within the Ex situ Programme,* this chapter). No infectious causes could be determined in the other stillborn, premature cubs.
- Three offspring died of aggression. In one case, the lesions had been caused by a littermate (Antonevich et al., this book), while the other two had been injured by the mother.
- One offspring had an idiopathic chylothorax (see *Discussion of clinical cases experienced within the Ex situ* Programme, this chapter).

- One animal died accidentally in quarantine after biting an electric wire of a surveillance camera.
- One animal died of enterotoxemia after ingesting meat contaminated with Clostridium toxin. It was also severely anemic and infested with fleas. Similar symptoms have been described in other captive non-domestic felids that were exposed to demographic explosions of flea populations (Carles J. Sallés, pers. comm.).
- A two-day-old offspring died suddenly. A non-traumatic perforation of the stomach wall and associated peritonitis were observed during necropsy. The initial cause of death could not be determined.

Development of technical reports

Every examination during anesthesia, necropsy or clinical intervention is thoroughly documented and the corresponding report is sent to the appropriate institutions and/or administrations. The GAAS also issues reports and recommendations on specific topics related to health aspects (*Cytauxzoon,* tuberculosis, FeLV, etc.), whenever they are considered necessary.

Creation and maintenance of a biomedical database

All clinical procedures and analytical results generate a significant amount of information that needs to be sorted and organized. A biomedical database (BDB) was therefore created and divided into different sections, including procedures, analytical results and clinical histories. It is a useful tool that can be used for research and management of the species. The BDB information is available to technicians, institutions and researchers that hold a collaborative agreement.

Courses, meetings, and workshops

Courses on health issues of the Iberian lynx and other felids have regularly been held with the ultimate purpose of revising all available information generated on Iberian lynx health issues and learning from other related programmes, as well as from each other. In addition, meetings and workshops have been held at the breeding centers to unify criteria and improve procedures and needs (workshops on pediatrics, cesarean procedures, hand-rearing, anesthesia, handling of severely injured patients, etc.). Also, GAAS members maintain active communication and discussions via an electronic forum and hold regular meetings to discuss problems and needs, while enhancing the communication between veterinarians and researchers.

Health management programme for captive Iberian lynx

Free-ranging animals that are captured and are candidates for incorporating to the *Ex situ* Programme are quarantined in specific facilities, which are equipped with surveillance cameras to allow continuous monitoring of the animals. Two anesthetic events, with complete clinical exams, radiographs and sample collections, are performed during the quarantine period. The first one is performed 10 to 14 days after the beginning of the quarantine period and the second a month later. The animals are identified with a microchip and vaccinated against FHV, FCV and FPV (Fevaxyn-i-CHP®, FortDodge) as well as FeLV (Purevax FeLV®, Merial). After quarantine and evaluation of the clinical exam, analyses and video-monitoring, the animals that are considered suitable for the *Ex situ* Programme are transferred to the breeding facilities (Vargas et al., this book).

The health management of Iberian lynx belongs to the Captive Breeding Programme is similar to that of other felid species (Meltzer, 1999) and follows the recommendations issued by the EAZA and AZA (Joslin et al., 1998; Blomqvist et al., 1999; Woodford, 2001; Krelekamp, 2004). The inherent chronic stress registered in certain captive-kept felid species seems to be related to a higher prevalence of certain diseases when compared to free-ranging populations (Munson et al., 2005). In addition to spacious facilities, limited entry to only center staff, environmental enrichment and a captive diet as similar as possible to the natural diet, the preventive medicine programme seeks to minimize stress and improve health through the following measures:

Biosecurity system: The procedures include footbaths, disinfection of the materials and changing clothes and footwear when entering the animal facilities.
Parasitology analysis: Feces are analyzed every three months. *Eimeria* (from rabbits offered as live prey to the

animals), *Strongylidae* and *Toxacara* are common findings in fecal exams. Antihelminthics are avoided unless a certain parasite load is detected or the animal shows signs of being parasitized. As part of the prevention measures for enteroparasite infection, daily husbandry includes removing feces from the enclosures on a daily basis.

Fecal cultures: Conducted to test for *Salmonella/Shigella/Yersinia/Campylobacter* are performed every six months. Only *Campylobacter* has been detected sporadically without symptoms. Fecal cultures also aim at detecting the potential zoonotic risk to the staff at the breeding facilities. Cultures of rectal swabs taken during anesthesia of free-ranging and captive individuals were also negative for the abovementioned bacteria.

Vaccinations: A trivalent inactivated vaccine against FHV, FCV and FPV, and a recombinant vaccine against FeLV are used as it was described previously in this chapter. The offspring born in the breeding facilities are vaccinated at eight weeks of age and then re-vaccinated a month later, and eventually boosted at a yearly basis. Although some protocols in non domestic felids recommend initiating a vaccination plan at an earlier age (around six weeks), and to revaccinate every three weeks until the animals are 12 weeks old, the current protocol is considered sufficient and safe for captive Iberian lynx, given the known prevalence of infectious agents, the biosecurity barrier and the quarantine system. In addition, no infection with these agents has ever been detected in the *ex situ* population. In any case, the evaluation of the safety and efficacy of the vaccines is a pending action required by the Iberian Lynx Captive Breeding Action Plan. Adult animals are revaccinated every two to three years, and breeding females are revaccinated two months before breeding season to maximize immunity transfer to the offspring.

Brush and weed clearing: Periodic clearing of the large, open enclosures reduces optimal ectoparasite habitats (mainly ticks) while it ensures proper control of the animals via the video surveillance system.

Capture: Whenever possible, the capture of an animal is programmed and done with a squeeze-cage specifically designed for Iberian lynx (Fig. 3a and Fig. 3b). Nets and darts are avoided to reduce stress, trauma and distrust of the animals towards the staff.

Complete physical exams: Adults are currently examined under anesthesia once a year. The objectives of the examinations are: 1) to periodically assess the health status of each individual; 2) to vaccinate and deworm if necessary; 3) to evaluate female reproductive health of by performing a sonographic examination of the reproductive tract (Göritz et al., this book); 4) perform radiographic examinations; 5) to evaluate male reproductive health of by studying sperm quality (Gañán et al., in press; Roldan et al., this book) and performing a sonographic examination of the testes and prostate (Göritz et al., this book); 6) to collect biological samples to complete the analyses considered in the protocols, and 7) to collect biological samples for the BRBs.

Elimination of external parasites: *Spot-on* pipettes containing fipronyl (Frontline®, Merial) or selamectine (Stronghold®, Pfizer) are routinely applied when animals are captured and anesthetized. Animals suspected to be infested with ectoparasites causing unspecific dermatological problems have been captured sporadically with squeeze-cages and pipettes have been applied.

Weighing: The animals are weighed regularly by entering tunnels or handling corridors with platform scales.

Diet: Food could be the port of entry for pathogens that could imperil the captive population, as has occurred in other species (Jauniaux et al., 2008) and in the Iberian lynx (see Clostridiosis in *Discusion of clinical cases experienced within the Ex situ Programme,* this chapter). The diet and supplements administered to the programme's animals are prescribed with the help of a nutritionist (Helena Marqués, ConZoolting©). Ninety percent of the captive lynx diet consist of rabbit (live or dead). The remaining 10% include quail and cow meat. Besides the vitamin mix provided when dead rabbits are fed to the captive colony, beta-carotene is also added to the regular diet starting six to eight weeks prior to breeding season and until the pairs have copulated.

Discussion of clinical cases experienced within the Programme

Infectious agents that have been identified through molecular biology studies and serological analyses are discussed elsewhere (see Meli et al., this book), and have been described in several articles (Willi et al., 2007; Luaces et al., 2008; Millán et al., 2008; Roelke-Parker et al., 2008). The results of histopathology and immunochemistry studies in Iberian lynx are exhaustively discussed in another chapter (Jiménez et al., this book) and in publications (Peña et al., 2006; Jiménez et al., 2008).

Despite the relative small size of the Iberian lynx *ex situ* population and the short period of time studied

Fig. 4a. Miscarried Iberian lynx (at 44 gestational days).

Fig. 4a. Aborto de lince ibérico (con 44 días de gestación).

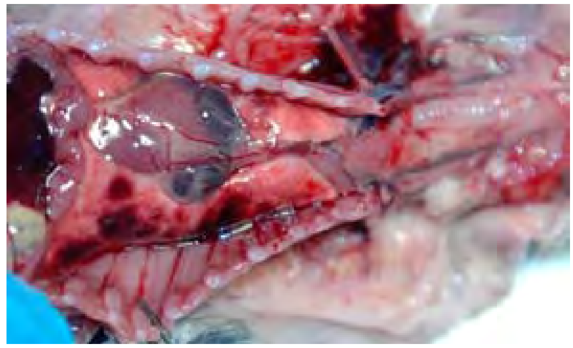

Fig. 4b. Post-mortem examination of the thoracic cavity of an Iberian lynx premature (61 days of gestation). Lung hemorrhage and generalized congestion. The animal received intensive medical care but died promptly. Septicemia by *Escherichia coli* was the final death reason.

Fig. 4b. Examen de necropsia de la cavidad torácica de un prematuro de lince ibérico (61 días de gestación). Se observa una hemorragia pulmonar y una congestión generalizada. El animal no fue atendido por la madre y necesitó cuidados intensivos, pero murió rápidamente. El diagnóstico final de la causa de muerte fue una septicemia por *Escherichia coli.*

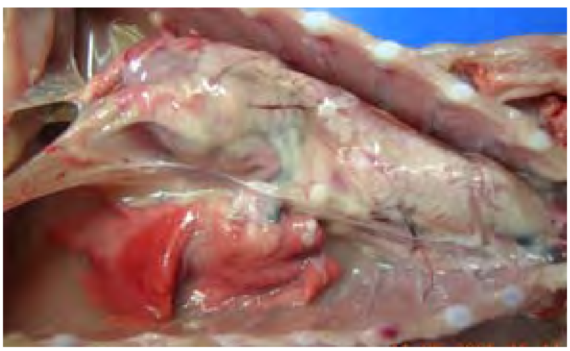

Fig. 5. Post-mortem examination of the thoracic cavity of an Iberian lynx with chylothorax.

Fig. 5. Examen de necropsia de la cavidad torácica de un lince ibérico con quilotórax.

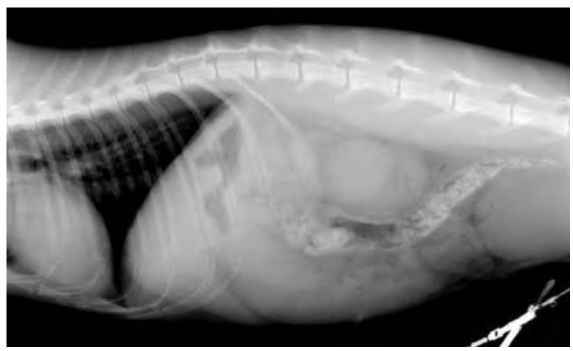

Fig. 6. Abdominal radiograph of an Iberian lynx with enteritis and fecal retention.

Fig. 6. Radiografía abdominal de un lince ibérico con un cuadro clínico de enteritis y retención fecal.

similar morbidity and mortality have been observed in a retrospective study in captive jaguars (Hope and Deem, 2006). This section focuses on the diseases observed in animals of the *Ex situ* Conservation Programme.

Miscarriages and premature births

Between 2005 and 2008, the *Ex situ* Programme has seen the birth of 34 offspring, seven of which were either premature or miscarried. The normal gestation period of captive Iberian lynxes seems to vary between 63 and 66 days (n=15; Vargas et al., this book). One female miscarried two fetuses after 42 days of gestation (Figure 4a); several analyses (PCR, histopathology, microbiology) did not allow determining the cause of miscarriage, and the female did not show any signs of disease. One female whelped two young after 56 days of gestation; one was dead, while the other was abandoned by the mother and died 24 hours later. The necropsies of these two individuals revealed a generalized congestion and hemorrhage without any other lesions or noteworthy laboratory results. Another female gave birth to two premature offspring after 61 days of gestation and abandoned them immediately thereafter. They died of septicemia caused by *E. coli* (Figure 4b). Finally, one female delivered two premature offspring after 60 days of gestation. One was born dead, while the other one with a septicemia survived thanks to intensive medical care.

Idiopathic chylothorax

A naturally reared male died at eight weeks of age. The animal had been vaccinated two days earlier, and although its body condition was poor at the time of capture, the surveillance cameras showed that its behavior and activity were normal. Necropsy revealed abundant lymphatic, reddish liquid in the thoracic cavity (Figure 5); diffuse interstitial pneumonia, enteritis with crypt necrosis, generalized lymphatic congestion and distention, and enterobacterial septicemia was detected. FPV was identified by means of PCR, but it was probably the inactivated vaccine virus. An analysis of vaccines of the same lot did not reveal any viral replication or bacterial growth. An idiopathic chylothorax was diagnosed due to the impossibility to determine the cause of the observed signs. The chylothorax may have been caused by trauma, because the primiparous mother had been hand-raised, so never been correctly socialized. Although her maternal instinct was good, she played roughly with her cub. The most plausible hypothesis regarding this specific case is that trauma might have caused a lesion of a lymphatic vessel, which could then have led to the chylothorax.

Clostridiosis

Several adult animals presented gastrointestinal signs in the spring of 2006. Some animals vomited food or only bile. In most affected animals, vomiting was sporadic, while they maintained their appetite and showed normal behavior. One female presented bouts of vomiting, colitis and slight depression for a day. Another female vomited once to three times per week during 11 weeks, sometimes with diarrhea. She had loss of appetite, slight weight loss and a scruffy appearance of her fur. The female was rearing two offspring during this period, but the young did not show any signs of disease. Chemical immobilization for full exam and sampling was not performed in the most severely affected females because they were still rearing their offspring, and because their general status allowed a conservative approach to continue excluding possible etiologies. The cubs did not present the signs described for the mothers. Finally, it was determined that the signs had been caused by rabbit meat contaminated with enterotoxins, and Clostridium was isolated in microbiological cultures. It is possible that a delayed evisceration of the rabbit carcasses led to contamination of the flesh with intestinal contents. Alternatively, the carcasses may not have been correctly refrigerated. In addition to changing the food source (individually packed rabbit meat for human consumption is currently used), all animals were treated with omeprazol and amoxicillin (Clamoxyl®, Pfizer) or amoxicillin with clavulanic acid (Synulox®, Pfizer).

Dermatitis

An adult male that was periodically anesthetized (Fig. 2a) to treat a digit abscess developed an acute alopecic, erythematous, bilateral lesion of the neck that seemed to be self-inflicted due to licking or continuous scraping. The etiology could not be determined, but the case was resolved by administering prednisone (Dacortin®, Merck) and marbofloxacine (Marbocyl®, Vétoquinol). A psychogenic dermatitis related to stress caused by repeated capture and immobilization was suspected.

Tooth abscess

Inflammation was observed on the left side of the snout of a male aged four months; the animal did not present any other signs. During chemical immobilization, the inflammation was attributed to the persistence of a deciduous canine, and an associated abscess from which *Proteus mirabilis* was isolated. The problem was resolved without complications by extraction of the canine, wound cleaning, and administration of spiramycin and metronidazole (Rhodogil®, Aventhis Pharma).

Dermatomycosis

An alopecic area was observed on the neck of a captive-born, 5-month-old female that shared an enclosure with two female littermates and the mother. The animal was examined under anesthesia, and additional alopecic and erythematous areas were found on face and extremities. It was assumed that the littermates were also affected, although no lesions were observed by means of the surveillance cameras, and the animals were not immobilized for examination. The mother did not seem to be affected. Cultures and biopsies revealed that the cause of alopecia was an infection with *Trichophyton mentagrophytes.* The fungi had probably been transmitted to the lynx from the live rabbits used as food items, but no cause-effect relationship could be proved. All the family unit –who was sharing the same enclosure– was treated with itraconazol (Itrafungol®, Esteve) administered in the food. Griseofulvine was not used because toxic effects have been described in other felids (Wack et al. 1992). The animals recovered without complications. Another hand-reared offspring of the 2008 breeding season, and two naturally-raised offspring, were affected by a dermatomycosis also caused by *T. mentagrophytes.* No systemic treatment was administered, and the animals recovered without complications. It is possible that these infections are self-limiting, as it occurs in the domestic cat.

Chlamydiosis

One free-ranging, apparently healthy adult male was captured for its incorporation to the Captive Breeding Programme. Conjunctivitis and a small corneal ulceration, without additional signs, were observed during the quarantine examination. *Chlamydophila felis* was identified by PCR in conjunctiva swabs, while additional tests did not reveal any other pathology. Oral treatment with doxycycline (Ronaxan®, Merial) eliminated the signs. A second PCR was negative for Chlamydophila, and for other agents that may cause oculo-respiratory syndromes in felids.

Enteritis with fecal retention

Two adult lynx kept in different breeding centers presented unspecific signs, including depression and gastrointestinal signs: vomiting in one and constipation in the other. One of the animals was anesthetized and underwent a complete examination with sample collection, radiographs and endoscopy of the stomach (Figure 2c). No noteworthy anomalies were identified in the laboratory analyses or biopsies (except for slight multifocal gastritis), while the radiographs revealed enteritis of the large intestine with fecal retention (Fig. 6). The animal recovered without complications after administration of an enema, passing of feces from the intestine, maropitant (Cerenia®, Pfizer), and fluid therapy.

The other animal was also anesthetized for examination and sample collection, but no anomalies were found during the exam. The radiographs revealed enteritis of the large intestine similar to the previous case. The animal was also treated with an enema and fluid therapy. It recovered without complications.

The origin of these unspecific symptoms of enteritis and fecal retention are not known. They do not seem to be associated with the diet, but rather with the reduced physical activity of some captive individuals. They may also be associated with the temporary holding of the animals in enclosures devoid of plants and weeds (e.g., quarantine facilities) which the lynx ingest sporadically.

Umbilical hernia

Three hand-reared and one naturally reared offspring of the 2008 breeding season presented an abdominal hernia caused by a persistent umbilical ring. Two of the animals required surgical intervention. It is possible that the way hand-reared animals were handled (Rivas et al., this book), specifically during stimulation for urination and defecation, could have forced them in such a way that it interfered with normal scarring of the umbilical ring. The mother-raised cub was also handled after a cub fight (see Antonevich et al., this book) to help it with the transition to solid food, and this handling might have caused the inadequate closing of the umbilical ring.

Perianal gland inflammation

Two hand-reared offspring of the 2008 breeding season presented inflammation/infection of the perianal glands. Accumulated purulent material (*Staphylococcus* sp. isolated) was removed from one animal. Both cubs were treated with topical application an ointment containing antibiotics and corticoids (Panolog®, Novartis), and oral administration of cephalexine (Cefacure®, Intervet), and both recovered without further complications.

Epilepsy in cubs

Three of the six hand-reared offspring born in the 2008 breeding season presented seizures starting at 71, 75 and 116 days of age, respectively. Two of them shared the same father whereas the other was unrelated to them. The cause of these signs remains unknown, in spite of a wide range of tests and studies (PCRs for infectious agents, toxicological studies, hematology, biochemistry, urinalysis, magnetic resonance (MR) in one cub and cerebrospinal fluid (CSF) analyses in two of the cubs). Physical exam, complementary tests and laboratory results did not shown abnormalities, so idiopathic epilepsy was therefore diagnosed. We postulated that the etiology was probably associated with hand-rearing (e.g., infant formula, oxygen therapy, vitamin or mineral supplements, etc.), which has led to some changes in the hand-rearing procedures to try to avoid potential future cases. Anticonvulsant therapy (phenobarbital) was effectively used from the beginning and, after 6 months of therapy, a half-dosage treatment was given. To date, no additional episodes have been observed in any of the three animals.

CONCLUSIONS

The Iberian Lynx *Ex situ* Conservation Programme has grown from four individuals in December 2003 to 60 in December 2008, of which 24 have been born and raised in captivity (Vargas et al., this book). During time years, and based on the nature of the observed clinical cases, necropsy results and screening for infectious agents, we consider that the overall captive population can be regarded as healthy and that our preventive medicine plan has been effective. Except for a few isolated cases, most clinical manifestations have been controlled and have never represented a significant threat to the *ex situ* population as a whole. A strict adherence to the preventive medicine protocols and management-targeted research can assist in continue to maintain the captive population in good health and then optimal for reintroduction.

Regarding the free-ranging population, one of the main priorities for its conservation is the implementation of an epidemiological surveillance programme for the Iberian lynx and associated species in its current range and in potential reintroduction sites. This plan should be coordinated with the relevant administrations, and should be granted long-term financial and institutional support. A fluid and rapid communication between the responsible technical staff, together with the systematic collection of the biomedical information generated during procedures and analyses, should complement the surveillance plan. In addition, the acquired scientific knowledge is applied to the management and conservation of the highly endangered Iberian lynx.

ACKNOWLEDGEMENTS

The authors would like to thank all the friends and colleagues from Hospital Veterinario Ávila (Cádiz), Guadiamar Servicios Veterinarios de Referencia (Sevilla), Clínica Veterinaria San Francisco (Jaén), Centro Veterinario Condado de Huelva (Huelva), Clínica Veterinaria Rocaberti (Barcelona), BanGes and Museo Nacional de Ciencias Naturales de Madrid, Universidad de Castilla-La Mancha (Albacete), Centro de Investigaciones Biológicas-CSIC (Madrid), Fundación Bioandina (Buenos Aires), Doñana National Park (Sevilla-Huelva), Centre for Fish and Wildlife Health (Berne), Institute for Zoo and Wildlife Research (Berlin), Red andaluza de CREAs, Facultad de Veterinaria de Córdoba, Facultat de Veterinària de Barcelona, Facultad de Veterinaria de Madrid, Hospital Clinic Veterinari (Barcelona), Zoological Pathology Programme (Illinois), Conzoolting (Barcelona), Parques Reunidos (Madrid), Instituto de Investigación en Recursos Cinegéticos (Ciudad Real), Estación Biológica de Doñana (Sevilla), Life-Nature Project for Iberian Lynx Conservation in Andalucia, Fundación BBVA (Madrid), Pfizer (Madrid), FortDodge (Madrid), Farmadiet (Barcelona), Merial (Barcelona), Fundación Tambre (Madrid), and many thanks to Mariella Superina and Roberto Aguilar for translating this manuscript.

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Photo: Antonio Rivas

La inutilidad de las cosas está en función de la inutilidad de la imaginación.

Guillermo Summers

Haematological reference values for the Iberian lynx

Valores de referencia hematológicos para el lince ibérico

Josep Pastor, Ester Bach-Raich, Montse Mesalles, Ignacio García, Fernando Martínez, Astrid Vargas, Rafaela Cuenca and Santiago Lavín

Resumen

La obtención de valores de referencia hematológicos y bioquímicos de una especie es importante para estudios biológicos y para la interpretación de los resultados de laboratorio. Sin unos valores de referencia, que son los resultados esperados para un animal sano, el valor analítico obtenido en un animal es muy difícil de interpretar. La información que disponemos sobre los parámetros hematológicos en el lince ibérico es escasa. Los objetivos principales del presente trabajo son: 1) establecer valores de referencia hematológicos para el lince ibérico y 2) evaluar si existen variaciones significativas en los parámetros hematológicos, dependiendo del sexo, edad (cachorro, joven, subadulto o adulto), hábitat (en libertad, en cuarentena o cautividad prolongada), método de captura (caja trampa, red, sujeción manual o mediante dardo), origen (poblaciones de Donaña o Sierra Morena) y ser positivo a Cytauxzoon sp. Se determinaron los valores hematológicos de 151 linces clínicamente sanos. Las muestras sanguíneas se recogieron en EDTA y fueron analizadas como muestras felinas con el analizador hematológico de rayo láser ADVIA 120. Se prepararon frotis sanguíneos teñidos con Diff-Quick para su estudio morfológico. Las hembras presentan recuentos eritrocitarios inferiores a los machos. La edad y el método de captura afectaron de forma significativa al hemograma, y deben de considerarse cuando se facilitan valores de referencia. El método de captura de caja trampa induce, en la mayoría de animales, un leucograma de estrés. El recuento de eritrocitos, leucocitos y plaquetas de animales subadultos y cachorros muestran diferencias significativas respecto a los animales adultos y deben considerarse como subgrupos separados al determinar valores de referencia.

Palabras clave

Lince ibérico, *Lynx pardinus*, valores hematológicos, valores de referencia

Abstract

Determination of baseline haematological and biochemical values for a species is important for biological studies and for the interpretation of laboratory data. Without reference values, which comprises the results expected to be found in healthy individuals, the analytical values of a single animal would be very difficult to interpret. The information on haematological parameters previously available for the Iberian lynx *(Lynx pardinus)* was scarce. The main objectives of the present study included: 1) to establish haematological reference values for the Iberian lynx and 2) to assess whether there were significant variations on these values depending on factors such as sex, age (cub, young, subadults and adults), habitat condition (free-ranging, quarantine and prolonged captivity), capture method (box-trap, net, physical restrain, darting by blow pipe), origin (Doñana or Sierra Morena populations) and infection by Cytauxzoon sp. Haematological values were determined from 151 clinically healthy Iberian lynxes. Blood samples were obtained in EDTA and analysed as feline sample within the first 24h hours with an automated laser flow analyzer ADVIA 120. Blood smears were stained with Diff-Quick and evaluated by optical microscopy. Females present a lower red blood cell count than males. Age and capture method strongly affect the blood count, so they need to be considered when a reference range is given. The box trap method induces a stress leukogram; red blood cells, leukocytes and platelets counts from subadult animals and cubs showed the most important differences when compared to adult animals. In conclusion, the reference values obtained through this study can be used as general guidelines in the assessment of the health status of the Iberian lynx.

Keywords

Iberian lynx, *(Lynx pardinus),* haematology values, reference values

Photo: Antonio Rivas

Haematological reference values for the Iberian lynx

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Introduction

Determination of baseline hematological and biochemical values for animal populations is important for biological studies and for the interpretation of laboratory data. Reference values are derived from an observed distribution of measurements of each parameter in an appropriate group of animals, with the central area containing 95% of the total distribution. An analytical result outside this reference value classifies an animal as abnormal, indicating an unusual or pathological condition.

Few reports describe hematological and biochemistry values for the critically endangered Iberian lynx (Beltrán et al., 1991; García et al., 2008), and the influence of different factors in blood values has never been fully addressed. The objective of this study is to determine the hematological reference values for the Iberian lynx and study the influence of sex, age (cub, juvenile, subadult and adult), habitat condition (free-ranging, quarantine and prolonged captivity), capture method (box-trap, net, physical restrain, darting by blow pipe), origin (Donaña or Sierra Morena population) and Cytauxzoon sp. Infection on various blood parameters.

Material and methods

Reference values were obtained from 151 Iberian lynxes from animals originating from two areas (Doñana and Sierra Morena), free-ranging or captive, and studied under coordination between the *ex situ* and *in situ* conservations programmes. All animals included in the study were captured (by box-trap, net, physical restrain or darting by blow pipe and anesthetized with a combination of ketamine (Imalgene 1000®, Bayer) and medetomidine (Domtor®, Pfizer) (Martínez et al., 2007; Martínez et al., this book).

Animals included for the hematological reference values study were clinically healthy and negative for feline pathogens (FeLV, FIV, FPV, FCV, FHV, CDV, Chlamydiophila, Mycobacterium bovis). They have been captured and sampled as part of a multidisciplinary programme coordinated by *in situ* and *ex situ* conservation actions. Animal's age ranged from two-months to 12 years-of-age. The presence of Cytauxzoon felis was studied by PCR (Meli et al, this book) and by routine blood smears were stained with Diff-Quick.

Blood samples were obtained from the jugular, saphene or the cephalic veins and collected into EDTA tubes from anesthetized animals. EDTA tubes were placed in a cooler container and transported by express mail to the haematology Laboratory at the Veterinary Department of the Universitat Autònoma of Barcelona. Complete blood counts were performed using the laser blood cell analyzer ADVIA 120 (Siemens diagnostics, SA.) within 24 hours from blood extraction. The following parameters have been studied: leukocyte count (WBC), red blood cell count (RBC), haemoglobin concentration (HGB), hematocrit value (HCT), mean corpuscular volume (VCM), mean corpuscular haemoglobin (MCH), mean cospuscular haemoglobin concentration (MCHC) and laser direct mean corpuscular haemoglobin concentration (CHCM), red cell distribution width (RDW), haemoglobin distribution width (HDW), neutrophil, lymphocyte, monocyte, eosinophil percentage and count , large unstained cells (LUC), myeloperoxidase index (MPXI), reticulocyte count and percentage, mean volume of reticulocytes (ret MCV), platelet count (PLT), plateletcrit, mean platelet volume (MPV) and platelet distribution width (PDW).

Age groups were classified as follow: 1) cubs (less than two months); 2) juveniles (from two to nine months); 3) subadults (from nine months to two years-of.age), and 4) adults (over two years-of-age). Habitat condition included three subgroups: 1) free-ranging animals (free living lynxes from either the Sierra Morena or the Doñana populations); 2) prolonged captivity (animals maintained captivity for more than two months, in the study only the first hemogram is included) and 3) quarantine (animal captured from the wild and kept in isolation during a six-week period; in the study only the haemogram from the first quarantine check-up is included).

Statistical analysis

Normal distribution has been evaluated using the Kolmogorov-Smirnov test and the Shapiro Wilk test. Influence between parameters such as sex, age, habitat condition, capture method, origin population and Cytauxzoon sp. infection were evaluated by multivariate analysis and multiple comparative studies performed by the post hoc Turkey test. Statistical analyses were performed using the SPSS software, version 15.0 (Statistical Package for Social Sciences, Chicago, IL, USA).

Results

All haematological parameters obtained with the ADVIA 120 followed a normal distribution; however, in order to use a more robust method to describe the population, median and percentiles have been used. Table 1 shows the number of animals, median and percentiles 25, 50, and 97.5 from the 151 Iberian lynx sub-classified according to age subgroup.

Reference values from "free-ranging", "quarantine" and "prolonged captivity" animals, subdivided according to each age group are presented at tables 2 to 4. Because only 14 cubs are included in the study, with only one of them from the free–ranging subgroup, they are presented in table 1 as a single group.

Table 5 shows statistical significant differences in the studied parameters according to its determined classification. Red blood cell parameters are influenced by sex. In general, females have lower RBC count, hemoglobin concentration and haematocrit value than males. The main difference between age groups is found in red blood cell parameters, with results showing that subadults have significantly higher values than cubs or juveniles. In cubs, the leukogram, as well as reticulocytes and platelets are statistically different than white blood cell parameters of juveniles, subadult and adults. Source population has an influence in the red blood cell counts, with higher values in Sierra Morena animals, also leukocyte count is influenced by the animal's population of origin with higher values in animals from Doñana. Habitat condition mainly influences leukocyte counts, with free-ranging animals displaying higher leukocyte, neutrophil, monocyte counts, as well as lower lymphocyte counts than captive or quarantined animals. The box trap method seems to strongly influence the haemogram leading to an increase in red blood cells and leucocytes, and inducing a "stress leukogram" when compared to the other capture methods. Presence of Cytauxzoon spp statistically increases the red blood cell index, as well as lymphocyte and platelet counts.

Figures 1-10 illustrate specific features of Iberian lynx blood cells, and also represent the presence of some infectious agents such as Hepatozoon sp and Babesia sp. Animals that presented these blood parasites were excluded from the study.

Discussion

Reference values are important aspects to address homeostasis in each species. The first step in the calculation of reference values is to define a healthy population of animals. This may seem a simple task, yet, in wild,

	ADULT (n=50)		SUBADULT(n=42)		JUVENILE (n=43)		CUB (n=14)	
	Median	Percentiles	Median	Percentiles	Median	Percentiles	Median	Percentiles
		(2,50 - 97,50)		(2,50 - 97,50)		(2,50 - 97,50)		(2,50 - 97,50)
WBCx10³ (cells/µL)	10,68	(3,72 - 33,11)	7,36	(2,43 - 25,57)	9,49	(3,71 - 32,21)	7,43	(5,27 - 18,78)
RBCx10⁶ (cells/µL)	9,32	(4,98 - 12,41)	9,63	(2,95 - 11,64)	8,76	(3,37 - 10,79)	8,10	(5,77 - 10,72)
HGB (g/dL)	13,35	(9,58 - 18,19)	14,15	(7,19 - 17,73)	12,70	(6,37 - 15,07)	12,20	(8,90 - 14,70)
HCT (%)	43,80	(23,36 - 60,64)	46,45	(13,26 - 57,79)	40,80	(15,52 - 49,82)	38,20	(27,30 - 46,00)
MCV (fL)	48,00	(40,82 - 52,94)	48,35	(43,36 - 54,53)	46,70	(41,66 - 50,84)	46,40	(42,40 - 50,10)
MCH (pg)	14,60	(12,21 - 27,32)	14,60	(13,92 - 26,21)	14,50	(12,85 - 23,52)	14,85	(13,30 - 17,80)
MCHC (g/dL)	30,90	(25,62 - 56,74)	30,30	(28,04 - 58,36)	31,10	(28,71 - 50,32)	31,80	(30,20 - 35,90)
CHCM (g/dL)	29,60	(25,88 - 32,26)	29,40	(24,83 - 33,04)	30,00	(26,23 - 33,85)	30,45	(26,70 - 32,70)
RDW	15,70	(13,69 - 19,24)	15,65	(14,32 - 20,34)	16,40	(15,01 - 20,50)	16,60	(14,70 - 20,10)
HDW (g/dL)	2,12	(1,71 - 4,89)	2,05	(1,67 - 2,77)	2,16	(1,60 - 2,65)	2,27	(1,70 - 2,98)
Neutrophil (%)	80,95	(35,95 - 94,51)	74,80	(35,35 - 94,87)	70,30	(38,71 - 94,41)	50,20	(35,40 - 88,30)
Lymphocyte (%)	16,00	(1,29 - 60,39)	18,15	(3,87 - 52,29)	21,90	(3,86 - 50,16)	39,55	(8,00 - 57,40)
Monocyte (%)	2,20	(1,10 - 5,65)	2,25	(0,54 - 5,26)	2,60	(0,95 - 5,80)	2,95	(1,50 - 6,90)
Eosinophil (%)	2,15	(0,00 - 15,92)	3,60	(0,00 - 19,75)	3,30	(0,02 - 8,90)	3,25	(1,20 - 17,00)
Basophil (%)	0,15	(0,03 - 0,55)	0,10	(0,00 - 0,40)	0,20	(0,00 - 0,89)	0,25	(0,00 - 0,60)
LUC (%)	0,10	(0,00 - 1,50)	0,10	(0,00 - 0,78)	0,10	(0,00 - 1,51)	0,10	(0,00 - 0,50)
Neutrophilx10³ (cells/µL)	8,21	(1,77 - 30,05)	5,75	(1,32 - 24,17)	6,10	(1,91 - 30,36)	3,41	(2,41 - 16,58)
Lymphocytex10³ (cells/µL)	1,35	(0,28 - 3,57)	1,51	(0,76 - 3,17)	1,91	(0,97 - 4,32)	3,31	(1,45 - 4,26)
Monocytex10³ (cells/µL)	0,26	(0,05 - 0,80)	0,18	(0,03 - 0,52)	0,25	(0,09 - 0,61)	0,31	(0,10 - 0,67)
Eosinophil x10³ (cells/µL)	0,20	(0,00 - 2,05)	0,20	(0,00 - 3,26)	0,32	(0,00 - 1,10)	0,24	(0,14 - 1,64)
Basophilx10³ (cells/µL)	0,01	(0,00 - 0,12)	0,01	(0,00 - 0,05)	0,02	(0,00 - 0,09)	0,02	(0,00 - 0,06)
LUCx10³ (cells/µL)	0,01	(0,00 - 0,22)	0,00	(0,00 - 0,10)	0,01	(0,00 - 0,14)	0,01	(0,00 - 0,04)
LI	0,00	(0,00 - 2,67)	0,00	(0,00 - 2,15)	0,00	(0,00 - 2,94)	0,00	(0,00 - 2,92)
MPXI	12,25	(-1,35 - 19,63)	12,05	(3,38 - 21,15)	11,70	(-5,87 - 22,73)	0,55	(-6,50 - 17,90)
Reticulocytex10⁹ (cells/µL)	24,80	(7,40 - 102,11)	28,85	(5,45 - 161,20)	35,40	(11,35 - 168,22)	39,90	(12,90 - 264,20)
Reticulocyte (%)	0,30	(0,10 - 1,31)	0,30	(0,10 - 1,99)	0,40	(0,10 - 1,88)	0,55	(0,10 - 3,80)
Reticulocyte MCV (fL)	59,00	(46,97 - 69,43)	62,10	(51,85 - 71,30)	59,90	(50,34 - 70,65)	58,15	(48,10 - 69,60)
PLTx10³ (cells/µL)	327,50	(116,95 - 759,65)	303,00	(123,80 - 695,20)	350,00	(93,10 - 691,60)	516,50	(279,00 - 702,00)
Plateletcrit (%)	0,41	(0,24 - 0,84)	0,40	(0,19 - 1,25)	0,45	(0,15 - 0,88)	0,53	(0,33 - 1,38)
MPV (fL)	12,60	(8,58 - 25,75)	12,85	(10,22 - 18,16)	13,50	(9,62 - 20,97)	11,30	(7,90 - 19,60)
PDW	66,50	(53,00 - 86,85)	64,30	(46,55 - 82,27)	61,50	(48,78 - 77,97)	66,05	(48,90 - 78,50)

Table 1. Reference CBC values for the Iberian lynx , classified by age.

Tabla 1. Valores de referencia hematológicos para el lince ibérico según edad.

endangered animals it is difficult to attain because we need to sample a large group of individuals so that the reference interval is useful for a broad group of patients. The ideal number of animals for such reference values must be around 120, but a more realistic number of 60 may be sufficient if a Gaussian distribution is present. Nevertheless, attempts to establish reference values with fewer number of animals frequently results in weak confidence intervals that are often questioned by clinical observations.

Various circumstances can influence the biochemistry and hematological values of wild animals. Some factors are connected to the sampling method and to the shipping and storage procedures. Other factors include the presence of serum constituents that influence the analytical methods, such as lipid content or hemolysis. Also, many factors affecting the 'normal' life of an animal may have significant influence on the hematological and

FREE-RANGING	ADULT (n=23)		SUBADULT (n=19)		JUVENILE (n=19)	
	Median	Percentiles	Median	Percentiles	Median	Percentiles
		(2,50- 97,50)		(2,50- 97,50)		(2,50- 97,50)
WBCx10³ (cells/μL)	14,41	(4,23 - 33,75)	12,77	(5,65 - 26,28)	11,94	(4,83 - 33,37)
RBCx10⁶ (cells/μL)	9,40	(4,40- 10,77)	9,61	(7,48- 11,40)	8,74	(6,36 - 10,29)
HGB (g/dL)	13,40	(11,00 - 16,30)	14,00	(10,80- 16,30)	12,60	(10,40 - 14,50)
HCT (%)	44,10	(20,80 - 51,00)	46,10	(35,00- 51,90)	40,80	(30,40 - 47,90)
MCV (fL)	47,50	(44,20 - 53,30)	48,00	(43,30- 53,60)	47,20	(42,20 - 50,10)
MCH (pg)	14,70	(13,40 - 29,60)	14,50	(14,20- 16,00)	14,70	(12,80 - 16,40)
MCHC (g/dL)	30,90	(29,80 - 62,60)	30,50	(28,50 - 33,20)	31,20	(29,70 - 34,30)
CHCM (g/dL)	29,60	(26,10 - 32,40)	29,80	(26,20 - 33,10)	30,00	(28,40 - 31,60)
RDW	15,60	(13,50 - 18,20)	15,80	(14,30 - 17,10)	15,90	(15,00 - 18,40)
HDW (g/dL)	2,09	(1,70 - 2,67)	2,07	(1,85 - 2,78)	2,24	(1,89 - 2,65)
Neutrophil (%)	84,90	(48,70 - 93,20)	81,40	(58,00 - 95,30)	80,10	(50,30 - 94,70)
Lymphocyte (%)	9,10	(2,30 - 41,40)	14,30	(3,60 - 24,60)	13,50	(3,60 - 43,10)
Monocyte (%)	2,30	(1,10 - 5,70)	2,20	(0,50 - 5,30)	2,40	(0,90 - 5,80)
Eosinophil (%)	2,60	(0,20 - 18,50)	2,40	(0,30 - 20,50)	3,30	(0,20 - 5,90)
Basophil (%)	0,10	(0,10 - 0,60)	0,10	(0,00 - 0,40)	0,20	(0,00 - 0,40)
LUC (%)	0,20	(0,00 - 1,80)	0,10	(0,00 - 0,80)	0,10	(0,00 - 0,70)
Neutrophilx10³ (cells/μL)	12,33	(2,38 - 31,33)	9,64	(4,52 - 25,04)	9,48	(2,43 - 31,59)
Lymphocytex10³ (cells/μL)	1,32	(0,74 - 3,62)	1,48	(0,80 - 2,99)	1,73	(0,96 - 4,23)
Monocytex10³ (cells/μL)	0,34	(0,05 - 0,87)	0,26	(0,06 - 0,53)	0,27	(0,16 - 0,52)
Eosinophil x10³ (cells/μL)	0,38	(0,03 - 2,36)	0,32	(0,03 - 3,41)	0,32	(0,03 - 0,70)
Basophilx10³ (cells/μL)	0,02	(0,00 - 0,14)	0,01	(0,00 - 0,05)	0,02	(0,00 - 0,05)
LUCx10³ (cells/μL)	0,02	(0,00 - 0,27)	0,01	(0,00 - 0,10)	0,02	(0,00 - 0,09)
LI	0,00	(0,00 - 2,68)	0,00	(0,00 - 0,00)	0,00	(0,00 - 2,96)
MPXI	13,50	(-1,90 - 19,90)	11,20	(5,80 - 19,30)	14,40	(-5,90 - 22,90)
Reticulocytex10⁹ (cells/μL)	24,40	(7,10 - 82,10)	23,00	(6,00 - 104,70)	31,60	(11,30 - 173,20)
Reticulocyte (%)	0,30	(0,10 - 0,80)	0,30	(0,10 - 1,40)	0,40	(0,10 - 1,90)
Reticulocyte MCV (fL)	59,20	(46,80 - 67,60)	62,50	(52,90 - 70,00)	60,20	(54,90 - 70,20)
PLTx10³ (cells/μL)	336,00	(79,00 - 706,00)	303,00	(122,00 - 417,00)	348,00	(103,00 - 588,00)
Plateletcrit (%)	0,44	(0,22 - 0,88)	0,41	(0,19 - 0,63)	0,53	(0,16 - 0,72)
MPV (fL)	12,60	(8,30 - 27,40)	13,20	(10,20 - 17,70)	14,10	(11,00 - 21,30)
PDW	65,90	(52,50 - 78,30)	63,90	(46,20 - 74,70)	63,10	(48,70 - 76,80)

Table 2. Reference CBC values for the free-ranging Iberian lynx, classified by age.

Tabla 2. Valores de referencia hematológicos para linces ibéricos de vida libre según edad.

serum chemistry parameters; some of these include food availability, animal density, seasonal changes, age, stress, geography (usually different populations), sexual maturity, reproductive status. Probably, stress is one of the major events that can affect blood parameters. When sampling a wild animal, the situation in itself obviously represents stress for the individual. It has also been shown that drugs commonly used for chemical immobilization may also influence hematology and serum chemistry parameters, as well as the time lapsed between immobilization and sampling.

Since all these situations can affect reference values, we believed that it would be more efficient and realistic to present the reference range using a more robust statistical method, dividing groups by age and specifying if the

PROLONGED CAPTIVITY	ADULT (n=21)		SUBADULT (n=16)		JUVENILE (n=15)	
	Median	Percentiles	Median	Percentiles	Median	Percentiles
		(2,50- 97,50)		(2,50- 97,50)		(2,50- 97,50)
WBCx10³ (cells/µL)	7,76	(3,56 - 18,77)	4,91	(2,41 - 7,86)	7,33	(3,62 - 16,25)
RBCx10⁶ (cells/µL)	9,18	(6,50 - 12,85)	9,81	(2,58 - 11,66)	8,82	(4,42 - 10,79)
HGB (g/dL)	13,60	(9,30 - 18,60)	14,45	(6,90 - 17,80)	12,70	(6,20 - 15,10)
HCT (%)	43,60	(30,10 - 62,40)	47,85	(11,50 - 58,20)	40,70	(18,40 - 50,00)
MCV (fL)	48,60	(40,30 - 51,90)	48,80	(44,60 - 51,10)	45,40	(41,60 - 50,90)
MCH (pg)	14,50	(12,10 - 21,30)	14,55	(13,90 - 27,00)	13,90	(13,30 - 16,50)
MCHC (g/dL)	30,50	(24,60 - 41,30)	30,15	(29,00 - 60,40)	30,90	(28,60 - 33,80)
CHCM (g/dL)	29,40	(25,80 - 31,60)	29,05	(24,80 - 30,60)	30,00	(27,40 - 34,10)
RDW	15,70	(14,20 - 19,40)	15,45	(14,50 - 20,60)	16,40	(15,10 - 20,70)
HDW (g/dL)	2,12	(1,74 - 5,67)	2,04	(1,67 - 2,34)	2,13	(1,59 - 2,60)
Neutrophil (%)	77,70	(34,00 - 95,00)	59,55	(43,00 - 79,80)	56,00	(37,70 - 80,70)
Lymphocyte (%)	17,20	(0,90 - 64,60)	32,90	(15,60 - 52,10)	35,70	(12,10 - 50,70)
Monocyte (%)	2,10	(1,10 - 3,60)	2,25	(1,20 - 4,70)	2,60	(1,40 - 4,70)
Eosinophil (%)	1,30	(0,00 - 4,90)	3,60	(0,00 - 9,80)	6,00	(0,00 - 8,90)
Basophil (%)	0,10	(0,00 - 0,40)	0,20	(0,00 - 0,40)	0,30	(0,10 - 0,90)
LUC (%)	0,10	(0,00 - 0,30)	0,00	(0,00 - 0,20)	0,10	(0,00 - 0,40)
Neutrophilx10³ (cells/µL)	5,54	(1,54 - 16,64)	3,08	(1,31 - 6,21)	4,06	(1,88 - 13,12)
Lymphocytex10³ (cells/µL)	1,40	(0,15 - 2,92)	1,66	(0,76 - 3,06)	2,49	(1,16 - 3,77)
Monocytex10³ (cells/µL)	0,18	(0,05 - 0,31)	0,10	(0,03 - 0,29)	0,22	(0,09 - 0,31)
Eosinophil x10³ (cells/µL)	0,09	(0,00 - 0,35)	0,20	(0,00 - 0,70)	0,32	(0,00 - 1,13)
Basophilx10³ (cells/µL)	0,01	(0,00 - 0,04)	0,01	(0,00 - 0,03)	0,02	(0,01 - 0,09)
LUCx10³ (cells/µL)	0,01	(0,00 - 0,04)	0,00	(0,00 - 0,01)	0,01	(0,00 - 0,04)
LI	0,00	(0,00 - 2,64)	0,00	(0,00 - 2,32)	0,00	(0,00 - 2,73)
MPXI	12,30	(0,90 - 17,70)	12,80	(4,40 - 21,30)	8,40	(-5,60 - 21,20)
Reticulocytex10⁹ (cells/µL)	27,80	(9,40 - 109,70)	48,10	(16,00 - 109,40)	46,90	(20,80 - 123,40)
Reticulocyte (%)	0,30	(0,10 - 1,50)	0,55	(0,20 - 2,00)	0,50	(0,20 - 1,70)
Reticulocyte MCV (fL)	57,80	(51,00 - 67,50)	60,80	(51,80 - 71,40)	59,30	(50,70 - 70,70)
PLTx10³ (cells/µL)	326,00	(229,00 - 780,00)	306,50	(236,00 - 715,00)	350,00	(92,00 - 698,00)
Plateletcrit (%)	0,39	(0,31 - 0,73)	0,39	(0,30 - 1,30)	0,42	(0,15 - 0,74)
MPV (fL)	12,00	(9,40 - 17,20)	12,60	(10,50 - 18,20)	11,00	(9,60 - 18,00)
PDW	66,30	(54,30 - 88,00)	69,50	(53,60 - 82,40)	61,50	(49,50 - 78,10)

Table 3. Reference CBC values for the Iberian lynx in prolonged captivity classified, by age.

Tabla 3. Valores de referencia hematológicos para el lince ibérico en cautividad según edad.

animal is free-ranging or captive. Using this division we decreased the number of animals used in the reference range tables, while we actually increased the accuracy and usefulness of the data provided in this paper.

Overall, the reference values obtained in our study are similar to those described for the same species by Beltrán et al., 1991 and other Felidae (Wack, 2003). The former authors described differences between sex for RBC and PCV but not for haemoglobin. Our study, with a larger number of animals, also showed that sex strongly affects red blood cell parameters, with females tending to have lower values than males. Our data on sex is similar to that described by other felids (Wack, 2003). Age is also an important factor to consider in reference values (Jain, 1993) and several statistically significant differences have been found in our study. Specifically,

QUARANTINE	ADULT (n=6)		SUBADULT (n=7)		JUVENILE (n=9)	
	Median	Percentiles	Median	Percentiles	Median	Percentiles
		(2,50- 97,50)		(2,50- 97,50)		(2,50- 97,50)
WBCx10³ (cells/µL)	10,80	(5,80 - 17,80)	7,90	(4,73 - 13,90)	8,91	(4,62 - 16,27)
RBCx10⁶ (cells/µL)	9,12	(7,13 - 10,52)	9,16	(7,89 - 11,04)	8,29	(3,25 - 9,72)
HGB (g/dL)	13,05	(10,30 - 15,50)	14,00	(11,60 - 16,90)	13,20	(7,90 - 14,80)
HCT (%)	41,80	(32,30 - 48,20)	47,90	(38,40 - 52,70)	41,10	(15,20 - 48,20)
MCV (fL)	46,55	(42,20 - 49,10)	48,80	(45,50 - 54,60)	48,20	(43,10 - 50,30)
MCH (pg)	14,60	(13,60 - 15,40)	15,30	(14,40 - 16,50)	15,00	(13,50 - 24,30)
MCHC (g/dL)	32,10	(30,30 - 32,60)	31,30	(28,00 - 32,10)	31,00	(29,80 - 52,10)
CHCM (g/dL)	30,25	(29,10 - 31,90)	29,30	(25,20 - 31,30)	30,20	(26,10 - 30,60)
RDW	16,05	(14,60 - 18,10)	15,20	(14,90 - 16,00)	16,70	(15,50 - 18,70)
HDW (g/dL)	2,14	(1,97 - 2,39)	2,19	(1,72 - 2,59)	2,21	(1,70 - 2,41)
Neutrophil (%)	72,50	(41,10 - 91,40)	60,50	(35,00 - 86,90)	68,50	(48,00 - 87,00)
Lymphocyte (%)	20,60	(4,70 - 49,30)	30,00	(9,20 - 52,30)	21,90	(7,90 - 45,30)
Monocyte (%)	2,25	(2,00 - 2,80)	2,70	(1,60 - 3,30)	3,60	(2,60 - 5,80)
Eosinophil (%)	5,25	(0,80 - 6,60)	5,90	(0,60 - 10,50)	2,40	(0,60 - 7,80)
Basophil (%)	0,20	(0,10 - 0,30)	0,20	(0,00 - 0,30)	0,20	(0,00 - 0,80)
LUC (%)	0,20	(0,00 - 0,60)	0,00	(0,00 - 0,30)	0,10	(0,00 - 1,60)
Neutrophilx10³ (cells/µL)	7,28	(2,46 - 16,27)	6,13	(1,88 - 12,07)	6,00	(2,69 - 14,16)
Lymphocytex10³ (cells/µL)	1,85	(0,83 - 3,00)	1,81	(1,11 - 3,18)	1,70	(1,23 - 4,33)
Monocytex10³ (cells/µL)	0,23	(0,13 - 0,50)	0,18	(0,07 - 0,37)	0,34	(0,14 - 0,62)
Eosinophil x10³ (cells/µL)	0,46	(0,14 - 0,79)	0,44	(0,06 - 0,61)	0,23	(0,03 - 0,47)
Basophilx10³ (cells/µL)	0,02	(0,01 - 0,03)	0,01	(0,00 - 0,03)	0,01	(0,00 - 0,05)
LUCx10³ (cells/µL)	0,02	(0,00 - 0,04)	0,00	(0,00 - 0,03)	0,01	(0,00 - 0,14)
LI	0,00	(0,00 - 0,00)	0,00	(0,00 - 0,00)	0,00	(0,00 - 2,52)
MPXI	4,25	(0,10 - 12,80)	10,70	(3,30 - 15,10)	12,30	(-1,60 - 16,90)
Reticulocytex10⁹ (cells/µL)	21,35	(8,20 - 52,20)	24,10	(5,40 - 165,40)	33,40	(12,90 - 120,70)
Reticulocyte (%)	0,30	(0,10 - 0,60)	0,20	(0,10 - 1,80)	0,40	(0,20 - 1,60)
Reticulocyte MCV (fL)	60,75	(52,90 - 69,70)	62,20	(57,80 - 66,60)	59,90	(50,30 - 68,10)
PLTx10³ (cells/µL)	347,50	(217,00 - 416,00)	303,00	(266,00 - 385,00)	384,00	(297,00 - 613,00)
Plateletcrit (%)	0,38	(0,29 - 0,44)	0,40	(0,32 - 0,47)	0,55	(0,40 - 0,90)
MPV (fL)	10,80	(9,30 - 15,30)	12,60	(10,70 - 14,80)	14,30	(13,00 - 16,30)
PDW	72,75	(58,90 - 78,40)	58,00	(54,40 - 65,10)	60,20	(54,00 - 69,10)

Table 4. Reference CBC values for the Iberian lynx in quarantine, classified by age.

Tabla 4. Valores de referencia hematológicos para el lince ibérico en cuarentena según edad.

subadults have significantly higher erythrocyte counts while cubs present a leukogram with statistically higher lymphocytes than any of the other age groups.

Free-ranging animals and those captured by box trap showed higher counts for leukocytes, lymphocytes, neuthrophils and monocytes, probably due to the stress induced by the capture method. On the other hand, animals kept in captivity did not show the same leukogram changes, indicating less stress due to the capture method. In addition, the same leukocyte trend is seen in animals associated to habitat, animals from Sierra Morena showed stress leukogram more often than Doñana ones; in fact, these may reflect the interaction between free-ranging and the method of capture, because box trap is the method most frequently used.

	Age	Sex	Origin population	Habitat Condition	Capture Method	Cyatuxzoon (PCR)
WBCx10^3 (cells/ L)			SM: 9,13 (9,45) ≠ D: 12,83 (14,26)	F:12,67 (12,00) ≠ C: 7,11 (6,88) and Q: 8,44 (7,35)	B:11,40 (12,20) ≠ N: 6,79 (6,00)	
RBCx10^6 (cells/ L)	SA: 9,36 (2,99) K: 8,24 (3,13)	F: 8,51 (3,24) ≠ M: 9,29 (2,56)	SM: 9,14 (3,08) ≠ CP: 8,16 (3,69)		B:9,05 (2,55) ≠ P: 7,94 (3,21)	
HGB (g/dL)	SA: 13,90 (3,67) ≠ Y: 12,42 (3,55) and K: 12,12 (3,42)	F:12,63 (4,10) ≠ M:13,67 (3,18)	SM:13,51 (3,88) ≠ CP: 11,80 (4,81)		B:13,36 (3,22) ≠ P: 11,63 (4,10)	
HCT (%)	SA: 45,29 (14,85) ≠ Y: 39,86 (13,97)	F: 40,51 (16,13) ≠ M: 44,01 (12,60)	SM: 43,66 (15,09) ≠ CP: 37,85 (17,67)		B:43,14 (12,51) ≠ P: 36,56 (14,25)	
MCV (fL)	SA: 48,37 (4,94) ≠ Y: 46,70 (4,50) and K: 46,16 (5,13)					N: 47,15 (4,68) ≠ P: 48,51 (4,90)
MCH (pg)						
MCHC (g/dL)					N:33,34 (17,07) ≠ P:31,99 (3,53)	N: 31,53 (7,15) ≠ P: 31,89 (12,03)
CHCM (g/dL)						
RDW	A: 15,81 (2,42) and SA: 15,74 (2,05) ≠ Y:16,53 (2,30)			F: 15,84 (1,82) and C: 16,07 (2,84) ≠ Q: 16,52 (2,62)	D:16,16 (3,71) ≠ P: 17,08 (3,46)	
HDW (g/dL)					B:2,13 (0,48) ≠ D: 2,75 (3,28)	
Neutrophil (%)	A: 76,65 (29,52) ≠ K: 50,89 (28,92)		SM: 68,64 (32,75) ≠ D: 76,35 (27,28) ≠ CP: 55,35 (26,31)	F: 77,64 (25,96) ≠ C: 65,28 (30,00) and Q: 62,00 (35,72)	B:75,08 (28,10) ≠ N: 57,16 (26,40)	
Lymphocyte (%)	A:17,79 (26,68), SA: 23,36 (27,30) and Y: 24,72 (26,73) ≠ K: 39,66 (26,80)		SM: 24,50 (29,00) ≠ D: 17,27 (24,49) ≠ CP: 36,48 (23,87)	F: 15,59 (21,18) ≠ C:28,45 (27,02) and Q: 30,00 (32,20)	B:18,61 (24,66) ≠ P: 35,29 (28,87)	
Monocyte (%)	A:2,34 (2,07), SA: 2,37 (1,97), and Y: 2,89 (2,02) ≠ K: 3,53 (3,13)	F: 2,80 (2,52) ≠ M: 2,43 (1,89)		F:2,48 (2,31) and C: 2,47 (1,80) ≠ Q: 3,13 (2,56)		
Eosinophil (%)						
LUC (%)				F:0,20 (0,54) ≠ C: 0,10 (0,18)		
Basophil (%)				F:0,16 (0,22) ≠ C: 0,23 (0,30)		
Neutrophilx10^3 cells/ L)	A: 9,71 (13,28) ≠ K: 4,69 (7,27)		SM: 6,70 (9,33) and CP: 5,20 (5,93) ≠ D: 10,48 (14,21)	F:10,97 (13,01) ≠ C:4,94 (6,65) and Q: 6,14 (8,10)	B:9,06 (12,07) ≠ N: 4,13 (5,62)	
Lymphocytex10^3 (cells/ L)	A: 1,54 (1,57), SA: 1,62 (1,28) and Y: 2,04 (1,61) ≠ K: 3,12 (1,82)		SM:1,80 (1,75) and D:1,62 (1,34) ≠ CP: 3,05 (1,50)	F:1,67 (1,53) and C: 1,79 (1,69) ≠ Q: 2,34 (2,03)	B:1,64 (1,54) ≠ P:2,88 (1,91)	N: 1,94 (1,72) ≠ P: 1,56 (1,59)
Monocytex10^3 (cells/ L)	A:0,27 (0,32), SA: 0,20 (0,25) and Y: 0,28 (0,25) ≠ K: 0,31 (0,34)	F: 0,27 (0,31) ≠ M: 0,24 (0,27)	SM: 0,23 (0,29) ≠ D: 0,30 (0,27)	F: 0,31 (0,29) and Q: 0,29 (0,33) ≠ C: 0,17 (0,17)		
Eosinophil x10^3 (cells/ L)						
LUCx10^3 (cells/ L)				F:0,03 (0,08) ≠ C: 0,01 (0,02)		
Basophilx10^3 (cells/ L)				F: 0,02 (0,04) ≠ C: 0,01 (0,03)		
LI					B:0,2 (1,40) and N: 0,43 (1,99) ≠ P:1,26 (2,75)	N: 0,43 (1,97) ≠ P: 0,06 (0,79)

Table 5: continue in the next page.

	Age	Sex	Origin population	Habitat Condition	Capture Method	Cyatuxzoon (PCR)
MPXI	A:10,52 (11,58), SA:12,05 (8,02) and Y: 10,91 (13,90) ≠ K: 3,15 (14,46)			F: 11,92 (11,75) and C: 10,72 (11,71) ≠ Q: 6,88 (13,11)	B:11,17 (11,40), D: 14,22 (9,96) and N: 10,11 (9,38) ≠ P: 5,45 (16,53)	
Reticulocytex10^9 (cells/ L)			SM: 42,54 (77,14) and D: 30,11 (51,90) ≠ CP: 74,05 (97,30)		B: 0,41 (0,68) ≠ P: 0,81 (1,27)	
Reticulocyte (%)	A:0,33 (0,47), SA: 0,45 (0,86) and Y: 0,55 (0,85) ≠ K: 1,01 (2,06)		SM: 0,50 (1,06) and D: 0,35 (0,59) ≠ CP: 0,95 (1,25)	F:0,37 (0,61) ≠ C: 0,52 (0,82)		
Reticulocyte MCV (fL)	A: 29,90 (40,41), SA: 38,95 (64,14) and Y: 44,95 (65,86) ≠ K: 78,52 (153,52)			F: 32,57 (52,63) ≠ C: 43,30 (54,95)		
PLTx10^3 (cells/ L)	A: 360,92 (253,47), SA: 313,69 (195,55) and Y: 359,30 (270,43) ≠ K: 511,36 (250,76)		SM:351,65 (248,31) and D: 350,00 (278,88) ≠ CP: 459,85 (231,79)		B:336,46 (230,19) and D: 320,60 (141,96) ≠ P: 469,24 (311,59)	N: 375,73 (281,28) ≠ P: 320,02 (182,44)
Plateletcrit (%)	A: 0,45 (0,25) and SA:0,41 (0,33) ≠ K: 0,60 (0,50)				B:0,43 (0,27) ≠ P: 0,58 (0,50)	
MPV (fL)			SM: 12,77 (4,29) ≠ D: 13,92 (6,85)			
PDW	A:67,33 (14,72) ≠ Y: 61,81 (14,20)					

Age:	**A: Adult;**	**SA: Subadult;**	**Y: Young;**	**K: Cub**
Sex:	**F: Female,**	**M: Male**		
Origin population:	**SM: Sierra Morena;**	**D: Doñana;**	**CP: Crossed Population**	
Habitat Condition:	**F: Free-ranging;**	**C: Prolonged captivity;**	**Q: Quarantine**	
Capture Method:	**B: Box-trap;**	**N: Net; D**	**B: Blow-pipe with dart;**	**P: Physical restraint**
Cytauxzoon (PCR):	**P: Positive;**	**N: Negative**		

Table 5. Statistical significant differences obtained comparing groups by age, sex, origin population, habitat condition, capture method and Cytauxzoon.

Tabla 5. Diferencias estadísticas significativas obtenidas tras la comparación entre grupos según la edad, sexo, población de origen, hábitat, método de captura y Cytauxzoon.

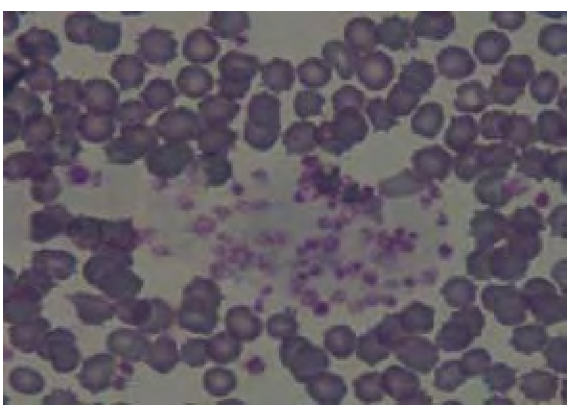

Figure 1. Normal erythrocytes and aggregated platelets (Diff-Quick stain, 100X).

Figura 1. Eritrocitos normales y plaquetas agregadas (Diff-Quick stain, 100X).

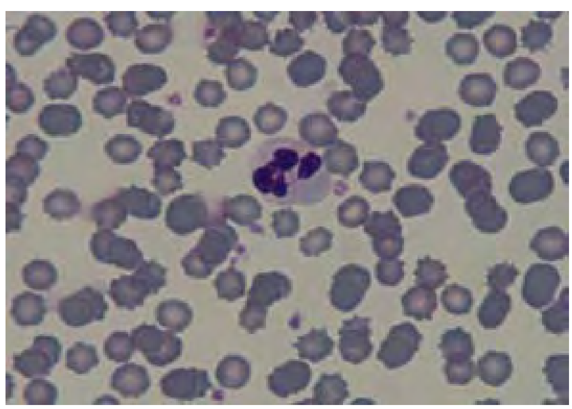

Figure 2. Neuthophil (Diff-Quick stain, 100X).

Figura 2. Neutrófilo (Diff-Quick stain, 100X).

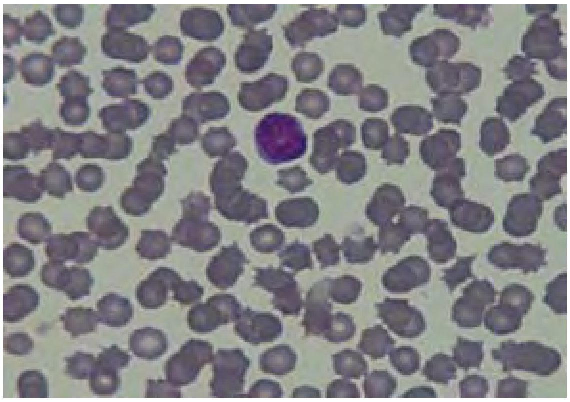

Figure 3. Lymphocyte (Diff-Quick stain, 100X).

Figura 3. Linfocito (Diff-Quick stain, 100X).

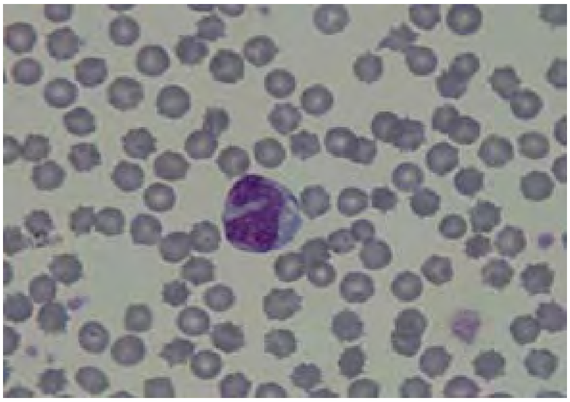

Figure 4. Monocyte (Diff-Quick stain, 100X).

Figura 4. Monocito (Diff-Quick stain, 100X).

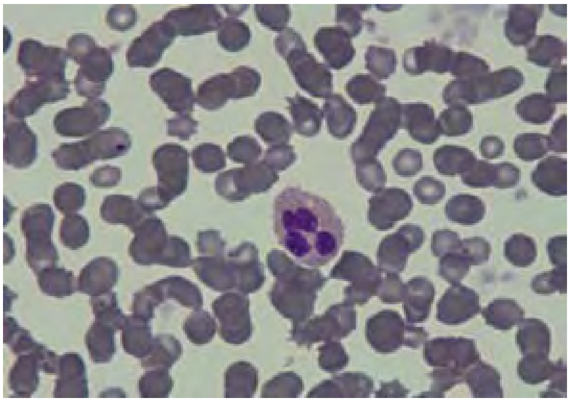

Figure 5. Eosinophil (Diff-Quick stain, 100X).

Figura 5. Eosinófilo (Diff-Quick stain, 100X).

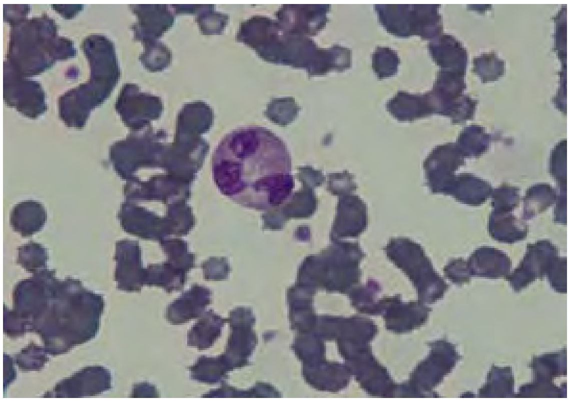

Figure 6. Basophil (Diff-Quick stain, 100X).

Figura 6. Basófilo (Diff-Quick stain, 100X).

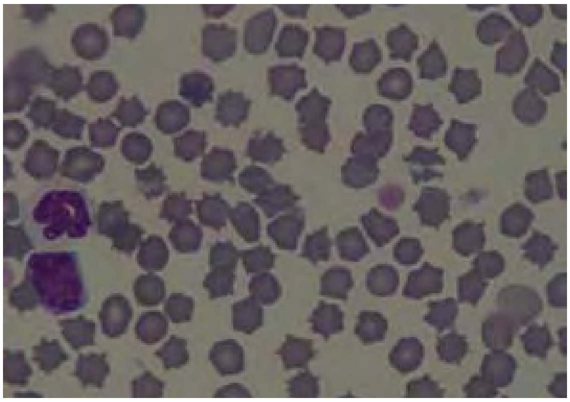

Figure 7. Lymphocyte and segmented neuthrophil to the left. Several erythrocytes shows Cytauxzoon sp microorganisms (Diff-Quick stain, 100X).

Figura 7. Linfocito y neutrófilo segmentado a la izquierda de la fotografía. Varios eritroctos muestran microorganismos de Cytauxzoon sp (Diff-Quick stain, 100X).

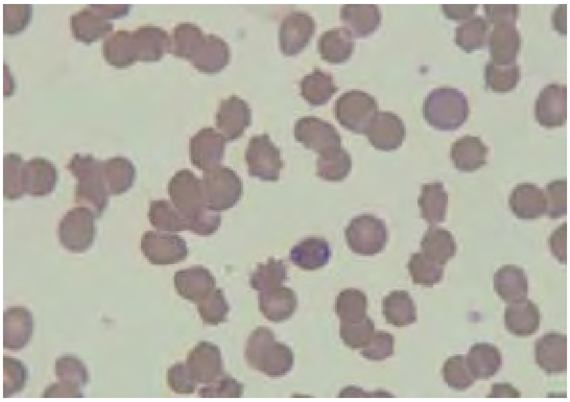

Figure 8. Two Babesia sp microorganism in one erythrocyte (Diff-Quick stain, 100X).

Figura 8. Babesia sp en el interior de un eritrocito (Diff-Quick stain, 100X).

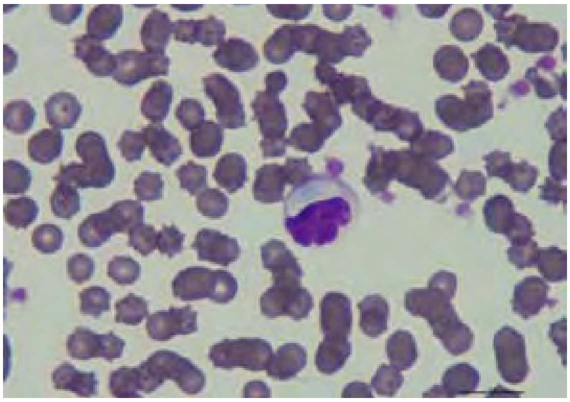

Figure 9. Hepatozoon sp in a neuthrophil (Diff-Quick stain, 100X).

Figura 9. Hepatozoon sp en un neutrófilo (Diff-Quick stain, 100X).

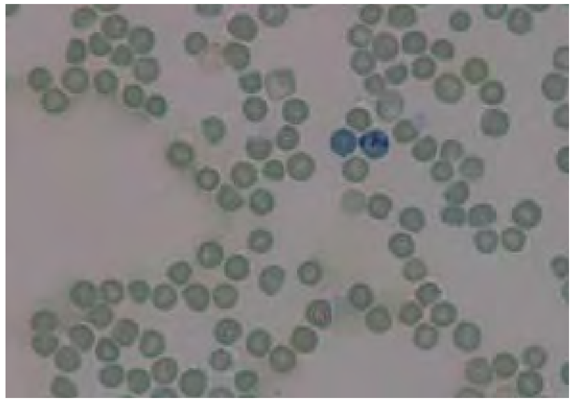

Figure 10. Supravital stain to show one punctatta and one aggregate reticulocyte (100x).

Figura 10. Tinción supravital que muestra un reticulocito punctatta y uno agregatta (100x).

Animals positive to Cytauxzoon spp presented higher MCV, as well as higher lymphocyte and platelet counts, however the presence of this blood parasite does not affect normal erythrocyte regeneration or shortens RBC lifespan because no statistical significant differences have been found.

In conclusion, the Iberian lynx reference values analyzed through this study have been obtained using a large number of individuals. Age, sex and environment have been considered as the major factors that influence the hemogram (see tables 1-6). Nevertheless, capture method also needs to be considered, since it affects the leukogram yielding a typical stress response.

Acknowledgements

We would like to thank the Environmental Council of the Andalusian Government for providing the Iberian lynx samples. We thank the staff from the LIFE-Nature Project, the Doñana Biological Station, the *Ex situ* Programme, and all the committed individuals that work for the conservation and recovery of the Iberian lynx.

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Photo: Pete Oxford

Todo lo que somos es el resultado de lo que hemos pensado; está fundado en nuestros pensamientos y está hecho de nuestros pensamientos.

Buda (563 AC-486 AC)

Serum biochemical parameters for the Iberian lynx *(Lynx pardinus)*: references values

Parámetros bioquímicos séricos en el lince ibérico *(Lynx pardinus)*: valores de referencia

Ignacio García, Fernando Martínez, Josep Pastor, Ester Bach-Raich,
Álvaro Muñoz, Astrid Vargas and Irene Zorrilla

Resumen

El conocimiento de los valores bioquímicos de referencia proporciona información sobre el estado de salud de una población. Esto es particularmente importante en el caso de las especies amenazadas para poder interpretar los datos de laboratorio, que a menudo constituyen el primer indicador de una enfermedad. No obstante, en la actualidad, se dispone de poca información sobre los parámetros bioquímicos en el lince ibérico *(Lynx pardinus)*. Los principales objetivos del presente estudio fueron: 1) establecer los valores de referencia de los parámetros bioquímicos en suero de lince ibérico; 2) comparar dichos valores con los del gato doméstico y otras especies de felinos, y 3) determinar si existen variaciones significativas en dichos valores, dependiendo de factores tales como el sexo, la edad (juveniles, subadultos o adultos) o la condición de hábitat (libres, cautivos o en cuarentena). Se determinaron los valores bioquímicos a partir de 104 ejemplares clínicamente sanos de lince ibérico. Se obtuvieron muestras de sangre de individuos de distintos sexos, edades, condiciones de hábitat, orígenes (metapoblaciones de Sierra Morena y Doñana) y métodos de captura (caja-trampa, red, dardo anestésico e inmovilización manual). La mayoría de los valores bioquímicos analizados fueron similares a los valores de referencia señalados para el gato doméstico y otras especies de felinos silvestres. Se encontraron niveles más altos de glucosa, lactato deshidrogenasa y creatina kinasa en comparación con el gato doméstico, lo cual concuerda con los resultados publicados en otras especies de felinos y podría estar asociado al estrés físico durante la captura.
Además, se observaron diferencias estadísticamente significativas en algunos parámetros en función del sexo, la edad y la condición de hábitat. Las concentraciones de fósforo fueron significativamente mayores en ejemplares machos comparados con las hembras, mientras que la urea presentó niveles más altos en las hembras. Se observaron niveles superiores de fosfatasa alcalina, fósforo y colesterol en ejemplares juveniles, mientras que los adultos presentaron niveles más altos de creatinina, proteínas totales y amilasa pancreática. Los subadultos presentaron valores superiores de triglicéridos en relación con los otros grupos de edades. Los niveles mayores de creatinina y

glucosa observados en ejemplares cautivos de lince podrían estar asociados a a la dieta. Por otra parte, las concentraciones más elevadas de encimas musculares en linces capturados en el medio silvestre se deben probablemente al estrés fisiológico. Los valores obtenidos pueden servir para establecer los intervalos de referencia para los parámetros bioquímicos séricos en el lince ibérico y deben ser tenidos en cuenta en cuenta en la evaluación del estado de salud de los animales.

Palabras clave
Lince ibérico, *Lynx pardinus*, valores bioquímicos séricos, intervalos de referencia

Abstract
Knowledge of baseline biochemical reference values provides information on the health status of a population. This is especially important when dealing with threatened species for the interpretation of laboratory data, which is often the first indicator of disease. However, the information on serum biochemical parameters currently available for the Iberian lynx *(Lynx pardinus)* is scarce. The main objectives of the present study were: 1) to establish the serum biochemical reference values for the Iberian lynx; 2) to compare these values with those of the domestic cat and other felid species and 3) to assess whether there were significant variations on these values depending on factors such as sex, age (juveniles, subadults and adults) or habitat condition (free-living, quarantine and prolonged captivity). Serum biochemical values were determined from 104 clinically healthy Iberian lynx. Blood samples were obtained from animals of different sexes, ages, habitat conditions, origin (Sierra Morena and Doñana metapopulations) and captured using different methods (trap-box, net, anaesthetic dart and manual immobilization). Most biochemical values analyzed were similar to the reported reference values for the domestic cat and other wild felid species. Higher glucose, lactate dehydrogenase and creatine phosphokinase levels in comparison to the domestic cats are in accordance with those published for other wild felids, and may be due to the physical stress experienced during the capture. Statistically significant differences between some parameters depending on sex, age and habitat condition were also observed. Phosphorus concentrations were significantly higher in males than in females, while urea was higher in females. The levels of alkaline phosphatase, phosphorus and cholesterol were higher in juvenile lynx, while creatinine, total proteins and pancreatic amylase were higher in adults. Subadults showed higher values of triglycerides with respect to the other age classes. The increased levels of creatinine and glucose observed in captive lynx may be attributed to the diet. On the other hand, the higher concentrations of enzymes in muscle obtained in free-living lynx were probably due to physiological stress. The values obtained in this study can serve to establish the reference intervals for the serum biochemical parameters in the Iberian lynx, and should be taken into account when assessing the health status of the animals.

Keywords
Iberian lynx, *Lynx pardinus*, serum biochemical values, reference intervals

Serum biochemical parameters for the Iberian lynx *(Lynx pardinus)*: references values

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Introduction

In recent years, numerous actions have been taken to conserve the Iberian lynx (Simón et al., this book; Vargas et al., this book; Calzada et al., this book). Furthermore, diverse studies have contributed towards expanding knowledge about the specie's biology (Ferreras et al., 2004; Johnson et al., 2004; Rodríguez and Delibes, 2004). Although the information regarding the conditions that affect the Iberian lynx has increased in recent years (Torres et al., 1998; Briones et al., 2000; Pérez et al., 2001; Vicente et al., 2004; Peña et al., 2006; Jimenez et al., 2008; Roelke et al., 2008), studies relating to the use of biochemical and blood parameters as indicators of health are still very limited in this species (Beltrán et al., 1991; García et al., 2008). Knowledge of biochemical values is especially important in non-domestic species for the interpretation of laboratory data, which is often used as a preliminary indicator of disease.

The objectives of the present study were: 1) to establish the serum biochemical reference values for the Iberian lynx; 2) compare these values with those of the domestic cat and other felid species, 3) to assess whether there are significant differences between these values, depending on factors such as sex, age (juveniles, subadults and adults) or habitat condition (free-living, quarantine and prolonged captivity).

Materials and methods

Animals analyzed

The reference values were established using blood plasma samples obtained from 104 clinically healthy animals (54:50) between the ages of one and 12 years, during the period 2006-2008.

The study includes lynx from the two existing metapopulations: 60 animals from Sierra Morena (the provinces of Jaén and Córdoba), and 37 from Doñana (provinces of Huelva and Seville), as well as seven young animals born in captivity, with parents from both metapopulations.

The age of the animals was established according to weight, dentition, fur and certain body characteristics. They were divided into kittens (<3 months), juveniles (between three and nine months), subadults (between nine months and two years) and adults (>2 years). However, due to the low number of kittens sampled (3), in order to establish biochemical values in the different ages classes, it was decided to group kittens with the juvenile class. So the study group was composed of 28 juveniles, 21 subadults, and 55 adults.

Fifty six samples came from lynx captured in the wild (54%), 42 from animals in captivity (40%), and six from animals still in quarantine (6%), in specific facilities for a period of two months to evaluate their health in terms of suitability for the *Ex situ* Captive Breeding Programme). Although the majority of the lynx were captured using cage traps (87), other methods included capture with nets (9), manual immobilization for kittens (6) and darts containing anaesthetic, fired using a blowpipe (2).

To determine the biochemical values for age groups within the different situations (free-living, captive or quarantined) samples were included from the same specimens provided that they were of different ages or

Value	n	Mean (±2DE)		Range	Percentiles (2.5; 50 and 97.5)			Normal range for domestic cat*
Glucose (mmol/l)	87	87.9	(56.1)	35.2-157.3	38.0	84.7	144.5	33-55
Cholesterol (mmol/l)	103	4.2	(3.0)	1.9-9.7	2.0	4.0	8.4	2-6
Triglycerides (mmol/l)	99	0.3	(0.4)	0.1-1.0	0.1	0.2	0.9	0.5-1.1
Uric acid (mmol/l)	90	33.0	(45.0)	5.9-110.6	5.9	28.0	105.3	< 59.50
Total proteins (g/l)	91	72.6	(16.8)	53.3-95.4	56.5	72.0	93.3	50-80
Albumin (g/l)	90	33.1	(14.3)	21.2-48.0	22.8	30.1	47.0	25-40
Phosphorus (mmol/l)	88	1.8	(1.0)	0.9-3.1	1.0	1.8	3.0	1-3
Iron (mmol/l)	86	16.6	(12.0)	4.1-28.8	4.3	16.7	28.4	12-39
Calcium (mmol/l)	86	2.5	(0.5)	1.7-3.2	2.0	2.5	3.2	2-3
Chloride (mmol/l)	88	32.6	(3.8)	26.2-38.7	26.8	32.7	37.1	32-37
Magnesium (mmol/l)	88	1.1	(0.4)	0.6-1.7	0.7	1.0	1.5	0.8-0.9
GGT (IU/l)	82	3.3	(3.9)	1.0-9.1	1.0	3.0	8.1	1-5
ALP (IU/l)	89	114.3	(172.5)	23.2-343.0	30.6	75.0	314.8	10-93
LDH (IU/l)	62	641.2	(1113.9)	27.0-1928.0	30.8	430.0	1903.9	10-273
Urea (mmol/l)	101	12.1	(6.4)	5.8-22.7	6.8	11.5	20.8	7-11
Creatinine (mmol/l)	99	118.7	(78.5)	50.4-221.0	54.8	114.9	204.7	43.0-128.9
AST (IU/l)	86	72.6	(122.0)	10.2-272.0	10.7	54.6	260.8	10-60
ALAT (IU/l)	88	52.6	(66.6)	9.0-154.9	10.9	44.3	140.5	6-83
Pancreatic amylase (IU/l)	89	1156.7	(562.9)	633.0-1805.0	669.0	1115.0	1689.5	40-1800
CK (IU/l)	81	846.3	(1842.4)	114.7-4697.0	115.8	488.0	3762.5	50-480
Lipase (IU/l)	84	14.7	(8.3)	8.2-26.2	8.3	13.8	23.9	<250

* From Kaneko et al,(1997), Bush (1991) and Jain (1993).

Table 1. Serum biochemical values from 104 Iberian lynxes *(Lynx pardinus)*.

Tabla 1. Valores de bioquímica sanguíneos obtenidos a partir de 104 linces ibéricos *(Lynx pardinus)*.

situations. Therefore, a total of 130 samples from 104 lynx were analysed to obtain the biochemical values in different situations according to age (tables 2, 3 and 4).

Most individuals were anaesthetised using a combination of ketamine (Imalgene 1000®) and medetomidine (Domtor®) (Martínez et al., this book). Males who underwent electroejaculation were anaesthetised with tiletamine and zolazepan. All anaesthetics were administered intramuscularly.

A complete physical examination was carried out on each animal, and the presence/absence of different clinical signs (anorexia, lethargy, depression, dehydration, anaemia, jaundice, dyspnoea, ocular or nasal discharge, vomiting or diarrhoea) was evaluated. Body temperature, respiratory rate, heart rate and pulse rate were also recorded. Only samples from healthy individuals that did not show any of the clinical signs previously mentioned, and whose anaesthetic parameters were within the normal range, were included in the study.

Obtaining the samples

Two millilitres of blood were taken from each animal via the cephalic vein using disposable syringes and 23G needles. The samples were collected in tubes containing lithium heparin (Becton-Dickinson®, Rutherford, New

	Free-living								
	Juveniles			Subadults			Adults		
Value	n	Mean (±2SD)		n	Mean (±2SD)		n	Mean (±2SD)	
Glucose (mmol/l)	16	75.4	(53.3)	15	62.3	(67.4)	20	77.2	(43.8)
Cholesterol (mmol/l)	21	5.2	(2.3)	17	5.2	(2.2)	30	3.3	(1.5)
Triglycerides (mmol/l)	20	0.4	(0.5)	15	0.2	(0.2)	29	0.4	(0.5)
Uric acid (mmol/l)	17	28.5	(43.5)	15	32.2	(47.3)	18	28.6	(43.2)
Total proteins (g/l)	17	70.6	(21.6)	16	76.7	(15.7)	21	76.0	(14.6)
Albumin (g/l)	17	33.8	(14.7)	16	35.7	(15.0)	20	35.6	(15.3)
Phosphorus (mmol/l)	17	2.5	(1.0)	16	2.2	(1.0)	20	1.6	(0.7)
Iron (mmol/l)	15	16.2	(15.9)	14	15.9	(12.1)	18	14.1	(12.8)
Calcium (mmol/l)	14	2.3	(0.5)	13	2.3	(0.8)	18	2.4	(0.5)
Chloride (mmol/l)	17	33.1	(6.7)	16	33.6	(5.2)	18	32.0	(4.0)
Magnesium (mmol/l)	15	1.0	(0.3)	14	1.1	(0.3)	19	1.0	(0.3)
GGT (IU/l)	15	3.1	(3.0)	16	2.5	(4.2)	19	3.2	(4.7)
ALP (IU/l)	16	175.2	(207.6)	16	142.2	(141.9)	20	59.3	(39.8)
LDH (IU/l)	14	757.0	(857.5)	12	762.8	(1322.2)	15	902.1	(1119.8)
Urea (mmol/l)	20	11.0	(6.2)	17	11.4	(4.7)	28	12.7	(7.3)
Creatinine (mmol/l)	21	88.6	(56.8)	17	96.8	(62.1)	27	123.8	(66.9)
AST (IU/l)	14	72.5	(73.5)	11	67.4	(88.5)	16	79.4	(71.9)
ALAT (IU/l)	15	54.4	(40.4)	14	63.7	(58.5)	20	56.8	(58.1)
Pancreatic mylase (IU/l)	16	917.6	(536.4)	15	1103.5	(344.9)	15	1103.7	(470.3)
CK (IU/l)	10	806.9	(876.0)	11	797.2	(1119.7)	14	989.3	(1350.9)
Lipase (IU/l)	14	15.0	(11.3)	14	16.2	(8.6)	17	15.1	(8.5)

Table 2. Serum biochemical values in free-ranging Iberian lynxes (*Lynx pardinus*).

Tabla 2. Valores de bioquímica sanguíneos de lince ibérico (*Lynx pardinus*) en libertad.

Jersey, USA) and within an hour, they were centrifuged at 400 g for 15 minutes. Finally, after separation of the serum, all the samples were sent by refrigerated courier and analysed within 24 hours of being extracted.

A total of 21 biochemical parameters were analysed using methods recommended by the IFCC (International Federation of Clinical Chemistry, Thomas, 1998; Grant et al., 1999; Schumann and Klauke, 2003) with the help of an automatic analyser (RA115000 - CLIMATE MC 15, RAL S.A., Spain). The mean, ± twice the standard deviation, maximum, minimum and percentiles (2.5, 50 and 97.5) were determined for each parameter.

Statistical analysis

The Pearson Correlation test was used to evaluate the collinearity between sex, age, population, habitat condition and capture method. Likewise, differences between groups were analyzed using the Tukey Post Hoc Multiple Comparison Test.

For each biochemical parameter, the normality in the distribution of values and the homogeneity of variance were determined using the Kolmogorov-Smirnov and Levene's tests, respectively. The parameters that showed a normal distribution and equal variances were analysed using the parametric one factor ANOVA test. When the

	Captive								
	Juveniles			Subadults			Adults		
Value	n	Mean (±2SD)		n	Mean (±2SD)		n	Mean (±2SD)	
Glucose (mmol/l)	13	113.0	(60.2)	12	105.3	(37.0)	25	93.0	(48.7)
Cholesterol (mmol/l)	12	6.5	(3.2)	12	4.9	(2.5)	25	3.8	(2.5)
Triglycerides (mmol/l)	13	0.3	(0.3)	12	0.3	(0.4)	23	0.2	(0.2)
Uric acid (mmol/l)	13	32.7	(35.1)	12	39.9	(44.5)	25	28.2	(34.7)
Total proteins (g/l)	13	66.9	(14.0)	12	70.3	(12.1)	24	72.8	(13.1)
Albumin (g/l)	13	30.7	(13.2)	12	37.0	(15.6)	24	32.7	(13.6)
Phosphorus (mmol/l)	13	2.5	(1.2)	12	1.9	(1.1)	25	1.5	(0.7)
Iron (mmol/l)	13	17.0	(8.9)	12	15.8	(9.8)	24	16.3	(12.3)
Calcium (mmol/l)	13	2.5	(0.7)	12	2.4	(0.5)	24	2.5	(0.3)
Chloride (mmol/l)	13	32.7	(3.5)	10	33.3	(3.2)	25	32.8	(2.8)
Magnesium (mmol/l)	13	1.1	(0.6)	11	1.0	(0.1)	25	1.0	(0.4)
GGT (IU/l)	12	3.1	(3.3)	11	2.4	(3.5)	23	2.9	(4.2)
ALP (IU/l)	13	275.0	(117.1)	12	117.4	(158.8)	24	62.3	(56.1)
LDH (IU/l)	11	258.7	(334.4)	10	374.8	(641.8)	11	281.6	(562.7)
Urea (mmol/l)	13	10.6	(2.6)	12	10.6	(4.6)	24	12.5	(5.0)
Creatinine (mmol/l)	13	91.6	(33.0)	12	122.1	(68.8)	23	142.3	(64.3)
AST (IU/l)	13	41.6	(40.6)	12	39.0	(33.9)	24	34.5	(58.4)
ALAT (IU/l)	12	48.6	(54.6)	12	45.4	(45.0)	25	38.2	(66.4)
Pancreatic amylase (IU/l)	13	1012.9	(360.9)	12	1035.2	(470.2)	24	1204.0	(527.2)
CK (IU/l)	13	484.5	(626.4)	12	367.8	(200.1)	25	374.1	(708.6)
Lipase (IU/l)	12	12.9	(4.4)	12	13.0	(7.5)	24	12.6	(6.6)

Table 3. Serum biochemical values in captive Iberian lynxes *(Lynx pardinus)*.

Tabla 3. Valores de bioquímica sanguíneos de lince ibérico *(Lynx pardinus)* en cautividad.

variable did not show a normal distribution and/or homogeneity of variance, the non-parametric Kruskal-Wallis test was selected. Values with $P<0.05$ were considered statistically significant. The statistical analyses were carried out using SPSS version 14.0 software (Statistical Package for Social Sciences, Chicago, IL, USA).

Results

The biochemical reference values of the 21 parameters analyzed are shown in Table 1. Likewise, biochemical values in the different age groups sampled in free-living, captivity and quarantine are shown in tables 2, 3 and 4, respectively.

The correlation analysis showed that sex and age were independent from the different variables included in the study. However a significant correlation was found between habitat condition, the population and the method of capture. Therefore, given the biological and clinical relevance, and the importance of the management of the situation in which the animal is found, the variables "population" and "capture method" were eliminated in order to determine statistical differences.

The levels of alkaline phosphatase, phosphorus and cholesterol were higher in young animals, whereas creatinine, total protein and pancreatic amylase were higher in adults. The subadults showed higher triglyceride

	Quarantine								
	Juveniles			Subadults			Adults		
Value	**n**	**Mean (±2SD)**		**n**	**Mean (±2SD)**		**n**	**Mean (±2SD)**	
Glucose (mmol/l)	3	120.8	(16.4)	3	105.5	(28.9)	5	68.3	(36.4)
Cholesterol (mmol/l)	3	6.3	(4.3)	3	3.1	(1.5)	5	4.4	(1.0)
Triglycerides (mmol/l)	3	0.4	(0.6)	3	0.2	(0.1)	5	0.4	(0.3)
Uric acid (mmol/l)	3	31.9	(54.3)	3	47.2	(58.3)	5	39.7	(47.2)
Total proteins (g/l)	3	66.6	(12.9)	3	69.9	(10.5)	5	80.5	(22.0)
Albumin (g/l)	3	32.9	(21.2)	3	36.0	(19.2)	5	31.7	(17.8)
Phosphorus (mmol/l)	3	2.1	(0.7)	3	2.0	(0.5)	5	1.5	(0.4)
Iron (mmol/l)	3	20.0	(9.5)	3	19.3	(12.8)	5	16.9	(9.4)
Calcium (mmol/l)	3	2.8	(0.8)	3	2.3	(0.3)	5	2.5	(0.4)
Chloride (mmol/l)	3	32.1	(2.2)	3	33.7	(5.1)	5	33.8	(5.2)
Magnesium (mmol/l)	2	1.0	(0.4)	3	0.9	(0.0)	4	1.2	(0.4)
GGT (IU/l)	3	1.8	(0.6)	3	4.3	(8.2)	4	3.8	(5.4)
ALP (IU/l)	3	212.9	(82.1)	3	80.7	(51.2)	5	38.4	(25.6)
LDH (IU/l)	3	450.8	(485.6)	1	1368.0	(0.0)	5	720.0	(1425.2)
Urea (mmol/l)	3	13.5	(8.4)	3	8.5	(3.5)	4	12.5	(7.7)
Creatinine (mmol/l)	3	123.8	(133.5)	3	136.4	(29.9)	5	106.8	(32.7)
AST (IU/l)	2	44.5	(35.4)	3	52.1	(54.7)	4	74.2	(68.5)
ALAT (IU/l)	2	62.0	(2.8)	3	32.1	(16.2)	4	73.1	(57.1)
Pancreatic amylase (IU/l)	3	1213.0	(791.0)	3	1028.3	(224.6)	5	1408.2	(538.6)
CK (IU/l)	3	763.7	(443.0)	3	860.7	(2011.7)	5	1033.1	(1324.9)
Lipase (IU/l)	3	14.2	(11.9)	3	14.3	(7.5)	5	17.0	(5.7)

Table 4. Serum biochemical values for Iberian lynxes *(Lynx pardinus)* in quarantine.

Tabla 4. Valores de bioquímica sanguíneos de lince ibérico *(Lynx pardinus)* en cuarentena.

values compared with other age groups. The concentration of phosphorus was significantly higher in males than in females, whereas urea was greater in females.

Animals sampled in different situations showed statistically significant differences in several biochemical parameters. The wild caught and quarantined lynx had higher levels of AST, ALT, CK, LDH, triglycerides and lipase and than those kept in captivity. Furthermore, they showed significantly higher concentrations of glucose in captive and quarantined individuals compared with the free-living lynx analyzed in this study.

Discussion

Most of the biochemical parameters analysed showed values similar to those reported in other species of lynx (Fuller et al., 1985; Weaver and Johnson, 1995; Miller et al., 1999), the domestic cat (Bush, 1991; Jain, 1993; Kaneko et al., 1997; O'Brien et al., 1998) and other species of wild feline (Currier and Russell, 1982; Hawkeye and Hart, 1986; Marco et al., 2000).

The differences observed between the values previously obtained by Beltrán et al., (1991) and our values are possibly related to sample size, habitat condition and the methodology employed. In the present study, the sample size could be considered representative of the total population due to the large number of animals analysed. The results are also similar to those published by our team in a previous study based on 31 individuals sampled between 2004 and 2006 (García et al., 2008).

In this study, we have obtained higher concentrations of glucose, AST, CK, LDH, ALP and urea than those established for the domestic cat. These results agree with those reported in other species of wild cat and are possibly a result of increased levels of stress suffered by these species during capture (Kocan et al, 1985; Hawkeye and Hart, 1986; Weaver and Johnson, 1995; Miller et al., 1999). Intraspecific variability, nutritional factors, muscle damage, muscle mass and/or breeding conditions are also possible factors involved in the differences between these parameters (Currier and Russell, 1982; Fuller et al., 1985; Meyer et al., 1992; Marco et al., 2000).

The high levels of glucose obtained in Iberian lynx in comparison with the domestic cat have also been described in other wild feline species (Currier and Russell, 1982; Fuller et al., 1985; Weaver and Johnson, 1995; Miller et al., 1999). The process of immobilisation and manipulation during capture has a greater adverse effect on wild species than on domestic cats. Stress and the use of anaesthetics during capture decrease the reduction of hepatic glycogen, and therefore, increase the concentration of glucose in blood (Miller et al., 1999).

Urea levels in the Iberian lynx are also above the established ranges in the domestic cat (Kaneko et al., 1997). Higher values of urea were also found in other species of wild cat (Fuller et al., 1985; Marco et al., 2000). The high concentrations found in Canada lynx *(Lynx canadensis)* adults by Weaver and Johnson (1995) were attributed to chronic renal dysfunction and, similarly, autoimmune membranous glomerulonephritis has recently been described in Iberian lynx (Jiménez et al., 2008; Jimenez et al., this book). Although this study revealed higher levels of urea in adults compared with juveniles and subadults, these differences were not statistically significant.

Young animals had significantly higher concentrations of ALP, phosphorus and cholesterol than adult animals. The differences with respect to the ALP are expected, given that the activity of this enzyme decreases as osteoblastic activity stops towards adulthood (Kaneko et al., 1997). These findings coincide with those previously observed in the Iberian lynx (Beltrán et al., 1991; García et al., 2008), Canada lynx (Weaver and Johnson, 1995) and other felid species (Paul-Murphy et al., 1994).

The concentration of serum creatinine is directly related to muscle mass, so it is normal for levels obtained in adults to be higher. Likewise, increased physical activity and nutritional factors are also associated with high levels of creatinine, total protein and pancreatic amylase found in adults (Weaver and Johnson, 1995; Dunbar et al., 1997; Miller et al., 1999). Recently, Jimenez et al,(2008) found elevated levels of creatinine in Iberian lynx affected by membranous glomerulonephritis, which is more prevalent in adult animals.

Females showed higher levels of urea and lower levels of phosphorus than males. These results do not match those previously reported for this species (Beltrán et al., 1991; García et al., 2008) and more specific studies are needed to explain these differences. The higher levels of albumin previously reported in female Iberian lynx (García et al., 2008) and European wildcat *(Felis sylvestris)* (Marco et al., 2000) were not observed in this study.

The increase in levels of creatinine and glucose in captive lynx could be related to diet. Captive animals are offered a diet based on domestic rabbits (85%), and a small proportion of quail and beef. On the other hand, high concentrations of muscle enzymes (AST, ALT, CK, LDH) found in free-living lynx are probably due to stress, intense physical exercise and/or muscle damage associated with the capture (Seal and Hoskinson, 1978; Currier and Russell, 1982; Fuller et al., 1985; Marco et al., 2000). Previous studies showed elevated levels of AST and ALT in free living Iberian lynx and bobcat *(Lynx rufus)* (Fuller et al., 1985; Beltrán et al., 1991; García et al., 2008) compared to Canada lynx in captivity (Weaver and Johnson, 1995).

Conclusions

The values obtained in this study may serve to establish the biochemical reference intervals in the Iberian lynx and should be taken into account when assessing the animal's physiological status. The data is representative of the total population as it has been obtained from more than half of the existing lynx population, including animals of different ages, sexes, habitat conditions and metapopulations.

Acknowledgments

We give thanks to Luis, Eva, Anabel and Mariló for their industrious cooperation and methodical work in the laboratory; to Caterina and Sebas for their help in drafting the chapter; to Cristina for the collection of data; to all LIFE lynx project staff, to the researchers and field staff of the Doñana Biological Station, and the staff of the *Ex situ* Conservation Programme for the capture of individuals and obtaining samples and information about the animals. We are indebted to Josie Dade for translating this manuscript. This work has been possible thanks to the Environment Ministry of the Andalusian Government, which has commissioned the study and provided samples. The work has been carried out thanks to the Environmental Management Corporation (EGMASA) and the Environment Ministry of the Andalusian Government.

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Photo: Antonio Rivas

Photo: Antonio Rivas

Joy in looking and comprehending is nature's most beautiful gift.

Albert Einstein
(1879-1955)

Diseases of the Iberian lynx *(Lynx pardinus)*: histopathological survey, lymphoid depletion, glomerulonephritis and related clinical findings

Enfermedades del lince ibérico *(Lynx pardinus)*: estudio histopatológico, depleción linfoide, glomerulonefritis y hallazgos clínicos relacionados

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Resumen

Entre los años 1998 y 2006, se realizó un estudio de investigación mediante evaluación post-mortem e histológica de linces ibéricos *(Lynx pardinus)* de las poblaciones de Doñana y Sierra Morena. Los estudios iniciales revelaron la presencia de depleción linfoide, glomerulonefritis y hialinosis folicular esplénica entre otros hallazgos menos frecuentes como tuberculosis y carcinomas de células escamosas. Muestras de tejidos fijados en formol de 40 linces ibéricos fueron procesadas, teñidas con hematoxilina y eosina y evaluadas. Además, se emplearon tinciones especiales, inmunohistoquímica y microscopía electrónica en los tejidos renales y linfoides de casos seleccionados. El estado inmune fue evaluado mediante técnicas de linfoproliferación y fenotipado de células mononucleares sanguíneas, utilizando muestras de sangre de 23 linces ibéricos. Para evaluar la función renal fueron recogidas muestras de sangre (n=23) y orina (n=17) de forma prospectiva. En la mayoría de los animales de este estudio se observó una depleción variable de células T y B en los tejidos linfoides periféricos. Por su parte, los ensayos de linfoproliferación y citometría de flujo mostraron una respuesta reducida y reducción en el número de las poblaciones linfocitarias, respectivamente, en la mayoría de los animales. También se observaron depósitos de hialina en muchos de los folículos esplénicos evaluados. En todos los animales se halló glomerulonefritis membranosa en diferentes grados de severidad. En 16 de los 23 animales vivos analizados se hallaron indicios de enfermedad renal crónica. La disfunción renal y las alteraciones inmunitarias pueden tener repercusiones en la conservación de las poblaciones de lince ibérico supervivientes y deberían tenerse en consideración.

Palabras clave

Histopatología, inmunidad, hialinosis folicular esplénica, glomerulonefritis membranosa, inmunocomplejos

Abstract

A research study was conducted, between the years 1998-2006, which involved thorough post-mortem and histological evaluations of Iberian lynxes from the two remaining, populations of Doñana National Park and Sierra Morena. Initial studies revealed lymphoid depletion, glomerulonephritis and splenic follicular hyalinosis among other less frequent findings such as tuberculosis and squamous cell carcinomas. Formalin fixed tissue samples of all organ systems from 40 Iberian lynxes were routinely processed, stained with hematoxilin-eosin and evaluated. Additionally, special stains, immunohistochemistry and electron microscopy were applied on renal and lymphoid tissues of selected cases. The immunity status was evaluated by means of lymphoproliferative stimulation and phenotyping of peripheral blood mononuclear cell types using whole blood samples from 23 Iberian lynxes. Blood (n=23) and urine (n=17) samples were prospectively collected for evaluation of renal function. Various degrees of both B and T cells depletion in peripheral lymphoid tissues were noted in the majority of animals. Lymphoproliferative assays and flow cytometry also revealed diminished responses and decreased numbers of lymphocytes respectively in the majority of animals. Dense hyaline material was noted in many of the spleens evaluated. Different stages of membranous glomerulonephritis (MGN) were present in all of the animals. Sixteen of the 23 live animals evaluated also contained some signs of chronic renal disease. Renal impairment and immunity alterations may impact conservation management of the surviving populations and should be considered.

Keywords

Histopathology, immunity, splenic follicular hyalinosis, membranous glomerulone–phritis, immune complexes

Photo: Antonio Rivas

Diseases of the Iberian lynx *(Lynx pardinus)*: histopathological survey, lymphoid depletion, glomerulonephritis and related clinical findings

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Introduction

Among the many aspects necessary for the study and conservation of endangered species, knowledge of the health status and diseases that can affect these species is highly relevant. One of the ways to carry out this premise with the Iberian lynx *(Lynx pardinus)* has been by means of post-mortem gross and histologic examination in search of lesions and diseases with possible repercussion in the survival of this critically endangered species. For this purpose, a research study involving exhaustive post-mortem and histologic evaluation of Iberian lynxes deceased between the years 1998-2006 was conducted. Initial studies revealed alarming indices of lymphoid depletion, glomerulonephritis and splenic follicular hyalinosis among other somewhat frequent findings such as tuberculosis and squamous cell carcinomas, which derived into a more in depth study of the morphology and pathogenesis of these lesions. Consequently due to these findings, clinical studies evaluating immunity status and renal function in the living populations were also conducted.

For the purpose of this chapter, those studies that derived from initial findings in necropsy and histopathology will be described separately from the clinical studies that were prospectively conducted on the living population as a result of the initial histological findings. This research has led to two scientific publications (Jiménez et al., 2008; Peña et al., 2006) and readers are referred to these articles for a more detailed description of the methodology and results obtained from these studies.

Materials and methods

Histological evaluation of formalin fixed Iberian lynx necropsy samples

Formalin fixed tissue samples of all organ systems were evaluated from 40 (19:21) Iberian lynxes from the areas of Doñana (n=24) and Sierra Morena (n=9) between 1998 and 2006. Thirty one animals were free-ranging and nine were held in captivity. Death was attributed in the majority of cases to car accidents (n=23), followed by diseases (tuberculosis, neoplasms, unknown, etc., n=8), trauma (n=3) and was undetermined in the remaining cases.

Samples were routinely processed and stained with hematoxilin-eosin (h-e) for examination. Additional special stains such as acid-fast, PAS, Masson's trichrome were employed when pertinent for thorough evaluation of lesions. For more profound studies on the immunity status, glomerulonephritis and splenic hyalinosis previously mentioned, additional stains, immunohistochemistry and electron microscopy were applied on pertinent tissues from the above cases as follows.

Lymphoid depletion

Peripheral lymphoid organs including lymph nodes, spleen, mucosa associated lymphoid tissue (MALT) and thymus were histologically evaluated and lymphoid depletion was categorized and scored considering decreases in numbers of lymphoid follicles and cell density, and the number and type of affected lymphoid tissues. Lymphoid cell types were categorized by means of immunohistochemistry using antibodies against CD3 (T-cell), CD79 (B-cell marker), MAC387 (myeloid and histiocytic marker), CD68 (macrophage marker) (Peña et al., 2006).

Splenic follicular hyalinosis

The presence of splenic follicular hyalinosis was evaluated using routine h-e and special stains (PAS, Masson's trichrome, immunohistochemistry with antibodies against laminin, fibronectin, type IV collagen and IgM, IgA and IgG). Electron microscopy (EM) and immunogoldlabelling (using antibodies against IgA, IgG and IgM) of selected formalin-fixed and glutaraldehyde fixed samples were also carried out (Jiménez et al., 2006).

Glomerulonephritis

In addition to h-e, processed renal tissues were also stained with PAS, Masson's trichrome, silver and immunohistochemical stains against laminin, fibronectin, type IV collagen, IgA, IgG and IgM. Glomerular lesions were characterized and scored according to severity (mild, moderate and severe). Electron microscopy and immunogoldlabelling (using antibodies against IgA, IgG and IgM) of selected formalin and glutaraldehyde fixed samples were conducted in a similar manner to the splenic samples (Jiménez et al., 2008).

Clinical evaluation of immunity status and renal function in the living population

Immunity status

The immunity status was evaluated by means of lymphoproliferative stimulation and phenotyping of peripheral blood mononuclear cell types using specific antibodies against the various lymphoid populations and flow cytometry analysis. Lymphoproliferative responses were assessed by stimulation with mitogen concavalin A (conA).For these experiments, serum and whole blood samples from 23 (11:12) Iberian lynxes, eight free-ranging and 15 captive, from the Doñana and Sierra Morena populations were prospectively collected.

Evaluation of renal function

Urinalyses from nine of the necropsied Iberian lynxes were available and revised for evaluation of renal function. Blood (n=23) and urine (n=17) samples were prospectively collected from live animals in both populations and used for the same purpose. Serum biochemistry parameters related to renal function such as total proteins, albumin, blood urea nitrogen (BUN), creatinine, calcium and phosphorus levels were determined and evaluated. Urinalyses included urine specific gravity (USG), albumin, glucose, leukocytes, blood, urine sediment and determination of protein/creatinine ratio. Chronic renal disease was categorized into four stages following adapted recommendations of the International Renal Interest Society (IRIS) (Jiménez et al., 2008).

Results

Histological evaluation of formalin fixed Iberian lynx necropsy samples

The most important findings noted in the necropsied animals were the lymphoid depletion, splenic hyalinosis and glomerulonephritis. Because of the importance and the research that resulted from these findings, these lesions will be addressed separately and further along in this chapter.

Among the remaining lesions noted within organ systems, it is important to remark the presence of tuberculosis in four of the examined animals *(Isabel, Pablo, Fermín* and *Piña)* (Figure 1) and three cases of cutaneous squamous

Figure 1. Bilateral uveitis in an Iberian lynx with tuberculosis.

Figura 1. Uveítis bilateral en un lince ibérico con tuberculosis.

Figure 2. Iberian lynx ear with squamous cell carcinoma.

Figura 2. Oreja de lince ibérico con carcinoma de células escamosas.

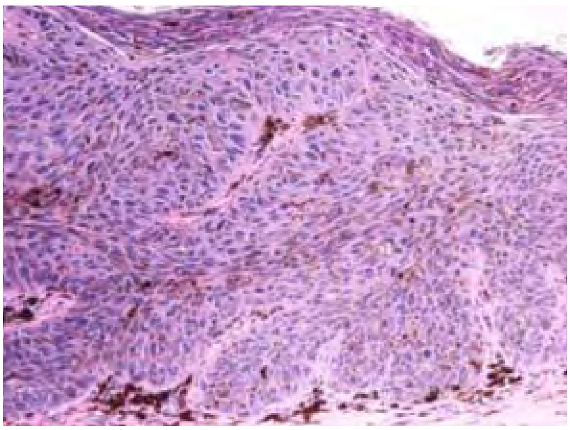

Figure 3. Skin; Iberian lynx, squamous cell carcinoma.

Figura 3. Piel; lince ibérico, carcinoma de células escamosas.

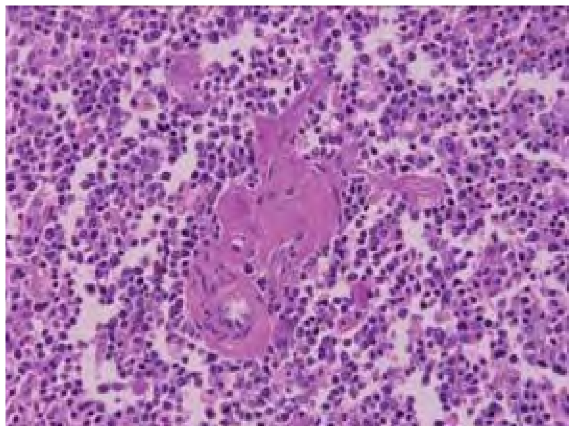

Figure 4. Spleen; Iberian lynx, splenic follicular hyaline deposits enveloping capillaries and surrounding the centroarteriolar tunica adventitia.

Figura 4. Bazo; lince ibérico, depósito de hialina folicular englobando capilares y rodeando la túnica adventicia centroarteriolar.

cell carcinomas (*Isabel, Celi* and *Ángeles*) (Figure 2).Three of the four animals with tuberculosis contained disseminated granulomas with intralesional acid-fast bacilli in organs such as eyes, lungs, liver, lymph nodes and adrenal glands among others, while the other case contained granulomas in lungs only.

Cutaneous squamous cell carcinomas were located in areas of pigmented skin in all three animals (Figure 1). In one animal *(Isabel)*, previous biopsies of the neoplasm had been submitted and histopathology revealed an *in situ* squamous cell carcinoma (Figure 3). In later necropsy samples, disruption of basement membranes was observed indicating progression from an *in situ* to a squamous cell carcinoma. In another animal, the neoplasm also contained features of an *in situ* squamous cell carcinoma with regional disruption of basement membranes, again suggesting progression from an *in situ* to a squamous cell carcinoma.

Lymphoid depletion

Categorization and scoring of peripheral lymphoid organs revealed some degree of both B and T cells depletion in peripheral lymphoid tissues in all animals. These findings were portrayed in a publication including animals from the years 1998 and 2003 (Peña et al., 2006). Similar findings with variable degrees of severity were noted in Iberian lynxes necropsied within the following years included in this survey.

Splenic follicular hyalinosis

Many splenic follicles contained central, dense accumulations of pale to bright eosinophilic, glassy, homogenous hyaline material rarely enveloping small capillaries (Figure 4). This dense hyaline material was noted in approximately 76% of the spleens evaluated for this study. Special stains and immunohistochemistry revealed diverse basement membrane and extracellular matrix components such as type IV and type VIII collagen, laminin and fibronectin, and silver stains confirmed the presence of capillaries within this material. Congo red stains were negative, ruling out amyloidosis.

Electron microscopy of selected samples confirmed the presence of collagen within this material. Additionally, electron dense deposits were noted within splenic arteriolar basement membranes. These structures were identified as immune complexes (IC) and immunogoldlabelling revealed IgG and IgM in these complexes. Other changes noted in these follicles were degeneration of arteriolar endothelial cells and medial smooth muscle degeneration (Jiménez et al., 2006).

Glomerulonephritis

The histopathological, immunohistochemical and ultrastructural studies revealed the presence of different stages of membranous glomerulonephritis (MGN) in all of the animals. The severity increased with age. Electron dense deposits compatible with immune complexes were identified and immunogoldlabelling and immunohistochemistry revealed the presence of IgG and IgM within the lesions. The urinalyses of 10 of the necropsied Iberian lynxes revealed a high prevalence of hypostenuria and proteinuria, indicating glomerular filtration impairment (Jiménez et al., 2008).

Clinical evaluation of immunity status and renal function in the living population

Immunity status

Lymphoproliferative responses were variable. In a low percentage of animals the response was considered adequate while in a high percentage (approximately 72%) of the animals analyzed, the response was diminished. Flow cytometry assays revealed reduced numbers of circulating T lymphocytes in 90% of the animals analyzed and reduced circulating B lymphocytes in 60% of the samples. Similar decreases were noted in subpopulations of CD4 and CD8 T lymphocytes.

Evaluation of renal function

According to the IRIS classification system (Polzin, 2004), 16 out of 23 animals contained some signs of chronic renal disease. Among the altered biochemical parameters noted as indicative of renal disease were uremia, hyperphosphatemia, hypercalcemia and elevated creatinine. Urine analyses revealed proteinuria and hypostenuria in many cases. These results have been published by Jiménez et al., 2008.

Discussion

These descriptive and clinical studies carried out between the years 1998 and 2006 have enlightened the current understanding of the health status of this endangered species. The elevated number of samples permitted a representative overview of the status of the remaining free-ranging populations, comprised of approximately 200 animals (Guzmán et al., 2002). These findings in themselves stress the importance of carrying on these studies in the free-ranging populations.

As we mentioned earlier in this chapter, the most significant findings were the lymphoid depletion noted in peripheral organ systems, the splenic follicular hyalinosis and the membranous glomerulonephritis, though the cause of death was not in any case directly related to these lesions.

The lymphoid depletion affecting both B and T cells noted in all animals could only be related in few cases to old age and/or concomitant diseases (tuberculosis, tumors) (Peña et al., 2006). Flow cytometry and lymphoproliferation assays from samples of the living population revealed a similar generalized decreaseof lymphocytes T and B as for T cell CD4 and CD8 subpopulations. These results correlate with the histological findings. The cause for this apparent lymphoid depletion was undetermined but viral, genetic or toxic origins remain plausible hypotheses (Peña et al., 2006).

As for the splenic follicular hyalinosis, the various techniques employed to evaluate this lesion revealed the existence of a sclerosing vasculopathy affecting splenic capillaries and centrofollicular arterioles. This lesion was characterized by thickening and disruption of vascular basement membranes and progressive scarring, possibly induced by circulating immune complexes, as evidenced in some splenic arterioles with electron microscopy (Jiménez et al, 2006). Ischemia as a consequence of this vascular scarring is plausible, causing degeneration and necrosis of the adjacent lymphoid tissue, impacting the number of leukocytes.

Membranous glomerulonephritis in various stages was the main renal lesion in the examined Iberian lynxes. Membranous glomerulonephritis is an immune mediated, degenerative disease characterized by irregular thickening of the glomerulocapillary basement membranes caused by deposition of circulating immune complexes or specific antibodies (Franklin et al., 1973; Valaitis, 2002; Newman et al., 2007; Jiménez et al., 2008). The most severe lesions in Iberian lynxes were significantly related with senescence, indicating a progressive degenerative disease.

The results from blood and urinalyses of the living population indicated some degree of chronic renal disease, possibly related to similar lesions as those noted histologically. For a more detailed discussion on these findings, see Jiménez et al., 2008.

The presence of immune complexes noted in glomerular basement membranes with EM along with the immune complexes in certain splenic arteriolar basement membranes suggest the existence of a systemic immune-complex disease of uncertain etiology. Serology and PCR tests were available for 37 of the 40 animals included in this study and showed that all but one (Arena, FeLV PCR positive) were negative for infectious agents normally related to membranous glomerulonephritis, suggesting that an infectious origin was unlikely. Autoimmune/genetic origins are speculated as possible causes for this immune-complex disease, however, infectious or toxic origins cannot be completely ruled out.

Other somewhat frequent histological findings worth mentioning here were the presence of tuberculosis and cutaneous squamous cell carcinomas. It is possible that the frequency of tuberculosis may have been exacerbated as a result of certain immunity alterations or due to increased exposure to the disease in domestic and wild ungulates that may be part, in low percentage, of the natural diet of the Iberian lynx (Aranaz et al., 2004; Peña et al., 2006).

The cutaneous squamous cell carcinomas observed in this study were all located in areas of pigmented skin and in some instances contained evidence of progression from *in situ* to squamous cell carcinomas. These features are also seen in the feline Bowenoid *in situ* squamous cell carcinoma (Gross et al., 2005). Cutaneus squamous cell carcinomas have been described in other felid species (Terio et al., this book).

New concerns regarding the remaining Iberian lynx populations have arisen as a result of these studies. It is important to emphasize the potential repercussion of renal impairment and immunity alterations in the conservation management of both wild and captive populations. Treatment options, vaccinations or mere handling must take into consideration the possibility of these underlying diseases, which may impact or interfere with the desired effects and health of the affected animals. Furthermore, ongoing research and continuous formal surveillance of these diseases should be pursued for further elucidation of the pathogenesis involved.

Acknowledgments

This study was supported by the Spanish Ministry of Environment, Research Project 90/2002 in agreement with the Andalusian Government. We would like to express our gratitude to Astrid Vargas, Fernando Martínez, Alvaro Muñoz, Irene Zorrilla, Ignacio García Bocanegra and all the professionals involved in the field conservation and health aspects at the Iberian Lynx Conservation Breeding Programme, Centro de Análisis y Diagnóstico de la Consejería de Medio Ambiente, Estación Biológica de Doñana, and LIFE Project. We acknowledge Celia Sánchez for her involvement prior to 2004 and would like to give a special thanks to the EM technicians Agustín Fernández and María Luisa García, and to the histopathology technician Pedro Aranda.

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Photo: Antonio Rivas

Truly, man is the king of beasts, for his brutality exceeds theirs.

Leonardo da Vinci
(1452-1519)

Photo: Antonio Rivas

Threats to the Iberian lynx *(Lynx pardinus)* by feline pathogens

Patógenos de felinos como amenazas para el lince ibérico *(Lynx pardinus)*

Marina L. Meli, Valentino Cattori, Fernando Martínez, Guillermo López, Astrid Vargas, Miguel A. Simón, Irene Zorrilla, Álvaro Muñoz, Francisco Palomares, José V. López-Bao, Josep Pastor, Ravi Tandon, Barbara Willi, Regina Hofmann-Lehmann and Hans Lutz

Resumen

Para determinar la importancia de distintos agentes infecciosos como amenazas potenciales para la conservación del lince ibérico, se realizaron análisis a 77 individuos en libertad entre noviembre de 2003 y septiembre de 2007. Se encontró evidencia de la presencia de 13 de los 14 agentes infecciosos posibles: se detectaron anticuerpos frente a calicivirus felino (FCV) en 29/74 (39.2%); parvovirus felino (FPV) en 22/74 (29.7%); coronavirus felino (FCoV) en 19/74 (25.7%); moquillo canino (CDV) en 12/74 (16.2%); herpesvirus felino (FHV) en 9/74 (12.2%), y *Anaplasma phagocytophilum* en 4/74 (5.4%) animales. Mediante PCR en muestras de sangre, se detectó parvovirus felino en 2/75 (2.7%); moquillo canino en 1/75 (1.3%); *Cytauxzoon felis* en 24/77 (31.2%); *Mycoplasma haemofelis* en 25/77 (32.5%); "*Candidatus* Mycoplasma haemominutum" en 27/77 (35.1%); "*Candidatus* Mycoplasma turicensis" en 10/77 (13%); *Chlamydophila felis* en 1/75 (1.3%), y *Bartonella henselae* en 16/75 (21.3%) muestras. Sólo se encontraron animales positivos al moquillo y parvovirus felino en la zona de Doñana, mientras que todos los individuos positivos a *C. felis* procedían de la zona de Sierra Morena. No se encontró evidencia de infección por el virus de la inmunodeficiencia felina (FIV). Durante la investigación, se observó un brote de infección por el virus de la leucemia felina (FeLV). Catorce linces eran provirus-positivos al virus de la leucemia felina (FeLV); once de éstos presentaban antígenos (FeLV p27 positivo). Seis de los linces provirus-positivos con antígenos murieron en seis meses en 2007. La secuenciación del gen de la glicoproteína de superficie de la leucemia felina reveló el origen común de 10 de las 11 muestras. A partir de estos hallazgos, se concluyó que las enfermedades infecciosas constituyen amenazas importantes para la supervivencia del lince ibérico y deben ser objeto de un seguimiento permanente. También se recomienda mantener medidas de control, tales como campañas de vacunación de linces y animales domésticos en zonas adyacentes al hábitat del lince.

Palabras clave

Lince ibérico, especies amenazadas, patógenos de felinos, FeLV, co-infección

Abstract
In order to determine the importance of various infectious agents as potential threats to Iberian lynx conservation, 77 free-ranging animals were screened for presence of 14 feline pathogens between November, 2003 and September, 2007. Evidence of presence of 13 out of 14 infectious agents was found: antibodies to feline calicivirus (FCV) were detected in 29/74 (39.2%); to feline parvovirus (FPV) in 22/74 (29.7%); to feline coronavirus (FCoV) in 19/74 (25.7%); to canine distempervirus (CDV) in 12/74 (16.2%); to feline herpesvirus (FHV) in 9/74 (12.2%), and to *Anaplasma phagocytophilum* in 4/74 (5.4%) animals. PCR of blood samples detected FPV in 2/75 (2.7%); CDV in 1/75 (1.3%); *Cytauxzoon felis* in 24/77 (31.2%); *Mycoplasma haemofelis* in 25/77 (32.5%); "*Candidatus* Mycoplasma haemominutum" in 27/77 (35.1%); "*Candidatus* Mycoplasma turicensis" in 10/77 (13%); *Chlamydophila felis* in 1/75 (1.3%), and *Bartonella henselae* in 16/75 (21.3%) samples. CDV- and FPV-positive animals were found only in the Doñana area, while all *C. felis*-positive lynxes originated from Sierra Morena area. No evidence of infection with feline immunodeficiency virus (FIV) was found. In 2007, an outbreak of feline leukemia virus (FeLV) infection was witnessed. Thirteen lynxes became feline leukemia virus (FeLV) provirus-positive; 11 of these were antigenemic. Sequencing of the FeLV surface glycoprotein gene revealed a common origin of the virus. Seven of the provirus-positive, antigenemic lynxes died within a few months, indicating that FeLV might be particularly virulent in Iberian lynxes. Our data on the prevalence and virulence of infectious diseases in the Iberian lynx suggest that infectious agents of felids may constitute important threats to the survival of Iberian lynxes and have to be constantly monitored. The implementation of control measures such as vaccination campaigns of lynxes and domestic animals in the surrounding lynx habitats should also be maintained over time.

Keywords
Iberian lynx, endangered species, feline pathogens, FeLV, co-infection

Photo: Antonio Rivas

Threats to the Iberian lynx *(Lynx pardinus)* by feline pathogens

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Introduction

The Iberian lynx *(Lynx pardinus)* is the most endangered felid species in the world (Nowell, 2002). Factors that may contribute to the reduced numbers of animals include habitat loss and the decline of the main prey base, the wild rabbit (Ferreras et al., 2009). In addition, a reduced genetic variability of the remaining Iberian lynxes has been reported (Johnson et al., 2004; Godoy et al., 2009). All these factors could contribute to render this species extremely susceptible to infectious agents, even those that are normally not highly pathogenic in more genetically diverse felids.

Viral pathogens of the domestic cat include the feline herpesvirus (FHV) and feline calicivirus (FCV) that are the causative agents of upper respiratory diseases (Povey, 1979). Feline parvovirus (FPV) affects all rapidly dividing cells and therefore causes diarrhoea, bone marrow depression and lymphoid depletion in the cat (Parrish, 1995). Feline coronavirus (FCoV) occurs in up to 80% of the domestic cat population and is causatively involved in the development of feline infectious peritonitis, the most frequent infection causing death (Pedersen, 1987). These viral infections are also known to affect wild felids to different degrees (Hofmann-Lehmann et al., 1996; Wasieri et al., 2009). Moreover, a canine distemper virus (CDV) outbreak in the Serengeti lion population in 1994 caused severe disease and death of about 1000 lions (Roelke-Parker et al., 1996) and demonstrated that CDV may also be pathogenic for felids. Another feline virus, the feline immunodeficiency virus (FIV), a lentivirus closely related to human immunodeficiency virus is known to occur in domestic cats and a number of wild felids (Brown et al., 1994). In the domestic cat, FIV causes severe immunosuppression by depletion of CD4+ T-lymphocytes (Torten et al., 1991). Feline leukaemia virus (FeLV), a gammaretrovirus of felids, occurs worldwide and causes anemia, immunosuppression and various forms of lymphoma (Jarrett et al., 1964; Hoover et al., 1975; Hardy et al., 1976). FeLV infection is bound to four different outcomes: progressive, regressive and focal infection; and abortive exposure (Torres et al., 2005; Hofmann-Lehmann et al., 2008; Levy et al., 2008). Cats with progressive infection eventually succumb to FeLV-associated diseases (McClelland et al., 1980; Hoover et al., 1991; Flynn et al., 2000; Flynn et al., 2002; Hofmann-Lehmann et al., 2007). Cats with regressive infection are capable to overcome viremia after a few weeks to months, and are not at high risk of dying of FeLV-related diseases. Their infected cells do not produce FeLV but expression of the virus might be reactivated, e.g. during a period of stress or immunosuppression (Rojko et al., 1982). FeLV focal infection is confined to tissues such as spleen, lymph nodes, small intestine and mammary glands (Lutz et al., 1980; Pacitti and Jarrett, 1985; Hayes et al., 1989). Cats with abortive infection produce effective early host immune responses, which abrogate viral replication and supposedly eliminate FeLV-infected cells, so that the virus remains undetected (Hofmann-Lehmann et al., 2001; Torres et al., 2005; Hofmann-Lehmann et al., 2007).

So far, only limited information is available about infectious diseases affecting the Iberian lynx. Recently, infections by *Cytauxzoon felis*, *Toxoplasma gondii*, hemotropic mycoplasmas, *Mycobacterium bovis* and several parasitic diseases have been described in individual animals (Torres et al., 1998; Vicente et al., 2004; Luaces et al., 2005; Martín-Atance et al., 2006; Millán et al., 2007; Sobrino et al., 2007; Willi et al., 2007; Roelke et al., 2008). Bovine tuberculosis has been reported to be the cause of death of five Iberian lynxes during the years 1998 and 2007. The affected lynxes had probably acquired this disease during consumption of fallow deer *(Dama dama),* deer *(Cervus elaphus)* or wild boars *(Sus scrofa)* living in the area (Briones et al., 2000; Aranaz et al., 2004; Gortázar et al., 2005; Parra et al., 2005; Peña et al., 2006; Naranjo et al., 2008). Histopathological studies revealed a generalized immune depletion in these animals (Peña et al., 2006), apparently not related to infectious agents or malnutrition, and glomerulonephritis (Jiménez et al., 2008; Jiménez et al., 2009). In order to save Iberian lynxes habitat protection and restoration, repopulation programmes for the major prey base and cryopreservation of genetic material (Léon-Quinto et al., 2009; Roldan et al., 2009) have been initiated. In addition, an *ex situ* conservation programme aiming at the reintroduction of the species was set up in November 2003. In this context, the present study was initiated in late 2003, which aimed at the systematic analysis of the prevalence and importance of pathogens known to occur in other felid species as threats for the surviving of the Iberian lynx.

Materials and methods

Animals and material collected

From late 2003 until September 2007 EDTA-anticoagulated blood, serum and faeces of 77 free-ranging Iberian lynxes were collected from both Doñana and Sierra Morena areas, in the Southwest Spain. Blood samples were collected from the cephalic, saphena or jugular veins in animals that were anesthetized for radiocollaring or other management purposes. Blood was taken from the thoracic cavity or from the heart in animals that were found dead.

Detection of specific infections and sequencing

Serological methods: presence of antibodies against FIV, FHV, FCV, FPV, FCoV and CDV and FeLV p27 antigen was determined as previously described (Meli et al., 2009). FHV, FPV, FIV and FeLV provirus, *B. henselae*, *Ch. felis*, *A. phagocytophilum* and hemotropic mycoplasmas were detected and quantitated by real-time TaqMan™ PCR. Presence of *C. felis* was detected by conventional PCR. FCV, FCoV, CDV and FeLV viral RNA were detected and quantitated by real-time TaqMan™ RT-PCR (Meli et al., 2009). Full-length FeLV-A *env* surface unit (SU) amplification, subcloning and sequencing as well as multiple sequence alignments (MSA) and percent identity (PID) value determination were performed as described elsewhere (Meli et al., 2009).

Statistical analysis

Correlation between infections was evaluated using the Fisher exact test, performed using GraphPad Prism version 3.00 for Windows (GraphPad Software, San Diego, USA). P-values <0.05 were considered as significant.

Results

Case history and clinical manifestations

From late 2003 to September 2007, 17 of the 77 Iberian lynxes that were part of this study died. Seven lynxes were found dead after being stuck by vehicles, one due to illegal hunting, and nine died for reasons related to infectious diseases. One of the latter nine lynxes exhibited high viral loads of CDV, suggesting that CDV infection was the cause of death (data not shown). In another animal, a juvenile, *M. bovis* was found by PCR in the

								Hematological parameters		
Name	**Sex**	**Age (years)**	**Habitat/ status**	**Clinical status**	**Collection Date**	**FeLV provirus**	**FeLV p27**	**PCV (%)**	**WBC (/µl)**	**PLT (x103/ µl)**
Arena	M	1	Doñana (CRS)/ free-ranging	Struck by vehicle. Necropsy: several traumatic lesions.	9/1/2004	pos	neg	n.p.	n.p	n.p
Román	M	4	Doñana (CRS)/ free-ranging	Clinically healthy in December 2006. Found dead in May 2007. Kachexia, dehydratation, bacterial necrosupurative abscess on cervical area and bacterial embolic pneumonia by *Streptococcus canis* and *Mycoplasma gateae*.	12/15/2006	pos	pos	n.p.	n.p.	n.p.
Arrayán	M	4	Doñana (CRS)/ free-ranging	Found dead. Dehydratation, fibrinous pericarditis, hemotorax, hemopericardium, blefaroconjuntivitis, *Plesiomonas shigelloides* septicemia.	3/13/2007	pos	pos	n.p.	n.p.	n.p.
Uda	M	11	Doñana (Dehesa gato)/ free-ranging	Euthanized. Cachexia, dehydration, atrophic left kidney, chronic cystitis, squamous carcinoma on ear, adrenalitis, enlarged parathyroides, enteritis, diffuse miliar bronchopneumonia, atrophic right mesenteric muscle, *Streptococcus canis* septicemia.	3/16/2007	pos	pos	34	31410	281
Nati II	M	9	Doñana (CRS)/ free-ranging	Found dead. Advanced autolysis.	4/26/2007	pos	pos	n.p.	n.p.	n.p.
Coca	M	1	Doñana (CRS)/ free-ranging	Clinically healthy. Transported to the rescue center.	7/22/2007	pos	pos	36	18410	348
Rayuela	F	4	Doñana (CRS)/ free-ranging	Chronic focal dermatitis at inter-shoulder area. Reported to the rescue center. The animal recover from infection (p27 neg) and was returned to the wild.	7/7/2007	pos	pos	38	11500	398
Inesperado	M	3	Doñana (Dehesa gato)/ free-ranging	Clinically healthy. Transported to the rescue center. Escaped.	5/21/2007	pos	pos	35	16160	278
Daphne	F	<1	Doñana (CRS)/ free-ranging	Died at the rescue center.	6/8/2007	pos	pos	n.p.	n.p.	n.p.
Dalia	F	<1	Doñana (CRS)/ free-ranging	Clinically healthy. Transported to the rescue center.	6/8/2007	pos	pos	n.p.	n.p.	n.p.
Cicuta	M	1	Doñana (CRS)/ free-ranging	Died at the rescue center.	7/212007	pos	pos	20	3950	96
Viciosa	F	7	Doñana (CRS)/ free-ranging	Clinically healthy.	7/14/2007	pos	neg	37	11480	470
Candalo	M	3	Sierra Morena (Valquemado)/ free-ranging	Clinically healthy.	7/13/2007	pos	neg	49	9380	266
Cacao	M	1	Doñana (CRS)/a free-ranging	Clinically healthy. Transported to the rescue center.	8/1/2007	pos	pos	33	9820	419

Table 1. Clinical signs, history, and hematological parameters of the 14 FeLV-infected Iberian lynxes

Tabla 1. Signos clínicos, historia, y parámetros hematológicos de los 14 linces ibéricos infectados con FeLV.
M= macho; F=Hembra; CSR=subpoblación de Coto del Rey; neg=negativo; pos=positivo; PCV= Volumen de células packed; WBC= células de la serie blanca; PLT=plaquetas; N.P.=no realizado

tracheal wash. This lynx displayed bilateral uveitis and poor body condition; after radiographic exam, a lung nodule was found. Upon necropsy, tuberculosis was diagnosed in the lungs, kidneys, and adrenal glands. One of the animals struck by a vehicle had been found to be FeLV provirus-positive in 2004. The remaining seven dead lynxes were all positive for FeLV provirus and FeLV p27 antigen (Table 1), and all died within six months during the spring-summer of 2007. Four animals died in the wild in the northern part of the Doñana area (Figure 1) and three more lynxes died in the quarantine rescue center (López et al., 2009).

In early December 2006, during a health and reproductive exam of free-ranging animals (n=16) in Doñana National Park, an adult male was found to be FeLV provirus- and p27-positive. The same animal was negative for FeLV when sampled during a procedure intended for radiocollaring one year before. This animal demonstrated no clinical symptoms and was released. In May 2007, the same lynx was found dead. In December 2006, the other 15 lynxes from the northern part of the Doñana area were FeLV-negative. Beginning in March 2007, seven lynxes died (some of the adult/juveniles sampled in December 2006 and some young-of-the year): all were positive for FeLV provirus and FeLV p27 (Table 1). Four adult males died in the field. In an effort to prevent the spread of the epizootic, many free-ranging lynxes were captured, tested for antigenemia (p27) and vaccinated and released if negative. FeLV p27-positive animals (n=5) were removed from the wild and transported to the Los Villares rescue center in order to reduce infectious pressure (López et al., 2009); three of them (two subadults and a cub-of-the year) died within few months due to a very acute anemic disease. The case characteristics, clinical signs, and available hematological data for the FeLV-infected lynxes are summarized in Table 1. Relationships and geographical distribution of the infected lynxes are depicted in Figure 1. See López et al,(this book) for specific details on management measures implemented in order to control the spread of the FeLV outbreak in Doñana.

The majority of the remaining lynxes (n=60) that were part of this study were clinically healthy. One animal showed scoliosis due to a resolved lumbar vertebral fracture, a few with resolved wounds (probably from fights) and most of the lynxes showed a mild ectoparasitosis (ticks and flies).

Prevalence of selected infections

Antibodies to FCV were detected in 29/74 (39.2%); to FPV in 22/74 (29.7%); to FCoV in 19/74 (25.7%); to CDV in 12/74 (16.2%); to FHV in 9/74 (12.2%), and to *A. phagocytophilum* in 4/74 (5.4%) animals (Table 2). Antibodies to FIV were not detected in any of the 74 samples tested. In the Doñana area antibodies titers to FHV and CDV were found to be higher than in the Sierra Morena area. In contrast, antibodies titers to FCoV and to FCV were higher in the Sierra Morena area. Eleven out of 75 lynxes tested positive for FeLV p27 antigen. All FeLV antigenemic lynxes originated from the Doñana area. PCR of blood samples detected FeLV in 14/77 (18.2%); FPV in 2/75 (2.7%); CDV in 1/75 (1.3%); *C. felis* in 24/77 (31.2%); *M. haemofelis* in 25/77 (32.5%); "*Candidatus* M. haemominutum" in 27/77 (35.1%); "*Candidatus* M. turicensis" in 10/77 (13%); *Ch. felis* in 1/75 (1.3%), and *B. henselae* in 16/75 (21.3%) samples. CDV- and FPV-positive animals were found only in the Doñana area, while all *C. felis*-positive lynxes originated from Sierra Morena. PCR of all blood samples did not detect FIV, FCoV, FHV, FCV, or *A. phagocytophilum* (Table 2). Feces tested negative for FCoV (0/68) and positive for FPV in 1/62 (1.6%) and CDV in 1/45 (2.2%) samples; the latter was obtained from the only animal in which CDV was found in the blood. Only six fecal samples from the 14 FeLV provirus-positive animals were available. Five samples originating from antigenemic animals tested positive, whereas one sample from a p27-negative animal tested negative by PCR (data not shown).

Association between FeLV and other infectious agents

Statistically significant associations (based on PCR results) were found between FeLV infection and infections with *M. haemofelis* (P_{Fisher}=0.0004) and "*Candidatus* M. turicensis" (P_{Fisher}=0.0205). The other pathogens did not show any significant association with FeLV infection.

FeLV and its possible origin

Fourteen lynxes (13 from the Doñana area and one from Sierra Morena) tested positive for FeLV provirus (Tables 1 and 2). FeLV provirus was amplified and sequenced from 11 positive lynxes from the Doñana area. Nucleotide sequence analysis of the surface unit (SU) of the env gene revealed a common origin for the provirus found in Doñana lynxes in 2007 (99.5-100% identity). The sequences clustered with and were 97.9-98.2% identical to the

Agent	Antibodies			Antigen		
	DN	SM	Total	DN	SM	Total
	pos/total tested (percent)					
Viral infections						
FeLV	11/45* (24.4%)	0/30*	11/75* (14.7%)	13/45 (28.9%)	1/32 (3.1%)	14/77 (18.2%)
FIV	0/44	0/30	0/74	0/45	0/32	0/77
FCoV	7/44 (15.9%)	12/30 (40%)	19/74 (25.7%)	0/45	0/32	0/77
FHV	7/44 (15.9%)	2/30 (6.7%)	9/74 (12.2%)	0/45	0/30	0/75
FPV	13/44 (29.5%)	9/30 (30%)	22/74 (29.7%)	2/45 (4.4%)	0/30	2/75 (2.7%)
FCV	15/44 (34.1%)	14/30 (46.7%)	29/74 (39.2%)	0/45	0/30	0/75
CDV	11/44 (25%)	1/30 (3.3%)	12/74 (16.2%)	1/45 (2.2%)	0/30	1/75 (1.3%)
Protozoan infections						
Cytauxzoon felis	n.a.	n.a.	n.a.	0/45	24/32 (75%)	24/77 (31.2%)
Bacterial infections						
M. haemofelis	n.a.	n.a.	n.a.	16/45 (35.6%)	9/32 (28.1%)	25/77 (32.5%)
*C.*M. haemominutum	n.a.	n.a.	n.a.	13/45 (28.9%)	14/32 (43.8%)	27/77 (35.1%)
*C.*M. turicensis	n.a.	n.a.	n.a.	6/45 (13.3%)	4/32 (12.5%)	10/77 (13%)
A. phagocytophilum	1/44 (2.3%)	3/30 (10%)	4/74 (5.4%)	0/45	0/30	0/75
B. henselae	n.a.	n.a.	n.a.	6/45 (13.3%)	10/30 (33.3%)	16/75 (21.3%)
Chlamydophila felis	n.a.	n.a.	n.a.	0/45	1/30 (3.3%)	1/75 (1.3%)

DN: Doñana area; SM: Sierra Morena area; FeLV: feline leukemia virus; FIV: feline immunodeficiency virus; FCoV: feline coronavirus; FHV: feline herpesvirus; FPV: feline parvovirus; FCV: feline calicivirus; CDV: canine distemper virus; M.=Mycoplasma; C.M.=Candidatus Mycoplasma; A.=Anaplasma; B.=Bartonella; *= p27 antigen; n.a.=not applicable.

DN: área de Doñana; SM: área de Sierra Morena; FeLV: virus de la leucemia felina; FIV: virus de la inmunodeficiencia felina; FCoV: coronavirus felino; FHV: herpesvirus felino; FPV: parvovirus felino; FCV: calicivirus felino; CDV: virus del moquillo canino; M.= Micoplasma; C.M. = Micoplasma candidatus; A. = Anaplasma; B. = Bartonela; *antígeno p27; n.a. = no aplicable.

Table 2. Overview of the prevalence of infection in Iberian lynxes.

Tabla 2. Resumen de la prevalencia de infecciones en el lince ibérico.

FeLV-A/61E strain (Figure 2) (Pedersen et al., 1987; Overbaugh et al., 1988), while the sequence obtained from the lynx found to be provirus-positive in 2004 *(Arena)* was only 94.8-95% identical to the other sequences and was more related to FeLV-A/Rickard (97.4% identity, Figure 2).

Discussion

In view of the endangered situation of the Iberian lynx, the central government of Spain, in conjunction with the autonomous government of Andalusia, has initiated an *ex situ* conservation programme comprising several captive breeding centers (Vargas et al., 2009). Animals that were selected as breeding stock had been caught in the wild and tested for the absence of pathogenic infections prior to integration into the captive breeding population. Noteworthy, some of the founder lynxes were found to be infected with *C. felis*, haemotropic mycoplasmas and *B. henselae*, and also to exhibit antibodies to other infectious agents; however, these animals were clinically healthy. Between late 2003 and 2007, substantial information was obtained regarding the prevalence and importance of infectious agents in Iberian lynx populations of the Doñana and Sierra Morena habitats. With the exception of FIV, evidence for all tested feline pathogens was found. Interestingly, the seroprevalence of FHV, FCV, FPV, and FCoV was substantially higher than that observed in free-ranging Eurasian lynxes *(Lynx lynx)* from Sweden (Ryser-Degiorgis et al., 2005). The increased seroprevalence of these feline pathogens in Iberian lynxes probably reflects a higher population density and closer contact with domestic cats when compared to lynxes in the Swedish population.

From late 2003 to late 2006, FeLV infection (provirus) was detected in one animal *(Arena)*. In a recently

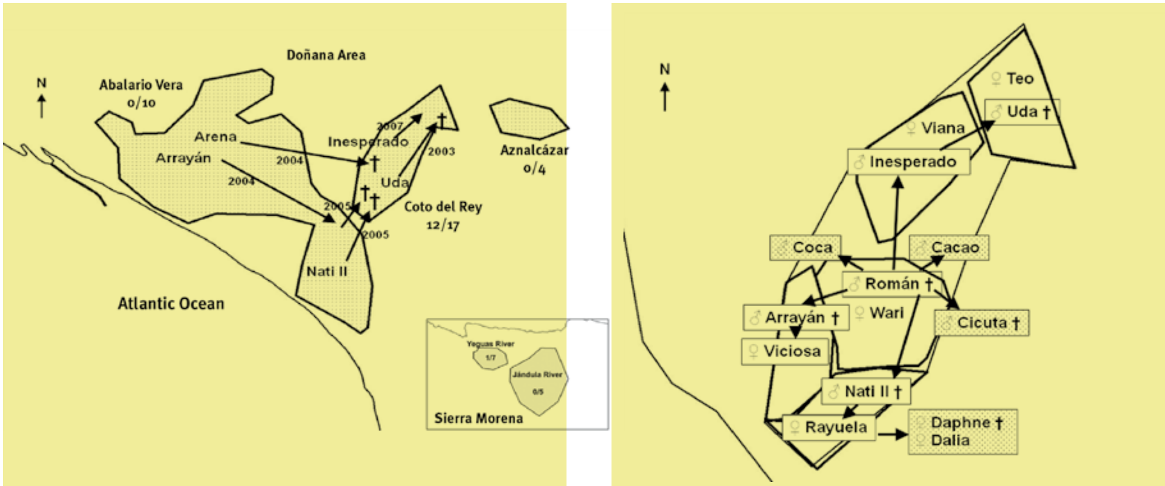

Figure 1. Schematic display of the supposed FeLV spreading between lynxes in Doñana area. (A). Map showing all migrations (arrows, accompanied with the year of migration) and the place of death (†) of infected lynxes in the Doñana area. All deaths occurred in the region of Coto del Rey. Animals are identified by their names (bold). In the areas of Abalario-Vera, Coto del Rey and Aznalcázar, numbers identify the number of FeLV positive (p27 and provirus) out of the total checked during a FeLV control programme in 2007. In the smaller rectangle as comparison the Yeguas River and Jándula River regions of Sierra Morena area at about 500 kilometers from the Doñana area, and actually non-communicated with each other. Again, numbers identify the number of FeLV positive (p27 and provirus) out of the total checked in 2007. (B). Coto del Rey territories represented schematically with the most probable route of spreading of FeLV. It is assumed that males fight during mating season and that copulations were the most plausible way of spreading the infection through the population. With our data, apparently *Román* was the first lynx infected and, if so, the probable origin of the outbreak. Frames indicate infected animals. Frames with dots represent cubs or subadults without a territory. Dead animals are indicated by a cross. Sex of animals is indicated by symbols.

Figura 1. Representación esquemática de la hipotética extensión del FeLV entre linces del área de Doñana. (A). Mapa que muestra los movimientos (flechas, acompañadas del año de la migración) y el lugar de la muerte (†) de los linces infectados en el área de Doñana. Todas las muertes ocurrieron en el área de Coto del Rey. Los animales están identificados por su nombre (en negrita). En las áreas de Abalario-Vera, Coto del Rey y Aznalcázar, los números identifican los animales positivos a FeLV (p27 y provirus) dentro del número total de linces que fueron examinados durante un programa de control del FeLV en el año 2007. El recuadro pequeño muestra, con fines comparativos, las regiones de los Ríos Yeguas y Jándula en el área de Sierra Morena, situadas a aproximadamente 500 kilómetros del área de Doñana y no conectadas entre sí. Una vez más, los números indican el número de animales positivos al FeLV (p27 y provirus positivo) del total de animales examinados en 2007. (B). Representación esquemática de los territorios de Coto del Rey, indicando la vía más probable de expansión del virus FeLV. Se supone que los machos se pelean durante la época de reproducción y que las cópulas son la forma más plausible de extender la infección por toda la población. Según nuestros datos, *Román* aparentemente fue el primer lince infectado, y si así hubiera sido, también sería el origen probable del brote. Los nombres enmarcados indican animales infectados. Los enmarcados punteados representan cachorros o subadultos sin territorio. Los animales muertos están indicados con una cruz. Se utilizan símbolos para indicar el sexo del animal.

published retrospective study (Luaces et al., 2008), six of 21 lynxes sampled between 1993 and 2003 were found to be FeLV provirus-positive, but not antigenemic. Although no clinical data were reported in that study, there was no indication that FeLV infection would represent a major risk for the lynx population. This situation changed acutely when, in 2007, several animals with severe clinical signs were found to be infected with FeLV and eventually died. The pathogenicity of the FeLV strain involved in the outbreak was rather unexpected, with seven of 12 infected animals from the Doñana area succumbing from March 2007 to July 2008. Six of the FeLV-infected lynxes that had died were living in the Coto del Rey nucleus, where three adult pairs and their offspring normally only use 700-1000 ha per territory (Palomares et al., 2001; Palomares, 2009). Consequently, contact between individuals may be frequent in this area (López-Bao et al., 2008). The other FeLV-positive dead lynx was living in the Gato nucleus, where animals have relatively high rates of contact –via dispersal– with those living in the Coto del Rey area (Ferreras, 2001). Several of the FeLV-infected lynxes showed clinical signs and/or hematologic abnormalities, such as anemia, lymphopenia or neutropenia, and bacterial infections compatible with immunosuppression; such symptoms are also observed in FeLV-infected domestic cats.

Sequencing of the complete FeLV envelope gene revealed that the virus present in lynxes affected in 2007 was clearly distinct from the virus detected in the FeLV-positive lynx found road-killed in 2004 *(Arena)*. This suggests that from late 2003 to late 2008 FeLV was introduced into the Doñana Iberian lynx population on at least two occasions. Nonetheless, all analyzed FeLV sequences were closely related to FeLV-A and, in particular,

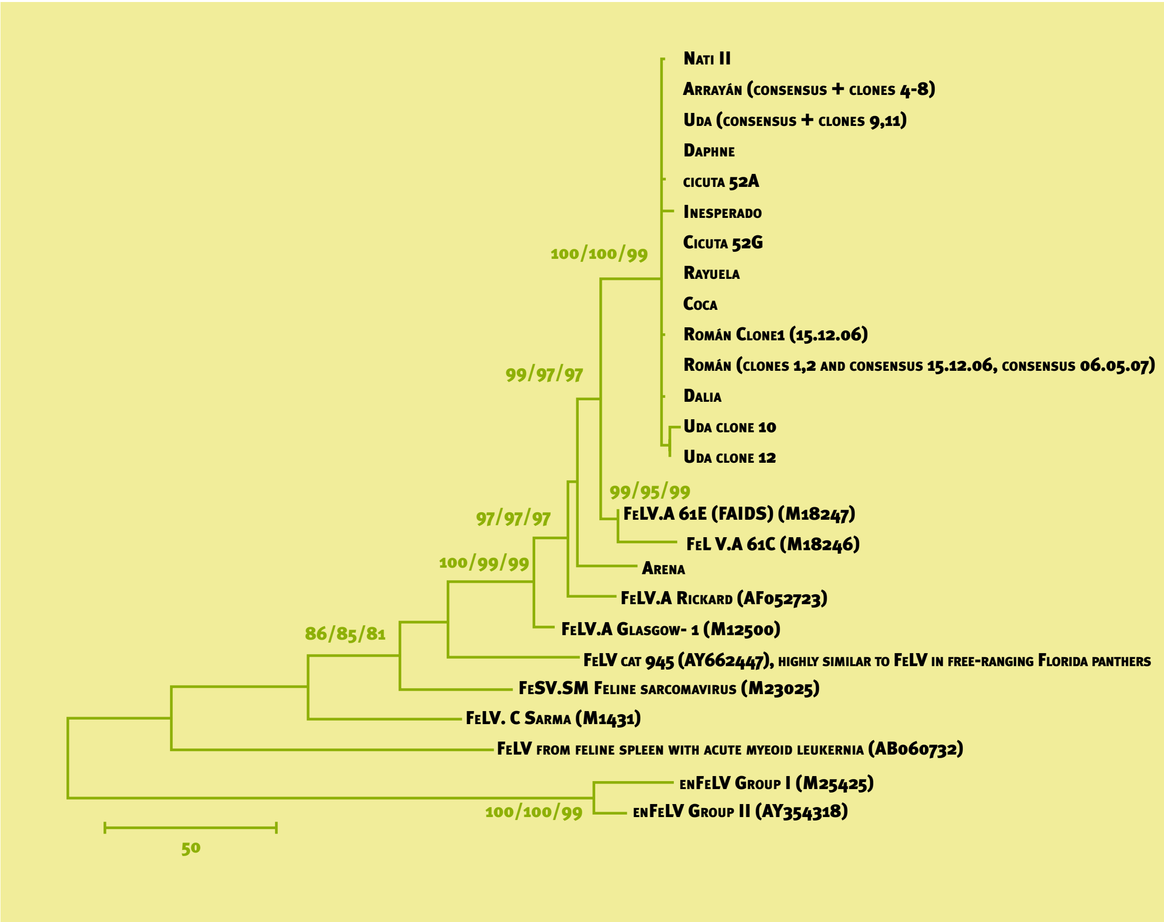

Figure 2.
Evolutionary relationships of Iberian lynx FeLV SU at the DNA level. The Maximum Parsimony (MP) tree is shown. Trees are drawn to scale; length is in terms of the number of changes over the entire sequence. MP tree length=643; consistency index=(0.744726); retention index=(0.827143); composite index=0.671491 (0.615995) for all sites and parsimony-informative sites (in parentheses). The codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 1424 base positions in the final dataset, of which 311 were parsimony-informative.

Figura 2. Relaciones evolutivas del FeLV SU en el DNA del lince ibérico. Se muestra el árbol de "Máxima Parsimonia" (MP). Los árboles están dibujados a escala; la longitud se muestra en términos del número de cambios en toda la secuencia. Longitud del árbol MP=643, índice de consistencia=(0,744726), índice de retención=(0,827143), índice compuesto=0,671491 (0,615995) para todos los elementos así como para los elementos informativos de la parsimonia (entre paréntesis). Se incluyeron las posiciones 1+2+3+no-codificante del codón. Hubo un total de 1424 posiciones de bases en la serie de datos final, de las cuales 311 fueron informativas sobre la parsimonia.

to FeLV-A/61E, a member of a highly conserved family representing prototype viruses of the horizontally transmitted, minimally pathogenic FeLV-A. These FeLV-A prototype viruses are assumed to be present in all naturally occurring FeLV infections in domestic cats. FeLV-A/61E in conjunction with FeLV-A/61C was found to cause severe immunodeficiency in domestic cats (FeLV-FAIDS) and was originally isolated from a domestic cat in Colorado (Hoover et al., 1987). Further sequence analyses yielded no additional variants in Iberian lynxes.

Moreover, the high pathogenicity observed in FeLV-infected Iberian lynxes might be due to the host, rather than to viral factors. The genetic diversity of the Doñana lynx population is lower than that of the Sierra Morena population (Johnson et al., 2004; Godoy et al., 2009), and therefore the pathogenic potential of FeLV could have been enhanced by inbreeding. In addition, an immune-mediated systemic disease of unknown origin has been recently postulated (Jiménez et al., 2009). The presence of co-infections could also have contributed to the high pathogenicity of FeLV in the lynxes. A significant association between FeLV infection and haemotropic mycoplasma infections, i.e., *M. haemofelis* and "*Candidatus* M. turicensis", was found. Based on temporal events, which suggest that hemotropic mycoplasma infections were present before FeLV infection, it is possible that pre-existing infection by these agents may favor FeLV infection. The increased frequency of FeLV infection in lynxes positive for hemotropic mycoplasmas could also reflect simultaneous transmission of these agents with FeLV during cat-to-lynx contact.

As only a few cases of FeLV infection were observed prior to 2007 (Luaces et al., 2008), it is likely that FeLV infection in lynxes is rare, is not carried within the lynx population, and most likely originates from domestic cats, which are increasingly frequent in Doñana (López et al., 2009). FeLV antigen prevalence of about 16% was reported in Spanish cats (Arjona et al., 2000); this is higher in villages around Doñana (G. López, pers. comm.). Therefore, a higher risk of transmission from domestic cats to lynxes is likely. Since some FeLV variants seem to be highly pathogenic in the Iberian lynx, further transmission from domestic cats to lynxes, with the consequent transmission among the free-ranging lynx population, must be controlled. To this end, conservation authorities in Andalusia have implemented a vaccination campaign in Doñana in order to vaccinate free-ranging lynxes against FeLV infection using a Canarypox-based FeLV vaccine (Tartaglia et al., 1993). Although vaccination did not induce sterilizing immunity in domestic cats, it was nonetheless able to stimulate the immune system to a degree that allowed the cats to overcome the infection rapidly and to clear most of the viral RNA from the blood (Hofmann-Lehmann et al., 2007). Therefore, even if immunization does not completely protect lynxes against transient infection, there is justified hope that the outcome of another FeLV infection would be less severe. In addition to vaccination, it is crucial to decrease the infectious pressure that domestic cats exert over the two Iberian lynx free-ranging populations. Domestic cats should be prevented from entering the lynx habitats and should also be vaccinated against FeLV infection to reduce the risk of transmission to lynxes. A long term epidemiological survey and specific measures must be implemented in Iberian lynx distribution areas and future areas targeted for reintroduction efforts.

Acknowledgements

The authors would like to thank Y. Gahler, E. Gönczi, T. Meili Prodan, B. Pineroli, E. Rogg, E. Schuler, and B. Weibel for excellent laboratory assistance and helpful support. The authors are indebted to the Environmental Council of the government of Andalusia, Southern Spain, for providing the Iberian lynx samples. We are grateful to our collaborators in the Iberian Lynx Life Project 2000-2005, Life Project 2006-2011, the Doñana National Park, and the *Ex situ* Breeding Programme for sampling the free-ranging Iberian lynxes, and to Laura Peña from the Departamento de Patología for her expertise in pathology. Some of the lynxes used in the present study were trapped and radio-tracked under research projects CGL2004-00346/BOS of the Spanish Ministry of Education and Science, and 17/2005 of the Spanish Ministry of the Environment under the National Parks research programme, with the collaboration of Land Rover España S.A. JVLB is an FPU fellow (Ministry of Education). Laboratory work was performed using the logistics of the Center for Clinical Studies at the Vetsuisse Faculty of the University of Zurich. R.H. L. is the recipient of a professorship by the Swiss National Science Foundation (PP00B 102866 and PP00P3-119136).

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Photo: Héctor Garrido

If you weep because the sun has gone out, your tears may blind you to the stars.

Stray Birds, Rabindranath Tagore
(1861-1941)


Photo: Antonio Sabater

A feline leukemia virus (FeLV) outbreak in the Doñana Iberian lynx population

Brote del virus de la leucemia felina (FeLV) en la población de lince ibérico de Doñana

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Resumen
El virus de la leucemia felina (FeLV) es un retrovirus que afecta a poblaciones de gatos domésticos en todo el mundo y ocasionalmente a otras especies de felinos. Su acción patógena incluye generalmente anemias, inmunodepresión y tumores. En el lince ibérico *(Lynx pardinus)* hay constancia de contacto esporádico con el virus, si bien antes de 2007 no se había detectado ningún brote epidémico. Durante la primera mitad de 2007, fue detectado un brote de infección por FeLV en el Coto del Rey, la subpoblación de Doñana con mayor densidad de individuos. El brote epidémico tuvo una difusión rápida, afectando al 66% de los ejemplares del núcleo durante el primer trimestre del año. Las infecciones mostraron un curso agudo y se registró una alta mortalidad. Ante la amenaza de epidemia para la exigua población de linces de Doñana, se adoptó un programa de manejo encaminado a detener su dispersión. Las medidas comprendieron la retirada del medio natural de los linces virémicos, la vacunación de los no infectados y la intensificación en la retirada del medio de gatos domésticos que suponían un riesgo de infección para los linces. Se pudo evaluar a alrededor del 80% de la población de Doñana y al 90% de la subpoblación afectada. El brote epidémico pudo ser controlado durante el transcurso del año 2007, probablemente debido a las medidas aplicadas en el programa de control. Se detectaron 12 linces infectados, que mostraron mayoritariamente cuadros de anemias no regenerativas con inmunosupresión asociada. Dos de ellos acantonaron el virus en la médula ósea y están libres en el medio natural, mientras que otros ocho han muerto.

Palabras clave
Leucemia felina, FeLV, lince ibérico, brote, enfermedades infecciosas, gatos

Abstract
The Feline Leukemia Virus (FeLV) is a retrovirus that affects domestic cats all over the world, occasionally affecting other felid species. Its pathogenic effects generally include anemia, immunosuppression and tumors. In the Iberian lynx *(Lynx pardinus)*, sporadic contact has been detected since the virus began to be monitored in the population, although no outbreak had ever been detected before 2007. During the first half of 2007, an FeLV outbreak was detected in Coto del Rey, the densest subpopulation within the Doñana Iberian lynx population. The outbreak showed a rapid dissemination, 66% of the individuals in the nucleus being infected during the first trimester of the year. The infection showed an acute response and high associated mortality. Given that the occurrence of the outbreak threatened the scarce Doñana Iberian lynx population, a management programme was carried out to stop the dispersion of the virus over the population. The management measures included in the control programme included the following: 1) removal of viremic lynxes; 2) vaccination of negative individuals and 3) reduction of the feral cat population (which is thought to be the primary vector for the FeLV infection in lynxes). Around 80% of the total lynx population and 90% of the outbreak focus subpopulation could be evaluated. Finally, the outbreak could be controlled during 2007, probably due to the application of the management measures. A total of 12 infected individuals were found, most of them showing signs of non-regenerating anemia and associated immunosuppression. Two of them sequestered the virus and live normally in the field, while eight of them have died.

Keywords
Feline leukemia, FeLV, Iberian lynx, outbreak, infectious diseases, cats

Photo: Antonio Rivas

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Introduction

FeLV is a feline retrovirus that infects domestic cats *(Felis catus)* worldwide (see Weijer et al., 1987; Burmeister, 2001), occuring in nature as a family of closely related viruses (Levy, 2008). Infected individuals can suffer a persistent viremia or develop an effective immune response that turns the virus into latency in the bone marrow (Pacitti, 1987; Hofmann-Lehmann et al., 1997), although viremia can be reactivated due to immunosuppression (Hofmann-Lehmann et al., 2008). Persistent viremic animals suffer malignant and proliferative diseases including lymphomas and leukemia, as well as degenerative diseases including anemia, leading to death within months to years (Rezanka et al., 1992).

Dissemination of the virus takes place mainly through direct contact (Barr and Bowman, 2006). There is no curative treatment for FeLV-infected cats (Hartmann et al., 1999), although some drugs seem to improve quality of life and prolong life expectancy (Mari et al., 2004). Vaccination can prevent the virus spread into a population, since it protects from persistent viremia (Hofmann-Lehmann et al., 2006, 2007). Current molecular methods for detecting FeLV are capable of detecting one copy of provirus per reaction (Cattori et al., 2008; Tandon et al., 2008; Torres et al., 2008). FeLV infection has sometimes been reported in non-domestic felids both in captivity (Rasheed and Gardner, 1981; Meric, 1984; Citino, 1986; Sleeman et al., 2001; Briggs and Ott, 1986; Marker et al., 2003) and in the wild (Rickard and Foreyt, 1992; Jessup et al., 1993; Daniels et al., 1999; Fromont et al., 2000; Ostrowsky et al., 2003; Cunningham et al., 2008). Interestingly, FeLV does not seem to represent an important health risk for most non-domestic felid populations (Fromont et al., 2000; Filoni et al., 2003; Filoni et al., 2006) and only the Florida panther *(Puma concolor coryi)* wild population has suffered a real FeLV outbreak (Cunningham et al., 2008).

The Iberian lynx *(L. pardinus)* is the most endangered felid in the world, as it has been cataloged as "critically endangered" by IUCN (IUCN, 2003). Endemic to the Iberian Peninsula (Ferrer and Negro, 2004), its population size severely decreased during the Twentieth Century (Rodríguez and Delibes, 1992) and, currently, only two isolated breeding populations survive in southern Spain (Guzmán et al., 2004). The species is eminently solitary, contact between individuals taking place mainly during the mating season (Ferreras et al., 1997; López-Bao et al., 2007; Palomares, this book). Iberian lynxes are commonly involved in both interspecific and intraspecific fights (see Palomares and Caro, 1999; LIFE conservation project, unpublished data). Some sporadic FeLV-latent infected Iberian lynxes had been found before 2007 (Luaces et al., 2008; Roelke et al., 2008), so lynx exposure to FeLV is thought to have been constant during the last decades. During the breeding season 2007, an FeLV outbreak was detected for the first time in the Doñana Iberian lynx population (see López et al., 2009; Meli et al., 2009; Meli et al., this book). An adult male was found positive to FeLV viremia in a routine health evaluation performed within the framework of a research project before the mating season. The rest of the individuals evaluated in Doñana tested negative for the virus. Subsequent sequencing of the virus showed that the most probable source of infection to the lynx was a domestic cat (Meli et al., this book). Based upon previous data regarding FeLV infection in non-domestic felids, only radio-monitoring of the infected individual was carried out. On March 13, 2007 a three-year-old male was found dead with FeLV viremia. The cause of death was a septicemia by *Plesiomonas shigelloides*. On March 17, an 11-year-old male inhabiting the same area was found dead because of a septicemia by *Streptococcus canis*. FeLV viremia was also detected by molecular analysis. Although the FeLV control programme was started in April, two more FeLV viremic adult males died before they could be trapped: one on April 24 (ultimate cause of death is unknown), and other on May 8 (septicemia by *Streptococcus canis*).

To design the FeLV control programme for the Iberian Lynx (FCP), a commission was created. Members included representatives from each institution with competence in the conservation of the Iberian lynx and an advisory group of national and international world-renowned experts in FeLV, non-domestic felids, Iberian lynx and wildlife diseases. The goals of the FCP were: 1) to control the FeLV focus to help stop the spread of the virus over the whole Doñana Iberian lynx population; 2) to remove the virus from the population, and 3) to minimize the possibility of occurrence of a new outbreak. In order to achieve these goals, the FCP implemented the following guidelines: 1) removal from nature of FeLV viremic Iberian lynxes; 2) FeLV vaccination of all naïve individuals; 3) modification of all management structures (supplementary feeding stations, water points, etc.) to minimize the contact between individuals, and 4) control of the feral cat population. Because they were not infectious, and due to the scarcity of individuals in Doñana, it was decided that latently infected individuals should be allowed to remain in the field under an intense monitoring.

The aim of this chapter is to describe the evolution of the FeLV outbreak, the management measures implemented for its control and to present the results obtained with this management. About 80% of the entire Doñana lynx population (estimated in 41 individuals by 2007) was captured, checked and vaccinated within a period of eight months. The outbreak was effectively controlled before the following breeding season, yet the risk of occurrence of a new outbreak still persists.

Material and methods

Fieldwork

To carry the FCP out, lynx captures lasted for eight months (April-December, 2007), whereas domestic cat captures are maintained through time. All Iberian lynxes were captured using double entrance, electro-welded-mesh-made boxtraps (2x0.5x0.5 m), with the exception of one animal that was trapped by means of a remote-controlled teleinjection system (Ryser et al., 2005). Traps were baited with a live domestic rabbit fixed to the trap with a harness. The effort to capture lynxes was 2350 trap/days during the capture period of the FCP. Traps were checked three to five times a day by at least two people trained in lynx handling. Whenever a lynx was captured, it was transferred to a stainless steel compression cage (1 x 0.5 x 0.25 m), in which it was transported to the Iberian lynx clinic. Domestic cats were trapped using both guillotine and Tomahawk box traps. Capture efforts were mainly focused on lynx distribution areas close to human populations. Traps were baited with dead chickens and sardines. A total of 15 traps were used at the same time, working five days a week. After a physical examination of the cats, 10 ml of blood were taken and some drops were employed to run the fast FeLV antigen ELISA test (IDEXX® Snap test), which detects viremia (p27 antigen). FeLV viremic domestic cats were euthanized using sodium pentobarbital (Dolethal®, Vetoquinol ®). FeLV-negative feral cats were transported to an animal protection society, where they stayed waiting for an owner.

The risk of infection to lynxes posed by supplementary feeding stations and man-made water points –as the two main structures thought to increase contact rate between individuals– was also evaluated. Regarding feeding stations, it was decided that only one rabbit should be released at a time to avoid different lynxes feeding on the same prey. In addition, weekly cleaning and disinfection of drinking points was implemented.

Lynx handling and laboratory methods

A routine standardized handling and health evaluation protocol was performed in all captured lynxes once they were transported to the clinic. After chemical immobilization with ketamine (Imalgene® 1000, Merial®) and medetomidine (Domtor®, Pfizer®) 12-24 ml of blood were collected from the cephalic vein in a serum separator, ethylenediaminetetraacetic acid (EDTA) and Li-heparin. Some drops of blood were used to run a FeLV ELISA test (IDEXX® Snap test ®). Subsequently, sandwich ELISA and real-time PCRs were performed in the Clinical Laboratory of the Veterinary Faculty of Zürich University, Switzerland (Meli et al., this book). Real-time PCR is a highly sensitive diagnostic method that detects viral DNA, so both viremic and latently infected animals are detected (Tandon et al., 2005; Meli et al., unpublished data). In addition, the sandwich ELISA detects viremia (Lutz et al., 1990; Meli et al., this book). Afterwards, 2 ml of blood were used to perform a hemogram (complete cell blood count and blood smear cytology) in the hematology unit of the Veterinary Clinic Hospital of the Veterinary Faculty of the Barcelona University, Spain (Pastor et al., this book). Also, other serum and blood samples were used for other routine diagnostic tests (biochemistry panel, protein electrophoresis, infectious serology and PCR panels) (Martínez et al., this book).

All viremic lynxes were transfered to the Los Villares Wildlife Rehabilitation Center, Córdoba, Spain, where a full exam and sampling were performed every two months. Infected lynxes received antibiotherapy (Cefovecin, Convenia®, Pfizer®) and antiviral drugs (Virbagen® feline interferon, Virbac®) when it was considered necessary. If a viremic individual became latently infected, it was returned to the wild. All lynxes found non-viremic were vaccinated subcutaneously with one dose PureVax FeLV® (Merial®) vaccine (kindly donated by Dr. J.C. Thibault, Merial®, Lyon, France) in the right shoulder. Afterwards they were released. This recombinant canarypox-vectored has shown to be the most protective vaccine against FeLV in domestic cats (Hofmann-Lehmann et al., 2006), and there is no danger of provoking FeLV disease. A booster vaccination was carried out with as many animals as we were able to re-trap (n=10).

Statistical analyses

First, we explored the differences in hemogram values due to sex or age through a MANOVA including hemogram values (total red blood cells counts, hemoglobin concentration, hematocrit, erythrocyte indexes: mean corpuscular volume –MCV–, mean cell hemoglobin concentration –MCHC–, mean corpuscular hemoglobin –MCH–; total white blood cells counts –leukocytes–, differential, white blood cells counts, thrombocytes counts, punctata reticulocytes and agregata reticulocytes) as dependent variables, and sex and age (expressed as one year or older) as factors, including their interaction in the model. There were no notable differences in any of the hemogram values due to sex, age or their interaction, so we excluded these factors in the final analysis. To explore the effect of the FeLV over the hemogram parameters, we performed a one-way ANOVA including hemogram values as dependent variables and FeLV viremia status as a factor.

Results

Lynx captures within the FCP took place between April and December 2007. Given that most of the population had been evaluated by December, the Programme was stopped to prevent interfering with next breeding season. A total of 30 Iberian lynxes (including the deceased ones) were evaluated to detect the presence of FeLV infection. This accounted for 83% of the total Doñana population. Eight lynxes (about 20% of the population) tested positive for FeLV (seven were viremic and one latently infected); all infected lynxes inhabited the Coto del Rey area (Figure 1). These FeLV positive lynxes together with the four dead males accounted for an FeLV prevalence value of 27% in the Doñana lynx population (35%) if considering only the handled animals whose FeLV status was known). Eight out of 12 total positives (67%) were males (Figure 2). The last positive animal was found in August 1, 2007. No FeLV-positive lynxes were found outside Coto del Rey. A total of 22 lynxes were negative and subsequently vaccinated, 10 of them (45% of those vaccinated) also were given a booster vaccination (Table 2).

Figure 1. Spatial distribution of Iberian lynx captures during the implementation of the FeLV control programme campaign in Doñana, by subpopulations. Green symbols represent negative individuals and red symbols represent positive ones. Blue symbols represent known FeLV cases before 2007.

Figura 1. Distribución espacial de las capturas de lince ibérico durante el programa de control del brote de leucemia felina en Doñana. Los símbolos verdes representan los individuos negativos y los rojos los individuos positivos. Los símbolos azules representan los casos conocidos de FeLV en lince ibérico antes de 2007.

Figure 2.

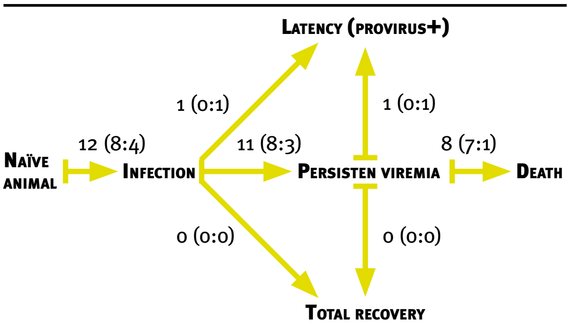


Figure 2. Diagram showing the connection and the evolution of the different status stages in the infection by FeLV. Numbers refer to the number of Iberian lynxes (males/females) that have followed every different step out of the found FeLV positive in the 2007 outbreak.

Figura 2. Diagrama de flujo mostrando la evolución de los diferentes estados de infección por FeLV. Los números marcan el número de linces ibéricos (machos/hembras) que han seguido cada paso, de los que fueron hallados virémicos en 2007.

Hemogram values	F(1,29)	p
Erythrocytes	20.81	<0.01
Punctata reticulocytes	18.87	<0.01
Hemoglobin	17.60	<0.01
Hematocrit	17.19	<0.01
Agregata reticulocytes	14.76	<0.01
HCM	7.34	0.01
VCM	6.53	0.02
CCMH	2.90	0.09
Leukocytes	2.37	0.13
Thrombocytes	0.49	0.49

Table 1. Results of the one-way ANOVA exploring the effect of FeLV viremia on hemogram values in various Iberian lynx captured during the FeLV Control programme. All values in bold showed a significant difference between infected and non-infected individuals.

Tabla 1. Resultados del ANOVA unifactorial explorando el efecto de la viremia de FeLV sobre los valores hematológicos en los linces ibéricos capturados durante el programa de control del brote de FeLV. Los valores en negrita mostraron diferencias significativas entre infectados y no infectados.

Name	Subpopulation	Sex	Age (Yr)	Vaccination	Booster
Teo	CRS	F	5	21-May-2007	28-Dec-2007
Durillo	CRS	M	0	13-Jun-2007	22-Oct-2007
Aliso	AB-VE	F	3	3-Jul-2007	N/A
Viana	CRS	F	5	15-Ago-2007	31-Oct-2007
Dardo	CRS	M	0	16-Ago-2007	30-Oct-2007
Dedalera	CRS	F	0	16-Ago-2007	22-Oct-2007
Bonares	AB-VE	F	2	21-Ago-2007	N/A
Clavo	AB-VE	M	1	22-Ago-2007	27-Sep-2007
Jabata	AB-VE	F	5	22-Ago-2007	24-Sep-2007
Boliche	AB-VE	M	2	24-Ago-2007	10-Oct-2007
Salado	AZN	M	4	29-Ago-2007	8-Nov-2007
Dulce	AZN	F	0	4-Sep-2007	6-Nov-2007
Damán	AZN	M	0	7-Sep-2007	N/A
Daro	CRS	F	0	17-Sep-2007	N/A
Wari	CRS	F	8	19-Sep-2007	N/A
Duquesa	AZN	F	0	20-Sep-2007	N/A
Drupa	CRS	F	0	26-Oct-2007	N/A
Dumbo	AB-VE	M	0	21-Nov-2007	N/A
Mata	AB-VE	F	5	21-Nov-2007	N/A
Bruma	AB-VE	F	2	28-Nov-2007	N/A
Bocacha	AB-VE	M	2	27-Nov-2007	N/A
Lula	AB-VE	F	>5	5-Dec-2007	N/A

Table 2. FeLV-naïve Iberian lynxes captured during the FeLV Control programme in Doñana and their vaccination date, by subpopulations: Coto del Rey (CRS), Abalario-Vera (AB-VE) and Aznalcázar (AZN). Booster vaccination could not given to every vaccinated individuals because some of them could not be re-trapped.

Table 2. Linces negativos a FeLV capturados durante el programa de control del brote de FeLV en Doñana, con su fecha de vacunación, por subpoblaciones: Coto del Rey (CRS), Abalario-Vera (AB-VE) y Aznalcázar (AZN). Algunos animales no pudieron ser revacunados al no poder ser recapturados.

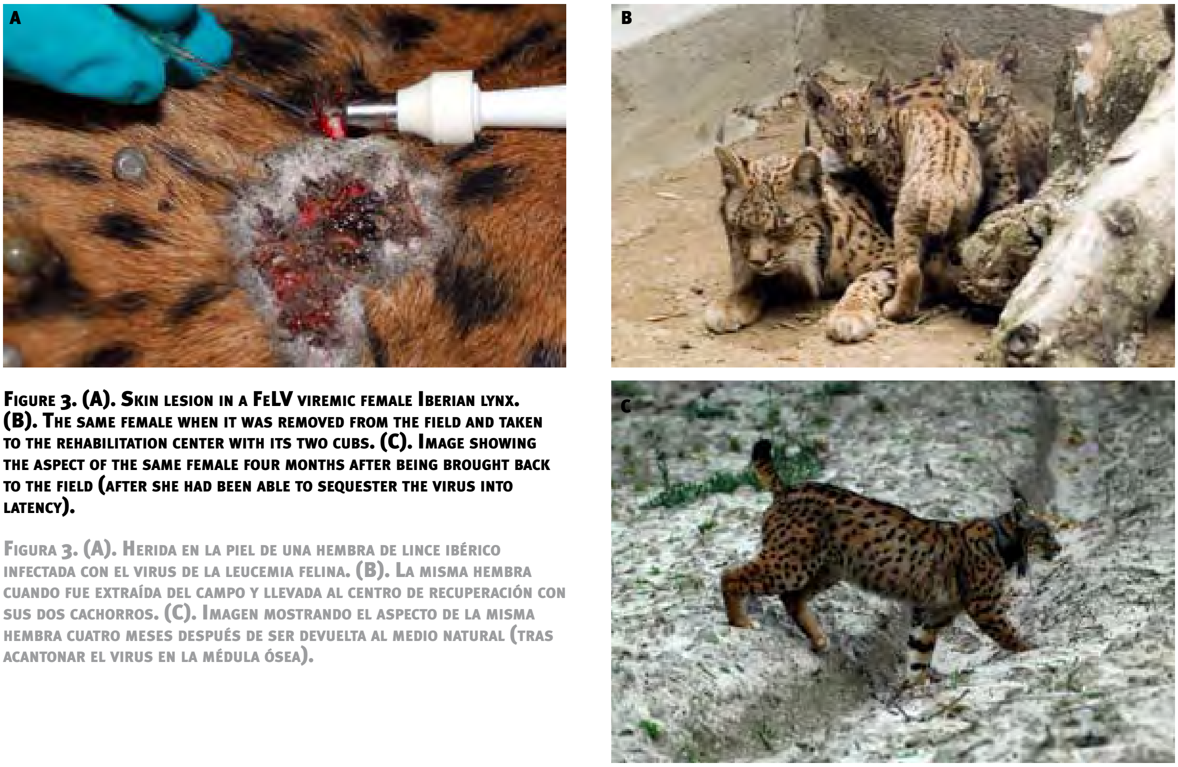


Figure 3. (A). Skin lesion in a FeLV viremic female Iberian lynx. (B). The same female when it was removed from the field and taken to the rehabilitation center with its two cubs. (C). Image showing the aspect of the same female four months after being brought back to the field (after she had been able to sequester the virus into latency).

Figura 3. (A). Herida en la piel de una hembra de lince ibérico infectada con el virus de la leucemia felina. (B). La misma hembra cuando fue extraída del campo y llevada al centro de recuperación con sus dos cachorros. (C). Imagen mostrando el aspecto de la misma hembra cuatro meses después de ser devuelta al medio natural (tras acantonar el virus en la médula ósea).

To carry out an intense monitoring programme of the lynx population over time and, thus, help detect rapidly any potential health problem, all trapped adult lynxes were equipped with a radio-collar (some of them were already radio-tagged).

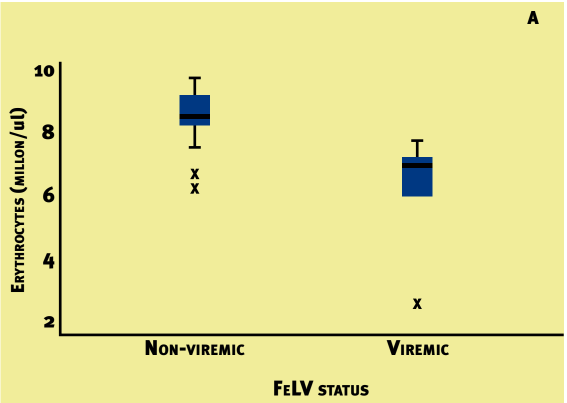
One adult viremic female that was removed to the rehabilitation center in early June had turned to be latently infected and non-viremic by early December 2007, so it was released back into her original territory, provided that there was no other female occupying this space. This female mated normally and whelped a litter in the next breeding season (Figure 3). The other six viremic lynxes have not sequestered the virus up to now. Only two of these six individuals (a young female and a subadult male) remain alive in January 2009.

Although the symptoms FeLV-infected lynxes were not obvious, they all showed signs of anemia. Viremic lynxes showed significantly lower values of erythrocytes, hemoglobin and hematocrit, whereas they showed significantly higher values of MCV, MCH and reticulocytes than those displayed by non-viremic individuals (Table 1; Figure 4). The mean corpuscular volume seemed to be an honest parameter to monitor the evolution of the disease, although sample size is not large enough to draw conclusions. All infected-individuals that eventually died, did so due to secondary processes, all of them presented different degrees of anemia and immunosuppression. Despite the fast course of infection, no signs of neoplasia were detected in FeLV viremic lynxes, as it was the case in the Florida Panther epizootic (Cunningham et al., 2008).

From May 2007 to September 2008, 74 domestic cats and three wildcats *(Felis sylvestris)* were trapped in Doñana. Seven domestic cats (11% of the total) were found FeLV-viremic, whereas the three wildcats were negative to the virus. Capturing effort for cats is maintained over time because villages surrounding Doñana area seem to be a constant source of feral cats that leave the villages and enter the protected area.

Conclusions

According to previous reports on FeLV in non-domestic felids, the source of FeLV infection in Iberian Lynx is thought to have been feral/domestic cats (Meli et al., this book). Nevertheless, further research is needed to address this issue. Vaccination, isolation of viremic lynxes, together with measures adopted at the supplementary



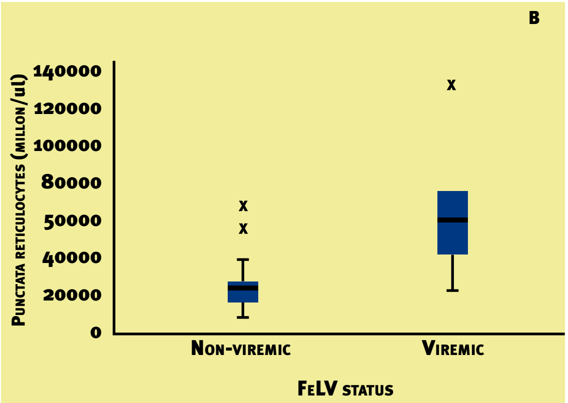
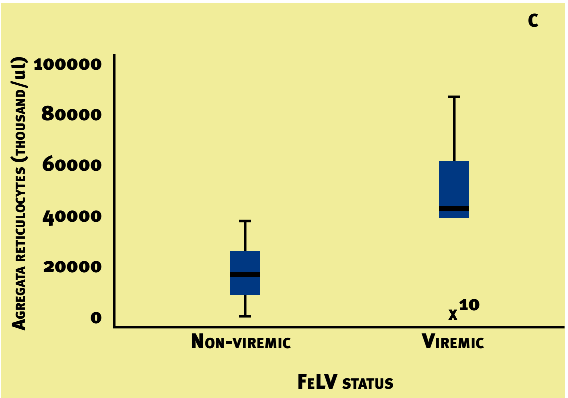


Figure 4. Boxplots representing differences in erythrocyte (A), punctata reticulocytes (B) and agregata reticulocytes (C) between viremic and non-viremic Iberian lynxes captured in Doñana during the FeLV control programme campaign, in 2007.

Figura 4. Diagrama de cajas representando las diferencias en valores de eritrocitos (A), reticulocitos punctata (B) y reticulocitos agregata (C) entre los individuos virémicos y los no virémicos capturados en Doñana durante el programa de control del brote de FeLV, en 2007.



feeding stations, are thought to have played an important role in preventing further FeLV dissemination over the Doñana Iberian lynx population. Moreover, the drastic reduction of the feral cat population might be very important to prevent future outbreaks. Fortunately, no more FeLV cases have been detected since August 2007. Nevertheless, the risk of reactivation of FeLV viremia in the two latently infected females still exists. To manage this situation, an intensive monitoring programme, that was recommended by the expert commission, was started by 2008. This programme involved: 1) annual evaluation of latently infected individuals and their offspring; 2) annual vaccination campaigns while those animals remain in the population, and 3) constant non-invasive monitoring of the population's FeLV status via PCR analysis of feces collected in the field. During 2008, both latently infected females and their offspring were evaluated. Both females remained non-viremic, and their offspring were negative. Eight adult Iberian lynxes were re-vaccinated in 2008, while five cubs were vaccinated for the first time. Moreover, 98 feces were tested for FeLV presence in 2008. No FeLV was detected in them.

Although the occurrence of FeLV in the Doñana Iberian lynx population is not new (Luaces et al., 2008), a severe outbreak had never been detected before 2007. Interestingly, dissemination of the virus over the Iberian lynx population in early 2007 was much faster than expected for a solitary species. The possible causes proposed to explain this spreading pattern include: 1) unexpectedly high pathogenicity of the FeLV strain infecting the lynxes (see Meli et al., this book); 2) timing of infection, which reached the lynx population just before breeding season, when more contact between individuals is known to occur, and 3) the high density of the affected subpopulation. Another potential factor underlying this virulent pattern is the level of inbreeding displayed Doñana lynxes (Godoy et al., this book). Inbreeding has shown to provoke depression of the immune system in other species (Merola, 1994; Ross-Guillespie et al., 2007). The idea that the FeLV strain affecting Iberian lynxes in Doñana was highly pathogenic is supported by the unusually fast spread of the infection. While FeLV infection usually lasts years before death occurs (Miyazawa, 2002; Barr and Bowman, 2006), six out of the 12 lynxes found infected died in less than six months after infection. Nevertheless, a generalized immunosuppression due to inbreeding and/or any other factor could be also responsible for those findings (Peña et al., 2006; Jiménez et al., 2008; Jiménez et al., this book).

According to previous studies carried out in the domestic cat (Lutz et al., 1990; Fuchs et al., 1994; Arjona et al., 2000; Levy et al., 2006), males show a higher infection rate than females, probably because of their aggressive behavior (see Barr and Bowman, 2006). Similarly, in the Iberian lynx, intraspecific fights in males have been detected at a much more frequent rate than those in females (LIFE conservation project, unpublished data). Supporting this idea, one male Florida panther was thought to have been infected after an aggressive encounter with a viremic male during an FeLV outbreak (Cunningham et al., 2008). Although FeLV has been found in the semen of viremic domestic cats, venereal transmission has not been considered important (Hoover and Mullins, 1991). The presence of infection in Iberian Lynx females, however, suggests that transmission also may occur during courtship and mating. In the Doñana Iberian lynx population, a sex ratio deviation towards adult females has been detected in the last decade (see Gaona et al., 1998). Given these results, we consider that FeLV might have been affecting the dynamics of the Doñana lynx population at least since the early 90s.

By January 2009, the Doñana Iberian lynx population is composed of about 25 adult territorial individuals (7 males and 18 females) out of a population of approximately 50 total individuals. Eighty per cent of the adult lynxes are radio-tagged, and a continuous monitoring programme is being carried out. This programme allows for early detection of mortality, including mortality due to infectious diseases, which is very difficult to detect otherwise (Martínez et al., this book). The FeLV ourbreak could be controlled throughout 2007 and no more cases have been detected in more than 18 months. During 2008, more than 20 lynx cubs were born in Doñana, 16 of which are still alive in early 2009. This cub production is the highest ever known for this population. Despite the high toll paid due to the FeLV outbreak in 2007, the Doñana lynx population continues to move forward, although the scarcity of adult males should be managed in the short-term to avoid fatal consequences (Simón et al., this book; Palomares, this book). We believe that a thorough monitoring of the two Iberian lynx free-ranging populations is essential to ensure the wellbeing and continuity of this critically endangered felid.

Acknowledgements

This work has been funded by the LIFE project LIFENAT 06/E/000209. Idexx® donated all combo FeLV/FIV tests (Snap test®). We are grateful to Dr. J.C. Thibault for donating the Merial® (Purevax® FeLV) vaccine doses. N. Viqueira, J.C. Capuz, M.J. Pérez, C.I. León, K. Reyes, M.P. Ryser-Degiornis, M. Roelke and J. Velarde collaborated with the chemical immobilizations and exams. G. Ruiz, J.A. Báñez, R. Sanabria, D. Palacios, R.B. Millán, J.A. Tena, E. Rojas, C. Gutiérrez and the rest of the LIFE project members conducted field work, radiotracking and lynx captures. The expert commission and the Iberian Lynx Veterinary Advisory Group (GAAS) led and facilitated the FeLV control programme. A. Ryser helped and advised with the capture of priority individuals. Most of the laboratory work was performed using the logistics of the Center for Clinical Studies at the Veterinary Faculty, University of Zürich. Los Villares Wildlife Rehabilitation Center staff provided treatment and care to all the viremic animals removed from field. Doñana National and Natural Park, Doñana Biological Station staff and researchers helped with fieldwork, with captures, and also detected two FeLV-dead individuals.

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The sun, with all those planets revolving around it and dependent upon it, can still ripen a bunch of grapes as if it had nothing else in the universe to do.

Galileo
(1564-1642)

Diseases of captive and free-ranging non-domestic felids

Enfermedades de felinos silvestres en cautividad y en libertad

Karen A. Terio

Resumen
Paradójicamente, tanto las enfermedades infecciosas como las no infecciosas pueden ayudar a mantener sana a una población, si su presencia permanece a niveles bajos. Salvo en muy pocos casos, las enfermedades no han sido un factor importante en la viabilidad de distintas poblaciones de felinos. No obstante, la fragmentación del hábitat y la mayor interacción con animales domésticos pueden modificar ciertos patrones de enfermedades que han sido históricamente estables. Gran parte de la mortalidad presente en las poblaciones de felinos silvestres está asociada a traumatismos (conespecíficos o inducidos por humanos) o a la falta de capacidad de supervivencia que se observa con frecuencia en animales juveniles o viejos, así como en los animales que se ven obligados a vivir en la periferia de un hábitat idóneo. Los agentes infecciosos son especialmente significativos en las poblaciones que están aisladas geográficamente, en las que están en contacto con animales domésticos, en animales que viven en hábitats marginales y en las poblaciones genéticamente empobrecidas. Las enfermedades infecciosas en las poblaciones en libertad suelen afectar sólo a unos pocos ejemplares, aunque en algunos casos han llegado a afectar a toda una población. Por otro lado, las enfermedades degenerativas y relacionadas con la edad son más frecuentes que las enfermedades infecciosas entre las poblaciones cautivas, generalmente como consecuencia de los programas de medicina preventiva. No obstante, una enfermedad infecciosa puede tener un impacto muy negativo sobre un programa de cría en cautividad, no sólo porque cause enfermedades en animales de gran valor genético, sino porque también limita el intercambio de ejemplares entre centros y la reproducción de los animales "positivos" o portadores. La cautividad, e incluso la contención durante un período corto, pueden afectar a la homeostasis fisiológica básica del animal y si no se mitigan los efectos del estrés, estos pueden afectar profundamente a la salud general de los animales. El control de las enfermedades debe ser un elemento imprescindible en cualquier programa de conservación, tanto *in situ* como *ex situ*. Lo ideal sería que los programas de seguimiento incluyesen, siempre que fuese posible, una evaluación post mórtem exhaustiva de todos los animales muertos para determinar los riesgos reales asociados a la exposición a enfermedades. Con el cambio de hábitats y del clima, el riesgo de transmisión de enfermedades en poblaciones anteriormente estables también podría cambiar y, por tanto, los programas de seguimiento son críticos para identificar las causas de los problemas, con el fin de poder responder a ellos a través de una gestión adecuada.

Palabras clave
enfermedad infecciosa, enfermedad degenerativa, viabilidad de la población, epidemiología, post mórtem

Abstract

Disease, infectious or non-infectious, when present at low levels, paradoxically can help keep populations healthy. With a few exceptions, disease has not been a significant factor in felid population viability. However, habitat fragmentation and increased interaction with domestic animals may alter previously stable disease patterns. Many mortalities in wild populations of felids are associated with trauma (conspecific or human-induced); or a failure to thrive, common in juveniles and aged animals as well as in animals forced to the periphery of suitable habitats. Infectious agents have heightened significance in geographically isolated populations, populations in contact with domestic animals, animals living in marginal habitats and in genetically impoverished populations. Infectious diseases in free-ranging populations typically only affect small numbers of individuals, but in a few instances have had effects at the population level. In contrast, degenerative and age-related diseases are more common than infectious disease in captive populations. This is generally owing to preventative medicine programmes. However, infectious disease can cripple a captive breeding programme not only by causing disease in genetically valuable animals but also by limiting movements and breeding of "positive" or exposed animals. Captivity, even short-term holding, can also impact an animal's basic physiological homeostasis and the effects of stress, if not mitigated, can have profound impacts on overall health. Disease monitoring should be a vital component of any *in situ* or *ex situ* conservation programme. Ideally monitoring programmes should include comprehensive post-mortem evaluation of available carcasses such that the true risks associated with disease exposure can be ascertained. As habitats and climates change, the risks of disease to previously stable populations of felids may change and these monitoring programmes will be critical to identifying the problem so that useful management solutions can be implemented.

Keywords

infectious disease, degenerative disease, population viability, epidemiology, post-mortem

Photo: Alexander Sliwa

Diseases of captive and free-ranging non-domestic felids

Karen A. Terio

Introduction

This review aims to concentrate on the major diseases that affect free-ranging and captive non-domestic felids. It should not be construed as comprehensive as it cannot describe every case of a disease entity in non-domestic felids, nor comment upon all diseases to which they might be susceptible. Additionally, it is beyond the scope of this review to cite the numerous serosurveys that have documented exposure to infectious agents in many species of felids across varied habitats (see Munson et al., in press, for a thorough review of the subject).

In wild populations, disease surveillance relies heavily on ante-mortem serological and parasitological testing. However, most serological assays detect antibodies and not antigen. Therefore, it is important to remember that seropositivity only implies previous or current exposure. From these serosurveys it is evident that many feline viruses are endemic in wild felid populations, with the notable exception of feline leukemia virus (Munson et al., in press; Meli et al., 2009; López et al., 2009). Correlation of exposure to infectious agents with actual disease, verified by post-mortem evaluation, serves to provide additional understanding of relative risk to a population.

Vaccinations are available for control of some infectious diseases and are used commonly in captive populations and in rare circumstances in free-ranging populations (Cunningham et al., 2008; López et al., 2009). The use of modified live vaccines is always a concern as their use in some non-domestic felids has resulted in disease (Crawshaw et al., 1996; Kennedy-Stoskopf, 2005). Killed vaccines are considered safer, however these are generally less efficacious.

Selected bacterial and fungal infections in felids

Mycobacterial infections

Tuberculosis is an important cause of morbidity and mortality in free-ranging lions living in Kruger National Park and Hluhluwe-uMfolozi Park (Keet et al., 1996; Keet et al., 2005; Michel et al., 2006) as well as in Iberian lynx (Briones et al., 2000; Pérez et al., 2001; Aranaz et al., 2004) (Figure 1). In felids, aerosolization of *M. bovis* during feeding on an infected carcass (cape buffalo, deer, fallow deer or wild boar) is considered to be the primary route of infection. Affected animals are thin to emaciated with numerous granulomas within the lungs and regional lymph nodes. Within some regions of Kruger National Park, up to 90% of the lions have been exposed to *Mycobacterium bovis* and the disease has spread to cheetahs and leopards presumably due to scavenging of buffalo carcasses (Keet et al., 1996). In the southern Iberian Peninsula, there is a high prevalence of tuberculosis in wild ungulates and wild boars related to the high density of animals in hunting or protected areas (Gortázar et al., 2008).

Photo: Héctor Garrido

Figure 1. Iberian lynx eating a fallow deer. Tuberculosis is highly prevalent in some deer populations in Spain, which entails a health risk for this endangered felid species.

Figura 1. Lince ibérico comiendo un gamo. La tuberculosis tiene una alta prevalencia en algunas poblaciones de ciervos en España, lo que supone un riesgo sanitario para este felino en peligro de extinción.

The epidemiology of tuberculosis has been best studied in lions (Figure 2). Frequent exposure to infected tissue and spread of disease within lion prides occurs in areas where bovine tuberculosis is prevalent in buffalo. Therefore, it has been difficult to discern whether lions can serve as a maintenance host. Morbidity and mortality due to tuberculosis may also be associated with social changes within prides, such as faster male coalition turnover with subsequent infanticide, which are contributing to decreased survival and breeding success (Michel et al., 2006).

Anthrax

Anthrax, caused by *Bacillus anthracis,* has been associated with sporadic mortalities in free-ranging and captive lions and cheetahs in Southern Africa (Figure 3). Typically, these predators are infected after eating an infected carcass (Jager et al., 1990). Felids commonly present with marked cervical edema (Jager et al., 1990). The typical anthrax lesions of splenomegaly and unclotted blood are also present.

Helicobacter sp.

Helicobacter spp. are spiral bacteria that commonly colonize the stomachs of free-ranging and captive felids (Kinsel et al., 1998a, b). *Helicobacter* spp. are generally an incidental finding with only rare individual cases of gastritis reported in most felid species (Schroder et al., 1998). In contrast, the majority (~95%) of captive cheetah have lymphoplasmacytic gastritis of variable severity (Eaton et al., 1993; Munson et al., 1993; Robert and Waltzer, this book). Clinical signs range from weight loss to chronic vomiting and regurgitation. Cheetahs with gastritis also commonly develop secondary amyloidosis with renal medullary deposition of amyloid and, in some cases, renal failure (Papendick et al., 1997). Although some treatments provide short-term relief, treatments for gastritis and *Helicobacter* have little long-term effect on disease progression. (Citino and Munson, 2005). The types of *Helicobacter* are similar in both free-ranging and captive cheetahs, however, free-ranging cheetahs rarely develop disease unless they are held in captivity (Terio et al., 2005). These findings along with minimal treatment efficacy suggest that the bacteria alone do not cause disease and that host factors are important in disease development. Current research has focused on understanding the host immune response to *Helicobacter* and whether stress in captivity is altering this response.

Salmonella sp.

Salmonella has been shown in one study of captive felids to be shed by ~90% of the examined cats (Clyde et al., 1997). *Salmonella typhimurium* was the most common isolate and was presumed to have been acquired from a

Figure 2. Lion (*Panthera leo*).

Figure 2. León (*Panthera leo*).

Figure 3. Cheetah (*Acinonyx jubatus*).

Figure 3. Guepardo (*Acinonyx jubatus*).

food source. Although there were rare cases of diarrhea, the majority of cats in this study were asymptomatic. Shedding of *Salmonella* poses a potential zoonotic threat and proper hygiene should be observed.

Cryptococcus neoformans

Cryptococcus neoformans is a fungal organism that causes both cutaneous and systemic disease in cats. While all felids are susceptible, there have been a number of reports of systemic *cryptococcosis* in captive and free-ranging cheetahs (Berry et al., 1997; Bolton et al., 1999; Millward and Williams, 2005). Cheetahs in these studies were serologically negative for immunosuppressive viruses (FIV and FeLV) suggesting that other causes of immune dysfunction may be important in disease pathogenesis.

Dermatophyte infections

Dermatophyte infections caused by *Trichophyton* or *Microsporum* spp. occur sporadically in captive felids. *T. mentagrophytes* has been isolated from Iberian lynx in captivity, from cubs with dermatologic lesions (Martínez et al., this book). Clinical dermatophytosis has also been diagnosed in a few free-ranging Florida panthers, infected with *T. mentagrophytes* and/or *M. gypseum* (Rotstein et al., 1999). Lesions varied from focal alopecia to more extensive alopecia with excoriation, ulceration, pyoderma and eventually lichenification of the skin. In some animals lesions resolved spontaneously, but in a single animal from this study intensive oral treatment was required.

Selected viral infections in felids

Canine distemper virus

One virus that has caused significant mortality in free-ranging felids is canine distemper virus (CDV). In 1994, a CDV epidemic within the Serengeti ecosystem was associated with a loss of approximately 30% of the lion population (Roelke-Parker et al., 1996). Affected lions either disappeared or presented in thin condition. Rarely, lions were observed with neurological signs including seizures. Histologically, animals had interstitial pneumonia, encephalitis, and/or lymphoid depletion with rare intracytoplasmic and intranuclear inclusions. Viral isolation and characterization suggests that this epidemic was caused by a new strain of CDV that could cross the species barrier but still retain its pathogenicity for canids (Carpenter et al., 1998a). In addition to free-ranging lions within the Serengeti ecosystem, there are reports of sporadic cases in other free-ranging felids (McBurney et al., 1998; Munson, 2001; Meli et al., this book) and captive felids have also succumbed to canine distemper infection (Appel et al., 1994).

Interestingly, since 1994, serosurveys have demonstrated multiple events during which naïve free-ranging lions have been exposed to canine distemper virus without significant mortalities. Recent research has determined

Photo: Alexander Sliwa

Figure 4. Pallas' cat *(Otocolobus manul).*

Figura 4. Gato de Pallas *(Otocolobus manul).*

that the CDV mortality events were associated with high levels of a co-pathogen, *Babesia* spp., levels of which were not elevated during exposures without mortality. High *Babesia* levels were also linked to climatic and ecological conditions that favored tick propagation. Therefore, CDV may not necessarily be a threat to populations but if circumstances favor co-pathogens then higher mortality rates are possible (Munson et al., 2008).

Feline upper respiratory tract disease

Feline herpesvirus (FHV) and feline caliciviruses (FCV) are both important viral causes of upper respiratory tract infections in non-domestic felids. Exposure to these viruses is common both in captivity and in the wild, however disease is typically mild and self-limiting. Clinical signs include oculonasal discharge, rhinitis and conjunctivitis.

Particularly severe disease associated with Herpesvirus has been noted in captive Pallas' cats (Ketz-Riley et al., 2003) and cheetahs (Junge et al., 1991). Herpesviral disease in Pallas' cats (Figure 4) has been associated with recent vaccination using modified live virus vaccines (Kennedy-Stoskopf, 2005). Although a mild upper respiratory tract disease is more common, some captive cheetahs develop a severe proliferative dermatitis around the eyes and at sites in contact with saliva and tears (Junge et al., 1991; Robert and Waltzer, this book) characterized histologically as a florid plasmacytic and eosinophilic dermatitis (Munson et al., 2004). Despite this unusual and severe presentation, antigenic studies have shown that the virus is similar to feline herpes type I, the common herpes virus of domestic cats (Scherba et al., 1988). The disease in cheetahs develops despite previous vaccination suggesting either inadequate titers or an inappropriate immune response.

Feline coronavirus

Infection with feline coronavirus (FCoV) is common in felids and can result in mild enteritis, chronic ulcerative colitis or rarely feline infectious peritonitis (FIP) (Kennedy et al., 2002). These different disease manifestations are associated with differences in viral biotypes of feline enteric corona virus (FECV) and FIPV, respectively. Although all felids are susceptible, FIP is most commonly manifested in captive cheetahs. FIP lesions in affected cheetahs have included the typical fibrinopurulent peritonitis, pleuritis and vasculitis as well as multifocal necrosis throughout many organs (Evermann et al., 1988; Munson, 1993). Serological testing is complicated as it cannot distinguish between FECV and FIPV, because there are two serotypes of FCoV (type I and type II), and each serotype has viruses of both biotypes (Kiss et al., 2000). Research has shown that serology does not always correlate to current infection and that some persistently infected cheetahs actively shedding FCoV are seronegative (Kennedy et al., 2001). Also, some infected cheetahs only shed virus intermittently in the feces (Gaffney and Munson, pers. comm.). Therefore, combining PCR on fecal samples and serology can provide a more accurate evaluation of infection status (Kennedy et al., 2001).

Parvovirus

Parvoviruses (both feline and canine) can affect non-domestic felids. Feline parvovirus (FPV/panleukopenia) causes disease similar to that in domestic cats and has been reported sporadically in captive (Fix et al., 1989;


Photo: Alexander Sliwa

Figure 5. Tiger *(Panthera tigris).*

Figura 5. Tigre *(Panthera tigris).*


Photo: Alexander Sliwa

Figure 6. Snow leopard *(Pantera uncia).*

Figura 6. Leopardo de las nieves (*Pantera uncia*).

Mochizuki et al., 1996; Valicek et al., 1993; Wasieri et al., 2009) and free-ranging non domestic felids (Schmidt-Posthaus et al., 2002; Stahl and Vandel, 1999). Canine parvovirus (CPV-2a and 2b) has been identified in felids. CPV-2b was identified in cases of necrotizing enteritis with crypt necrosis in cheetahs while CPV-2a was identified in a Siberian tiger with diarrhea. Some of the affected cheetahs had been vaccinated for feline parvovirus suggesting that FPV vaccines may not provide protective immunity (Steinel et al., 2000).

Feline papillomavirus

Papillomaviruses are present in all felids studied to date, but lesions are most common in lions, tigers (Figure 5), snow leopards (Figure 6), and cheetahs (Sundberg et al., 2000). Infected species can develop multicentric oral and cutaneous papillomas that spontaneously regress. However, in captive snow leopards, papillomas have been documented to transform into aggressive, often multicentric squamous cell carcinomas (Ott-Joslin et al., 2001).

Feline immunodeficiency virus

Feline immunodeficiency virus (FIV) is prevalent worldwide and many species of felids, both free-ranging and captive, have serological evidence of exposure. Exposure has not, to date, been identified in Iberian lynx (Roelke et al., 2008; Meli et al., 2009). Of the non-domestic felids, FIV infection has been most extensively studied in African lions. Domestic cats infected with FIV can be asymptomatic or can develop disease that ranges from

Photo: Alexander Sliwa

Figura 7. Clouded leopard (*Neofelis nebulosa*).

Figura 7. Pantera nebulosa (*Neofelis nebulosa*).

lymphoid hyperplasia in the early stages to depletion of the lymphoid follicles and paracortical regions of lymph nodes (Bendinelli, 1995). In contrast to domestic cats, there is controversy as to whether disease is associated with FIV infections in non-domestic felids with only rare reports of disease possibly associated with infection (Poli et al., 1995). Alterations in lymphocyte subsets have been noted with depletion of CD4 cells in both captive and free-ranging lions and pumas infected with FIV (Bull et al., 2003; Roelke et al., 2006). In addition to lions, Mountain lions (pumas), Pallas' cats, leopards, and cheetahs can be infected with their own lentiviruses (Brown et al., 1994; Carpenter et al., 1996; Barr et al., 1997; Carpenter et al., 1998b; Troyer et al., 2005).

Feline leukemia virus

Unlike many felid viruses, infection or exposure to feline leukemia virus (FeLV) is rare in free-ranging felids. Recently however, a subset of FeLV ELISA positive free-ranging Florida Panthers have been diagnosed with lymphadenopathy, anemia and lymphopenia due to infection (Brown et al., 2006; Cunningham et al., 2008). The virus strain suggests infection from a domestic cat with subsequent spread within the Florida panther population. Iberian lynx, a previously naïve population (Roelke et al., 2008), have recently been exposed to FeLV (Luaces et al., 2008; Meli et al., this book; Meli et al., in press) with a recent epizootic in 2007 (López et al., this book; Meli et al., this book; Meli et al., in press).

Influenza A viruses

Avian influenza or the "bird flu" is caused by an influenza type A virus of the *Orthomyxoviridae* family. There are multiple types of influenza A viruses which vary substantially in their pathogenicity. Multiple experimental studies on domestic cats have shown that cats exposed to various influenza A viruses can become infected, develop disease, and spread virus between cats with some viral strains (Paniker and Nair, 1972; Hinshaw et al., 1981; Kuiken et al., 2004; Rimmelzwann et al., 2006). Virus can be shed not only through respiratory secretions and aerosolizations but also by the gastrointestinal tract (Rimmelzwann et al., 2006).

In late 2003-early 2004, captive clouded leopards (Figure 7), leopard cats and tigers died of avian influenza (H5N1) after being fed infected chicken and quail (Keawcharoen et al., 2004). In late 2004, during an outbreak of H5N1 at a zoo there was evidence that after the initial viral infection, spread between tigers was possible (Thanawonguwech, 2005). Clinical signs in affected felids are primarily high fever and respiratory distress. The impact of the recent viral disease outbreak on wild populations of felids is unknown, however this and future avian influenzas have the potential to spread into individual wild felids when the cats kill infected birds or scavenge on carcasses.

Bluetongue virus

Bluetongue is an insect-transmitted orbivirus that infects wild and domestic ruminants. Previous research indicated that carnivores (Canidae, Felidae and Hyenidae) could become infected after ingestion of infected prey species (Alexander et al., 1994), however no disease was reported. Recently, Bluetongue virus serotype 8 was isolated from two captive Eurasian lynx that died with anemia, hemorrhages and pulmonary congestion or pneumonia (Jauniaux et al., 2008). Infection was presumed secondary to ingestion of fetuses and stillborn ruminants fed to the lynx rather than direct infection from the insect vector.

Selected parasitic infections in felids

Ectoparasitism: mange

There have been several reports of notoedric and sarcoptic mange infecting free-ranging felids, such as those documented in Eurasian lynx in several countries and cheetahs within the Serengeti ecosystem (Mwanzia et al., 1995; Ryser-Degiorgis et al., 2002; Ryser-Degiorgis, this book). Affected animals can have hair loss and or crusting dermatitis. Although *Sarcoptes scabiei* was implicated in both cases, *Notoedres* were also found in Eurasian lynx.

Hemoparasites

Hemoparasites are common in free-ranging felids and are usually incidental findings. Captive felids housed in warmer climates are also susceptible. Piroplasms including *Babesia*, *Theileria,* and *Cytauxzoon* spp. have been reported in a variety of felids, including Iberian lynx, as have hemoplasma (see review Munson et al., in press; Meli, in this book; Luaces et al., 2005; Willi et al., 2007). Primary disease is less common with one report of fatal *Cytauxzoonosis* in a bobcat cub (Nietfeld and Pollock, 2002). There is a high prevalence of *C. felis* in Florida panthers (35%) and while there are some hematological changes including decreased mean cell hemoglobin, overall hematological parameters are within reference ranges (Rotstein et al., 1999; Yabsley et al., 2006). *Babesia* spp. have been implicated as a co-pathogen explaining high mortality rates with CDV infection in wild lions as discussed in this chapter (Munson et al., 2008).

Toxoplasma gondii

Toxoplasma gondii is a protozoal parasite that commonly infects both captive and wild felids, including Iberian lynx (Roelke et al., 2008), but is rarely associated with disease. In contrast, captive Pallas' cats are uniquely susceptible to infection with *Toxoplasma* resulting in high neonatal mortality (Swanson, 1999; Kenny et al., 2002). Infection commonly results in a necrotizing encephalitis, pneumonia and/or hepatitis but can cause necrotizing to granulomatous inflammation in many other organs such as the spleen and kidney as well as within adipose (Terio, unpubl. observation). The reason for this unique susceptibility is not known. A survey of wild Pallas' cats in Mongolia found that ~13% are seropositive and organisms could not be identified in the feces or in tissues (Brown et al., 2005) suggesting that the Pallas' cat may have evolved without exposure to this parasite and thereby did not develop a commensal relationship. Others have suggested that immune deficiencies may contribute to the susceptibility (Ketz-Riley et al., 2003).

Metazoan parasites

Metazoan parasites commonly infect free-ranging felids and are less common in captive cats. In general, these parasites whether nematodes, cestodes, or trematodes are incidental findings. Rarely, high parasite burdens are associated with ill-thrift.

Selected Degenerative Diseases

Chronic renal disease

Renal disease is extremely common in older felids in captivity. In many species, chronic renal disease is of unknown cause, however there are a few distinct renal diseases for which the cause is known. Acute renal disease due to oxalates has been sporadically reported (Silberman et al., 1977; Stoskopf et al., 1978; Spelman et al., 1998). This disease manifests similar to ethylene glycol poisoning and has been presumed to be associated with contaminated feed. *Leptospira* spp. infection has been noted as a cause of interstitial nephritis in free-ranging mountain lions. Another primary renal disease whose pathogenesis has been elucidated is glomerulosclerosis in captive cheetahs (Bolton and Munson, 1999). This disease only rarely occurs and is typically of mild severity in wild cheetahs (Munson et al., 2005). The reason for the high prevalence of this disease is not known but is hypothesized to be due to either diet or metabolic changes (hyperglycemia) associated with chronic stress. Renal amyloidosis is common in black-footed cats many of which also have amyloid deposition in other organs (Terio et al., 2008). Renal amyloidosis has also been seen in some free-ranging populations of African lions (M. Kinsel, pers. comm.). A unique membranous glomerulonephritis has been reported in Iberian lynx (Jiménez et al., 2008) and is covered elsewhere in this book (Jiménez et al., in this book).

Arthritis

Vertebral spondylosis is common in older, larger felids (Kolmstetter et al., 2000). Although more common in captivity, it has been noted in free-ranging lions (M. Kinsel, pers. comm.).

Telangiectasia

In addition to biliary cysts and neoplasms (see next section), telangiectasia is a common post-mortem finding in the larger felid species (Pettan-Brewer and Lowenstine, 1999). Lesions vary in degree from mild sinusoidal ectasia to large areas of hemorrhage. These lesions are not thought to be clinically significant, but the pathogenesis is not known.

Neoplasia

There are a variety of neoplastic diseases that can affect non-domestic felids including lymphoma, biliary tumors, gastrointestinal adenocarcinomas, pulmonary bronchoalveolar carcinomas and leiomyomas. These neoplasias have been noted primarily in captive felids, likely due to longer life-spans in captivity. Biliary tumors are common in older large felids, particularly lions and tigers (Pettan-Brewer and Lowenstine, 1999). Uterine and ovarian leiomyomas are common in felids (Chassey et al., 2002) and occur spontaneously.

Captive fishing cats have a high incidence of transitional cell carcinomas (urinary bladder cancer) (13% of adult fishing cat deaths) (Sutherland-Smith et al., 2004). Mean age in affected cats is 10.8 years and no sex predilection has been noted. The cause of this high incidence is not known, but may be related to low levels of Vitamin E. Invasive portions of the neoplasms express cyclo-oxygenase 2 (COX-2) and therefore treatment with COX-2 inhibitors may slow disease progression (Landolfi and Terio, 2006).

Jaguars have a high prevalence of ovarian cystadenocarcinomas, an ovarian neoplasm not commonly noted in other felids (Munson, 1994; Kazensky et al., 1998; Hope and Deem, 2008). In these studies, the occurrence of this tumor was not related to progestin (melengestrol acetate: MGA) contraception. The occurrence of this neoplasm only in jaguars suggests a species predilection for this form of ovarian cancer.

Adverse effects of contraceptives

Contraceptives have been utilized in captive populations to regulate fertility. The most commonly used contraceptive is melengestrol acetate (MGA), a progestin. However, studies have suggested that prolonged exposure to progestins in felids can have deleterious effects. Felids with long-term (≥4 yrs) exposure to MGA have increased prevalence of severe hyperplastic and degenerative uterine diseases including adenomatous and cystic endometrial hyperplasia, hydrometra and endometrial mineralization (Munson et al., 2002). Additionally, MGA exposure is associated with higher prevalences of endometrial carcinoma as well as mammary gland neoplasia (Munson et al., 1995; Harrenstien et al., 1996). Ovarian lesions have not been associated with progestins (Kazensky et al., 1998).

Conclusion

The pathogenesis and epidemiology of the diseases briefly summarized in this review are based on the current state of knowledge. However, disease dynamics will likely be changing in the future as suitable habitats diminish, and humans and carnivores struggle to co-exist. Global climate change may also alter many disease patterns through changes to habitats as well as changes in the geographic distribution of infectious disease vectors. Continued disease monitoring will be critical to understanding the risks these changes pose to felid populations. Monitoring needs to be comprehensive including not just prevalence surveys for infectious agents but complete post-mortem examinations to assess the real risk of these exposures. Post-mortem evaluations are also critical for early identification of diseases with a possible genetic basis in both free-ranging and captive breeding populations. Critical assessment of monitoring data combined with current knowledge of disease pathogenesis and epidemiology needs to be a valued component in conservation programmes for all species.

Acknowledgments

The author wishes to thank Drs. Linda Munson and Michael Kinsel for critical review and comments on this manuscript.

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Photo: Alexander Sliwa

Photo: Alexander Sliwa

You can't do anything about the length of your life, but you can do something about its width and depth.

Henry Louis Mencken (1880-1956)

Pathological disorders in captive cheetahs

Patologías de guepardos en cautividad

Nadia Robert and Chris Walzer

Resumen

En todo el mundo, los guepardos *(Acinonyx jubatus)* en cautividad padecen varios problemas sanitarios que rara vez afectan a guepardos en libertad y son poco comunes en otras especies, sobre todo en los felinos. Entre estos problemas se incluyen las enfermedades del sistema nervioso central (SNC) y otras enfermedades que no afectan al SNC.

Entre las enfermedades neurológicas, la encefalomielopatía representa una seria amenaza para la población cautiva europea del European Endangered Species Plan (EEP), mientras que la leucoencefalopatía sólo afecta a la población cautiva norteamericana del Species Survival Plan (SSP). Ambas enfermedades son trastornos degenerativos de la sustancia blanca del sistema nervioso central (SNC) que afectan a la médula espinal o al cerebro, respectivamente. Además, en guepardos en cautividad han sido diagnosticados varios casos de encefalopatía espongiforme felina (EEF), enfermedad causada por priones y que se considera relacionada con la encefalopatía espongiforme bovina (EEB). La mayoría de los guepardos afectados por la EEF nacieron en el Reino Unido y probablemente fueron alimentados con carne bovina infectada.

Entre las enfermedades que no afectan al SNC, la gastritis linfoplasmocítica asociada a *Helicobacter* spp. es prevalente en guepardos cautivos en todo el mundo (Europa, Norteamérica, Sudamérica y Japón); la gastritis leve también ha sido diagnosticada en guepardos de vida libre. Otra enfermedad importante en la población cautiva de guepardos es la glomeruloesclerosis. La amiloidosis sistémica de proteína amiloide AA con afección al riñón, al hígado y a otros órganos también aparece con frecuencia en todas las poblaciones cautivas, y existe una alta correlación entre esta enfermedad, la gastritis crónica y la glomeruloesclerosis. Otras enfermedades renales diagnosticadas con frecuencia son la nefropatía por oxalatos y la pielonefritis. La enfermedad veno-oclusiva hepática (VOD), que causa una insuficiencia hepática, es frecuente en la población del SSP, pero no así en las poblaciones del EEP o en África austral. Sin embargo, el mielolipoma, siendo una de las lesiones más comunes que se observan en el bazo y, en ocasiones, también en el hígado, no es clínicamente relevante.

Entre las enfermedades infecciosas, el herpesvirus felino (FHV) está ampliamente extendido en la población cautiva y, a menudo, causa conjuntivitis,

rinitis y dermatitis facial crónica. Aunque se han descrito casos de peritonitis infecciosa felina (FIP, causada por FCoV), también hay cuadros de colitis causada por coronavirus entéricos felinos (FECV), que necesitan una mejor y mayor atención epidemiológica. Entre los parásitos, la infestación masiva de *Ascaris* sp. es un problema común en la población cautiva, a pesar de llevar a cabo una desparasitación regular, y también han sido descritas neumonías por *Aelurostrongylus abstrusus.*
En la mayoría de los casos, la causa primaria de estas enfermedades atípicas, pero con una alta prevalencia en la población cautiva, no ha sido identificada. No obstante, la baja incidencia de estas enfermedades en los guepardos de vida libre sugiere que existen causas extrínsecas que actúan como factores que predisponen a ellas. (Munson, 2005).

Palabras clave
Guepardos en cautividad, enfermedades del SNC, enfermedades que no afectan al SNC, EEP, SPP

Abstract
Captive cheetahs *(Acinonyx jubatus)* worldwide suffer from a number of health problems rarely observed in free-ranging ones, and unusual in other species, especially felids. These include diseases of the central nervous system (CNS) as well as non-CNS diseases. Among the neurological diseases, the encephalomyelopathy represents a serious threat to the European Endangered Species Plan (EEP) cheetah population, whereas the leucoencephalopathy affects only the Species Survival Plan (SSP) North-American population. Both are degenerative disorders of the CNS white matter, affecting the spinal cord or the cerebellum, respectively. Futhermore, several cases of feline spongiforme encephalopathy (FSE), a disease caused by a prion and considered to be related to the bovine spongiforme encephalopathy (BSE), have been diagnosed in captive cheetahs. Most of the FSE-affected cheetahs were born in the United Kingdom (UK) and probably were fed with infected bovine carcasses.
Among the non-CNS diseases, lymphoplasmacytic gastritis associated with *Helicobacter* spp. is prevalent in captive cheetahs worldwide (Europe, North-America, South-Africa, Japan). Mild gastritis has also been diagnosed in free-ranging cheetahs. Another important disease in the captive cheetah population is glomerulosclerosis. Systemic AA amyloidosis affecting the kidneys, liver and other organs is also frequently diagnosed in all captive populations. There is a high correlation between amyloidosis and chronic gastritis and glomerulosclerosis. Oxalate nephrosis and pyelonephritis are other frequently diagnosed renal diseases. Veno-occlusive disease of the liver resulting in progressive liver failure is a frequent disease in the North-American population but not in the European and South-African populations. Myelolipoma are common lesions seen in the spleen, sometimes also in the liver, but are however clinically not relevant. Among the infectious diseases, the clinical feline herpes virus (FHV) infection is widespread in captive cheetahs and frequently causes conjunctivitis, rhinitis and chronic facial dermatitis. Feline infectious peritonitis (FIP-caused by feline coronavirus [FCoV]) has been reported in cheetahs, but colitis caused by feline enteric corona virus (FECV) may deserve increased attention. Among parasites, in captive populations, massive infestation with *Ascarid* sp. is a common problem despite regular deworming. Pneumonia by lungworms *(Aelurostrongylus abstrusus)* has been reported.
The primary cause of these unusual diseases is mostly unidentified and the reason for their high prevalence in captive cheetahs is unknown, but the low level of these disorders in free-ranging cheetahs suggests extrinsic causes as predisposing factors (Munson, 2005).

Keywords
Captive cheetahs, CNS and non-CNS diseases, extrinsic factors, EEP, SSP

Pathological disorders in captive cheetahs

Nadia Robert and Chris Walzer

Introduction

In 2004 the cheetah EEP population included 345 cheetahs within 75 institutions. Two hundred and seventy cheetahs in Europe originated from Southern Africa (Republic of South Africa [RSA] and Namibia). Seventy five captive cheetahs held in the United Arabic Emirates, were originally from Northern Africa (Chad, Sudan, Ethiopia and Somalia). The European Cheetah Disease Working Group was established in 2002. The main goals of the group were: a) to centralize data management; b) to standardize disease description; c) to carry out comparative disease description and prevalence – USA/RSA; d) to conduct research on cheetah ataxia and encephalomyelopathy, and e) to comply with the Global Cheetah Conservation Plan. As of December 2004 the necrospy database included 136 cheetahs from which we have samples. The material comes from 26 different institutions in 10 countries. An EEP Cheetah Necropsy Protocol has been established and sent to all EEP cheetah institutions. In this chapter we present information regarding diseases affecting the captive cheetah population. We will first discuss diseases affecting the Central Nervous System (CNS), followed by information regarding the incidence of non-CNS diseases. For general information regarding diseases in captive and free-ranging felids, see also Terio, this book.

Central Nervous System (CNS) disorders in cheetahs

Cheetah encephalomyelopathy

The cheetah encephalomyelopathy, a neurological disease characterized by degenerative lesions of the spinal cord and cerebellum that cause ataxia and paresis, has emerged in the past 20 years in the EEP cheetah population. The disease accounts for 25% of all deaths and represents a limiting factor in the growth of the European captive population. Cheetahs of every age group are affected and often several or all cheetahs of the same litter will eventually develop the disease, either simultaneously or successively over a period of several months or years. The course of the disease is variable, from rapid onset of ataxia to a slower progressive development with stabilization and acute relapsing episodes (Figure 1). Pathologically, the disease is characterized by bilateral symmetrical degeneration of the white matter of the spinal cord (Figure 2), with loss of myelin exceeding axonal

Photo: N. Robert

Figure 1. Encephalomyelopahy: typical standing position of an ataxic cheetah with abducted hind legs and reduced support of the tail.

Figura 1. Encefalomielopatía: postura típica de un guepardo con ataxia, con las patas traseras separadas y el rabo más débil.

Photo: N. Robert

Figure 2. Cut section through the spinal cord in a cheetah with encephalopathy showing bilateral symmetric, peripheral, whitish discoloration corresponding to the foci of white matter degeneration.

Figura 2. Corte transversal de la espina dorsal de un guepardo mostrando una decoloración bilateral simétrica y periférica, que corresponde a los foci de la degeneración de la sustancia blanca.

loss, suggesting a primary myelin disorder. Changes in the cerebellar white substance characterized by myelin and axonal loss associated with astrogliosis and microgliosis, as well as with degeneration of Purkinje and granular cells are also frequently observed. The etiology of the disease is still unknown and investigations to determine the causes of this cheetah disorder have been based on known causes of encephalomyelopathy in human and domestic animals that are characterized by white matter demyelination. Several causes have been considered, including genetic, environmental, toxic, nutritional (especially copper [Cu]) and viral factors. The pattern of incidence does not indicate a major genetic basis for this disease, however, a genetic component leading to general disease predisposition and response cannot be ruled out, and multifactorial inheritance might also play a role. Extrinsic factors, either related to the management or the environment have to be considered; however, no "common denominator" in nutrition, holding and environmental conditions, husbandry, deworming and/or vaccination regimen has been identified to date (Walzer 1995, 2003; Palmer 2001; Robert, 2008).

Cheetah leucoencephalopathy

Leucoencephalopathy is a serious degenerative disease affecting SSP cheetahs and has never been observed in the EEP and South-African populations despite thorough investigations. The most distinctive clinical signs are blindness or visual abnormalities, lack of responsiveness to the environment, behavioral change, incoordination or convulsions. The disease emerged in 1996, peaked between 1998-2001, and is now declining. About 70 animals have been affected to date in about 30 different facilities. Most affected animals are at least 10-year old. The pathological lesions are restricted to the cerebral cortex and characterized by loss of white matter with associated bizarre astrocytosis. The cause is unknown, but epidemiological features suggest exposure to an exogenous agent through diet or medical management (Munson 1999b).

Feline spongiform encephalopathy

Feline spongiform encephalopathy (FSE), affecting domestic cats and captive non-domestic felids, is a prion disease considered to be related to bovine spongiform encephalopathy (BSE). FSE has been reported in cheetahs, pumas, ocelots, tigers, lions and cougars, but the relatively high incidence in captive cheetahs suggests that they may be more susceptible than other captive felids. To date nine cases of FSE have been diagnosed in cheetahs. All affected cheetahs were older than five years of age, and with the exception of two cheetahs born in France, all were born in the UK. Clinically, chronic progressive ataxia initially involving the hind limbs but later the forelimbs as well was consistently seen. Further clinical signs appear with variable

Photo: N. Robert

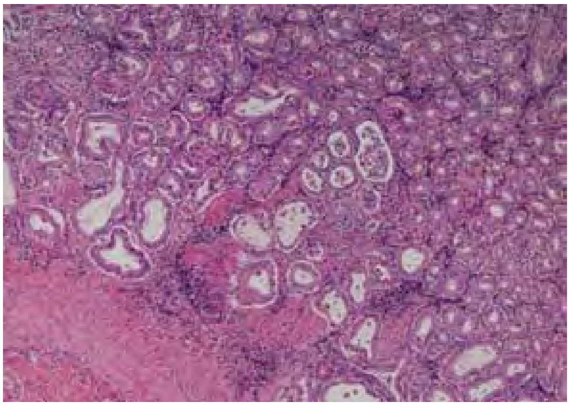

Figure 4. Histological section of a stomach with gastritis characterized by marked lymphoplasmacytic infiltration (H&E stain).

Figura 4. Corte histológico de un estómago con gastritis caracterizada por una marcada infiltración linfoplasmática (tintura h-e).

Photo: N. Robert

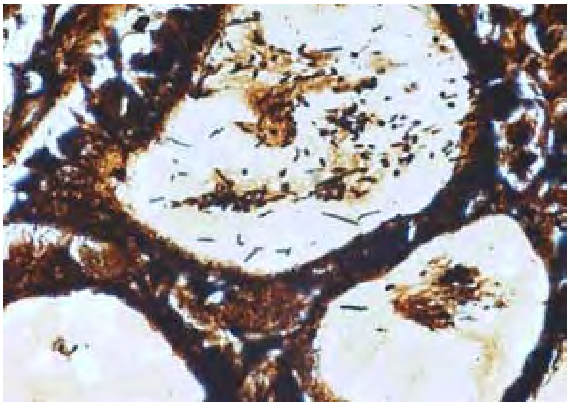

Figure 5. Histological section of a stomach with gastritis showing dilated gastric glands containing argyrophilic spiral-shaped bacteria (Silver stain).

Figura 5. Corte histológico de un estómago con gastritis. Se observan dilatadas las glándulas gástricas que contienen bacterias argirófilas de forma espiral (tintura de plata).

frequency and include postural difficulties, hypermetria, muscle tremors (particularly affecting the head), changes in behavior (aggressiveness/anxiety), hyperesthesia, ptyalism and blindness. The diagnosis of FSE requires histopathological examination of the brain and the finding of characteristic vacuolation in the neuropil and neurons. It is broadly accepted that FSE is the result of BSE infection in felids, probably from the ingestion of infected bovine carcasses, and the incubation period appears to be 4.5-8 years in cheetahs (Robert, 2008).

Non-CNS diseases in cheetahs

Gastritis

Lymphoplasmacytic gastritis associated with *Helicobacter* spp. causes significant morbidity and mortality in captive cheetahs worldwide (Europe, North-America, South-Africa, Japan). Despite abundant spiral bacteria colonization, free-ranging cheetahs have been shown to develop only mild gastritis in few cases, suggesting that a direct cause-effect is unlikely. In the EEP population, gastritis was observed in 81% of the samples, ranging from mild to severe, characterized mainly by lymphoplasmacytic inflammation of the mucosa, at times associated with neutrophilic infiltration. Spiral bacteria consistent with *Helicobacter* spp. were detected in most cases (Figure 3), but there was no correlation between the severity of the gastritis and the amount of bacteria in stomach glands. An altered immune response to a commensal bacteria related to chronic stress is postulated (Munson, 1993, 1999a; Terio et al., 2005; Walzer, 2006).

Amyloidosis

Systemic protein AA amyloid deposition in liver, kidney and other organs (adrenals, thyroid, gastrointestinal tract) is a common finding in all captive populations worldwide. Mild to marked amyloid deposition was recorded in 17 cases (48% of EEP cheetahs older than 1 year of age) mostly in kidneys and liver (Figure 4). In three cases amyloid was also seen in the adrenals, thyroid and/or spleen. In 16 cases amyloidosis was associated with glomerulosclerosis/nephrosclerosis and in 13 cases with gastritis (Munson, 1993, 1999a; Walzer, 2006).

Glomerulosclerosis and other renal diseases

The most prevalent renal disease was glomerulosclerosis, affecting 80% of the cheetahs older than one year of age. The disease was severe in about 40% of the animals older than six years, making it one of the main cause of mortality in adult cheetahs. Glomerulosclerosis is characterized by progressive thickening of the glomerular basement membrane that leads to glomerular ischemia and sclerosis (Figure 5). The lesion resembles that

Figure 6. Spleen with multiple myelolipomas.

Figura 6. Bazo con mielolipomas múltiples.

of diabetic nephropathy and is often accompanied by some degree of interstitial fibrosis and nephritis, glomerulonephritis and calcifications. Other renal pathological findings were pyelonephritis and/or papillary necrosis, presence of crystals in the tubular lumen (oxalate crystals), and amyloidosis (Munson, 1993, 1999a; Walzer, 2006).

Lesions of the spleen

Multiple splenic myelolipomas were present in 39 cheetahs (54% of the examined spleens) (Figure 6). The youngest cheetah affected was one year old. These lesions are not clinically important, but should be recognized because they have been misdiagnosed as metastatic cancer. The cause is not known, but dietary or stress-induced metabolic changes are suspected. More than 50% of the cheetahs had lymphoid depletion (Walzer, 1996).

Lesions in the liver

Veno-occlusive disease (VOD) is caused by fibrous occlusion of the efferent blood supply of the liver (central and sublobular veins), resulting in progressive liver failure and ascites. The cause is not known. Whereas the prevalence in the SSP population is high (63%), no VOD could be observed in the 76 liver samples examined (animals older than one year) in the EEP. Only mild increase of collagen fibers and reticulin fibers were observed around the central veins and in the sinusoids (Munson, 1993, 1999a; Walzer, 2006).

Infectious diseases

Viral diseases

- Feline coronavirus (FCoV): As reported in the cheetah SSP population, feline infectious peritonitis (FIP) seems to be a rare problem in captive populations despite frequent exposure to FCoV. In the EEP population, only two cheetahs with granulomatous lesions consistent with FIP were recorded. The viral etiology of these cases still need to be confirmed by molecular techniques. However, feline enteric corona virus FECV induced colitis may be an emerging disease of concern and therefore enhanced attention should be given to possible FeCoV infection in case of diarrhea problems (see also Terio, this book).
- Feline herpesvirus: Infection with feline herpes virus (FHV) is widespread in all captive populations. Occasionally neonatal cubs may die from acute infection (i.e., pneumonia) or may develop severe and persistent lesions such as corneal scars, prolapsed third eyelids, chronic epiphora or ulcerative dermatitis. All infected animals become chronic FHV carriers. Rarely, chronic carriers develop severe ulcerative dermatitis at sites of exposure to lacrimal and salivary secretions, or persistent, non-resolvable, ocular signs such as prolapsed third eyelids or corneal scarring. In the EEP population, two adult cheetahs (six and seven years of age) were reported to have chronic conjunctivitis with typical histological lesions associated with intranuclear inclusion bodies. The herpesvirus genom has been sequenced from one conjunctival swab; the gene sequence has >99% overlapping with FHV-1 (Genebank entry).

- Parvovirus: Chronic diarrhea and mild necrotizing enteritis have been associated with canine parvovirus (CPV) and feline parvovirus (FPV, that caused feline panleukopenia) virus in cheetahs. In the EEP population two cases of feline panleukopenia were observed in a seven week-old cub and in a one year-old animal (Walzer, 2006).

Bacterial diseases

Only occasional bacterial infections have been reported as major cause of disease or as cause of death in the EEP population:

- One bronchopneumonia by *Pasteurella* sp. infection in a one year-old animal.
- *Clostridium perfringens* was isolated from colon content in animals. In one case a perforating enterocolitis was caused by *C. perfringens* type A.
- *Campylobacter* spp. and *Salmonella* spp. are regularly isolated in cases of diarrhoea in cubs and also in adult cheetahs. Some of the cases are food associated.
- Two institutions had reported deaths related to *Hemobartonella felis* infection (now M*ycoplasma haemofelis*) (Walzer, 2006).


Photo: N. Robert

Figure 7. Stunted and cachectic cub with "Peaugres-syndrome", presenting diffuse alopecia and seborrhea.

Figura 7. Cachorro poco desarrollado y caquéctico con "Síndrome de Peaugres", que presenta alopecia difusa y seborrea.

Parasitic infections

- Massive infestation with *Ascarid* worms (*Toxascaris leonina, Toxocara* sp.) is a frequent problem in young and adult captive cheetahs despite regular deworming (up to six times a year in some institutions).
- Lungworms *(Aelurostrongylus abstrusus)* are frequently detected in feces. Two adult cheetahs showed severe parasitic pneumonia at post-mortem (Walzer, 2006).

Genetic diseases. The "Peaugres-Syndrome"

This "syndrome" might be one of the first "true" genetic diseases in cheetahs. Twenty seven cubs born in five litters from two normal dams which were sisters (*Fanny* and *Rina*) and one unrelated normal male (Fota). Twenty six cubs died between one and 134 days-old. The cubs were more or less affected and presented with various pathological lesions including poor hair coat, heart malformations (aortic aneurysma and heart hypertrophy), liver fibrosis, stunted growth, osteoporosis and encephalitis (Figure 7). The etiology of the disease remains unclear, however a genetic cause is probable. Similar lesions are described in a human multisystemic genetic disease known as Menkes disease, related to a defect in the copper transport proteins (Walzer, 2006).

Conclusion

- Myelopathy accounts for 25% of all deaths, including young and adult animals, and represents a limiting factor in the cheetah population growth within the EEP.
- Gastritis, glomerulosclerosis and amyloidosis are the most important non-CNS diseases.
- Most adult cheetahs in captivity are dying from a combination of gastritis and kidney disease, often with additional amyloidosis. It is therefore difficult to estimate the true prevalence of gastritis, renal failure or amyloidosis as main cause of death.
- VOD has not been observed in the EEP population, but is present in the SSP and the South African captive populations. Only mild centrilobular liver fibrosis is often recorded in the EEP cheetahs, but this is considered to be an incidental finding.
- Myelolipomas are common lesions seen in the spleen, sometimes also in the liver, but are not clinically relevant.

- While infectious diseases mostly cause mild clinical signs, they do not appear to be “major” causes of disease or death in the cheetah captive populations. FHV frequently causes transient sneezing and conjunctivitis in cubs. Rarely, chronic FHV-carriers may develop severe ulcerative dermatitis or conjunctivitis. FIP has rarely been reported in captive animals; however, attention should be given to FECV as a potentially emerging disease. Despite regular and frequent deworming, captive cheetahs tend to have significant *Ascarid* sp. infestation.
- Cheetahs in captivity frequently suffer of a number of diseases which are unusual in other animal species, especially in felids, and the reason for this high prevalence is still unknown, but the low level of these disorders in wild cheetahs suggest extrinsic causes, associated with the captive environment, as potential predisposing factors.

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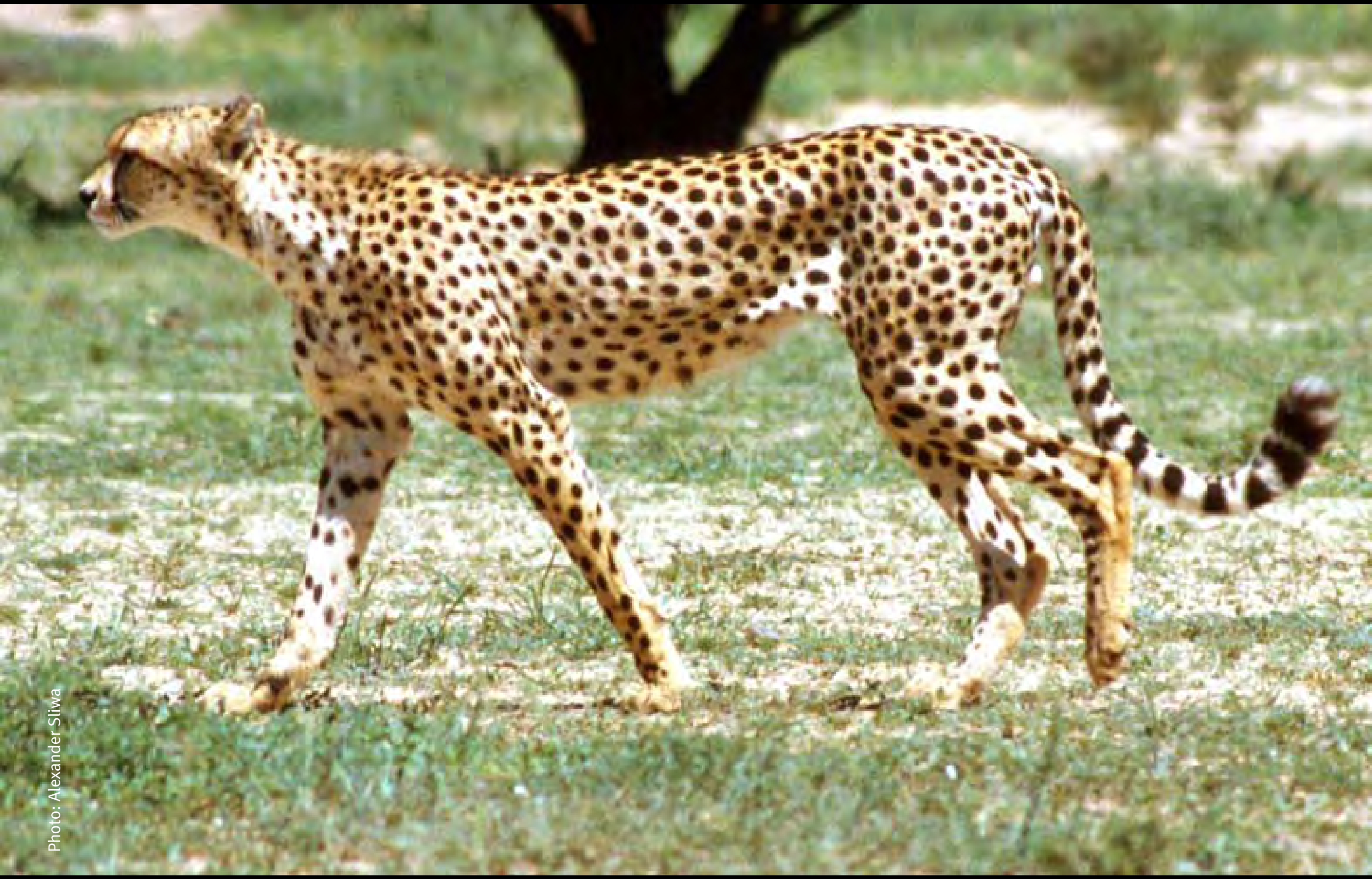
Photo: Alexander Sliwa

What makes a forest beautiful is that somewhere it hides animals.

Adapted from
Antoine de Saint-Exupéry
(1900-1944)

Photo: Urs Breitenmoser

Causes of mortality and diseases of Eurasian lynx *(Lynx lynx)*

Causas de mortalidad y enfermedades del lince boreal *(Lynx lynx)*

Marie-Pierre Ryser-Degiorgis

Resumen

Los linces boreales en libertad mueren principalmente por causas no infecciosas, como accidentes de tráfico, caza y furtivismo. Por consiguiente, la mortalidad relacionada con el hombre es muy significativa; según el país y el período de estudio, puede variar entre el 54% y el 96.5%. En comparación, las enfermedades sólo representan entre el 3.5 y el 25% de las causas de muerte. No obstante, es probable que se haya infravalorado la importancia de las enfermedades infecciosas, teniendo en cuenta que los estudios sobre mortalidad utilizan principalmente los datos correspondientes a los linces encontrados muertos al azar.

Se ha descrito una amplia gama de enfermedades infecciosas y no infecciosas que afectan al lince boreal. Como felino, el lince probablemente sea susceptible a la mayoría de las enfermedades que afectan a los gatos domésticos; el lince boreal, además, está expuesto a agentes infecciosos a través de sus presas. Aún así, los brotes epidémicos no parecen ocurrir en las poblaciones en libertad. Debido a su comportamiento solitario, el lince boreal tiene muy pocas oportunidades de transmitir patógenos antes del desenlace mortal o la recuperación de la enfermedad, aunque es posible que una enfermedad de larga duración o con un período prolongado de incubación pudiese ser la excepción a esta norma. La ausencia de anticuerpos o antígenos en los estudios de población indica que las poblaciones estudiadas no habían tenido contacto reciente con estos agentes o que posiblemente la especie sea especialmente susceptible a infección (porque los ejemplares infectados no sobreviven), o ambos. En comparación, una alta prevalencia indica que los agentes en cuestión no causan problemas graves de salud en la especie, sobre todo, si no se ha observado una mortalidad relacionada y las poblaciones infectadas están estables. Por ejemplo, se han documentado prevalencias elevadas en el caso de *Toxoplasma, Trichinella* y *Cytauxzoon*, que suelen ser apatogénicos para el lince.

La enfermedad que se diagnostica con mayor frecuencia en el lince boreal en libertad es la sarna sarcóptica; no obstante, hasta ahora ni la sarna ni ninguna otra enfermedad parece ser una amenaza para las poblaciones de lince. De todos modos, las pérdidas por enfermedad podrían tener un impacto si se agravasen por otros problemas graves, como la caza ilegal o el deterioro del hábitat, o ambos. Además, los problemas emergentes aparentes, tales como las malformaciones congénitas o lesiones cardíacas del lince en Suiza, destacan la necesidad de tener un seguimiento veterinario meticuloso a largo plazo de las poblaciones en libertad y en cautividad, así como la recopilación de muestras abundantes para estudios inmediatos o posteriores. En este contexto, es imprescindible la colaboración estrecha entre biólogos de campo y veterinarios, tanto en la recopilación de datos como en su interpretación.

Palabras clave
Infección, control sanitario, parasitología, furtivismo, necropsia, serología

Abstract
Free-ranging Eurasian lynx mostly die of non-infectious causes such as traffic accidents, poaching and hunting. Thus, human-related mortality is of major importance; depending on the country and study period, it varies from 54 to 96.5%. In contrast, diseases represent only 3.5-25% of the causes of death. However, the importance of infectious diseases is probably underestimated since mortality studies mostly rely on data from lynx found dead by chance.

A wide range of infectious and non-infectious diseases has been reported in Eurasian lynx. As a felid, the lynx is probably susceptible to most diseases affecting domestic cats. Furthermore, the Eurasian lynx is exposed to infectious agents through its prey. Nevertheless, epidemic outbreaks do not seem to occur in free-ranging populations. Because of its solitary behaviour, the Eurasian lynx has only rare opportunities to transmit pathogens before a fatal outcome or recovery from the disease – although a disease with long duration and/or incubation period might represent an exception.

The absence of detection of antibodies or antigens in population surveys indicate that the investigated populations either did not have any recent contact with these agents, and/or possibly, that the species is highly susceptible to infection (i.e., infected individuals do not survive). In contrast, a high prevalence is an indication that the concerned agents do not cause serious health problems in the species, especially if related mortality has not been observed and infected populations are stable. For example, high prevalences have been documented for *Toxoplasma, Trichinella* and *Cytauxzoon*, which are normally apathogenic to lynx.

The disease most commonly diagnosed in free-ranging Eurasian lynx is sarcoptic mange, but neither mange nor other diseases do appear as a threat to free-ranging lynx populations so far. Nevertheless, losses due to diseases might have an impact if added to serious problems such as poaching and/or habitat destruction. Furthermore, apparently emerging problems such as congenital malformations and heart lesions in Swiss lynx underline the need for a long-term, careful health monitoring of free-living and captive populations, together with extensive sample collection for immediate or later studies. In this context, close collaboration between field biologists and veterinarians is essential, both for data collection and interpretation.

Keywords
Infection, health monitoring, parasitology, poaching, post-mortem, serology

Causes of mortality and diseases of Eurasian lynx *(Lynx lynx)*

Marie-Pierre Ryser-Degiorgis

Introduction

The Eurasian lynx *(Lynx lynx)* is the largest felid in Europe and the closest relative of the Iberian lynx. Information on causes of death and diseases of Eurasian lynx used to be scarce, but interest in health aspects regarding this species clearly increased in the past decade. Health monitoring of Eurasian lynx is mainly performed by means of systematic post-mortem examinations and parasitological investigations of faecal samples. Additional data originate from physical exams of animals caught in the frame of ecological studies, and from screenings of blood samples for selected infectious agents or antibodies (serosurveys).

The aims of this review are 1) to give an overview of the actual knowledge on causes of mortality and diseases in Eurasian lynx, thus providing data on the susceptibility of Eurasian lynx to various infectious agents; 2) to discuss the importance and potential impact of infectious and non-infectious causes of mortality on free-ranging Eurasian lynx populations, and 3) to briefly assess the role of the Eurasian lynx in the epidemiology of infectious diseases. The data from Eurasian lynx can also provide useful baseline information for the planning of epidemiological surveys in Iberian lynx, and for the interpretation of data gathered in this highly endangered species.

Non-infectious causes of mortality

Human-related casualties

Traffic accidents due to collisions with cars, trains, and less commonly motorcycle, often occur, accounting for 20-50% of mortality causes of lynx found by chance (Stahl and Vandel, 1999; Schmidt-Posthaus et al., 2002; Ryser-Degiorgis et al., 2005a, c). According to the data presented by Schmidt-Posthaus et al.,(2002), subadult lynx die significantly more often due to traffic accidents than adults (x^2, $P<0.001$), which could be explained by inexperience and dispersal movements over long distances (Breitenmoser and Breitenmoser-Würsten, 2008).

Controlled hunting or harvest has taken place for many years mainly in Fennoscandia and Estonia, but also in eastern European countries (von Arx et al., 2004); in these lynx populations, hunting is largely the main cause of death (Ryser-Degiorgis et al., 2005a; von Arx et al., 2004). Legal shooting of Eurasian lynx that cause repeated damages on domestic livestock occasionally occurs (Ryser-Degiorgis et al., 2005a, c; Schmidt-Posthaus et al., 2002).

Poaching was recorded to cause 46% of the mortality of adult radio-collared lynx in Scandinavia (Andrén et al., 2006). It was reported as the most common cause of death in lynx from other countries (Jedrzejewski et al., 1996; Wölfl et al., 2001), and it accounts for up to 23% of the dead lynx found by chance in France (Stahl and Vandel, 1999). Indeed, a recent survey of lynx status identified poaching as the most important threat across all European populations (von Arx et al., 2004). Among poaching methods, shooting appears to be most the common one (Schmidt-Posthaus et al., 2002; Andrén et al., 2006). Interestingly, up to 22% of Swiss lynx have been shown to harbour lead pellets from non-fatal shooting attempts (Ryser-Degiorgis, 2006), indicating that poaching is much more common than it appears from the recorded deaths caused by illegal shooting. Poisoning has been observed several times in Switzerland: single individuals and family groups were poisoned through their kills, which served as bait. Identified poisons were alpha-chloralose and cyanide (Ryser-Degiorgis et al., 2005c). Secondary poisoning with bromadiolone was recorded in France (Stahl and Vandel, 1999).

Trapping accidents have been observed in the course of ecological studies. They were either directly linked to the capture method, occurred during/following anaesthesia, or were due to subsequent problems with the radio-collar (Stahl and Vandel, 1999; Schmidt-Posthaus et al., 2002; Ryser-Degiorgis et al., 2005c; Arnemo et al., 2006).

Dog attacks *(Canis familiaris)* have been recorded on juvenile and subadult lynx (Schmidt-Posthaus et al., 2002; Ryser-Degiorgis and Mörner, unpubl. data). In most cases, the victims were weakened, diseased lynx.

Natural accidents

Natural accidents are rarely observed or reported. Specific cases of lynx natural mortality included falling from rocky slopes (Stahl and Vandel, 1999; Schmidt-Posthaus et al., 2002), being caught between tree branches (Ryser-Degiorgis et al., 2005c), and drowning (Schmidt-Posthaus et al., 2002). Dog attacks *(Canis familiaris)* have been recorded on juvenile and subadult lynx (Schmidt-Posthaus et al., 2002; Ryser-Degiorgis and Mörner, unpubl. data). In most cases, the victims were weakened, diseased lynx.

Intraspecific fights with fatal consequences have been observed in the wild (Schmidt-Posthaus et al., 2002; Andrén et al., 2006) and in captivity, including siblicide (Naidenko, 2000; Naidenko and Antonevich, this book). Attacks from wolverines *(Gulo gulo)* have been suspected in Scandinavia (Andrén et al., 2006) and in eastern countries, where also the wolf *(Canis lupus)* has been identified as an occasional lynx predator (Matjuschkin, 1978). However, there are also indications that Eurasian lynx can survive traumatic injuries such a severe bone fractures (Ryser-Degiorgis, unpub. data), similarly to observations made in Iberian lynx in Spain (García-Perea, 2000; Martínez, pers. comm.).

Starvation

Starvation is a common cause of debilitation and an eventual death in juvenile Eurasian lynx during the fall (Stahl and Vandel, 1999; Schmidt-Posthaus et al., 2002; Ryser-Degiorgis et al., 2005c). These animals are regarded as orphans, still too young to hunt by themselves. Typically, they are found severely emaciated in human settlements, where they look for easy food sources.

Matjuschkin (1978) also mentioned mortality due to harsh climatic conditions making hunting of prey difficult.

Non-infectious diseases

Several malformations have been recorded in Eurasian lynx. Some were considered as direct or indirect causes of death, others were secondary findings without clinical significance: skeletal deformities affecting the vertebral column and/or the long bones, peritoneo-pericardial hernia, hiatal hernia, prognathismus inferior, monorchidia, subaortic stenosis (Ryser-Degiorgis et al., 2004), pelvic asymmetry (Fig. 1), and abnormally small but functional kidney (Ryser-Degiorgis and Robert, 2006).

A clinical cardiac disease was recorded in an adult free-ranging male from Switzerland that died of a circulatory failure attributed to a cardiomyopathy (Ryser-Degiorgis et al., 2004). Main histological lesions were extensive myocard fibrosis and severe arteriosclerosis. Subsequently, a retrospective histological study showed

Photo: FIWI Bern

Figure 1. Malformation of the pelvic bone of a Eurasian lynx. The pelvis presents a severe asymmetry and a one-sided fusion with the vertebral column.

Figura 1. Malformación de la pelvis de un lince boreal. La pelvis muestra una asimetría severa y fusión unilateral con la columna vertebral.

that such histological lesions are very common in Swiss lynx, especially subadult and adult males (Ryser-Degiorgis and Robert, 2006). The aetiology of the lesions is unknown to date, and the possible role of inbreeding needs to be elucidated. The sanitary relevance of the observed lesions also is unclear, however, if compared to similar findings in other species, either chronic cardiac disease or sudden death without previous clinical symptoms can be expected in affected individuals.

Tumors seem to occur rather rarely in lynx. In Eurasian lynx, at least two tumor types have been reported, both in captive lynx: a pancreas tumor (Kirchhof and Geiss, 1995) and a benign giant cell tumor of tendon sheats (Malatesta et al., 2005).

Iron deficiency anemia, characterized by low serum iron concentration and microcytic hypochromic anaemia, and attributed to a combination of flea infestation and inadequate diet, was diagnosed in a captive Eurasian lynx (Harvey and Coleman, 1982).

Infectious diseases

(For general information on the mentioned infectious agents, see also Terio, this book).

Ectoparasites

Mange is a highly contagious debilitating skin disease caused by mites. It was already mentioned as a known disease in free-ranging Eurasian lynx by Matjuschkin in 1978. Mange caused by *Sarcoptes scabiei* or sarcoptic mange was later reported in captivity in China (Jeu and Xiang, 1982), as well as in the wild in Norway (Holt and Berg, 1990), Sweden (Mörner, 1992), Switzerland (Ryser-Degiorgis et al., 2002a) and Germany (Kuhr, 2007), in association with outbreaks of sarcoptic mange in red foxes *(Vulpes vulpes)*. Mangy red foxes are considered as the major source of infection for lynx (Mörner, 1992; Bornstein et al., 1994; Ryser-Degiorgis et al., 2002a), but intraspecific transmission has been suspected within family groups (Ryser-Degiorgis et al., 2002), and might also occur between adults especially during mating season. Lesions

Photo: M. P. Ryser-Degiorgis

Figure 2 (A). Close up of the ear of a Eurasian lynx affected by sarcoptic mange. There are very thick crusts with deep fissures, in which mild hemorrhages are visible.

Figura 2 (A). Primer plano de la oreja de un lince boreal afectado por sarna sarcóptica. Se aprecian costras de mucho grosor con fisuras profundas en las que se observan hemorragias leves.

Photo: M. P. Ryser-Degiorgis

Figure 2 (B). Eurasian lynx affected by sarcoptic mange. The animal is emaciated. Due to the thick fur, the generalized thick crust formation is not evident. Hair loss is only focally present.

Figura 2 (B). Lince boreal afectado por la sarna sarcóptica; el animal está emaciado. Debido al espeso pelaje, no se observa claramente la formación de una costra gruesa generalizada. La presencia de pelo es sólo focal.

consist of an extensive encrusting dermatitis that covers the entire body but is usually more prominent on the head, ears, feet and tail. Typical macroscopical changes are thick crusts with deep fissures (Fig. 2a). If present, alopecia is spotty and never generalized (Fig. 2b) and mites are very numerous in scrapings and in histological sections (Ryser-Degiorgis, pers. comm. cited in Pence and Ueckermann, 2002). Lymph nodes are generally enlarged. Towards the end stage of the disease, animals are cachectic and often harbour a large amount of *Ascarid*s in the intestine. Experimental infections in red foxes and Eurasian lynx indicate that the incubation lasts from 10 to 72 days (Mörner and Christensson, 1984; Bornstein et al., 1994; Bornstein et al., 1995), and that several months can pass by between the first mange symptoms and death. A Swiss lynx that appeared healthy at time of capture died of mange three months later (Ryser-Degiorgis et al., 2002a). Sarcoptic mange is the most frequent infectious disease in Eurasian lynx, reaching up to 22% of non-hunted dead lynx in Sweden (Ryser-Degiorgis et al., 2005a). Nevertheless, mange does not appear as a threat for the long-term survival of affected lynx populations (Ryser-Degiorgis et al., 2005a, c).

Mange caused by *Notoedres cati* or notoedric mange is rarely observed in lynx in zoos (Dobiàs, 1981) and has been reported in free-ranging lynx from Switzerland, where also a mixed infection with *N. cati* and *S. scabiei* has been documented (Ryser-Degiorgis et al., 2002). Lesions of notoedric mange are similar to those observed in cases of sarcoptic mange. Affected domestic cats are suspected to be the primary source of infection. In contrast to sarcoptic mange, notoedric mange is rarely observed in Eurasian lynx in the wild.

Otodectes cynotis is a non-burrowing mite commonly causing otitis externa in domestic cats. Otodectic or ear mange has been reported in free-ranging Eurasian lynx from Sweden (Degiorgis et al., 2001) and Switzerland (Schmidt-Posthaus et al., 2002; Ryser-Degiorgis et al., 2005c). In contrast to Sweden, it appears to be very common in lynx from Switzerland.

Ixodid ticks are commonly, louse flies and fleas occasionnally found on free-ranging Eurasian lynx (Schmid-Posthaus et al., 2002; Ryser-Degiorgis et al., 2005a, c).

Endoparasites

Gastro-intestinal parasites are very common in Eurasian lynx, and normally do not cause clinical disease. In Sweden, 71% of more than 200 lynx had gastro-intestinal parasites, most commonly *Ascarid*s such as *Toxocara cati* (Fig. 3; Ryser-Degiorgis et al., 2005a). Investigations from Switzerland and Poland reveal similar results with 63% and 73%, respectively, of the lynx affected by gastro-intestinal parasites (Ryser-Degiorgis et al., 2005c; Szczęsna et al., 2008). *Ascarid*s have been reported as a cause of death in a lynx kitten (Schmid-Posthaus et al., 2002). Cestodes (*Taenia* spp.) and protozoans (*Isospora* sp., *Cystoisospora* sp.), which are transmitted by intermediary or paratenic hosts, are less commonly found in lynx in Switzerland and Sweden. In northeastern Europe, infestation with *Toxocara* spp. is frequent too (93% in Lithuania; 76% in Latvia, and 68% in Estonia) but also cestodes are common, especially in Latvia and Estonia, where all investigated lynx harboured *Taenia pisiformis* (Bagrade et al., 2003; Valdmann et al., 2004). In an earlier review from Matjuschkin (1978), *T. mystax* and *T. pisiformis* were also mentioned as the most common helminths of the lynx in Eastern countries. In contrast, prevalence of *T. cati* was reported as low in lynx from Poland (Górski et al., 2006; Szczęsna et al., 2008), where the cestode *Spirometra janickii* was recently identified as the most common parasite (Szczęsna et al., 2008). Possibly, the helminth fauna reflects the food habits of the lynx (Valdmann et al., 2004) as well as geographical differences regarding the occurrence of the parasites.

Photo: M. P. Ryser-Degiorgis

Figure 3. Open section of the small intestine containing numerous *Ascarid* worms (Eurasian lynx).

Figura 3. Sección abierta del intestino delgado con numerosos áscaris. (Lince boreal).

Lungworms are generally less common than gastro-intestinal parasites. *Capillaria* sp. was found in less than 2% of lynx from Sweden (Ryser-Degiorgis et al., 2005a), and *Aelurostrongylus* sp. with associated bronchopneumonia was diagnosed twice in lynx from

Switzerland (Schmidt-Posthaus et al., 2002; Ryser-Degiorgis and Robert, 2006). In Bialowieza Forest (Poland) *A. abstrusus* was identified in 17% of the investigated samples (Szczęsna et al., 2008). However, *Capillaria (Thominx) aerophilus* was found in the bronchi and trachea of 33% lynx from Latvia (Bagrade et al., 2003). *Thominx* sp. was even reported to be the most common parasite in lynx from Bialowieza Forest, with a prevalence reaching up to 75% (Miniuk, 1996). Nematodes were further observed in the urinary bladder *(C. felis-cati)* and in the gall bladder (*Nematoda* sp.) of lynx from Estonia (Bagrade et al., 2003).

Toxoplasma gondii is a coccidian parasite with felids as definitive hosts, in which infection usually does not lead to disease symptoms. In Fennoscandia, seroprevalence in lynx reaches 70-75% and is significantly higher in subadult and adult lynx than in juveniles (Oksanen and Lindgren, 1995; Ryser-Degiorgis et al., 2006). Prevalence of infection appears to be highest in southern regions of Sweden, which are more densely populated by humans, possibly due to the presence of domestic cats shedding oocysts to the environment, to climatic differences (i.e., to survival of oocysts) and/or to variations in prey availability, since prevalence of infection greatly vary between different prey species. Seropositivity has also been reported in captive lynx (Sedlák and Bártová, 2006). No clinical disease has been reported in Eurasian lynx so far, but sporadic cases have been reported in young bobcats *(Lynx rufus)* (Dubey et al., 1987; Smith et al., 1995), although asymptomatic infections are also very common in this species (Oertley et al., 1980).

Antibodies to *Neospora caninum* have been identified in few captive Eurasian lynx (Sedlák and Bártová, 2006). A life cycle of *N. caninum*, a coccidian parasite that may cause high abortion rates in cattle and fatal neurological disease in dogs, has been reported to also exist in wild animals (Gondim, 2006).

Members of the Felidae normally are definitive hosts for protozoan parasites of the genus *Sarcocystis*. Intermediate hosts are usually herbivores or omnivores. However, sarcocysts can occasionally be found in the muscles of felids. They appear to be quite common in bobcats (Andersen et al., 1992) and have been found in a Eurasian lynx from Sweden (Ryser-Degiorgis et al., 2005a).

Trichinella spp. are small parasitic nematode worms that infest the intestines of various mammals and whose larvae move through the bloodstream, becoming encysted in muscles. *Trichinella* is mainly found in carnivores (fox, bear, wolf, etc.) and omnivores (wildboar, domestic pig) but can also affect humans. Infection occurs through consumption of meat containing larvae. In Europe, *Trichinella* is common in foxes that are considered as an important infection source for other wildlife. Prevalence in Eurasian lynx is usually high: 30-50% (Brglez, 1989; Järvis et al., 2001; Bagrade et al., 2003; Valdmann et al., 2004; Frey et al., 2008). In Finland, prevalence varies from 5-70%, depending on the geographical region (Oksanen et al., 1998; Oivanen et al., 2002), and in Sweden, it is generally very low with 5% prevalence (Pozio et al., 2004). Interestingly, prevalences in red fox populations show similar geographical variations (Oivanen et al., 2002), suggesting an epidemiological relationship between those two species: the red fox is indeed an important prey species of lynx in some geographical regions and has to be considered as a source of infection for lynx. Data suggest that *Trichinella* accumulate in lynx, which thus appear to be a good indicator for the presence of the parasite in the environment (Frey et al., 2008). *Trichinella* species identified in Eurasian lynx are *T. pseudospiralis* in Sweden (Pozio et al., 2004), *T. nativa* in Finland (Oivanen et al., 2002) and Estonia (Järvis et al., 2001), and *T. britovi* in Switzerland (Frey et al., 2008). Lynx harbour *Trichinella* without developing a disease condition.

Cytauxzoon felis infection has been demonstrated in various non-domestic felids including the Eurasian lynx, with a prevalence of 26% in Switzerland (Meli et al., 2006). In wild felids, *C. felis* infection is usually subclinical, but fatal disease has been reported in bobcats under experimental and natural conditions (Kier et al., 1982; Nietfeld and Pollock, 2002).

The presence of *Anaplasma phagocytophila*, which causes tickborne fever in domestic ruminants, has been reported in a number of domestic and wildlife species. Seroprevalences lower than 10% have been demonstrated in Eurasian lynx in absence of clinical signs (Ryser-Degiorgis et al., 2005a, c).

Virus

Rabies is a contagious and fatal disease of virtually all mammals. Sporadic cases have been diagnosed in Eurasian lynx (Kolar, 1976; Matjuschkin, 1978; Jedrzejewski et al., 1996; Stahl and Vandel, 1999; Tschirch, 2001). In Slovakia, six of 1000 lynx (0.6%) that were caught or killed within 10 years had rabies (Fernex, 1976). The susceptibility of animals to rabies virus varies a lot, depending, among other factors, on the animal species. Felids are less susceptible to the fox rabies virus than the fox itself (Fernex, 1976; Pastoret et al., 2005). Furthermore, they usually get infected through perforating bite wounds rather than through ingestion of an infected prey (Bell and Moore, 1971; Lutz, 2005). Thus, the lynx is not thought to play a significant role in the epidemiology of rabies in Europe (Tschirch, 2001). Fernex (1976) reported that all six rabid lynx diagnosed in Slovakia showed the paralytic signs of the disease, with absence of aggression ("dumb" rabies). However, a case of a rabid lynx showing aggression ("furious" rabies) was also described (Kolar, 1976).

Feline panleucopenia or parvovirosis is a highly infectious disease caused by a feline parvovirus (FPV), which is remarkably able to survive in the environment. The disease considerably varies in severity, ranging from subclinical infection to a severe, fatal syndrome. In Eurasian lynx, three fatal cases of parvovirus infection have been reported, both in free-ranging and captive lynx (Stahl and Vandel, 1999; Schmidt-Posthaus et al., 2002; Wasieri et al., 2008). Pathological changes consisted mainly in fibrinous enteritis. Serological studies showed various prevalences depending on the country: 1% in Sweden and 18.5% in Switzerland (Ryser-Degiorgis et al., 2005b, c). The presence of antibodies in clinically healthy animals shows that *lynx* are able to survive a parvovirus infection.

Feline coronavirus (FCoV) infection can lead to feline infectious peritonitis (FIP), enteritis or no obvious clinical signs at all. FIP has been observed in a captive lynx (Hyslop, 1955) and in a free-ranging Eurasian lynx from France (Joubert and Blancou, 1987). Serological studies in free-ranging populations revealed low prevalences: 0% in Sweden and 4.6% in Switzerland (Ryser-Degiorgis et al., 2005b, c).

Serological investigations for feline leukaemia virus (FeLV) in captive and free-ranging Eurasian lynx from Sweden and Switzerland did not reveal any positive animal (Lutz et al., 1992; Ryser-Degiorgis et al., 2005b, c) and clinical disease has not been reported in this species. However, FeLV-associated disease has been observed in a captive bobcat following close contacts with an infected domestic cat (Sleeman et al., 2001) and an FeLV outbreak with several fatal cases has been recorded in Iberian lynx (Meli et al., 2009; Meli et al., this book; López et al., this book), indicating that *Lynx spp.* are susceptible to FeLV infection.

Antibodies to feline immunodeficiency virus have been demonstrated in a variety of captive and free-ranging wild felid species, including the bobcat (Brown et al., 1993; Franklin et al., 2007). In contrast, investigations in free-ranging Eurasian lynx gave negative results (Ryser-Degiorgis et al., 2005b, c).

Serological studies in free-ranging Eurasian lynx indicate that seroprevalence is apparently low for feline herpesvirus (0% in Sweden; 3% in Switzerland) but that it shows considerable variation for feline calicivirus (0% in Sweden; 26.2% in Switzerland) (Ryser-Degiorgis et al., 2005b, c).

Seroprevalence for canine distemper virus (CDV) in Eurasian lynx was shown to be 1% in Sweden and 24.3% in Switzerland (Meli et al., 2006). Clinical disease has not been reported in Eurasian lynx so far, but CDV-associated encephalitis has been reported in Canada lynx *(Lynx canadensis)* and bobcats (Daoust et al., in press). CDV prevalence in the Iberian lynx is 16.2%, with one reported as a fatal case (Meli et al., 2009; Meli et al., this book).

Borna disease is a severe neurological syndrome caused by Borna disease virus. The host spectrum of BDV is very broad and includes domestic cats and humans. BD has been diagnosed in a free-ranging lynx from Sweden, which presented an abnormal, apathetic behavior (Degiorgis et al., 2000).

Cowpox virus is an Orthopoxvirus that can affect several mammal species including man. In cats, it usually causes skin lesions, but a lung form of the disease has been described in wild felids (Marennikova et al., 1977). A captive Eurasian lynx died of a cowpox infection in the UK (M. Bennet, pers. comm. cited in Tryland et al., 1998). Although clinical cases have not been reported in the wild, a study performed in Fennoscandia revealed a seroprevalence for orthopoxvirus in free-ranging lynx of 1% and 29%, respectively, depending on the geographical area (Tryland et al., 1998). In Sweden, more than 7.5% of the lynx investigated by PCR were positive for orthopoxvirus (Ryser-Degiorgis et al., 2005a).

Feline syncitium-forming virus (FeSFV) is a member of the Spumavirinae (Retroviridae). Spumaviruses have been isolated from many species but generally do not appear to be pathogenic. Nevertheless, the possibility still exists that FeSFV may produce or predispose animals to disease, perhaps in conjunction with other agents or in cats of a particular histocompatibility type (Gaskell and Bennet, 1994). Antibodies to FeSV were detected in several captive felid species, including three lynx (Lutz et al., 1992).

Photo: M. P. Ryser-Degiorgis

Figure 4. The lower canines of this Eurasian lynx orphan kept in captivity are worn out and present a greyish discoloration following a severe bilateral inflammation of the dental pulp and root. Such lesions are the result of intensive biting on hard materials in the enclosure at the wildlife rescue station.

Figura 4. Los caninos inferiores de este lince boreal huérfano, mantenido en cautividad, están desgastados y presentan manchas grisáceas como consecuencia de una inflamación bilateral severa de la pulpa dental y de la raíz. Dichas lesiones fueron causadas por morder intensivamente los materiales duros del cercado en el centro de rescate de vida silvestre.

Bluetongue virus (BTV) is transmitted by several species of *Culicoides* biting midges and causes bluetongue in ruminants. One year after the introduction of BTV serotype 8 (BTV-8) to northern Europe, two captive Eurasian lynx died of a BTV-8 infection in Belgium (Jauniaux et al., 2008). A transmission of the virus by the oral route was suspected.

Bacteria

Clinical disease and/or mortality due to bacterial infection in Eurasian lynx is apparently mostly due to ubiquitous bacterias: infected wounds; pulpitis following canine teeth injuries (Fig. 4), associated with alveolar periostitis, osteomyelitis and/or septicaemia in lynx orphans kept in rescue station facilities (Schmidt-Posthaus et al., 2002; Ryser-Degiorgis et al., 2005c); a dental abcess in carnassial tooth associated with *Arcanobacter pyogenes* infection (Rostami et al., 2007); purulent bronchopneumonia due e.g. to *Streptococcus* spp., *Pasteurella* sp., and occasionnally associated with pyothorax and/or pericarditis; purulent cystitis and pyelonephritis due to an ascending urinary tract infection with haemolytic *Escherischia coli* (Schmidt-Posthaus et al., 2002).

Salmonellosis *(Salmonella arizonae)* associated with septicaemia was reported in a captive Eurasian lynx (Macri et al., 1997). Pseudotuberculosis *(Yersinia pseudotuberculosis)* was observed in lynx from Switzerland, both in the wild and in rescue station facilities. In the first case, it was a chronic form with multifocal necrotic foci in inner organs (Boujon, pers. comm.); in the second case, an acute form with diarrhoea, anorexia and apathetic behaviour followed by septicaemia was observed (Ryser-Degiorgis and Robert, 2006).

Helicobacter sp. infection has been demonstrated in free-ranging, apparently healthy lynx from Sweden (Mörner et al., 2008). Possibly, these organisms are either commensals or opportunistic pathogens, as suggested in the bobcat.

Borrelia burgdorferi is the agent causing borreliosis or Lyme disease. Although cats do produce specific antibodies, it is unclear whether they develop clinical symptoms. Antibodies to *B. burgdorferi* were demonstrated in one of two investigated free-ranging lynx from France (Doby et al., 1991). A recent study on borreliacidal effect of carnivore's serum complement indicated that wolf and lynx probably are competent reservoir for *Borrelia spp.* (Bhide et al., 2005).

"Bartonella henselae" causes "cat scratch disease" in humans but cats are usually asymptomatic. Transmission from cat to cat is mainly by fleas. No systematic study has been performed on prevalence of this bacterium in Eurasian lynx so far, but one investigated animal from Switzerland was positive (Ryser-Degiorgis and Robert, 2006). Free-ranging wild felids from North America, including bobcats, were shown to be seropositive (Chomel et al., 2004), indicating that wild felids including *Lynx* spp. may act as reservoir for this bacteria.

Francisella tularensis is a highly infectious bacterium causing tularemia. It is primarily a disease of lagomorphs and rodents but has been reported from numerous mammalian species. However, many mammals are only carriers. Antibodies to *F. tularensis* were detected in free-ranging bobcats and Canada lynx (Heidt et al., 1988; Biek et al., 2002); however, none of the investigated Eurasian lynx from Sweden tested positive (Ryser-Degiorgis et al., 2005b).

Feline haemotropic mycoplasmas, the causative agents of infectious anaemia, are cell wall-less bacteria, which parasitize red blood cells of several mammalian species including cats. Of 36 free-ranging Eurasian lynx from Switzerland, four (11%) were PCR-positive for *Mycoplasma haemofelis*, 14 (39%) for *"Candidatus M. haemominutum"*, and two (6%) for *"Candidatus M. turicensis"*. The pathogenic potential of these agents is unclear (Willi et al., 2007). However, a significant association was found between FeLV infection and haemotropic mycoplasma infection in Iberian lynx (Meli et al., this book).

Dermatophytes

Dermatomycosis (also known as "ringworm"), mostly due to *Microsporum* or *Trichophytum* spp. is a sporadic skin disease of captive wild felids, including Eurasian lynx (Gass, 1987) and Iberian lynx (Martínez et al., this book). Typically, there are multiple round hairless areas on legs or head. Stress, presence of ectoparasites and traumatic skin lesions are some of the factors predisposing to an infection.

Diseases of unknown aetiology

Schmidt-Posthaus et al.,(2002) mention an acute necrotizing pancreatitis with fibrinous and plasmacellular serositis in an adult lynx, and a metastatic calcification in lung, kidney and vessels of all organs concomitantly with a mild membranous glomerulonephritis in a juvenile. A chronic inflammatory myopathy associated with similar lesions in the myocard and liver was observed in a subadult animal from Switzerland (Ryser-Degiorgis and Robert, 2006). A lynx affected with a kidney necrosis of unknown origin was reported from France (Stahl and Vandel, 1999).

Discussion

The most common causes of death in free-ranging Eurasian lynx appear to be non-infectious (70-87%) and mostly of traumatic origin, such as traffic accidents, poaching, hunting and legal shooting of animals causing damages to livestock (Jedrzejewski et al., 1996; Schmidt-Posthaus et al., 2002; Stahl and Vandel, 1999; Ryser-Degiorgis et al., 2005a, c; Breitenmoser and Breitenmoser-Würsten, 2008). Overall, human-related mortality is of main importance: depending on the country and study period, it varies from 54 to 96.5%. In contrast, diseases represent only 3.5-25% of the causes of mortality. Due to its feeding behaviour (it returns to a kill for several consecutive nights, depending on the kill's weight; Jobin et al., 2000), Eurasian lynx is particularly exposed to poaching through shooting or poisoning. Indeed, poaching is considered as the most important threat across all European populations (von Arx et al., 2004). Interestingly, there does not seem to be a simple relationship between an increased legal harvest and decreased poaching as is commonly expected (Andrén et al., 2006).

Regarding the potential impact of diseases, caution is however recommended: while most animals dying in traffic accidents will be found, many diseased lynx will not. The importance of infectious diseases might thus be largely underestimated since mortality studies mostly rely on data from lynx found dead by chance. Indeed, Schmidt-Posthaus et al.,(2002) showed that 18% of the animals they included in their study died of an infectious disease, but if only radio-tagged animals (that were supposed to be a more representative sample of the population) were considered, the percentage of mortality due to infections raised to 40%. Similar results have been found in Iberian lynx (Martínez et al., this book). Furthermore, apparently emerging problems such as congenital malformations and heart lesions compatible with cardiomyopathy in Swiss lynx definitely require particular attention, and underline the need of a long-term, careful veterinary monitoring of free-living populations.

A wide range of infectious and non-infectious diseases has been reported in Eurasian lynx, in free-ranging and captive animals. As a felid, the lynx is probably susceptible to most, if not all diseases affecting domestic cats. Furthermore, as a large predator, the Eurasian lynx is exposed to infectious agents through its prey (red fox, hares, roe deer, rodents). Epidemic outbreaks do not seem to occur in free-ranging Eurasian lynx populations. Eurasian lynx are solitary living animals, males and females being usually separated in space and time even if their home ranges overlap. A family group consists only of a female and her cubs of the year (Breitenmoser and Breitenmoser-Würsten, 2008). Because of this solitary behaviour, there are only rare opportunities for intraspecific transmission of pathogens before a fatal outcome or recovery from the disease, although a disease with long duration and/or incubation period, such as feline leukemia, might represent an exception.

The absence of detection of antibodies or antigens in population surveys indicate that the investigated lynx populations either did not have any recent contact with these agents, and/or possibly that the species is highly susceptible to infection (i.e., infected individuals do not survive an infection and are thus not detected in serosurveys). In contrast, a high prevalence is an indication that the concerned agents do not cause serious health problems in the species, especially if related mortality has not been observed and infected populations are stable. For example, high prevalences have been documented for *Toxoplasma*, *Trichinella* and *Cytauxzoon,* which are normally not pathogenic to lynx.

The disease most commonly diagnosed in free-ranging Eurasian lynx is sarcoptic mange. Though, long-term population monitoring in Sweden and Switzerland indicate that this disease is not a threat for the long-term survival of affected lynx populations. Furthermore, it only affects lynx in areas where it is endemic in red foxes.

Overall, diseases do not appear as a significant threat to lynx populations so far. One can however not exclude that an emerging disease could have a devastating effect in a small population, especially if added to serious additional problems such as poaching and/or habitat destruction. Furthermore, knowledge on disease susceptibility and carrier role of lynx is necessary to propose adequate health management measures, e.g., in the frame of translocation programmes. Long-term health monitoring of free-living and captive populations, together with extensive sample collection for immediate or later analysis, is therefore recommended. The combination of radio-telemetry studies and veterinary investigations including detailed necropsies provides the most reliable information. In this context, close collaboration between field biologists and veterinarians is essential, both for data collection and interpretation. Managers also have to be part of this interdisciplinary approach, in particular when considering the implementation of adequate management measures suggested by the data.

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Photo: Héctor Garrido

Reproductive physiology

FISIOLOGÍA REPRODUCTIVA

Merely the attempt to solve the biodiversity crisis offers great benefits never before enjoyed, for to save species is to study them closely and to learn them well.

E.O. Wilson
(The Diversity of Life, Harvard University Press, 1992)

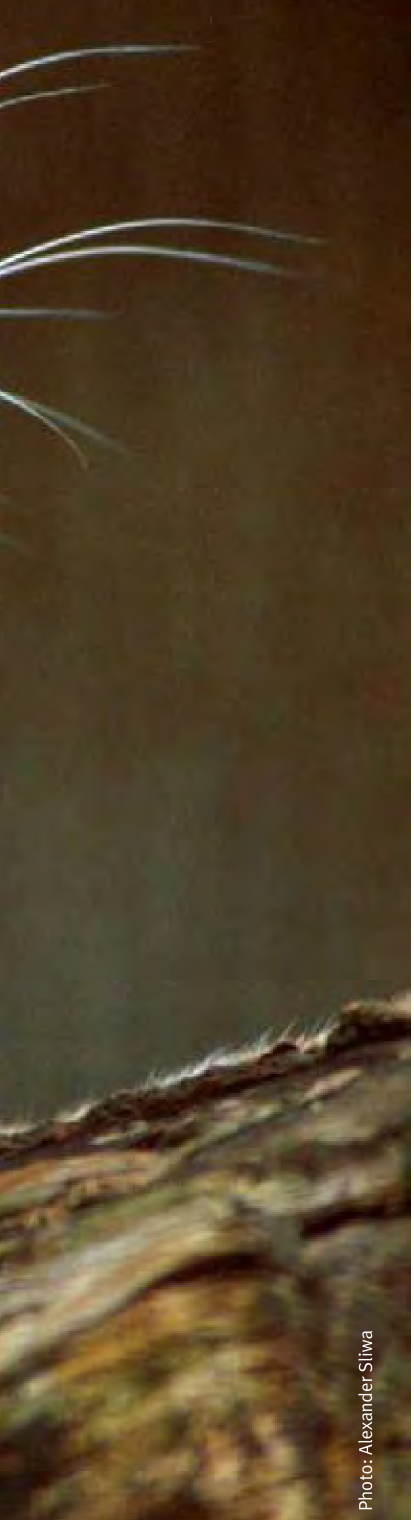
Photo: Alexander Sliwa

Contributions of reproductive science to wild felid conservation

Contribuciones de la ciencia de la reproducción a la conservación de felinos silvestres

David E. Wildt, Jo Gayle Howard, Katey Pelican, Janine Brown and Budhan Pukazhenthi

Resumen

En la ciencia de la reproducción se han producido notables adelantos gracias a tecnologías innovadoras que han permitido, entre otras cosas, superar problemas de infertilidad y aumentar el número de crías. Los incentivos para estos avances se derivan principalmente de los esfuerzos comerciales dirigidos a mejorar la producción ganadera y también a resolver problemas sanitarios en humanos. En el sector ganadero, por ejemplo, la inseminación artificial ha permitido una amplia distribución de genes (esperma) procedentes de padres particularmente aptos por ser genéticamente superiores. Mediante la transferencia de embriones, se ha conseguido que hembras reproductoras que normalmente sólo tenían algunas crías a lo largo de su vida produzcan una gran progenie. En los humanos, la fecundación *in vitro* (FIV), combinada con la transferencia de embriones, ha permitido a miles de parejas superar la infertilidad y tener hijos sanos. La crioconservación del esperma y los embriones ha permitido que, tanto en el ganado como en la especie humana, se pueda regular el momento de la reproducción, en ocasiones incluso a lo largo de varias generaciones.

Comparada con la reproducción asistida en especies ganaderas y en humanos, la gestión y conservación de especies silvestres implica unas ideas y una logística más complejas. Para las especies amenazadas en colecciones *ex situ*, el objetivo no es sólo obtener un mayor número de crías, sino crías con un origen conocido y un genotipo adecuado para conservar la integridad de la especie, subespecie o población. Esto a menudo implica cruzar dos individuos que tienen el genotipo adecuado pero tal vez sean sexualmente incompatibles o estén separados físicamente por grandes distancias. También puede ser ventajoso conservar a largo plazo los genes de un individuo valioso para incorporarlos periódicamente a la colección con el fin de maximizar su vigor genético. Por la misma razón, puede ser útil cruzar individuos o poblaciones en centros de cría con individuos que viven en estado silvestre. Los responsables de la gestión de especies silvestres deben tener en cuenta todas estas cuestiones.

Desde hace ya bastante tiempo, las tecnologías reproductivas se consideran como una forma de ayudar al manejo genético y la reproducción de las especies amenazadas. Hasta el momento, las técnicas de reproducción asistida utilizadas de forma habitual en especies domésticas no han sido fáciles de adaptar a las especies silvestres. Las diferencias entre especies en cuanto a la forma (anatomía/morfología) y la función de la reproducción (mecanismos que regulan el éxito reproductor) limitan la aplicabilidad práctica de dichas técnicas para la obtención de progenie. Por lo tanto, el factor limitante es la falta de conocimientos básicos sobre miles de especies que no han sido objeto de estudio, lo cual constituye la base esencial para la mejora de la reproducción. Actualmente, existen claras evidencias de que las tecnologías reproductivas son de gran utilidad para estudiar cómo se reproducen las distintas especies, sobre todo cuando se definen mecanismos nuevos y únicos. En este capítulo, se analiza la situación y relevancia de distintas tecnologías reproductivas útiles o prometedoras para felinos silvestres, con el fin de conseguir mejoras en su reproducción, gestión y conservación. Nuestra experiencia de más de 25 años muestra claramente que los mecanismos de reproducción de las especies de la familia Felidae presentan grandes variaciones entre sí. Las tecnologías reproductivas son particularmente valiosas para esclarecer dichas diferencias fundamentales en las funciones biológicas básicas. Una vez que se han generado los conocimientos adecuados, existen ejemplos que muestran cómo la reproducción asistida y, particularmente, la inseminación artificial, se han utilizado para aumentar la producción de crías y mejorar el manejo genético de las poblaciones *ex situ*.

Palabras clave
reproducción asistida, inseminación artificial, esperma, fertilización, embrión

Abstract
Innovative technologies have advanced the reproductive sciences, including overcoming infertility and increasing offspring numbers. Incentives largely have been derived from commercial efforts to increase livestock production and addressing human health issues. For example, artificial insemination (AI) allowed the wide scale distribution of cattle genes (sperm) from outstanding, genetically superior sires. Embryo transfer (ET) permitted large numbers of young to be produced from dams normally capable of producing only a few offspring during a lifetime. *In vitro* fertilization (IVF) combined with ET allowed thousands of humans to overcome infertility and produce healthy children. The ability to cryopreserve sperm and embryos permitted livestock managers and human couples to regulate the timing of offspring production, sometimes spread over generations.

Compared with assisting livestock and humans, management and conservation of wildlife species have more complex expectations and logistics. For endangered species maintained *ex situ*, the aim is not only to produce more young, but offspring of known provenance and genotype that will preserve species integrity. This often requires the breeding of individuals of appropriate genotype, but which may be sexually incompatible or physically separated by distance. It also may be advantageous to preserve the genes of an individual for long durations to be periodically infused back into the collection to maximize genetic vigor.

Reproductive technologies have long been considered a means of assisting in the genetic management and propagation of endangered species. So far, techniques that are routine in domesticated species are not easily adapted to wildlife. Species differences in reproductive form (anatomy/morphology) and function (mechanisms regulating reproductive success) limit practical applicability. The limiting factor is the lack of basic knowledge about thousands of unstudied species, the essential foundation to allow reproduction to be enhanced. There is excellent evidence that reproductive technologies are most useful as tools for studying how different species reproduce, especially defining unique mechanisms. Here, we review the relevance of various reproductive technologies for improving reproduction, management and conservation of wild felids. It is clear that species within the Felidae family vary markedly in reproductive mechanisms. Reproductive technologies are most valuable for elucidating these fundamental differences in basic biological function. And, when adequate knowledge is generated, there are examples of how assisted breeding, especially AI, has been used to enhance offspring production and genetic management of *ex situ* populations.

Keywords
reproduction, assisted breeding, artificial insemination, sperm, fertilization, embryo

Contributions of reproductive science to wild felid conservation

David E. Wildt, Jo Gayle Howard, Katey Pelican, Janine Brown and Budhan Pukazhenthi

Introduction

Twenty-three of the 38 known cat species (or subspecies) are threatened or endangered with extinction. The causes of population declines largely are related to habitat loss and fragmentation, although some wild cat species continue to be relentlessly persecuted by humans. While declines in wild felid numbers are significant, cats in general have the advantage of being charismatic, and their predatorial features (even the homicidal trait of some of the "great cats") intrigues us. In short, people generally like or at least tolerate felids, and this interest is advantageous to scientists who study and conserve wild felids. The public often is sympathetic to saving the world's wild felids, and this attention often can be translated into helpful conservation policy or even financial support.

Because reproduction is a key factor to species success, understanding how cats reproduce helps to produce strategies for conservation and management. This is important for wild felids, but also certain domestic cat genotypes. Much of the work in our laboratory is driven by the value of the domestic cat as a human biomedical model, especially for metabolic defects analogous to human congenital errors (e.g., mucopolysaccharidosis, mannosidosis, diabetes mellitus, among others). However, most interesting have been the comparisons in reproductive phenomena between the domestic cat and its wild counterparts, as well as examining the utility of reproductive technologies for propagating and improving genetic management.

Reviews of the roles of the reproductive sciences and related technologies for wild animal species are available from many sources, the most complete being a compendium for diverse taxa by Holt et al. (2003b). There are two recently published articles on the value of conventional reproductive technologies for wildlife (Pukazhenthi and Wildt, 2004) as well as emerging techniques (Pukazhenthi et al., 2006a). Overviews on felid reproductive biology (Wildt et al., 1998) and the value of assisted reproduction (Wildt and Roth, 1997; Howard and Wildt, 2009) also are available. In this chapter, we share highlights of more than 25 years of experience on the value of such technologies for understanding, managing and conserving wild felids.

Evolution of wild felid studies

As recently as the mid-1970s, most of our biological knowledge about wild felids was derived largely from 1) behavioral ecology studies of a few species in nature 2) behavioral viewing of zoo animals and 3) examinations of skulls and other museum specimens. The physiological factors regulating reproductive success had gone unstudied. This was a particularly serious gap in information because cats evolved throughout the world from within varied habitats and into wonderfully diverse morphotypes. Everyone assumed that all cats reproduced in a similar fashion, that is, males produced sperm throughout the year, but females were seasonal breeders and "induced" ovulators (the ova released in response to copulation). As recently as 1980, there were no clues about hormonal control of reproductive events in wild cats. Most scientists believed that it would be virtually impossible to study hormonal patterns in, for example, tigers. How would one ever be able to collect serial blood samples for hormonal analysis?

Near the same time, there was growing interest in developing hedge populations of wild felids for captive management. Zoos were realizing the need to become "conservation catalysts" rather than "wildlife exploiters". It suddenly was anathema to take from nature, but rather to focus on developing self-sustaining wildlife populations that, in turn, were "ambassadors" for the wild that served to educate the public. If no more animals were to be removed from nature, then managers soon realized the need to develop state-of-the-art approaches for maintaining healthy populations of these complex species in "artificial environments". The concept of Species Survival Plans® emerged, essentially the formation of zoo consortia that shared animals for breeding to maximize genetic heterozygosity. While some species (lions and tigers) seemed to breed at whim, others defied reproduction. Computer matchmaking of genetically "ideal" pairs often was compromised by sexual partner preferences. Cats can be like people in that simply introducing a male and female does not necessarily ensure breeding success due to sexual incompatibility. For instance, the clouded leopard (Fig. 1) male often kills or severely maims designated female partners, even those in estrus. Additionally, managers soon learned the insidious effects of poor husbandry, especially the adverse impacts of inbreeding.

Besides behavioral challenges, other factors were emerging as important regulators of reproductive success. Early studies revealed inherently low amounts of genetic diversity in some wild felid species unrelated to zoo breeding but originating from population bottlenecks in nature. The cheetah (Fig. 2) and certain populations of African and Asian lions and North American pumas (i.e., the Florida panther) had low genetic variability while producing high proportions of abnormally shaped (pleiomorphic) spermatozoa (Fig. 3). The impact of this condition, teratospermia (Pukazhenthi et al., 2006b), was unknown. Nutritional inadequacies (e.g., overfeeding, imbalanced diets and the lack of appropriate vitamin and mineral supplements), disease (especially viruses)

Photo: Jessie Cohen, Smithsonian's National Zoological Park

Figure 1. The clouded leopard, a felid typically difficult to naturally or artificially breed in captivity.

Figura 1. La pantera nebulosa, un felino difícil de criar en cautividad, tanto de modo natural como artificial.

Photo: Jessie Cohen, Smithsonian's National Zoological Park

Figure 2. The cheetah, one of several felid species/subspecies with low genetic variation that routinely ejaculates high proportions of pleiomorphic (malformed) spermatozoa (photograph b).

Figura 2. El guepardo, una de las muchas especies/subespecies con poca variabilidad genética, que de forma rutinaria produce eyaculados con alta proporción de espermatozoides pleiomórficos (con malformaciones), (fotografía b).

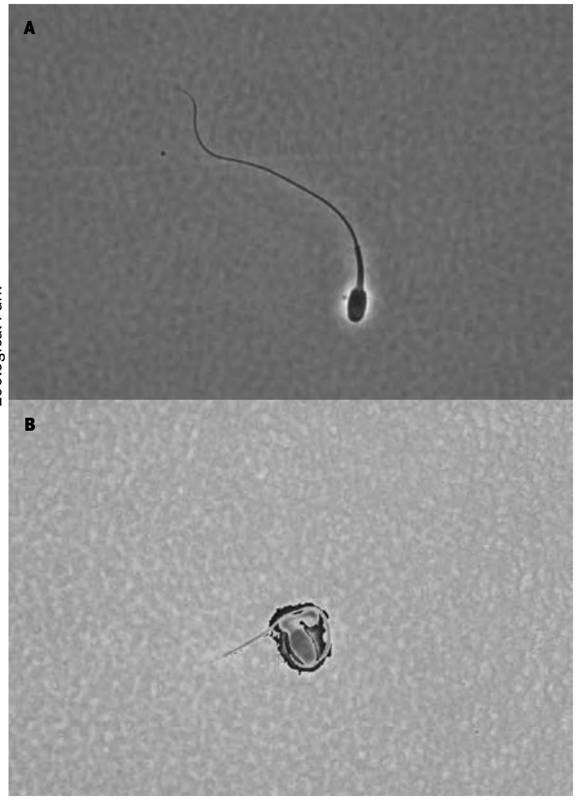

Figure 3. Structurally normal (a) and malformed (b) felid spermatozoa with the latter having a tightly coiled flagellum and abnormal midpiece.

Figura 3. Espermatozoides de felino: estructuralmente normales (a) y malformados (b). Estos últimos presentan un flagelo enroscado y la pieza central también presenta una forma anormal.

and "stress" also were potentially adversely affecting reproductive capacity, especially in zoo-held felids.

Thus, there was a broad menu of factors to study and significant motivation from two directions, one being simply intellectual curiosity and the other being the need for knowledge that would have practical management and conservation application.

Highlighted Discoveries

In retrospect, our laboratory's contribution to the study of wild felids was grounded in extensive prerequisite studies of the reproductive biology of the domestic laboratory cat. Begun in 1975, our early investigations of gonadal and gamete function, hormonal profiles, behavior and assisted breeding provided us the fundamental information (and confidence) to expand studies to many wild felid species. Efforts were bolstered through partnerships with zoos and wildlife reserves throughout the world. We encountered a common interest wherever we went, agreement that these magnificent species deserved immediate research attention.

The details of the important findings in wild felid reproductive biology are beyond the scope of this chapter. However, areas of significant discovery by us and other laboratories included:

The importance of species-specificity

Cats evolved unique reproductive traits that no doubt maximize reproductive fitness within their normal wild niche. There is an amazing diversity among species in, for example, sperm quality, seasonality, the duration of the female reproductive cycle, the amounts of endogenous hormone produced and type of ovulation (see review, Wildt et al., 1998). For instance, spontaneous (non-copulatory induced) ovulation is not uncommon in certain wild species (i.e., the clouded leopard; Fig. 1) or even certain genotypes of domestic cat. The female ocelot and margay show distinctive ovarian cycles throughout the year, contrasted to the tightly regulated Pallas' cat that generally cycles for only two months annually. In giving exogenous gonadotropins to artificially stimulate ovarian activity and ovulation, there is no relationship between species body mass and hormone dosage required. For example, the ocelot is rather tolerant to these exogenous hormone treatments (i.e., a substantial dose is required), whereas the clouded leopard is exquisitely sensitive (i.e., a small dose is used to avoid ovarian hyperstimulation).

The phenomenon of teratospermia

Fifteen felid species routinely produce more than 50% abnormally shaped sperm (Fig. 3) in the ejaculate (see reviews, Pukazhenthi et al., 2001, 2006b). Certain domestic cats experience this same condition. Defects range from simple bending of the midpiece or flagellum to extensive derangements of the mitochondrial sheath and acrosome. Etiology is not well understood, although malformed sperm are prevalent in species or populations that have diminished genetic diversity. These males have normal pituitary function, but often low circulating testosterone. Sperm (including normal appearing cells) from these teratospermic species are compromised in ability to undergo the acrosome reaction, bind, penetrate and decondense in the cytoplasm of the oocyte. In short, abnormal and even normal appearing sperm from teratospermic males do not participate in fertilization. While the cellular defect is now clearly understood, there is a need to better understand the etiology of teratospermia, a condition that interestingly is common in the human.

Tools for the study and evaluation of felid reproduction

Wild cats generally are intractable, and, thus, physiological studies require novel, creative approaches that maximize safety for the animal as well as the scientist (see review, Howard, 1999). The study of male reproductive potential has benefited from the ability to safely anesthetize and collect sperm by electroejaculation. Cat semen, however, requires special medium and handling to optimize *in vitro* viability and evaluation. For females, much has been learned by direct ovarian observation via laparoscopy. It also is possible to recover oocytes from the ovary and evaluate their function *in vitro* by a host of methods, including, recently, by metabolic uptake of substrates (Spindler et al., 2000). One of the most powerful assessment tools for contemporary wild felid studies is the monitoring of steroidal metabolites longitudinally in voided feces (Brown et al., 2001; Brown, 2006, 2009 [this book]). It now is clear that excretory patterns clearly reflect physiological function. This noninvasive approach has allowed fascinating studies of seasonality and estrous cycle variability among species. It also has been useful for improving the chances of successful assisted breeding and beginning studies of the impact of stress on felid welfare and reproductive success. For example, one study has demonstrated that the pairing of female cheetahs in zoos results in reproductive (ovarian) suppression of both individuals due, in part, to subtle, inter-individual aggression (Wielebnowski et al., 2002). Simply separating the animals (even into adjacent pens) allows resumption of reproductive cyclicity (Fig. 4). A priority is extending noninvasive fecal hormone monitoring to all felid species, including testing its utility in wild populations (see review, Monfort et al., 2003).

Assisted breeding and cryopreservation

Assisted breeding refers to an array of techniques that includes AI, IVF and ET, as well as miscellaneous tools ranging from hormonal therapies (to stimulate ovulation) to nuclear transfer (cloning). Again, what is clear is that species differences and having adequate basic knowledge dictate the successful application of any of these techniques, most of which have their most value in simply improving fundamental knowledge about the reproductive biology of each species (Pukazhenthi and Wildt, 2004). However, with the exception of AI, most have little value to practical management and conservation of wild felids, at least today. The usual challenge is largely related to sexual incompatibility and the need for transfer of genetic material among breeding institutions. Thus, the technique of greatest value is AI with fresh or frozen-thawed spermatozoa. Significant advances have been made in the use of AI in felids, including the production of offspring in eight species (leopard cat, cheetah, tiger, puma, ocelot, clouded leopard, little spotted cat and snow leopard) (Howard, 1999; Howard and Wildt, 2009). Young have been produced in three species (leopard cat, ocelot and cheetah) with thawed sperm. The most consistent AI success has been in the cheetah with 11 litters produced, including using cryopreserved sperm imported to the USA from Africa (Wildt et al., 1997; Fig. 5).

The value of cryopreserving germplasm and embryos from wildlife species has been reviewed extensively (Wildt et al., 1997; Holt et al., 2003a). Furthermore, there are multiple ongoing investigations involving the

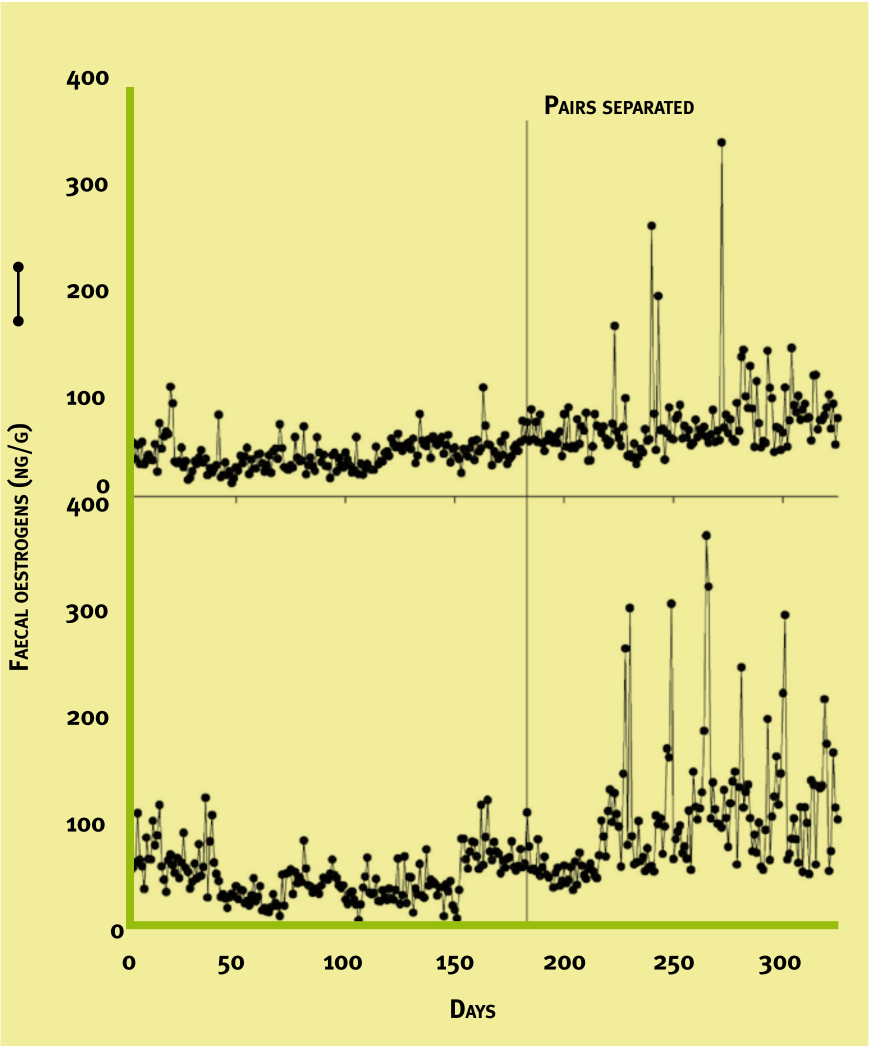

Figure 4. Fecal estrogen profiles in cheetahs while housed in pairs (with ovarian suppression) followed by re-initiation of regular cyclic activity when separated into individual enclosures (from Wielebnowski et al., 2002).

Figura 4. Perfiles de estrógenos fecales en guepardos mantenidos en parejas (con supresión ovárica), seguido por una reiniciación de la ciclicidad normal tras separar a los individuos en instalaciones independientes (de Wielebnowski et al., 2002).

cryobiology of sperm and, to a lesser extent, oocytes and embryos of wildlife species. Carnivore sperm do not freeze well using standard livestock methodologies. There has been slow progress in using cryopreserved sperm and AI in wild carnivores, although overall results remain unsatisfactory (Howard and Wildt, 2009). Felids are particularly challenging in cryobiology studies because ejaculates contain comparative few sperm, many of which are malformed. These sperm also have an exquisite sensitivity to simple cooling (before freezing) that can cause massive acrosomal damage that, in turn, reduces fertilizing capacity (Pukazhenthi and Wildt, 2004). Thus, a contemporary high priority in felid research is determining ways to reduce acrosomal damage during sperm cooling. Domestic cat kittens have been produced using frozen-thawed embryos (and ET), with the same techniques also successfully applied to the African wild cat (Pope et al., 2000). A similar strategy has been used recently to produce ocelot kittens through IVF followed by embryo cryopreservation and transfer to a conspecific recipient (Swanson, 2007). Even with these milestones, embryo cryopreservation is not being used as a management tool, and there are no large-scale embryo banks for any wild felid species.

There is potential value in IVF, although much more work is needed to identify species specificities for generating healthy embryos under laboratory conditions. In general, given the availability of good quality oocytes and sperm, then gamete interaction can occur readily *in vitro*, and embryos can form. The common

Figure 5. Cheetah cub produced by artificial insemination using frozen sperm collected from a wild cheetah and then imported into the U.S.A. from Namibia.

Figura 5. Cachorro de guepardo nacido mediante inseminación artificial utilizando espermatozides congelados obtenidos de un guepardo de vida libre y después importados a los EE.UU. desde Namibia.

condition of teratospermia in felids can compromise embryo production. Furthermore, there always is the question: What should be the recipient for embryos produced *in vitro* (Pukazhenthi and Wildt, 2004)? And, although occasionally successful, including in felids, interspecies embryo transfer is now known to be much more complex than originally believed (Loskutoff, 2003; Pukazhenthi and Wildt, 2004; Gómez and Pope, 2005). Therefore, for IVF/ET to have practical, large-scale value for managing wild felids there would be a need to maintain significant groups of conspecifics surrogates (i.e., individuals of the same species). For example, to use this technique to benefit lions (or any cat species) would require maintaining many lion embryo recipients, an expensive, labor-intensive undertaking that would take up valuable captive breeding space. It also must be remembered that ET largely was developed as a tool for rapidly expanding the genotype of a few select individuals that normally produce only a single young per year (e.g., the cow). In the case of wild carnivores, the management goal is different – to sustain all existing gene diversity.

Nonetheless, embryo studies (especially those involving IVF) are particularly valuable for generating new scholarly information about felid gametes, including on topics pertaining to maturation, functionality, interaction (fertilization), embryogenesis and early developmental biology. There has been substantial progress, especially involving *in vitro* maturation of intraovarian oocytes recovered by laparoscopy or at necropsy (see review, Gómez and Pope, 2005).

There also has been substantial discussion and research in felids pertaining to nuclear transfer (cloning). The relevance (and irrelevance) of nuclear transfer for wildlife has been thoughtfully addressed by Critser et al. (2003). We ascribe to these author's argument that, rather than asking "is nuclear transfer applicable to wildlife", that it is more prudent to ask "as the technology evolves, how can nuclear transfer become a useful tool in a repertoire of assisted breeding technology?" Current cloning approaches have negligible practical value because of extreme inefficiency, tendency to produce abnormal offspring and failure to contribute to the most sought after goal: maintaining gene diversity as the procedure perpetuates individuals and, thus, homozygosity. Nonetheless, nuclear transfer studies have value for increasing scholarly knowledge, including understanding cellular and molecular aspects of nuclear reprogramming and/or producing embryonic stem cells. Furthermore, in cases of near extinction, nuclear transfer could be the only recourse for preventing species loss. There have been notable successes, including the first domestic cat produced via cloning (Shin et al., 2002). This was followed by the production of cloned and living African wild cat kittens born to domestic cat surrogates (Gómez and Pope, 2005; Gómez et al., 2006). Pregnancies also have been established after transferring cloned black-footed cat embryos to domestic recipients, but offspring were not produced. Fetal resorption and abortion have been frequently observed at various stages of pregnancy after transfer of African wild cat cloned embryos into domestic cat recipients (Gómez et al., 2006). Abnormalities, such as abdominal organ exteriorization and respiratory failure and septicemia have been the main causes of mortality in neonatal cloned kittens.

Summary and conclusions

Our years of experience have revealed one unequivocal truth: reproductive biology in a cat is unlike that of a rat, cow, human or even another species of cat. Furthermore, studying the reproductive physiology of wild felids, although challenging, is possible including in a controlled, experimental fashion. Without this hypothesis-driven approach, most of what we know about these fascinating species is little more than anecdotal. Thus, our first priority should always be the need for characterizing these species and producing more fundamental data. This indeed is a major task given the sheer numbers of species in the family Felidae and limited accessibility to research animals and funding. Nonetheless, it is essential.

Once generated, we also have discovered the utility of applying scholarly knowledge to the improved management and conservation of wild felids. The cheetah is a useful model, although even this species requires substantially more research to ensure that reproductive capacity is maximized in zoos. Additionally, whenever possible, the new knowledge needs to be applied to species in nature. In the case of the cheetah, one of our contemporary projects is the development of a genome (sperm) bank in a country of origin, Namibia in southern Africa (Crosier et al., 2006). Expanding the cheetah example to the many other deserving felid species is a high priority.

Lastly, it cannot be emphasized enough that investigations of wildlife are so very different from traditional studies in academe. These advances in wild felid biology have only been possible through partnerships – collaborations among managers of wild felids and diverse scientific disciplines across many institutions. The authors are grateful to the hundreds of individuals and organizations worldwide who have cooperated to understand the biology of wild felids to enhance knowledge and management capability.

Acknowledgments

The authors also thank the many current and former staff from the Department of Reproductive Sciences for expertise, dedication and contributions to felid research and conservation, especially Karen Goodrowe, Annie Donoghue, Leslie Johnston, Mark Barone, Terri Roth, Bill Swanson, Julie Long, Barbara Wolfe, Linda Penfold, Laura Graham, Nadja Wielebnowski, Rebecca Spindler, Adrienne Crosier, Pierre Comizzoli and Rose Bauer.

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Photo: Alexander Sliwa

What we have to learn to do, we learn by doing.

Aristotle
(384 a.C-322 a.C.)

Photo: Antonio Rivas

A Genetic Resource Bank and assisted reproduction for the critically endangered Iberian lynx

Un Banco de Recursos Genéticos y reproducción asistida para el críticamente amenazado lince ibérico

Eduardo R. S. Roldan, Montserrat Gomendio, J. Julián Garde, Natalia Gañán, Raquel González, Cristina Crespo and Lucía Arregui

Resumen

Aunque la mejor estrategia para la conservación de la biodiversidad es la preservación del medio natural, las posibilidades de implementar esta estrategia son a veces reducidas o inviables. Por ello, es importante considerar estrategias adicionales para la conservación de la variabilidad genética de poblaciones amenazadas. Las tecnologías reproductivas ofrecen nuevas soluciones para facilitar el manejo genético e incluyen el desarrollo de bancos de recursos genéticos para conservar gametos, embriones y tejidos somáticos que pueden ser de gran apoyo a programas de conservación *in situ* y *ex situ*. Para el desarrollo de tecnologías reproductivas hemos empleado el gato doméstico, el lince rojo y el lince euroasiático como modelos. Hemos caracterizado parámetros seminales y factores que afectan a la criopreservación de espermatozoides en lince ibérico empleando dos diluyentes de congelación. Los espermatozoides se refrigeraron en pajuelas cortas, utilizando un sistema programable, y se congelaron en vapores de nitrógeno. Los espermatozoides criopreservados de lince ibérico fueron capaces de fecundar oocitos de gata doméstica *in vitro*, lo que constituye un método conveniente para evaluar la capacidad fecundante en el laboratorio. Se obtuvieron espermatozoides de machos de dos años y, en ocasiones, de calidad suficiente para criopreservación, aunque la calidad del semen en machos de menos de tres años fue menor que la de machos de mayor edad. Se recuperaron espermatozoides de epidídimos de animales muertos (5/14 machos), que se han criopreservado, y se han conservado fragmentos de testículos. Se pudieron recuperar oocitos a partir de ovarios de hembras muertas y se logró maduración *in vitro* hasta el estadio de metafase II. Se han conservado tejidos y células somáticas de necropsias de 25 individuos y de biopsias de más de 70 individuos de lince ibérico cautivos o de ejemplares de vida libre. Los resultados de este proyecto representan un avance muy importante para el conocimiento de la biología reproductiva del lince ibérico y para la conservación de germoplasma y tejidos somáticos de esta especie críticamente amenazada. Con esta información será posible asistir en los esfuerzos de conservación facilitando el flujo de material genético entre subpoblaciones cautivas, entre poblaciones cautivas y en libertad, y también entre poblaciones naturales.

Palabras clave
Células somáticas, espermatozoides, oocito, fecundación, testículo, ovario

Abstract

Although the best strategy for biodiversity conservation is the preservation of the natural habitat, implementing this statregy is not always possible or viable. It is thus important to consider additional strategies for the conservation of the current genetic variability of endangered populations. Various reproductive technologies offer new solutions for the genetic management of endangered populations. Among them, the preservation of biological materials (gametes, embryos, somatic tissues) in genetic resource banks (GRBs) may play an important role in both *in situ* and *ex situ* conservation. We have developed reproductive technologies for the Iberian lynx using the domestic cat, the bobcat and the Eurasian lynx as models. Seminal parameters in the Iberian lynx have been characterized, and factors affecting semen cryopreservation have been examined. Two different cryodiluents have been tested, refrigerating spermatozoa in short straws, using a programmable system, and freezing them in nitrogen vapours. Crypreserved Iberian lynx spermatozoa were capable of fertilizing *in vitro* domestic cat oocytes that were matured *in vitro*; this represents a useful and convenient method to assess fertilizing ability in the laboratory. Spermatozoa could be collected from two year old males and, on some occasions, were of sufficient quality for cryopreservation. However, the quality of spermatozoa from males younger than three years old was lower than of older males. Spermatozoa could also be collected from dead individuals (5/14 males) and they were cryopreserved; testis tissue was also preserved. Oocytes were collected from ovaries of dead females and they were matured *in vitro* up to the metaphase II stage. Somatic tissues and cells have been collected and preserved from necropsies of 25 individuals and biopsies were obtained from over 70 individuals, in both captive and free-ranging animals. The results of this project represent an important advance in our knowledge of the reproductive biology of the Iberian lynx and have led to the preservation of germplasm and other biomaterials of this critically endangered species. With these tools it will be possible to assist in conservation efforts facilitating the flow of genetic material between captive sub-populations, between natural and captive populations and also between natural populations.

Keywords
Somatic cells, spermatozoa, oocyte, fertilization, testis, ovary

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Introduction

The best strategy for biodiversity conservation is the preservation of the natural habitat. However, implementing this strategy is not always possible or viable. It is thus important to consider additional strategies for the conservation of the current genetic variability of populations. This could be achieved through the organization of Genetic Resource Banks (GRBs) that allow the preservation of biological materials from and for captive breeding programmes and natural populations. These GRBs will be essential for conservation allowing for an interchange of genetic materials between individuals of threatened populations. Furthermore, they would allow individuals that die before reaching reproductive age an opportunity to reproduce. The role of GRBs in conservation and the potential of reproductive technologies for the preservation and management of genetic diversity in endangered species has been reviewed extensively (Wildt, 1992; Wildt and Wemmer, 1999; Watson and Holt, 2001; Pukhazenthi and Wildt, 2004; Pukazhenthi et al., 2006a, b; Roldan and Garde, 2004; Roldan et al., 2006; Swanson et al., 2007; Roldan and Gomendio, 2009; Wildt et al., 2009).

With the general aim of conserving biodiversity through the preservation and use of reproductive biomaterials, we have developed work on three main areas. First, we have concentrated, since 1995, on the negative effects of inbreeding on male reproduction of three species of endangered gazelles (Gomendio et al., 2000; Roldan et al., 2006) and have developed methods for assisted reproduction in these three species (Garde et al., 2003, 2008). Sperm cryopreservation has been achieved successfully for two species, and we have used cryopreserved semen for artificial insemination which has been followed by the birth of the first live calf born using frozen semen (Garde et al., 2006). We have also examined the feasibility of oocyte *in vitro* maturation and fertilization using cryopreserved semen, and the effect of long-acting neuroleptics on the success of *in vitro* maturation and fertilization (Berlinguer et al., 2008; González et al., 2008). This work and the potential of other reproductive technologies in gazelle conservation have been reviewed recently (Roldan et al., 2006; Roldan and Gomendio, 2009). Second, in 2003 we started a new initiative to organize a germplasm and somatic tissue bank for endangered Iberian species, with special emphasis on the Iberian lynx (see below).

Third, in 2005 we initiated a project for the development of assisted reproductive techniques and conservation of biomaterials for South American felids.

Among the endangered Iberian species, our efforts are focused, in a first stage, on the Iberian lynx and on European mink. In subsequent stages we aim to include also the monk seal and brown bear, although this may prove to be a more difficult task. Since it is difficult to have access to individuals from endangered species, work also relies on the study of model animals for ungulates (sheep and deer), felids (domestic cat, Eurasian lynx and bobcats) and mustelids (American mink). Efforts concentrate on the development of protocols for the cryopreservation of ejaculated or epididymal spermatozoa and the collection, maturation, fertilization and development of oocytes *in vitro*; we are also exploring methods for the cryopreservation of oocytes and embryos. It is also of great interest to collect and cryopreserve testes and ovaries of individuals that die in road accidents (or for other reasons) since they could be used in the future by means of xenotransplantation. Finally, we also carry out a routine cryopreservation of somatic tissues and cells collected during biopsies or necropsies; they could, perhaps, be used in the future via somatic cell nuclear transfer. An updated summary of results will be presented in this chapter.

Somatic cell and tissue preservation

The cryopreservation of somatic tissues and cells would allow the preservation of a maximum of genetic diversity, especially if biomaterials from animals that have failed to reproduce are collected and banked. Such somatic cells may be used in the future with the help of assisted reproductive techniques, as has already been demonstrated with the use of somatic cell nuclear transfer (cloning) for some endangered species (Loi et al., 2001; Gómez et al., 2006, 2009). The use of cloning in endangered species has generated considerable debate but we believe there are many advantages to be obtained with this approach (Roldan and Garde, 2004). We have implemented a system to collect, transport and process in the laboratory samples from dead animals and biopsies from live individuals (Roldan et al., 2005). Currently, there is an active health and reproductive screening programme that involves routine examinations of Iberian lynx in both the Captive Breeding Programme and in natural populations in order to monitor for the prevalence of infectious diseases (Martínez et al., 2009) and this has generated an excellent opportunity to collect samples from many animals. Samples from over 25 deceased animals have been obtained through necropsies, and over 70 samples have been collected through biopsies of live captive and free-ranging individuals.

Tissue is collected by biopsy, during routine examinations (Figure 1a) or during necropsies, and samples are transported by us or are sent to the laboratory by courier in culture medium in refrigerated styro-foam containers. Tissue sub-samples are both cryopreserved and processed for cell proliferation (Figure 1b) by incubation in D-MEM with 10% bovine serum, L-glutamine, antibiotics and antifungal agents at 37 °C under 5% CO2 in air (Figure 2a). Cells grow out of explants until confluency (with a maximum of one to three passages) and are then cryopreserved in D-MEM with bovine serum and 10% DMSO, or in bovine serum-10% DMSO and stored in liquid nitrogen (Figure 2b). Cryopreserved tissues and cells maintain viability and can grow in culture after several months of storage in liquid nitrogen (Figure 2c). Occasional problems arise due to contamination of tissues (perhaps due to insufficient cleaning during collection) or to lack of viability during incubation due to a long delay between death of the animal and collection of tissues. Thus, it is sometimes necessary to repeat the biopsies to secure banking of valuable material. We have examined a variety of factors that affect the success of incubation and banking as well as the influence of the source of samples such as sex, population, or cause of death. Using this methodology, somatic tissues and cells have been cryopreserved in our laboratory from Iberian lynxes, Eurasian lynxes and bobcats (Crespo et al., 2007a, b).

Cryopreservation of cat spermatozoa as a model for wild felids

The success of semen cryopreservation varies between species and methods for successful freezing and thawing, with a maximum recovery of sperm motility and acrosomal integrity, should be examined and validated for the species of interest. This is usually difficult for endangered species for which there are always a limited number of individuals, and due to the high genetic value of each sample. For these reasons, it is necessary to resort to phylogenetically related model species that allow the provision of sufficient number of samples for

Figure 1. Collection of skin biopsies and fibroblasts in culture. (A) Skin biopsies are collected aseptically, placed in transport medium and shipped to the laboratory for culture or cryopreservation. (B) Fibroblasts from Iberian lynx grown in culture.

Figura 1. Obtención de biopsia de piel y fibroblastos en cultivo. (A) Las biopsias de piel se obtienen en forma aséptica, se colocan en un medio de transporte y se envían al laboratorio para cultivo o criopreservación. (B) Fibroblastos de lince ibérico en cultivo.

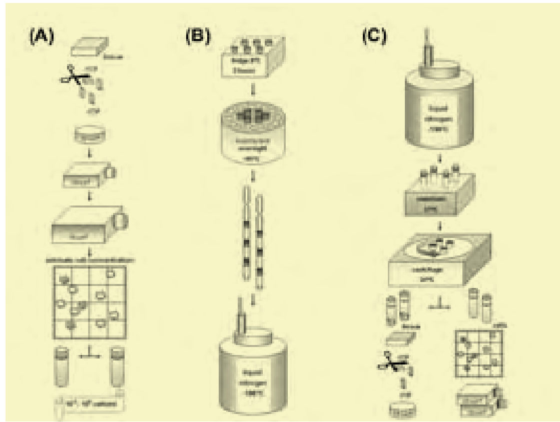

Figure 2. Procedure for tissue culture. (A) Skin (or other tissue) is cut and placed in a small Petri dish, covered with culture medium and cells allowed to grow from the tissue explant. Cells are "passaged" to larger containers until the desired amount of cells is obtained and confluence is reached. Cell concentration is estimated and cells are then suspended in cryopreservation medium. (B) Cells in cryopreservation medium are cooled at 5 °C during 2 h and then frozen overnight in a container with isopropanol, after which vials are transferred to liquid nitrogen and stored. (C) Cells are thawed in a water bath, centrifuged to remove the cryopreservation medium and resuspended in culture medium. Cell concentration is verified and culture initiated. If intact tissue is cryopreserved, it is sliced and cultured as for fresh tissue.

Figura 2. Procedimiento utilizado para cultivo de tejidos. (A) La piel (u otro tejido) se corta y se coloca en una placa de Petri pequeña, se cubre con medio de cultivo y las células crecen a partir del explante tisular. Las células se transfieren a un recipiente de mayor tamaño y se continúa el cultivo hasta que se obtiene el número deseado de células y se alcanza confluencia. Se estima la concentración celular y las células se suspenden en medio de criopreservación. (B) Las células en medio de criopreservación se enfrían a 5 °C durante 2 h y se congelan durante toda la noche en un recipiente con isopropanol y, posteriormente, los viales se transfieren y almacenan en nitrógeno líquido. (C) Las células se descongelan en un baño maría, se centrifugan para eliminar el medio de criopreservación y se resuspenden en medio de cultivo. Se verifica la concentración celular y se inicia el cultivo. Si se ha criopreservado tejido intacto, el tejido se corta en trozos y se cultiva tal como se realiza con el tejido fresco.

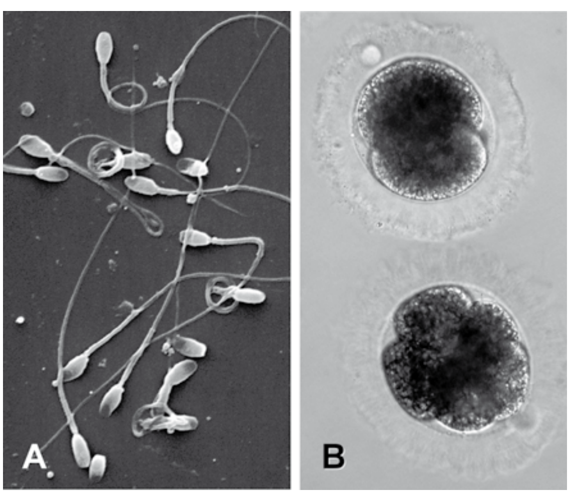

Figure 3. Iberian lynx spermatozoa and domestic cat oocytes fertilized by Iberian lynx sperm. (A) Iberian lynx spermatozoa examined using scanning electron microscopy. Note the presence of various abnormal sperm forms. (B) Domestic cat oocytes matured *in vitro* and fertilized *in vitro* by cryopreserved Iberian lynx spermatozoa. This method allows for the evaluation of sperm potential fertility in the laboratory.

Figura 3. Espermatozoides de lince ibérico y oocitos de gato doméstico fecundados por espermatozoides de lince ibérico. (A) Espermatozoides de lince ibérico examinados mediante microscopía electrónica de barrido. Nótese la presencia de varios espermatozoides anormales. (B) Oocitos de gato doméstico madurados *in vitro* y fecundados *in vitro* por espermatozoides de lince ibérico. Este método permite evaluar la capacidad fecundante en el laboratorio.

the development of adequate methods of sperm evaluation and protocols of sperm cryopreservation. We use the domestic cat as model for endangered felids (Gañán et al., 2005). Epididymal spermatozoa have been collected from subadult and adult cats. Variations were observed throughout the year both in the availability and quality of the samples. Mean values observed have been: 55% motile sperm, 24 x 10^6 total spermatozoa, 80% sperm with normal morphology and 75% sperm with intact acrosomes. Samples with at least 40% motile sperm and a total of 20 x 10^6 sperm/ml have been used in studies of factors affecting cryopreservation (Gañán et al., 2006a).

For cat sperm cryopreservation, we have tested two diluents, Tes-Tris (TEST) and Biladyl, both with 20% egg yolk and 4% glycerol, two cooling rates (0.5 °C/min and 0.125 °C/min), two packaging methods (pellets and straws) and timing of glycerol addition (before and after refrigeration) (Gañán et al., 2006a, b). Spermatozoa were evaluated for motility, viability and acrosome integrity (using Coomassie blue staining) at different stages during the cryopreservation protocol: fresh, after refrigeration and after freezing-thawing. In addition, fertilizing capacity of cat spermatozoa was evaluated using *in vitro* fertilization of *in vitro* matured cat oocytes. Results revealed no differences between the two diluents employed when spermatozoa were examined upon thawing. However, after IVF, spermatozoa cryopreserved in TEST showed higher fertilization rates.

Semen collection from Iberian lynxes and related species

In order to use a more phylogenetically close model, studies were carried out on semen collection and cryopreservation in bobcats and Eurasian lynx. While a limited number of samples could be obtained from the latter, bobcat semen was obtained from various males and factors affecting cryopreservation were analyzed (Gañán et al., 2008a). Diluents such as TEST and Biladyl (both with 20% egg yolk and 4% glycerol) were again examined in cryopreservation, and the ability of bobcat sperm to fertilize domestic cat oocytes was employed as a test of fertilizing ability. The capacity of spermatozoa from various felid species to fertilize *in vitro* matured oocytes from the domestic cat has been used previously as a laboratory test (Swanson et al., 2006, 2007; Thiangtum et al., 2006; Stoops et al., 2007). No differences were found upon thawing between spermatozoa cryopreserved in both diluents. However, fertilization rates of spermatoza cryopreserved in Biladyl were lower than that of spermatozoa cryopreserved in TEST.

Semen was collected by electroejaculation from captive Iberian lynx males kept in the Captive Breeding Programme. Ejaculates contained on average (mean ± SEM) 3.3 ± 0.6 x 10^6 total spermatozoa and 73 ± 4.6 motile spermatozoa (Gañán et al., 2008b). Sperm abnormalities were found in all animals (Figure 3a) in agreement with observations made in other felids (e.g., Wildt et al., 1983; Wildt et al., 1987a, b; Pukazhenthi et al., 2006a, b). On average, ejaculates contained 24 ± 4.0% normal sperm and 41 ± 2.3% spermatozoa with intact acrosomes (Gañán et al., 2008b). In general terms, average values of semen parameters in Iberian lynx were not very different to those found by us in Eurasian lynxes and bobcats sampled at Spanish zoos and animal parks. These results suggest that the presumed loss of genetic variability in the Iberian lynx has not yet significantly affected its seminal parameters. However, it could also mean that semen traits in Eurasian lynxes and bobcats examined may be low due to some inbreeding in the individuals sampled.

We have analyzed the ability of semen extenders to support cryopreservation of Iberian lynx spermatozoa. Two diluents have been evaluated so far: TEST and Biladyl, both with 20% egg yolk and 4% glycerol. After cooling from 22 °C to 5 °C during 2 h, results were similar between TEST and Bildayl with regards to the proportion of motile sperm and the proportion of spermatozoa with intact acrosomes. On the other hand, there were differences between diluents with regards to survival after freezing and thawing. Oocytes collected from domestic cats and matured *in vitro* were used to test the fertilizing capacity of Iberian lynx spermatozoa. Results showed that heterologous fertilization of cat oocytes by Iberian lynx spermatozoa can take place (Figure 3b), although at a lower rate than that found for other felid species (unpublished results). In any case, fertilization rates differed between spermatozoa cryopreserved in TEST and Biladyl (Gañán et al., 2008b) thus confirming the results obtained by examination of motility post-thawing.

We have also obtained and cryopreserved epididymal spermatozoa from five out of 14 dead Iberian lynx males. Most males from which spermatozoa could not be recovered were young or died outside of reproductive season. Testis fragments were cryopreserved for future use. These results demonstrate the importance of rescuing male germplasm for possible future uses via assisted reproduction.

Preservation of testicular tissue and xenotransplantation

In addition to the cryopreservation of spermatozoa, it is important to preserve testicular tissue and testicular cell suspensions from young animals and adults, especially from those that die before having the opportunity to reproduce. Xenotransplantation of testis grafts is successful even when done between distant species (Honaramooz et al., 2002; Dobrinski, 2007) and, furthermore, testicular sperm suspensions are capable of organizing testicular tissue after xenotransplantation (Arregui et al., 2008b). However, although tissue from immature individuals survive and develop after transplantation, leading to the generation of spermatozoa that can be used by means of intracytoplasmic sperm injection, our results revealed that tissue from adult individuals of several species seldom survive and multiply after transplantation (Arregui et al., 2008a). Therefore, alternatives that would allow proliferation and differentiation of testes from mature individuals should be explored in more detail (Arregui et al., 2008c). In any case, if testicular tissue and cells are to be useful in the future, one critical factor to bear in mind is the method for recovery of testicular cells and tissues. For this reason it is essential that testes are collected and transported to the laboratory as soon as possible and under appropriate conditions after the death of individuals. Initial work on domestic cats shows that this approach is indeed promising (Snedaker et al., 2004; Kim et al., 2007).

In vitro maturation, fertilization and culture of oocytes

Oocytes recovered post-mortem as well as those collected after hormonal treatments from animals in captive breeding programmes represent a good source of female germplasm to maximize the preservation of genetic diversity. For the development of *in vitro* maturation and fertilization techniques, as well as for the required methods for oocyte and embryo cryopreservation, it is necessary to resort to domestic cat oocytes as models. With the collaboration of various veterinary clinics in the city of Madrid, Spain, cat ovaries were collected, transported to the laboratory, and sliced to recover immature oocytes. Oocytes have been matured *in vitro*, fertilized with fresh epididymal spermatozoa, and cultured up to the blastocyst stage (González et al., 2005).

Oocytes matured *in vitro* can be used to test the fertilizing ability of spermatozoa. Cat spermatozoa cryopreserved in Tes-Tris with 20% egg yolk and 4% glycerol were used for *in vitro* fertilization of *in vitro* matured cat oocytes. Comparisons between sperm prepared using swim-up and sperm dilution after thawing showed similar fertilization rates (about 50%), and similar rates of blastocyst development (about 50%).

Preservation of Iberian lynx female germline

To allow for a maximum preservation of genetic diversity, female germline of Iberian lynxes should also be preserved. Ovarian slices can be cryopreserved for future use in xenotransplantation. In addition, primary follicles could be collected and saved for future *in vitro* maturation (when reliable techniques become available). At the moment, efforts in our group are directed towards ensuring an adequate collection and *in vitro* maturation of oocytes from Iberian lynx females killed in road accidents (González et al., 2007). This has some limitations due to the fact that animals die outside the reproductive season, and that there is usually a delay between the moment when the animal dies, the timing of necropsy and the opportunity to transfer one ovary to the laboratory. In spite of this, we have managed to achieve *in vitro* maturation of Iberian lynx oocytes in our laboratory and we anticipate that it may be possible to advance towards *in vitro* fertilization, culture and cryopreservation of *in vitro*-produced Iberian lynx embryos. Ovaries from five females that died due to different reasons were transported to the laboratory and processed for oocyte collection. Oocytes could be collected in two occasions and they were incubated for *in vitro* maturation. In one occasion, a proportion of the oocytes matured to the mataphase II stage (González et al., 2007). These results are the first example of *in vitro* maturation of oocytes from this species and they are most encouraging because they suggest that a routine rescue and preservation of female germplasm may be possible for its future use by means of assisted reproduction.

Conclusions

Work carried out in our laboratory has shown that it is feasible to rescue and cryopreserve somatic tissues and germplasm from critically endangered Iberian lynxes. The preservation of such biomaterials represents a valuable opportunity to maximize the conservation of genetic diversity in this species. In addition, they allow us to carry out studies to characterize the reproductive biology of this species in order to develop adequate assisted reproductive techniques. With these tools it will be possible to assist in conservation efforts facilitating the flow of genetic material between captive sub-populations, between natural and captive populations and also between natural populations.

Acknowledgements

Our work on felids is made possible through the generous collaboration and support of veterinary clinics in Madrid, Spanish zoos and parks, the *Ex situ* and *In situ* Conservation Programmes for the Iberian lynx, and the Environmental Council of the Andalusian Government, who provides access to all Iberian lynx samples. We are particularly indebted to Astrid Vargas, Fernando Martínez, María José Pérez Aspa, Iñigo Sánchez, José María Aguilar and Francisco Palomares for all their help and for facilitating the access to captive and free-ranging lynxes, and to all the people in both *Ex situ* and *In situ* Programmes that in one way or another contribute to this conservation effort. Continuous encouragement from Miguel Aymerich, Borja Heredia and Miguel Ángel Simón is acknowledged. The support of the Ministry of the Natural, Rural and Marine Environment and Andalusian Government is deeply appreciated. Our studies are funded by the Ministry of the Natural, Rural and Marine Environment and CSIC and by the Fundación BBVA. Ford España (Novauto) kindly supplied a vehicle for field work.

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Photo: Antonio Rivas

El carácter de un hombre es su destino, según Heráclito. Según la sabiduría popular, la unión hace la fuerza. Y según parece, por fin hemos empezado a unir esfuerzos, para que en un futuro cercano sea, la fuerza del carácter de este felino, lo que principalmente marque su propio destino.

T. León-Quinto

An Iberian lynx Biological Resource Bank and its applications to the *in situ* and *ex situ* conservation of the species

Un Banco de Recursos Biológicos para el lince ibérico y sus aplicaciones en la conservación *in situ* y *ex situ*

Trinidad León-Quinto, Miguel A. Simón, Rafael Cadenas, Jonathan Jones, Verónica Ruiz, Juan M. Moreno and Bernat Soria

Resumen

El Banco de Recursos Biológicos (BRB) del Lince Ibérico Junta de Andalucía-Universidad Miguel Hernández fue creado como una herramienta de apoyo complementaria para la conservación de la especie. Los principales objetivos fueron: evitar la pérdida irreversible de biodiversidad que se produce con la muerte de cada individuo; disponer de una alta representación genética y biológica de la diversidad poblacional, y preservar biomateriales que representen una interfase entre las estrategias de conservación *in situ* y *ex situ*. Al objeto de proporcionar futuras oportunidades reproductoras mediante cualquier técnica posible, se procesaron y criopreservaron tanto células y tejidos germinales como células y tejidos somáticos, obtenidos de animales muertos y vivos. Para estudiar el mejor procedimiento de criopreservación de gametos y gónadas se consideró una especie filogenéticamente cercana no amenazada como modelo animal, el gato doméstico. Utilizando gónadas de gatos domésticos se han desarrollado diferentes estudios, extrapolando los mejores resultados al lince ibérico. Las células somáticas se consideraron para desarrollar estudios bio-sanitarios que pudieran ayudar a preservar la especie dentro de su hábitat, y también para proporcionar futuras oportunidades reproductoras mediante técnicas modernas de biotecnología como la transferencia nuclear, si se considera pertinente en un futuro y después de realizar la investigación necesaria. Con el objeto de intentar mejorar la eficiencia de la técnica de transferencia nuclear, nuestro grupo introdujo una aproximación pionera, la búsqueda de células madre, independientemente de su potencial de diferenciación: uni, multi o pluripotencial, ya que todos los tipos de células troncales tienen una mayor plasticidad que las células somáticas y esta característica puede facilitar la reprogramación nuclear que debe tener lugar. Muestras de sangre entera y derivados de ésta, pelos con raíz, orina y heces procedentes de numerosos individuos también fueron preservados. La correcta preservación de estas muestras es requerida para realizar, cuando sea necesario, estudios epidemiológicos para testar diferentes hipótesis etiológicas o, en general, para desarrollar cualquier estudio dirigido a mejorar la conservación de la especie. Los BRBs completos deberían ser seriamente considerados como un apoyo a las estrategias de conservación *in situ* y *ex situ* para especies en peligro de extinción. En este sentido, el BRB del Lince Ibérico podría ser de utilidad como modelo para el desarrollo de bancos similares para otras especies amenazadas.

Palabras clave
Reproducción, estrategias de conservación, especies amenazadas, lince ibérico, Banco de Recursos Biológicos

Abstract
The Iberian Lynx Biological Resource Bank (BRB), co-managed by the Environmental Council of Andalusia and the Miguel Hernández University, was created as a complementary supporting tool for endangered species conservation, including the Iberian lynx. The main goals of this BRB were: to avoid the irreversible biodiversity loss from each dead individual, to count with a high representation the population's diversity and to preserve biomaterials representing an interface between the *in situ* and *ex situ* conservation strategies. To provide future reproductive opportunities through any possible technique, we processed and cryopreserved germinal cells and tissues as well as somatic cells and tissues obtained from dead and live animals. In order to study the best gamete and gonad cryopreservation procedures we considered a non-threatened, phylogenetically related species as a model, the domestic cat. By using gonads from domestic cats we carried out different studies and extrapolated the best results for the Iberian lynx. Somatic cells were considered to allow for any bio-sanitary studies that can help preserve the species within their habitat, and also to provide future reproductive opportunities by means of modern biotechnology techniques such as nuclear transfer, if considered pertinent in the future and after proper research. In order to try to improve the efficiency of the nuclear transfer technique we introduced a pioneering approach, the search for stem cells, independently of their differentiation potential: uni, multi or pluripotential as all types of stem cells have greater plasticity than somatic cells and this feature can be useful for the nuclear re-programming that must take place. Samples of whole blood and its derivatives, pulled hairs, urine and feces from many individuals were also preserved. Proper storage of such samples is required to allow epidemiological studies to be performed when necessary for the testing of different hypotheses or, in general, to develop any study focused on improving lynx conservation. Complete BRBs should be seriously considered as a means of supporting *in situ* and *ex situ* conservation strategies for endangered species. In this sense, the present Iberian Lynx's BRB could be a useful model for the development of similar banks for other threatened species.

Keywords
Reproduction, conservation strategies, threatened species, Iberian lynx, Biological Resource Bank

An Iberian lynx Biological Resource Bank and its applications to the *in situ* and *ex situ* conservation of the species

Trinidad León-Quinto, Miguel A. Simón, Rafael Cadenas, Jonathan Jones,
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Introduction

The aim of wildlife conservation is to maintain or, if possible, increase biodiversity. The ideal approach to species conservation is by *in situ* strategies that pursue the preservation of animals in their natural habitats, which is always the main objective (e.g. Wildt et al., 1997; Pukazhenthi and Wildt, 2004). Nevertheless, in spite of efforts, sometimes the propagation of small populations is difficult and *ex situ* conservation becomes necessary. Within *ex situ* conservation, the most relevant strategy is captive breeding, but newer *ex situ* conservation strategies have been developed, such as biological resource banks (e.g. Wildt, 1997; Comizzoli et al., 2000), also termed genome or genetic resource banks. Such biological reserves are repositories of collected, processed and stored biological material and serve as an insurance policy in small populations. Collection and storage of blood and other biomaterials was already considered by Wildt (Wildt, 1992; Wildt et al., 1997). However, in spite of that, most of the biological resources banks are mainly or exclusively specialized in gametes, primarily containing semen samples (Harnal et al., 2002; Crosier et al., 2006).

With the aim of providing a supporting tool for conservation programmes, we created in 2002 a Biological Resource Bank for Spain's endangered wildlife, including mammals, fishes and many avian species (León-Quinto et al., 2005). This bank began as a reserve of cryopreserved somatic cells, and was later extended in the case of the most endangered species to also include tissues and somatic cells, gametes, gonads as well as other biological samples such as whole blood, serum, plasma, urine, etc.

In this work we will focus on the Iberian Lynx Biological Resource Bank generated by our group during the 2003-2006 periods, in collaboration with the Environmental Council of the Regional Government of Andalusia and with members of the Captive Breeding Programme (León-Quinto et al., 2008). The samples taken by us are divided into two categories: cells from which we can obtain, at least potentially, individuals, and biomaterials that allow studies to be performed that can help preserve the species within their habitat (see below), which includes cells/tissues as well as other biological samples.

Resource sampling: we have implemented protocols to collect and send samples from dead and living animals and provided sampling kits with appropriate media. The samples are taken from recent cadavers, or in the case of live animals, millimetre-sized biopsies are isolated. The samples, once isolated, are placed in specific media depending on the somatic or germ tissue and transported in refrigerated styro-foam containers. These specific media represent the first step towards aseptic conditions and its composition varies depending on whether it is a somatic tissue or gonad.

Cell bank: the cell reserve is mainly focused on obtaining new individuals. To this end, we consider all possible cell types, in which not only mature germ cells are taken into account but also immature germ cells and somatic cells.

Germ Cell Bank: since August 2005 one gonad isolated from each deceased animals is sent to our laboratory.

Experimental model: domestic cat

Since each germ sample of endangered wild animals is of great importance, we considered a non threatened phylogenetically related species as animal model to study the best suited procedure of gamete and gonad cryopreservation. We thus selected the domestic cat as felid model. By using gonads from domestic cats castrated at local veterinary clinics, we have carried out different studies and extrapolated the best results to the Iberian lynx.

For the cryopreservation of sperm cells obtained from cat epididymus, we tested two of the most utilized glycerol concentrations for feline sperm, 7% and 4% (e.g. Tsutsui et al., 2000; Zambelli et al., 2002; Pukazhenthi et al., 2006a), by using Tes-Tris buffer diluent and 20% egg yolk. The samples were evaluated for sperm viability, motility and acrosomal integrity after thawing. The best results were obtained in 4% glycerol. Further experiments are currently in course considering different cooling rates, other glycerol concentrations as well as using other additives.

Sperm cells and testes from Iberian lynx

Based on our results for sperm cells from cat epididymus, we used for the Iberian lynx a freezing medium that contained 4% glycerol as cryoprotectant, Tes-Tris buffer as diluent and 20% egg yolk. Sperm cells were also isolated from part of the testicular tissue (Devroey and Van Steirteghem, 2004; Comizzoli et al., 2006) and preserved in the same freezing medium. Each gonad was cryopreserved by using different media. The proper storage of the testicular architecture of each gonad can also be useful for their *in vivo* maturation when the xenografting technique is further developed, or for their *in vitro* maturation.

From August 2005 to the end of 2006 we received testes from seven males. We have successfully isolated living sperm cells in two cases, although in small amounts and with little or no movement. Such a modest rate of success could be explained by factors like the long post-mortem interval and the fact that most of the individuals were subadults or died outside the reproductive season. We nevertheless preserved them because special techniques can be used with immobile but mature sperm cells, such as the intra-cytoplasmic sperm injection (Comizzoli et al., 2006), whereas immature sperm cells can be latter matured *in vitro* or *in vivo* (Axner, 2006). In this sense, research is under way in our laboratory on *in vitro* and *in vivo* maturation of the frozen testicular tissue and testicular cell suspensions.

Oocytes and ovaries

In the case of ovaries, part of the gonads was processed to release the follicle population by slicing with a scalpel blade. We isolated and froze immature oocytes without undergoing maturation as several reports showed that mature oocytes are more sensitive to the freezing and thawing process (Comizzoli et al., 2004; Luvoni, 2006), mainly due to the delicate metaphase II spindle in mature oocytes. Furthermore, it seems that the rescue of female germ cells from ovaries of rare and endangered felid species is currently best performed by cryopreservation of isolated immature preantral follicles or ovarian slices (Jewgenow and Paris, 2006). We also used the cat model to test the cryopreservation procedure for oocytes and maturation after thawing.

Recently, a great interest in the storage of intragonadal gametes by ovarian or testicular tissue cryopreservation (Luvoni, 2006; Pukazhenthi et al., 2006b; Jewgenow and Paris, 2006) has emerged, despite that it is still considered strictly experimental. This represents an important strategy for preserving germ cells of young individuals that die before reaching sexual maturity or without offspring. However, there is a gap of species-specific knowledge in this respect and more studies are needed.

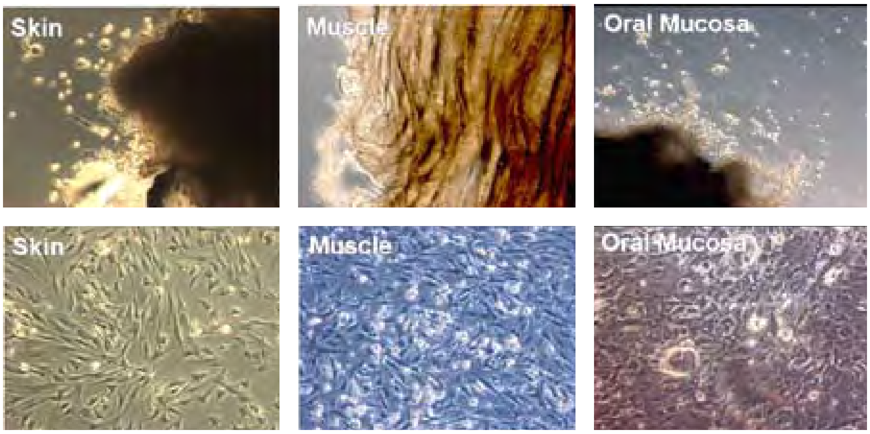

Figure 1. Cell populations isolated from Iberian lynx tissues. The top three images are tissue samples of skin, muscle and oral mucosa. The bottom images represent the cell cultures isolated from the tissues.

Figura 1. Poblaciones celulares aisladas de tejidos de lince ibérico. Las tres imágenes superiores corresponden a muestras de tejido de piel, músculo y mucosa oral. Las imágenes inferiores representan los cultivos celulares aislados de dichos tejidos.

Figure 2. Potential stem cells present in the cell cultures from Iberian lynx tissues.

Figura 2. Células madres potenciales presentes en los cultivos celulares de los tejidos de lince ibérico.

Somatic Cell Bank

The collection and processing of somatic tissues allows the preservation of a maximum of genetic biodiversity, from prematurely dead adults, cubs, juvenile individuals and even fetuses. Somatic cells are considered to allow for any bio-sanitary studies that can help preserve the species within their habitat, and also to provide future reproductive opportunities by means of modern biotechnology techniques such as nuclear transfer, if considered pertinent, in the future and after proper research.

The first cloned mammal was obtained only 10 years ago and, therefore, such a technique is still in a developing stage and presently has a low efficiency rate. In spite of this, 16 different mammalian species have been cloned (see Cibelli, 2007 for a review), including the domestic cat (Shin et al., 2002) and the African wildcat (Gómez et al., 2004). Fundamental research in the field is needed (Campbell et al., 2005; Cibelli, 2007) as well as new approaches to try to improve the effectiveness of this technique.

In any case, when future progress has been achieved in the field, we will have a somatic cell reserve that reflects the widest biodiversity possible. In order to try to improve the efficiency of the nuclear transfer technique we introduced a new approach. Many reports (e.g., Tuan et al., 2002; Wagers and Weissman, 2004; Yang et al., 2007) have recently shown the presence of stem cells in adult tissues. Stem cells undergo more replication cycles and have a greater plasticity than fully differentiated somatic cells (Soria et al., 2000; Berná et al., 2001; Tuan et al., 2002; León-Quinto et al., 2004; Wagers and Weissman, 2004; Yang et al., 2007). We search for stem cells, independently of their differentiation potential –uni, multi or pluripotential– as all types of stem cells have a greater plasticity than somatic cells and this feature can be useful for the nuclear re-programming that must take place. Moreover, stem cells can also be used in future therapeutic approaches when previous proper investigations have been made.

To this end, the tissues are broken down to cellular components (Figure 1) and placed in media and culture methods that favour the growth of stem cells, if they are present in the tissue fragment we receive. When colonies are detected along with indications of possible stem cells present (Figure 2) they are isolated and the purification phase is commenced, combining various media, methods and techniques. The search for stem cells is performed considering various protocols simultaneously. However, we receive small fractions of tissue from adult specimens, which are few centimetres in size, and thus do not necessarily

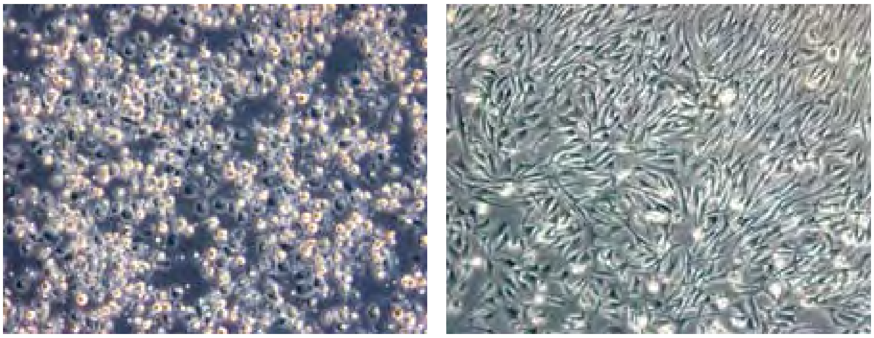

FIGURE 3. CELL CULTURES FROM IBERIAN LYNX TISSUES WITHOUT ANY SIGNS OF CONTAINING STEM CELLS.

FIGURA 3. CULTIVOS CELULARES DE TEJIDOS DE LINCE IBÉRICO SIN INDICIOS DE CONTENER CÉLULAS MADRE.

have to contain stem cells. In spite of this, it was possible to isolate and purify stem cells in various samples from different Iberian lynx individuals.

In general, the majority of samples do not present any signs of containing stem cells (Figure 3). In such cases, the cells are grown in the least amount of time possible, and cryopreserved for their use in any bio-sanitary study for different aspects of Iberian lynx conservation. Variations in the composition of the culture medium allows cells, fundamentally fibroblasts and miocytes, to appear in 2-4 days, obtaining millions of cells in a maximum of 21 days in culture and with 1-2 passages, with no significant difference observed among the different type of tissues.

Other cells and biological reserves

Our main objective is to create a biological reserve that supports global conservation, both *ex situ* and *in situ*. A reserve of cryopreserved cells opens up the possibility for studies in toxicology or phylogeny, among others, with the obtained results being useful to take decisions concerning animal management from which samples were taken, from either *in situ* or *ex situ* populations. Besides cells and somatic tissue, we have also considered necessary to contemplate the specific preservation of other biomaterials such as whole blood, urine, pulled hair and feces from which to obtain derivatives such as serum and plasma. Blood and urine are used in epidemiological studies (Hayes et al., 2002) and routinely taken to general sanitary check-ups. Samples of feces or hair are frequently used as noninvasive methods of genetic identification (Palomares et al., 2002; Pilot et al., 2007). Fecal samples are also used to identify gastrointestinal parasites (Rodríguez and Carbonell, 1998) or monitoring reproductive activities through the measurement of different metabolites (Comizzoli et al., 2000). When necessary, from the samples preserved, we will be able to analyze the presence of antibodies or levels of sexual hormones, biochemical analyses, lymphocyte populations, urianalyses, etc., that will help with retrospective studies. In this sense, we have recently started a new study concerning the intestinal parasites present in the Iberian lynx population from the fecal samples preserved in the bank.

Summary and conclusions

The Iberian Lynx Biological Resource Bank presented in this work is an important tool to avoid the irreversible loss of biodiversity from each dead individual and to count with a high representation the population's diversity. To provide future reproductive opportunities through all possible techniques, we globally processed and cryopreserved germinal cells and tissues obtained from dead animals, seven males and six females, and somatic cells and tissues from 69 different individuals, obtained from necropsies and biopsies, respectively. The somatic cell reserve reflects a very important fraction of the population's diversity which, in the future, will allow to extrapolate the results from specific studies to most of the population. Samples of whole blood and derivatives, hair, urine and feces were processed as well. Collection, processing and proper storage of such samples are needed in epidemiological studies in order to provide material for testing different hypotheses or, in general, to develop any bio-sanitary study to improve the Iberian lynx conservation efforts.

Acknowledgements

We would like to give thanks to the Environmental Council of the Andalusian Government and General Directorate for Natural Environment and Forest Policy of the National Ministry of the Natural, Rural and Marine Environment. Our recognition to Dr. Wildt and his collaborators for their guidance thanks to their pioneering work in germ cell banks in particular, and in biological resource banks in general. Special thanks to Astrid Vargas and Fernando Martínez, from the *Ex situ* Conservation Programme; to Guillermo López, Álvaro Muñoz and Isabel Molina from the Environmental Council of the Andalusian Government, and to Fernando Bornay, Raquel Pomares, Encarna Fuster and Nestor Vicente from Bioengineering Institute and Miguel Hernández University. Our work has been funded by Environmental Council of the Andalusian Government, Universidad Miguel Hernández, Diputación de Alicante and Bancaja.

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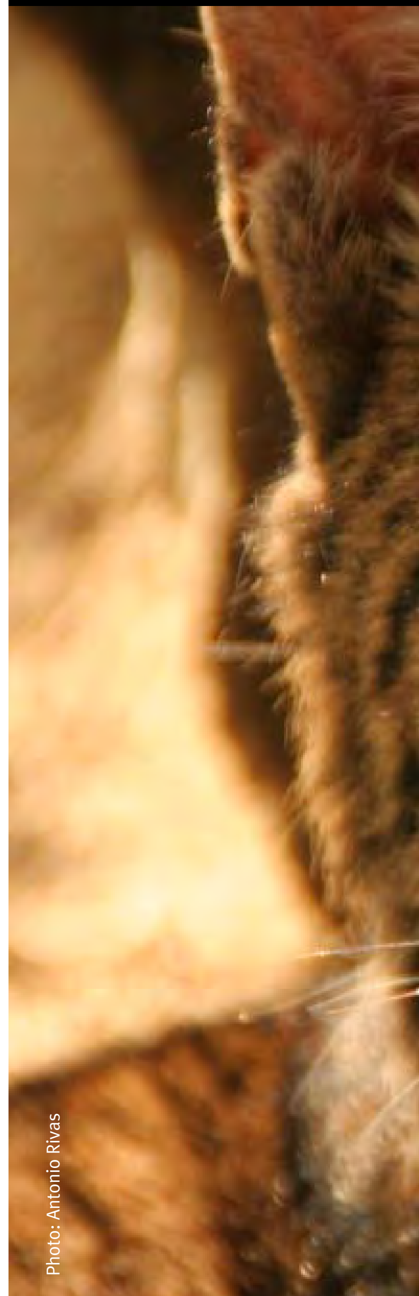

One day a wise, older man was walking along the seashore. As he looked down the beach, he saw a young man picking up something and very gently throwing it into the ocean. As he got closer, he called out, –Good morning! What are you doing? / The young man paused, looked up and replied. –Throwing starfish into the ocean. / I guess I should have asked, –Why are you throwing starfish into the ocean? / –The sun is up and the tide is going out. And if I don't throw them in they'll die. / –But young man, don't you realize that there are miles and miles of beach and starfish all along it. You can't possibly make a difference! The young man listened politely. Then he bent down, picked up another starfish and threw it into the sea, past the breaking waves. –It made a difference for that one!

Adapted from The Star Thrower, by
Loren Eiseley

Comparative endocrinology of domestic and non-domestic felids

Endocrinología comparada de felinos domésticos y silvestres

Janine L. Brown

Resumen

La capacidad de realizar un seguimiento de la actividad gonadal y suprarrenal a través de las hormonas es clave para optimizar la salud y la reproducción. A lo largo de décadas de estudio, se ha aprendido mucho sobre la biología de las gatas domésticas, incluyendo la función endocrina. Además, investigaciones comparativas recientes sobre endocrinología han ampliado considerablemente nuestros conocimientos sobre felinos silvestres. Esto ha sido posible en gran medida gracias al desarrollo de técnicas no invasivas de análisis de metabolitos de esteroides en heces, que constituye el método de elección para el seguimiento de la función endocrina en especies silvestres, incluyendo a los felinos. Se reconoce ampliamente que entre los felinos existen distintos patrones endocrinos, de los cuales muchos rasgos y mecanismos son poco comunes o incluso únicos. Existe una gran variabilidad en el tipo de ovulación (espontánea frente a inducida) en este taxón. Incluso en una misma especie, algunas hembras sólo presentan ovulación inducida, mientras que en otras también se da la ovulación espontánea. El metabolismo de esteroides también presenta diferencias: los metabolitos se excretan casi exclusivamente en las heces, con muy poca presencia de esteroides en la orina. Las distintas especies presentan grandes diferencias en cuanto a las influencias estacionales y sociales sobre la reproducción, las respuestas suprarrenales al manejo de la especie en cautividad y la respuesta ovárica a los procedimientos de reproducción asistida. Por consiguiente, el desarrollo de estrategias para la mejora de la salud y la reproducción de los felinos se debe realizar caso por caso. Este capítulo resume el estado actual del conocimiento sobre la endocrinología reproductiva de las hembras de felinos domésticos y silvestres, además de describir cómo la base de datos sobre endocrinología, en rápido crecimiento, está contribuyendo a los esfuerzos de gestión *ex situ*.

Palabras clave

Reproducción, esteroides gonadales, seguimiento no-invasivo, hormonas fecales

Abstract
The ability to track gonadal and adrenal activity via hormones is key to optimizing health and reproduction. Through decades of study, a great deal has been learned about the biology of female domestic cats, including endocrine function. More recently, comparative endocrine studies have greatly expanded our knowledge base of non-domestic felids as well. The latter has been possible largely through the development of non-invasive fecal steroid metabolite analysis techniques, which currently is the method of choice for monitoring endocrine function in wildlife species, including felids. It now is well-recognized that a range in endocrine patterns exists among Felidae, with many traits and mechanisms being uncommon, if not unique. There is a high degree of variability in the type of ovulation (spontaneous vs. induced) expressed across the taxon. Even within species, some individuals exhibit ovulation that is only induced, whereas others ovulate spontaneously as well. Steroid metabolism also differs in that metabolites are excreted almost exclusively in feces, with very little steroid found in urine. Across species there are marked differences in seasonal and social influences on reproduction, adrenal responses to husbandry practices, and ovarian responses to assisted reproductive procedures. This means that developing strategies to improve health and reproduction of felids must be done on a species by species basis. This presentation summarizes current knowledge on the reproductive endocrinology of female domestic and non-domestic cats, and describes how the rapidly growing endocrine database is aiding *ex situ* management efforts.

Keywords
Reproduction, gonadal steroids, non-invasive monitoring, fecal hormones

Photo: Alexander Sliwa

Comparative endocrinology of domestic and non-domestic felids

Janine L. Brown

Introduction

Practical methods for monitoring endocrine activity are essential for assessing the reproductive potential of individual animals and for developing and using assisted reproductive technologies when natural breeding fails. Hormones are the essence of reproduction; thus, understanding the factors that influence endocrine function is key to maximizing reproductive success. Systematic studies of felids began in the mid-1970s, focusing primarily on fundamental patterns of hormonal activity in the female domestic laboratory cat *(Felis catus)* (Goodrowe et al., 1989; Wildt et al., 1981; Wildt, this book). Blood samples collected over time and analyzed for pituitary gonadotropins and ovarian steroids provided a foundation for understanding the duration of the reproductive cycle, time of ovulation in relation to mating, and dynamics of hormone secretion during pregnancy. Early reports based on repeated anesthesia and blood sampling also described estrous cycle hormone patterns in a few wild felid species (Schmidt et al., 1979; Schmidt et al., 1988; Schmidt et al., 1993; Bonney et al., 1981; Seal et al., 1985; Schramm et al., 1994). Scientific papers began emerging in the 1980s demonstrating that steroid metabolites could be measured in urine and/or feces in an array of species (Lasley and Kirkpatrick, 1984). For most species, the decision to measure fecal or urinary hormones is determined by which material is easiest to collect, process and analyze. However, for some species, mode of excretion also is a consideration. In the case of felids, urinary analysis of reproductive steroids is not a viable option because steroids are excreted almost exclusively in feces (Shille et al., 1984; Shille et al., 1990; Brown et al., 1996a).

Non-invasive steroid monitoring

Injection of radiolabelled steroids (e.g., 14C, 3H) into domestic cats revealed that 85-95% of metabolites are excreted in feces within 1-2 days (Shille et al., 1990; Brown et al., 1996a; Graham and Brown, 1996). High performance liquid chromatography and gas chromatography/mass spectrometry determined that: 1) estradiol-17 β is voided in nearly equal amounts as unconjugated estradiol and non-enzyme-hydrolyzable estrogen conjugates (3-sulfate, 17 α -sulfate and 17 β -sulfate) (Shille et al., 1990); and 2) progesterone is metabolized and excreted as very polar metabolites, possibly non-enzyme-hydrolyzable conjugates (~75%), and unconjugated pregnanes (Brown et al., 1996a; Möstl et al., 1993). Cortisol metabolism differs somewhat in that some native steroid is excreted into urine (~14%). However, the majority of radioactivity is excreted into feces as polar metabolites, presumably non-hydrolyzable conjugates, with no measurable free cortisol or corticosterone present (Graham and Brown, 1996). Extracting steroid metabolites from feces generally involves boiling, vortexing or shaking samples in combinations of organic (e.g., ethanol, methanol) and aqueous solvents. Methods should strive for an extraction recovery of ≥80%. Because some metabolites are conjugated, the inclusion of 10% water can increase extraction efficiency significantly over organic solvents alone. Within sample variability can be reduced by drying feces and mixing the powder before extraction (Brown et al., 1996a); however, accurate results have been obtained using well-mixed wet samples (e.g., Moreira et al., 2001). Because estradiol-17 ß is excreted in its native form and as conjugates, both estradiol-specific and broad spectrum (e.g., total estrogens or estrogen conjugates) immunoassays are effective for monitoring follicular dynamics. By contrast, luteal activity is best defined using group-specific antisera that crossreact with excreted free and presumed conjugate pregnane metabolites.

Comparative reproductive profiles

The domestic cat has traditionally been described as an induced ovulator (Shille et al., 1979). The classic response consists of mating-induced neuronal stimulation of the medial basal hypothalamus, a reflex release of gonadotropin-releasing hormone (GnRH) and subsequent luteinizing hormone (LH) surge from the pituitary gland (Wildt et al., 1980; Banks and Stabenfeldt, 1982; Shille et al., 1983). Based on regular fluctuations in estrogens, unbred domestic cats have an estrous cycle length of ~2 weeks, with estrus lasting 3-7 days. On Day 3 of estrus, behavioral signs are strongest in association with peak estrogen secretion (Tsutsui and Stabenfeldt, 1993). Frequent matings are usually needed to stimulate sufficient LH to cause final follicular maturation and ovulation, which occurs 24-48 hours post-coitus depending upon the time of mating onset. The ovulatory LH surge occurs within minutes of copulation, peaks in a few hours and returns to baseline within 4-16 hours (Shille et al., 1983; Johnson and Gay, 1981).

Reproductive ovarian steroid cycle patterns now have been published for half of the 36 non-domestic felid species (tiger; cheetah; clouded leopard; snow leopard; leopard, *Panthera pardus*; Pallas' cat; fishing cat; margay; tigrina; ocelot; leopard cat; black-footed cat, *Felis nigripes*; *serval, Leptailurus serval*; caracal, Caracal caracal; bobcat, *Lynx rufus*; lion, *Panthera leo*; Iberian lynx, *Lynx pardinus*) (Brown et al., 1996a; Moreira et al., 2001; Moreland et al., 2002; Brown et al., 1995; Brown et al., 1996b; Shille et al., 1976; Czekala et al., 1994; Graham et al., 1995; Pelican et al., this book), with fecal steroid analyses being used in over three quarters of the studies (Brown et al., 1996a; Brown et al., 2002; Moreland et al., 2002; Brown et al., 1995; Brown et al., 1996b; Shille et al., 1976; Czekala et al., 1994; Graham et al., 1995). Surges of fecal estrogens distinguish estrous from interestrous periods, and in general females cycle at 2 to 4-week intervals with estrus lasting 3-10 days (Figure 1). One thing noted in our laboratory is that in some species (ocelot; lynx; jaguar, *Panthera onca*) fecal progestagens are increased during estrogen surges, correlating as high as r=0.4 (for example, Figure 1 ocelot). This is not observed in all species, however (e.g., Figure 1 tigrina, margay), and the functional significance of this covariance is unknown. The progestagens are probably of follicular origin, because concentrations are only a fraction of those observed after ovulation.

Another finding is the occurrence of variable periods of follicular inactivity not associated with season (e.g., cheetah, 20; ocelot, 30; fishing cat, Bauer, Moreland and Howard, unpubl). In each of these species, cycles have been observed in every month of the year, but for many individuals not continuously. In cheetahs, periods of alternating estrous cyclicity in some group-housed females suggested that enforced social living might be suppressing reproductive activity (Brown et al., 1996b). Cheetahs in the wild are solitary, yet many facilities house females in groups because intra-species aggression is relatively low. A study was conducted to evaluate behavior and fecal estrogen patterns in paired cheetahs and found that although serious altercations were rare, subtle agonistic behaviors often were observed (growl, hiss, chase, attack) (Wielebnowski et al., 2002b). Average fecal estrogen concentrations were lower in paired cheetahs, with subordinant females being more suppressed than dominants. Separation of the pairs resulted in a reinitiation of normal cyclic ovarian activity (Wielebnowski et al., 2002b). The one exception was a bonded pair where only affiliative behaviors were observed, and there was no suppression of follicular estrogenic activity in either female. These results suggest that estrous cyclicity may be inhibited in paired cheetahs even when aggressive interactions are relatively minor, and that social compatibility is important for maximizing reproductive success. Results of a study on ocelots (Moreira et al., 2001; Figure 1) differed from that of cheetahs in that periods of acyclicity were observed in females housed alone and, thus, were not due to social suppression (Moreira et al., 2001). Rather, other management or husbandry conditions might be contributing factors. Understanding the cause(s) of ovarian inactivity is important for

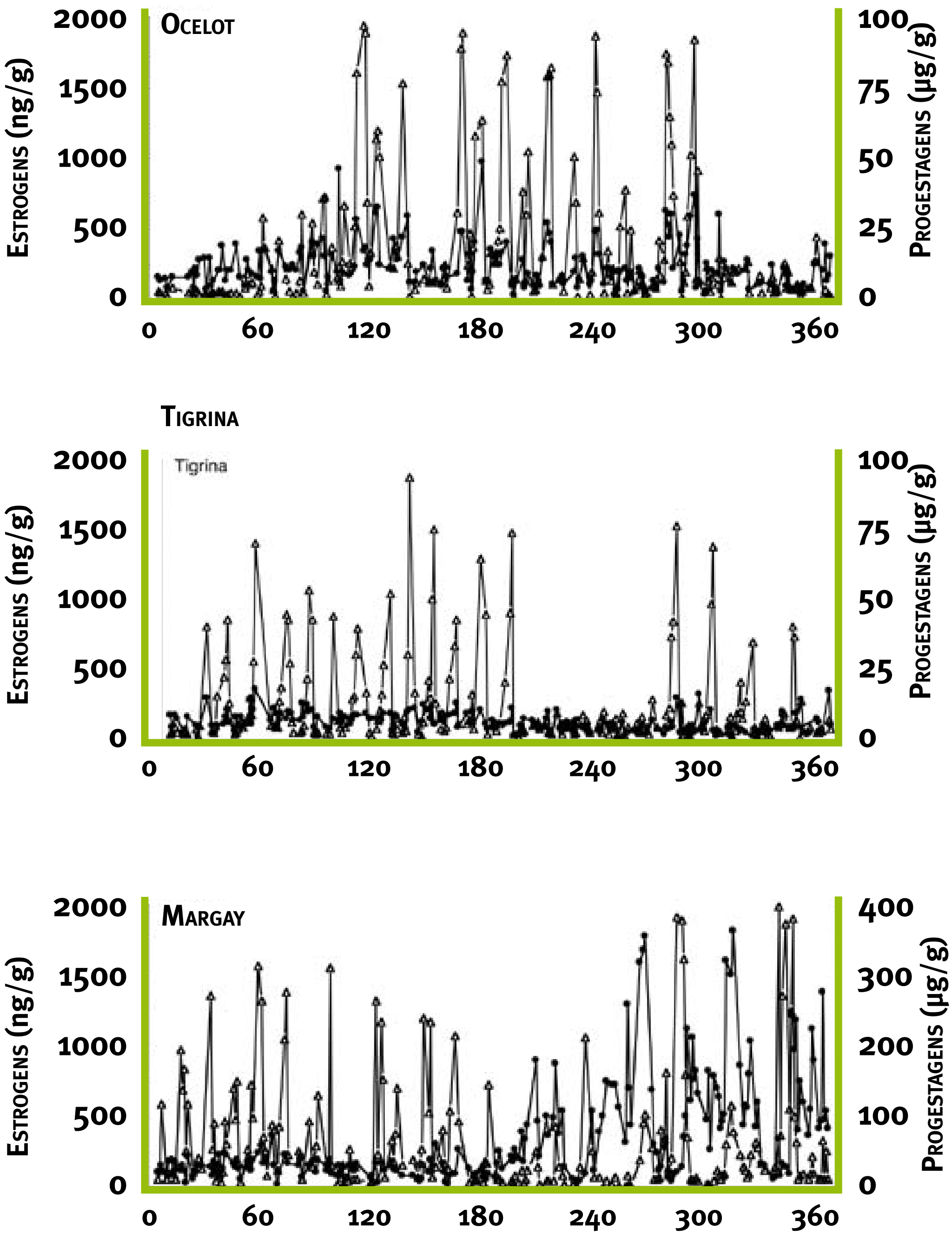

Figure 1. Longitudinal profiles of fecal estrogens (open triangle) and progestagens (closed circle) in a singly-housed female ocelot, tigrina and margay. Inexplicable periods of acyclicity, unrelated to season, were evident in the ocelot at the beginning and end of the sampling period, and in the tigrina during the middle of the sampling period. The margay exhibited periods of regular estrous cyclicity for the first half of the sampling period, followed by several, non-mating-induced spontaneous ovulations during the latter half (adapted from Moreira et al., 2002).

Figura 1. Perfiles longitudinales de estrógenos fecales (triángulo abierto) y de progestágenos (círculo cerrado) de hembras de ocelote, tigrina y margay, alojadas individualmente. Se observaron períodos de aciclicidad inexplicables, no relacionados con la estacionalidad, al principio y al final del período de muestreo en el caso del ocelote, y a mitad del período de muestreo en el caso de la tigrina. La hembra de margay mostró períodos de ciclicidad estral regular durante la primera mitad del período de muestreo, seguida de varias ovulaciones espontáneas –no inducidas por la reproducción– durante la segunda mitad del muestreo (adaptado de Moreira et al., 2002).

Photo: Alexander Sliwa

Photo: Alexander Sliwa

Serval (*Leptailurus serval*).

Black-footed cat
Gato de pies negros
(Felis nigripes).

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Photo: Alexander Sliwa

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Caracal (*Caracal caracal*) .

Tiger/ Tigre
(Panthera tigris).

Ocelote
(Leopardus pardalis).

Bobcat/Lince rojo
(Lynx rufus).

determining why so many felid species reproduce poorly in captivity despite intensive breeding efforts. With this information, it should be possible to identify mitigating solutions, and fecal steroid monitoring will be key to assessing efficacy.

Hormonal changes throughout the post-mating diestrus period in the domestic cat have been well characterized. Starting 1-2 days after ovulation (2-3 days after mating), progesterone secretion from corpora lutea increases and remains elevated for 64-67 days in pregnant cats, and approximately half that (36-38 days) in non-pregnant females (Tsutsui and Stabenfeldt, 1993; Paape et al., 1975; Wildt et al., 1981; Schmidt et al., 1983). Luteal LH receptor and progesterone content increases after 100 hours in parallel with the rise in circulating progesterone (Roth et al., 1995). After ovulation, serum estradiol-17ß periodically fluctuates above basal levels, indicating steroidogenic activity occurs even during gestation. Estrogens increase significantly and steadily during the latter half of gestation, with a distinct surge occurring about a week before parturition (Verhage et al., 1976). Peripheral prolactin concentrations increase about Day 35 and again just before parturition (Banks et al., 1983). Gestational prolactin is luteotropic because administering the dopamine agonist, cabergoline, after mid-gestation reduces progesterone to baseline and causes fetal absorption or abortion (Tsutsui and Stabenfeldt, 1993; Jochle and Jochle, 1993). Circulating relaxin from the fetoplacental unit increases about Day 20 and continues to rise throughout gestation before declining around the time of parturition (Stewart and Stabenfeldt, 1985). Relaxin helps soften pelvic connective tissue to facilitate parturition (Tsutsui and Stabenfeldt, 1993). Circulating prostaglandin $F2\alpha$, produced by the fetoplacental unit and endometrium increases at about Day 30, plateaus around Day 45 and spikes at the end of gestation to facilitate parturition (Tsutsui and Stabenfeldt, 1993). This hormone has a direct luteolytic effect and can induce abortion and fetal expulsion if administered after Day 30 of gestation (Verstegen et al., 1993). Diagnosing pregnancy based on progesterone analysis during the first half of gestation is not possible because luteal phase profiles are indistinguishable. However, because the non-pregnant luteal phase is shorter, it is technically possible to diagnose pregnancy if progesterone is still elevated after ~40 days post-mating. Relaxin and prolactin measured in serum or plasma also are dignostic of pregnancy after mid-gestation (Jewgenow et al., this book) because neither are secreted during non-pregnant luteal phases.

In non-domestic felids, progestagen concentrations during pregnant and non-pregnant luteal phases are quantitatively similar, just as in the domestic cat. In felids where comparative serum (lion, Schmidt et al., 1979; Briggs et al., 1990; puma, Bonney et al., 1981; snow leopard, Schmidt et al., 1993) or fecal (cheetah, clouded leopard, Pallas' cat, Brown et al., 1995, 1996a, b, 2002; tiger, Schmidt et al., 1993; and fishing cat, Bauer, Moreland and Howard, unpublished) data are available, length of the non-pregnant luteal phase is about one third to one half that of pregnancy. There is a species difference in estrogen excretory patterns throughout gestation. Estrogens increase after midgestation in the cheetah (Brown et al., 1996b), Pallas' cat (Brown et al., 2002) and fishing cat (Bauer and Howard, unpublished) similar to the domestic cat, but remain stable in the clouded leopard (Brown et al., 1995) and tiger (Graham and Brown, unpublished). It is technically possible to diagnose pregnancy in non-domestic felids based on fecal progestagens that remain elevated past the normal length of a non-pregnant luteal phase. The difficulty in collecting longitudinal blood samples has precluded determining if prolactin or relaxin would be reliable diagnostic tests for pregnancy in non-domestic cats.

The domestic cat has typically been characterized as a reflex ovulator; however, spontaneous ovulations are known to occur in laboratory-maintained females, often frequently, depending upon the individual, housing conditions, proximity to a male and possibly genetics (Concannon, 1991; Lawler et al., 1993; Gudermuth et al., 1997; Graham et al., 2000). Most wild felids mate frequently during estrus; thus, it has been assumed they too are induced ovulators. Indeed, spontaneous increases in progestagens after estrogen surges are non-existent or rare in the tiger (Seal et al., 1985), puma (Bonney et al., 1981), snow leopard (Schmidt et al., 1993), cheetah (Brown et al., 1996b, Czekala et al., 1994), tigrina and ocelot (Moreira et al., 2001). However, many felids are now known to exhibit spontaneous ovulations, not unlike those observed in some domestic cats. So far, spontaneous ovulations have been documented in the lion (Schmidt et al., 1979; Schramm et al., 1994), clouded leopard (Brown et al., 1995), leopard (Schmidt et al., 1988), Pallas' cat (Brown et al., 2002), fishing cat (Moreland et al., 2002) and margay (Moreira et al., 2001). In some species, non-mating induced increases in progestagens were more prevalent when females were housed together (lion, Schmidt et al., 1979; Schramm et al., 1994; leopard, Schmidt et al., 1988), whereas in others spontaneous ovulations occurred in singletons as well (clouded leopard, Brown et al., 1995; margay, Moreira et al., 2001; fishing cat, Moreland et al., 2002). Thus, within this taxon ovulatory mechanisms vary, regulated to a greater or lesser degree by species –and even individual– specific responses to as yet unidentified stimuli of a physical and/or psychosocial nature.

The domestic cat is seasonally polyestrus in naturally fluctuating photoperiods, but will cycle year round when maintained in 12-14 h of artificial light per day (Shille et al., 1979; Wildt et al., 1998). Melatonin appears to be the signal by which the female domestic cat measures photoperiod, with ovarian activity ceasing under decreasing photoperiod and resuming with increasing photoperiod (i.e., a long-day breeder). Melatonin secretion is controlled by the prevailing photoperiod (as in other mammals) with concentrations being highest during the dark phase (Leyva et al., 1984; Leyva and Stabenfeldt, 1989a, b). Exogenous melatonin administered intravenously or orally can suppress ovarian activity in domestic cats maintained under a variety of lighting regimens (Leyva and Stabenfeldt, 1989a, b; Graham et al., 2004). Females appear to exhibit a graded response to melatonin, with follicular suppression occuring more rapidly as the daily duration of melatonin elevation is increased (Leyva et al., 1989a; Graham et al., 2004).

Reproduction is at least somewhat seasonal in some non-domestic felids like the tiger (Seal et al., 1985), clouded leopard (Brown et al., 1995), Pallas' cat (Brown et al., 2002) and snow leopard (Schmidt et al., 1993). Conversely, follicular activity in captive lions (Schmidt et al., 1979), leopards (Schmidt et al., 1988), pumas (Bonney et al., 1981), margays, ocelots and margays (Moreira et al., 2001) and fishing cats (Moreland et al., 2002) is not influenced by season. Like the domestic cat, photoperiod appears to control seasonal reproduction in non-domestic felids. For example, clouded leopards housed indoors with continuous exposure to 12 h of artificial light per day will cycle year round (Brown et al., 1995). Pallas' cats are highly seasonal and females exhibit ovarian activity for only ~3 months of the year (Jan-Mar) (Brown et al., 2002). However, sudden transitions to "long days" can stimulate premature follicular steroidogenesis. In one example, ovarian activity was stimulated in a Pallas' cat female when she was moved to an outdoor exhibit and then again during a month-long event in November where zoo lights were left on for an additional 5 hours to facilitate night viewing by the public ("Festival of Lights") (Brown et al., 2002). When the normal seasonal increase in photoperiod occurred, the female responded with an increase in fecal estrogen excretion, but no breeding occurred. Only after she was moved away from the festival lights the following year did conception occur (Brown et al., 2002).

One of the most important uses of hormone monitoring is to assess ovarian responses to ovulation induction and artificial insemination (AI) protocols. The gonadotropins eCG and hCG are typically used to stimulate follicular development and induce ovulation, respectively. Unfortunately, the success rate of AI remains inconsistent for many felid species (Pelican et al., 2006; Swanson, 2006). Recent studies suggest that standard chorionic gonadotropin regimens induce ovarian hyperstimulation, resulting in estrogen concentrations that are several-fold higher than those observed during natural estrus (clouded leopard, Brown et al., 1995; tiger, Graham et al., 1996; domestic cat, Graham et al., 2000; Swanson et al., 1997). The etiology of excessive estrogen production after gonadotropin treatment appears to be due, in part, to the development of ancillary ovarian follicles (Pelican et al., 2006). Hyper-estrogenism creates an abnormal endocrine environment that is detrimental to fertilization, embryogenesis and implantation. One exception is the cheetah, where fecal estrogen concentrations after gonadotropin ovulation induction are not different from those associated with natural estrus (Brown et al., 1996b). It is in this species that pregnancy success after AI is among the best for felids (Howard et al., 1992).

In the domestic cat, pregnancy rates are highest when gonadotropins are administered during the interestrus period, when ovarian activity is minimal (Goodrowe and Wildt, 1987; Goodrowe et al., 1988; Donoghue et al., 1992). One problem for non-domestic felids is that most females rarely exhibit clear signs of behavioral estrus, so ovulation induction and AI are generally scheduled randomly with respect to ovarian cycle stage. The relatively high AI success rate in the cheetah is probably due to the frequently quiescent state of the ovaries. This inactivity could allow the ovary to be more consistently responsive to eCG/hCG because there are few active follicles and no luteal tissue producing endogenous steroids to disrupt exogenous gonadotropin action. Studies are now underway to identify approaches to temporarily shut-down ovarian activity before ovulation induction, which hopefully will result in more normal responses without hyperstimulation (Pelican et al., 2006; Swanson, 2006).

In recent years there has been a growing demand to develop methods to assess stress in zoo animals, and

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Snow leopard/leopardo de las nieves *(Panthera uncia).* **Lion/León *(Panthera leo).***

Leopard/Leopardo *(Panthera pardus).* **Cheetah/Guepardo *(Acinonyx jubatus).***

determine how captive conditions affect reproduction and health. The difficulty is determining what constitutes "stress" because not all stressors have negative impacts. It is only a concern when stress causes deleterious effects (Moberg, 1987; Moberg and Mench, 2000). Indeed a lack of stimuli can lead to boredom, with equally detrimental consequences (Vanrooijen, 1991). For felids, failure to reproduce has long been attributed to stress ensuing from suboptimal housing and husbandry (Mellen, 1991; Lindburg and Fitch-Snyder, 1994; Manteca, this book; Martos, this book). However, objective experimental proof has been lacking. Analyses of circulating or excreted corticosteroids can provide a physiological indicator of adrenal activity and overall levels of stress. For example, urinary cortisol was increased in domestic cats exposed to a stressful caretaking routine (Carlstead et al., 1993) and in domestic cats and leopard cats after translocation (Carlstead et al., 1992, 1993). Conversely, elevated urinary cortisol concentrations in leopard cats were reduced after barren cages were enriched with branches and hiding places (Carlstead et al., 1993). However, because urine collection is difficult in felids and most corticoids are excreted in feces, fecal metabolite assays were developed as a more practical approach to assessing adrenal activity (Graham and Brown, 1996; Schatz and Palme, 2001). Appropriate assays need to be broad spectrum to crossreact with the variety of corticoid metabolites found in cat feces. The most common method used today is a commercially available corticosterone RIA (ICN Biomedicals, Costa Mesa, CA) (Graham and Brown, 1996; Terio et al., 1999; Wasser et al., 2000; Wielebnowski et al., 2002a). However, a cortisol EIA developed by Coralie Munro (No. R4866; University of California, Davis) also has proven effective in measuring fecal corticoids in other carnivore species, like the black-footed ferret (Young et al., 2001) and red wolf (Walker, 1999). Recently, a study was conducted to compare the cortisol EIA with the corticosterone RIA for monitoring adrenocortical activity in a number of carnivore species, including felids (domestic cat, cheetah and clouded leopard) (Young et al., 2004). Based on HPLC analyses, metabolic forms differed between suborders, but among felid species the elution patterns were similar, suggesting that like ovarian steroids (Brown et al., 1996a), glucocorticoid metabolism is conserved within the taxon. However, the two assays detected different glucocorticoid metabolites within each species. Despite that, longitudinal profiles were qualitatively similar and data were highly correlated, suggesting both systems were equally effective in monitoring adrenal activity. Another assay used in felids is an EIA that measures 11,17-DOA (group of cortisol metabolites formed by side-chain cleavage) (Schatz and Palme, 2001). Thus, a number of options exist for monitoring stress status via adrenal steriods in felids.

Studies now are in progress to use fecal corticoid analyses in combination with evaluations of behavior and physiology to provide more meaningful indicators of stress. Fecal corticoids have been shown to be transiently increased following anesthesia (domestic cat, Smith et al., 1999; cheetah, Terio et al., 1999), translocation and introduction to a male (cheetah, Terio et al., 1999). Cheetahs identified by keepers as being more "nervous" also had higher fecal corticoid concentrations than "calm" individuals (Terio et al., 1999; Jurke et al., 1997). In clouded leopards, comparisons across temperament categories indicated that "nervous" animals had higher corticoid concentrations than "calm" individuals (Wielebnowski et al., 1999). In another clouded leopard study, cats housed in enclosures with more vertical space or off exhibit had lower fecal corticoid levels, whereas higher corticoids were found in cats housed in close proximity to other large predators and in those displaying self-mutilating behaviors (Wielebnowski et al., 2002a). In a study of Brazilian small cats, females were subjected to three enclosure conditions over successive time periods: Phase I - large, enriched enclosures for three months; Phase II - small, empty enclosures for six months; Phase III - the same small enclosures enriched with branches and nest boxes for six months (Moreira et al., 2007). Margay and tigrina females exhibited distinct elevations in corticoid concentrations after transfer from large enriched enclosures to smaller barren cages that corresponded with agitated behavior, especially soon after transfer. Fecal corticoid concentrations were then reduced following cage enrichment in tigrinas, but not in margays indicating a species difference in response to enrichment approaches.

Research priorities

It is clear that felids express slight to marked variations in reproductive mechanisms, and that a better understanding of their fundamental reproductive physiology could facilitate breeding, management and conservation activities. What we lack for many Felidae species are clearly defined normative data, ranging from the onset of puberty through to reproductive senescence. Identifying the type of ovulation (induced versus spontaneous) and effect of seasonality on reproduction for each species is important because these two characteristics impact both natural and assisted breeding efforts. The ability to easily and safely assess reproductive status, especially through non-invasive means, will allow identifying reproductive problems and developing mediating solutions. One of the most useful benefits of steroid metabolite monitoring will be assessing causes of poor fertility in response to assisted reproductive procedures, eventually allowing these tools to more reliably contribute to species propagation. A high priority is developing ovulation induction protocols that result in consistent responses, without ovarian hyperstimulation, to provide an optimal maternal environment for fertilization and embryo development. Along with this effort is the need to control the reproductive cycle, including down-regulating endogenous ovarian activity and synchronizing estrus (Pelican et al., 2006; Swanson, 2006). We also need a quick and reliable single sample test for diagnosing pregnancy, preferably one that is non-invasive. Particularly important will be systematic assessments of zoo habitats and determining how they impact animal health. Long-term evaluations of adrenal activity in the context of exhibits and management strategies should allow identifying the optimal captive environment compatible with welfare needs and maximal reproductive potential. Ultimately, by utilizing a variety of endocrine techniques in conjunction with physiological and behavioral assessments, zoo managers will be in a better position to determine which environmental factors are harmful, benign or stimulating to individual animals. If we are to continue maintaining wild cats *ex situ*, we no longer can ignore our obligation to assess the adequacy of environmental and husbandry conditions for optimal behavior, health, reproduction and wellbeing.

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There are two ways to live: you can live as if nothing is a miracle; you can live as if everything is a miracle.

Albert Einstein
(1879-1955)

Unusual gonadal hormone profiles in the Iberian lynx as determined by fecal monitoring

Perfiles poco habituales de hormonas gonadales en el lince ibérico según análisis de muestras fecales

Katharine M. Pelican, Teresa Abaigar, Astrid Vargas, José Manuel Rodríguez, Juana Bergara, Javier López, Anastasio Vázquez, Juan Matías Chaparro, Janine Brown and David E. Wildt

Resumen

El lince ibérico *(Lynx pardinus)* es una especie en peligro crítico que se reproduce de forma estacional en el medio silvestre, y cuyos cachorros nacen en primavera. Se dispone de muy poca información sobre la función endocrina en esta especie. El análisis de hormonas en heces ha sido adaptado con éxito en nueve especies de felinos silvestres para caracterizar su función endocrina normal y se esperaba que esta técnica se pudiera adaptar con facilidad al lince ibérico. Para ello, se trabajó con animales procedentes del centro de cría de El Acebuche, en el Parque Nacional de Doñana (España), y se analizaron muestras fecales obtenidas a diario de hembras adultas (n=4) y machos adultos (n=4) entre abril de 2004 y junio de 2006, utilizando inmunoensayos enzimáticos validados para el lince ibérico.

Todas las hembras mostraron pronunciados cambios estacionales en los niveles de estrógenos, con concentraciones superiores a los valores de referencia a partir del mes de enero y disminuciones hasta los niveles más bajos anuales entre los meses de mayo y agosto. Las hembras también mostraron un aumento en las concentraciones de estrógenos antes o durante las cópulas en seis de siete eventos reproductivos. En cambio, se observó que las fluctuaciones en las concentraciones de los metabolitos de los progestágenos no se correspondían con la temporada de cría, sino que disminuían ligeramente entre octubre y diciembre para aumentar de nuevo en enero. No se observaron diferencias en los patrones de estrógenos o progestágenos entre hembras de lince preñadas y hembras que habían copulado sin quedar preñadas. En los machos, se observó una estacionalidad moderada, con las mayores concentraciones de andrógenos en heces entre diciembre y junio, aunque los niveles eran lo suficientemente elevados en todos los meses para respaldar la posibilidad de producción de semen a lo largo de todo el año. Los resultados confirman que la estacionalidad reproductora en la hembra de lince ibérico se puede demostrar mediante la observación de cambios en la excreción de metabolitos de los estrógenos en las heces. Los machos sólo muestran una leve estacionalidad en las hormonas gonadales, lo cual parece concordar con su capacidad de engendrar progenie a lo largo de todo el año. A diferencia de los análisis de estrógenos, los progestágenos en heces no son buenos indicadores del estado reproductivo en el lince ibérico, dado que los metabolitos 1) mantienen concentraciones altas durante más de nueve meses al año, y 2) no muestran un aumento claro durante la gestación. Por lo tanto, el análisis de hormonas en heces es menos informativo en el lince ibérico que en otros felinos ya estudiados.

Palabras clave
Hormonas fecales, estacionalidad, cría en cautividad, felino, reproducción

Abstract
The critically endangered Iberian lynx *(Lynx pardinus)* is a seasonal breeder in the wild, with cubs born in the spring. There is minimal information available on endocrine function in this species. Fecal hormone monitoring previously has been adapted successfully to nine wild felid species to characterize normative endocrine function. Our expectation was that this technique could be easily adapted to the Iberian lynx. The source of study animals was the El Acebuche breeding population within the Doñana National Park, Spain. Daily fecal samples collected from April 2004 through June 2006 from adult females (n=4) and males (n=4) were analyzed using enzyme-immunoassays validated for the Iberian lynx. All females showed marked seasonal changes in estrogen metabolites with concentrations increasing above baseline in January and declining to nadir from May through August. Females also exhibited increased estrogen concentrations before or during copulation in six of seven breeding events. In contrast, fluctuations in progestogen metabolite concentrations did not correspond to the breeding season, but rather decreased slightly from October through December before increasing again in January. There was no difference in either estrogen or progestogen patterns between pregnant lynx and females that copulated but failed to conceive. Males showed modest seasonality with the highest fecal androgen concentrations measured from December through June, although levels were sufficiently high in all months to support the possibility of year-long sperm production. Results confirm that reproductive seasonality in the female Iberian lynx can be affirmed by changes in fecal estrogen metabolite excretion. Males show only mild gonadal hormone seasonality, which appears consistent with the ability to produce offspring throughout the year. Contrary to estrogen analyses, fecal progestogen is a poor indicator of reproductive status in the Iberian lynx as metabolites 1) are sustained at high concentrations for more than nine months of the year and 2) fail to show clear elevations during pregnancy. Thus, fecal hormone monitoring is less informative in the Iberian lynx than in previously studied felids.

Keywords
Fecal hormone, seasonality, captive breeding, felid, reproduction

Unusual gonadal hormone profiles in the Iberian lynx as determined by fecal monitoring

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Introduction

The Iberian lynx is the world's rarest felid species, being listed as critically endangered by the IUCN-World Conservation Union (IUCN, 2008). As a component of current recovery efforts, an *ex situ* management programme was established to develop a reservoir population to produce animals for eventual reintroduction. However, these animals also are invaluable for developing a base of biological information for this rare species, including in the area of reproductive physiology and endocrinology.

Detailed data on the reproductive biology of the Iberian lynx is lacking. The species is well known to be a seasonal breeder that generally produces one to three cubs from March through June after a gestation of ~60 Days (Palomares et al., 2005). A powerful tool for generating knowledge about the endocrinology of reproduction is monitoring hormonal metabolites in voided feces. This technology has revolutionized our understanding of the similarities and differences in reproductive mechanisms within the diverse species of the family Felidae (Brown, 2006; Brown et al., 2001). To date, fecal hormone monitoring techniques have been validated and proven effective for assessing reproduction and stress status in diverse felid species, ranging from the Pallas' cat (Brown et al., 2005) to the tiger (Graham et al., 2006) (see review, Brown, 2006; Brown, this book). Especially important has been understanding the fundamental reproductive biology of species to allow enhanced natural breeding management or the development of artificial insemination or *in vitro* fertilization/embryo transfer (Brown et al., 1995; Pelican et al., 2008; Swanson et al., 1996). This monitoring approach also has been applied successfully to certain free-ranging wild carnivores, including for studying how social dominance and dynamics alter stress response and survival in Kalahari meerkats (Young et al., 2007) and determining stress hormone fluctuations associated with radiocollaring African wild dogs (Creel et al., 1997).

Thus, a logical step in the recovery of the Iberian lynx was to apply this previously successful hormonal tracking technology to understand the dynamics of seasonality in both sexes and the specifics of the female's reproductive cycle and pregnant luteal phase. Our findings also complimented a parallel investigation by colleagues who compared endocrine profiles in the male Iberian lynx to those of the closely related Eurasian lynx *(Lynx lynx)* (Dehnhard et al., 2008). In that study, there was a tendency for fecal testosterone concentrations to be slightly elevated in both species during the months of March and April, which coincided with the presumed breeding season and the peak in sperm production in the Eurasian lynx (Jewgenow et al., 2006). Given these preliminary findings, and because our laboratory had previously used non-invasive hormonal monitoring successfully in eight other felid species (Brown et al., 1994; 1995; 1996a; 1996b; 2002; Brown and Wildt, 1997; Moreira et al., 2001; Swanson et al., 1996), we proceeded to study gonadal hormone profiles in Iberian lynx at the El Acebuche Center. The goal was to document seasonal hormone patterns, identify normative estrous cycle patterns and determine if there were differences in excretion patterns between pregnant and copulating, but non-pregnant females.

Materials and methods

Animals and fecal sampling

Fresh fecal samples were collected from the ground of individual enclosures of four adult females (three to 14 years at study onset) and four adult males (1.5 to three years) from April 2004 through June 2006. Each sample was placed in a labeled plastic bag and stored at -20 °C until analysis. All animals were wild-caught, six from the Sierra Morena Mountains and two from Doñana National Park. Diets were comprised of live rabbit (85%), quail, ungulate meat and dead rabbit. Male and female lynxes were kept in separate, but adjacent enclosures until breeding season onset. Breeding pairs were established taking into account genetic and behavioral factors (Vargas et al., this book; Fernández et al., this book). Once the selected pairs were proven to be compatible (Figure 1) they were allowed to remain together for as long as possible after copulation, but never longer than two weeks prior to the expected day of parturition. Husbandry procedures during the breeding season at El Acebuche Breeding Center are described in Vargas et al. (2005).

Fecal processing and steroid extraction

Whole fecal samples (2004-2005) or steroid hormone extracts (2005-2006) were shipped frozen from Spain to the Conservation & Research Center of the Smithsonian's National Zoological Park for endocrine evaluation. Samples in 2004-2005 were processed so that hormonal metabolites were extracted from lyophilized feces, whereas wet feces were used in 2005-2006. Steroids were extracted by following a standardized protocol that has been highly effective in previous felids studies (Brown et al., 1994). In brief, each sample was mixed thoroughly and 0.18 to 0.22 g of fecal material vortexed in 5 ml of an ethanol: water (90:10) solution for 30 min. After centrifugation (500 x g, 20 min), the supernatant was recovered and the pellet resuspended in 5 ml of

Photo: Antonio Rivas

Figure 1. One of the Iberian lynx breeding pairs featured in this study.

Figura 1. Una de las parejas reproductoras objeto del presente estudio.

90% ethanol, vortexed and re-centrifuged. Combined supernatants then were dried completely under air and re-dissolved in 1 ml methanol. Each extractant was vortexed for 1 min and sonicated for 20 min. The extract was diluted in dilution buffer and stored frozen until analyzed by enzyme immunoassay (EIA) to quantify estrogen and progesterone (female) or testosterone (male) metabolites.

Specifics for hormonal determination followed the EIA procedures of Munro et al. (1991) and previous detailed studies of felids from our laboratory. Antibodies for progesterone (monoclonal progesterone antibody CL425, 1:10,000 dilution), estrogen (polyclonal estradiol antibody R4972, 1:10,000 dilution) and testosterone (polyclonal testosterone antibody R-156/7, 1:7,500 dilutions) were provided by Coralie Munro (University of California, Davis, CA, USA). The CL425 cross-reacted with various progesterone metabolites, including 4-pregnen-3,20-dione (100%), 4-pregnen-3 α -ol-20-one (188%), 4-pregnen-3 β -ol-20-one (172%), 4-pregnen-11 α -ol-3,20-dione (147%), 5 α -pregnan-3 β -ol-20-one (94%), 5 α -pregnan-3 β ,20-dione (64%), 5 α -pregnan-3,20-dione (55%), 5 β -pregnan-3 β -ol-20-one (12.5%), 5-pregnan-3,20-dione (8.0%), 4-pregnen-11 β -ol-3,20- dione (2.7%) and 5 β -pregnan-3 α -ol-20-one (2.5%) (Graham et al., 2001). The R4972 cross-reacted with estradiol 17 β (100%) and estrone (3.3%). The R-156/7 cross-reacted with testosterone (100%) and 5 α -dihydrotestosterone (57.4%). Before analysis, fecal extracts were diluted in dilution buffer (1:10 to 1:1,400 for estrogens, 1:100 to 1:48,000 for progesterone and 1:20 for testosterone). Each enzyme-immunoassay was validated for Iberian lynx by demonstrating: 1) parallelism ($P<0.05$) between binding inhibition curves of serial dilutions of pooled fecal extracts and the appropriate steroid standard (Fig. 2); 2) accuracy of enzyme-immunoassays for fecal steroids, and 3) significant recovery of exogenous steroid added to fecal samples. Mean ± standard error of the mean extraction efficiency was 83.1 ± 0.004% as determined by recovery of ^{3}H-estradiol and ^{14}C-progesterone, or ^{3}H-testosterone added to feces before extraction. There was no difference ($P>0.05$) between extraction efficiency in wet versus dry extraction methods. Inter-assay and intra-assay variation (CV) was ≤15% and 10%, respectively. Absorbance was measured at 405 nm with an automatic microtiter plate spectrophotometer. Hormone concentrations were expressed as ng/g feces. Endocrine patterns were compared to reproductive behaviors, including onset of estrus and mating in females and subsequent parturition in pregnant individuals.

Data analysis

To determine parallelism between standards and samples, a Pearson correlation analysis was performed. To determine the breeding-associated rise in estrogen, baseline estradiol concentrations were calculated using an

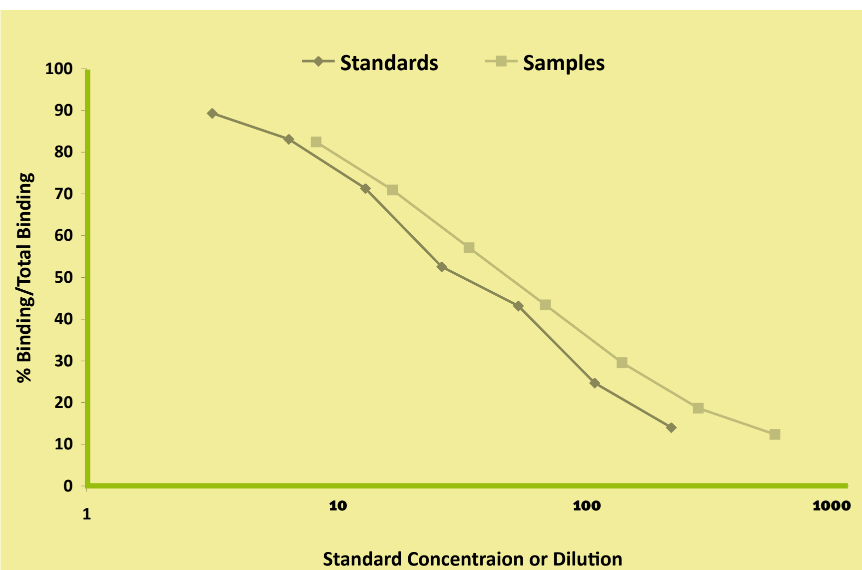
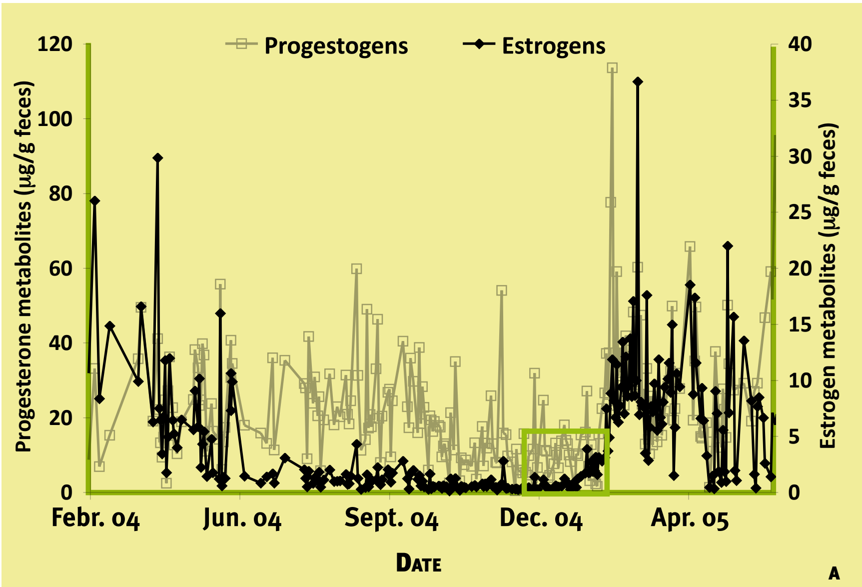


Figure 2. Parallelism (P<0.05) between the pregnane enzyme immuno assay and a serially diluted pooled fecal extract from female Iberian lynx.

Figura 2. Paralelismo (P<0.05) entre el enzimoinmunoensayo de la pregnona y una dilución seriada de un extracto conjunto de heces de hembra de lince ibérico.



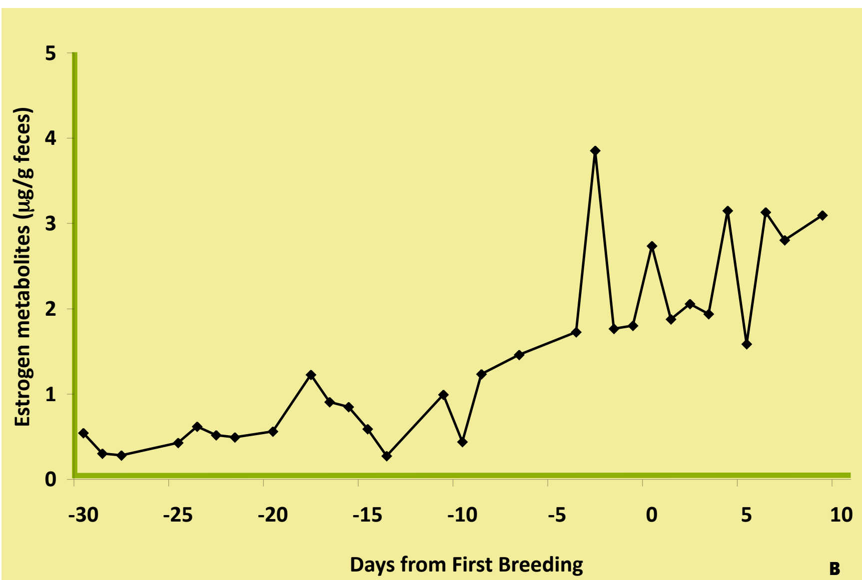


Figure 3. (a) Representative fecal hormone profiles from a female Iberian lynx, illustrating the clear and marked seasonal increase in estrogen excretion (solid diamonds). In contrast, progestogen (open squares) production generally remained elevated throughout the year, with the exception of lower concentrations during late fall and early winter. (b) A magnified view of the boxed area in profile "A" demonstrating the peri-copulatory increase in estrogen excretion in the same female in January 2005. Note the difference in scale between the two profiles. Asterisk represents the first day of estrogen metabolite elevation above baseline. Day 0 is the day of copulation.

Figura 3. (a) Perfiles representativos de hormonas fecales de una hembra de lince ibérico en los que se observa un aumento estacional claramente marcado en la excreción de estrógenos (rombos sólidos). En comparación, la producción de progestágenos (cuadrados abiertos) generalmente se mantiene en un nivel elevado durante todo el año, salvo por las concentraciones más bajas observadas a final de otoño y principios de invierno. (b) Imagen aumentada del recuadro del perfil hormonal "A" que muestra un aumento peri-copulatorio de la excreción de estrógenos en la misma hembra en enero de 2005. Obsérvese la diferencia en escala entre los dos perfiles. El asterisco representa el primer día de elevación del metabolito de estrógeno respecto de la línea basal. El día 0 corresponde al día de la copulación.

iterative process in which values that exceeded two standard deviations (SD) above the mean were excluded. The average then was recalculated and the elimination process repeated until no values exceeded the mean plus two SD (Brown et al., 1994). The average of the remaining values was considered "baseline" for that animal. Values greater than twice the SD were considered "elevated".

Results

When estrogen metabolite profiles were plotted in individual female Iberian lynx, there was a trend for increased excretion to occur during the breeding season (January through June) (Figure 3a for a representative female). Compared to other times of the year, females produced more estrogen beginning in January or February with peaks occurring in January through June followed by a return to baseline from May through August, which then remained at nadir through the end of the year (Figure 3a). During this study, the only recorded estrous behavior

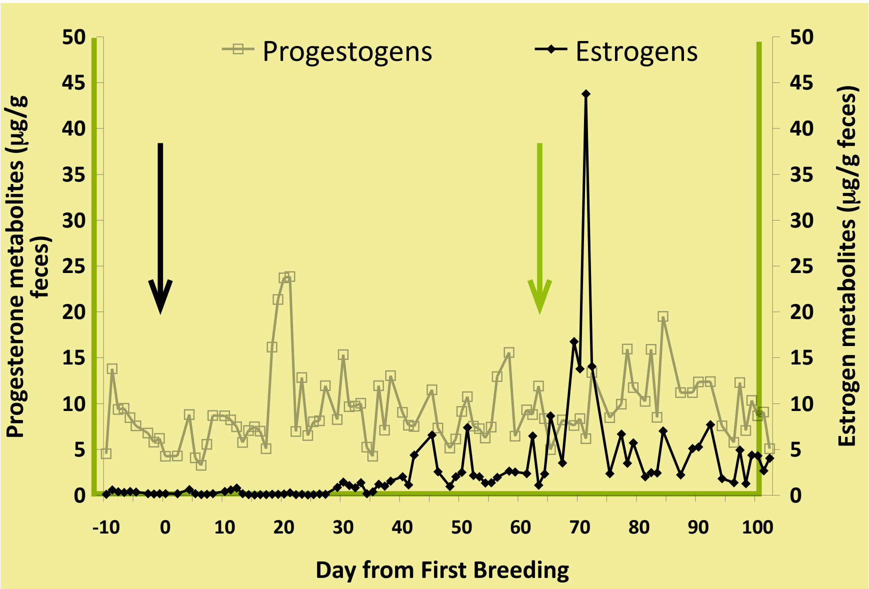


Figure 4. Representative fecal hormone profile in a pregnant Iberian lynx. Black arrow represents the day of first breeding, with a lack of a discernible estrogen surge or a sustained post-ovulatory rise in progestogens. Interestingly, a distinctive estrogen surge occurred after parturition (designated by the green arrow).

Figura 4. Perfil representativo de las hormonas fecales de una hembra gestante de lince ibérico. La flecha negra representa el primer día de apareamiento; no se observa un aumento apreciable de estrógenos ni un incremento sostenido de los progestágenos después de la ovulación. Curiosamente, se observa un aumento claro de estrógenos después del parto (señalado con la flecha verde).

(rolling, vocalization, lordosis) occurred at breeding season onset, during the first obvious and sustained rise in estrogen metabolite concentrations. During six of the seven recorded copulation events at the start of the breeding season, there was a pre-mating rise in estrogen metabolites (Figure 3b). However, this estrogen metabolite increase was modest compared to the subsequent seasonal rise (Figure 3a, b). During the latter time, estrogen metabolites increased at least 10-fold above estrual concentrations with no readily distinguishable cyclic pattern. This elevated, rather varied profile occurred in females that became pregnant (n=3) or remainded non-pregnant (n=4). Of the three monitored pregnancies, two subsequent parturitions were followed by a marked short-term increase in estrogen metabolites within 15 days (Figure 4). Although it appeared that this surge might have been associated with a post-partum, lactational estrus, no sexual behavior was observed in either individual, both of which were nursing cubs.

The progesterone assay that has been so effective in monitoring time of ovulation and the duration of the luteal phase in other felid species was essentially uninformative in the Iberian lynx. Increased excretion of progestogen metabolites occurred beginning in January and were sustained most of the year through October when there was a marginal year-end decrease before a rise coincident with next year's breeding season onset (Figure 3a). Confirmed pregnancy with the subsequent birth of cubs and lactation had no discernible impact on the trajectory of progestogen production (Figure 4). Likewise, progestogen patterns were indistinguishable between the pregnant and non-pregnant individuals.

Each adult male produced excretion profiles that reflected a trend towards slightly higher testosterone during the known breeding season (January through May) that was followed by a 10 to 50% decrease during summer and fall months (Figure 5). However, spikes in testosterone metabolite production occurred throughout the year, including during summer months.

Discussion

The Iberian lynx presents unique challenges to its conservation. Its numbers in nature have fallen precipitously due to habitat loss/fragmentation as well as decline of prey (Calzada et al., this book; Calvete, this book). The establishment of a captive breeding programme has not been without controversy, but is proving to be highly successful despite an initially small founder size and the discovery of species oddities such as siblicide (Vargas et al., 2006; 2008; this book). The uniqueness of the species now extends to its gonadal endocrinology. We anticipated a simple project that would easily characterize the extent of seasonality in the female and male

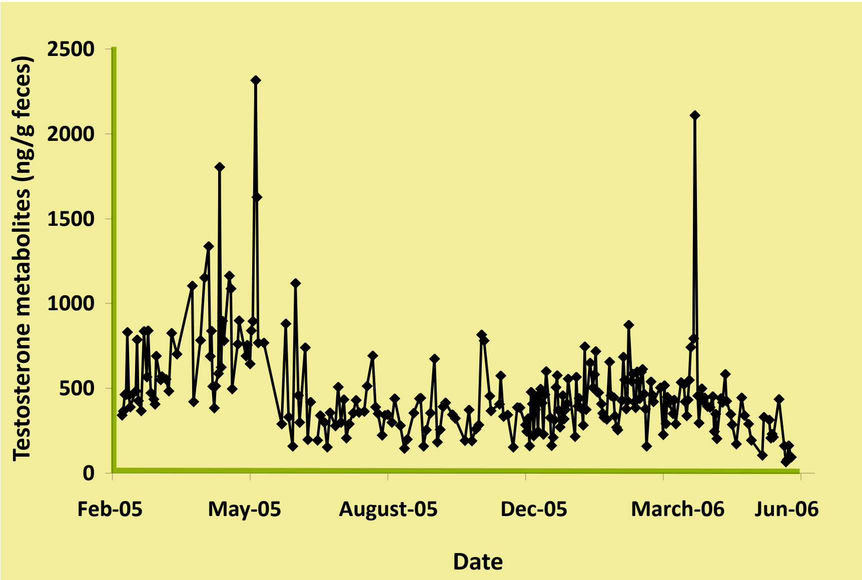


Figure 5. Representative fecal testosterone profile in an adult male Iberian lynx. Although there appeared to be a slight trend indicating a seasonal variation in androgen production in general, excretion of this gonadal hormone did not vary markedly with season.

Figura 5. Perfil representativo de testosterona fecal en un macho de lince ibérico. Aunque aparentemente se muestra una ligera tendencia que indica una variación estacional en la producción general de andrógenos, la excreción de esta hormona gonadal no varió significativamente en las distintas épocas.

Iberian lynx as well as the duration of the ovarian cycle, including tracking corpus luteum activity (the source of progesterone) post-ovulation and during pregnancy. Besides being of scholarly interest, such information has implications for management, including preparing for pairing and separating animals and assembling resources for anticipated births. For example, previous studies have discovered essentially no or marginal seasonality in the margay (Moreira et al., 2001) and tiger (Graham et al., 2006), respectively, in contrast to extremely brief annual periods of sexual activity in the Pallas' cat (Brown et al., 2002; 2006). Still other similar studies in the cheetah have identified frequent (and short) ovarian cycles interrupted inexplicably by periods of complete ovarian quiescence (Brown et al., 1996b). In essentially all cases to date, non-invasive monitoring has been effective at tracking biological activity in both of the major ovarian steroids. Thus, it was surprising to encounter challenges with the Iberian lynx. The conventional estrogen and testosterone EIAs were useful for recognizing seasonal variations in ovarian and testicular activity in this species as well as to identify elevations coincident with estrus. However, there were odd hyper-elevated estrogen patterns post-copulation that require more study. Furthermore, there was a lack of useful information on luteal steroid patterns from these traditional and previously effective assays.

Jewgenow and colleagues recently monitored fecal estrogen and progesterone metabolites in 15 pregnant and seven non-pregnant Eurasian lynx, a closely related, but distinctive species from the Iberian lynx (Dehnhard et al., 2008; Dehnhardt, this book). Eurasian lynx also failed to produce informative progesterone profiles, and metabolite patterns were indistinguishable between pre- and pregnant individuals. Additionally, estrogen profiles in pregnant Eurasian lynx were similar to non-pregnant counterparts. Interestingly, there was close correlation between the estrogen and progesterone patterns over time, with rises in the former failing to be clearly associated with estrus and breeding (Dehnhard et al., 2008). In contrast, we observed an increase in excreted estrogen in the Iberian lynx that coincided with behavioral estrus on six of seven recorded copulatory occasions.

All previous observations, including those associated with the Captive Breeding Programme, have confirmed that the female Iberian lynx is highly seasonal. Our observations indicated that increased sexual activity was associated with a detectable rise in excreted estrogen. More specifically, we observed high estrogen concentrations occurring in these captive animals from March through April, a time which coincided with previous observations of breeding in free-living lynx in the same geographic region (Palomares et al., 2005). In contrast, although there were slight trends for increased testosterone production in males during the breeding season, these elevations were not marked, suggesting that the testes were likely producing significant amounts of androgen throughout the year. This assertion was consistent with recent observations by Jewgenow et al. (this book) who have demonstrated sperm production of Eurasian lynx via electroejaculation during the non-breeding season.

We also observed overall greater estrogen excretion in females during periods of sexual activity, including copulation, compared to the non-breeding season. With nearly daily fecal collections, it was possible to detect distinctive estrogenic (ovarian) surges at the time of copulatory events. However, these increases were dwarfed by the dramatic estrogen rise observed during the weeks following breeding. These hyper-elevations were not associated with clear cyclic patterns (as described for follicular cycles in other felids) and occurred regardless of the female being pregnant or non-pregnant. This estrogen likely was of ovarian origin as it consistently followed copulations. Although residual ovarian follicles are known to occur in certain felids during pregnancy (Schmidt et al., 1983), the extreme estrogen concentrations found in the Iberian lynx remain inexplicable and require further study. It was interesting to observe a distinctive estrogen surge in two of three nursing females, indicating early post-partum ovarian follicular activity, but in the absence of sexual behavior.

Although there are challenges in interpreting some of the estrogen data, the real enigma was the total lack of value in monitoring fecal progestogen metabolites in the lynx. All females appeared to produce elevated concentrations consistent with onset of the breeding season followed by comparatively high levels until late fall, well beyond the known breeding season.

Numerous studies in other felids have determined that progestogen metabolites are highly reflective of post-ovulatory events, rising soon after ovulation and then remaining sustained for a species-specific duration during pregnancy or, in the case of failed conception, the end of a luteal phase (Brown et al., 1995, 1996b, 2001, 2002; Moreira et al., 2001). In general, the duration of increased progesterone production during gestation is twice as long as during a "pseudopregnancy" (Brown et al., 1995, 1996b, 2001, 2002; Moreira et al., 2001). Until now the only exception has been the Eurasian lynx, a species recently shown to have equivocal fecal progestogen production in pregnant versus non-pregnant individuals (Dehnhard et al., 2008). Perhaps these two species in the lynx genus have evolved different steroid metabolism mechanisms that somehow prevent capturing the progestogen metabolites sequestered in feces that normally are quantifiable by typical EIAs in other felids. However, high performance liquid chromatography (HPLC; after infusing a Eurasian lynx with radiolabelled progesterone) has revealed metabolite profiles no different from those published for other felid species (Brown et al., 1994). Furthermore, the assay antibodies being used have been found to effectively bind to a wide range of progestogen metabolites in the Eurasian lynx (Dehnhard et al., 2008). It also is possible that biologically active progestogen metabolites also are being produced by other sources (e.g., placenta, ovaries, adrenal glands) depending on female reproductive status, and that these similarly crossreact with the EIA antibody masking biologically relevant changes. This is a valid possibility as HPLC during various reproductive stages in the Eurasian lynx have revealed changes in the ratio of polar-to-non-polar progestogen metabolites between the pregnant and lactating female (Dehnhard et al., 2008; Dehnhardt et al., this book). Additionally, ultrasonography during lactation in the Eurasian lynx has indicated the presence of corpora lutea suggestive of long-term sustainability of these structures and/or post-partum ovulation (Göritz et al., this book). The latter option does perhaps relate to our observation of an early lactational estrogen surge, which even may have resulted in spontaneous and "silent" (no sexual behavior) ovulation. The concept of protracted luteal viability or occasional spontaneous corpus luteum formation on the ovaries also would be consistent with our observations of prolonged progesterone elevations into October (Göritz et al., this book). The potential of unusual luteal physiology in both species of lynx compared to other felids warrants more investigation.

In conclusion, although there were some unexpected challenges to adapting non-invasive gonadal hormone monitoring to the Iberian lynx, it was possible to confirm that increases in estrogen metabolite content in feces was reflective of the reproductive season. Furthermore, in general, the evaluation of estrogen metabolites in daily fecal samples correlated with behavioral estrus in most, but not all copulating females. It appeared that male lynx are much less markedly variant in gonadal androgen production throughout the year, which means that reproductive seasonality in this species is more a feature of females. Most interesting is the peculiar lack of distinctive fecal progestogen patterns indicative of unique luteal activity that appears a characteristic of both the Iberian and Eurasian lynx.

Acknowledgments

We thank Eva Vázquez, David Rodríguez and all participating staff and volunteers at the El Acebuche Breeding Center for outstanding efforts in collecting daily fecal samples and for monitoring the lynxe's behavior via the surveillance camera system. We also thank Katherine MacKinnon and Bernardo Mesa Cruz of the Conservation & Research Center, and Miguel Angel Domené at the Estación Experimental de Zonas Aridas who worked tirelessly during the lynx breeding season to generate data and provide in a timely fashion to the Iberian Lynx Captive Breeding Center El Acebuche. The work was partially supported by the Spanish Ministry of Education and Research (CGL2004-00603) and FEDER funds. The Environmental Council of the Andalusian Government provided the Iberian lynx fecal samples and the Ministry of the Environment of Spain provided logistical support.

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Photo: Pete Oxford

My grandmother always told me "The cleverer give in". This is completely wrong. We have to enlighten the ignorants and to keep the fools within bounds. Otherwise we will loose the Iberian lynx and other endangered species.

M. Dehnhardt

Fecal steroid hormones analysis in captive Eurasian and Iberian lynxes. Comparison of hormone metabolism in the two sister taxa

Análisis de esteroides sexuales en heces de lince ibérico y de lince euroasiático en cautividad. Comparación del metabolismo hormonal en ambas especies

Martin Dehnhard, Frank Göritz, Antje Frank, Sergey Naidenko,
Astrid Vargas and Katarina Jewgenow

Resumen

El seguimiento no invasivo de hormonas en heces se ha convertido en una herramienta importante para el manejo reproductor de animales cautivos. La finalidad de este estudio consistió en: 1) identificar los metabolitos de esteroides sexuales en heces más relevantes en el lince euroasiático *(Lynx lynx)* mediante técnicas de Cromatografía Líquida de Alta Resolución (HPLC) y, 2) evaluar la especificidad de los inmunoensayos de testosterona y progestágenos para caracterizar la actividad reproductiva estacional en los machos y hembras de lince euroasiático en cautividad. Se escogió al lince euroasiático como modelo para el lince ibérico *(Lynx pardinus)* con el fin de proporcionar la necesaria validación biológica de los análisis de hormonas en heces que se realizasen posteriormente en el Programa de Conservación *Ex situ* del lince ibérico. Durante un periodo de tres años, se tomaron muestras fecales de cuatro machos y 10 hembras de lince euroasiático mantenidos en cautividad en una estación de investigación ubicada cerca de Moscú. Las muestras se analizaron mediante enzimoinmunoensayo (EIA) con el fin de determinar los metabolitos de testosterona así como de estrógenos y progestágenos, en heces de machos y hembras, respectivamente. Además, se realizaron estudios de radiometabolismo mediante HPLC, en un macho ([^{3}H]testosterona) y una hembra ([^{3}H]progesterona) de lince euroasiático para caracterizar los metabolitos más relevantes de los esteroides eliminados por heces. Para poder realizar comparaciones entre las especies, también se analizó una muestra fecal de un macho y de una hembra de lince ibérico al análisis por HPLC. En ambas especies de lince, los metabolitos de esteroides (progestágenos y testosterona) presentes en heces se caracterizan por tener perfiles idénticos en la HPLC, que se pueden explicar aludiendo a un metabolismo de esteroides similar en ambas especies de lince. Los metabolitos de testosterona en heces reflejan la actividad testicular en machos de lince euroasiático (estacionalidad) y de lince ibérico (madurez sexual), mientras que los metabolitos de progestágenos en heces no son eficaces para diagnosticar el celo o la gestación en hembras de lince euroasiático. No obstante, el programa de cría en cautividad de lince ibérico depende de un diagnóstico de gestación fiable y no invasivo para evitar pérdidas perinatales de cachorros. Por lo tanto, es necesario desarrollar métodos alternativos, tales como el seguimiento de las hormonas en la orina.

Palabras clave
Hormonas en heces, lince, testosterona, progesterona, radiometabolismo

Abstract

Non-invasive fecal hormone monitoring has become an important tool for the reproductive management of captive populations. The aim of the present study was twofold: 1) identify relevant fecal steroid metabolites in the Eurasian lynx *(Lynx lynx)* using High Performance Liquid Chromatography (HPLC) analysis and 2) evaluate the specificity of testosterone and gestagen immunoassays to characterize seasonal reproductive activity of captive male and female Eurasian lynx. We chose the Eurasian lynx as a model for the Iberian lynx *(Lynx pardinus)* to provide the necessary biological assay validation to be applied in the *Ex situ* Programme established for this endangered species. Fecal samples were collected over a three year period from four male and 10 female Eurasian lynx maintained at a research station near Moscow. Samples were extracted and subjected to enzyme immunoassays (EIA) to determine fecal testosterone in males and estrogen and gestagen metabolites in females. In addition, radiometabolism studies were performed in one male ($[^3H]$testosterone) and one female ($[^3H]$progesterone) using HPLC analysis to characterize the most relevant fecal steroid metabolites. For species comparison, a fecal sample from a male and female Iberian lynx was also subjected to HPLC analysis. In both lynx species, fecal steroid metabolites (gestagens and testosterone) are characterized by identical HPLC metabolite profiles, which basically may be traced back to a similar steroid metabolism in both lynx species. Fecal testosterone metabolites reflect the testicular activity in male Eurasian (seasonality) and Iberian lynx (sexual maturity), whereas fecal progestagen metabolites are ineffective for estrous and pregnancy diagnosis in both lynx species. The Iberian lynx captive breeding programme, however, depends on a reliable and non-invasive pregnancy diagnosis to prevent peri-natal losses of cubs. Therefore, alternative approaches, like monitoring urinary hormones, need to be developed.

Keywords
Fecal hormones, lynx, testosterone, progesterone, radiometabolism

Fecal steroid hormones analysis in captive Eurasian and Iberian lynxes. Comparison of hormone metabolism in the two sister taxa

Martin Dehnhard, Frank Göritz, Antje Frank, Sergey Naidenko, Astrid Vargas and Katarina Jewgenow

Introduction

The genus Lynx includes four species: the Eurasian lynx *(Lynx lynx)*, the Canada lynx *(Lynx canadensis)*, the bobcat *(Lynx rufus)* and the Iberian lynx *(Lynx pardinus)*. Phylogenetic analyses of mtDNA sequence variation supports the hypothesis that the Iberian lynx Eurasian lynx, and Canada lynx diverged within a short time period around 1.53-1.68 million years ago, and that the Iberian and Eurasian lynx are sister taxa (Johnson et al., 2004).

All lynx species are distributed over the Northern Hemisphere in Europe, Asia and America. The Iberian lynx was always restricted to the Iberian Peninsula south of the Pyrenees (Calzada et al., this book), whereas the Eurasian lynx was found in the forested areas throughout most of Europe, the Middle East and Asia. Today, the range of the Eurasian lynx has been drastically reduced in Europe. Recently, this species has been reintroduced to parts of Germany, Switzerland and Austria, where it is beginning to re-establish (Breitenmoser and Breitenmoser-Würsten, 1990; von Arx et al., this book).

All four lynx species have some general common features (Table 1), which are typical of the Felidae (Breitenmoser et al., 1993; Heptner and Sludskii, 1972). Data presented in this table are mostly based on skinned carcasses collected from trappers or on reports of captive animals (Hayssen et al., 1993; Kvam, 1990; Parker and Smith, 1983). The reproductive parameters are similar between the four species. All except for the bobcat are monestrous breeders, starting their mating period in January. The Iberian lynx has the narrowest breeding season, lasting for about one month (Palomares et al., this book; Vargas et al., this book), with the other three species being less restricted. The bobcat is supposed to be seasonally polyestrous until July. It has also the shortest gestation length and can have more than one litter per year. In the Iberian and Eurasian lynx, litter sizes of 1-4 kittens have been reported. The gestation length is approximately 65-70 days for the Eurasian lynx and 63-66 days for its Iberian counterpart (Vargas et al., this book). Male lynxes reach sexual maturity at three years-of-age. In Eurasian lynx females, the first ovulation may occur at the age of 10 months (Kvam, 1990), while actual reproduction occurs at age of 22 months, with the females giving birth for first time at approximately two years of age (Naidenko, 1997).

	L. lynx	*L. pardinus*	*L. canadensis*	*L. rufus*
Body length	80-130 cm	75 – 100 cm	90-100 cm	72 – 98 cm
Ad. weight	18 –35 kg	8-17 kg	9 – 18 kg	11 – 14 kg
Neonatal weight	250-360 g	150-220 g	200 g	112-226 g
Litter size	2-3 (1-5)	2-3 (1-4)	2 (1-4)	3.5 (1-6)
Sexual maturity	2 – 3 y	3 y	2 y	?
Oestrus length	2-7 d	2-7 d		2 d
Cycle length	-	-	-	44 d
Gestation	68-72 d	63-66 d	60-65 d	50-60 d
Lactation (solid food)	3m (6 w)	3-4m (8-9 w)		3m (7-8 w)
Litter/year	1	1	1	>1
Breeding season	Jan-Apr	Jan-Feb	Jan-Feb	Jan-July

Table 1. Parameters of reproduction in four lynx species.

Tabla 1. Parámetros de reproducción en cuatro especies de lince.

Considering the tight relationship between both sister taxa (Johnson et al., 2004), a similar hormone pattern and metabolism was suggested for both, the Eurasian and the Iberian lynx. A prerequisite for the establishment of methodology for non-invasive hormone monitoring is the biological validation of the analytical method. As this validation is impossible to perform in the highly endangered Iberian lynx, the Eurasian lynx was suggested as a model for the establishment of hormone monitoring in captive male and female lynxes. The aim of the presented study was to characterize relevant steroid hormone metabolites in captive Eurasian lynx to assist with non-invasively monitoring of male and female reproductive performance. The ultimate goal was to provide a biological validation of fecal hormone assays to be applied in the Iberian lynx captive breeding programme.

Material and methods

Animals

Four male and 10 female Eurasian lynx were housed at the scientific field station Tchernogolovka of the A.N. Severtsov Institute (Naidenko and Antonevich, this book). Average annual temperature varied from +3.5° to +4.3 °C, average temperature July is +19 °C, January is -11 °C. The animals were kept within six enclosures (74 m^2) and in one large fenced enclosure (7.500 m^2), that is part of the natural mixed forest providing a semi-natural environment. Animals were housed separately and males and females were put together only for mating. Mating season took place in March, and animals reproduced every year. Fecal samples were collected monthly throughout a two-year period and stored at –20 °C within 1 h after defecation until analyses. From February to April (prospective mating season) the frequency of collection was increased to 1-2 times per week. For comparative assessment of hormone metabolites, fecal samples from captive Iberian lynxes were provided from the Iberian Lynx Captive Breeding Center, El Acebuche, in Doñana National Park (Vargas et al., this book).

Fecal samples (0.5 g) were extracted for 30 min by shaking with 4.5ml of 90% methanol. After centrifugation (15 min at 1200 g) the supernatant was transferred into a new tube. Aliquots of the fecal extracts were subjected either to High Performance Liquid Chromatography (HPLC) analyses, or diluted 1:1 with water and added directly to the respective steroid enzyme immunoassay (EIA). All hormone measurements were carried out in duplicates to assess the coefficient of variation. Results were expressed as immunoreactive steroid metabolites in µg/g of fecal wet weight.

Female reproduction (adapted from Dehnhard et al., 2008)

Progesterone and estrogen immunoassays

Progesterone (P4) analyses were carried out with an in-house microtitre plate enzyme immunoassay procedure as described earlier (Göritz et al., 1997) using a commercial P4 antibody (Sigma P1922, generated in rat) against progesterone and 4-pregnen-3,20-dione-3-CMO-peroxidase label. Fecal estrogen analyses were also carried out with an in-house microtitre plate enzyme immunoassay using as a polyclonal antibody (rabbit) against 1,3,510)-estratrien-3,17 α -diol-17-HS-BSA and 1,3,510)-estratrien-3,17 β -diol-17-HS-peroxidase label (Meyer et al., 1997). The cross-reactivity's of both antibodies and their inter- and intra-assays as coefficients were as described before (Dehnhard et al., 2008).

Radiometabolism study

To identify relevant progesterone metabolites we carried out a radiometabolism study. To a solution (0.25 ml) containing ~250μCi [^{3}H]progesterone (70–110Ci/mmol, TRK413, Amersham Bioscience, UK) in ethanol sterile 0.9% NaCl solution (2.25 ml) was added and the total volume was injected into the cephalic vein of a 15-year-old female lynx. Prior to injection, the animal was sedated by IM injection with 3 ml of a 3:1 mixture of Rometar® (2%- solution of xylazine hydrochloride) and ketamine hydrochloride. Following the radiolabelled injection, all excreted fecal samples were separately collected from the enclosures immediately after defecation during a 4-day period, placed in a plastic bag and stored at -20 °C. Aliquots of each sample were extracted for gestagen determination and for radioactivity counting. The second sample, collected on day 2 after injection, contained the highest amount of radioactivity was used for HPLC analyses. All radioactive counting was conducted in a Perkin Elmer MicroBeta Trilux counter (Perkin Elmer, Germany).

HPLC analysis of metabolites

For separation and characterisation of fecal steroid metabolites, 50 μl portions of fecal extracts were used. For progesterone metabolite analysis a reversed-phase Ultrasep ES100/RP-18/6 μm HPLC column (4 x 250 mm, Sepserv, Berlin) was used. Metabolites were separated with a methanol:water mixture (78:22) at a flow rate of 1 ml/min. Fractions of 0.33 ml were collected at 20 sec intervals and diluted with 1 volume of water, before 20 μl of the fractions were added into the assay systems. The elution positions of authentic progesterone (4-pregnen-3,20-dione; P4), 5 α -pregnan-3,20-dione (DHP), 5 α -pregnan-3 β -ol-20-one (5 α -P), 5 β -pregnan-3 α ,20 α -diol (pregnanediol, PD) on this column had been previously determined in separate HPLC runs.

For fecal estrogen metabolite separation an Allure Biphenyl 5μm HPLC column (3.2 x 150 mm; Restek, Bad Homburg, Germany) was used. Metabolites were separated with an acetonitril: water mixture (43:57) at a flow rate of 1 ml/min. Fractions were collected and added to the assay as described above. The elution positions of authentic 1,3,510)-estratrien-3,17-one (estrone), 1,3,510)-estratrien -3-ol-17-one α -diol (17 α -estradiol), and 1,3,510)-estratrien-3,17 β -diol (17 β -estradiol) on this column had been determined in separate HPLC runs after their injection.

Male reproduction (adapted from Jewgenow et al., 2006)

Testosterone

Fecal testosterone was measured with an automated chemiluminometric high sensitivity testosterone assay (T, Immulite, Diagnostic Products Corporation, Los Angeles, CA) using an Immulite® automated analyser (DPC Biermann, Germany) as described before (Jewgenow et al., 2006). A single determination uses 25 μl of fecal extracts. According to the manufacturer the functional sensitivity for the T assay on this system is 0.49 ng/ml (1.7 nmol/liter) and the average interassay coefficient of variation is 13.7% at a concentration of 4.27 ng/ml (14.8 nmol/liter). A test for parallelism was performed for feces from both the Eurasian and the Iberian lynx.

Radiometabolism study

The testosterone radiometabolism study was performed with a solution (0.25 ml) containing ~ 250 μCi [^{3}H] testosterone (70-105 Ci/mmol, TRK921, Amersham Bioscience, UK) in ethanol. Sterile 0.9% NaCl solution (2.25 ml) was added to the radiolabelled solution and the total volume was injected into the cephalic vein of a 15 years old male Eurasian lynx. Sedation and sample collection was performed as described for females (see above). Aliquots of each sample were extracted for testosterone determination and radioactivity counting.

To prove whether testosterone or its metabolites were conjugated to glucuronides or sulfates, the fecal extract was also subjected to enzyme hydrolysis and solvolysis (Jewgenow et al., 2006) before HPLC separation.

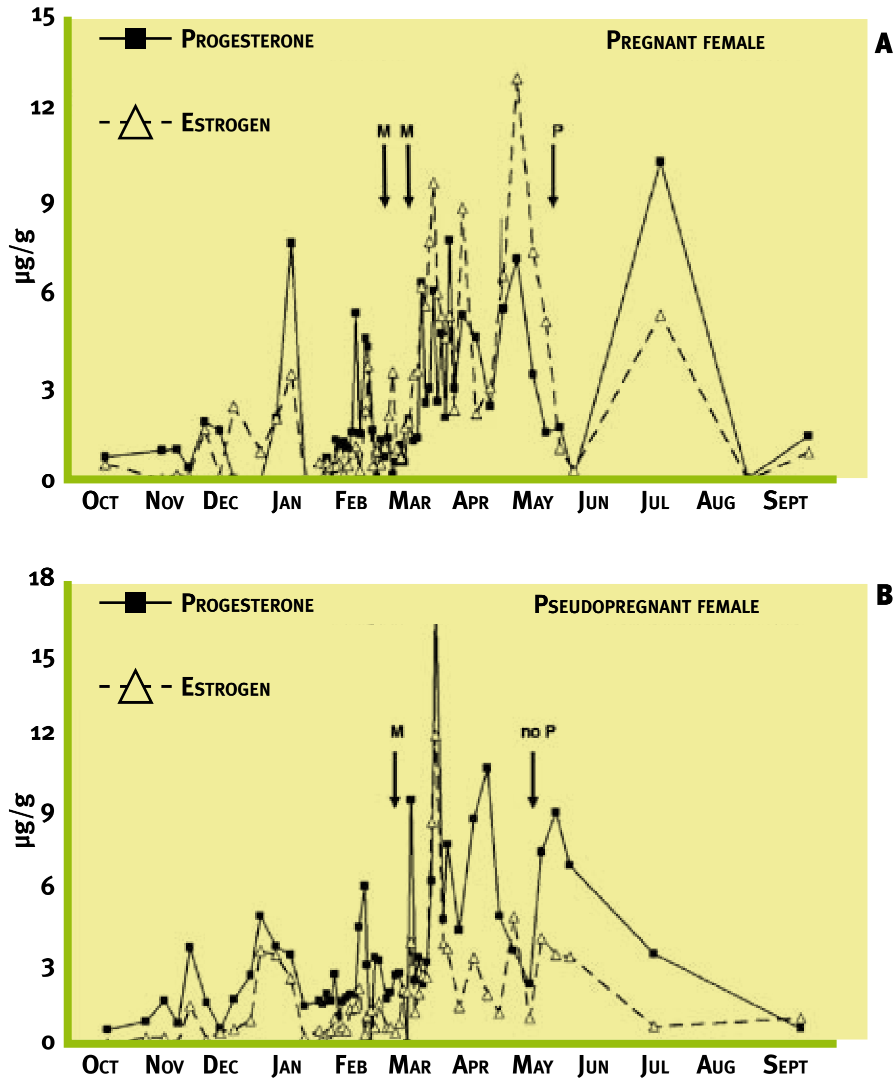


Figure 1. Course of fecal progesterone and estrogen metabolites in a pregnant (a) and pseudopregnant (b) Eurasian lynx. Samples were collected over one year, beginning in October (M: mating; P: parturition).

Figura 1. Evolución de los metabolitos de la progesterona y los estrógenos fecales en una hembra (a) gestante y (b) pseudogestante de lince boreal. Las muestras fueron recogidas durante un año, empezando en el mes de octubre (M: apareamiento; P: parto).

All radioactive counting was conducted in a Packard TRI-CARB 1900 TR liquid scintillation counter (Canberra-Packard GmbH, Germany).

HPLC analysis of testosterone metabolites in males

For separation and characterisation of androgen metabolites, 50 µl of fecal extracts were loaded on a reverse-phase Ultrasep ES100/RP-18/6 µm HPLC column (4 x 150 mm, Sepserv, Berlin). A linear gradient was generated at a flow rate of 1 ml per min: 0–11 min, 60% to 75% methanol; 11-20 min, 75%; 20-25 min, 75% to 100% methanol. Fractions of 0.33 ml were collected at 20 sec intervals over a period of 21 min and diluted with 1 volume of water, before 20 µl of the fractions were transferred into an in-house testosterone EIA (see Jewgenow et al., 2006 for details). The elution positions of authentic testosterone and dihydrotestosterone (Sigma Chemie GmbH, Deisenhofen, Germany) on this column had been previously determined in separate HPLC runs.

Data analysis

For each animal, means ± standard error (SEM) were calculated at each time period and differences were assessed by multiple ANOVA. Female samples analyzed for gestagens and estrogens were tested for linear correlation (Pearson r). Calculated P values < 0.05 were considered to be significantly different. The statistical procedures were performed with the software programme Instat Version 3 (Graphpad Software Inc.).

Results and discussion

Seasonal patterns of reproduction

Female reproduction: estrogen and gestagen metabolites

We analysed fecal samples of pregnant (n=15) and pseudo-pregnant (n=7) female Eurasian lynxes during a 3-year study period. Figures 1a and 1b display the courses of fecal progesterone (P4) and estrogen (E2) metabolites in a pregnant and a pseudopregnant female. There is a tendency towards higher progestagen and estrogen metabolite concentrations during pregnancy. However, no distinct differences between profiles from pregnant (a) and pseudo-pregnant (b) females were obtained. As shown for pregnant and pseudopregnant females, a distinct estrogen peak was absent at mating time. Both steroid metabolites showed a post-partum increase with no difference between the pregnant (a) and pseudo-pregnant (b) females. Surprisingly a highly significant (n=310, r^2=0.8131, p< 0.0001) correlation between E2 and P4 metabolites was obtained, when calculated for all females.

The results from the Eurasian lynx revealed that the measurement of fecal progesterone metabolites led to irregular patterns, which were different from expected profiles derived from data of other felid species (Brown et al., 2001; Brown et al., 1994; Brown et al., this book), but similar to fecal gestagen metabolite analysis in the Iberian lynx (Pelican et al., 2006; Pelican et al., this book). Thus, fecal estrogen and progesterone metabolites were ineffective for estrus and pregnancy diagnosis in Eurasian and Iberian lynx (see also Pelican et al., this book).

Therefore a radiometabolism study was necessary to investigate whether our enzyme immunoassay could detect the relevant metabolites reflecting the biologically active hormones. In addition, a biological validation of the presumed luteal activity was needed. This involved the characterisation of immunoreactive metabolites during and after pregnancy, the determination of blood serum hormones (Jewgenow et al., this book) and, finally, an ultrasound examination to verify the functional activity of Corpus luteum (C.l.) outside breeding season (Göritz et al., this book).

Male reproduction: fecal testosterone metabolites

There were no differences in fecal testosterone metabolite excretion between males (P>0.05) and between years (P>0.05). Because of the limited sample size for each month, testosterone metabolite concentrations were combined every two months before, during and after breeding season, and every three months during the non-breeding period. Figure 2 presents the means ± SEM determined by the Immulite testosterone assay in four captive Eurasian males. In all four males, a similar pattern of fecal testosterone metabolite concentration were obtained with highest concentrations during the breeding season (March-April) and lowest values in January/February. However, only in one male this difference was significant (P< 0.05).

Radiometabolism study and characterization of relevant steroid metabolites in female and male Eurasian lynx feces

Female reproduction: radiometabolism of progesterone

To identify the relevant progesterone metabolites, which reflect C.l.-activity in the Eurasian lynx, a radiometabolism study was performed. For the development of techniques for fecal steroid analysis, experiments on the metabolism of radiolabelled steroids have provided a valuable insight into the metabolism and the excretion of hormone metabolites via faeces and urine. After injection of ^{3}H (tritiated) labelled hormone, the excreted metabolites of a particular steroid can be analysed by high-performance liquid chromatography (HPLC).

Figure 3a shows the distribution of radiolabelled progesterone metabolites in a fecal extract of a female Eurasian lynx. When passing through the non-polar (reversed phase) column, the metabolites are retained and separated based on differences in their polarity. The extract of female lynx faeces is composed of four major polar radiolabelled gestagen metabolites detectable in fractions 6-8, 10, 12-13, and 16–17. Only minor amounts of radiolabelled progesterone metabolites were detectable at positions of unpolar substances corresponding

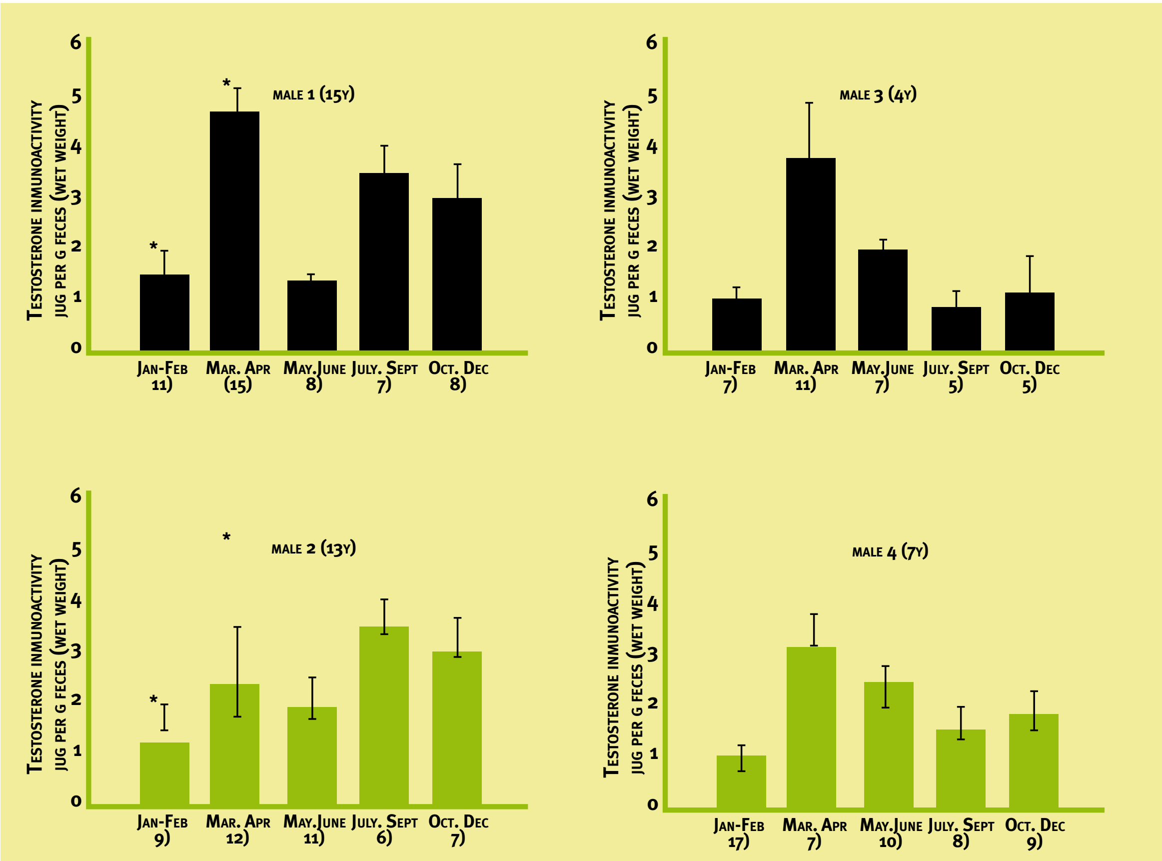


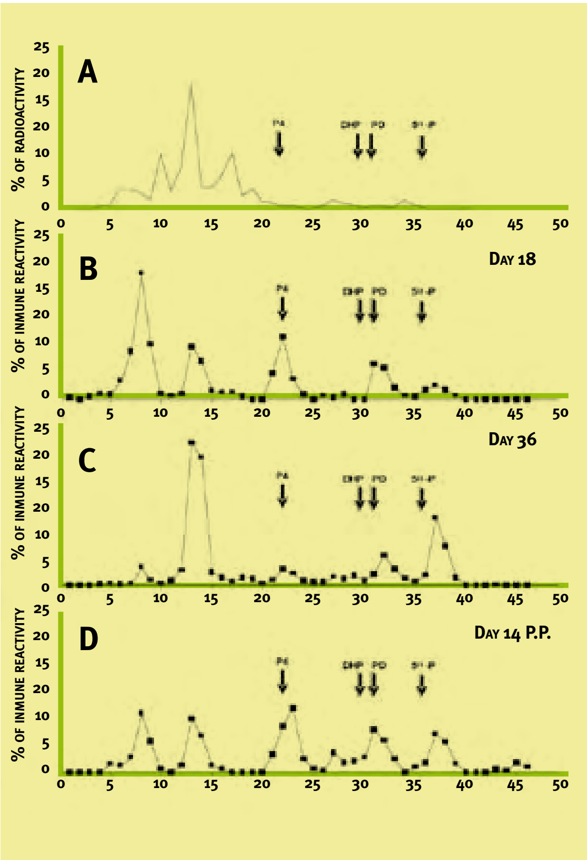
Figure 2. Fecal testosterone metabolites in four captive Eurasian males. Means ± SEM are presented bimonthly for the period before and after breeding season (March–April) and quarterly for the rest of the year. *Significant differences in testosterone content ($P < 0.05$).

Figura 2. Metabolitos de testosterona en heces de cuatro machos de lince boreal en cautividad. Los valores medios ± ESM se presentan bimensualmente para el período anterior y posterior a la época de reproducción (marzo-abril) y trimestralmente para el resto del año. *Diferencias significativas en el contenido de testosterona ($P<0.05$).

to progesterone and one of the other gestagen standards. This suggests that the circulating hormone itself is merely present in minor quantities in feces.

Usually the pattern of radiolabelled metabolites in HPLC-analysis and those of immunoreactive hormone metabolites does not coincide due to different immunological cross-reactivities of commercial, custom-made, or in-house antibodies, which are directed towards binding with original hormones or known derivates. It is extremely important to consider that the peak seen in an HPLC immunogram does not reflect the quantity of fecal hormone metabolites but is a result of the percentage of cross-reactivity of the antibody, together with the amount of actual metabolite in a particular HPLC fraction. In contrast, the radioactivity within a fraction directly reflects its quantitative amount and, thus, the composition of metabolites even if their detailed chemical structure remains unknown.

The progesterone immunoassay demonstrated five different immunoreactive progesterone metabolites (Figure 3b, c, d). Two of them were consistent with the two major radiolabelled metabolites (fractions 8–10 and 13-14), whereas three immunoreactive metabolites that eluted after fraction 20, corresponded to the elution positions of progesterone, DHP and 5 α -P, respectively. The broad-shouldered peak at fractions 7-9 indicates a cluster of polar



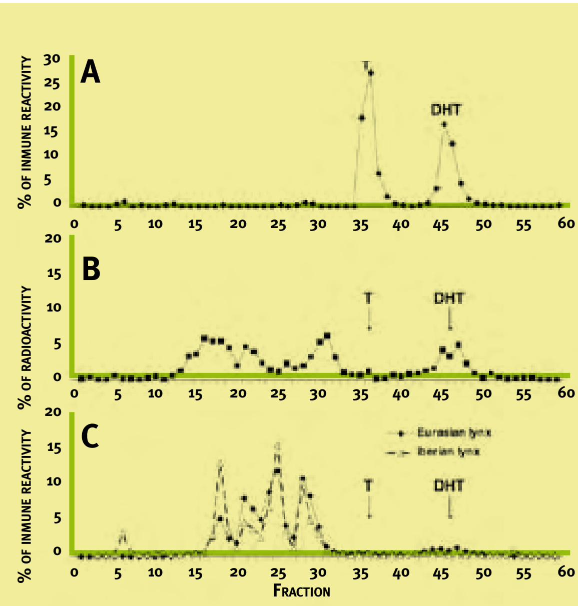


Figure 4. Elution profile of testosterone (T) and dihydrotestosterone (DHT), radiolabelled testosterone metabolites, and immunoreactive testosterone metabolites in male Eurasian and Iberian lynx.

Figura 4. Perfil de elución de testosterona (T) y de dihidrotestosterona (DHT), metabolitos de testosterona radiomarcados y metabolitos de testosterona inmunorreactivos en machos de linces boreales e ibéricos.

Figure 3. Elution profile of radiolabelled progesterone metabolites and immunoreactive progesterone metabolites in a female Eurasian lynx. Fecal extracts chosen from days 18 and 36 of pregnancy and 14 days post-partum were subjected to HPLC separation. Arrows indicate the elution positions of progesterone (4-pregnen-3,20-dione, P4), 5 α -pregnane-3,20-dione (DHP), 5-pregnen-3 β -ol-20-one (pregnenolon, PD), and 5 α -pregnane-3 β -ol-20-one (5 α -P). For methodological details see Jewgenow et al. 2006. (Radiometabolism study and detection of immunoreactivities were carried out in different animals).

Figura 3. Perfil de elución de metabolitos de progesterona radiomarcados y metabolitos inmunorreactivos de progesterona en una hembra de lince boreal. Los extractos fecales seleccionados en los días 18 y 36 de embarazo y a los 14 días posparto fueron separados por cromatografía líquida de alta resolución (HPLC). Las flechas indican las posiciones de elución de progesterona (4-pregnen-3,20-diona, P4), 5 α pregnano-3 β -ol-20-diona (DHP), 5-pregnano-3 β -ol-20-ona (pregnenolona, PD), and 5 α -pregnano-3 β -ol-20-ona (5 α -P). Ver Jewgenow et al., 2006, para los detalles metodológicos. (Se realizaron estudios radiometabólicos y de detección de inmunorreactividades en distintos animales).

conjugated steroid metabolites. Enzymatic hydrolysis of samples (Jewgenow et al., 2006) changed the elution pattern resulting in the disappearance of conjugated metabolites towards an increase of free steroids.

Male reproduction: radiometabolim of testosterone

The elution positions of the standards testosterone (T) and dihydrotestosterone (DHT), and the HPLC profile of radiolabelled testosterone metabolites in Eurasian lynx feces in combination with data on elution positions of immune reactivity determined in the immunoassay in the Eurasian and Iberian lynx are shown in Figure 4a, b, c. The extract of male Eurasian lynx feces is composed of several radiolabelled metabolites (Fig. 4b). The majority was detected in fractions 14–18, 21-23, 29-32, and 45-48 (see double peak). Two minor radioactive peaks co-eluted with T and DHT at fraction 36 and 45, respectively. The broad peak at fractions 14-18 indicates a cluster of polar metabolites. Hydrolysis did not changed the elution pattern of polar radiolabelled metabolites, only after acid treatment (solvolysis) the double peak in fraction 45-48 increased from 14% to 26% at the expense of the polar metabolites in fractions 14-18 (data not shown). Further analyses of fecal samples were carried out on untreated extracts.

In the Eurasian lynx, the immunoassay detected testosterone immunoactivity (Fig. 4c) consisting of two major peaks (fractions 24–25 and 28–29), which were not identical to the major radiolabelled metabolite peaks. In addition, two immune reactive peaks co-eluting with major peaks of radioactivity were detected in fractions 17–19 and 21–22. No substantial immune reactivity was associated with the elution position of testosterone whereas a small amount of reactivity was associated with the position of dihydrotestosterone, and the respective peak of radioactivity.

We also investigated immunoactive testosterone metabolites in fecal samples from the Iberian lynx (Figure 4c), revealing four major peaks at the same elution positions as in the Eurasian lynx (fractions 17-19, 21-22, 24-25, 28-29). As in the Eurasian lynx, only minor portions of immunoactivity were detectable at the elution positions of T and DHT.

Biological validation

Female reproduction: estrous cycle and pregnancy monitoring

Biological validations aim to demonstrate that hormone metabolite measures reflect the physiological event in question. One possibility to validate a non-invasive hormone assay is to compare and correlate fecal metabolite levels with blood concentrations. Even if blood sampling contradicts the non-invasive approach, veterinary check-ups and treatments should be used to collect blood and fresh fecal samples simultaneously. However, the time lag between secretion and excretion had to be considered, making such correlations often difficult to attain. Short-term changes in plasma hormone levels may be dampened in excreted samples, whereas improved correlations can be expected when blood levels are constant over longer periods of time.

The strongest methods to physiologically validate non-invasive methods are pharmacological stimulations or inhibitions of steroid hormone release. These methods typically involve the administration of high doses of releasing hormones, such as gonadotrophic releasing hormone (GnRH), to stimulate the production of gonadal sex steroids (Kretzschmar et al., 2004) and adrenocorticotrophic hormone (ACTH) to stimulate the adrenal gland to produce corticosteroids (Wasser et al., 2000).

A biological validation can also be based on data analysis. The measured hormone pattern in the lynx should mirror the reproductive events. In females, the predicted hormone pattern (derived from other related felid species) should include an estradiol peak around mating, elevated progesterone levels during pregnancy, followed by a decrease towards basal levels after 4 weeks in pseudopregnant females and, a more or less immediate decrease down to baseline prior to parturition in pregnant females (Brown et al., this book). In males, changes in testosterone metabolites should follow sexual maturity during puberty or annual fluctuations in seasonally active animals.

Our results, however, revealed differences from these predicted patterns (Figure 1): Lynx estrogens did not reflect follicular activity by peaking around ovulation (mating), but were strongly correlated to the excretion of gestagen metabolites. Therefore, we assume that fecal estradiol immunoreactivity reflect the activity of corpora lutea. Gestagen metabolite profiles in the Eurasian lynx were also not in accordance to typical felid hormone patterns. We analysed elevated progesterone (and estradiol) metabolite levels throughout pregnancy and thereafter. However, the composition of hormone metabolites after parturition was different from those during pregnancy. Particularly, the relation between progesterone metabolites eluting between fractions 7-9 and 13-14 differed markedly during pregnancy and the post-partum period (Figures 3b, c, d). This might be due to different hormone sources during and after pregnancy (C.l., placenta, or adrenals). To confirm this, an additional validation for C.l. function had to be performed. In case of the Eurasian lynx, we performed a transrectal ultrasound investigation and we found corpora lutea (Göritz et al., this book). This is in agreement with the above mentioned high p.p. progesterone values, altogether supporting the hypothesis of a post partum luteal activity.

Male reproduction: puberty and seasonality of testicular function

In contrast to female hormones, biological validation of testosterone metabolites reflects the seasonal nature of sexual activity in Eurasian lynx males (Göritz et al., 2006). We demonstrated that serum testosterone concentrations in male Eurasian lynx were characterized by seasonal fluctuation with its highest levels in March (1.96 ng/ml testosterone) and lowest in June (0.75 ng/ml testosterone) (Figure 2, Jewgenow et al., 2006). This is consistent with the mating season of the Eurasian lynx (Kvam, 1990; Naidenko and Erofeeva, 2004). Low concentrations were obtained during January and February. There were some inconsistencies during the period from May to December, probably indicating a second increase of testosterone levels at the end of the year. An increase in testicular activity several months prior to mating season may not be unusual. In the roe deer, a small testosterone rise during the month of in May took place before the maximum levels of androgen production during the August/September rut season (Roelants et al., 2002; Schams and Barth, 1982).

Our preliminary data on fecal hormone concentrations in captive Iberian lynx suggests that the Immulite assay tested on Eurasian lynx is also applicable in its Iberian counterpart. This basically may be traced back to the similar metabolism of testosterone in both lynx species. The determination of fecal testosterone metabolites in a juvenile Iberian male reflected his gonadal immaturity by significant lower levels compared to the two adult males (see also Pelican et al., this book). The seasonal pattern of both adult Iberian lynx males indicated a tendency towards increased testosterone secretion during Spring time, however, more data are necessary to confirm this statement. Nevertheless, fundamental knowledge of basic reproductive physiology is essential to improve reproduction in captivity, as well as to provide support for assisted reproduction and gamete banking.

Summary and conclusions

Our comparative approach is very useful to get new insights on reproductive physiology of both the Eurasian and the Iberian lynx. Since many examinations procedures, such as radiometabolism studies, are not possible to be performed in the highly endangered Iberian lynx, the Eurasian lynx can serve as a model species.

Our results show that fecal steroid metabolites (progesterone and testosterone) are characterized by an identical pattern of HPLC profiles, which basically may be traced back to a congruent steroid metabolism in both lynx species. Fecal testosterone metabolites reflect testicular seasonality in male Eurasian and sexual maturity in Iberian lynx, whereas fecal gestagen metabolites are ineffective for estrus and pregnancy diagnosis in both lynx species. This finding underlines that, for each species, new analytical methods need to be established mainly due to the species-specific hormone metabolism. Specifically, for the development of an effective breeding programme, the ability to diagnose early pregnancy and to differentiate it from pseudopregnancy is urgently required. Currently, the most reliable pregnancy-specific method is the transabdominal ultrasonographic or radiographic imaging of the uterus, but this technique usually requires handling and anesthesia of the female, potentially stressing both the queen and developing fetuses. Alternative approaches, like urinary hormones or blood collected by bugs are currently being used to monitor pregnancy in captive Iberian lynx (Jewgenow, this book). Non-invasive pregnancy monitoring continues to be of highest priority for the reproductive management of the Iberian lynx captive population.

Acknowledgements

We thank all the staff and volunteers of Tchernogolovka Research Station, in Russia, and at El Acebuche Breeding Center (T. Rivas, J. Bergara, T. Váquez, J. Pardo, E. Vázquez J. López, M. Chaparro, J.M. Rodríguez, and D. Rodríguez) in Spain, for collecting fecal samples of Eurasian lynx and Iberian lynx, respectively. We also thank A. Frank, K. Paschmionka and M. Rohleder (IZW) for their excellent technical assistance. We appreciate the support of Russian students within DAAD Leonard Euler Programme.

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Prof. Dr. Roger Short,
University of Melbourne, Australia

Ultrasonographical assessment of structure and function of the male and female reproductive organs in the Eurasian and the Iberian lynx

Evaluación ecográfica de la estructura y función de los órganos reproductivos masculinos y femeninos de linces boreales e ibéricos

Frank Göritz, Astrid Vargas, Fernando Martínez, Thomas B. Hildebrandt, Sergey V. Naidenko, Francisco Palomares, José Vicente López-Bao, Maria José Pérez, Miguel A. Quevedo and Katarina Jewgenow

Resumen

Con el fin de poder criar de modo eficiente al lince ibérico en cautividad, consideramos imprescindible conocer bien su biología reproductiva y disponer de métodos fiables para el seguimiento de su reproducción. Debido al número limitado de linces ibéricos, en el presente estudio se trabajó paralelamente con especies menos sensibles, como el lince boreal y el lince rojo. Se evaluaron distintos parámetros morfológicos y funcionales de los órganos reproductores masculinos antes, durante y después de la época de reproducción de linces boreales adultos en cautividad (n=3). El tamaño y la morfología del tracto reproductivo fueron estudiados mediante ecografía transcutánea (testículos) y transrectal (glándulas sexuales auxiliares). En el caso del lince ibérico, se trabajó con varios machos del Programa de Cría en Cautividad (n=7; 4 adultos y 3 juveniles), así como con ejemplares de las poblaciones de vida libre (n=4 adultos); todas las ecografías se realizaron en época no reproductiva (finales de noviembre/principios de diciembre). Los testículos y la próstata del lince boreal mostraron cambios de tamaño y de textura relacionados con la época del año. Los volúmenes testiculares máximo y mínimo fueron 2,8 ± 0,76 cm^3 y 1.5 ± 0,3 cm^3 (Media ± EE), respectivamente. Se observaron las concentraciones más elevadas de testosterona en febrero (1240 ± 393 ng/g heces) con un segundo incremento en mayo (971 ± 202 ng/g heces). Las concentraciones más bajas de testosterona se detectaron en enero (481 ± 52.9 ng/g heces). En el lince ibérico, el volumen testicular medio osciló entre 0,4 y 2,0 ± 0,2 cm^3 en juveniles y adultos, respectivamente. La concentración media de testosterona en sangre fue más alta en el lince ibérico (0,32 ± 0,07 ng/ml) que en el lince boreal (0,16 ± 0,04 ng/ml). En las hembras de lince boreal e ibérico, así como en las de lince rojo, se utilizó la ecografía transrectal para visualizar las estructuras ováricas (folículos, cuerpos lúteos) y para evaluar la actividad ovárica. Además, se realizaron análisis de progesterona y estradiol en sangre. La presencia de cuerpos lúteos activos durante la época no reproductiva fue confirmada mediante ecografía y también mediante análisis hormonal, denotando unos niveles elevados de progesterona en sangre: una media de 3,56 ± 1,3 ng/ml en las hembras de lince boreal y de 6,1 ± 0,26 ng/ml en las de lince ibérico. Los resultados ecográficos de la estructura ovárica indican que los cuerpos lúteos formados a partir de la ovulación permanecen activos hasta noviembre y regresionan antes del inicio del siguiente estro.

Palabras clave
Ecografía, estacionalidad, evaluación reproductiva, calidad espermática

Abstract
Knowledge on lynx reproduction biology and reliable methods for reproductive monitoring are imperative to assist and propagate the Iberian lynx in captivity. Because of limited access to Iberian lynxes the less endangered Eurasian lynx and the bobcat were included as a surrogate species in this comparative study. We examined morphological and functional parameters of the male reproductive organs prior to, during and after the breeding season in adult captive Eurasian lynxes (n=3). Size and morphology of the reproductive tract was monitored by transcutaneous (testes) and transrectal (accessory sex glands) ultrasonography. Captive (n=7; 4 adult and 3 juvenile animals) and free ranging (n=4 adult animals) male Iberian lynxes were available for ultrasound examination out of breeding season (late November/early December). Testes and prostate of Eurasian lynx showed seasonal-related changes in size and texture. The maximum and minimum testicular volume was 2.8 ± 0.76 cm^3 and 1.5 ± 0.3 cm^3 (Mean ± SEM), respectively. The highest testosterone concentrations were found in February (1240 ± 393 ng/g feces). A second increase was documented in May (971 ± 202 ng/g feces). The lowest testosterone concentrations were measured in January (481 ± 52.9 ng/g feces). In the Iberian lynx the mean testicular volume ranged from 0.4 cm^3 in juvenile to 2.0 + 0.2 cm^3 in adult animals. Mean testosterone concentration measured in blood serum was higher in the Iberian lynx (0.32 ± 0.07 ng/ml) than in the Eurasian lynx (0.16 ± 0.04 ng/ml). In female Eurasian and Iberian lynxes and in bobcats, transrectal ultrasonography was implemented to visualize ovarian structures (follicles, corpora lutea) and to assess ovarian activity in addition to analysis of serum progesterone and estradiol. The presence of active corpora lutea during the non breeding season was confirmed by ultrasonography and by elevated serum levels of progesterone averaging 3.56 ± 1.3 ng/ml in Eurasian and 6.1 ± 0.26 ng/ml in Iberian lynx, respectively. The ultasonographical findings on the ovarian structure suggests strongly that corpora lutea developed after ovulation stay active at least until November and regress before the onset of the next estrus.

Keywords
Ultrasonography, seasonality, reproductive assessment, reproductive hormones, sperm quality

Ultrasonographical assessment of structure and function of the male and female reproductive organs in the Eurasian and the Iberian lynx

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Introduction

All species comprised within the genus Lynx: the Eurasian lynx *(Lynx lynx)*, the Canada lynx *(Lynx canadensis)*, the bobcat *(Lynx rufus)* and the Iberian lynx *(Lynx pardinus)* present similar reproductive parameters (Parker and Smith, 1983; Kvam, 1991; Hayssen et al., 1993; Dehnhard et al., this book). In recent studies on Eurasian lynx we have shown that male reproduction is seasonal corresponding to the female estrus (Jewgenow et al., 2006a). Fecal testosterone concentration, volume of ejaculates, percentages of motile spermatozoa and intact sperm were maximized during the breeding season in February/March (Göritz et al., 2006b; Dehnhard et al., this book). In several felid species, pregnancy diagnosis based on faecal hormone metabolites became almost a routine procedure. However, it has been shown that progesterone metabolites in lynxes did not follow the typical pregnancy pattern and made pregnancy diagnosis unattainable by faecal sampling (Jewgenow et al., 2006b; Pelican et al., 2006) as well as by urinary progesterone (Dehnhard et al., 2008; Jewgenow et al., this book). Surprisingly, progesterone measurement suggestive of luteal activity was also detected after the weaning of cubs (Göritz et al., 2009). This does not occur in domestic cats (Tsutsui and Stabenfeldt, 1993).

Because information on reproductive physiology in lynxes is limited, our recent studies focused on implementation of transrectal ultrasonography to assess their reproductive health and to monitor ovarian and testicular activity of the two sister taxa: the Iberian and Eurasian lynx. Due to limited access to the endangered Iberian lynx, where frequent examinations were not possible, Eurasian lynx and bobcats were also examined for comparative purposes.

Material and methods

Animals

Iberian lynxes (IL) in captivity were managed under the Iberian Lynx *Ex situ* Conservation Programme and housed at three different locations in Southern Spain (El Acebuche Captive Breeding Center in Doñana National Park, Huelva; Jerez Zoo, Cádiz, and La Olivilla Captive Breeding center, in Jaén) (Vargas et al., this book). Almost all females from the breeding centers were examined in late October 2006 (n=10) and late November 2007 (n=18). Captive males (n=4) were examined in November 2005 and 2006. Additionally, seven males and six females captured from the free ranging populations in Doñana National Park were also examined to assess their health and reproductive status in late November/early December 2006. Eurasian lynxes (EL) were kept in a field research station situated 50 km northeast of Moscow, surrounded by the natural vegetation of the Russian South Taiga forests (Naidenko et al., this book). Eurasian females were examined in June 2002/2005 (n=11), July (n=7) and November 2003 (n=3). In addition, males were examined prior to (November), during (March) and after

(April and June) breeding season between 2002 and 2004. Examined bobcats (BC) were kept in Russia at the same station (n=1 female, June 2007) and at the Jerez Zoo in Spain (n=3 females, November 2007). All females were kept in separate enclosures. During the breeding season adult females were allowed to mate. Mating was observed, either by direct observation (in Russia) or by 24 hours surveillance using remote controlled camera systems (in Spain). For data evaluation, the females were grouped according to there different age and breeding status: (i) juvenile (<2 years); (ii) adult (>2 years), no mating; (iii) adult, mating, no birth (pseudopregnant); and (iv) adult, mating, birth.

Ultrasonography

Eurasian lynxes were immobilized by IM administration (via blow pipe) of 3.5 to 4.0 mg/kg xylazine hydrochloride (Rompun™ 10%; Bayer, Leverkusen, Germany) and 3.0 to 3.5 mg/kg ketamine hydrochloride (Ketamine 10%™; Essex, Munich, Germany). In the Iberian lynxes and bobcats we used 50 µg/kg medetomidine (Domitor™, Pfizer, Karlsruhe, Germany) and 5 mg/kg ketamine hydrochloride (Imalgene 1000™). After examination and sample collection, anaesthesia was reversed with 0.2 mg/kg atipamezol hydrochloride (Antisedan™; Pfizer, Karlsruhe, Germany). The entire genital tract of each female was examined from caudad to craniad by transrectal ultrasonography in lateral recumbency (Göritz et al., 1997; Hildebrandt et al., 2000). A real-time, B-mode ultrasound scanning system (CS 9100 Oculus, Picker International GmbH, Espelkamp, Germany) equipped with a 7.5 MHz curved linear transducer (EUP-F 334) was used. The transducer was fitted with of two specific adaptors (15 and 25 cm in lengths, A. Schnorrenberg Chirurgiemechanik, Schönwalde, Germany) and introduced into the rectum using ultrasound gel. The dimensions of the ovaries were measured using a calliper system integrated into the ultrasound machine and their volume calculated as that of a simple spheroid (Göritz et al., 2003). Corpora lutea (Cll) were counted. The diameter of the smallest and largest corpus luteum (Cl) was measured and the mean volume of total luteal tissue (mm^3) calculated per animal ($V=4/3\ \pi$ * mean Cl $radius^3$ * number of Cll). For follicle counts, follicles were defined as anechoic structures >1 mm in diameter.

In males, ultrasonography of testes and accessory glands was performed as described before (Göritz et al., 2006b). Accessory glands (prostate) were visualized transrectally and the testes and epididymis by transcutaneous ultrasound using a handheld 7.5 MHz curved linear transducer. The dimensions of the accessory glands and testes were measured. The volume of the prostate and testes were calculated according to the volume of a simple rotation ellipsoid ($V=1/6\ \pi$ * length * height * width) accommodating the spherical and symmetrical shape of these organs.

Endocrinology

Blood samples were taken during each ultrasound investigation by venepuncture. Additionally, blood was collected using the same method during pregnancy from one BC (in June) and two EL (May). Serum was separated after centrifugation. Also, IL blood samples were obtained in March (n=14) non-invasively using blood-suckling bugs *(Dipetalogaster maxima)* as described and validated earlier (Voigt et al., 2004). The bugs were placed into cork plates, which were installed at the lynxe's preferable resting sites. The ingested blood was withdrawn from bugs and centrifuged (Braun et al., 2008; Jewgenow et al., this book).

The obtained serum (syringe collection during anesthesia) and plasma (bug ingested blood) samples were kept frozen until hormone assessment. Progesterone, estradiol (females) and testosterone (males) were measured in the blood serum or plasma by enzyme immunoassays with double antibody technique (Meyer et al., 1997) after extraction as described and validated before (Göritz et al., 1997; Dehnhard et al., 2008; Jewgenow et al., 2009; Dehnhard et al., this book).

Statistics

Data are presented as means ± standard errors of the mean (SEM). Comparisons of mean values were performed by Welch corrected unpaired t-Test, when the number of examinations or samples exceeded n=6. Relationship between volume of luteal tissue and progesterone concentration in blood serum was determined by Spearman correlation. All statistical tests were based on a 5% level of significance. The statistical procedures were performed with the software programme Instat Version 3 (Graphpad Software Inc.).

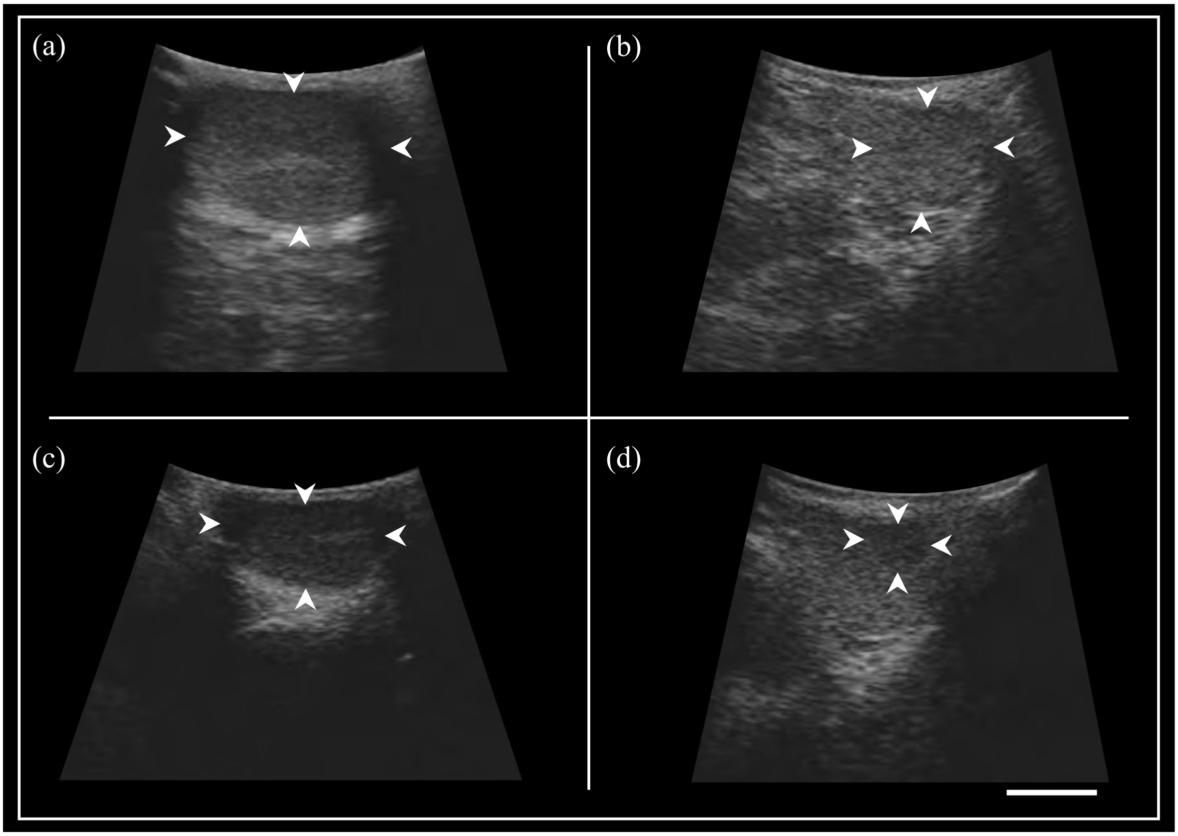

Figure 1. Sonograms of testis (a and c) and prostate (b and d) of a single male Eurasian lynx during the breeding season (March; a and b) and out of breeding season (November; c and d). Note distinct changes in size of the testis and prostate and texture of parenchyma. Arrow heads indicate organ borders. Scale bar represent 1 cm.

Figura 1. Ecografía de los testículos (a y c) y de la próstata (b y d) de un macho de lince euroasiático durante la época de reproducción (marzo; a y b) y fuera de ésta (noviembre; c y d). Obsérvense los cambios en el tamaño de los testículos y de la próstata así como en la textura del parénquima. Las cabezas de flecha indican los bordes de los órganos representados. La barra representa una escala de 1 cm.

Results and Discussion

Figure 1 and Figure 2 represent typical sonomorphology of urogenital tracts of male (Figure 1) and female (Figure 2) lynxes. In all lynx species, sonomorphology of urogenital tracts was similar to other carnivores (Göritz et al., 1997) and followed the typical, size-related structure of other felids (Göritz et al., 2005, 2006a). In male Eurasian lynx, seasonal fluctuation of male reproductive organs was evident. The testes and prostate showed changes in size and texture (Figure 1). They were most active in March and June, while in April and in November they appeared inactive. Seasonal changes were most pronounced in the testes, with a maximum and minimum volume of 2.8 ± 0.76 cm^3 and 1.5 ± 0.3 cm^3, respectively. Examination of the IL was not performed during the breeding season; therefore a seasonal pattern could not be shown. In the Iberian lynx the mean testicular volume ranged from 0.4 cm^3 in juvenile to 2.0 ± 0.2 cm^3 in adult animals. The volume of prostate ranged from 0.05 ± 0.02 to 0.2 ± 0.05 mm^3 in juvenile and adult animals, respectively.

The examination of female ovaries was of special interest; because luteal hormone activity was measurable not only during pregnancy, but also in mated non-pregnant females and in lactating females (Dehnhard et al., 2008; Jewgenow et al., in this book). By transrectal ultrasound, luteal activity after parturition was confirmed by detection of corpora lutea (Figure 2) and by elevated serum levels of progesterone (Table 1) in EL and IL, in June/July and November/December, respectively. These findings are in agreement with our recent results on steroid hormone metabolite concentrations measured in faeces and urine (Jewgenow et al., 2006b; Dehnhard et al., 2008; Dehnhard et al., this book; Jewgenow et al., this book). A typical faecal ovarian hormone profile

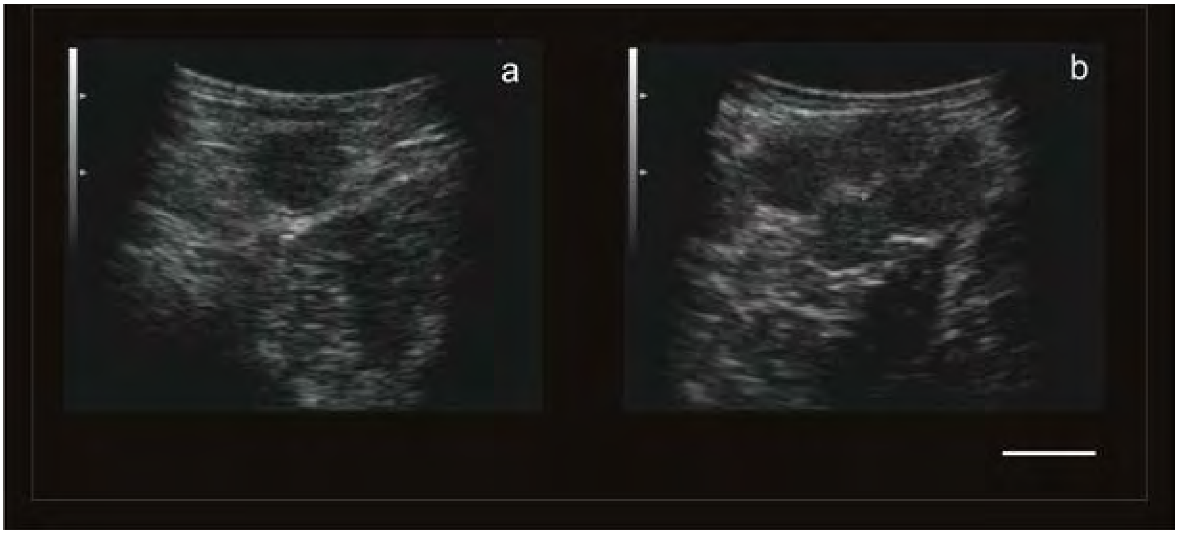

Figure 2. Sonograms of ovaries of two different adult Iberian lynxes out of breeding season in late November. The ovary of an animal in which no mating was observed during the breeding season appears inactive (a). The homogeneous parenchyma does not show any corpora lutea. The ovary of an animal in which mating, birth and lactation was observed (b) is characterised by multiple corpora lutea. They appear as spherical moderate echoic structures. Ovarian border and corpora lutea are indicated by arrowheads and numbers, respectively. The scale bar represents 1 cm.

Figura 2. Ecografía de los ovarios de dos hembras de lince ibérico fuera de la temporada de reproducción (a finales de noviembre). El ovario de una hembra que no se apareó aparece inactivo (a). El parénquima homogéneo no muestra ningún cuerpo lúteo. El ovario de un animal que se ha apareado, ha gestado y ha mostrado un periodo de lactación (b) se caracteriza por los múltiples cuerpos lúteos. Estos aparecen como estructuras esféricas moderadamente ecóicas. Los bordes del ovario y de los cuerpos lúteos aparecen indicados por cabezas de flecha y números respectivamente. La barra representan una escala de 1 cm.

in the Eurasian lynx included an absence of a significant estrogen or progesterone elevation before mating, a positive correlation between faecal progesterone and estradiol metabolites increases in both hormones during pregnancy, decreases towards parturition, and increases again during lactation period. To our knowledge, the existence of elevated progesterone during lactation and presumably active corpora lutea throughout much of the year is unique for felid species. A moderate serum progesterone concentration of about 5 ng/ml was measured out of breeding season in the present study (Table 1), although it was about four to 10 times higher during pregnancy (EL=61.1 ± 13.3 ng/ml; IL=17.0 ± 10.1 ng/ml; BC=28.4 ng/ml). The existence of large corpora lutea and increased volume of luteal tissue several months after parturition detected by ultrasound examination (Table 1) allows us to assume that progesterone is of luteal origin, although additional studies are required to confirm that the source is ovarian and not adrenal.

The low but steady progesterone concentration may function to induce a negative feed back to inactivate folliculogenesis. That would represent a mechanism to turn the normally polyoestric cycle seen in most felids into in a monoestric cycle in the lynx and thus contribute to the seasonality of its breeding pattern so as to match the seasonal sperm production in the males (Göritz et al., 2006b; Jewgenow et al., 2006b). It is known that long term application of exogenous progesterone derivates, frequently used in cats for contraception (Concannon and Lein, 1983; Concannon and Meyers-Wallen, 1991) suppress ovarian function, although they can also induce uterine pathology (Munson et al., 1993). The apparent correlation between estrogen and progesterone secretion suggested by current results may reflect the important role of estrogens, which might help maintain progesterone receptors and sensitivity for progesterone.

Juvenile animals presented small ovaries (mean ovarian volume in mm^3: 110.4 ± 70.5 in BC; 160.7 ± 91.3 in IL; 288.0 in EL) without corpora lutea and very low (<0.5 ng/ml) progesterone concentrations (Table 1). Adult lynxes without observed mating also had no corpora lutea (EL, BC) or only few (1.6) (IL), indicating that the most likely normal mechanism would be that of induced (reflex) ovulation. Whereas the EL may be an "obligate" induced ovulator, the IL seems able to ovulate without copulation in some instances. Mean number of corpora

Species	Age class (No. of animals)	Recent breeding history #	Mean (±SD) ovarian volume per animal [mm³]	Mean (±SD) No. of follicles per animal	Mean (±SD) No. of Cll and (cubs born) per animal	Mean (±SD) volume of luteal tissue [mm3] (No. of animals)	Mean (±SD) P4 [ng/mL] (No. of samples)	Mean (±SD) E2 [pg /mL] (No. of samples)
Bobcat	juvenile (n=1)		110.4 ± 70.5	0	0	0	0.4	0.1
	adult (n=2)	no mating	739.0	0	0	0	4.4 ± 2.5 (2)	1.7 ± 1.7 (2)
	adult (n=1)	mating, birth	2477.8 ± 2108	0	1.5 ± 0.7 (1.5 ± 0.7)	109.2 (1)	6.3	1.8
	adult (n=1)	pregnancy* (3th week, June)	n.d.	n.d.	n.d.	n.d.	28.4	1.15
European lynx	juvenile (n=1)		288	0	0	0	n.d.	n.d.
	adult (n=2)	no mating	1703.7 ± 1216.7	0	0	0	n.d.	n.d.
	adult (n=1)	mating, no birth	2843.8 ± 1637	2 ± 1.4	4 ± 1.4	1342.5 ± 286.6 (2)	3.9 ± 0.9 (2)	1.2 ± 0.1 (2)
	adult (n=17)	mating, birth	2315.8 ± 1131.1	0.5 ± 1.1	3.3 ± 0.8 (2.6 ± 1.2)	2067.3 ± 743.4 (8)	4.9 ± 2.3 (16)	0.8 ± 0.4 (10)
	adult (n=2)	pregnancy* (3th week, May)	n.d.	n.d.	n.d.	n.d.	61.1 ± 13.3 (2)	n.d.
Iberian lynx	juvenile (n=4)		160.7 ± 91.3	0	0	0	0.5 ± 1.0 ^{a} (15)	0.6 ± 0.6 (13)
	adult (n=3)	no mating	221.3 ± 167.7	0.3 ± 0.6	1.6	0	2.3 ± 0.2 (2)	n.d.
	adult (n=9)	mating, no birth	994.9 ± 929	0	3.8 ± 1.2 ^{a}	1082.0 ± 584.5 (6)	4.3 ± 2.1 ^{b} (9)	3.7 ± 6.4 (8)
	adult (n=19)	mating, birth	1319.7 ± 825.4	0.7 ± 0.5	5.3 ± 1.2 ^{b} (2.1 ± 0.3)	1441.8 ± 581.8 (14)	4.3 ± 1.4 ^{b} (19)	2.8 ± 3.5 (14)
	adult (n=14)	pregnancy* (3th-8th week, March)**	n.d	n.d.	n.d.	n.d.	17.0 ± 10.1 ^{c} (14)	n.d.

\# breeding history in the year of examination; no ultrasound examination was conducted during pregnancy
* blood collection by venepuncture under general anaesthesia
** non invasive blood collection with blood suckling bugs (Braun et al., 2008)
§ volume of luteal tissue calculated for each animal ($V=4/3\,\pi$ * mean Cl radius^3 * No of Cll); Cl=corpus luteum, Cll=corpora lutea
n.d. not determined
a, b, c different superscript indicate significant differences between the means estimated by Welch unpaired t-test corrected

Table 1. Individual morphological and endocrinological parameters (Mean ± SD) describing the ovarian activity of the bobcat, the Eurasian and the Iberian lynx at the time of ultrasound examinations conducted out of breeding season (from June to November).

Tabla 1. Parámetros morfológicos y endocrinológicos (media y ± DE) que describen la actividad ovárica del lince rojo, del lince euroasiático y del lince ibérico durante las ecografías realizadas fuera de la época reproductora (de junio a noviembre).

lutea detected ultrasonographically after parturition was 3.3 and 5.3 in EL and IL, respectively (Table 1). While in the EL the number of corpora lutea was generally consistent with the number of cubs born, there were always more corpora lutea detected than cubs born in the IL (mean litter size in EL=2.6 and in IL=2.1, respectively). Whether this is caused by absence of fertilization of oocytes after ovulation or by early embryonic resorption or possibly by lutenization of antral follicles and subsequent development of accessory corpora lutea is yet uncertain. Further investigations are necessary to reveal this aspect of IL reproductive physiology.

Conclusions

Fundamental knowledge of basic reproductive physiology is essential to improve reproduction in captivity by assisted reproduction and gamete banking (Roldan et al., this book). Our comparative approach is very useful to gain new insights into the reproductive pecularities of both Eurasian and Iberian lynx. Since frequent examination procedures are not possible in the endangered Iberian lynx, the Eurasian lynx can serve as a model species. Assessment of reproductive health by transrectal ultrasound was applied to study seasonal changes in male and female Eurasian lynx. This knowledge was essential to assess the reproductive soundness and physiology of the Iberian lynx. The ultrasonographical findings strongly suggest that corpora lutea developed from ovulations in mid-winter and stayed active until at least late November. Their functional role on lynx reproduction is still unknown, but we hypothesize that the associated elevated progesterone may support lactation, prevent a new estrus cycle and thus restrict the breeding season to midwinter (as opposed to the strategy used by the polyoestric bobcat). Further comparative longitudinal ultrasound and hormone evaluations on all species within the lynx family are needed to further elucidate their unique reproductive patterns.

Acknowledgements

We thank the Andalusian Goverment for providing the samples and all the staff and volunteers at the El Acebuche, Jerez Zoo and La Olivilla Breeding Centres for their every day engagement with the Iberian lynx Conservation Breeding Programme. Our research with free-raging Iberian lynx was partially funded by the projects CGL2004-00346/BOS (Spanish Ministry of Education and Science) and 17/2005 (Spanish Ministry of the Environment; National Parks Research Programme). JVLB was supported by a FPU fellow (Ministry of Education). We also thank K. Paschmionka, A. Frank and M. Rohleder (IZW) for their excellent technical assistance.

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Photo: Antonio Rivas

It takes two days to learn everything about a man;
to know animals you will need more time.

Iranian Proverb

Pregnancy diagnosis in Iberian lynx *(Lynx pardinus)* based on urinary and blood plasma hormones

Diagnóstico de gestación en el lince ibérico *(Lynx pardinus)* basado en la presencia de hormonas en orina y plasma sanguíneo

Katarina Jewgenow, Beate C. Braun, Frank Göritz, Christian C. Voigt, Fernando Martínez, Lourdes Anaya, Astrid Vargas and Martin Dehnhard

Resumen

El Programa de Conservación *Ex situ* del Lince Ibérico constituye una parte esencial de un Plan de Acción coordinado para conservar a este felino amenazado. Para llevar a cabo con éxito el Programa de Cría en Cautividad es necesario disponer de sistemas de diagnóstico de gestación fiables y no invasivos. Durante tres años de estudio, se tomaron muestras de orina de seis hembras de lince ibérico en cautividad (un ciclo sin gestación, un ciclo de pseudogestación y 11 ciclos de gestación). Se determinaron los niveles de progesterona y estradiol en extractos de orina y se caracterizaron los metabolitos relevantes de estrógenos en orina mediante HPLC. Además, se utilizó el test comercial Witness® Relaxin para detectar la presencia de la hormona relaxina en orina, así como en muestras de sangre obtenida mediante chinches hematófagos.

En el lince ibérico el perfil de la progesterona en orina no mostró el patrón habitual de aumento durante la gestación que se observa generalmente en otras especies de felinos. Sin embargo, sí se observó un incremento de estrógenos en orina, que aumentaron de 3.8 ± 0.6 a 8.6 ± 0.5 ng/mg creatinina ($P<0.001$) durante la gestación. Los niveles de estrógenos fueron más elevados en las hembras gestantes que en las que presentaban pseudogestación ($P<0.05$). En una hembra que no copuló no se observó ninguna diferencia en los niveles de estrógenos entre la estación reproductiva y la no reproductiva. Aunque el test Witness®Relaxin fue positivo en muestras de plasma obtenidas mediante chinches hematófagos a partir de hembras entre los 32 y 56 días de gestación, la prueba no funcionó en muestras de orina obtenidas durante el mismo intervalo de tiempo. Tras concentrar la orina por ultrafwiltración (>50 x), se observó una leve reacción en el test Witness®Relaxin en muestras obtenidas entre los 29 y los 46 días de gestación. En muestras de orina concentrada obtenidas a partir de hembras no embarazadas y de hembras al inicio de la gestación los resultados de la relaxina fueron negativos. Sin embargo, se debe juzgar con precaución cualquier resultado negativo en la prueba de relaxina, dado que los niveles de esta hormona podrían situarse por debajo del umbral de detección del test.

Palabras clave

Relaxina, lince ibérico, diagnóstico de gestación, orina, suero, test Witness Relaxin®

Abstract
The Iberian lynx *Ex situ* Conservation Programme is an essential part of a coordinated Action Plan to conserve this endangered felid. Successful Captive Breeding demands reliable methods for monitoring reproduction, including reliable non-invasive pregnancy diagnosis. During a three year study, urine samples from six captive Iberian lynx females were obtained (1 non-pregnant, 1 pseudo-pregnant and 11 pregnant cycles). Progesterone and estradiol were determined in urinary extracts and relevant urinary estrogen metabolites were characterized by HPLC. Additionally, the Witness® Relaxin test was used to determine relaxin in urine samples and blood plasma collected by blood suckling bugs.

Urinary progesterone did not follow the typical pregnancy-related course of felids. In the Iberian lynx we failed to demonstrate a progesterone elevation during pregnancy. In contrast, urinary estrogens increase from 3.8 ± 0.6 to 8.6 ± 0.5 ng/mg creatinine ($P<0.001$) throughout the gestation period. A comparison of pseudo-pregnant with pregnant cycles revealed a further increase of estrogens caused by implantation ($P<0.05$). In one female that failed to mate, no difference was detected between estrogen levels during breeding and non-breeding season. Although the Witness®Relaxin test was positive in plasma samples collected from animals between days 32-56 of pregnancy, it failed to detect relaxin in urine samples collected from the same stage of pregnancy. A weak relaxin reaction in urine samples collected from animals between days 29-46 of pregnancy was detectable after urines were concentrated by ultrafiltration (>50 x). Concentrated samples obtained from non-pregnant and early pregnant animals yielded negative test results. In conclusion, urinary estrogens and the Witness® Relaxin test can be applied for pregnancy diagnosis in the Iberian lynx. For estrogens, serial sampling is necessary, so we recommend relaxin as the hormone of choice to detect pregnancy in the Iberian lynx, since a single positive signal already indicates the presence of a placenta and, thus, an ongoing gestation. A negative relaxin test, however, must be judged carefully because hormone levels might be below the detection level.

Keywords
Relaxin, Iberian lynx, pregnancy diagnosis, urine, serum, Witness® Relaxin test

Photo: José María Pérez de Ayala

Pregnancy diagnosis in Iberian lynx *(Lynx pardinus)* based on urinary and blood plasma hormones

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Introduction

The Iberian lynx *Ex situ* Conservation Programme is an essential part of a coordinated Action Plan to conserve this highly endangered species (Vargas et al., 2008). Besides ensuring the existence of the Iberian lynx in captivity, the *Ex situ* Programme has the benefit of allowing the study of various aspects of the specie's biology and physiology that could not easily be studied in the wild. One of such aspects is the understanding of reproductive physiology and the development of methods for non-invasive monitoring of the reproductive status (Braun et al., 2008; Dehnhard et al., 2008; Jewgenow et al., 2006; Dehnhardt et al., this book; Pelican et al., this book). Pregnancy diagnosis by monitoring ovarian physiology is of particular importance in this respect, particularly as a management tool for the Captive Breeding Programme. In lynx, however, fecal steroids do not follow the typical pregnancy pattern of felids (Brown et al., 1994b; Pelican et al., this book; Dehnhardt et al., this book) and, thus, have been considered an inappropriate method for monitoring reproductive activity.

Alternatively to fecal hormone metabolites, urine has been often used for tracking sexual hormones. Steroid hormones are secreted via the kidney into urine, mainly conjugated as sulfates or glucuronides. Those products are more water-soluble than the parent steroids (Dehnhard et al., 2006; Taylor, 1971). Additionally, several peptide hormones, like luteinizing hormone (LH), human chorionic gonadotropin (hCG) and relaxin, have been detected in urine and could be related to sexual activity or pregnancy status (Canfield et al., 1987; Steinetz et al., 1996; Cole, 1997; Jeffcoate and England, 1997). Relaxin, which is produced mainly by the placenta (Addiego et al., 1987), is a useful marker for pregnancy diagnosis. In the domestic cat, relaxin increases at the beginning of the second trimester (day 20 to 25) reaching a plateau and a subsequent decrease 10 to 15 days before parturition (Stewart and Stabenfeldt, 1985). Recently, de Haas van Dorsser et al. (2006) have shown that relaxin is detectable in urine of pregnant domestic cats and leopards. Furthermore, a bench top kit (Witness® Relaxin) was successfully used for pregnancy detection in urine collected from domestic cats (Haas van Dorsser et al., 2007). Since urine collection from captive Iberian lynx was established to supply urine for camera trapping and identification of free-ranging lynxes and it is performed on a regular basis at the Ex situ Breeding Programme, the aim of the present study was to test whether urine could be effectively used for pregnancy diagnosis. Our study also focused on looking for a relaxin signal in serum obtained using a minimally invasive method that involved the use of triatomine, blook-sucking, insects (Voigt et al., 2004).

Material and methods

The Iberian lynx maintained under the umbrella of the *Ex situ* Conservation Programme are kept in three breeding centers (Vargas et al., 2008; Vargas et al., this book). This study took place at the El Acebuche Captive Breeding Center in Doñana National Park, in Southern Spain. The captive population consisted of 16 animals (seven males, nine females) in 2006 and of 52 (28 males, 24 females) in 2008 (Vargas et al., this book). The first birth in captivity was in 2005, since then 24 cubs have been raised in the Breeding Programme. Animals were kept in separate enclosures (550 m^2), and during mating season (January to February) all females were allowed to mate by introducing a male into the female's enclosure. Depending upon the female's behavior, the couples were maintained together for several weeks or just for the copulatory period. All behavioral interactions were recorded by remote video cameras (Vargas et al., this book). Mating was documented in all females, and the cycle was considered to involve a pseudo-pregnancy if no parturition or abortion was observed (Göritz et al., this book). Delivery of cubs was the final indication of pregnancy.

Blood plasma collection (adapted from Braun et al., 2008)

During two breeding seasons, plasma samples from five pregnant females were collected with triatomine, blood-suckling bugs (Voigt et al., 2004). Prior to the test, Iberian lynx females were encouraged to use cork plates as resting spots. As part of the daily routine, keepers would handle the corks so females would not be suspicious when the bugs were finally placed inside the corks (Figure 1). Since all enclosures were equipped with cameras, the time span during which each individual rested on the cork plate was monitored (Figure 2). After 30 min of uninterrupted resting (generally sleeping) on the plates, females were removed from the enclosure and bugs were taken out of the cork holes. Blood samples obtained from each bugs ranged from 0.5-2 ml (Figure 3). If samples were drawn carefully, bugs could be reutilized for other purposes, although never to draw blood again from another potentially pregnant Iberian lynx female. Blood was centrifuged to obtain plasma, a few drops of which were used for pregnancy diagnosis via Witness® Relaxin, while the remaining sample was sent for additional hormone and antibody analyses.

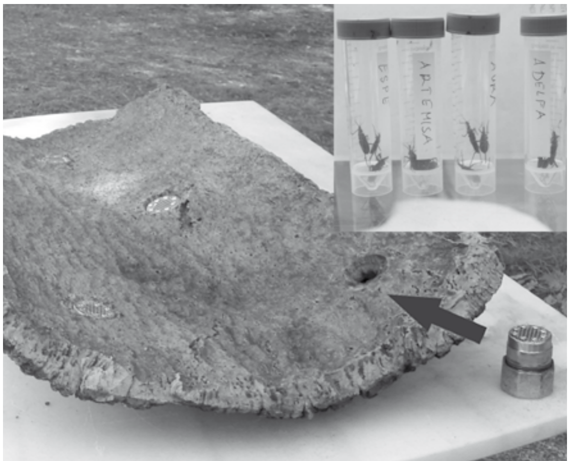

Figure 1. Cork plates, which lynxes use as resting spots, are equipped with holes for special containers containing blood-suckling bugs (arrow). As part of the daily routine, keepers would handle the corks so females would not be suspicious when the bugs were finally placed inside the corks. After installing the bugs into the cork plate, the bugs pierce its proboscis through the mesh, which closes the top of the container, to draw blood from a lynx resting on the cork plate.

Figura 1. Las planchas de corcho que los linces utilizan como lugares de descanso se modifican con agujeros donde se insertan contenedores especiales que sirven para ubicar a los insectos triatóminos (flecha). Como parte de la rutina diaria, los cuidadores manejan los corchos para que los linces no "sospechen" en el momento en el que finalmente se introducen los insectos. Una vez que los insectos están en las planchas de corcho, los insectos sacan su probóscis a través de la malla que cubre la parte superior del agujero y obtienen una muestra de sangre del lince mientras éste descansa en su corcho.

Figure 2. Iberian lynxes were monitored with cameras during the "bug sampling" procedure. On the computer monitor four different "houses" of the enclosures are visible. The upper screens show two resting sites equipped with a cork plates. The lower screens show females on the cork plates. The female on the left is resting. The one on the right lower screen is being released to the outside enclosure after 30 min motionless resting.

Figura 2. Durante el procedimiento "muestreo con insectos", se hizo un seguimiento de los linces mediante videovigilancia. En el monitor del ordenador se observan cuatro compartimentos diferentes de las instalaciones. En las dos pantallas superiores se ven las planchas de corcho con los agujeros que contienen los insectos. Las pantallas inferiores muestran las hembras sobre las planchas de corcho. La hembra de la izquierda está descansando; la de la derecha está saliendo hacia el cercado exterior después de un descanso de 30 minutos sin movimiento.

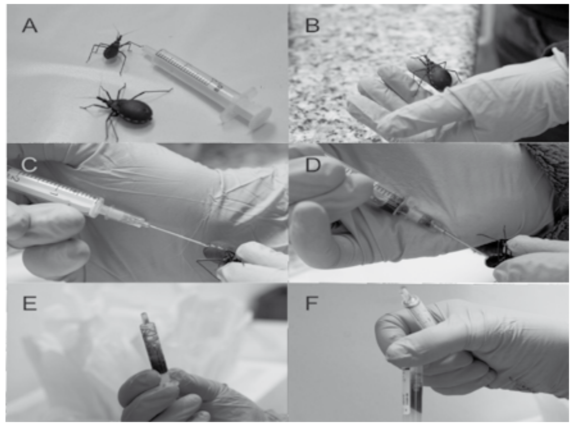

Figure 3. Sampling procedure from the filled blood suckling bug (A-F). (A): Triatomine bugs before (in the back) and after (in front) blood suckling. (B): Bug filled with ca. 1 ml blood. (C-D): Collection of ingested blood was performed with a 2 ml syringe. (E): After determination of volume, the samples was transferred to a serum tube (F) and centrifuged for plasma collection. An aliquote of "Bug" plasma was directly subjected to the Witness® Relaxin test.

Figura 3. Procedimiento de muestreo para extracción de sangre del insecto triatómino (A-F): (A): Chinche antes (al fondo) y después (al frente) de succionar la sangre. (B): Insecto tras ingerir 1 ml de sangre. (C-D): La extracción de sangre se lleva a cabo con jeringas de 2 ml. (E): Tras determinar el volumen, las muestras se transfieren a un tubo de desuerado (F) y se centrifugan para la recolección del plasma.
Una alícuota de plasma obtenido mediante el insecto se utiliza para realizar el test Witness® de relaxina.

Urine sampling and reproductive status (adapted from Jewgenow et al., in press)

Urine was collected from six different females by placing homemade collectors in their enclosures. The collectors consisted of vertical stainless steel plates (60 x 60 cm) ending in gutters at the bottom and a slight v-shape inclination that allowed the urine to run into a collector cup. Lynxes used these plates to mark their territories (Figure 4). Urine samples (10 to 50 ml) were collected daily and stored at -20 °C. During the breeding season (January to April), samples were obtained 2-3 times per week, for the rest of the year at least one sample per month per animal was used. Table 1 presents the samples available for this study.

For steroid analysis, urine aliquots of 100 µL were hydrolysed, extracted and subjected to hormone assays

Figure 4. An Iberian lynx marks the urine collector in its enclosure. The urine was freshly collected for relaxin determination.

Figura 4. Un lince ibérico marca el colector de orina ubicado en su instalación. La orina se recoge en fresco para realizar el test de relaxina.

	Number of females (n=number of samples)			
Status	non-pregnant	pregnancy	pseudo-pregnancy §	non-breeding
Season	breeding	breeding	breeding	outside breeding season
Mating	no mating	mating	mating	-
Parturition	no	cubs born	no cubs	-
2006	-	2 (n=48)	1 (n=18)	3 (n=45)
2007	-	6 (n=143)*	-	6 (n=240)
2008	1 (n=20)	3 (n=105)	-	4 (n=62)
Σ	1 (n=20)	8 (n=237)# 3 (n=59)*	1 (n=18)	13 (n=347)

§ The pseudo-pregnancy might be the result of either an infertile mating or early embryonic death.
*** Three females were kept together with their mates during the entire pregnancy.**
 # Number of females kept during sample collection alone (n=pure female urine samples).

Table 1. Collection of urine samples from female lynx at the El Acebuche Iberian Lynx Captive Breeding Center during breeding (January-April) and non-breeding (May-December) seasons 2006 to 2008 (Jewgenow et al., in press).

Tabla 1. Recogida de muestras de orina de hembras de lince en el Centro de Cría en Cautividad El Acebuche durante las épocas reproductivas (enero-abril) y no reproductivas (mayo-diciembre) entre 2006 y 2008 (Jewgenow et al., en imprenta).

as described before (Jewgenow et al., in press). Urinary hormone concentrations are expressed as ng per mg creatinine in order to control for differences in urine concentration.

Progesterone (P4) determination

Urinary progesterone was determined by enzyme immunoassays based on a rat antibody (Sigma P1922, generated to progesterone) together with 4-pregnen-3,20-dione-3-CMO-peroxidase as label (Göritz et al., 1997; Dehnhardt et al., 2006). The cross-reactivity's of both antibodies and their inter- and intra-assays coefficients were as described before (Braun et al., 2008).

Estrogen (E2) determination in urine samples

Estrogen analyses were carried out with an in-house microtitre plate enzyme immunoassay using a polyclonal antibody (rabbit) against 1,3,5(10)-estratrien-3,17ß-diol-17-HS-BSA and 1,3,5(10)-estratrien-3,17ß-diol-17-HS-peroxidase label (Meyer et al., 1997). The cross-reactivities of antibodies and inter- and intra-assay coefficients were as described before (Jewgenow et al., in press).

Ultrafiltration of urine samples for Relaxin determination

Urine samples were subjected to ultrafiltration (Braun et al., 2008). In brief, microcon YM50 unit (Millipore®, USA) were used to separate high molecular proteins by centrifugation (14000 x g, 4 °C). The flow through (low molecular peptides) was subjected to a second ultrafiltration step with a centricon YM3 (Millipore®, USA) separating proteins larger then 3 kDa molecular weight (7500 x g, 4 °C). At this stage, relaxin and other 3-50 kDa proteins were supposed

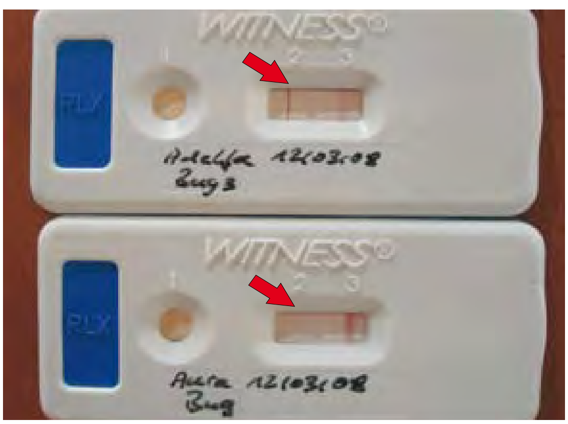

Figure 5. Witness® Relaxin tests were performed with bug ingested lynx blood plasma or highly concentrated urine samples (x 50). The positive lines (arrows) are an indication for relaxin in the sample, whereas a missing signal in line 2 must be judged carefully, because the hormone levels might below the detection level.

Figura 5. Las pruebas Witness® de relaxina se llevaron a cabo con plasma obtenido a través de sangre de lince ingerida por insectos triatóminos o mediante muestras de orina altamente concentradas (x 50). Las líneas positivas (flechas) indican la presencia de relaxina en la muestra, mientras que la falta de señal en la línea 2 debe ser interpretada con cautela.

to be retained on the membrane. Samples were centrifuged (after washing with PBS) until almost no further volume decrease was attained. Finally, urine samples were concentrated up to ninety times of the original urine volume.

Test with Witness® Relaxin (Synbiotics Corporation)

Blood plasma samples drawn from bugs were directly subjected to Witness® Relaxin test according to the manufacture's instructions. In brief, two drops of plasma were transferred to the sample well followed by two drops of the provided buffer. Within the reading window, the result of the test was visible after at least 15 minutes (Figure 5). The appearance of a specific relaxin and a control band was judged as an indication of relaxin in blood serum. A missing relaxin band after a prolonged time (1 hour) was an indication of a negative test result.

Urine samples were processed as described by de Haas van Dorsser et al. (2007). Thus, urine was mixed with equal amounts of lynx blood serum (serum from a non-pregnant Iberian lynx female obtained during chemical immobilisation in November 2006) before it was subjected to the Witness® Relaxin test. In case of concentrated urine samples, they were diluted with of blood serum from a non-pregnant Eurasian lynx and 64 µL of the urine-serum mixture (estimated volume of two normal blood drops) was added to the sample well followed by two drops of the provided buffer. Positive relaxin signals were seen in the reading window within at least 20 minutes after application; control bands were visible in every test.

Statistics

The results of urinary steroid hormone determination were expressed as immunoreactive steroid in ng per mg creatinine. Data presented as means ± standard errors of the mean (SEM). Comparisons of means values were performed by Welch corrected unpaired t-Test. All statistical tests were based on a 5% level of significance. The statistical procedures were performed with the software programme Instat Version 3 (Graphpad Software Inc.).

Results and discussion

Urinary P4 levels did not reveal a distinct increase during pregnancy compared to non-breeding season levels (Table 2). Also, no difference was found in progestin concentrations when non-pregnant (no mating), pregnant

Immune reactive steroid ng per mg creatinine (number of samples)	Outside breeding season Means (± SEM)	Pregnancy (day 1 to 64) Means (± SEM)	Unpaired t test with Welch correction
Progesterone (n)	1.8 ± 0.05 (347)	2.4 ± 0.1 (237)	t =1.93, P > 0.05
Estrogens (n)	8.6 ± 0.6 (342)	8.6 ± 0.5 (237)	t=14.28, (P<0.001)

Table 2. Determination of steroid concentration in urine samples collected from female Iberian lynx outside the breeding season, either before or after pregnancy, versus during pregnancy. Mean values were calculated for all samples available not discriminating between individuals (Jewgenow et al., in press).

Tabla 2. Determinación de la concentración de esteroides en las muestras de orina recogidas de hembras de lince ibérico en época no reproductiva, antes o después de la gestiación, comparado con las recogidas durante la gestación. Se calculó el valor medio de todas las muestras, sin distinguir ejemplares. (Jewgenow et al., en imprenta).

		Progesterone in ng/mg creatinine (n samples)	Estrogens in pg/mg creatinine (n samples)
Artemisa	Outside breeding season (May – Dec)	2.0 ± 0.2 (47)	2.8 ± 0.2 (47) [A,B]
	# Pregnancy 2007	1.0 ± 0.7 (16)	46.8 ± 2.7 (15)[A,C]
	Breeding season 2008 (Jan – April)	1.5 ± 0.3 (20)	0.8 ± 0.2 (20) [B,C]
Aura	Outside breeding season (May – Dec)	1.9 ± 0.1 (97)	3.9 ± 0.3 (96) [D,E]
	Pseudo-pregnancy (mating, no birth)	4.4 ± 1.4 (18)	9.2 ± 1.0 (18) [D,F]
	Pregnancy 2007/2008	3.0 ± 0.2 (40)	12.1 ± 1.3 (38) [E,F]

 # Sample collection was performed in the presence of male.
a-f Identical superscripts indicate statistical differences between estrogen concentrations estimated by unpaired t test with Welch correction.

Table 3. Urine estrogens and progesterone in urine samples of a non-mated *(Artemisa)* and pseudo-pregnant *(Aura)* in relation to samples collected outside breeding season and pregnancy (Jewgenow et al., in press).

Tabla 3. Estrógenos y progesterona urinaria de una hembra no apareada *(Artemisa)* y una pseudogestante *(Aura)*, comparadas con las muestras recogidas fuera de la época de reproducción y de embarazo (Jewgenow et al., en imprenta).

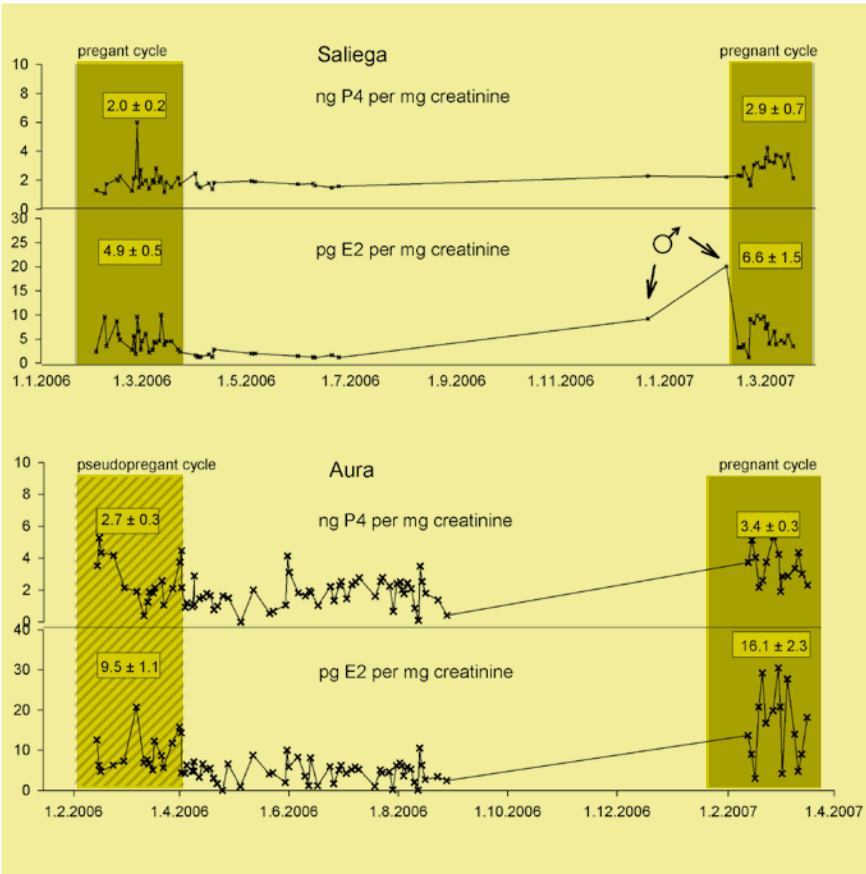

Figure 6. Determination of progesterone (P4) and estradiol (E2) in extracts of urine collected from female Iberian lynx. As an example, seasonal patterns of two adult females –*Saliega* (upper panel) and *Aura* (lower panel)– are presented. *Saliega* was pregnant (grey block) in 2006 and 2007, whereas *Aura* mated in 2006, but did not conceive (pseudo-pregnancy, lined block). In 2007, she delivered three healthy cubs after 64 days of pregnancy (green block). Arrows indicate the presence of a male sharing the enclosure with the female.

Figura 6. Determinación de la progesterona (P4) y el estradiol (E2) en extractos de orina de hembras de lince ibérico. Como ejemplo se muestran los patrones estacionales de dos hembras adultas –*Saliega* (panel superior) y *Aura* (panel inferior). *Saliega* quedó gestante (bloque gris) en 2006 y 2007, mientras que *Aura* se apareó en 2006 pero no quedó gestante (pseudogestación, bloque con rayas). En 2007, *Aura* tuvo tres cachorros sanos tras 64 días de gestación (bloque verde). Las flechas indican la presencia de un macho compartiendo instalación con la hembra.

and pseudo-pregnant lynx cycles were compared directly within the same animals in different years (Table 3, Figure 6, *Aura*). The mean progesterone concentrations were slightly lower, when no mating occurred (Table 3, *Artemisa*), but progesterone levels of pseudo-pregnant and pregnant cycles did not differ significantly. Thus, urinary progesterone may indicate the existence of corpora lutea after induced ovulation, but a reliable pregnancy diagnosis based on progesterone was unattainable. In this respect, urinary progesterone follows the pattern described for fecal progestagen excretion in Iberian lynx females (Pelican et al., this book). In contrast to many other felid species (Brown et al., 1994a), pregnancies in Iberian lynx are not characterized by elevation of either fecal (Pelican et al., this book) or urinary progesterone concentration (this study). We suggest that this might be the consequence of the prolonged presence (and function) of corpora lutea throughout most of the year (Kvam, 1990; Göritz et al., this book). Thus, a permanent progestin production may ensure the strong seasonality in lynx (but masks any changes associated with the luteal phase of pregnancy).

In most felids, increased estradiol excretion is associated with estrus behaviour or exogenous gonadotropin treatment (Brown et al., 1994b). Unfortunately, the collection of urine samples from individual females during the period of mating was impossible, since the husbandry scheme allows breeding pairs to share enclosures until two weeks before parturition. Therefore, we were unable to detect a mating-related urinary estrogen peak. Urine samples collected from females with males were characterized by several-fold higher ($t=35.2$, $P<0.0001$) estrogen concentrations (66.8 ± 5.7 ng E2 per mg creatinine; $n=59$) in comparison to pure female urine samples (means of all females: 5.7 ± 0.2 ng E2 per mg creatinine, $n=611$). The elevated estrogen concentrations in mixed male and female urine were found not only during co-housed pairs immediately before and during the mating period (and thus possibly related to estrus of the female), but was also seen when the couples were left together during pregnancy (three females in 2007, Table 1; *Artemisa*, Table 3). It is likely that urine from male

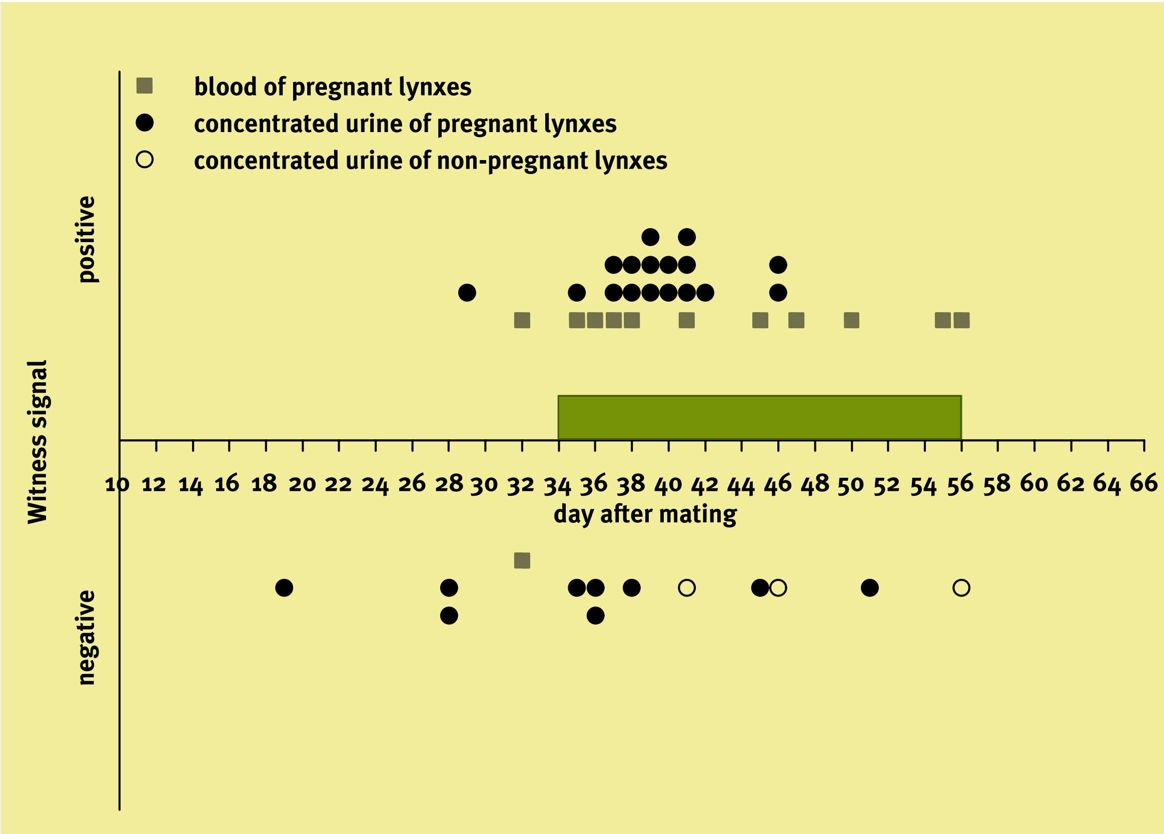

Figure 7. Witness®Relaxin test results of urine and blood samples of pregnant and non-pregnant Iberian lynxes. Pregnancy could by diagnosed with blood samples –obtained via triatomine bugs– between days 31 and 52 after the first mating. The one pregnant animal which shows a negative signal at day 31 (marked by asterisk) was later detected as pregnant via urine test in day 42. Her urine sample of day 38 after mating (marked by an asterisk) was negative too. Urine samples of other pregnant females showed positive signals with samples that were collected between days 37 to 47 after mating. Urine samples of non-pregnant lynxes in this period also yielded negative results.

Figura 7. Resultados de la prueba Witness® Relaxin realizada en muestras de orina y sangre de hembras de lince ibérico gestantes y no gestantes. La gestación de las hembras de lince pudo ser diagnosticada en muestras sanguíneas –obtenidas mediante insectos triatóminos– entre los días 31 y 52 después del primer apareamiento. La única hembra gestante que dio un resultado negativo en el día 31 tras la primera cópula observada (marcada con asterisco) dio posteriormente un resultado positivo a la relaxina en una muestra de orina tomada el día 42, siendo negativa también su muestra de orina del día 38 después del apareamiento (marcado con asterisco). Las muestras de orina de otras hembras gestantes fueron positivas a la relaxina cuando fueron recogidas entre los días 37 y 47 después del apareamiento. Las muestras de orina de las hembras no gestantes durante este período dieron resultados negativos.

lynx contains estrogen concentrations that could be up to 10-fold higher than female urine. Within the Iberian Lynx Captive Breeding Programme the collection of samples to monitor the onset of estrus in the absence of males was not possible, since all females were sharing enclosures with males prior to entering estrus. Yet, such information would be valuable for further characterization and for better understanding the onset of the Iberian lynx breeding season.

Nevertheless, in the absence of males, estrogens were still significantly elevated ($P<0.001$) during pregnancy (8.6 ± 0.5 ng E2 per creatinine, mean of all animals, Table 2) when compared to samples collected out of the breeding season (3.8 ± 0.2 ng E2 per mg creatinine). Figure 6 also demonstrates the considerable day-to-day variability of urinary estradiol concentrations and emphasizes the need for frequent sample collection in order to detect Iberian lynx pregnancies and to avoid potentially false negative results (E2 below 5 ng per mg creatinine).

The results from the female *Artemisa* (Table 3) showed a significant difference ($P<0.001$) in urinary estrogens between her non-breeding (2.0 ± 0.2 ng E2 per mg creatinine) and breeding (1.0 ± 0.7 ng/mg creatinine) seasons, a year in which she did not accept any male for copulation and, thus, did not have the chance to get pregnant.

Unfortunately, during her pregnancy in 2007, this female was kept together with a male and data on urinary estrogens (46.8 ± 2.7 ng E2 per mg creatinine, Table 3) were not suitable for comparative analysis. Although samples from only one female that failed to copulate were available, the low estrogen levels in *Artemisa* during the 2008 breeding season are an indication of the missing ovulation and corpora lutea (CL) formation.

We conclude that Iberian lynx are induced ovulators as described for many other felid species (Brown and Wildt, 1997). In contrast, the direct comparison of pregnant cycles (12.1 ± 1.3 ng E2 per mg creatinine) with pseudo-pregnant cycles (9.2 ± 1.0 ng E2 per mg creatinine) revealed a further increase of urinary estrogens caused by implantation (*Aura*, Table 3, Figure 6). Thus, if mating occurs, corpus luteum (Cl) formation is evident by elevated estrogens and ultrasound examination (Göritz et al., this book). The increase in estrogens in pregnant versus pseudo-pregnant cycles, might be either explained by a shorter half-life of the Cl in pseudo-pregnant cycles, as described for domestic cats, (Tsutsui and Stabenfeldt, 1993) or by estrogen production by fetal structures (placenta), which is physiological in other species, such as cattle (Hoffmann et al., 1997), pigs (Knight and Kukoly, 1990) and horses (Möstl, 1994).

The Witness®Relaxin test (Figure 5) yielded positive results in plasma samples in four out of five animals in 2007 and in all seven animals tested in 2008 (Figure 7). The positively tested animals were all between day 32 and 56 of pregnancy, of a 63-66 day gestation period (Vargas et al., this book). The animal whose plasma yielded negative Witness®Relaxin test results, was at day 32 after her first mating, although later she was proven to be pregnant. In contrast to urine of domestic cats (de Haas van Dorsser et al., 2006), Witness®Relaxin test failed in both fresh and frozen native urine samples. Only when urine samples were concentrated by ultrafiltration at least 50x, a weak relaxin positive reaction was observed between day 29 and 46 of the pregnancy (n=17). No urinary relaxin signal from pregnant animals was detected in some occurrences (n=5); these urines were collected on days 35, 36, 38 and 45 from pregnant animals (Figure 7). These failed pregnancy diagnosis might be explained by the ongoing degradation of relaxin within the urine samples (late collection) and/or insufficient level of the ultrafiltration procedure. Thus, the urinary relaxin was still under the detection level of Witness®Relaxin test. All concentrated urine samples from non-pregnant females yielded negative relaxin results, as well as did those urine samples of pregnant females in early (before day 29) and late (after day 50) gestation (Figure 7).

Conclusions

The present results suggest that pregnancy-related hormone activity in Iberian lynx can be monitored by urinary estrogens if samples are collected frequently and in the absence of males in the enclousure. In contrast to urinary progestagens, estrogens reflect Cl formation after ovulation. Furthermore, the observed increase of urinary E2 after mating and during late pregnancy suggest either an E2 secretion from the lynx placenta and/or a pregnancy-specific enhanced luteal secretion of estrogen, a point for additional study. In addition, pregnancy diagnosis is attainable by applying the Witness® Relaxin bench top test, if blood plasma or highly concentrated urine samples are used. Relaxin can be detected in a certain period during pregnancy, which ranges between 34 to 50 days after mating for blood plasma, and day 37 to 46 post-mating days for urine samples, respectively.

Acknowledgements

We thank all the staff (T. Rivas, J. Bergara, T. Váquez, J. Pardo, E. Vázquez, J. López, M. Chaparro, J.M. Rodríguez, and D. Rodríguez) and volunteers at the El Acebuche Breeding Centre for their every day engagement within the Iberian Lynx Conservation Breeding Programme. We also thank A. Frank, K. Paschmionka and M. Rohleder (IZW) for their excellent technical assistance. We appreciate the support of the Environmental Council of the Andalusian Government and the Spanish Ministry of the Environment.

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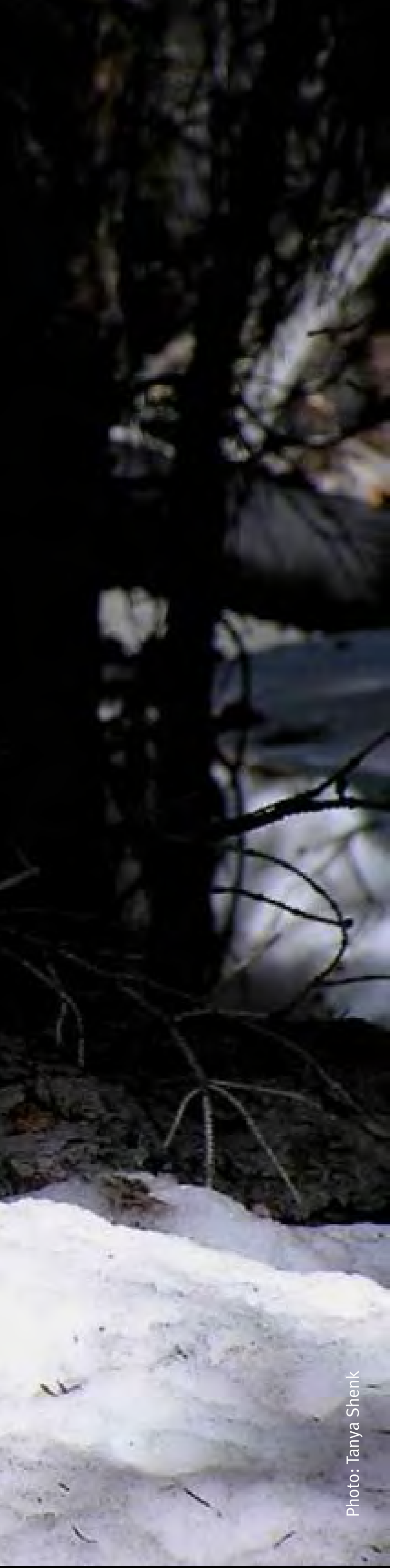
Study nature, love nature, stay close to nature.
It will never fail you.

Frank Lloyd Wright
(1867-1959)



Reproductive physiology of Canada lynx *(Lynx canadensis)*

Fisiología reproductiva del lince canadiense *(Lynx canadensis)*

Kerry Fanson, Nadja Wielebnowski and Jeffrey Lucas

Resumen

Este estudio tiene como objetivo validar las pruebas para la detección de distintos metabolitos hormonales en heces de lince canadiense y obtener conocimientos básicos sobre su fisiología reproductiva. Mediante las técnicas de laboratorio empleadas en este estudio se consiguió validar la metodología para detectar los andrógenos fecales en los machos, y los análisis revelaron un incremento estacional claro en los niveles de andrógenos antes y durante la época de reproducción. La validación de ensayos para estrógenos y progestágenos no fue completamente convincente, aunque sí informativa. En las hembras se observó un aumento significativo en los niveles de estrógenos durante la época reproductora. La validación del ensayo no fue concluyente en parte debido a la persistencia inusualmente prolongada de cuerpos lúteos, lo que posiblemente produzca distintos perfiles hormonales en las hembras de lince comparadas con las de otros mamíferos. Asimismo, se validó una prueba para detectar glucocorticoides en heces de machos y hembras. Encontramos indicios preliminares de que las hembras tienen una respuesta fisiológica más marcada ante el estrés que los machos, aunque esto no significa necesariamente que las hembras sean más sensibles al estrés. El estrés crónico (por ejemplo, por translocación o contención) parece suprimir la expresión de andrógenos en los machos. Sería necesario llevar a cabo más estudios para obtener un conocimiento más claro sobre cómo afectan los factores estresantes presentes en el entorno a la reproducción del lince canadiense y, por ende, a su tamaño poblacional. Un mayor conocimiento sobre la fisiología reproductiva del lince podría ayudar a mejorar las prácticas de conservación.

Palabras clave

Hormonas fecales, estacionalidad, estrés

Abstract

Knowledge about the reproductive physiology of lynx could help improve conservation practices. The goal of this study was to validate fecal hormone metabolite assays for Canada lynx and develop a basic understanding of their reproductive physiology. Fecal androgen assays were validated for males, and analysis revealed a clear seasonal increase in androgen expression immediately prior to and during the breeding season. The validation of fecal estrogen and progestagen assays for females was not entirely convincing, but nevertheless informative. A significant rise in fecal estrogens was observed during the breeding season for females. Assay validation may be partially confounded by the unusually long persistence of corpora lutea in Lynx species, which could produce different hormone profiles compared to other female mammals. Lastly, a fecal glucocorticoid metabolite assay was validated for both males and females. We found preliminary evidence that females may have a more pronounced physiological stress response than males, although this does not necessarily imply that females are more sensitive to stress. Chronic stress (e.g. translocation and holding) does appear to suppress androgen expression in males. Further research is needed to gain a clearer understanding of how environmental stressors may impact Canada lynx reproduction, and thereby affect their population size.

Keywords

Fecal hormones, seasonality, stress

Photo: Tanya Shenk

Reproductive physiology of Canada lynx *(Lynx canadensis)*

Kerry Fanson, Nadja Wielebnowski and Jeffrey Lucas

Introduction

Canada lynx *(Lynx canadensis)* are the most abundant felid species inhabiting North America's boreal forest. However, southern populations of lynx have declined dramatically in the last century, and in 2000, the species was listed as "threatened" by the US Fish and Wildlife Service (USFWS, 2000). While lynx historically extended well into the northern continental US, anthropogenic activities (e.g. trapping, habitat destruction) and climate change have dramatically reduced most US populations (Ruggiero et al., 2000; Poole, 2003). In response to this decline, the state of Colorado initiated a lynx reintroduction effort in 1999 (see Shenk, this book). However, poor reproductive success of reintroduced individuals has threatened the success of this effort.

There are two characteristics of lynx reproduction that may contribute to their diminishing population size and hinder reintroduction efforts. First, Canada lynx are highly seasonal breeders; they have only one chance per year to reproduce (Poole, 2003). They breed from late February to early April and give birth primarily in May, with a gestation period of 60-70 days (Nowak, 1999; Ruggiero et al., 2000). Even in the southern part of their range and in captivity, females only breed once per year and most kittens are born in May. Although the breeding season lasts about two months, individual females may have a much more restricted window of mating opportunity. Captive females exhibit signs of estrus or receptive behavior for about one week or less. Little is known about estrus in wild lynx, but behavioral indicators suggest that females are only receptive for a short period. Mating pairs only remain together for several days and females presumably mate with only one male (Ruggiero et al., 2000). There is no published information about the duration or the length of estrus in captive or wild Canada lynx (Nowak, 1999). Furthermore, it is unclear whether this strong seasonality is mediated solely by females, or if both females and males experience physiological changes that may restrict their ability to mate throughout the year.

The second characteristic is that lynx recruitment fluctuates dramatically with snowshoe hare abundance, especially in the northern part of their range (Ruggiero et al., 2000). Pregnancy/conception rates, birth rates, average litter size and kitten survival, all correlate positively with prey abundance, whereas female age at sexual maturity correlates negatively (Ruggiero et al., 2000; Poole, 2003). Biologists have also speculated that females ovulate spontaneously when prey densities are high, but become induced ovulators when prey

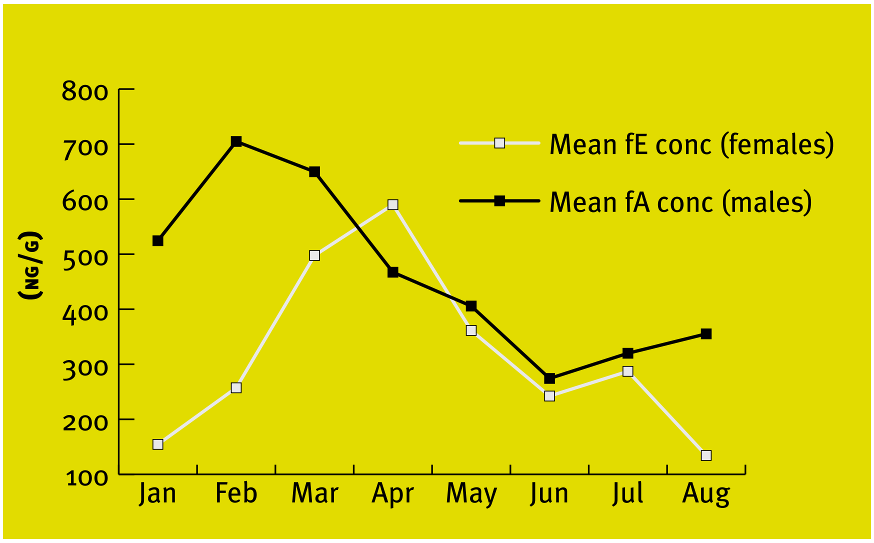


Figure 1. Seasonal changes in fecal androgen metabolites (fA) for males (♂), and fecal estrogen metabolites (fE) for females (♀). Means are plotted for intact, adult lynx. Breeding season is primarily during March.

Figura 1. Cambios estacionales en metabolitos de andrógenos (fA) en heces de machos (♂) y metabolitos de estrógenos (fE) en heces de hembras (♀). Se trazaron los valores medios para linces adultos. La época de reproducción tiene lugar principalmente durante el mes de marzo.

densities are low (Ruggiero et al., 2000). The reproductive physiology of lynx is therefore closely linked to the health of the snowshoe hare population, to the point that there is little to no recruitment during the low part of the cycle (Ruggiero et al., 2000; O'Donoghue et al., 2001; Poole, 2003). Consequently, lynx experience both annual and decadal restrictions to breeding.

Given these constraints to Canada lynx reproduction, it is unclear how climate change or anthropogenic activities may impact lynx reproduction. In order to develop effective conservation strategies and management plans for lynx, it is critical that we develop a stronger understanding of their reproductive physiology. To this end, we have initiated a research project focusing on the endocrine physiology of both captive and wild Canada lynx using fecal hormone analysis. The goal of the study described here was to 1) validate assays used to quantify fecal reproductive and glucocorticoid ("stress") hormone metabolites; 2) establish normative patterns of reproductive hormone expression in captive Canada lynx, and 3) provide a preliminary examination of the effects of stress on reproductive physiology.

Fecal samples were collected from two populations: 1) lynx in captivity and 2) lynx in holding pens. "Captive" lynx were permanently housed at captive institutions (zoos and breeding centers) and had lived in captivity most, or all, of their life. "Holding pen" lynx had been trapped in Canada or Alaska, transported to Colorado and housed in holding pens for ~2 months before they were reintroduced. Samples were collected 3-6 times per week. Hormone metabolite extraction and enzyme-immunoassay procedures have been previously described (Atsalis et al., 2004; Cavigelli et al., 2006).

Male reproductive physiology

Samples were collected from eight adult males housed at seven institutions. Two males, both housed alone, had been castrated. The remaining six males were all housed with intact females. Ages ranged from 4-18 years. Samples were also collected from two juvenile (<9 months) males.

Validation – Fecal androgen metabolites (fA) are reliable indicators of testicular activity in lynx. Both juvenile and castrated males had much lower fA concentrations than intact males (mean ± SE: 35.4 ± 30.7, 225.4 ± 63.2, 637.1 ± 116.3 ng/g feces, respectively). However, we still detected a moderate amount of fA in castrated males. There are several possible explanations for this. First, another source of androgens could contribute to circulating levels, and thereby excreted quantities, of androgens in castrated males. In humans and primates, the adrenal cortex produces a number of androgens (e.g. DHEA and DHEA-S, Rehman and Carr, 2004). Secretions of the adrenal cortex in lynx have not been studied, but could contribute to fA levels in castrated males. Second, hepatic metabolism or bacterial activity could convert other steroid molecules into androgens (Touma and Palme, 2005). A final possibility is that the antibody used cross-reacts with other steroid metabolites, because a non-specific, relatively broad-spectrum antibody was used. Regardless of which explanation is true, the assay is reliably detecting significant and biologically relevant signals, and thus can be successfully employed to track androgen excretion in male Canada lynx.

Effect of Season – Male lynx exhibit strong seasonal variation in fA expression (Figure 1). Just prior to and during the breeding season (Jan.–Mar.), fA concentrations are ~4-times higher than during the summer. Although we do not have data for the fall, we can speculate that fA concentrations (and thus testicular activity) begin to increase late in the fall (Nov. or Dec.). Living at high latitudes and/or elevations, lynx experience very harsh winters. Therefore, in order to ensure optimal kitten survival, strong selection pressure has probably shaped not only female patterns of reproduction, but has constrained male reproduction, as well.

Eurasian lynx exhibit similar patterns of seasonality. However, captive male Eurasian lynx have a second increase in testosterone expression and testicular size in May and June (Göritz et al., 2006). This may possibly be driven by the fact that if a female Eurasian lynx loses a litter, she can begin cycling again in June or July (Göritz et al., 2006). There is limited anecdotal evidence that female Canada lynx can also give birth in August, which means there would be a second period of estrous in May or June (J. Vashon and J. Tremblay, pers. comm.). However, we did not see a second increase in fecal androgens in May or June for any of the males in the study. The frequency of this "second estrous" in females is not known for either species, although it seems very rare for Canada lynx. Given the differences in androgen expression between Eurasian and Canada lynx, we can speculate that a second estrous may be somewhat more likely to occur in Eurasian lynx.

Female reproductive physiology

Samples were collected from nine females housed at seven institutions. One female had been spayed and was housed alone. Six of the females were housed with intact males and given the opportunity to breed, but only three females got pregnant. The remaining two females were housed with each other. Ages ranged from 3-17 years.

Validation – The evidence that fecal estrogen metabolites (fE) reflect ovarian activity is somewhat equivocal. The spayed female did show significantly lower fE concentrations than intact females (mean ± SE: 324.4 ± 57.1, 802.7 ± 182.2 ng/g feces, respectively) suggesting that the assay does detect biologically relevant changes in estrogen expression. Furthermore, there are also seasonal increases in fE expression that coincide with the breeding season (Figure 1). However, we have failed to detect fE peaks associated with documented estrous behavior or known ovulations/matings. Lynx defecate about once per day, so if ovulatory estrogen spikes are very short-lived, they may be muted in the fecal sample. Therefore, it is possible that fE can be used to measure gross differences in ovarian activity, but not to monitor distinct ovarian events for this species.

A common progestogen EIA assay, which has been validated successfully for several other felid species, did not pick up relevant hormone metabolites for monitoring pregnancy in Canada lynx. We were only able to obtain data from three successful pregnancies, but the expected increase in fP concentrations could not be detected in any of the females with the initial assay (Figure 2). To further determine whether improper antibody binding (e.g. high cross-reactivity) was interfering with our ability to detect a rise in hormone concentrations generally expected during pregnancy, we then tested four other progesterone antibodies. While some antibodies indicated a slight increase in fP towards the end of the pregnancy, none show the characteristic elevation seen in some other felid species (e.g., domestic cat, tiger, cheetah, clouded leopards – J. Brown, this book).

However, while fPs could not be used to accurately detect pregnancy in Canada lynx, the measured concentrations may nevertheless accurately reflect luteal activity. There is evidence that corpora lutea (CLs) persist for extensive periods of time in Canada lynx (Nellis et al., 1972). In fact, this phenomenon is common to all Lynx species; CLs can persist for several months, and probably years (bobcats – Duke, 1949; Eurasian and Iberian lynx – Göritz et al., this book). Duke (1949) proclaims "[t]he life history of the corpus luteum of the bobcat is an intriguing puzzle." Indeed, this puzzle seems to be relevant to the entire Lynx genus. It is unclear how long the CLs remain capable of producing progesterone, but this could explain the difficulty that other Lynx researchers have also had in identifying pregnant lynx using fecal hormone analysis (Pelican et al., this book; Jewgenow et al., this book; Dehnhardt et al., this book). It is also unclear how females might accommodate this prolonged elevation of circulating progesterone while maintaining normal patterns of reproduction.

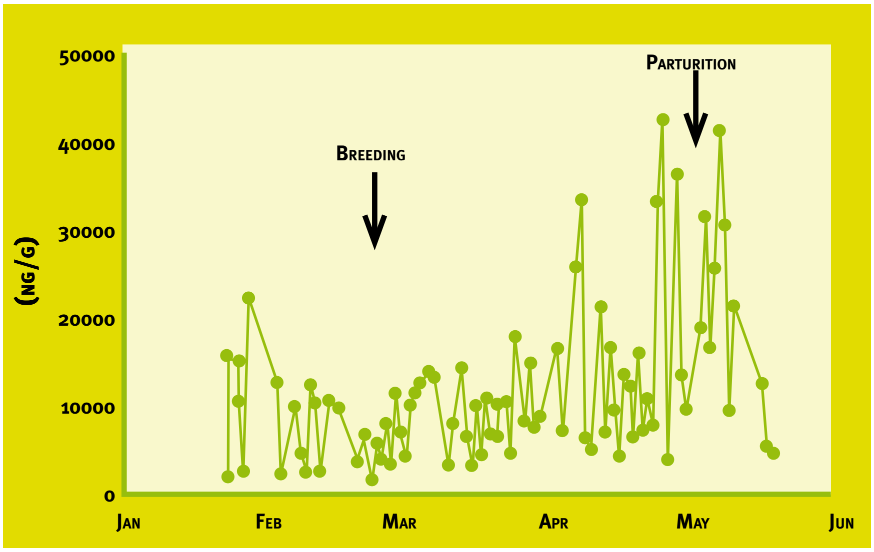


Figure 2. Representative profile of fecal progestogen metabolite (fP) expression in a pregnant lynx.

Figura 2. Perfil que representa de la expresión de los metabolitos de la progesterona (fP) en una hembra gestante de lince.

Another unexpected finding is that fE and fP show correlated patterns of expression. This is contrary to what we would expect to see, because luteal production of progesterone typically suppresses estrogen secretion, so these hormones should be inversely related. At this point, it is unclear what is causing this trend, but there is a possibility that it is related to the unusual CL characteristics of lynx.

Stress & reproduction

Normative patterns of reproductive physiology can be dramatically altered by stress. A prominent component of the physiological stress response is the hypothalamic-pituitary-adrenal (HPA) axis. Chronic up-regulation of the HPA axis can negatively affect the hypothalamic-pituitary-gonadal (HPG) axis, and thus suppress reproduction (Wingfield and Sapolsky, 2003). However, the interaction between the HPA and HPG axes is highly variable between species (Wingfield and Sapolsky, 2003). Understanding how environmental stressors may affect reproduction requires a species-specific understanding of how the HPA and HPG axes interact. Here we provide a preliminary examination of how stress might affect reproduction in lynx.

The glucocorticoid assay was validated by monitoring patterns of hormone expression during ACTH challenges (Figure 3), transfers/translocations, and exams. Results revealed that female lynx have significantly higher fecal glucocorticoid (fGC) concentrations than males. Furthermore, the relative difference in fGCs between "captive" and "holding pen" lynx (see Introduction for description) is much greater for females than males (Figure 4). Gender differences in fGC expression have been documented for a wide range of species (Touma and Palme, 2005). One explanation, which has been confirmed in some species, is that there are gender-based differences in steroid hormone production and/or metabolism (Touma and Palme, 2005). Another explanation, which is not necessarily mutually exclusive with the first, is that these differences may reflect differences in stress sensitivity. It remains unclear which is true for Canada lynx.

It is of course difficult to experimentally examine the effect of chronic stress on reproduction in a threatened species, and data collection is mostly opportunistic. However, using data collected from the reintroduced lynx population in Colorado, we have preliminary evidence that chronic elevation of the HPA axis may suppress the HPG axis, at least in males. The winter during which lynx are trapped, transported, examined, and held in holding pens, fA levels are lower and fGC levels are higher than in subsequent winters, after they have been released. We do not yet have data for females since this study is still ongoing. However, especially given the tremendous impact that hare density has on lynx reproduction, it will be key to develop a better understanding of how environmental factors and potential stressors impact reproductive physiology in this species.

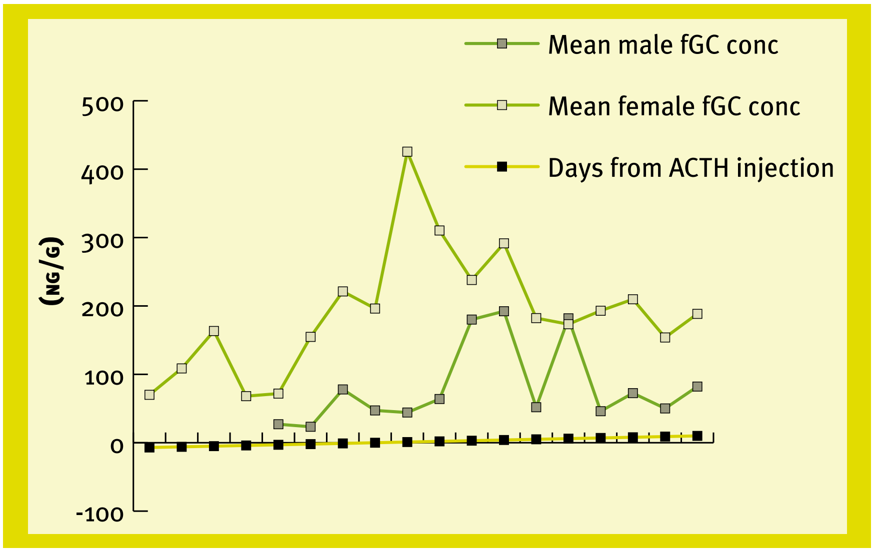


Figure 3. Mean fecal glucocorticoid metabolite (fGC) concentrations for two males (♂) and three females (♀) following an ACTH challenge (Day 0).

Figura 3. Concentración media de los metabolitos de glucocorticoides en dos machos (♂) y tres hembras (♀) de lince canadiense tras el desafío con la hormona ACTH (Día 0).

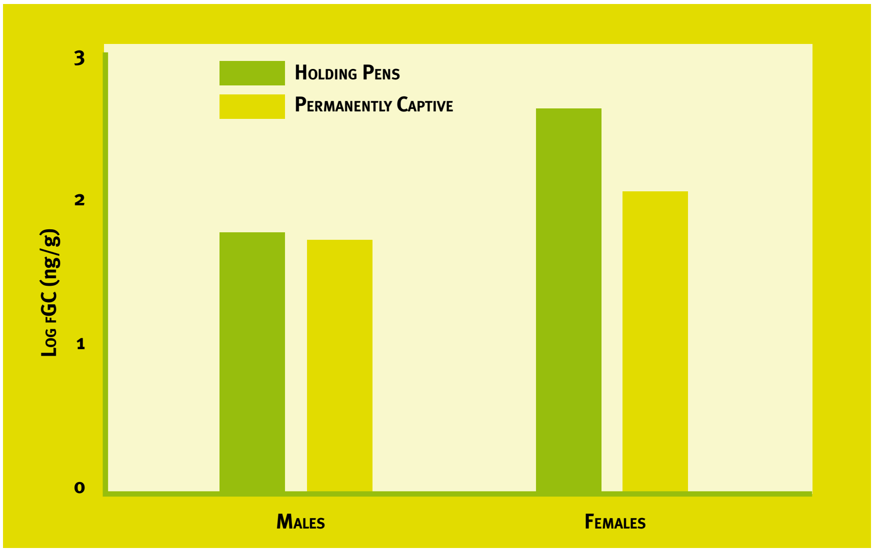


Figure 4. Gender differences in fecal glucocorticoid metabolite (fGC) concentrations. Note, the y-axis is log-transformed, which diminishes the difference between males and females, but shows the proportional difference between populations. "Permanently captive" lynx were housed at captive institutions and had lived in captivity most, or all, of their lives. "Holding pen" lynx had been trapped from the wild, transported hundreds of miles, and housed in holding pens for ~2 months.

Figura 4. Diferencias entre machos y hembras en los niveles de glucocorticoides (fGC). Se debe tener en cuenta que el eje de las y ha sufrido una transformación logarítmica, lo que reduce la diferencia entre machos y hembras aunque muestra la diferencia proporcional entre ambas poblaciones. Los linces "en cautividad permanente" se alojaban en centros de cautividad y habían vivido toda su vida –o la mayor parte de ella–en cautividad. Los linces en "instalaciones de presuelta" fueron capturados en el medio silvestre, transportados a cientos de millas de su lugar de origen y alojados en instalaciones de presuelta durante aproximadamente dos meses.

Conclusions

Understanding the basic reproductive physiology of Canada lynx, including the degree of plasticity and the impact of environmental stressors, is critical for 1) developing successful captive breeding programmes; 2) designing sound conservation strategies and 3) understanding the reproductive limitations of this species in the face of environmental and climatic changes. While our current findings provide only a small start towards a better understanding, and numerous questions remain yet unanswered, continued study of these questions becomes even more urgent given the close relationship and physiological similarities of Canada lynx to other, even more threatened, Lynx species. All four Lynx species have been declining, with the most substantial decline being in the Iberian lynx population, which is now listed as "critically endangered" (IUCN, 2008). Given the biological and physiological similarities already evidenced, any information we can glean about the basic biology of one species may prove to be helpful in aiding conservation efforts for the other Lynx species as well.

Acknowledgements

We appreciate the hard work and assistance provided by everyone at the participating institutions (Assiniboine Park Zoo, Brec's Baton Rouge Zoo, Cincinnati Zoo and Botanical Garden, Exotic Feline Breeding Compound, N.O.A.H. Feline Refuge Center, Utah's Hogle Zoo, Wild Trax Feline Refuge, and Zoo America). Thanks to Astrid Bellem and Jocelyn Bryant for technical support. Funding for this project was provided by the Chicago Zoological Society/ Chicago Board of Trade Endangered Species Fund, Purdue University, Sigma Delta Epsilon, and PEO International.

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Photo: Tanya Shenk

Reintroduction: case studies
and other aspects

REINTRODUCCIÓN: CASOS DE ESTUDIO Y OTRAS CONSIDERACIONES

If people think that nature is their friend, then they sure don't need an enemy.

Kurt Vonnegut
(1922-2007)

Lessons from the reintroduction of the Eurasian lynx in Central and West Europe

Lecciones aprendidas a partir de la reintroducción del lince boreal en Europa Central y Occidental

Manuela von Arx, Christine Breitenmoser-Würsten and Urs Breitenmoser

Resumen

En este capítulo revisamos las reintroducciones del lince boreal *(Lynx lynx)* en Europa Central y Occidental, así como el estado actual de las poblaciones. Para ello, hemos utilizado los requisitos establecidos en la “Guía para Reintroducciones” de la Unión Internacional para la Conservación de la Naturaleza (IUCN), con el fin de evaluar los proyectos de reintroducción e identificar los indicadores que potencialmente pueden afectar al éxito o al fracaso de las reintroducciones de lince. Las primeras reintroducciones de lince boreal comenzaron en el año 1970 y continúan hasta la fecha. En cada caso, sólo se liberó a un número pequeño de linces, entre dos y 31 ejemplares por suelta. El 57% de los linces reintroducidos fueron capturados en la naturaleza, en las montañas de los Cárpatos de Eslovaquia; el 40% fueron linces nacidos en cautividad y el resto de los ejemplares fueron de origen desconocido. En la mayoría de los casos, la evaluación de los programas de reintroducción del lince boreal se ve afectada por la escasez de datos y por la falta de un seguimiento adecuado (a largo plazo); incluso, en algunos de los proyectos más recientes no se han utilizado métodos científicos apropiados para hacer un seguimiento de la evolución de los animales liberados o del grado de éxito del programa. No obstante, el estudio comparativo de los distintos proyectos nos permite sacar algunas conclusiones generales sobre los cambios de las amenazas históricas, sobre el número y origen de los animales, sobre la elección de los lugares de suelta, sobre la diseminación de información y participación del público, así como sobre el control y seguimiento de las poblaciones reintroducidas. Estos datos nos pueden proporcionar lecciones que nos sirvan para la recuperación del lince ibérico.

Palabras clave

reintroducción, lince boreal, *Lynx lynx*, Europa, evaluación, directrices

Abstract

We review the Eurasian lynx *(Lynx lynx)* reintroductions in Central and West Europe and the present status of the populations. The requirements defined by the IUCN Reintroduction Guidelines are used to evaluate the reintroduction projects and to identify important factors for successful lynx reintroductions. Reintroduction of Eurasian lynx started as early as 1970 and continues to present. In all attempts, only a small number of animals, ranging from two to 31 animals, were released. Fifty-seven percent of the animals were wild-caught in the Carpathian Mountains of Slovakia; 40% were captive-born lynx and some were of unknown origin. The assessment of the Eurasian lynx reintroduction programmes suffers from lack of information and insufficient (long-term) follow-up monitoring for most projects. Even some of the recent projects failed to apply adequate scientific methods to monitor the fate of the released animals and the success of the Programme. The comparison of the different projects however still allows for some general conclusions regarding the reversal of historic threats, number and origin of the animals, the choice of release sites, information and public involvement, and monitoring and follow-up programmes which might provide some lessons for the recovery of the Iberian lynx.

Keywords

reintroduction, Eurasian lynx, *Lynx lynx,* Europe, evaluation, guidelines

Lessons from the reintroduction of the Eurasian lynx in Central and West Europe

Manuela von Arx, Christine Breitenmoser-Würsten and Urs Breitenmoser

Introduction

The Eurasian lynx *(Lynx lynx)*, once widespread across Europe, has reached the minimum of its historic distribution during the first decades of the 20th century. Due to human persecution in addition to habitat destruction and loss of prey, the species only survived in Russia, the Carpathian Mountains, Western Balkans and in small numbers in Scandinavia and Finland. Around 1950, the general downward trend came to a halt and the autochthonous populations started to recover. Not only the ecological conditions had improved, but also the general attitude of people towards large carnivores had changed and they were granted legal protection or at least some form of controlled hunting (Breitenmoser, 1998). A natural re-colonisation of former habitats in central, southern and western Europe from remnant populations was, however, no longer possible (the lowlands between the forested mountain ranges were too heavily encroached by man).
Therefore, several lynx reintroduction attempts into suitable areas were initiated from the 1970s to present (Figure 1; Table 1). Forty years ago, nobody was aware of the various and long-lasting problems to be faced when reintroducing carnivores. First, the return of large predators provoked massive opposition from people regarding them as competitors. Then, large carnivores need extended living spaces, and no protected area in Europe is large enough to host a viable population. Finally, implementing a reintroduction programme is a difficult, expensive and long-lasting task due to the slow turnover and elusiveness of the animals, and needs long-term commitments of all partners (Breitenmoser et al., 2001). These constraints have to be taken into account when planning such a project. For a successful reintroduction many additional factors, listed in the Guidelines of the IUCN Re-introduction Specialist Group, are important, such as number, age, sex ratio and origin of the animals to be released, the choice of release sites and the duration of the project (IUCN, 1998). Finally, a sound knowledge on the species' biology (life history, ecology, behaviour, genetics) and a robust scientific surveillance of the Programme helps define and adjust the optimal strategy.

Overview and evaluation on Eurasian lynx reintroductions

From 1970 to present 14 lynx reintroduction projects in Germany, Switzerland, Slovenia, Italy, Austria, Czech Republic, France and Poland have taken place (Table 1). A total of 172-177 lynx were released. For the time being, we consider four of the projects as successful, six as a failure and four cannot be assessed yet. Many of these reintroductions were poorly prepared and documented. However, a comparison of the requirements for reintroductions, as defined in the IUCN Reintroduction Guidelines (IUCN, 1998), considering the information available, still provides important insights into the reasons for success or failure of reintroduction projects.

Reversal of threats leading to extinction

The Guidelines list as first requirement the removal of (historic) threats that lead to the extinction of the species. This condition was fulfilled in all reintroduction projects for the Eurasian lynx in regard to the ecological settings: Habitat (forests) and prey populations (roe deer) have recovered across Central and West Europe throughout the 20th century (Breitenmoser, 1998; Breitenmoser and Breitenmoser-Würsten, 2008). However, human density and traffic network are nowadays extremely high in some of these regions, both of which are considered an obstacle for the reintroduction of lynx. Increased fragmentation of the landscape, together with a biologically low dispersal potential of the species, hinders the expansion into new areas and the establishment of larger meta-populations.

One threat was only partly reversed: the negative attitude of people. Although the general public welcomes the return of the lynx, the rural population –with a less romantic view of nature– is more sceptical about predators, and important target groups such as hunters and sheep breeders strongly oppose (Hunziker et al., 2001). The last survey on the Eurasian lynx in Europe (von Arx et al., 2004) revealed illegal killing to be believed the main threat for the species. This is especially true for the reintroduced populations. Between 1996 and 2001, the yearly number of illegally killed lynx in the Bohemian-Bavarian population –where the topic has been thoroughly studied (Cerveny et al., 2002)– was seven individuals. In other reintroduced populations, cases discovered per year ranged from one to five (von Arx et al., 2004). Considering that the majority of illegal acts never become public, we can assume that this is only the tip of the iceberg. The problem goes far beyond financial and rational reasons and, to mitigate the conflict, not only public education, but also involvement of interest groups are key (see also "Information and public involvement" below).

Number and origin of animals

Although an assessment of carnivore reintroductions in general demonstrates that success is a function of the number of animals released and that wild-caught animals have a higher survival rate than captive-born candidates (Breitenmoser et al., 2001), this pattern is not entirely true for the Eurasian lynx. Normally, fewer than 20 lynx, often much less, have been released at a few sites (Table 1). One of the most successful programmes the reintroduction in Slovenia, which formed the basis of the Dinaric population, originated from only six released lynx. We do, however, not know yet whether the limited genetic variability may have a negative impact in the long-run in terms of an enhanced vulnerability to diseases or environmental changes.

Where wild-caught animals were used, they were taken from the Slovakian Carpathian Mountains, the geographically nearest autochthonous lynx population. Although carnivore projects using wild-caught animals have a higher ratio of success (Breitenmoser et al., 2001) and captive-born animals show a higher post-release mortality (Jule et al., 2008), captive-born lynx seem to adapt quite well to living in nature and catching wild prey. Two reintroduction projects, in the Kampinos National Park in Poland and in the Harz Mountains in Germany (Table 1), have used captive-born lynx. The monitoring of the population development was however so insufficient that a proper assessment is difficult (von Arx and Breitenmoser, 2004; von Arx et al., 2004). The Guidelines provide further requirements for using captive stock and state clearly that whenever wild individuals are available they should be preferred over captive ones and "if captive animals are to be used, it must be from a population which has been soundly managed, both demographically and genetically, according to the principles of contemporary conservation biology" (IUCN, 1998). Lynx used for the Kampinos and the Harz reintroductions were taken from European zoos without genetic management. They were partly inbred, partly hybrids of several subspecies.

Choice of release site

The selection of the release site is crucial. Beyond the obviously important local conditions in regard to adequate ecological resources, the geo-strategic situation of the release site is important. The reintroduction area should, on one hand, be "closed" so that animals dispersing from the release site do not risk loosing contact with conspecifics. Yet, on the other hand, the connection to neighbouring existing or potential nuclei must be considered in regard to the long-term development of the (meta-) population. While, for example, lynx released in Austria spread into different directions and were soon too far from each other to set up a socio-spatial system, natural and anthropogenic barriers helped to establish a core population in Switzerland (Breitenmoser and Breitenmoser-Würsten, 2008).

Information and public involvement

All reintroduction programmes of Eurasian lynx were controversial, but there is no correlation between short-term success and public information or involvement of stakeholders. Paradoxically, the three most successful projects, those in the North-western Alps, the Jura and in southern Slovenia, were among those with the poorest public information. Nevertheless, public relation seems to be important for the long-term acceptance of the returning predator. The recovery of lynx in Switzerland, for instance, still suffers from the fact that all releases in the 1970s were done in a very clandestine way (Breitenmoser and Breitenmoser-Würsten, 2008).

Monitoring and follow-up programme

Monitoring is an ultimate requirement for the control and the adaptive management of the Programme. Regardless of how careful a reintroduction project is planned and carried out, it will remain a stochastic endeavour with many uncontrollable parameters. The general set-up of a project is never enough to explain successes and failures; knowledge of individual fates is indispensable for a thorough assessment. A successful reintroduction programme must be an adaptive process, where a serious monitoring of all parameters allows the correction of parts of the project as it goes on.

Insufficient post-release monitoring was a deficiency in all early (and also some newer) Eurasian lynx reintroduction projects. Apart from the Vosges reintroduction and the newest project in eastern Switzerland, the fate of the released animals was not consequently surveyed. In Switzerland, a scientific follow-up of the reintroduced lynx was only established in 1980. In spite of all these shortcomings, in approximately half of the reintroduction attempts, released animals established well, reproduced and expanded their distribution range. The remaining projects, however, failed (Table 1).

Status of the established populations

At present, we distinguish between the Bohemian-Bavarian, Dinaric, Alpine, Jura, and Vosges-Palatinian populations founded through reintroductions (Table 1; Fig. 1). The last Pan-European inquiry (von Arx et al., 2004) revealed that, approximately 30 years after the reintroductions, all populations are still considered to be "Endangered" to "Critically Endangered" at a local scale, according to the IUCN/SSC Red List Criteria (IUCN, 2003). Population sizes range from 20-37 (Vosges-Palatinian) to 130 (Dinaric). To be upgraded to "Vulnerable", an effective population size of at least 250 mature individuals would be necessary. Although a surprising number of the small-scale reintroduction projects were "successful" in founding a local population, it will still require a considerable amount of time –and possibly additional efforts– to establish viable populations. However, in many cases, this will never be possible because of the limited potential expansion of some of these populations.

Photo: C. Angst

Figure 1. Release of a Eurasian lynx in Switzerland.

Figura 1. Suelta de un lince euroasiático en Suiza.

Population/ Occurrence	Location of the re-introduction	Years	Number of animals (m/f)*	Origin of animals	Fate **
Bohemian-Bavarian	Bavarian Forest (DE)	1970-74	5-10	3 wild, 2 captive	failed
	Sumava Mts. (CZ)	1982-89	18 (11/7	wild	(success)
Dinaric	Kocevje (SI)	1973	6 (3/3)	wild	(success)
Alpine	Western Swiss Alps	1971-76	12 (7/5)	wild	(success)
	Engadin (CH)	1972/80	4 (2/2)	wild	failed
	Gran Paradiso NP (IT)	1975	2 (2/0)	wild	failed
	Austrian Alps	1977-79	9 (6/3)	wild	failed
	Eastern Swiss Alps	2001-08	12 (6/6)	wild	uncertain
Alpine/Jura	Swiss Plateau	1989	3	unknown	failed
Jura	Swiss Jura Mts.	1972-75	10 (5/5)	wild	(success)
Vosges-Palatinian	Vosges Mts. (FR)	1983-89	21 (12/9)	19 wild, 2 captive	uncertain
Podyji	Podyji NP (CZ)	1993-94	6 (2/2)	captive	failed
Kampinos occ.	Kampinos NP (PL)	1992-99	31 (14/17)	captive	uncertain
Harz occ.	Harz Mts. (DE)	since 2000	28 (9/15)	captive	uncertain

* m/f= males/females. Sometimes the information is not or only partly available.
** Fate: "Success" in brackets as these populations have up to now been surviving for 20-30 years with reasonable numbers of animals, however their long-term survival is not yet secured.
* m/f= machos/hembras. En ocasiones la información no está disponible o sólo se dispone de datos parciales.
** Evolución: "Éxito", entre comillas, porque estas poblaciones llevan sobreviviendo ya entre 20 y 30 años, con un número razonable de animales; no obstante, su supervivencia a largo plazo no está asegurada todavía.

Table 1. Lynx *(Lynx lynx)* reintroductions in Central and West Europe. Data compiled from Breitenmoser et al., 2001; von Arx et al., 2004; Breitenmoser and Breitenmoser-Würsten; 2008, Linnell et al., 2009.

Tabla 1. Reintroducciones del lince *(Lynx lynx)* en Europa Central y Occidental. Datos recopilados de Breitenmoser et al., 2001; von Arx et al., 2004; Breitenmoser y Breitenmoser-Würsten; 2008, Linnell et al., 2009.

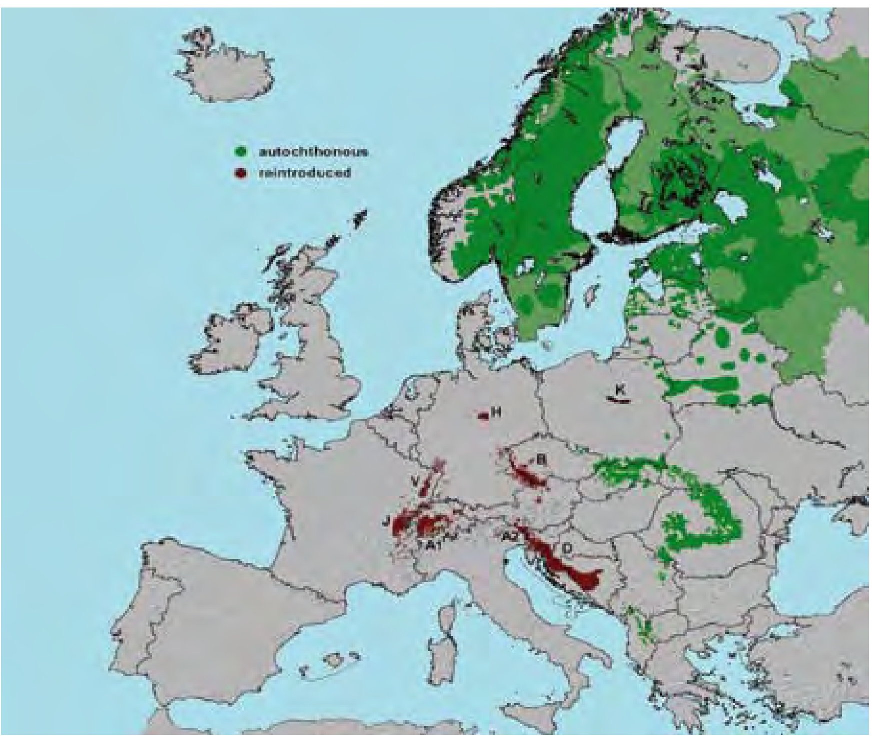

Figure 2. Distribution of the Eurasian lynx in Europe (von Arx et al., 2004). Constantly occupied area and single observations (brighter shade). Reintroduced populations: B=Bohemian-Bavarian: D=Dinaric; A=Alpine [with a western 1) and eastern 2) subpopulation]; J=Jura; V=Vosges-Palatinian; K=Kampinos occurrence; H=Harz occurrence.

Figura 2. Distribución del lince boreal en Europa (von Arx et al., 2004). Área de ocupación constante y observaciones individuales (color más claro). Poblaciones reintroducidas: B=Bohemia-Bavaria; D=Dinaric; A=Alpes [con subpoblaciónes occidental 1) y oriental 2)]; J=Jura; V=Vosges-Palatina; K=presencia en Kampinos; H=presencia en Harz.

In order to improve their conservation status, the populations in West and Central Europe should be considered under a metapopulation concept, which would involve improving the connectivity and consequently exchange of individuals between populations. As all potential or extant metapopulations are transboundary, cross-border collaboration has to be improved and coherent strategies should be developed and applied according to the European guidelines for population level management of large carnivores (Linnell et al., 2008).

Conclusions

Given the unprofessional approach in most of the Eurasian lynx reintroduction projects carried out in the 1970s and 1980s, the establishment of free-living populations was rather astonishing (Figure 2). However, the assessment of the reintroductions depends on the definition of success and the time frame applied. All populations are still small in size and extension, which makes them not only vulnerable to human-induced mortality, but also to genetic and stochastic processes. Obviously, 20 to 30 years are not enough to ensure the long-term persistence of lynx in Central and Western Europe, and further active support is needed. Even if the early attempts could not be considered brilliant and successful, they have at least allowed us to learn about the reintroduction of controversial animals such as the lynx, and to develop better schemes.

Reintroducing carnivores is a serious business that requires a long-term commitment of all partners involved, also –and especially– from government organisations (see Jiménez, this book). All these projects are controversial, and diverging interests can only be mitigated through a clear and long-term concept regarding reintroduction goals, together with a sound management strategy that grants the long-term survival of the reintroduced lynx populations.

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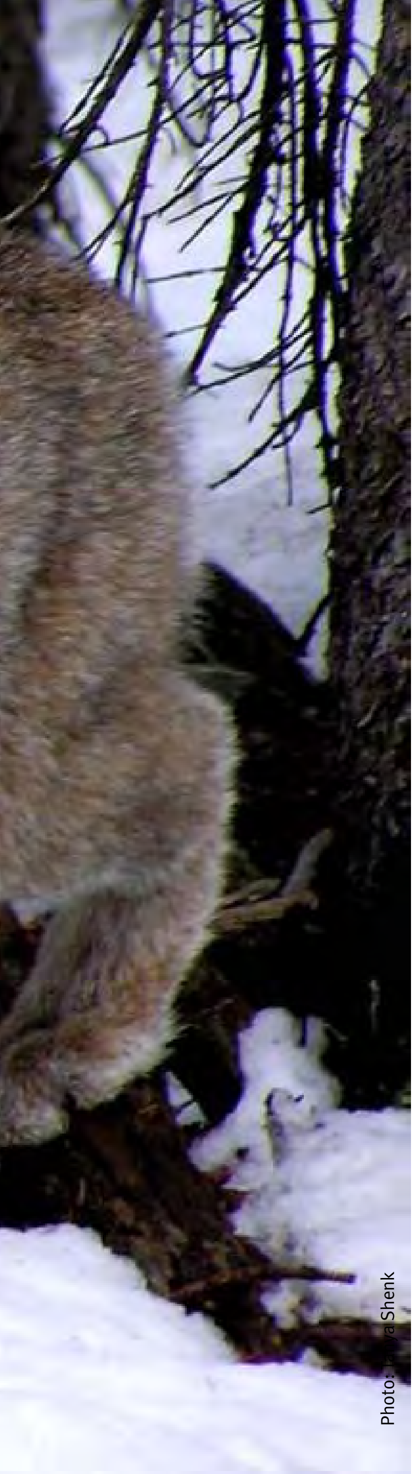
We have a rare opportunity here and with the help of the British Columbia trappers, we can bring back a native that has been missing from the Colorado mountains for a long time. ..... OK then. Let's do it, and let's make it work.

John Mumma,
Colorado Division of Wildlife Director
(1995-2000)



Canada lynx *(Lynx canadensis)* reintroduction in Colorado

Reintroducción del lince canadiense *(Lynx canadensis)* en Colorado

Tanya M. Shenk, Rick H. Kahn, Gene Byrne, David Kenvin, Scott Wait, John Seidel and John Mumma

Resumen

El lince canadiense *(Lynx canadensis)* habita en todos los bosques boreales del norte de Norteamérica y su distribución histórica más meridional se encuentra en el estado de Colorado, donde la especie campeaba en los bosques montañosos de mayor altura. A finales de los 70, los linces se habían extinguido en Colorado o sólo quedaban unos pocos ejemplares. Teniendo en cuenta el aislamiento de este estado respecto de las poblaciones más cercanas del norte, la División de Flora y Fauna de Colorado consideró que la reintroducción de la especie era la única opción para intentar restablecerla dentro de su territorio. El programa de reintroducción del lince canadiense en Colorado tiene como objetivo establecer una población viable de linces. Para valorar si el trabajo de reintroducción está teniendo éxito se utiliza la evaluación periódica de los logros alcanzados como método provisional. Consideramos que hay siete criterios necesarios para lograr una población viable: 1) creación de protocolos de suelta que impliquen una supervivencia inicial alta tras la liberación de los ejemplares reintroducidos; 2) supervivencia a largo plazo del lince en Colorado; 3) fomento de la filopatría (fidelidad al lugar de suelta) del lince en densidades suficientes para conseguir la reproducción en aquellas áreas que ofrezcan un buen hábitat; 4) los linces reintroducidos deben reproducirse; 5) la reproducción debe continuar con la reproducción de los cachorros supervivientes; 6) los linces nacidos en Colorado deben llegar a la edad de reproducción y reproducirse con éxito, y 7) la natalidad debe ser igual o superior a la mortalidad. Los primeros linces fueron soltados en Colorado en febrero de 1999. Se documentaron tanto la filopatría como la supervivencia mediante un seguimiento intensivo de los ejemplares, utilizando telemetría. La primera reproducción fue observada en la época de cría del año 2003. Las épocas de reproducción exitosa fueron documentadas en los años 2004, 2005 y 2006. La madre de una de las camadas que correspondían a la primera repoblación de linces nacidos en Colorado fue una hembra de lince nacida en Colorado en el año 2004. Hasta la fecha, los resultados han demostrado que la División de Flora y Fauna de Colorado ha creado protocolos de suelta que garantizan una alta tasa de supervivencia inicial tras la liberación, seguida de una supervivencia a largo plazo también alta, así como la filopatría, reproducción y repoblación de linces nacidos en Colorado en la población reproductora del estado. Queda por demostrar si Colorado es capaz de fomentar un nivel de repoblación que permita compensar la mortalidad anual y, con el tiempo, obtener una población viable de linces. El trabajo de seguimiento continúa con el fin de documentar si esto puede ser viable.

Palabras clave
Colorado, lince, *Lynx canadensis*, reintroducción

Abstract
The Canada lynx *(Lynx canadensis)* occurs throughout the boreal forests of northern North America. Colorado represents the southern-most historical distribution of lynx, where the species occupied the higher elevation, montane forests in the state. Lynx were extirpated or reduced to a few animals in the state by the late 1970's. Given the isolation of Colorado to the nearest northern populations, the Colorado Division of Wildlife considered reintroduction as the only option to attempt to reestablish the species in the state. The goal of the Colorado lynx reintroduction programme is to establish a viable population of lynx in this state. Evaluation of incremental achievements is an interim method of assessing if the reintroduction effort is progressing towards success. There are seven critical criteria for achieving a viable population: 1) development of release protocols that lead to a high initial post-release survival of reintroduced animals; 2) long-term survival of lynx in Colorado; 3) development of site fidelity by the lynx to areas supporting good habitat in densities sufficient to breed; 4) reintroduced lynx must breed; 5) breeding must lead to reproduction of surviving kittens; 6) lynx born in Colorado must reach breeding age and reproduce successfully, and 7) recruitment must be equal to or greater than mortality. The first lynx were released in Colorado in February 1999. Site fidelity and survival were documented through intensive monitoring of individuals through telemetry. Reproduction was first documented during the 2003 reproduction season. Successful breeding seasons were documented in 2004, 2005 and 2006. A female lynx born in Colorado in 2004 was the mother of one of these litters which documented the first recruitment of Colorado-born lynx into the Colorado breeding population.
Results to date have demonstrated that the Colorado Division of Wildlife has developed release protocols that ensure high initial post-release survival followed by high long-term survival, site fidelity, reproduction and recruitment of Colorado-born lynx into the Colorado breeding population. What is yet to be demonstrated is whether Colorado can support sufficient recruitment to offset annual mortality for a viable lynx population over time. Monitoring continues in an effort to document such viability.

Keywords
Colorado, lynx, *Lynx canadensis*, reintroduction

Canada lynx *(Lynx canadensis)* reintroduction in Colorado

Tanya M. Shenk, Rick H. Kahn, Gene Byrne, David Kenvin, Scott Wait, John Seidel and John Mumma

Introduction

The Canada lynx occurs throughout the boreal forests of northern North America. Colorado represents the southern-most historical distribution of lynx, where the species occupied the higher elevation, montane forests in the state. Little was known about the population dynamics or habitat use of this species in their southern distribution. Lynx were extirpated or reduced to a few animals in the state by the late 1970's due, most likely, to predator control efforts such as poisoning and trapping. Given the isolation of Colorado to the nearest northern populations, the Colorado Division of Wildlife (CDOW) considered reintroduction as the only option to attempt to reestablish the species in the state.

A reintroduction effort was begun in 1997, with the first lynx released in Colorado in 1999. To date, 218 wild-caught lynx from Alaska and Canada have been released in southwestern Colorado. The goal of the Colorado lynx reintroduction programme is to establish a self-sustaining, viable population of lynx in this state. Evaluation of incremental achievements necessary for establishing viable populations is an interim method of assessing if the reintroduction effort is progressing towards success. There are seven critical criteria for achieving a viable population: 1) development of release protocols that lead to a high initial post-release survival of reintroduced animals; 2) long-term survival of lynx in Colorado; 3) development of site fidelity by the lynx to areas supporting good habitat in densities sufficient to breed; 4) reintroduced lynx must breed; 5) breeding must lead to reproduction of surviving kittens; 6) lynx born in Colorado must reach breeding age and reproduce successfully, and 7) recruitment must equal or be greater than mortality over an extended period of time.

The post-release monitoring programme for the reintroduced lynx has two primary goals. The first goal is to determine how many lynx remain in Colorado and their locations relative to each other. Given this information and knowing the sex of each individual, we can assess whether these lynx can form a breeding core from which a viable population might be established. From these data we can also describe general movement patterns and habitat use. The second primary goal of the monitoring programme is to estimate survival of the reintroduced lynx and, where possible, determine causes of mortality for reintroduced lynx. Such information will help in assessing and modifying release protocols and management of lynx once they have been released to ensure their highest probability of survival.

Additional goals of the post-release monitoring programme for lynx reintroduced to the southern Rocky Mountains included refining descriptions of habitat use and movement patterns and describing successful hunting habitat once lynx established home ranges that encompassed their preferred habitat. Specific objectives for the site-scale habitat data collection include: 1) describe and quantify site-scale habitat use by lynx reintroduced to Colorado; 2) compare site-scale habitat use among types of sites (e.g., kills vs. long-duration beds), and 3) compare habitat features at successful and unsuccessful snowshoe hare chases.

Documenting reproduction is critical to the success of the Programme and lynx are monitored intensively to document breeding, births, survival and recruitment of lynx born in Colorado. Site-scale habitat descriptions of den sites are also collected and compared to other sites used by lynx.

Lynx is listed as threatened under the Endangered Species Act (ESA) of 1973, as amended (16 U. S. C. 1531 et. seq.) (U.S. Fish and Wildlife Service, 2000). Colorado is included in the federal listing as lynx habitat. Thus, an additional objective of the post-release monitoring programme is to develop conservation strategies relevant to lynx in Colorado. To develop these conservation strategies, information specific to the ecology of the lynx in its southern Rocky Mountain range, such as habitat use, movement patterns, mortality factors, survival, and reproduction in Colorado is needed.

Methods

Study area

Southwestern Colorado is characterized by wide plateaus, river valleys, and rugged mountains that reach elevations over 4200 m. Engelmann spruce-subalpine fir is the most widely distributed coniferous forest type at elevations most typically used by lynx. The Core Release Area is in southwestern Colorado (Figure 1). The lynx-established core area is in central Colorado and includes areas of continuous use by lynx, including areas used during breeding and denning (Figure 1).

Reintroduction effort

All 2006 lynx releases were conducted under the protocols found to maximize survival (see Shenk, 2001). Estimated age, sex and body condition were ascertained and recorded for each lynx prior to release (see Wild, 1999). Specific release sites were selected based on land ownership and accessibility during times of release (Byrne, 1998). Lynx were transported from the Frisco Creek Wildlife Rehabilitation Center, where they were held from their time of arrival in Colorado, to their release site in individual cages.

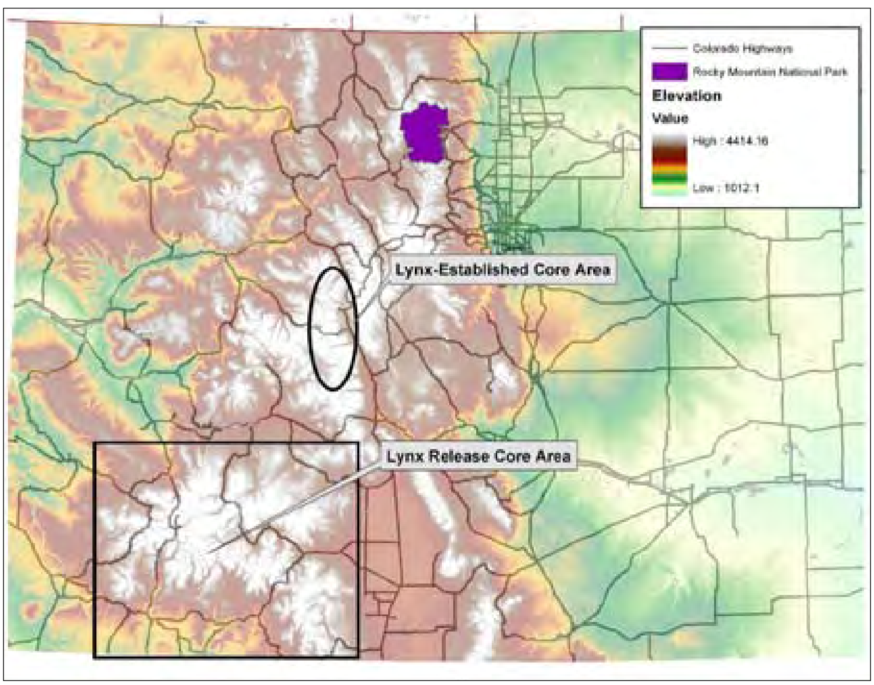


Figure 1. Lynx are monitored throughout Colorado and by satellite throughout the western United States. The lynx core release area, where all lynx were released, is located in southwestern Colorado. A lynx-established core use area has developed in the Taylor Park and Collegiate Peak area in central Colorado.

Figura 1. Se realiza un seguimiento de los linces en todo Colorado y, mediante telemetría satélite, en todo el oeste de los Estados Unidos. El corazón del área de suelta de los linces, donde fueron soltados todos los ejemplares, está situado en el suroeste de Colorado. Los linces han establecido un área vital que ha ido creciendo en la zona del Parque Taylor y Collegiate Peak en el centro de Colorado.

Distribution and movement patterns

All lynx released in 1999 were fitted with Telonics™ radio-collars. All lynx released since 1999, with the exception of five males released in spring 2000, were fitted with Sirtrack™ dual satellite/VHF radio-collars. To determine general movement patterns of reintroduced lynx, regular locations of released lynx were collected through a combination of aerial, satellite and ground radio-tracking. Locations were recorded in UTM coordinates and general habitat descriptions for each ground and aerial location were recorded.

Home range

Annual home ranges were calculated as a 95% utilization distribution using a kernel home-range estimator for each lynx we had at least 30 locations for within a year. A year was defined as March 15 – March 14 of the following year. Locations used in the analyses were collected from September 1999 – January 2006 and all locations obtained for an individual during the first six months after its release were eliminated from any home range analyses as it was assumed movements of lynx initially post-release may not be representative of normal habitat use. All locations used within a single home range analysis were taken a minimum of 24 hours apart.

Home range estimates were classified as being for a reproductive or non-reproductive animal. A reproductive female was defined as one that had kittens with her; a reproductive male was defined as a male whose movement patterns overlapped that of a reproductive female. If a litter was lost within the defined year a home range described for a reproductive animal were estimated using only locations obtained while the kittens were still with the female.

Survival and mortality factors

When a mortality signal was heard during either satellite, aerial or ground surveys, the location (UTM coordinates) was recorded. Ground crews then located and retrieved the carcass as soon as possible. The immediate area was searched for evidence of cause of death. All carcasses were transported to the Colorado State University Veterinary Teaching Hospital for a post mortem exam to 1) determine the cause of death and document with evidence; 2) collect samples for a variety of research projects, and 3) archive samples for future reference (research or forensic).

Reproduction

Females were monitored for proximity to males during each breeding season. We defined a possible mating pair as any male and female documented within at least 1 km of each other in breeding season through either flight data or snow-tracking data. Females were then monitored for site fidelity to a given area during each denning period of May and June. Each female that exhibited stationary movement patterns in May or June were closely monitored to locate possible dens. Dens were found when field crews walked in on females that exhibited virtually no movement for at least 10 days from both aerial and ground telemetry.

Kittens found at den sites were weighed, sexed and photographed. Each kitten was uniquely marked by inserting a sterile passive integrated transponder (PIT, Biomark, Inc., Boise, Idaho, USA) tag subcutaneously between the shoulder blades. Time spent at the den was minimized to ensure the least amount of disturbance to the female and the kittens. Weight, PIT-tag number, sex and any distinguishing characteristics of each kitten was also recorded. Beginning in 2005, blood and saliva samples were collected and archived for genetic identification.

During the den site visits, den site location was recorded as UTM coordinates. General vegetation characteristics, elevation, weather, field personnel, time at the den, and behavioral responses of the kittens and female were also recorded. Once the females moved the kittens from the natal den area, den sites were visited again and site-specific habitat data were collected (see Habitat Use section below).

Site-scale habitat use

Gross habitat use was documented by recording canopy vegetation at aerial locations. More refined descriptions of habitat use by reintroduced lynx were obtained through following lynx tracks in the snow (i.e., snow-tracking) and site-scale habitat data collection conducted at sites found through this method to be used by lynx.

Diet and hunting behavior

Winter diet of reintroduced lynx was estimated by documenting successful kills through snow-tracking. Prey species from failed and successful hunting attempts were identified by either tracks or remains. Scat analysis also provided information on foods consumed. Scat samples were collected wherever found and labeled with location and individual lynx identification. Only part of the scat was collected (approximately 75%); the remainder was left in place in the event that the scat was being used by the animal as a territory mark. Site-scale habitat data collected for successful and unsuccessful snowshoe hare kills were compared.

Results

Reintroduction effort

From 1999 through 2006, 218 lynx were reintroduced into southwestern Colorado (Table 1). All lynx were released in the Core Release Area of southwestern Colorado at or near previously used release sites in southwestern Colorado. Lynx were released with dual VHF/satellite radio collars so they could be monitored for movement, reproduction and survival.

Distribution and movement patterns

A total of 8680 aerial VHF locations for all 218 reintroduced lynx have been collected to date (June 30, 2006). An additional 18,963 satellite locations have been collected. Most lynx released in 2006 remained in southwestern Colorado. The majority of surviving lynx from the entire reintroduction effort continue to use high elevation (>2900 m), forested areas from New Mexico north to Gunnison, west as far as Taylor Mesa and east to Monarch Pass. Most movements away from the Core Release Area were to the north.

Numerous travel corridors have been used repeatedly by more than one lynx. Lynx appear to remain faithful to an area during winter months, and exhibit more extensive movements away from these areas in the summer. Such movement patterns have also been documented by native lynx in Wyoming and Montana (Squires and Laurion, 1999).

• Home range

Reproductive females had the smallest 90% utilization distribution annual home ranges ($\bar{x}$=75.2 km^2, SE=15.9 km^2, n=19), followed by attending males ($\bar{x}$=102.5 km^2, SE=39.7 km^2, n=4). Non-reproductive females had the largest annual home ranges ($\bar{x}$=703.9 km^2, SE=29.8 km^2, n=32) followed by non-reproductive males ($\bar{x}$=387.0 km^2, SE=73.5 km^2, n=6). Combining all non-reproductive animals yielded a mean annual home range of 653.8 km^2 (SE=145.4 km^2, n=38).

• Survival and mortality factors

As of June 30, 2006, CDOW was actively tracking 95 of the 138 lynx still possibly alive. There are 43 lynx that we have not heard signals on since at least June 30, 2005 and these animals are classified as "missing". One of these missing lynx is a mortality of unknown identity, thus only 42 are truly missing. Possible reasons for not locating these missing lynx include 1) long distance dispersal, beyond the areas currently being searched, 2) radio failure or 3) destruction of the radio (e.g., run over by car). CDOW continues to search for all missing lynx during both aerial and ground searches. Two of the missing lynx released in 2000 are thought to have slipped their collars.

Of the total 218 adult lynx released from 1999-2006 there are 80 known mortalities as of June 30, 2006. Causes of death are listed in Table 2. Starvation was a significant cause of mortality in the first year of releases only. Mortalities occurred throughout the areas through which lynx moved. Approximately 31.3% were human-induced which were attributed to collisions with vehicles

Year	Females	Males	TOTAL
1999	22	19	41
2000	35	20	55
2003	17	16	33
2004	17	20	37
2005	18	20	38
2006	6	8	14
TOTAL	115	103	218

Table 1. Lynx released in Colorado from February 1999 through June 30, 2006.

Tabla 1. Linces liberados en Colorado entre febrero de 1999 y el 30 de junio de 2006.

Cause of Death	Number of Mortalities
Unknown	26
Hit by Vehicle	11
Starvation	10
Shot	9
Other Trauma	7
Probable Shot	5
Plague	5
Predation	3
Probable Predation	2
Illness	2
Total Mortalities	80

Table 2. Causes of death for lynx released into southwestern Colorado from 1999-2006 as of June 30, 2006.

Tabla 2. Causas de la muerte de linces soltados en el suroeste de Colorado entre 1999 y 2006, (datos hasta el 30 de junio de 2006).

or gunshot. Malnutrition and disease/illness accounted for 21.3% of the deaths while 32.5% of the deaths were from unknown causes (Table 2).

Reproduction

Reproduction was first documented in 2003 when six dens and a total of 16 kittens were found in southwestern Colorado. Reproduction was also documented in 2004, 2005 and 2006 (Table 3). We weighed, photographed, PIT-tagged the kittens and recorded sex. We also took blood samples from the kittens for genetic work in an attempt to confirm paternity. While we were working with the kittens the females remained nearby, often remaining visible to us. The females generally continued a low growling vocalization the entire time we were at the den. In all cases, the female returned to the den site once we left the area. At all dens the females appeared in excellent condition, as did the kittens. The kittens weighed from 250-770 grams. Lynx kittens weigh approximately 200 grams at birth and do not open their eyes until they are 10-17 days old. Mean number of kittens per litter from 2003-2006 was 2.78 (SE=0.05) and sex ratio of females to males was equal ($\bar{x}$=1.14, SE=0.14).

The percent of tracked females found with litters in 2006 was lower (0.095) than in the three previous years (0.413, SE=0.032, Table 3). However, all demographic and habitat characteristics measured at the four dens that were found in 2006 were comparable to all other dens found. In addition, a female lynx born in Colorado in 2004 was the mother of one of the 2006 litters which documented the first recruitment of Colorado-born lynx into the Colorado breeding population.

Den sites. A total of 37 dens have been found from 2003-2006. All of the dens except one have been scattered throughout the high elevation areas of Colorado, south of I-70. In 2004, one den was found in southeastern Wyoming, near the Colorado border. Dens were located on steep ($\bar{x}_{slope}$=30°, SE=2°), north-facing, high elevation ($\bar{x}$=3354 m, SE=31 m) slopes. The dens were typically in Engelmann spruce/subalpine fir forests in areas of extensive downfall of coarse woody debris. All dens were located within the winter use areas used by the females.

Site-scale habitat use

Landscape-scale daytime habitat use was documented from 7421 aerial locations of lynx collected from February 1999-June 30, 2005. Throughout the year Engelmann spruce-subalpine fir was the dominant cover used by lynx.

Year	# Females Tracked	# Dens Found in May/June	% Tracked Females with Kittens	Additional Litters Found in Winter	Mean # Kittens/Litter	Total Kittens Found	Sex Ratio M/F
2003	17	6	0.353		2.67 (SE=0.33)	16	1.0
2004	26	11	0.462	2	2.83 (SE=0.24)	39	1.5
2005	40	17	0.425	1	2.88 (SE=0.18)	50	0.8
2006	42	4	0.095		2.75 (SE=0.47)	11	1.2
Mean			0.334 (SE=0.083)		2.78 (SE=0.05)	TOTAL 116	1.14 (SE=0.14)

Table 3. Lynx reproduction summary statistics for 2003-2006.

Tabla 3. Cifras resumidas correspondientes a la reproducción del lince durante los años 2003-2006.

A mix of Engelmann spruce, subalpine fir and aspen *(Populus tremuloides)* was the second most common cover type used throughout the year. Various riparian and riparian-mix areas were the third most common cover type where lynx were found during the daytime flights. Use of Engelmann spruce-subalpine fir forests and Engelmann spruce-subalpine fir-aspen forests was similar throughout the year. There was a trend in increased use of riparian areas beginning in July, peaking in November, and dropping off December through June.

The most common tree species documented in the site-scale habitat plots was Engelmann spruce. Subalpine fir and aspen were also present in >35% of the plots. While Engelmann spruce and subalpine fir occurred in similar densities for kills, long beds and travel sites, den sites had twice the density of subalpine firs found at all other sites.

Diet and hunting behavior

Winter diet of lynx was documented through detection of kills found through snow-tracking. Prey species from failed and successful hunting attempts were identified by either tracks or remains. Scat analysis also provided information on foods consumed. A total of 400 kills were located from February 1999-April 2005. We collected 671 scat samples from February 1999-April 2004 for content analysis. In each winter, the most common prey item was snowshoe hare, followed by red squirrel (Table 4).

A comparison of percent overstory for successful and unsuccessful snowshoe hare chases indicated lynx were more successful at sites with slightly higher percent overstory, if the overstory species were Englemann spruce, subalpine fir or willow. Lynx were slightly less successful in areas of greater aspen overstory. Higher density of Engelmann spruce and subalpine fir increased hunting success while increased aspen density decreased hunting success.

Discussion

In an effort to establish a viable population of lynx in Colorado, CDOW initiated a reintroduction effort in 1997 with the first lynx released in winter 1999. From 1999 through spring 2005, 204 lynx were released in the Core Release Area. The reintroduction effort was augmented with the release of 14 additional animals in April 2006, bringing the total to 218 lynx reintroduced to southwestern Colorado.

Locations of each lynx were collected through aerial- or satellite-tracking to document movement patterns and to detect mortalities. Most lynx remain in the high elevation, forested areas in southwestern Colorado. Dispersal movement patterns for lynx released in 2000 and subsequent years were similar to those of lynx released in 1999. However, more animals released in 2000 and subsequent years remained within the Core Release Area than those released in 1999. This increased site fidelity may have been due to the presence of con-

		Prey (%)			
Field Season	n	Snowshoe Hare	Red Squirrel	Cottontail	Other
1999	9	55.56	22.22	0	22.22
1999-2000	83	67.47	19.28	1.20	12.05
2000-2001	89	67.42	19.10	8.99	4.49
2001-2002	54	90.74	5.56	0	3.70
2002-2003	65	90.77	6.15	0	3.08
2003-2004	37	67.57	27.03	2.70	2.70
2004-2005	78	83.33	10.26	0	6.41

Table 4. Number of kills found each winter field season through snow-tracking of lynx and percent composition of kills of the three primary prey species.

Tabla 4. Número de carcasas detectadas, mediante rastreo de huellas de lince en la nieve, en distintos períodos de trabajo de campo en invierno y porcentajes de las tres presas principales que conformaban dichas carcasas.

specifics in the area on release. Numerous travel corridors have been used repeatedly by more than one lynx. Lynx appear to remain faithful to an area during winter months, and exhibit more extensive movements away from these areas in the summer. Most lynx currently being tracked are within the Core Release Area. During the summer months, lynx were documented to make extensive movements away from their winter use areas. Extensive summer movements away from areas used throughout the rest of the year have been documented in native lynx in Wyoming and Montana (Squires and Laurion, 1999). Human-caused mortality factors such as gunshot and vehicle collision are currently the highest causes of death.

Reproduction is critical to achieving a self-sustaining viable population of lynx in Colorado. Reproduction was first documented from the 2003 reproduction season and again in 2004, 2005 and 2006. Reproduction in 2006 included a Colorado-born female giving birth to two kittens, documenting the first recruitment of Colorado-born lynx into the Colorado breeding population. Additional reproduction is likely to have occurred in all years from females we are no longer tracking, and from Colorado-born lynx that have not been collared. The dens we find are more representative of the minimum number of litters and kittens in a reproduction season. To achieve a viable population of lynx, enough kittens need to be recruited into the population to offset the mortality that occurs in that year and hopefully even exceed the mortality rate for an increasing population.

Mowat et al., (1999) suggest lynx and snowshoe hare select similar habitats except that hares select more dense stands than lynx. Very dense understory limits hunting success of the lynx and provides refugia for hares. Given the high proportion of snowshoe hare in the lynx diet in Colorado, we might then assume the habitats used by reintroduced lynx also depict areas where snowshoes hare are abundant and available for capture by lynx in Colorado. From both aerial locations taken throughout the year and from the site-scale habitat data collected in winter, the most common areas used by lynx are in stands of Engelmann spruce and subalpine fir. This is in contrast to adjacent areas of Ponderosa pine, pinyon juniper, aspen and oakbrush. The lack of lodgepole pine in the areas used by the lynx may be more reflective of the limited amount of lodgepole pine in southwestern Colorado, the Core Release Area, rather than avoidance of this tree species.

In winter, hares browse on small diameter woody stems (<0.25"), bark and needles. In summer, hares shift their diet to include forbs, grasses, and other succulents as well as continuing to browse on woody stems. This shift in diet may express itself in seasonal shifts in habitat use, using more or denser coniferous cover in winter than in summer. The increased use of riparian areas by lynx in Colorado from July to November may reflect a seasonal shift in hare habitat use in Colorado. Major (1989) suggested lynx hunted the edge of dense riparian willow stands. The use of these edge habitats may allow lynx to hunt hares that live in habitats normally too dense to hunt effectively. The use of riparian areas and riparian-Engelmann spruce-subalpine fir and riparian-aspen mixes documented in Colorado may stem from a similar hunting strategy. However, too little is known about habitat use by hares in Colorado to test this hypothesis at this time.

Lynx also require sufficient denning habitat. Denning habitat has been described by Koehler (1990) and Mowat et al., (1999) as areas having dense downed trees, roots, or dense live vegetation. We found this to be in true in Colorado as well. In addition, the dens used by reintroduced lynx were at high elevations and on steep north-facing slopes. All females that were documented with kittens denned in areas within their winter-use area.

Snow-tracking of released lynx provided information on hunting behavior and diet through documentation of kills, food caches, chases, and diet composition estimated through prey remains. Snow-tracking results indicate the primary winter prey species are snowshoe hare and red squirrel, with other mammals and birds forming a minor part of the winter diet. In winter, lynx reintroduced to Colorado appear to be feeding on their preferred prey species, snowshoe hare and red squirrel in similar proportions as those reported for northern lynx during lows in the snowshoe hare cycle (Aubry et al., 1999). Caution must be used in interpreting the proportion of identified kills. Such a proportion ignores other food items that are consumed in their entirety and thus are biased towards larger prey and may not accurately represent the proportion of smaller prey items, such as microtines, in lynx winter diet. Through snow-tracking we have evidence that lynx are mousing and several of the fresh carcasses have yielded small mammals in the gut on necropsy. All evidence suggests reintroduced lynx are finding adequate food resources.

From results to date it can be concluded that CDOW has developed release protocols that ensure high initial post-release survival, and on an individual level lynx have demonstrated they can survive long-term in areas of Colorado. It has also been documented that reintroduced lynx could exhibit site fidelity, engage in breeding behavior and produce kittens that are recruited into the Colorado breeding population. What is yet to be demonstrated is whether current conditions in Colorado can support the recruitment necessary to offset annual mortality for a population to sustain itself. Monitoring of reintroduced lynx will continue in an effort to document such viability.

Acknowledgements

The lynx reintroduction programme involves the efforts of literally hundreds of people across North America, in Canada and the U.S. Any attempt to properly acknowledge all the people who played a role in this effort is at risk of missing many people. Personnel from the following agencies were critical to the implementation and support of the project. Colorado Division of Wildlife (CDOW) CLAWS Team (1998-2001), CDOW, Lynx Advisory Team (1998-2001), U.S. Forest Service, U.S. Fish and Wildlife Service, National Park Service, Colorado State University, Colorado Natural Heritage Programme, Wildlife Agencies in the following US States (Washington, Alaska, Montana, Maine, Minnesota, Wyoming, Utah, New Mexico), and Government and Trapping Associations in the Canadian Provinces. Special acknowledgements are given to personnel ant the Colorado Holding Facility, the CDOW Pilots and Field Crews (1999-2006). Funding was provided by CDOW, Great Outdoors Colorado (GOCO), Turner Foundation, U.S.D.A. Forest Service, Vail Associates and the Wildlife Heritage Foundation.

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Photo: Tanya Shenk


Photo: Joe Zammit-Lucia

The tree which moves some to tears of joy is in the eyes of others only a green thing that stands in the way. Some see nature all ridicule and deformity... and some scarce see nature at all. But to the eyes of the man of imagination, nature is imagination itself.

William Blake (1757-1827)

Restoration of bobcats to Cumberland Island, Georgia, USA: lessons learned and evidence for the role of bobcats as keystone predators

Reintroducción del lince rojo en la isla de Cumberland, Georgia, EEUU: lecciones aprendidas y evidencia del papel del lince rojo como predador clave

Duane R. Diefenbach, Leslie A. Hansen, Robert J. Warren, Michael J. Conroy and M. Greg Nelms

Resumen

Entre 1988 y 1989, durante un proyecto de reintroducción, reubicamos a 32 linces rojos (*Lynx rufus*; 3,1 linces rojos/10 km^2) en una isla costera de Estados Unidos, la isla de Cumberland, en Georgia, con el fin de estudiar la reintroducción de un predador nativo. La supervivencia anual de los adultos fue del 93% (Error standard=2.6%) durante los tres primeros años. Los linces rojos recapturados tuvieron un aumento medio de peso de 0,8 kg (un incremento del 12% desde el momento de la suelta). Se documentó la reproducción de los ejemplares reintroducidos. Las presas principales fueron los conejos *(Sylvilagus palustris)*, los ciervos de cola blanca *(Odocoileus virginianus)* y las ratas de algodón *(Sigmodon hispidus)*. Entre 1997 y 1998 hubo un cambio en el tipo de presa y la presencia de ciervos y conejos en heces fue menos frecuente que la de las demás especies. Ningún lince rojo llegó a mantener un área de uso exclusivo, sin solapamiento con otros congéneres. Las estimaciones e índices de abundancia de ciervos indicaron que tras la reintroducción de los linces rojos hubo una disminución de la población de ciervos, que se mantuvo en niveles bajos, aunque el peso corporal medio de los ciervos aumentó una media de 11 kg entre 1989 y 1997. En nueve parcelas, que contenían un total de 87 robles, la regeneración de cada árbol fue medida entre 1990 y 1997; el número de árboles con plantones o brotes de raíces se incrementó de 52 a 86 y el número medio de plantones por parcela aumentó en 153,5. En aquellas parcelas que contenían plantones y brotes, tanto en 1990 como en 1997, la altura media se incrementó en 4,6 cm (95% IC=4.0–5.2). Nuestras observaciones sobre el consumo de ciervos como presa primaria de los linces rojos, la disminución de la abundancia de ciervos y la mayor regeneración del roble indicaban que los linces rojos reintroducidos causaron un efecto de cascada trófica de la isla. Los estudios previos a la reintroducción de los linces rojos indicaron que los ciervos eran abundantes y que su ramoneo impedía la regeneración de los árboles. Según los mismos estudios, los ciervos eran presas idóneas para los linces rojos por su abundancia y su tamaño reducido. El seguimiento posterior a la suelta de una especie reintroducida proporciona información que nos permite comprender las razones por las cuales un proyecto de reintroducción pueda tener éxito o fracase. Asimismo, en los proyectos de reintroducción de poblaciones de predadores debe plantearse el posible seguimiento de las características relacionadas con los niveles tróficos del ecosistema, teniendo siempre en cuenta el escaso conocimiento que tenemos sobre el papel de los predadores en los ecosistemas, sobretodo el papel de los predadores vertebrados. Si se creasen programas de seguimiento para probar las teorías de la dinámica de las poblaciones comunitarias, habría mayores posibilidades de entender mejor las redes tróficas de los ecosistemas terrestres, así como sus interrelaciones.

Palabras clave
Predador clave, lince rojo, *Lynx rufus*, reintroducción, organización espacial, cascada trófica

Abstract
We translocated 32 bobcats (*Lynx rufus*; 3.1 bobcats/10 km^2) to a coastal barrier island, Cumberland Island, Georgia, USA, during 1988-1989 to restore a native predator. Annual survival of adults was 93% (SE=2.6%) for the first three years and recaptured bobcats exhibited an average weight gain of 0.8 kg (12% increase), and we documented reproduction. Marsh rabbits *(Sylvilagus palustris)*, white-tailed deer *(Odocoileus virginianus)* and hispid cotton rats *(Sigmodon hispidus)* were the principal prey species. By 1997-1998, prey use changed, in which white-tailed deer and marsh rabbits occurred less frequently in scats and all other species occurred more frequently. No bobcats retained areas of exclusive use from conspecifics of the same sex. Estimates and indices of deer abundance indicated that following reintroduction of bobcats the deer population declined and remained low but body weights of deer averaged 11.0 kg greater in 1997 compared to 1989. On nine plots containing 87 oak trees, where oak regeneration at each tree was measured in 1990, the number of trees with seedlings or root sprouts increased from 52 to 86 and the average number of seedlings per plot increased by 153.5. On plots that contained seedlings and sprouts in both 1990 and 1997, average height increased 4.6 cm (95% CI=4.0–5.2). Our observations of bobcat use of deer as a primary prey species, a decline in deer abundance, and an increase in oak regeneration indicated that bobcats caused a trophic cascade effect on the island. Research prior to the restoration of bobcats indicated deer were abundant and deer browsing suppressed tree regeneration, and apparently deer were suitable prey for bobcats because of their abundance and small size. Post-release monitoring of a reintroduced species provides information to understand why a reintroduction project succeeds or fails. Moreover, restoration projects of predator populations should consider monitoring trophic level characteristics of ecosystems because the role of predators in ecosystems in poorly understood, especially vertebrate predators. If monitoring programmes were developed to test theories of community population dynamics, there would be potential to better understand food webs of terrestrial ecosystems and trophic level inter-relationships.

Keywords
Food habits, keystone predator, *Lynx rufus,* reintroduction, spatial organization, trophic cascade

Restoration of bobcats to Cumberland Island, Georgia, USA: lessons learned and evidence for the role of bobcats as keystone predators

Duane R. Diefenbach, Leslie A. Hansen, Robert J. Warren, Michael J. Conroy and M. Greg Nelms

Introduction

The United States national park system is a network of nearly 400 natural, cultural and recreational sites across the nation designated to preserve and protect unique natural and cultural features. National parks designated for their natural resources have been mandated by the United States Congress to maintain the abundance, diversity, and ecological integrity of native plants and animals (16 United States Code 1, 2-4). The enabling legislation for Cumberland Island National Seashore (CINS) included directives to the National Park Service (NPS) on how the island should be managed, which included providing for public outdoor recreational use (including hunting, fishing and trapping), preserving related scenic, scientific and historic values, and conserving its primitive state together with its flora and fauna. Consequently, the Resources Management Plan for CINS (National Park Service, 1983) specifically identified extirpated species and the possibility of their reintroduction. Among extirpated predators, Harris (1984) identified the bobcat *(Lynx rufus)* as a species to receive highest priority for reintroduction because it would be least likely to cause human-related conflicts.

In 1988, the National Park Service funded the reintroduction of bobcats to Cumberland Island. However, because this project involved federal funding and public land, an Environmental Assessment (EA) was required under the National Environmental Policy Act (Public Law 91-190) to consider the environmental effects of reintroduced bobcats. We prepared the EA for the NPS (Warren et al., 1990) and justified the project from the standpoint of restoring ecological control over several species of native and exotic herbivores. We cited evidence of grazing and browsing effects from white-tailed deer *(Odocoileus virginianus)*, feral horses *(Equus caballus)*, and feral hogs *(Sus scrofa)* (Hillestad et al., 1975; Ambrose et al., 1983; Turner, 1986) and noted that bobcats can kill healthy, adult deer (McCord and Cardoza, 1982; Anderson, 1987). Furthermore, studies in the southeastern United States indicated that bobcats were effective predators on white-tailed deer fawns (Epstein et al., 1985). Other potential prey species on the island included hispid cotton rats *(Sigmodon hispidus)*, cotton deermice *(Peromyscus gossypinus)*, eastern gray squirrels *(Sciurus carolinensis)*, marsh rabbits *(Sylvilagus palustris)*, nine-banded armadillos *(Dasypus novemcinctus)*, and wild turkey *(Meleagris gallapavo)*. Of these species, wild turkeys were not expected to be an important component of bobcat diets.

During fall of 1988 and 1989 we released a total of 32 bobcats throughout Cumberland Island. All bobcats were captured from the coastal plain of Georgia and fitted with a radio-collar to monitor post-release movements, survival, and reproduction. This translocation of bobcats to Cumberland Island afforded the opportunity to conduct a reintroduction experiment for a mid-sized felid, in which failure would have no adverse effect on the global status of the species. Furthermore, post-release monitoring provided an opportunity to study the genetics and population viability of an insular predator population, test a population monitoring technique with a known population size, monitor the social organization and spatial distribution of bobcats, study predator-prey dynamics, and monitor changes in community structure and trophic-level interactions.

In this paper we 1) identify key lessons we learned that could be useful for future felid reintroductions, 2) demonstrate the importance of post-reintroduction monitoring to learn more about the role of predators in ecosystem functioning and 3) summarize insights we have gained about bobcat prey selection and social organization in a solitary felid. Also, we present previously unpublished data we collected that provide evidence for bobcats initiating a top-down trophic cascade on the Cumberland Island forested ecosystem.

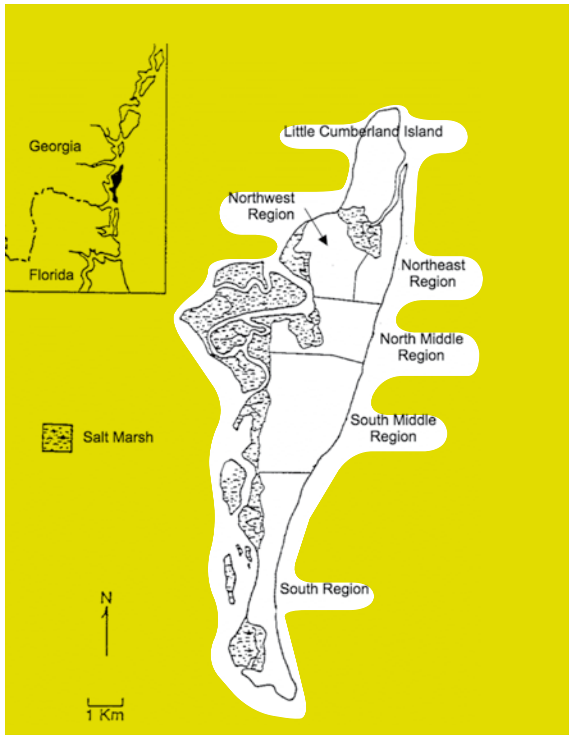

Figure 1. Location of Cumberland Island along the Georgia-Florida, USA coastline (inset) and regions of the island delineated for food habits analyses.

Figura 1. Situación geográfica de la isla de Cumberland en la costa estadounidense de Georgia-Florida (recuadro) y delimitación de las regiones de la isla para el análisis de costumbres alimentarias.

Materials and methods

Study area

Cumberland Island, a coastal barrier island 0.5 km north of the Georgia-Florida border (30°48' 16"N, 81°27'36"W), is the largest among a series of barrier islands that extend along the Atlantic Ocean seaboard from Cape Hatteras, North Carolina south to Talbot Island, Florida (Figure 1). The island is 25 km long and varies in width from 1 to 6 km. It is separated from the mainland by 2–4 km of salt marsh and open water. To the north is Little Cumberland Island, which is separated from Cumberland Island by <0.25 km of salt marsh and a tidal creek.

Immediately behind the eastern shore and primary dunes of the island is an interdune meadow, ≤200 m in width, dominated by grasses, sedges, and waxmyrtle *(Myrica cerifera)*. The interior of the island is dominated by live oak *(Quercus virginianus)* and pine *(Pinus)* forests with much of the understory dominated by stands of sawtooth palmetto *(Serenoa repens)*. Freshwater wetlands follow natural depressions between former dune ridges in the interior of the islands. The western edges of the islands are salt marsh.

Cumberland Island contains five major vegetation associations covering 84.5 km^2: sandy beach and interdune meadow (14.7 km^2); maritime forest, including lowland hardwoods (38.7 km^2); scrub-shrub thickets that developed after natural fires (7.0 km^2); freshwater wetlands (6.7 km^2); and salt marsh (17.4 km^2). Little Cumberland Island is 9.0 km^2 and contains sandy beach and interdune meadow (1.3 km^2), maritime forest (4.8 km^2), and salt marsh (4.8 km^2). Hereafter, we refer to the two islands together as Cumberland Island.

The climate is warm temperate to subtropical, with normal mean temperature ranging from 12 °C in January to 28 °C in July (Johnson et al., 1974). The average annual rainfall is 134 cm, with the wettest months being June through September ($\bar{x}$=16 cm/month) and the driest months being October, November, and April ($\bar{x}$=7 cm/month). Hillestad et al. (1975) described the study area in detail.

Capture and translocation

Details of the capture, handling, and translocation of bobcats are described in Diefenbach et al. (1993). We captured bobcats using hunting dogs *(Canis familiaris)*, foot-hold traps, and cage traps from the coastal plain of Georgia in the hope that these bobcats would have gene complexes adapted to the environment on Cumberland Island (Templeton, 1986). Captured bobcats were held in a facility for ≤1 month to monitor behavior and detect illness or injury. Only adult bobcats (≥1 year old) were reintroduced to Cumberland Island and all reintroduced bobcats were vaccinated for feline panleukopenia, rhinotracheitis, and calicivirus. Also, we held bobcats in captivity until we had 4-6 individuals to release so we could evaluate the scent-station population monitoring technique with a controlled increase in population size (Diefenbach et al., 1994).

Our goal was to release approximately 30 bobcats because this would result in a density similar to maximum densities on the mainland (1 bobcat/2.6 km^2). All releases of bobcats were "hard releases" in which bobcats were transported to the release site and freed. Release sites occurred throughout the island that were easily accessible by vehicle and likely were outside the home range of previously reintroduced bobcats.

Post-release monitoring

We began trapping on Cumberland Island to recapture bobcats a few months after the first bobcats were released on the island (Diefenbach et al., 1993). This recapture effort provided information on the physiological status of bobcats, allowed us to replace radio-collars before batteries failed, and capture bobcats born on the island to assess recruitment and survival of juveniles. We used only cage traps because they were less controversial than foot-hold traps even though they were inefficient and less effective. Captured juveniles were not vaccinated and were fitted with a radio-transmitter attached to a harness (Jackson et al., 1985) rather than a collar.

We monitored survival and locations of bobcats via triangulation of radio signals from the ground or by locating bobcats with fixed-wing aircraft throughout the year and the 24-hour day (Diefenbach et al., 2006). These data were used to assess habitat use (Baker et al., 2001; James, 1992) and spatial organization (Diefenbach et al., 2006). We monitored reproduction by conducting intensive telemetry monitoring of females during the denning season (see Ragsdale [1993] for details) to located dens and document reproduction.

To monitor food habits and prey selection we collected bobcat scats and measured prey abundance (Baker, 1991; Baker et al., 1993; Nelms, 1999; Baker et al., 2001). From November 1988 through July 1990 we surveyed prey abundance during a three-week period in November, March and July. We defined four habitats for purposes of analysis: woodlands with understories of saw palmetto (oak-palmetto), woodlands with understories not dominated by saw palmetto (open woodland), interdune meadow and an area that burned in 1981 (scrub thicket). These habitat types composed 26%, 37%, 9% and 10% of the island area, respectively, with the remainder of the island being bottomland hardwoods/shrubs, beach and residential areas.

Whenever possible, we used distance sampling methods (trapping webs or line transect surveys, Buckland et al., 2001) to estimate prey density (Baker et al., 2001). We used spotlight transects to estimate island-wide abundance of white-tailed deer and raccoons *(Procyon lotor)*. We walked eight permanent transects during early morning and late afternoon to assess habitat-specific abundance for white-tailed deer, eastern gray squirrels, nine-banded armadillos, marsh rabbits, feral swine, and raccoons. In November 1988 and March 1989 we walked each transect twice and alternated the starting point on consecutive days; four times thereafter. Each transect traversed multiple habitat types and pooled transect lengths in each habitat type were 11.5 km in oak-palmetto, 20.9 km in open woodland and 6.8 km in interdune meadow; vegetation in the scrub-thicket was too dense to observe animals. If we obtained too few observations or could not meet the assumptions of distance sampling we calculated indices of abundance (captures per 100 trap nights or numbers seen per km).

We collected bobcat scats by walking roads, trails, and dune/forest edges during a six week period encompassing each of the prey abundance surveys. Methods for identification of prey remains were described by Baker et al. (1993). Also, frequency of occurrence was estimated (no. of occurrences of a species/total no. of scats) and diet diversity was calculated using (1 – Simpson's Diversity Index), in which greater values indicated greater diet diversity (Hall et al., 1984). We used prey abundance and bobcat diet data to examine differences in diet (prey use among species and spatial differences across the island) and changes in diet over time. Also, we tested predictions of functional relationships of bobcat diets, and if diets differed between males and females. Details of the analytical methods used are described in Baker et al. (2001).

The scat collection and analysis procedures used by Baker (1991) and Baker et al. (2001) were replicated during November-December 1997, March 1998, and August 1998 (Nelms, 1999). Seasonal diets during 1997-98 were compared with the corresponding 1988-90 data (Baker et al., 2001) using chi-square tests of homogeneity (Conover, 1980).

Trophic level changes

We used weight, sex and age data collected from deer harvested during public hunts on CINS from 1980 to 1997, in which most deer were harvested on the northern end of CINS. Because the final bobcat releases were in fall 1989, we designated the years 1980-89 as the prerelease (PRE) period and 1990-98 the postrelease (POST) period.

We aged deer via the tooth wear and replacement method (Severinghaus, 1949) except all deer estimated to be ≥4.5 years were classified as 4.5+ because of small sample sizes and greater error associated with aging older deer. Age distributions for harvested male and female deer were compared using chi-square tests for homogeneity. We conducted a nested ANOVA (PROC GLM, SAS Institute, 2003) to test for changes in mean eviscerated weight between 1984-89 and 1990-97, in which age-sex class and bobcat release period (PRE or POST) were main effects and year was nested within time period. To test the effect of bobcat release period, the mean square error for the nested year effect was the divisor for the F test. Also, we estimated the change in mean eviscerated weights for each age-sex class in the PRE versus POST periods via t-tests with means of individual years as the sample unit.

We conducted deer spotlight surveys using protocols established for CINS by Ford (1987) and modified by Baker (1991) to obtain estimates of deer density using distance sampling (Buckland et al., 2001). Deer density estimates were converted to abundance estimates assuming 6,110 ha of upland habitat. However, because deer population estimates based on distance sampling were available only for four years during 1980-98, other methods were used to obtain estimates to demonstrate population trends. We estimated annual deer abundance using hunt data with the DeLury technique (Roseberry and Woolf, 1991; Appendix I) using the number of hunters and deer kill from all hunts in each year.

Lieske et al. (1990) established plots in spring 1990 immediately following the final bobcat releases. They chose 10 sites throughout the island and at each site located 10 oak (*Q. virginiana* and *Q. hemispherica*) trees, five with associated root sprouts or seedlings and five without. A 2x4 m plot was established on opposite sides of each tree. The center line of each plot was treated as a transect, and they measured heights of the closest 10 oak sprouts and seedlings along each transect line at 0, 1 2, 3, and 4 m from the base of each tree. Seedlings and sprouts were counted in subplots but the total 16 m^2 was treated as a single sample unit. We repeated these measurements in spring 1997 at 9 of the 10 plots and conducted an ANOVA to test whether the number of seedlings and sprouts differed between 1990 and 1997 with year, plot, and tree nested within plot as explanatory variables in the model (PROC GLM, SAS Institute, 2003). We used McNemar's test to test whether the number of plots with seedlings and sprouts changed between 1990 and 1997. We used the difference in mean sprout height at each plot to calculate the change in mean sprout heights between 1990 and 1997 at each plot.

Results

The initial justification of the reintroduction for the EA, to control herbivores, was a mistake (Warren et al., 1990) because it elicited a variety of negative comments from the public. Some environmental organizations and individuals questioned whether herbivores should be controlled in national parks, whereas hunters voiced concern about bobcats preying on wild turkeys, a game species, and recommended that deer population control could be accomplished more cost-effectively using hunters. Consequently, in subsequent news releases and public meetings we emphasized the purpose of the project was to reintroduce a formerly native species to restore biological diversity. This approach to justifying the reintroduction was successful in reducing much of the controversy, and subsequent newspaper articles de-emphasized the controversial aspects and emphasized the broader ecological significance of the project (Warren et al., 1990).

Following the public comment period, the NPS issued a Finding of No Significant Impact, which meant the project had no significant human impact and the project could move forward without further review. In hindsight, we underestimated public support for a reintroduction for its own sake, that of restoring a native predator. Had we conducted public scoping or human dimensions surveys prior to preparing the EA, we might have identified the diversity of public opinions that surrounded the proposed bobcat restoration project. Furthermore, a proactive role with the media could have minimized misconceptions about the project and resulting controversy, and personal contacts with influential people in the local community could have allowed us to identify opposition to the project prior to formally releasing the EA.

Capture and translocation

In fall 1988, we released 14 bobcats on the island (3 males, 11 females); four on 13 October, six on 3 November, and four on 28 November. Details of the capture, handling, and transporting of bobcats was described in detail by Diefenbach et al. (1993), and most aspects of this part of the project were successful. One bobcat died in captivity when it slipped its jaw through the radio-collar and suffocated; subsequently, bobcats were not fitted with a radio-collar until immediately prior to release. This resulted in additional handling of bobcats, but allowed us to assess bobcat condition immediately prior to release. Of those bobcats released in 1988, one female returned to the mainland in February 1989 and another died in January 1989, possibly due to injuries inflicted by a feral hog. In fall 1989, we released 18 bobcats (12 males, 6 females); six on 5 October, six on 25 October and six on 4 December. One male released in the interdune area swam into the Atlantic Ocean and presumably drowned.

Post-release monitoring

The effort to recapture bobcats on the island resulted in recapturing eight of 12 bobcats from the first year's release and nine of 15 bobcats from the second year's release. Thus, for the first three years of the project we knew the fate of all but one female bobcat, due to transmitter failure. Annual survival of adult bobcats was 93% (SE=2.6%, Diefenbach, 1992). Diefenbach et al. (1993) reported that most bobcats exhibited weight gains, in which bobcats increased an average of 0.8 kg (12.3%). Therefore, we have no evidence that food was limiting during this time period.

In 1989 we documented 10 kittens born in four litters, of which we monitored three from 4-10 months of age, captured and radio-collared three as adults in 1990, and recovered the carcass of one that died at two years of age. In 1990, we located one den with two kittens, and in 1991 we found no evidence that any females denned, although later in the year we observed two 3- to 4-month-old kittens. Recaptures of females for which we did not find dens with kittens indicated that they were not lactating and were unlikely to have produced young (Ragsdale, 1993).

Bobcat prey abundance differed among regions and varied seasonally, but we detected no effect of season or year on diet composition during 1989-1990 (Baker et al., 2001). On an annual basis, marsh rabbits composed the largest proportion of the diet in three of four regions of the island in both years, whereas deer composed the largest proportion of the diet in the northwest region (Baker et al., 2001). Bobcats consumed marsh rabbit, white-tailed deer, cotton rat, grey squirrel, raccoon, unidentified bird species, cotton mouse and feral hogs (Table 1). Marsh rabbit, white-tailed deer and cotton rat were the only prey species identified in bobcat diets during all surveys; thus, we considered these species the principal prey.

Only marsh rabbits (Spearman's $r=0.83$, $n=6$, $P=0.043$) and hispid cotton rats (Spearman's $r=0.84$, $n=6$, $P=0.036$) in bobcat diets were correlated with their abundance, which suggests bobcats had a functional response to these prey species. Diet species diversity (Spearman's $r=-0.87$, $n=6$, $P=0.023$) and species richness in bobcat diets (Spearman's $r=-0.82$, $n=6$, $P=0.046$) were negatively correlated with marsh rabbit abundance. This agrees with the predictions of a diet optimization model, in which increased use of alternate prey species (raccoons, feral hogs, and cotton mice) increases with decrease in abundance of a preferred prey species. Finally, changes in bobcat density or sex ratio within regions were not correlated with prey use (Baker et al., 2001), which did not support the hypothesis of interference or differences in prey use by male and female bobcats.

	November/December			March/April			July/August		
Species	1988 (n=36)	1989 (n=84)	1997 (n=86)	1989 (n=39)	1990 (n=69)	1998 (n=82)	1989 (n=64)	1990 (n=65)	1998 (n=37)
Marsh rabbit	58	43	21	64	33	22	55	39	21
Deer	47	26	12	23	38	7	44	43	31
Cotton rat	3	7	11	15	19	5	3	9	5
Grey squirrel	0	16	13	5	10	11	3	6	5
Raccoon	3	7	10	3	3	5	2	5	12
Bird spp.	3	12	11	0	15	3	5	6	2
Cotton mouse	3	11	14	3	12	23	2	3	5
Feral hog	3	5	9	3	7	24	0	0	19

Table 1. Number of scats analyzed (n) and percent occurrence of prey species in seasonal bobcat diets on Cumberland Island, Georgia, USA, 1988-90 and 1997-98.

Tabla 1. Número de excrementos analizados (n) y porcentaje de existencia de las especies de presa en las dietas estacionales de los linces rojos en la isla de Cumberland, Georgia, EEUU, durante 1988-90 y 1997-98.

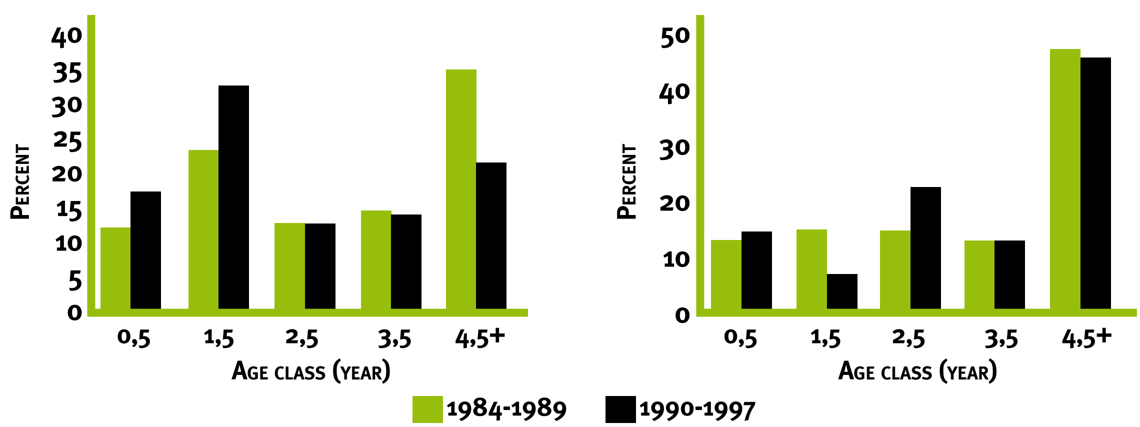

Figure 2. Age-sex structure of male (left) and female (right) white-tailed deer harvested on Cumberland Island, Georgia, USA during 1984-1989 and 1990-1997.

Figura 2. Distribución por edad-sexo de los ciervos de cola blanca capturados en la isla de Cumberland, Georgia, EEUU, durante los años 1984-1989 y 1990-1997: machos(izquierda) hembras (derecha).

We found bobcats consumed the same prey species during 1997-98, but detected important changes in prey use compared to 1988-90 (Table 1). Prey use differed in 1997-98 in fall (x^2_{14}=40.0, P < 0.001), spring (x^2_{14}=67.8, P <0.001), and summer (x^2_{14}=42.5, P <0.001). By 1997, marsh rabbit and white-tailed deer occurred less frequently in scats and occurrence of all other species increased in scats (Table 1) and this pattern was consistent among all three seasons. Overall, relative occurrence of prey species in scats was more evenly distributed during 1997-98 compared to 1988-90 (Table 1).

Female bobcats reintroduced during the first year of the reintroduction exhibited little change in the location or size of their home range, but failed to exclude newcomers from either their home range or core areas (Diefenbach et al., 2006). No bobcats retained areas of exclusive use from conspecifics of the same sex. We observed increasing intrasexual overlap among females during 1989-91, such that overlap of home ranges (95% fixed kernel utilization distributions) was equivalent to each female sharing her home range with >2 other

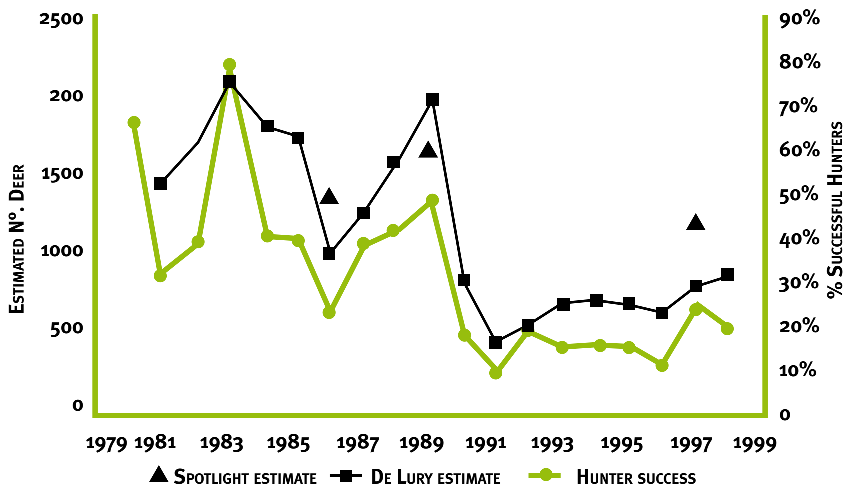

Figure 3. Population estimates (DeLury population estimate and spotlight surveys, see Methods) of white-tailed deer and an index of abundance (hunter success rate) on Cumberland Island, Georgia, USA, 1980-1998.

Figura 3. Estimaciones poblacionales de ciervos de cola blanca (utilizando el método DeLury y censos nocturnos con focos, véanse Métodos) e índice de abundancia (porcentaje de éxito de caza) en la isla de Cumberland, Georgia, EEUU, 1980-1998.

females. Overlap of core areas (50% fixed kernel utilization distributions) was equivalent to each female sharing her core area with nearly one other female.

Trophic level changes

The POST age distribution of harvested male deer was different from the PRE distribution (x^2_{14}=11.1, P=0.025), in which the proportion of fawns and yearlings increased, 2.5- and 3.5-year-old deer did not change, and 4.5+-year-old deer decreased (Figure 2). The POST age distribution of harvested female deer did not change (x^2_4=8.0, P=0.093; Figure 2). Body weights of deer increased after the reintroduction ($F_{18,819}$=100.93, P <0.001) and the effect of release period was significant ($F_{1,12}$=13.82, P=0.003). Mean eviscerated body weights increased 5.0–7.6 kg for males and 2.0–4.9 kg for females between the PRE and POST years Eviscerated body weights were 11.0 kg greater in 1997 compared to 1989 (average difference of means by age-sex class).

Estimates and indices of deer abundance indicated that following reintroduction of bobcats the population declined and remained low (Figure 3). The DeLury population estimates and hunter success rates declined following the bobcat reintroduction, even though the number of hunters during the PRE period ($\bar{x}$=270 hunters/year) differed little from the POST period ($\bar{x}$=278 hunters/year).

Of 10 plots established by Lieske et al. (1990), we re-visited nine plots and except for two trees (one dead and one not found) counted seedlings and sprouts. In 1997, 53 of 87 trees had ≥1 seedling or sprout within the 16-m^2 plot, but in 1990, 87 of 88 plots had ≥1 seedling or sprout (McNemar's test, x^2_1=33.0, P <0.001). The ANOVA indicated the overall F statistic was significant ($F_{87,88}$=3.44, P <0.001), the number of seedlings on plots increased an average of 153.5 between 1990 and 1997 ($F_{1,87}$=39.04, P <0.001) and we detected no differences among plots ($F_{8,79}$=0.64, P=0.742).

Discussion

We were able to successfully capture and translocate approximately 15 bobcats per year, and the complete reintroduction was completed in a short period of time and at low cost. Diefenbach et al. (1993) identified three important factors that reintroduction efforts should consider: 1) the source and genetic relatedness of individuals, 2) the age-sex structure of restored populations and 3) the method of releasing translocated individuals.

We captured bobcats from throughout the coastal plain of Georgia to obtain bobcats with gene complexes adapted to regional environmental conditions (Templeton, 1986), although multiple bobcats were captured from the same area. Consequently, some bobcats may be closely related genetically and may result in significant genetic founder effects on the population. We collected genetic samples (blood) from all bobcats, but these samples have not been analyzed. However, the opportunity exists to investigate the genetic changes in the population over time, which should be a priority of restoration efforts of an endangered species.

Photo: Duane Diefenback

Figure 4. Leslie Hansen releasing a bobcat on Cumberland Island.

Figura 4. Leslie Hansen liberando a un bobcat en la isla de Cumberland.

We were not able to capture enough bobcats on the mainland to control the sex ratio of the population of reintroduced bobcats. Consequently, at the conclusion of the translocations in 1989 the reintroduced population consisted of 14 males and 15 females, although it was female-biased (11 females, 3 males) after the translocations in 1988. These changes in the sex ratio of the population introduced potential confounding effects when interpreting results of our post-release monitoring of food habits and spatial organization (Baker et al., 2001; Diefenbach et al., 2006). Obviously, reintroductions of an endangered species should take greater care in the age-sex structure of the reintroduced population as well as the genetic relatedness of individuals.

We strongly recommend that reintroduction projects establish a means of conducting "slow-releases" whereby animals are held in captivity at the release site and allowed to leave captivity following a holding period. We believe that slow releases might have prevented the disorientation of the one bobcat that swam into the Atlantic Ocean and presumably drowned. However, the logistics of such an effort for our project were prohibitive (Diefenbach et al., 1993). We do not believe hard releases are justified in most reintroduction situations; for example, Brocke et al. (1991) reported movements of hundreds of kilometers for Canada lynx *(Lynx canadensis)* reintroduced to the Adirondacks in New York, USA.

Post-release monitoring is critical to evaluating the success or failure of a reintroduction project. We believe monitoring the physiological condition of animals after release and monitoring their movements, spatial distribution, survival and reproduction should be a mandatory aspect of any reintroduction effort. Future efforts to restore native species should endeavor to increase our knowledge about the effects of spatial distribution, genetics, demography, population size, mechanisms of population regulation, behavior and environmental conditions on the viability of populations. Most of our post-release monitoring occurred within three years of the reintroduction, which may not have been long enough to detect important changes in the bobcat population or its effects on the island. In their evaluation of prey selection, Baker et al. (2001) noted "Further studies of prey abundance and prey use…are needed to determine the long-term effect of the bobcat reestablishment on the island's fauna". To a great extent, research by Nelms (1999) conducted eight years post-reintroduction provided important insights into the effect of bobcats on the island ecosystem.

A 14-fold population decline in marsh rabbits, caused by above-normal rainfall from a hurricane, allowed us to detect changes in bobcat diets and identify a functional response to prey abundance and evidence for diet optimization (Baker et al., 2001). The frequency of occurrence of deer in bobcat diets year-round (23–47%) was greater than reported for other studies in the southeastern United States (0–8%; Maehr and Brady, 1986). Although we did not have sufficient data to identify the shape of these functional relationships, bobcat diets in 1997-1998 had lower occurrence of marsh rabbits and deer and a more even distribution of occurrence of all prey species in their diet (Table 1). Also, there was no evidence the frequent occurrence of deer in the diet was

Photo: Duane Diefenback

Figure 5. Interdune meadow and maritime forest of Cumberland Island (and Atlantic Ocean).

Figure 5. Pradera interdunar y bosque marítimo en la isla de Cumberland (con el océano Atlántico de fondo).

because of a lack of food availability because bobcat survival was high, recaptured bobcats exhibited weight gains and bobcats maintained normal home range sizes (Diefenbach et al., 1993, Diefenbach et al., 2006).

Observation of the spatial organization of bobcats was consistent with the hypothesis that bobcats maintain home ranges via a system of land tenure established by prior rights (Diefenbach et al., 2006). However, we observed significant intrasexual overlap of both home ranges and core areas. Furthermore, we observed declining reproduction with an increase in home range overlap. Similarly, Lembeck and Gould (1979) observed a negative relationship between population density and reproduction, in which they reported 100% of females produced young when population densities were least and only 50% produced young when densities were greatest. We believe that successful reproduction in bobcats may be related to access by females to exclusive use areas even under conditions of adequate or good food availability. Under the conditions of this study (moderate bobcat density, adequate food availability and limited dispersal) bobcats exhibited no evidence of an ability to exclude other adult individuals from their home ranges or core areas. Given the inverse relationship Diefenbach et al. (2006) observed between home-range overlap and reproduction, we suggest that establishment of exclusive use areas by females may be important for successful reproduction, and that the social conditions necessary for maintaining these exclusive areas are less likely to occur at greater population densities or in the absence of dispersal opportunities, thereby reducing population productivity even without food limitation.

Previous researchers noted the adverse effects of grazing and browsing by feral horses, white-tailed deer and feral hogs (Hillestad et al., 1975; Ambrose et al., 1983; Turner 1986; Miller 1988). Bourdeau and Oosting (1959) reported live oak seedling densities of 0.28 seedlings/m^2 on Smith Island, North Carolina, USA and noted "the understory and shrub layers were very dense," whereas Hillestad et al. (1975) reported a seedling density of 0.055 seedlings/m^2 on CINS. Eight years after bobcats were reintroduced to CINS, we estimated an oak seedling density of 0.76 seedlings/m^2 on the same plots that Lieske et al. (1990) noted seedlings were rare or absent. We believe the changes we observed in oak regeneration are related to a decline in the abundance of white-tailed deer.

Consequently, our observations of bobcat use of deer as a primary prey species following their reintroduction, a decline in deer abundance, and an increase in oak regeneration suggest that bobcats caused a trophic cascade effect on the island. We did not expect to observe such strong trophic level changes on the island ecosystem because deer generally are not considered primary prey for bobcats (Maehr and Brady, 1986; but see Epstein et al., 1985). However, deer on CINS were suitable prey for bobcats because of their abundance and small size.

A keystone species is defined as one whose abundance is relatively low but whose effect on the ecosystem is relatively large (Power et al., 1996), and there are few examples of mammals as keystone species (Estes, 1996). McLarin and Peterson (1994) documented changes in vegetation via wolf *(Canis lupus)* predation on moose

(Alces alces), but there are few examples of trophic cascades involving mammalian predators, herbivores, and plants in terrestrial ecosystems (Shurin et al., 2002). We believe bobcats on CINS act as keystone predators. Given that Breitenmoser and Haller (1993) reported deer populations declined following a reintroduction of European lynx *(Lynx lynx)*, and our evidence of a trophic cascade caused by bobcats on CINS, any restoration of a felid population should consider trophic cascade effects as a real possibility.

In conclusion, we believe our research on bobcats on CINS provides strong justification for post-release monitoring of a reintroduced species. Not only does post-release monitoring provide data to better understand why a reintroduction project may have succeeded or failed, but it also can provide fundamental knowledge regarding population and community dynamics. In general, the role of predators in ecosystems is poorly understood, especially vertebrate predators (Shurin et al., 2002), and restoration projects of predator populations should consider monitoring trophic level characteristics of ecosystems. If such a monitoring programme were developed to test theories of community population dynamics, there would be potential to better understand food webs of terrestrial ecosystems and trophic level inter-relationships.

Acknowledgements

Funding and support for this research was provided by NPS, University of Georgia, U.S. Fish and Wildlife Service, Georgia Department of Natural Resources, Kentucky Department of Fish and Wildlife Resources and Atlanta Falcons Foundation. Also we thank the many volunteers, technicians and NPS staff who assisted with fieldwork.

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Cierro, con un rápido paseo de retorno, el largo álbum desdoblado, saco la cabeza por el último cuadro, y me pongo, deslumbrado, a proyectar mi futuro en el ocaso.

Juan Ramón Jiménez
(1881-1958)


Photo: Antonio Sabater

Considerations for planning Iberian lynx translocations into Doñana National Park

Consideraciones para la planificación de la translocación de lince ibérico en el Parque Nacional de Doñana

Francisco Palomares

Resumen

El lince ibérico *(Lynx pardinus)* es la especie de felino más amenazada en el mundo. Una de las poblaciones supervivientes de lince ibérico más conocida se encuentra en el Parque Nacional de Doñana y en sus alrededores, en el entorno natural más protegido que existe dentro del área de distribución histórica de esta especie. En este lugar, el lince ibérico se ha mantenido estable durante los últimos 50 años, con una población de entre 40 y 50 ejemplares, la mayoría de los cuales vive actualmente fuera de los límites del parque. Por esta razón, la población de linces de Doñana es especialmente vulnerable a la extinción. Si dentro del parque nacional desapareciese un solo territorio de lince más, esta población podría extinguirse en los próximos 15 años. La recuperación de las áreas fuente de reproducción dentro de Doñana, además de la translocación de algunos ejemplares procedentes de Sierra Morena, así como la restauración de su hábitat y la mayor capacidad de carga de las áreas fuente dentro del parque nacional, reducirían la probabilidad de extinción de toda la metapoblación de linces de Doñana por debajo del 5% en los próximos 100 años. En este capítulo se tratan las consideraciones específicas a tener en cuenta en la selección de áreas para la translocación de linces ibéricos dentro del parque nacional, así como las características biológicas de los candidatos para la reintroducción (edad y estado del ejemplar) y las fechas más adecuadas para que ésta tenga lugar.

Palabras clave

Lince ibérico, reintroducción, extinción, Doñana

Abstract

The Iberian lynx *(Lynx pardinus)* is the most highly endangered felid species in the world. One of the best known surviving lynx populations lives in and around Doñana National Park, within the most highly protected natural setting left of the lynx's historic distribution. The population has remained stable at around 40-50 individuals for the past 20-25 years, the majority of which presently live outside Park boundaries. This makes the Doñana lynx population particularly vulnerable to extinction. If one more lynx territory disappears inside the National Park, model results indicate that extinction of the Doñana lynx population could happen within the next 15 years. Recovery of the reproductive source areas inside the national park, coupled with the translocation of a few lynxes from the Sierra Morena population, and supported by the restoration and increased carrying capacity of the source areas inside the park, would reduce the probability of extinction of the entire Doñana lynx metapopulation to below 5% in the next 100 years. In this chapter we discuss specific considerations for the selection of areas for Iberian lynx translocation inside the national park, together with the biological characteristics of translocation candidates (lynx age and status) and best timing for the actual translocation.

Keywords

Iberian lynx, translocation, extinction, Doñana

Photo: Antonio Rivas

Considerations for planning Iberian lynx translocations into Doñana National Park

Francisco Palomares

Introduction

The Iberian lynx *(Lynx pardinus)* is the most threatened felid in the world (Novell and Jackson, 1996). Endemic to the Iberian Peninsula, at present only 200 individuals remain in two populations (Doñana and Sierra Morena) of the south of Spain (Palomares et al., 2002; Guzmán et al., 2004; Simón, this book; Calzada, this book). The Doñana population, the smallest of the two, with about 40-50 lynx distributed in different nuclei over an area of approximately 2 500 km^2, is the best known and it is located in a protected area, the most sheltered from human influence when considering the present and potential distribution area of Iberian lynx in Spain and Portugal. Nevertheless, although the Doñana population has remained almost stable from the last decades, the spatial location of lynx has changed thoroughout the last 20 years. While in the late 80's most lynxes were settled inside the national park (Palomares y col., 1991), presently most of them roam outside the protected area (Román et al., 2006). This is particularly important for the viability of the Doñana population since nuclei from inside the national park act as sources for the overall population, whereas nuclei outside the protected area are sink areas for the species (Gaona et al., 1998).

Spatially explicit models indicate that viability of the current Doñana population is very low. There is a 95% probability of extinction in the next 100 year, and average extinction time could be in 32 years. Thus, if a single territory is lost inside the national park, extinction could take place in only 15 years (Revilla et al., 2007). Nevertheless, from a demographic standpoint, increasing the source populations (i.e., those inside the national park) thorough the translocation of four lynx in two years –at a rate of two lynx per year– or 10 lynx in five years –also at a rate of two lynx per year–, extinction probabilities for the Doñana population in the next 100 years would be less than 4% and 1%, respectively (Revilla et al., 2007).

On the other hand, genetic health of the Doñana lynx population is poor (Jiménez et al., this book; Meli et al., this book; López et al., this book). The first cue is that during the last decades the pelage of lynx from Doñana has lost two spot patterns, and now only lynx with large spots are found (Beltrán and Delibes, 1993). Furthermore, recent genetic studies indicate that the Doñana population has a 30% lower genetic variability than the lynx from the other remaining lynx population in Sierra Morena (Johnson et al., 2004; Godoy, this book). Breeding among close relatives (sibling, father-brother) is not unusual within the Doñana population (F. Palomares et al., unpubl.).

Therefore, there are both demographic and genetic reason to urgently translocate lynx into the source nuclei of the Doñana population. Here we present some basic considerations to help in the decision-taking process, regarding where to translocate lynx into the Doñana area. Such considerations also include indications regarding ecological and behavioural traits of the lynx, which might affect the results of the translocation.

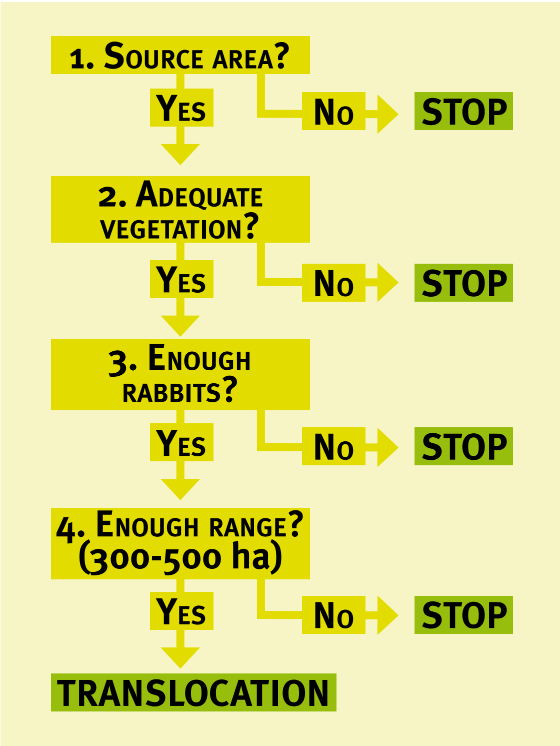

Figure 1. Hierarchy tree portraying conditions needed to be met to carry out an Iberian lynx translocation programme in the Doñana area.

Figura 1. Arbol de jerarquías mostrando las condiciones necesarias para llevar a cabo un programa de translocación de lince ibérico en Doñana.

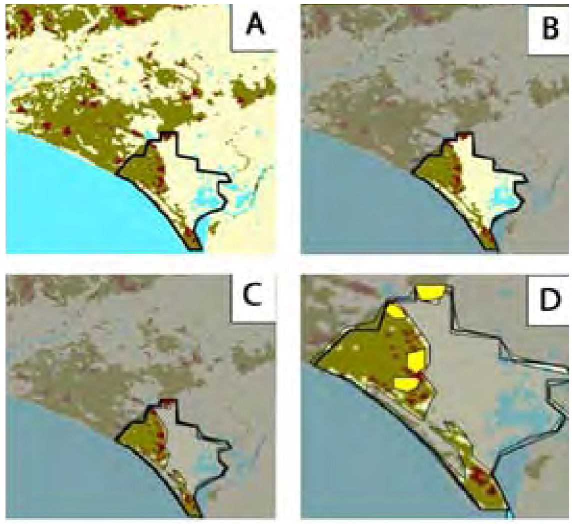

Figure 2. (a) Map of the study area showing the limits of the Doñana National Park (black line), and matrix (yellow), dispersing (green), and reproductive (red) habitats for lynx in the Doñana area; (b) Source areas (non-shadowed areas); (c) Suitable vegetation area (non-shadowed areas), and (d) Adequate areas for rabbits (yellow areas).

Figura 2. (a) Mapa del área de estudio mostrando los límites del Parque Nacional de Doñana (línea negra), y los hábitats de matriz (amarilla), dispersión (verde) y reproducción (rojo) en el área de Doñana; (b) Áreas fuente (áreas no sombreadas); (c) Áreas de vegetación adecuada (áreas no sombreadas), y (d) áreas adecuadas para conejos (áreas en amarillo).

Considerations to carry out lynx translocations in Doñana

Where to translocate lynx: spatial and habitat conditions

Some basic ecological and metapopulation conditions should be met to carry out lynx translocation in Doñana (Figure 1). These conditions are, in order of importance, that the area 1) should be a potential ecological source (i.e., where natality surpasses mortality); 2) should have adequate vegetation structure; 3) should have enough rabbit, and 4) should present enough adequate habitat to accommodate at least to a couple of reproductive lynx. Specific details for the justifications of these factors can be found in Gaona et al. (1988), Ferreras et al. (2001), Palomares (this book) and references therein, but briefly are justified because lynx are specialized in the use of Mediterranean scrubland, they prey on European rabbits *(Oryctolagus cuniculus)*, and hold home ranges of at least 300 ha for areas of best habitat quality. Thus, if lynx are not living in a potential source area –with the above mentioned characteristics– even if we increase the number of individuals, it will have no net effect on metapopulation viability.

In the Doñana area, the only part with the potential to be a source is the protected area inside the national park (Figure 2). There is adequate vegetation in approximately 22 000 ha out of the 55 000 ha that comprise the national park (Figure 2). However, of these 22 000 ha, there are enough rabbit for lynx in only four patches, which altogether represent approximately 2 500 ha (Figure 2). Therefore, at present, translocations should be carried out in these areas if other lynx are not occupying them.

An additional consideration to bear in mind is that only those prime habitat patches located in the south of the national park have the potential to be expanded as a source area, provided that adequate habitat management is undertaken to recover rabbits. Therefore, these patches should be considered first when thinking about potential sites for lynx translocation in Doñana.

Lynx origin

To account for both demographic and genetic aspects, translocated lynx should stem from the Sierra Morena population. However, from a demographic point of view re-locations of individuals from the Doñana population that live in sink nuclei, or surplus individuals from other areas within the Doñana area source nuclei, would also be considered. Nevertheless, Sierra Morena individuals are preferable for this conservation management strategy.

Lynx age and sex ratio

Both adult males and females are intrasexually territorial, and males may overlap several females within their territories (Palomares, this book). Therefore, males might quickly include released females into their territories. If male lynx are also released into recipient patches with resident adult males, then there is a high probability that released males might have to move away due to competitive interactions with resident males.

Therefore, there are three questions that needed to be address in order to provide recommendations regarding this point. First, we need to know if there are any lynx in the recipient area. If not, at least one male and one female (depending of the available range) of any age-class should be released. Another possible scenario is that there is presence of adult males; in such case young or adult females should be released. And finally, if there is no adult male in the recipient area, young or adult lynx of both sexes could be released.

Season for translocations

If adult male or female are going to be released, timing for translocation should be scheduled for several months before mating (September-October). This strategy will ensure that both lynx become familiarized with the new environment before the onset of breeding season and that they also have the opportunity to make contact with other adjacent lynx. This is particularly important if animals are coming from Sierra Morena. If released lynx are re-located from other areas of Doñana, release could take place as late as December, closer to mating season, since they are already acclimated to the Doñana environment. Mating normally occurs in December-January (Palomares, this book). However, if translocated lynx have not attained reproductive age (<3 years old), releases should take place out of breeding season in order to prevent strong competitive interactions with other resident lynx.

Translocation methods

The two main methods of releasing carnivores include soft and hard release. Soft release involves a variable pre-conditioning period in the recipient area before releasing the target species. In contrast, hard release consists on releasing animal directly into the recipient area without any previous acclimatization phase. If lynx to be released are from Sierra Morena, soft release is recommended, since both landscape and weather are rather different between Sierra Morena and Doñana. If lynx to be released are re-located from other Doñana areas, hard release might be attempted, particularly if dispersing individuals are going to be used, since ecological factors will be identical.

Final considerations

Individuals to be released should be in perfect health and body condition. Therefore, a thorough health check up should be carried out before releasing the animal into Doñana (Ryser, this book). Furthermore, there is information on the presence of some parasites in the Sierra Morena population that are absent in the Doñana population (Millán et al., 2007; Meli et al., this book), and although the effect on Doñana lynx is not clear, introduction on new parasites into a “clean” population should be avoided when possible.

On the other hand, the genetic characteristics of lynx could also be considered (Fernández et al., this book) as some individuals might be more suitable than others to address the genetic problem of the Doñana population. Thus, lynx from Sierra Morena with alleles that are absent in Doñana might be more adequate for translocation than animals with a similar genetic structure as that of the Doñana population. Finally, rigorous post-release monitoring and a sound scientific study should be undertaken to help with the decision making process at least during the first years of the Programme. Therefore rigorous information on factors affecting the success or failure of the translocation programme should be quickly incorporated and analyzed at all stages of the process.

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Photo: Antonio Sabater

Todo lo que yo hago es una gota en el océano
así de pequeño es lo que podemos hacer, y
sin embargo,
si yo no lo hiciera
al océano le faltaría una gota.

Federico García Lorca
(1898-1936)

Short communication on the first Iberian lynx translocation from Sierra Morena to the Doñana population

Comunicación breve sobre la primera translocación de un lince ibérico de Sierra Morena a la población de Doñana

Gema Ruiz, Marcos López, Leonardo Fernández, Juan Antonio Franco, Guillermo López and Miguel A. Simón

Resumen
Esta comunicación breve tiene la finalidad de relatar la primera translocación de un lince ibérico al Parque Nacional de Doñana. La meta de esta medida de manejo fue el reforzamiento genético, para lo que se seleccionó un macho adulto de la población de Sierra Morena. La liberación se planteó en un núcleo de Doñana donde todos los ejemplares adultos eran hembras, debido a la muerte o retirada de todos los machos residentes tras un brote del virus de la leucemia felina (FeLV) que tuvo lugar en la primavera de 2007. Este suceso promovió un manejo de crisis cuya finalidad fue abordar los tres siguientes objetivos: 1) Asegurar que las tres hembras establecidas en el área de suelta no se dispersasen de la zona en búsqueda de un macho; 2) Evitar una disminución en la productividad global de la población de Doñana; 3) Evitar que aumentara la probabilidad de extinción a corto plazo de la población de Doñana. El macho seleccionado, conocido como *Baya*, fue ubicado en una instalación de presuelta en diciembre de 2007 y liberado en enero de 2008. *Baya* se apareó con las tres hembras residentes y tuvo un total de ocho cachorros mixtos (mezcla de genes de Sierra Morena y Doñana), los cuales se consideran importantes para aumentar la variabilidad genética de la población de Doñana. Seis meses tras su liberación, el área de campeo de *Baya* continuó solapándose con los respectivos territorios de las tres hembras residentes y, por tanto, su asentamiento en la zona se consideró un éxito.

Palabras clave
Translocación, instalaciones de presuelta, extinción, liberación, área de campeo, diversidad genética

Abstract

This short communication aims at describing the first translocation of an Iberian lynx into Doñana National Park. The purpose of this management approach was to help increase the genetic diversity of the Doñana population and, for this reason, we selected a specific male from the Sierra Morena population. The aim was to release this male in a nuclei where all the territorial lynxes were females –because an outbreak of Feline Leukemia (FeLV) that took place in the in the Spring of 2007 had killed all the territorial males in that area. Altogether, the goals of this crisis management approach were threefold: 1) To ensure that the three established females would not leave the core population in the process of searching for a mate; 2) To avoid a decrease in global productivity within the Doñana population; 3) To avoid augmenting the potential probability of short-term extinction in the Doñana population. The new male, known as *Baya*, was translocated into an acclimatization pen in December 2007 and released in January 2008. *Baya* mated with the three resident females during breeding season and produced eight offspring of mixed Sierra Morena-Doñana origin, which are considered important to increase the genetic diversity of the Doñana population. Six months after release, *Baya's* homerange still overlapped the respective territories of the three resident females and the male was considered to be successfully settled in the area.

Keywords

Translocation, acclimatization pen, extinction, release, homerange, genetic diversity

Photo: E. Abad

Short communication on the first Iberian lynx translocation from Sierra Morena to the Doñana population

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Introduction

The first translocation of an Iberian lynx into Doñana, which took place in December 2007 under the framework of the LIFE Nature Project (Simón et al., this book), had the purpose of strengthening this remnant population from a genetic and a demographic point of view. Even though the original restocking project was scheduled to take place in a different part of the Protected Area, a crisis management decision led to reconsider the initial translocation site and opt for releasing this valuable male into the Coto del Rey nuclei, where a Feline Leukemia (FeLV) outbreak had wiped out the four territorial males in the area (López et al., this book).

The purpose of this crisis management decision was three-fold:

1) To ensure that the three established females would not leave Coto del Rey in the process of searching for a mate.

2) o avoid a decrease in global productivity within the Doñana population, which would result if the Coto del Rey metapopulation would not have adult males to breed with the established, territorial females.

3) To decrease the potential for short-term extinction in the Doñana population.

Population models predicted that the loss of a single territory in Coto del Rey would increase by 10% the global probability of extinction of the Doñana Iberian lynx population (Palomares et al., this book). Natural re-colonization of the Coto del Rey nuclei by free-ranging males was hard to predict but, in all likelihood, given the scarcity of adult males in Doñana, the most probable scenario would have been the settlement of a juvenile male, with lower reproductive potential than that of an adult. In fact, this has proven to be the case, since only one subadult male has naturally recolonized the area after the FeLV outbreak took place.

The translocation of lynxes from Sierra Morena to Doñana was originally thought out as a means to increase the already diminished genetic variability of this population. After the FeLV outbreak, the need for re-stocking was not only genetic, but also demographic. The situation encountered after the loss of all territorial males in Coto del Rey left three breeding females available in Doñana's main "population source" (Palomares et al., this book) which, paradoxically, was a situation that would allow for a more rapid gene flow of Sierra Morena genes through the Doñana population.

Pre-release considerations

The male selected for translocation, *Baya*, belonged to the 2005 cohort. One of the selection criteria included that the animal would be *Cytauxzoon*-free, since no *Cytauxzoon* has ever been detected in the Doñana free-ranging population (Meli et al., this book). After a 6-week quarantine period that ensured that *Baya* was disease-free, he was transferred to an acclimatization pen in Coto del Rey. *Baya's* translocation took place on December 21st, 2007, via soft-release in a 2

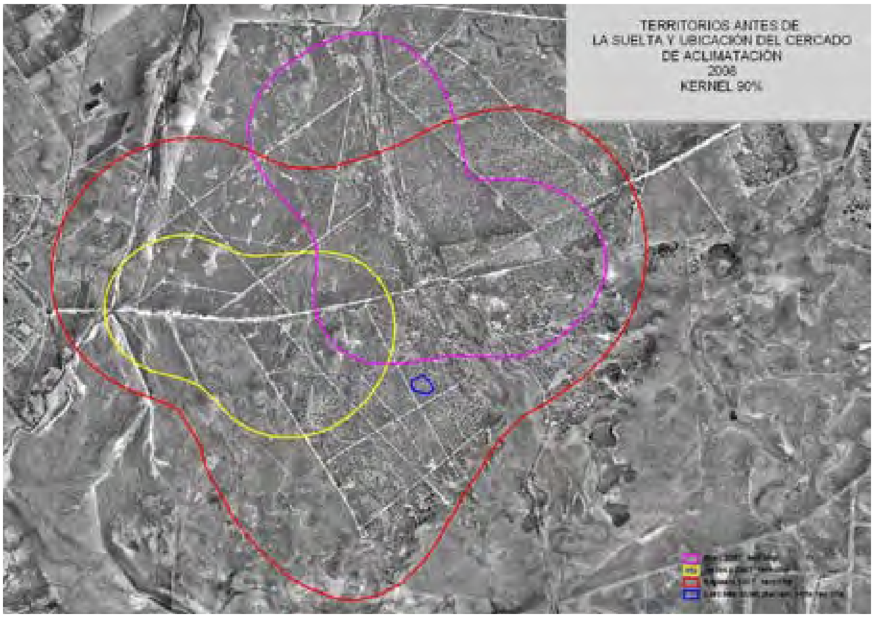

Figure 1 (a). Territories of the resident breeding females (red, blue and green). The arrow indicates the placement of the acclimatization pen, which is represented by an orange square.

Figura 1 (a). Territorios de las hembras reproductoras residentes (rojo, azul y verde). Con una flecha se indica la ubicación del cercado, que aparece representado esquemáticamente en color naranja.

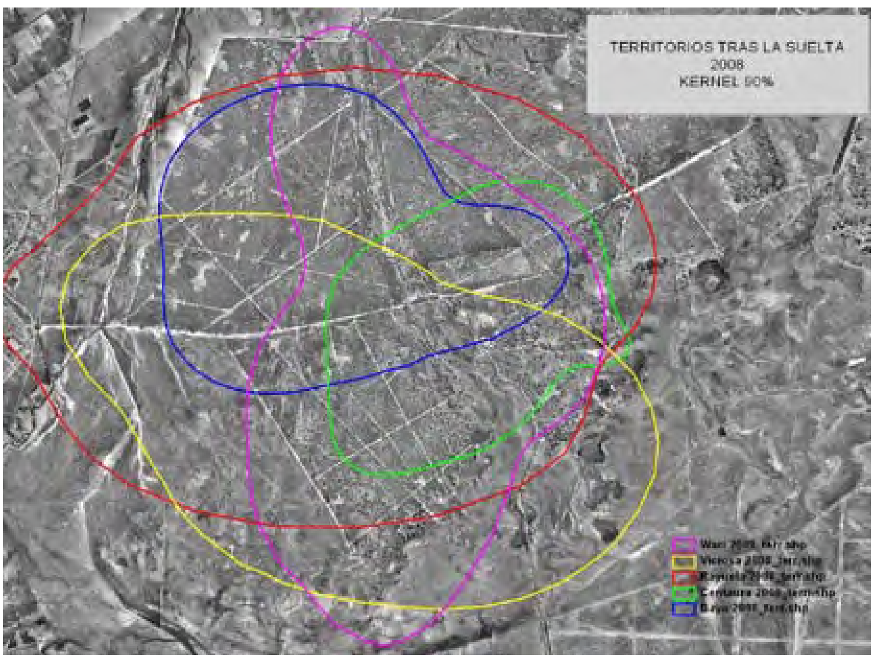

Figure 1 (b). Redistribution of territories six months after release.

Figura 1. (b). Redistribución de territorios seis meses después de la suelta.

Ha acclimatization pen. The chain link enclosure was 3.85 m high with an additional 0.5 m buried underground. Inside the enclosure there were Suplementary Feeding Stations (SFS) as well as a den site. Prior to *Baya*'s acclimatization to the release site, his own scats as well as scats from the three resident females were scattered in specific locations of the enclosure. Actual placement of the acclimatization pen was carefully chosen after evaluating the home-range overlap (using a 95% kernel) of the three territorial females (Figures 1a and b).

The final decision regarding the moment of actual release was determined taking the three following factors into account:

- Positive and continuous interactions (through the wire mesh) of *Baya* with the territorial females.
- Estimation of the timing of estrous of the three territorial females based on their birthing periods in previous years.

Figure 2. Moment of interaction between *Baya* and *Wari*, which marked the end of the acclimatization period.

Figura 2. Momento de interacción entre *Baya* y *Wari*, que marcó el final del periodo de aclimatación.

Figure 3. *Baya* (above) and *Rayuela* (sitting inside the Suplementary Feeding Station).

Figura 3. *Baya* (arriba) y *Rayuela* (sentada dentro del Cercado de Alimentación Suplementaria).

- Establishing a maximum limit for holding the male in the pen if no interactions with females had been observed. It was decided that Jaunaury 10th would be the maximum waiting period, always considering the observed behaviours during the acclimatization phase.

In order to detect the different behaviours, the acclimatization enclosure was equipped with a robotized (365° movement) infrared camera that was operated by project personnel 24 hours per day. One of the main objectives to be attained with this effort was to learn as much as possible from this release in order to improve techniques future reintroductions.

Baya was released on January 1st, 2008 (Figure 2). Project personnel estimated that the animal was properly acclimated to the site, considering that there have been a large number of positive interactions through the mesh with the three territorial females present in the area. The actual release took place after one of the females spent more than a day in the vicinity of the enclosure, vocalizing towards the male and showing signs of estrous.

Post-release monitoring

Intensive post-release monitoring efforts were conducted by the project's specialized staff in order to learn as much as possible from this release. These efforts continue to date but in a less intensive way.

Radiotelemetry

After release, at least two daily locations were obtained from *Baya*. The animal was followed for a while if it presented activity and, at the same time, all three territorial females were also located. After two months, and given that *Baya* had established himself in the area, he was included in the regular radio-monitoring protocol, with at least 2-3 locations per week.

Phototrapping and tracking

Suplementary Feeding Stations and the bounder of the enclosure are equipped with photo-trapping cameras, which have allowed for a precise control of the animals and their use of the enclosures (Figure 3). Altogether, a total of 212 lynx photographs have been obtained from the six photo-trapping stations during a 6-month period (January through June).

Monitoring gene flow

This genetic aspect of the project is monitored through two kinds of efforts: sampling of mixed-origin cubs for paternity tests (Godoy et al., this book), and identifying individual lynxes via scat collection. The latter method could become an effective and non-invasive way of studying gene flow throughout the Doñana population.

Conclusions

Six months after his release, we consider that *Baya* is effectively established in Coto del Rey, since he has never dispersed outside this nuclei. During the 2008 breeding season, *Baya* mated with the three adult females of this nuclei (Figure 1.B), although not with the 2-year-old subadult. Three of the eight mixed-origin cubs he has fostered have already reached dispersal age. We consider that this first stage of the re-stocking project, which involved ensuring the settlement of a Sierra Morena male in Doñana, has been successful. More time is needed to assess the genetic impact of this management action at the population level.

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Photo: E. Abad

Photo: Marianne Hartmann

If a cat does something, we call it instinct; if we do the same thing, for the same reason, we call it intelligence.

Will Cuppy

Breeding European wildcats *(Felis silvestris silvestris, Schreber 1777)* in species-specific enclosures for reintroduction in Germany

Cría del gato montés europeo *(Felis silvestris silvestris, Schreber 1777)* en recintos específicos para su reintroducción en Alemania

Marianne Hartmann-Furter

Resumen

Generalmente, se considera que el hábitat natural de una especie es aquel en el que se satisfacen mejor las necesidades del animal en cuestión. Por lo tanto, el comportamiento de los animales que viven en libertad se puede utilizar como base para el diseño de instalaciones en zoológicos. En un estudio experimental a largo plazo desarrollado en Suiza, se diseñó un recinto específico para el gato montés europeo, destinado a proporcionar a los animales todas las estructuras y estímulos importantes para la expresión de sus diversos comportamientos en función de todos sus ciclos funcionales. En dicho recinto los animales no desarrollaron ningun comportamiento anormal y mostraron un patrón de actividad similar a la de sus congéneres silvestres. Mediante un dispositivo electrónico para el suministro de alimento especialmente adaptado a las necesidades de la especie, los animales pudieron expresar prácticamente todo su rango de comportamientos naturales de caza. Las estructuras básicas se colocaron en el recinto siguiendo una disposición minuciosamente estudiada, y el comportamiento del cuidador se tuvo en cuenta como factor de igual importancia. Este recinto, que se ha adoptado en distintos zoológicos, ha servido de referencia en una serie de experimentos realizados para determinar los límites de adaptabilidad de la especie.

Desde 1993, las crías obtenidas de los ejemplares que viven en estos recintos se han destinado al proyecto de reintroducción del gato montés en Baviera (Alemania). Entre 1984 y 2008, un total de 580 ejemplares criados en cautividad fueron liberados en tres regiones distintas de Baviera. En 1999, se realizó el seguimiento de 11 ejemplares con radiotransmisor en un estudio piloto. Después de ser liberados de su jaula en el bosque, los gatos monteses deambularon entre tres y 20 días antes de establecerse en algún lugar. Tres individuos fueron atropellados al cruzar carreteras en las dos semanas posteriores a su liberación y otros dos no pudieron ser localizados. Todos los demás gatos monteses sobrevivieron, al menos tanto como las baterías de sus radio-collares y se probó que dos de ellos seguían vivos 11 meses después de su liberación. Además, se obtuvieron pruebas sobre el terreno que indicaban que los gatos monteses no tuvieron problemas a la hora de conseguir suficientes presas en el medio silvestre. En este sentido, este trabajo muestra que los gatos monteses criados en cautividad que crecen en recintos específicos para la especie están bien adaptados para sobrevivir tras su liberación en el medio silvestre.

Palabras clave
Cría en cautividad, dispositivo para el suministro de alimento, comportamiento del cuidador, radio-seguimiento

Abstract
The natural habitat of a species is commonly regarded as the environment where the needs of the animals are best met. Therefore, the behaviour of animals living in the wild can be used as a basis for designing zoo environments. In the course of a long-term experimental study in Switzerland, a species-specific enclosure for European wildcats was developed in order to provide the animals with all the structures and stimuli relevant to their behaviours as they relate to all functional categories of behaviour. In this enclosure the wildcats were free from behavioural disturbances and showed an activity rhythm similar to the one of their wild conspecifics. An electronic feeding device, specifically tailored to meet the wildcats' needs, enabled them to express almost their entire range of natural hunting behaviours. The behavioural results showed that the essential structures must be placed in a particular arrangement within the enclosure, with the keeper's appropriate behaviour towards the animals as the second and equally important factor for the animals' welfare. This enclosure design, which has been adopted by several zoos and wildlife parks, served as a baseline for a series of experiments that were conducted to determine the limits of the species' adaptability.
Since 1993 the offspring of the cats living permanently in these enclosures have been provided to the wildcat reintroduction project in Bavaria, Germany. Between 1984 and 2008, 580 captive-bred wildcats were released in four different regions in Bavaria. In the course of a pilot study in 1999, we were able to radio-track 11 wildcats. After leaving their cages in the forest, the cats were wandering around between three and 20 days before they settled down. Three cats were killed crossing roads within the first two weeks after their release and two animals could not be tracked. All other cats survived at least as long as the batteries of their collars kept working and there was proof for two of them still being alive 11 months after their release. We have obtained evidence indicating that the cats had no problems with procuring enough prey in the wild. This work shows that wildcats raised in species-specific enclosures are well adapted for survival after being released into the wild.

Keywords
Captive breeding, feeding device, keeper behaviour, radio-tracking

Breeding European wildcats *(Felis silvestris silvestris, Schreber 1777)* in species-specific enclosures for reintroduction in Germany

Marianne Hartmann-Furter

Introduction

Wildcats are kept in many zoos and wildlife parks. In traditional enclosures wildcats are often invisible, because they are hiding all day long. However, field studies have shown that wildcats in their natural habitat are active during the day as well (Stahl, 1986; Liberek, 1999). On the other hand, when some of the wildcats in traditional enclosures are active, they often carry out pronounced pacing (Hartmann, 2000). Not only stereotypical walking, but apathetic resting is also an abnormal behaviour (Böer and Dittrich, 1982; Wiepkema, 1983). Both types of behavioural disturbances can indicate that the housing and caring system is inadequate to satisfy the behavioural needs of the animals and therefore reflects a poor welfare situation (Fraser, 2008; Hosey et al., 2009). Since the European wildcat is a species that is currently being reintroduced into areas where it became extinct, the quality of the housing systems for rearing release candidates may play a crucial part in their successful reintroduction.

The goal of this project was to develop a species-specific enclosure and housing system for the European wildcat, where animals would not develop any abnormal behaviours and would be able to perform the whole range of their natural behaviour patterns. The problem of inadequate enclosures is usually approached through environmental enrichment, which is a suitable method of improving the living conditions of wild animals in captivity (Young, 2003; Martos, this book; Manteca, this book). However, in some cases, this method may prove inefficient for reaching the goal of species-specific housing conditions. For instance, some of the animals living for an extended time under inadequate conditions develop serious behavioural anomalies, which, to some extent, are irreversible (the author's own observations). These animals may no longer be able to make use of an enriched environment nor to react to the respective stimuli in a species-typical way. In order to avoid these problems, I used a different approach by developing an adequate housing system right from the beginning.

Enclosure design and behavioural results

An enclosure was constructed where the cats were provided with a variety of structures and stimuli so that a wide choice existed for the animals in every functional category of behaviour (Figure 1). Sometimes the best approach to designing species-specific environments for captive animals is supposed to be that of imitating a section of the natural habitat to the greatest detail. However, if a small section of a wildcats' natural environment is cut out, not all the structures essential to the animals' behaviour may be found within this section. Therefore, an enclosure should not be an imitation of a section, but rather a distilled version of an entire wildcat home range with all elements needed to perform a wildcats' natural behaviours. The most important behaviour-releasing

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Figure 1. The species-specific enclosure contains all the structures and stimuli essential for the wildcats' natural behaviour.

Figura 1. Las instalaciones específicas para gato montés contienen todas las estructuras y estímulos esenciales para que los gatos desarrollen comportamientos naturales.

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Figure 2. (a) Just as free-living wildcats wait in front of a mousehole, so do the cats in the enclosure in front of the foodboxes and (b) catch food items as soon as they are released.

Figura 2. (a) Al igual que los gatos monteses esperan enfrente de la ratonera, los gatos criados en estas instalaciones esperan frente a las cajas de Madera y (b) cogen los alimentos conforme caen de la caja.

stimuli to be provided in a wildcat enclosure are those of the behavioural categories of resting, climbing and hunting. In the experimental enclosure I offered resting places of different qualities such as degree of exposure, shelter, view, etc., as well as climbing structures of different heights and spatial alignment, different diameters, and of various types of ramifications with or without cover. Hunting is the predominant activity of free-ranging wildcats (Stahl, 1986; Liberek, 1999). This behaviour is reduced to almost zero under housing conditions where the animals are fed every day at the same time and in the same place. Offering wildcats live prey is not sufficient to meet their behavioural needs, because only a fraction of their natural hunting behaviour is exhibited, mainly catching and killing (Hartmann, 2000). However, wildcats spend most of their hunting time waiting, listening, being alert and exploring (Stahl, 1986). In order to simulate the natural hunting situation of a wildcat in the wild, an electronic feeding device was developed (Hartmann, 2000), which provided the cats with the same stimuli and in a similar temporal and spatial regime as it is encountered by their wild conspecifics when hunting. Up to 20 boxes of different sizes for different food items were installed in various places within each of the 150 - 370 m^2 enclosures. Everyday some of the boxes were randomly chosen to be filled with dead mice, rats or chickens. The boxes were opened at random times by an electronic control, so that the cats never knew where or when a food item would appear: the same situation as with wildcats hunting in the wild (Figure 2). From some of the boxes, the food items were pulled out by an elastic cord, and the cats had to catch and pull the mouse or rat off the cord. The latter was accomplished by a bite analogous to the killing bite: the cat had to keep biting as long as it felt any resistance. As a result of this feeding regime, random in time and space, the cats showed a significantly higher level of overall alertness than cats fed in a traditional manner (Hartmann, 2000).

The wildcats living in the specifically designed enclosure and being fed by the electronic feeder did not develop any abnormal behaviours such as stereotypies or apathy (Hartmann, 2000). Furthermore, the animals in this enclosure showed activity patterns similar to those of their wild conspecifics (Stahl, 1986; Liberek, 1999). The reproduction rate of these animals was higher than the one known for cats living in traditional cages (Hartmann, 2001). Although breeding success per se cannot be used as a parameter for good housing conditions, it can

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Figure 3. (a) Female leading her kittens on their first excursion through the enclosure; (b) After weaning, kittens spend a lot of time with their father.

Figura 3. (a) Hembra llevando a sus crías en su primera excursión por la instalación; (b) Tras el destete, las crías pasan mucho tiempo con su padre.

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Figure 4. (a) Captive female in an unusual position after her enclosure had been entered by a careless keeper; (b) Three weeks after the keeper had stopped working at the cat station the same female stays relaxed on the ground.

Figura 4. (a) Hembra de cautividad en una posición poco usual tras la entrada de un cuidador poco cuidadoso en su instalación; (b) Tres semanas después de que el cuidador haya dejado de trabajar en el centro, la misma hembra se mantiene relajada en el suelo.

still be an indicator when compared with traditional housing systems and with the breeding success of animals living under natural conditions.

The wildcats in this enclosure showed a highly developed social behaviour. This was also due to the fact that breeding pairs were permanently kept together and sometimes with other family members as well (Hartmann, 2001) (Figure 3).The richly structured enclosure with the electronic feeder served as a baseline for a series of experiments that were conducted to test how far the complexity of this environment could be reduced without causing any changes in the cats' behaviour and without provoking any abnormal behaviours; and thus to determine the limits of the species' adaptability. The results of these experiments showed that in a species-specific wildcat enclosure, the relevant structures and stimuli not only had to be available, but also had to be placed in a particular arrangement.

The results of the behavioural observations also showed that there is a second and equally important factor for the animals' welfare, although this factor is very often neglected: this is the appropriate behaviour of the keeper (Hartmann, 2008). A good keeper makes the animals feel safe in their artificial environment, even in his or her presence. The experimental design of the project required that the animals continued the behaviours they were engaged in when someone entered the enclosure. Otherwise the behavioural data would have been biased by the reactions to the keeper, as it was observed that behavioural changes could persist for several hours after the keeper had left the enclosure. This was demonstrated when a specific keeper did not follow the guidelines issued to all keepers concerning their correct behaviour towards the cats. From the panic reactions shown by the cats it was assumed that this person did not talk to the animals and chased them away for cleaning their enclosures. Two juvenile females even developed stereotypical behaviour starting as soon as someone entered their enclosure (Hartmann, 2008). The stereotypies persisted for about two hours even after the person had left the enclosure. The two females were the first wildcats ever to develop stereotypies in the species-specific enclosures. Both had been particularly confident cats before and it is therefore possible that the keeper had tried to touch or even grasp them. Some cats thereafter made a habit of hiding early in the morning, before a keeper arrived, and of not reappearing until the late evening. The dramatic behavioural changes, displayed by

Figure 5. (a) Mouse-cage in the wildcat enclosure in Wiesenfelden for live prey feeding; (b) Enclosure at the release site in the Bavarian Spessart.

Figura 5. (a) Caja de ratones para alimentación con presa viva en las instalaciones de Wiesenfelden; (b) Instalaciones en el lugar de reintroducción en el Spessart de Baviera.

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Photo: Marianne Hartmann

all of the cats living in the cat station at that time, disappeared only a few weeks after this keeper had stopped working at the cat station (Hartmann, 2008) (Figure 4).

Conclusions

The incident with one specific keeper showed that even in a well-designed species-specific enclosure the inadequate behaviour of a keeper may have a strong negative impact. From my behavioural observations I conclude that the following two factors are of equal importance for the welfare of European wildcats under captive conditions: a species-specific enclosure design as well as the keepers' appropriate behaviour towards the animals.

If planning to provide captive-bred animals for reintroduction programmes it has to be kept in mind that the keepers' behaviour may influence a successful release later on. European wildcats can be habituated to a single person, which means that the habituated cats do not generalize, but hide as soon as they perceive an unknown person (Hartmann, 1994). This feature possibly contributes to a successful release of captive-bred wildcats as well. With animal species that are more confident with humans in general than the European wildcat, the behaviour of their keepers and other humans in their captive environment has to be considered carefully in view of a possible impact on the animals' later survival in the wild.

Reintroduction

Introduction

The wildcat had probably become extinct in Bavaria (Germany) by the end of the 19th century (Büttner and Worel, 1990). In 1984, the Bund Naturschutz in Bayern started a project under the direction of Guenther Worel and Hubert Weinzierl to reintroduce this species. As a first step, educational campaigns were conducted for several years and an evaluation of the suitability of habitats was carried out (Worel, 2009). Between 1984 and 2008, 580 wildcats were released in four different Bavarian regions, namely the Spessart, the Steigerwald, the Hassberge and the Vorderer Bayerischer Wald.

Material and methods

In order to avoid endangering autochthonous populations by removing individuals for translocations, captive-bred cats were exclusively used for the reintroduction programme. Some of them were bred at the Bund Naturschutz wildcat station in Wiesenfelden, in enclosures offering naturalistic structures. Others were donated to the reintroduction project by more than 30 different zoos and wildlife parks in Europe. Since 1993 the offspring of the cats living in the Swiss Bockengut enclosures have been provided as well.

Cats coming from zoos were collected at the Wiesenfelden facilities where they received training for several months before being transported to the release sites. To a large extent these cats were trained by feeding them live prey. Mice and rats were released into a richly structured, escape-proof cage inside the wildcat enclosure, where they were able to hide (Figure 5a). Larger animals such as pigeons and rabbits were released directly into the cat enclosure. This method offers good opportunities for zoo-bred cats to be trained to catch and kill: in the absence of people, also shy cats are given the opportunity to catch and kill prey. However, as the wildcats learn quickly about the practice by the keeper of putting mice into the mouse cage, which becomes the signal for them to start hunting, this method is not suitable for long-term use and cannot keep the cats busy with hunting for hours as it is the case in the wild (Hartmann, 2000). Only the cats originating from Tierstation Bockengut, Switzerland, were released without any further training, because they had been trained extensively in their rearing enclosures by means of the electronic feeder described above.

After having been transported to the reintroduction area, the cats were kept for several days in enclosures situated in the forest in order to let them recover from transportation (Figure 5b). Finally, the cage doors were opened to allow the cats to leave the enclosures in the absence of people. Some of the cats were released from specifically designed and arranged spots called "biotopes", to which they were transferred in their familiar sleeping boxes from the Wiesenfelden enclosures (Worel, 2009). At that point, they had the choice to leave anytime. In both release procedures food was provided as long as there were signs of wildcat presence at the release site. The feeding period thereby varied from one day to several weeks.

Post-release monitoring and results

Several questionnaire surveys as well as road-kill analyses showed that captive-bred wildcats managed to survive in the wild and established core populations from where animals dispersed into surrounding areas (Büttner, 1991; Eppstein, 1995; Knapp, 2002). Furthermore, there was proof of wildcat reproduction in the Spessart and in the Vorderer Bayerischer Wald (Büttner, 1991; Eppstein, 1995). However, apart from a radio-tracking study at the beginning of the reintroduction (Heinrich, 1992), no comprehensive monitoring studies have been carried out so far in order to measure the success of the reintroduction programme. Therefore, the number of wildcats currently living in Bavaria is unknown. Lately, the question arose as to whether self-sustaining populations already exist, so that releases could gradually be stopped, or whether further releases would be necessary in the respective reintroduction areas.

In 1999, we were able to conduct a pilot study in the reintroduction area of Spessart, Germany (Nabulon and Hartmann, 2001). Our main interest was focused on the behaviour and the survival of the released cats. Eleven wildcats were equipped with radio collars and were released from two sites in the Spessart in different seasons (Figure 6). Eight of these cats had been bred in the Bockengut enclosures in Switzerland, whereas three cats came from zoos. Five of the cats from Tierstation Bockengut were released in June. They were wandering around for three to 20 days before they settled down. One male travelled almost 100 km in three weeks. The principal movements of the cats were recorded at night. After their dispersal period had finished, all five cats remained in their respective home ranges and did not relocate at least as long as the batteries of their collars kept working. Two cats were seen and identified 11 months after their release. One of them was captured, in excellent shape, equipped with a new radio collar and released again.

Six more cats were released at a different site within the Spessart in September. Three of them were killed while crossing roads within the first two weeks after their release. We assume that the high mortality rate in autumn was mainly due to seasonal effects. With dusk setting in earlier in autumn, movements of the cats were registered already in the earlier evening, coinciding with a time of heavy traffic. Therefore, the risk for a cat of being run over by a car was much higher in autumn than in summer, when the cats started to move at later hours. Our direct field observations as well as the stomach analyses of the animals killed by traffic showed that the cats were able to catch enough prey after their release. The behaviour of the released cats did not seem to differ from that of wildcats in autochthonous populations.

Photo: T. Nabulon

Figure 6. Wildcat with radio collar.
Figura 6. Gato montés con radio-collar.

Conclusions

The results of this pilot study showed that the wildcats bred in the species-specific enclosures were able to survive in the wild without any further training. This means that the cats in these enclosures find all the structures and stimuli necessary to perform their natural behaviours, to the extent that they are well adapted for surviving in the wild. Our conclusion is that the first three weeks after being released are critical for the cats' survival: they have to orient themselves, to get acquainted with their new environment and to catch enough prey while trying to find an unoccupied and suitable territory.

Whether zoo-bred cats trained with live prey at the Wiesenfelden Station have the same survival rate after release compared to cats bred in the species-specific enclosures still needs to be determined. After the pilot phase, a three-year study was planned, but it could not be implemented up to now. Following the IUCN Guidelines for Re-introductions (IUCN, 1998), data on the behaviour and survival of the released animals, on population density as well as on successful reproduction of the reintroduced population are to be collected for measuring the success of a reintroduction programme.

Captive-bred animals are not necessarily less suitable for reintroduction programmes than wild-caught and relocated individuals, provided that they receive proper training and are given the opportunity to acquire foraging skills and other essential abilities. Furthermore, rearing conditions should prevent animals from developing behavioural disturbances, which may disable them later on to react adequately in unfamiliar or critical situations. Removing individuals from autochthonous populations for relocation programmes might disturb local social systems, and thus impair the source population. Particularly, in the more solitary cat species, hardly any data are available yet on the effects of removing individuals from the breeding stock and on whether this would have an impact on the reproduction rate of the respective populations.

Acknowledgements

The enclosure studies were mainly supported by the Zurich Animal Protection Society (Zürcher Tierschutz), which also provided the Tierstation Bockengut for my wildcat research. Further financial support was received from the Swiss Federal Veterinary Office, the Haldimann-Foundation, the Dr. Berthold Suhner-Foundation, the Graf Fabrice, von Gundlach und Payne Smith-Foundation, the G & B Schwyzer-Foundation, the Ernst Hadorn-Foundation, Martin Hoch and many other private sponsors. Migros Zürich donated food for many years. I am very grateful to the Zürcher Tierschutz, Prof. Bernhard Nievergelt and Dr. Peter Weilenmann for their dedicated help and support in difficult situations. The pilot field study was made possible by the Bernd Thies-Foundation. I thank Günther Worel for the excellent collaboration and for his tremendous support in all situations. All involved forestry offices in Bavaria and Hesse and their foresters supported the pilot study with great enthusiasm. Julia Altmann from the Senckenberg Museum, Frankfurt am Main, carefully analysed the road-kills. I also want to thank Prof. Dr. H. Kummer and Prof. Dr. B. Nievergelt who provided me with the opportunity to carry out these projects in their departments at the University of Zurich. I thank Siegfried Weisel for reviewing and commenting on the manuscript as well as Dora and Prof. Willy Furter and Michael Hartmann for their support in all phases of the projects. Finally, I am very grateful to the many assistants, keepers and friends who contributed to the success of these studies by their careful and dedicated work in the field as well as in the cat station.

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Climb the mountains and get their good tidings. Nature's peace will flow into you as sunshine flows into trees. The winds will blow their own freshness into you... while cares will drop off like autumn leaves.

John Muir
(1838-1914)


Photo: Wildlife Conservation Society; (ISUNR camera trapping)

Breeding Far Eastern leopards for reintroduction: the zoo programme perspective

Cría del leopardo de Amur para su reintroducción: perspectiva del programa de cría en zoológicos

Sarah Christie

Resumen
El leopardo de Amur *(Panthera pardus orientalis)* es una de las dos especies de felinos de tamaño grande a mediano para cuya recuperación los expertos del Grupo de Especialista de Felinos de la IUCN considera necesaria la reintroducción de individuos procedentes de zoológicos. La otra especie para la que esto se considera necesario es el lince ibérico *(Lynx pardinus)*. Además de la planificación habitual destinada a atajar los problemas relacionados con la liberación de grandes predadores, es necesario establecer estrategias adecuadas de gestión de la cría y la liberación de la población de origen cautivo. En ambos ámbitos, los aspectos sociopolíticos suelen ser mucho más problemáticos que los biológicos. Este capítulo analiza los problemas biológicos y sociopolíticos asociados a la obtención de leopardos de Amur aptos para programas de reintroducción a partir de los individuos que se encuentran en distintos zoológicos del mundo. Además, se tratan algunos aspectos relevantes respecto a la selección de áreas de suelta, de estrategias de cría y liberación, así como al diseño de las instalaciones donde se mantiene a los animales. La estrategia de cría para la reintroducción se encuadra bajo el contexto del esfuerzo global por conseguir apoyo para la conservación del leopardo de Amur por parte de diversos zoológicos del mundo, aunque en este capítulo no se pretenden describir todos los factores implicados en el desarrollo del plan de reintroducción para esta especie, que actualmente no está finalizado ni oficialmente aprobado.

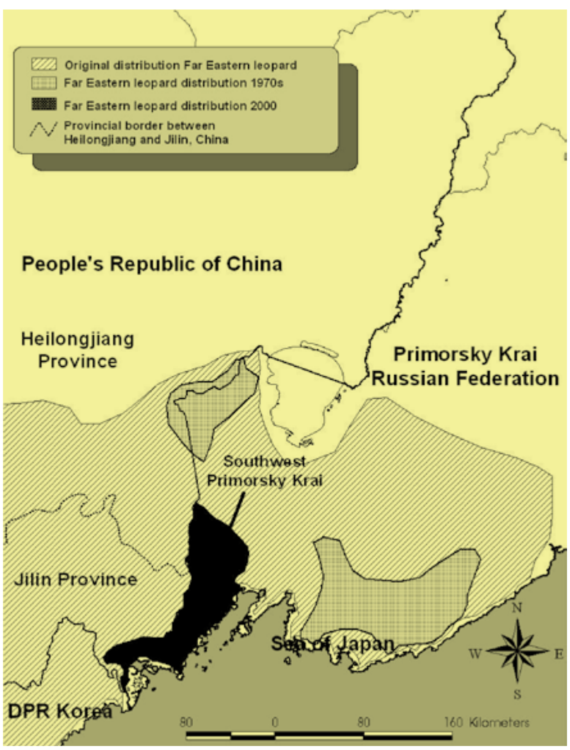
Palabras clave
Leopardo de Amur, conservación, cría en cautividad, liberación, reintroducción

Abstract

The Amur leopard, *Panthera pardus orientalis*, is one of only two large/medium cats for which a reintroduction from zoo stocks is currently judged a necessary conservation action by experts, such as the IUCN/SSC Cat Specialist Group; the other one being the Iberian lynx, *Lynx pardinus*. In addition, to the customary planning to address the problems associated with the release of translocated large predators at a given location, it is also necessary to establish appropriate breeding and release-management strategies for the relevant zoo population. In both these arenas, the socio-political issues are far more problematic than the biological ones. This chapter examines the biological and socio-political problems associated with producing Amur leopards suitable for reintroduction from the stocks present in the world's zoos, and also considers some relevant aspects of site selection, breeding and release strategy, and enclosure design. It places the reintroduction breeding strategy within the context of overall generation of conservation support for Amur leopards from the world's zoos, but does not attempt to give an account of all factors involved in production of the Amur leopard reintroduction plan, which is currently neither complete nor officially approved.

Keywords

Amur leopard, conservation, captive breeding, release, reintroduction



Source/ Fuente: WCS/Tigris Foundation.

Photo: Wildlife Conservation Society; (ISUNR camera trapping)

Figure 1. Amur leopard current and historical range in SW Primorye, Russia.

Figura 1. Área de distribución actual e histórica del leopardo de Amur en el suroeste de Primorye, Rusia.

Breeding Far Eastern leopards for reintroduction: the zoo programme perspective

Sarah Christie

Introduction

The Amur or Far Eastern leopard, Panthera pardus orientalis, is arguably the world's most endangered big cat, with only 25–34 animals remaining in the wild (Pikunov et al., 2007) in southwest Primorye in the Russian Far East (Figure 1). The vast majority of its current range lies in Russia, although individuals do range over the border into neighbouring China and there are still sporadic, unverified reports from North Korea. The population has been recognized as a distinct subspecies (Miththapala et al., 1996; Uphyrkina et al., 2002) and deserves protection as a unique genetic contribution to the species and to the region. As a top-order predator, it is also a significant indicator of ecosystem health and integrity (Ray, 2005). Its current range lies in a region of importance to Russia's economic development and also of considerable biological significance in terms of both biodiversity richness and endemism; 31% of Russia's endangered species are found there and half of these are unique to the region. The area is bounded by China, North Korea and the Sea of Japan on three sides and by human-dominated areas of Russia on the fourth. Potential for expansion exists only over the border with China, and only if wildlife- and habitat-protection laws there are effectively enforced.

The extant population of Far Eastern leopards is at risk from a multitude of threats, including illegal killing of leopards and their prey, habitat loss due to fires and infrastructure development, disturbance and poacher access from road construction through leopard habitat, a decline in the effectiveness of protected area management in southwest Primorye and the potential ill effects of small population size such as inbreeding depression, disease or demographic imbalance. Genetic analyses (Uphyrkhina et al., 2002) indicate a loss of genetic diversity, but as yet there is no evidence of a consequent loss of fitness such as a decline in fertility or cub survival.

A range of conservation actions have been put in place by relevant non-governmental organizations working with the Russian authorities. The Amur Leopard and Tiger Alliance (ALTA, see www.amur-leopard.org) has a comprehensive, but under-resourced, programme to address poaching and habitat loss as a result of human-induced fires and wildlife–human conflicts, to monitor leopard numbers, to evaluate wildlife disease status in the region and to improve local understanding of, and attitudes towards, the leopards. The key implementing agencies in ALTA are the Wildlife Conservation Society (WCS) and the Phoenix Fund, with other partners involved in Russia to a lesser degree (Moscow Zoo, the Zoological Society of London [ZSL], the International Fund for Animal Welfare, the Tigris Foundation and Wildlife Vets International) or providing relevant funding (ZSL, the Tigris Foundation, AMUR, 21st Century Tiger, David Shepherd Wildlife Foundation, Wildlife Alliance and Helsinki and Minnesota zoos). The other key agency is the World Wide Fund for Nature (WWF Russia), which has an Amur leopard programme covering anti-poaching, public education and the establishment of necessary protected areas, and is a partner in WCS's ecological monitoring. ALTA and WWF Russia coordinate leopard-conservation activities on the ground and are also working together on reintroduction planning.

To safeguard against the potential loss of Far Eastern leopards in the wild, experts have concluded that it is highly desirable that a second wild population be established in the near future (WCS, 2001). This view is supported by the IUCN/SSC Cat Specialist Group. Achieving this second population would not reduce the need to protect the original population in southwest Primorye, but would provide an opportunity to increase the numbers and genetic representation of this subspecies and would also increase the probability of survival in the wild if one or some combination of the many threats in southwest Primorye should force that population to extinction.

A reintroduction plan for this leopard is currently being prepared by a group of Russian and international scientists and conservationists for submission to the Russian government, and discussion on the subject with government authorities in Moscow has begun, facilitated by WWF Russia. I stress at this point that nothing will happen on the ground until the necessary political support and substantial funding have been obtained. This chapter is not intended to outline the entire plan, as it is not yet completed let alone authorized. Instead, I discuss the biological and political factors associated with managing the zoo population to produce cats suitable for release and with related factors, such as selection of release site and design of the proposed breeding and release centre.

Source of stock for release

In general, it is widely considered preferable to use wild-caught, translocated individuals for reintroduction projects rather than captive-bred stock. For carnivores, Jule et al. (2008) have recently reviewed information on the difference in success rates, which confirms this statement. Factors affecting success include lack of experience in hunting, lack of experience in exploring and establishing a home range, lack of humanamoun other predator-avoidance behaviours and vulnerability to disease.

As Jule et al. (2008) note, it would be of interest to further analyse the effects of each of these factors. While some projects have provided captive-bred carnivores with experience with live prey, there have been few efforts to ensure that captive-bred individuals slated for release do not become accustomed to humans, a much more problematic characteristic than lack of hunting experience. Release programmes for social species may have the option of pairing a captive-born individual with a mate accustomed to life in the wild in order to facilitate the learning process, at least in circumstances where there are such animals available (i.e., supplementations of existing wild populations or ongoing reintroductions in which some animals have already become established). This would not be possible for the Amur leopard, which is not social and would be released into an area with no existing leopards, making the learning period prior to release at dispersal age even more important.

The wild population of Amur leopards, at about 35 individuals, is far too small to withstand removal of sufficient animals to establish a new population. It is also genetically depauperate in comparison to the zoo population (Uphyrkhina et al., 2002). Leopards for release would therefore be sourced from the managed conservation breeding programmes in the world's zoos, and pre-release breeding and management protocols are being designed to mitigate all the above factors as far as possible (see below).

The zoo population

The zoo population of this taxon is held almost exclusively in North America, Europe and Russia and has, since 1998, been managed to produce maximum conservation support for its wild conspecifics in terms of fund-raising, public awareness, generation of useful data and skills, and maintenance of a genetic "lifeboat" (see Christie, in press for a fuller account of the conservation support roles of zoo animals, which extend well beyond reintroduction). The European/Russian zoo breeding programme (EEP, European Endangered species Programme) for Amur leopards is coordinated jointly by myself at ZSL and Tanya Arzhanova at Moscow Zoo, and the North American/Canadian programme (PMP, Population Management Programme) is coordinated by Diana Weinhardt at Minnesota Zoo. ZSL has established a centralized service for EEP participant zoos and anyone else wishing to support the ALTA Amur leopard-Conservation Programme; a website at www.amur-leopard.org and a regular email service provide supporters with news, pictures and reports from anti-poaching, outreach, camera-trapping, fire-fighting and veterinary projects run by WCS, Phoenix, ZSL and the Tigris Foundation, and ZSL acts as a collection point for funds raised. The PMP has played a similar role, gathering funds from participants and forwarding them to ZSL. Overall, zoos have provided a significant proportion of the funds so far spent on the conservation of this leopard in the wild.

Numbers and genetics

In early 2008, there were ~130 leopards in the European Species Plan (EEP) and ~97 in the Population Management Plan (PMP). There are a few additional leopards in zoos in Japan, Korea and China, but these are inaccessible to the conservation breeding programme and/or of inappropriate genetic makeup to be useful. The EEP population currently retains 88.9% of wild-gene diversity, which is just below the generally accepted desirable level of 90% (see Leus and Lacy, this book). The PMP population contains no genetic lines that are not also present in the EEP population. Little can be done to improve gene diversity retention other than to breed from as many optimal pairings as possible on both sides of the Atlantic and, perhaps, if the necessary artificial reproductive techniques (ART) develop sufficiently and if the wild population proves to contain significant diversity not present in the captive stock, to use semen collected from wild male leopards to fertilize oocytes from the captive population.

To date, there have been very few instances of using ART to bring about genetically useful matings with real conservation implications from pairings that could not have mated naturally if given the chance, in felids or in any other group (see Wildt et al., this book). However, the Berlin Institute of Zoo Biology and Wildlife Research is now able to store feline sperm, ovarian tissue and early embryos produced by IVF and is researching application of these techniques to exotic cats with particular emphasis on the Amur leopard. If they are successful, their work could bring real conservation benefits. It could enable breeding from zoo cats that have died since their gametes were harvested and hence extend the life of the captive gene pool; it could transfer genetic material from the wild to the captive population without removing any leopards from the wild; and it could also enable careful planning of breeding for release. Embryos of appropriate genetic makeup and sexes could be stored and implanted as necessary into one or two females held in the *in situ* breeding centres, which would eliminate the need for long-distance transfers of pairs for breeding and would also ensure a balanced sex ratio in cubs produced for release, something that cannot be assured by any other means. However, these methods are both expensive and experimental; there is still a very long way to go in this arena, and current plans remain based on natural mating.

Breeding strategy in the Amur leopard EEP and the Founder 2 problem

As if this leopard did not already have sufficient difficulties, the managed zoo population in both the American and European regions includes a considerable contribution of genes from one founder –Founder 2– who was not an Amur leopard. (NB: Founder 89 has also been shown to have non-Amur leopard genes; however, his genetic contribution to the captive population is rather small in comparison and is not of sufficient significance to merit special attention in the genetic management strategy). Founder 2 is long dead, but molecular genetics on samples from his descendants (Uphyrkhina et al., 2002) indicates that he was probably of the geographically adjacent *japonensis* subspecies from northern China. If this is so, he was simply from the other end of what

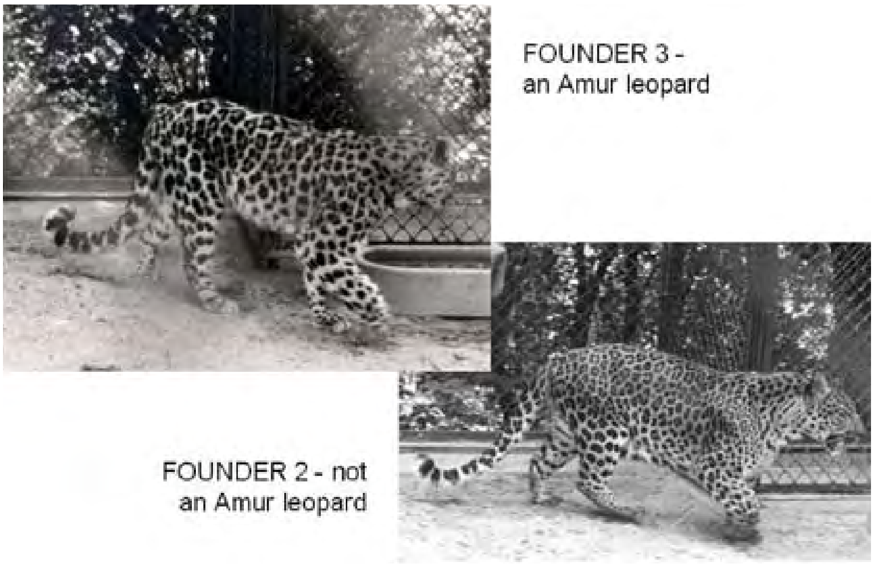


Figure 2. Founder 2 (not an Amur leopard) and his life-long mate Founder 3 (an Amur leopard) at Frankfurt Zoo in the 1970s). No copyright known.

Figura 2. Progenitores nº 2 (no leopardo de Amur) y nº 3 (leopardo de Amur), emparejados de por vida el uno con el otro en el Zoológico de Frankfurt en los años 70. Sin copyright conocido.

was quite recently a cline. Taxonomists favouring a morphological approach (who suggest his appearance is very different; see Figure 2) consider him more likely to be *pernigra* from the Himalayas. In the end though, this debate is purely academic. Taking a pragmatic approach, whatever his origin, his genes are so widespread within the zoo population, and the remaining wild population is so small and genetically impoverished that, as for the Prezwalski's horse population and its domestic horse genes (Bowling et al., 2003), there is no realistic option other than to retain his genes in the zoo and reintroduced population.

The debate over Founder 2 might be thought relatively inconsequential in biological terms, especially given the documented success in improving Florida panther, *Puma concolor*, viability using Texas puma genes (Pimm et al., 2006). However, relevant events clearly demonstrate that socio-political factors can often override biological ones in decision-making processes (Jiménez et al., this book). Initially, in the mid-1990s, representatives of European and Russian zoos were strongly opposed to retaining representation of Founder 2, largely on philosophical grounds, and strenuous efforts were made to breed the few available pure-bred leopards exclusively to each other. These pure-bred cats had all come from Pyongyang zoo in North Korea during the 1980s and 1990s, and were variously reported as wild caught or captive bred without any substantiating material. Genetic investigations by Uphyrkhina et al., (2002) concluded that they were closely related to the wild population in Russia and were also sufficiently related to one another to be the result of three generations of brother–sister pairings (Uphyrkhina, personal communication). Breeding attempts within this group largely failed to produce healthy offspring and for the past five years these cats, which where still alive and in breeding condition, have instead been paired with leopards containing low levels of Founder 2.

It was clear in 1998, and was further underlined at a meeting in Vladivostok, "Workshop on Conservation of the Far Eastern Leopard 2001" (WCS, 2001) –despite arguments that evolution might be expected to act on any undesirable genes present– that leopards with high levels of Founder 2 genes would not be acceptable to many scientists and conservationists for release. Hence, rather than simply treating Founder 2 as another founder on the grounds of his probable nearby geographical origin (according to molecular genetics), as the PMP has done, EEP strategy has been to manage breeding so as to minimize his contribution as far as possible while still maintaining acceptable overall levels of genetic diversity. The usual method of selecting cats for breeding on the basis of their mean kinship ranking (simply put, the level of unrelatedness to the rest of the population) has been balanced with consideration of their Founder 2 content, so that a cat with a high mean kinship rank but with more than 25% Founder 2 content might not get a breeding position while one with a lower mean kinship rank but only 12.5% Founder 2 is certain to do so. All leopards with more than 41% Founder 2 (simply a convenient cut-off point in the distribution of percentages) have been excluded from breeding since 1999 and, in recent years, those with more than 30% have also been mostly excluded as numbers of animals with lower levels have risen.

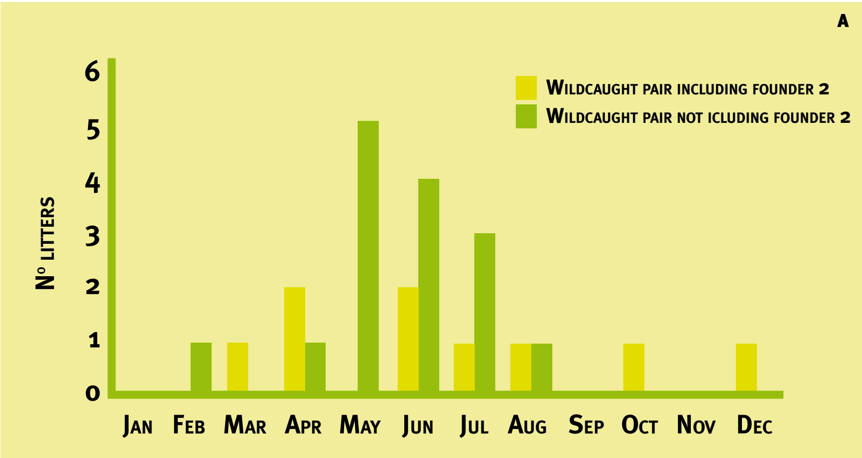
This policy has resulted in an overall decrease in the prevalence of Founder 2 genes and an increase in the number of leopards with low percentages of them. It is unfortunate that it was not possible to adopt this approach earlier, as several pure-bred leopards died without surviving offspring before the new policy was agreed. As of early 2008, 20% of the total EEP population (13 male and 13 female leopards) contained a Founder 2 contribution of 20% or less, as did 4% (two male and two female leopards) of the PMP population. Breeding from animals of between 20% and 30% Founder 2 will continue to some extent in order to conserve a wide gene pool in the population overall, but it is the pool of young leopards carrying a Founder 2 contribution of less than 20% that would provide the necessary breeding pairs for production of cubs for release in the Russian Far East.

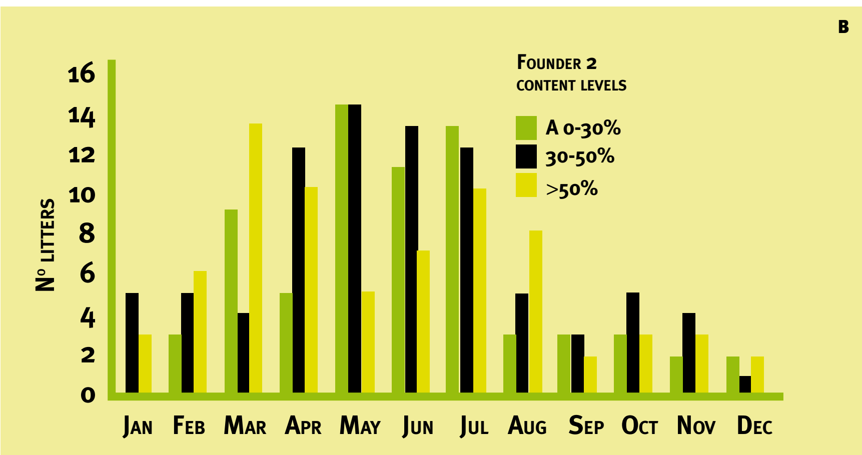
The PMP programme has recently recognized the political realities of breeding for possible reintroduction –i.e., that whatever the real biological consequences may be, many relevant scientists remain fundamentally opposed to Founder 2's inclusion– and has adopted the EEP approach of minimizing his contribution. Two young leopards with low Founder 2 content are being identified for shipment from Europe to Minnesota to assist in reducing overall Founder 2 levels in the PMP.

Melanism is present in the population, thanks partly to the enthusiasm with which its initial appearance was greeted by some participating zoos, which actively sought out and bred from these cats to the extent of importing them to Canada from Europe for the purpose. There are no records of the melanism gene in the wild population of Amur leopards, but it is not possible to attribute the gene in the zoo population exclusively to Founder 2 because all leopards with a genetic contribution from him also contain genes from his life-long mate, Founder 3. While the melanism gene could conceivably –given the snowy winters in the region– exert an impact on survival in the wild, being a recessive gene it would be likely to be expressed in only one cub per litter at most and to be rapidly selected against in the wild if it did increase mortality rates. The fact is, however, that when expressed it is visible and so, like the fox colour gene in the Prezwalski horse, is likely to attract intense hostility from those who are philosophically opposed to the inclusion of Founder 2 genes in the first place. Once again, socio-political factors outweighed biological ones in the management decision process and melanistic cats are now excluded from the breeding pool, although not all possible melanism gene *carriers* are excluded, as to do that would effectively cripple the Programme. Invisible genes (e.g., those affecting physiological processes such as temperature control) might have a more significant impact on survival in the wild. As we cannot detect them, however, the only possible strategy to mitigate their possible effects is to minimize Founder 2's overall contributions.

The matter of birth seasonality is an interesting one in this context. This is a factor that could compromise survival and could be affected by genes from another geographical location. It is also one which we can detect in zoo leopards. There are few data on birth seasonality from Amur leopards, but Amur tigers *(Panthera tigris altaica)* in the wild do not show a pronounced peak in birthing during the spring, in fact, most births are in late summer or autumn, with births reported in nearly every month of the year (Kerley et al., 2003). This could be considered surprising given the severe winters in the region. Captive Amur tigers, with a dataset of well over 4 000 births, do show a significant birth peak in April–June, with 45% of births occurring in those months (Traylor-Holzer, 2003), though births do occur throughout the year. Additionally, in two pure-bred Amur leopards in Prague Zoo, the quality of semen collected varied with season, with better-quality ejaculate obtained in spring and early summer than in December (Katarina Jewgenow, personal communication).

With this in mind, along with the need to allay as far as possible the fears of those objecting to Founder 2's genes, birth seasonality in the Amur leopard EEP with and without Founder 2 was investigated in 2001. Figure 3a shows the distribution of litters born to wild-caught pairs with and without Founder 2; there is a 2-month difference in average birth date between the two groups. Figure 3b compares birth distribution in leopards with low, medium and high Founder 2 content; the difference between the low and medium Founder 2 groups is statistically significant ($p>0.025$; $x^2 2 >20.12$, $df=11$, $p <0.05$ for each), indicating that Founder 2's genes do affect the birth season in this population.





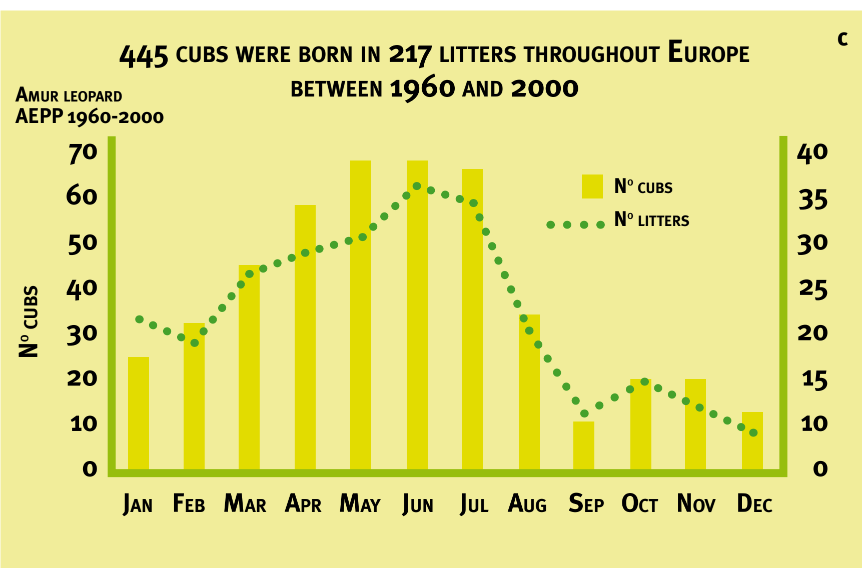


Figure 3. (a) Birth seasonality in pairs of wild-caught Amur leopards not including Founder 2, contrasted with births to Founder 2 and his life-long mate Founder 3. (b) Birth seasonality in leopards with low, medium and high Founder 2 content. (c) Birth seasonality in the whole population between 1960 and 2000. Source: ZSL.

Figura 3. (a) Estacionalidad de nacimientos en parejas de leopardos de Amur capturados en el medio silvestre, sin incluir al Progenitor nº 2, comparadas con los nacimientos del Progenitor nº 2 y su pareja de por vida, el Progenitor nº 3. (b) Estacionalidad de nacimientos en leopardos con bajo, medio y alto contenido del Progenitor nº 2. (c) Estacionalidad de nacimientos en toda la población entre 1960 y 2000. Fuente: ZSL.

However, the subsequent analysis of the whole population's birth distribution shown in Figure 3c clearly indicates that, overall, there is a peak in spring. As field scientists currently do not believe that this leopard is a seasonal breeder in the wild, they will presumably be less concerned about this factor in leopards for release than about melanism; but in any case, it seems that provided Founder 2 levels remain below 20%, there is unlikely to be an effect on birth seasonality. Some genetic lines in the zoo population have experienced high levels of inbreeding in the past, although management strategy over the past 10 years has ensured that current inbreeding coefficients are below 0.1. Past problems have been due partly to a shortage of founders, but also to a lack of active management prior to the mid-1990s, particularly in the pure-bred (and inbred) lines originating in Pyongyang zoo. At present, the EEP is collecting and collating data on any possibly inheritable defects, which have included deformed leg bones, cryptorchidism and "manx" tails, and ensuring that any affected animals are not included in the breeding pool. The bulk of the population is free from such problems. A veterinary team has been assembled by the EEP's veterinary advisor, John Lewis, to investigate specific factors where necessary, and a protocol for data collection has been circulated to participating zoos.

It is worth noting that in one instance of a possibly inherited defect –cryptorchidism– in a pure-bred cat, the strength of feeling among EEP participants about purity was such that attempts to breed from the animal in question continued despite clear veterinary advice pointing out that the problem is often associated with other damaging genes. As this leopard has also failed to show normal mating behaviour, ART has been used as well as natural mating attempts. However, no pregnancy has yet resulted.

Preparations in the field

Release-site selection

There is general consensus among stakeholders that the first effort should focus on establishing a second population rather than on supplementing the existing wild one given the potential veterinary, behavioural and genetic risks of adding captive-bred cats to the wild population and the lack of any evidence of loss of fitness resulting from small population size in the wild as yet. The release site for this second population should have the following characteristics:

- Be within the historical range of the Amur leopard (Figure 1).
- Be sufficiently separated from the current range to ensure no possibility of genetic mixing with the existing population.
- Be as far away as possible from human habitation in order to minimize chances of conflicts.
- Contain a high prey base of appropriate species.
- Contain cliffs and rocky areas to facilitate the leopard's use of refuges on release.
- Contain adequate infrastructure (roads, electricity and water supply) for management and monitoring activities.
- Be within a well-managed area with sufficient protection.

Additional criteria, including those related to the attitudes and reactions of local residents and other stakeholder groups, do of course need to be met before a reintroduction can proceed (see IUCN, 1998); here we are considering only criteria relating to site selection.

Lazovsky Zapovednik (zapovedniks are IUCN Category 1 protected areas) is located within the historical range and at the same latitude as the existing population (see Figure 4). Its physical characteristics also resemble the current range in that it is situated on the coast, which tends to create slightly warmer weather conditions, it contains similar cliffs and rocky areas, and the habitat types are also similar. Sika deer *(Cervus nippon)* occur at the relatively high density of 8.6 head/km^2, but species within the leopard's preferred prey-weight range (Hayward et al., 2007b) are less abundant (Alexander Myslenkov, personal communication). This is due partly to effective protection work carried out by the reserve staff with support from Phoenix Fund, but also to the fact that at the time the leopard became extinct in south Sikhote Alin, sika deer could legally be hunted there, while they are now fully protected. In the leopard's current range, it remains possible to legally hunt sika deer.

Tigers would present a risk to a small, reintroduced founder population of Amur leopards, in that intraguild predation (Palomares and Caro, 1999) has been demonstrated in the region (Aramiliev, personal communication).

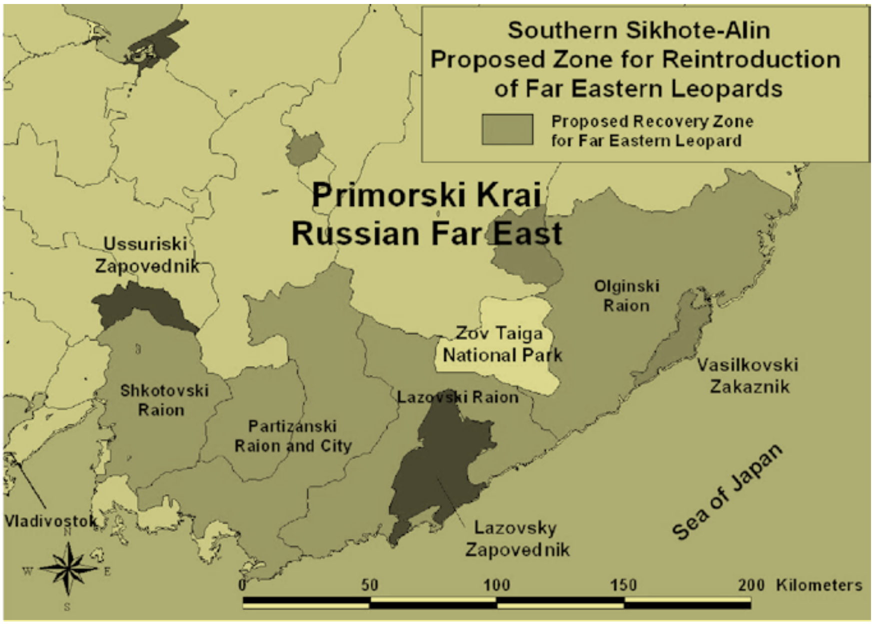


Figure 4. SW Primorye showing current leopard range and protected areas including Lazovsky Zapovednik.

Figura 4. Suroeste de Primorye, indicando el área de distribución actual del leopardo y las áreas protegidas, incluyendo Lazovsky Zapovednik.

Lazovsky contains tigers, as do all other potential release sites. Their presence is unavoidable and all that can be done is to take mitigating action, e.g. providing leopards for release with aversion training in relation to tigers and/or ensuring that site topography favours separation between the two as far as is possible. Spatial separation appears to exist in southwest Primorye, where tigers and leopards currently co-exist (Miquelle and Murzin, unpublished data), and is the basis for including "cliffs and rocky areas" which are assumed to provide refuge for leopards. Red deer, which are generally a preferred prey item for tigers but are too large for leopards, are present in Lazovsky but at low density (0.4 head/km^2, Alexander Myslenkov, personal communication), so the sika deer population there may be targeted by both tigers and leopards. As red deer are almost unknown in southwest Primorye, clearly it is possible for the two big cats to co-exist with sika deer as the primary prey item. Leopards take more small prey such as badgers and raccoon dogs than do tigers, so prey selection will not overlap completely.

Other factors

Other significant factors that must be addressed early in the planning process include: disease status of wildlife in general in the release area and of zoo leopards that may be used for breeding stock, local and political support for such a plan, and potential funding sources.

Acceptance of a reintroduction programme by local communities will clearly be critical to success, and social research to assess attitudes of local people is in the pipeline. While Phoenix Fund and ZSL staff have carried out studies in existing leopard range and Phoenix and WWF Russia have had considerable success in influencing attitudes towards tigers and leopards among children in southwest Primorye, work in the proposed release area is yet to begin, and Durham University is seeking funding for a 3-year study designed to assess local attitudes as a first step.

Steps must be taken to ensure that released cats do not harbour pathogens likely to adversely affect wildlife in the area and that they are not themselves vulnerable to pathogens already present in the environment. Towards this end, since 2006 ZSL and the Primorskii State Agricultural Academy, in partnership with WCS, have been implementing a 3-year programme focused on increasing wildlife health-monitoring capacity in the region, as well as collecting baseline data on wildlife diseases in the area and disease status in zoo leopards genetically suitable for production of cubs for release. Wildlife vets and students in the region participate in workshops that include lectures and hands-on training sessions, taught by international experts, with training programmes held in a local rehabilitation centre (Utyos) in Khabarovski Krai and in Moscow and Novosibirsk zoos. Training sessions in the zoos have served the additional function of collecting samples from Amur leopards for disease analysis, as these are the only two zoos in Russia holding cats genetically suitable for reintroduction.

Other suitable stock in European and American zoos is also being sampled as part of the disease testing programme, with additional specialist investigations such as MHC testing and expert analysis of heart murmurs being coordinated by John Lewis. Meanwhile, samples are being collected in and around the proposed release area from tigers, wild ungulates, small carnivores, and domestic ungulates and carnivores. In Ussurisk, a laboratory belonging to the Academy is being renovated and outfitted to enable in-country sample analysis in the long term, although samples are at present being analysed in UK and European laboratories as well.

The draft reintroduction plan has so far been the subject of one governmental meeting in Moscow, facilitated by WWF Russia, and comments made have influenced subsequent work. When the plan is complete and officially approved, it will then be possible to approach potential funding sources. However, finding sufficient support for such a major effort represents one of the great challenges of the reintroduction effort.

Breeding and release strategy

If this reintroduction is to succeed, it is clear that the design of the breeding and release centre, and the management of the leopards within it, must focus strongly on overcoming the difficulties imposed by the captive origin of the cats. In addition, a "soft" rather than "hard" release strategy should be used (i.e. the breeding enclosure should be located at the point of release). Three necessary behaviours should, if possible, be acquired prior to release: hunting and killing of live natural prey, avoidance of humans and avoidance of tigers. (The leopards will of course also need to learn exploratory behaviour within the home range, but this is not a factor that can be addressed in the breeding enclosure other than by placing it within the release habitat).

This section of the reintroduction plan is not yet complete, and there appear to be no models. While various projects have experimented with hard- and soft-release sites and with varying levels of experience of hunting for zoo-bred cats before release, there appear to have been no serious efforts to date to avoid contact with humans and instil human-avoidance behaviours, let alone avoidance of larger predators, in such cats. Avoiding contact with humans involves a complete reversal of accepted zoo enclosure-design principles which, as well as ensuring a good viewing experience for the public, tend to focus strongly on ensuring that the animals can be observed, moved around, given treatments and captured by staff with maximum ease for the keepers and with minimum stress for the animals.

The outline presented here is therefore experimental, is still under discussion, and is in no way intended to provide a final or complete account of intended protocols.

Breeding-centre design

The breeding centre will need to include a short-term holding facility, a veterinary examination room, storage for equipment and supplies, and staff housing, offices and meeting facilities, as well as several breeding enclosures. The breeding enclosures would be located at a considerable distance from the rest of the centre to ensure that the animals in the enclosures could not smell, see or hear the humans present there. This stands in stark contrast to the reintroduction of African top-order predators that are intended for ecotourism purposes. Feeding live prey to the release candidates from birth is a highly desirable tactic and, if this strategy is to be used, thought will also need to be given to provision of breeding facilities for prey items, e.g. deer and rabbits. This approach, although likely to attract criticism from animal-welfare agencies, would have the significant benefit of ensuring that released animals are able to identify and kill appropriate prey species. Nonetheless, relevant Ethics Committees would need to carefully consider the costs and benefits. Provision of a couple of "leopard display enclosures" –located at the main part of the centre, well out of sight, sound and smell of the breeding enclosures might also be desirable, as the project would be bound to attract interest, and good public relations will be of paramount importance, yet people must be kept away from leopards intended for release. One of the display enclosures might perhaps contain a tiger, and it is conceivable that a tiger housed there could be useful in aversion training for release stock.

The centre, and particularly the breeding enclosure(s), would be located within the release site to allow a "soft" rather than "hard" release. Pairs of physically and behaviourally healthy zoo leopards of suitable genetic

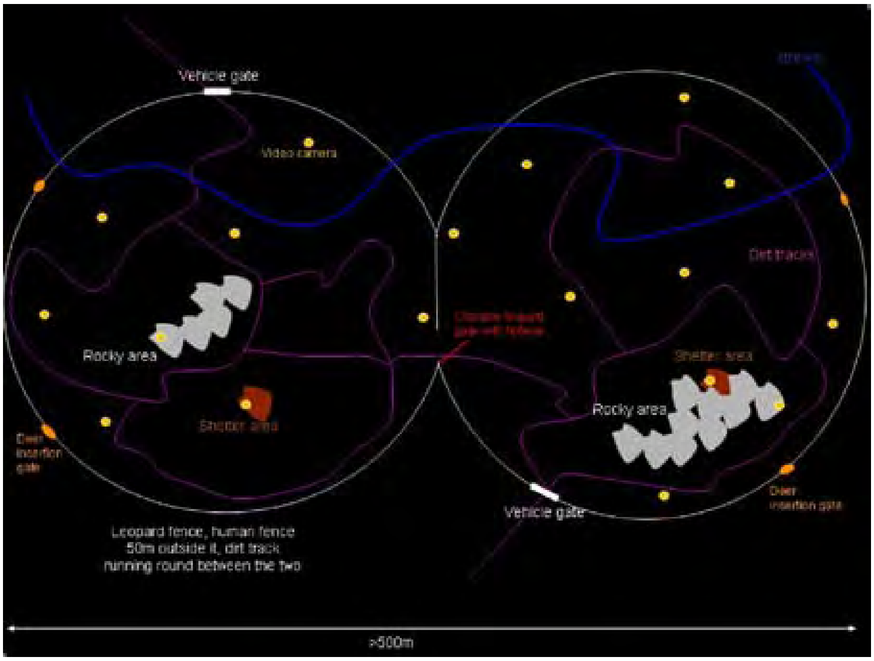


Figure 5. Amur leopard breeding and release enclosure design outline (not to scale).

Figura 5. Esquema del diseño de las instalaciones de cría y liberación del leopardo de Amur (no está a escala).

makeup, which had proven themselves to be successful breeders and rearers of cubs in zoo conditions, would be transferred to the facility for breeding purposes and returned to their home zoos when sufficient offspring from their genetic line had become established in the wild. Cubs would be born and reared with as little interference from humans as possible, with routine monitoring done long-range by video. The male would be removed as soon as pregnancy was confirmed. When the cubs were old enough to begin to learn to hunt, live prey would be supplied in the enclosure; basic hunting behaviour is instinctive in cats, and it is assumed that if cubs were given the opportunity to learn from experience while young, they would develop sufficient hunting skills to form a basis for a life in the wild.

When the cubs reached dispersal age, the mother would be removed, the cubs given a veterinary check-up and fitted with adult-sized radio collars, and the gates opened so that they could move out into the surrounding forest but still return to a known area for shelter and, if necessary, supplementary feeding.

More than one breeding and release enclosure should be established within reach of the centre, and consideration should be given to setting up one or more additional enclosures at a different location within the overall release area. Given that releases will need to be continued over a period of years, if leopards settle into home ranges near the release point there could be potential for aggressive encounters if further animals –particularly of the same sex– were released at the same site. The other components of the centre need not be duplicated to achieve this, but the holding facility at the centre should be designed to hold sufficient adult leopards to serve several breeding enclosures.

Design of breeding enclosures

It is imperative that leopards destined for release grow up without familiarity with humans and learn to fear humans as much as possible. The leopard breeding enclosure(s) associated with the centre should therefore be situated within reach of transport, water supply and electrical power, but out of sight, sound and smell of the other buildings necessary for the operation of the centre and of all associated human activities.

Breeding enclosures should be as large as can be achieved with available funds and should be in a wooded area so that the leopards have plenty of cover; they should not be visible to a casual observer unless at the edges of the enclosure. Enclosures should take advantage of natural features in the landscape and, if at all possible, should include rocky areas, a natural stream for water, and natural shelter areas such as caves, overhangs or fallen/hollow trees. If natural shelter is not available in the area, it will need to be constructed from natural materials, i.e. trees and rocks. As few man-made items as possible should be present, and capture of leopards for radio collaring or final medical assessments would be performed by darting from vehicles, not by the normal zoo methods of simply shutting the animal into its night den for darting or accustoming it to sleeping in the intended transport crate. Monitoring of the cats would be carried out via video cameras scattered around the enclosures and linked to viewing screens in the staff areas.

Enclosures could be shaped in a rough figure of eight with the central connection containing a leopard-proof closable gate (Figure 5). This design would allow the leopards to be separated if necessary, or to be shut into one end to facilitate catching them or while live prey, if used, is released in the other end. The figure-of-eight shape would also allow for live prey to be chased around without being cornered, which would help the leopards improve hunting skills. Chasing deer along the fences would be to some extent inevitable, but it is at least possible to avoid corners. Leopards can climb, and their enclosures in zoos have traditionally been roofed, although larger modern facilities have departed from this model. Serious escape attempts are unlikely in a large densely wooded enclosure with plenty of food, but nevertheless the fence would need to be high, perhaps 5 m, with a substantial overhang. Use of hotwire is also desirable both to discourage escape and in the hope of conditioning leopards against approaching man-made structures. Although a wild-caught African leopard is known to have scaled a 3-m high electric fence in order to escape from a boma (Hayward et al., 2007a), that animal had a far stronger motivation for crossing the fence than leopards in these enclosures would have. Large gates into each half, and dirt roads within, would permit vehicle access for captures of leopards and release of live deer, if used.

Management of leopards

Leopards selected for breeding would undergo a final set of health tests before shipment from their home zoos, and would arrive in spring to allow plenty of time for acclimatization before the onset of winter. They would initially go into the holding facility for a quarantine and acclimatization period before transfer to the breeding enclosure.

All cats in the enclosure would be radio-collared at all times, to assist in locating them within the enclosure and with recapture should there be an escape. Expandable collars allowing for growth are available for young cats. Live prey, if used, would be introduced into the enclosure regularly via vehicles or through specially constructed gates, with the leopards shut into the opposite part of the enclosure each time to keep them as unaware as possible of the association between humans and food supply.

Monitoring via video cameras would help to establish that the female was pregnant and, once this was achieved, the male would be removed to the holding facility or perhaps a display enclosure. The cubs would be captured, collared and given a veterinary check-up as soon as they began to show independence from their mother. The mother would be removed at some point prior to the cubs reaching dispersal age, at which time they would again be captured, fitted with an adult-sized radio collar and given a final health check. All gates would then be opened and perhaps also the fence breached in a few places, the supply of live food stopped and, if necessary, bait used to tempt the young leopards out. The cats would be monitored as they dispersed, but supplemental food would still be provided, probably for several months.

Breeding leopards would be held in the centre until they had contributed sufficient cubs and would then be returned to their home zoo and replaced by other cats of different genetic lines. It is expected that the process of establishing a stable population would take at least 10 years.

Next steps

It is intended that the plan will be completed and submitted to the Russian authorities during 2008, after which final discussions on the work will take place and the plan will be amended accordingly. Health evaluations of zoo leopards will also continue during this period and should be completed in 2009. As and when official endorsement is forthcoming, it will be possible to seek funding, which will be the next big hurdle for the project.

In the meantime, all involved will continue their efforts to protect the existing wild leopards, and the European and American conservation breeding programmes in zoos will continue to be managed so as to produce the maximum possible conservation support for their wild relations, as well as experienced young breeding pairs suitable for *in situ* breeding for release.

Acknowledgements

The reintroduction plan, and hence this chapter, is dependent on the dedicated work of many individuals including Dale Miquelle (WCS), Alexy Kostyria (Institute of Biology and Soils, Far Eastern Branch of the Russian Academy of Sciences), Vladimir Aramilev (Institute for Sustainable Use of Natural Resources), Dimitri Pikunov (Institute of Geography, Far Eastern Branch of the Russian Academy of Sciences), Yuri Darman (WWF Russia), Tanya Arzhanova (Moscow Zoo), Michiel Hötte (ZSL and Tigris Foundation) and John Lewis (Wildlife Vets International). Useful comments on the chapter itself were kindly provided by Matt Hayward, John Lewis, Ron Tilson, Dale Miquelle, Michiel Hötte, Josh Ginsberg and Richard Reading. Thanks are also due to all those who have provided and/or continue to provide support for activities relevant to reintroduction planning; WCS, WWF Russia, Phoenix, ZSL, Tigris Foundation, AMUR, the UK government's Darwin Initiative, the Trust for Mutual Understanding, Wildlife Vets International, Twycross Zoo Conservation Fund and Lucie Bergers Fund.

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Photo: Wildlife Conservation Society; (ISUNR camera trapping)

Wilderness is not a luxury but a necessity of the human spirit.

Edward Abbey
(1917-1989)

Reintroduction of the Black-footed ferret to the great plains of North America: *Do we really have the capabilities, resources and socio/political will to recover critically endangered species in the United States?* An opinion

Reintroducción del turón de patas negras en las grandes llanuras de Norteamérica: *¿realmente disponemos de las capacidades, recursos y voluntad sociopolítica para recuperar las especies en grave peligro de extinción en los Estados Unidos?* Una opinión

Mike Lockhart

Resumen
Aunque en algún momento se pensó que era una especie extinguida, en 1981, se descubrió una población pequeña de turones de patas negras (*Mustela nigripes*; turón) cerca de Meeteetse, en el oeste de Wyoming (Estados Unidos). En 1987, sólo quedaban 18 ejemplares que fueron llevados a cautividad con el fin de evitar su extinción mediante un programa de cría en cautividad. El turón depende de tres especies de perritos de la pradera *(Cynomys* spp.*)* para su supervivencia; utiliza los perritos de la pradera como presa y aprovecha las madrigueras de éstos para sus refugios y guaridas. Las causas de que los turones estuvieran al borde de la extinción fueron la conversión de los pastizales naturales del altiplano en tierras de cultivo y las décadas de envenenamiento intensivo de los perritos de la pradera durante la primera mitad del siglo XX. Desde 1987 hay un programa internacional para la recuperación del turón que ha superado grandes retos y ha tenido un éxito notable. Entre los años 1987 y 2007, se criaron aproximadamente 6000 turones en cautividad y fueron reintroducidos más de 2400 en 17 espacios de distintos proyectos en el oeste de los Estados Unidos y México. No obstante, la reintroducción ha tenido distintos grados de éxito y algunos proyectos se vieron afectados por el impacto sobre las presas o el hábitat de una enfermedad introducida (la peste bubónica), la sequía y la política (con los correspondientes fracasos en la

gestión del suelo). La estima más reciente de la población indica que las poblaciones de turones silvestres representan sólo un 20% del objetivo original que se estimó para lograr que su clasificación bajara a un nivel de protección menor (mejoras en la conservación de la especie que modificarían su clasificación, pasando de estar "en peligro crítico" a "amenazada"). A pesar de que la Ley sobre Especies Amenazadas y otras leyes estadounidenses sobre el medio ambiente contemplan mandatos y directrices para la recuperación de especies en peligro de extinción y para la gestión correspondiente de suelo público de propiedad federal, posiblemente el mayor obstáculo para los avances recientes en la recuperación del turón haya sido la intervención política y el abandono de las leyes y políticas medioambientales (comportamiento mostrado por la Administración de los Estados Unidos entre 2000 y 2008). Tenemos la experiencia técnica y la capacidad, así como el hábitat básico, para conseguir la total recuperación del turón en el medio silvestre. De todos modos, la plena recuperación sólo se puede lograr si las agencias estatales y federales se comprometen firmemente a cumplir los principios previstos por la Ley sobre Especies Amenazadas, y si actuamos para mejorar las condiciones del hábitat en el área de distribución histórica del turón. El presente ensayo crítico ofrece un ejemplo específico de cómo la intervención política ha afectado la recuperación del turón e incluye recomendaciones que, quizás, sean aplicables a la recuperación del lince ibérico y otras especies amenazadas.

Palabras clave
Turón de patas negras, *Mustela nigripes*, perrito de la pradera, programa de recuperación, Ley sobre Especies Amenazadas

Abstract
Once thought to be extinct, a small population of black-footed ferrets (*Mustela nigripes*; ferret) was discovered in 1981 near the town of Meeteetse, western Wyoming. By 1987, only 18 individual ferrets remained, all of which were taken into captivity in an effort to avert extinction and initiate a captive breeding programme. The ferret depends on three prairie dog species *(Cynomys* spp.*)* for survival, and use prairie dogs as prey and prairie dog burrows for shelter and dens. It was the conversion of native grasslands to agriculture and decades of intensive prairie dog poisoning in the 1900s that originally brought the ferret to the brink of extinction. Since 1987, an international ferret recovery programme has overcome significant challenges to experience many remarkable successes. From 1987 to 2007, about 6000 ferrets have been produced in captivity and over 2400 have been reintroduced at 17 separate project sites in the western U.S. and Mexico. Still, reintroduction success varied widely and some efforts were compromised by prey/habitat impacts from an introduced disease *(sylvatic plague)*, drought and politics (with attendant land management failures). The most recent population estimate indicates that wild ferret populations are only about 20% of a long-standing "downlisting" objective (population improvements that would change species status from "critically endangered" to "threatened"). Although the Endangered Species Act and other environmental law in the U.S. provides mandates and guidance for recovery of endangered species, and associated management of federal public lands, perhaps the biggest obstacle to recent recovery progress stemmed from political interference and neglect toward environmental law and policy (as displayed by the U.S. 2000-2008 Administration). The technical expertise, capabilities and raw habitat exist to fully recover the ferret in the wild. However, complete recovery can only achieved with strong recommitments by state and federal agencies to principles set forth in the Endangered Species Act and action to improve habitat conditions over the ferret's historical range. A specific example of how political interference impacted ferret recovery is addressed in this opinion paper, as well as a recommendations perhaps applicable to recovery of the Iberian lynx and other imperiled species.

Keywords
Black-footed ferrets, *Mustela nigripes,* prairie dog, recovery programme, Endangered Species Act

Reintroduction of the Black-footed ferret to the great plains of North America: *Do we really have the capabilities, resources and socio/political will to recover critically endangered species in the United States?* An opinion

Mike Lockhart

Introduction

Dramatic habitat loss and human persecution over decades preceding passage of U.S. Endangered Species Act (16 U.S. C 1531-1544, 87 Stat.884, as amended) caused the near extirpation and/or dramatic decline of many North American wildlife species. After the 1970s disappearance of the last known wild black-footed ferrets (*Mustela nigripes*; ferret) from Mellette County, South Dakota, the ferret was widely regarded as extinct. However, in 1981, another small population was found near the town of Meeteetse, in western Wyoming. Since then, ferret recovery has followed something of a roller coaster track, bringing the species perilously close to extinction by 1987 (when only 18 surviving animals remained and were all brought into captivity), and then to greater stability by 1999, when captive population objectives were first met and substantial progress was made in reestablishing wild populations.

The early history and direction of the ferret recovery programme was accentuated by controversy and sharp management disputes between involved agencies and other parties (Miller et al., 1996). Interpretation of the actual nature, severity and consequences of early programme disputes vary, but despite disagreements and real threats to species survival, significant recovery progress was realized (Lockhart et al., 2006). Indeed, the black-footed ferret programme is now often viewed as something of a model of partnerships and cooperation in endangered species recovery and involves numerous state and federal agencies, Indian Tribes, conservation organizations, and private interests across the U.S., Canada and Mexico. And certainly, since ferrets were relegated to captivity in 1987, the coalition of recovery partners has weathered difficult times to achieve some rather remarkable successes (see previous Endangered Species Bulletin articles; Vol. XXI, No.6; Vol. XXV, No. 3; Vol. XXVII, No.2; Vol. XXVIII, No. 3).

But, where does the black-footed ferret recovery programme really stand today? What are the prospects for downlisting to threatened status, and for full recovery? After years of resigned loss, rediscovery, research, intensive management and numerous advances and setbacks, what lessons have been learned about restoring such a critically endangered species in the face of ever-pressing demands on the natural resources needed to sustain it? These are difficult questions, but necessary to address head-on if recovery challenges are to be overcome.

The black-footed ferret is an obligate carnivore of three North American prairie dog species *(Cynomys* spp.*)* with an original range that largely overlaid prairie dog distribution from southern Canada, through the grasslands and steppes of 12 western states, to northern Chihuahua, Mexico (Figure 1). Although nocturnal, secretive and not well known to science until recent years, the ferret was undoubtedly a numerous and highly successful

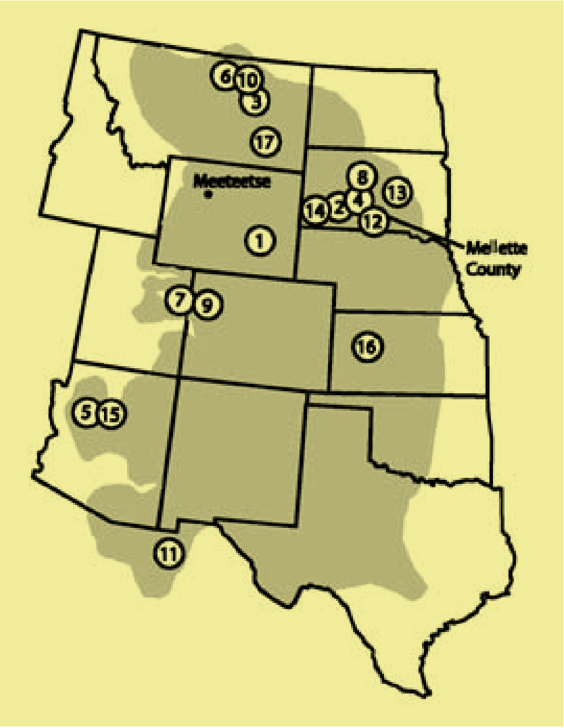

Figure 1. Probable historical range of the Black-footed ferret as defined by composite ranges of black-tailed, white-tailed and Gunnison's prairie dogs (shaded area). The locations of the last extant wild ferret populations near Mellette, County, SD and Meeteetse, WY are also shown. Finally, the locations of specific reintroduction efforts and/or established wild ferret populations are portrayed in their chronological order of implementation as follows: 1) a reintroduced and established wild population in Shirley Basin, WY (1991); 2) a reintroduction effort in Badlands National Park, SD (1994); 3) a reintroduction attempt at UL Bend NWR, MT (1994); 4) a well established wild population in Conata Basin, SD; 5) a reintroduced population in Aubrey Valley, AZ (1996); 6) a reintroduction effort on Fort Belknap Indian Reservation, MT (1997); 7) a reintroduced population at Coyote Basin, UT (1999); 8) a reintroduced and well established population at Cheyenne River Indian Reservation, SD (2000); 9) a reintroduction effort at Wolf Creek, CO (2001); 10) a reintroduction attempt on the BLM "40-Complex", MT (2001); 11) a reintroduced population in Janos, Chihuahua, Mexico (2001); 12) a reintroduced population on the Rosebud Indian Reservation, SD (2003); 13) a reintroduction effort on the Lower Brule Indian Reservation, SD (2006); 14) a new reintroduction effort at Wind Cave National Park, SD (2007); 15) a new reintroduction effort on the Espee Ranch, AZ (2007); 16) a new reintroduction site at Logan County, KS (2007); and, 17) a new reintroduction effort on the Northern Cheyenne Indian Reservation, MT (2008). (From: a revised draft of the Black-footed Ferret Recovery Plan, U.S. Fish and Wildlife Service, unpublished. Some reintroduction sites are doing well, others no longer support ferrets. About 900 ferrets, both kits and adults, were counted on these sites in 2007).

Figura 1. Probable ocupación histórica del turón de patas negras, definida según la combinación de las áreas de distribución de los perritos de las praderas de cola blanca, de cola negra y de Gunnison (área en gris). Las áreas donde existieron las últimas poblaciones de turones en el "Condado de Mellette", Dakota del Sur, y "Meteetsee" Wyoming, también se muestran en el mapa. Finalmente, las áreas de reintroducción y las zonas donde ya existen poblaciones de turones establecidas se muestran en el orden cronológico de los diferentes proyectos de reintroducción: 1) población reintroducida y establecida en "Shirley Basin" (1991); 2) proyecto de reintroducción en el Parque Nacional de "Badlands", Dakota del Sur (1994); 3) proyecto de reintroducción en "UL Bend NWR", Montana; 4) población reintroducida y exitosamente establecida en "Conata Basin", Montana; 5) población reintroducida en "Aubrey Valley", Arizona (1996); 6) proyecto de reintroducción en la "Reserva India de Fort Belknap", Montana (1997); 7) proyecto de reintroducción en "Coyote Basin", Utah (1999); 8) población reintroducida y exitosamente establecida en la "Reserva India del Río Cheyenne", Dakota del Sur (2000); 9) proyecto de reintroducción en "Wolf Creek", Colorado (2001); 10) proyecto de reintroducción en "BLM Complejo-40", Montana (2001); 11) población reintroducida en "Los Janos", Chihuahua, México (2001); 12) población reintroducida en la "Reserva India de Rosebud", Dakota del Sur (2003); 13) proyecto de reintroducción en la reserva india de "Lower Brule", Dakota del Sur (2007); 14) nuevo proyecto de reintroducción en el Parque Nacional de "Wind Cave", Dakota del Sur; 15) nuevo proyecto de reintroducción en el "Rancho Espee", Arizona (2007); 16) nuevo área de reintroducción en el "Condado de Logan", Kansas (2007); y, 17) nuevo proyecto de reintroducción en la reserva india de "North Cheyenne", Montana (2008). (Fuente: borrador revisado del Plan de Recuperación del Turón de Patas Negras, Servicio de Pesca y Vida Silvestre; no publicado. Algunas áreas de reintroducción van progresando bien, mientras que otras ya no necesitan recibir más turones. Aproximadamente 900 turones, incluyendo adultos y crías, fueron censados en conjunto en todas estas áreas de reintroducción en el año 2007).

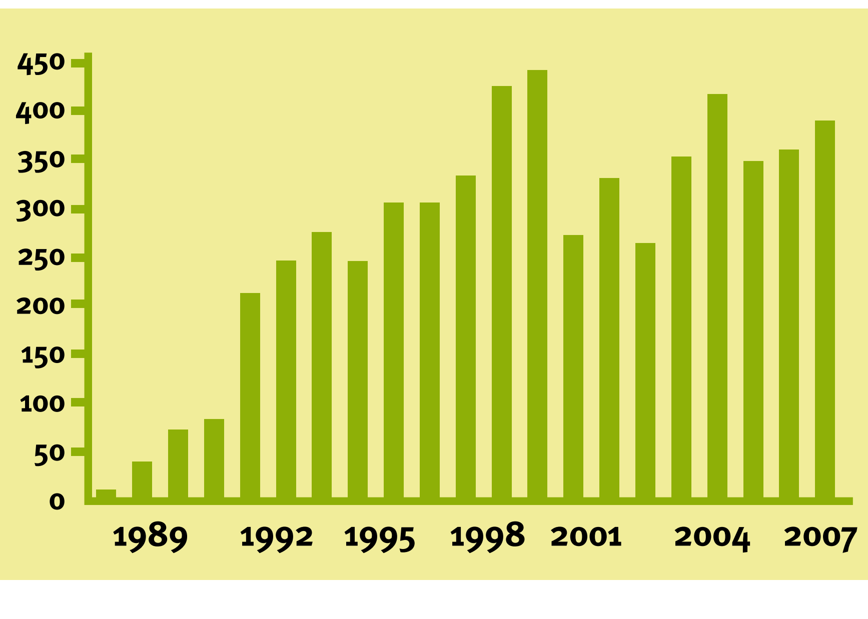

Figure 2. Births of Black-footed ferrets in state and federal breeding facilities and zoos, 1987 to 2007. (Counting ferrets born in a few field breeding programmes, over 6,000 ferrets have been born in captivity since 1987).

Figura 2. nacimientos de turones de patas negras en instalaciones de cría nacionales y en zoológicos entre 1987 y 2007. (Teniendo en cuenta turones nacidos en algunas instalaciones *in situ*, más de 6000 turones han nacido en cautividad entre 1987 y 2007).

species with seemingly inexhaustible habitat and prairie dog prey. That was, of course, before European settlement of North America and eventual conversion of much of the nation's native prairies (and prairie dog colonies) to cultivated lands, and decades of intensive, large scale prairie dog poisoning campaigns waged by western livestock producers over much of the 1900s. While diminished in scope, both factors continue to affect prairie dog populations and limit ferret recovery today! Prairie dogs are still widely regarded as agricultural vermin competitors with cattle for grazing and the cause of rangeland ruin (which is contrary to the evolutionary history of grassland ecosystems and the greater impacts of domestic herbivores, managed pasture systems and drought on range degradation).

The black-tailed prairie dog *(C. ludovicianus)*, the most numerous and widespread of all prairie dog species, suffered the greatest population impacts over the 20th century. The historical range of the black-tailed prairie dog was subjected to extensive habitat conversion and poisoning, resulting in an estimated species population reduction of between 90 and 95% (Forrest, 2005). Impacts to black-tailed prairie dogs were so great that in 2000, the species was designated a candidate for listing as threatened under the Endangered Species Act; a classification later withdrawn by the U.S. Fish and Wildlife Service (USFWS, 2004). Although, new data suggested that the black-tailed prairie dog was not threatened over its historical range, it nevertheless experienced enormous population declines and, with few exceptions, exists today in comparatively small and highly fragmented colonies, colonies largely unsuitable for ferrets.

In addition to threats imposed directly by humans, sylvatic plague (the rodent borne form of bubonic plague) was introduced to the West coast in the early 20th century (via shipboard rats) and has since spread through most western states. Plague has had locally devastating effects on prairie dogs and reintroduced ferrets and is perhaps the single greatest obstacle to ferret recovery today. Only portions of the eastern third of the black-tailed prairie dog range remain free from the effects of sylvatic plague and the entire ranges of the more westerly white-tailed prairie dog *(C. leucurus)* and Gunnison's prairie dog *(C. gunnisoni)* have been impacted.

Thanks to collaborative efforts of numerous state and federal agencies, Indian Tribes, conservation organizations and private partners (many represented on a Black-footed Ferret Recovery Implementation Team established in 1996), ferret recovery has indeed experienced enormous progress. Since ferrets were first successfully produced in captivity in 1987, some 6000 ferrets have been born in Black-footed Ferret Species Survival Plan facilities (six zoos and a Wyoming Game and Fish Department/Fish and Wildlife Service breeding center) and a few field breeding projects (Figure 2). And since 1991, about 2400 ferrets have been reintroduced into Wyoming; South Dakota (six sites), Montana (four sites), Arizona (two sites), two project areas overlaying adjoining portions of northeastern Utah and northwestern Colorado, Kansas and northern Chihuahua, Mexico (Figure 1).

Black-footed ferret reintroduction projects have achieved varying levels of success. The most successful, a reintroduction project covering portions of the Conata Basin district, Buffalo Gap National Grasslands (U.S. Forest Service), and adjoining habitats within Badlands National Park, South Dakota, support a moderately sized and productive wild ferret population. The Forest Service's Conata Basin portion of the recovery area has proven so successful that it is used as a donor site where excess wild-born kits are captured and translocated to start or augment other reintroduction projects. Conversely, several other reintroduction projects have struggled and have, thus far, failed to establish viable populations due to the effects of plague, drought and other habitat constraints.

Steady progress is being made to address biological limitations of wild ferret recovery at the field level. Over the years, a wealth of expertise and capabilities have been developed by programme partners, including some recent and promising results in development of a plague vaccine and other plague management applications. In essence, programme cooperators know how to produce substantial numbers of ferrets in captivity and what is needed to successfully establish wild populations. And it is well demonstrated that, given proper habitat conditions, reintroduced ferrets are capable of rapidly establishing viable, wild populations.

However, that's where the rub in recovery lies today. Presently, there are too few prairie dog complexes (aggregations of colonies distributed about 1.5 km or less from each other) across North America that are large enough, and with suitable densities, to readily support ferret populations. And worse, although there are millions of "unbroken" (uncultivated) hectares of prairie lands with potential to restore prairie dog complexes and ferret populations, and although there is an enormous base of federal public lands in the west that could be managed more proactively to advance recovery, there remains: 1) a continuing intolerance of prairie dogs by the agricultural community; 2) growing apprehension over sharing lands, especially private lands, with endangered species; 3) an inability to allocate and/or dedicate sufficient financial incentives necessary to develop effective partnerships to offset concerns over managing lands for ferrets and other sensitive prairie wildlife; 4) cumbersome, costly and time consuming administrative processes to establish recovery sites and reintroduction projects, and 5) a growing and troubling trend in devaluing our natural resource legacy in favor of socio-economic factors (especially under the 2000-2008 U.S. Administration).

Recovery events in 2004 exemplified the tenuous nature of ferret recovery; a year which saw high captive breeding output and encouraging progress at reintroduction sites in Arizona, Wyoming, and Cheyenne River Sioux Tribal lands, South Dakota. However, much of the West was also under the grip of an extended drought, and both prairie dogs and ferrets were impacted at reintroduction sites in Mexico, Colorado/Utah and Conata Basin/Badlands, South Dakota. The drought elevated conflicts over forage between cattle and prairie dogs in Mexico and South Dakota. And in South Dakota, the drought caused dispersal of prairie dogs from the highly successful Conata Basin area onto adjoining private lands. That dispersal touched off a fiery political debate and prompted a U.S. Forest Service review of management practices at Conata Basin in an attempt to provide better protections for neighboring ranchers who did not want prairie dogs on their lands.

The process of reducing prairie dog encroachment around Conata Basin included poisoning of prairie dogs both on adjoining ranches and within national grassland "buffer zones" adjacent to private lands, areas which also supported wild ferrets. Although it was unlikely that ferrets were killed from secondary poisoning (the Environmental Protection Agency approved rodenticide, 2%, zinc phosphate bait has limited secondary hazards to carnivores), the added loss of prey in an already drought stressed year undoubtedly increased impacts on ferrets. Moreover, the Forest Service subsequently amended its land use management plan to more assertively promote a "good neighbor" policy that included options for continued lethal control on Forest Service border lands; and more significantly, set the stage for a future plan amendment to control prairie dogs within the heart of the Conata Basin ferret recovery area itself.

The Conata Basin site supports the most successful reintroduced black-footed ferret population to date. Yet, this population is small and still vulnerable (only about 100 breeding adults). Without some ability to offset prairie dog habitat reductions on boundary areas of Conata Basin, and if further restrictions are imposed on natural habitat expansion and prairie dog populations within the core of Conata Basin itself, the full potential of this recovery site would be severely limited and could substantially setback overall ferret recovery prospects. And just recently, an active plaque episode was documented on the southern side of Conata Basin, the worst possible news and gravest management challenge to this increasingly threatened recovery area.

It remains to be seen what effects renewed prairie dog control and plague will ultimately have on the stability and long range potential of the Conata Basin ferret population. However, this issue goes directly to the heart of the most fundamental obstacle in ferret recovery, making room for ferrets and prioritizing long range management of adequate lands and prairie dog complexes to support viable populations of ferrets and other prairie dog associate wildlife species. The current Black-footed Ferret Recovery Plan includes a "downlisting" objective of 1500 breeding adults occupying 10 or more populations scattered over the historical range of the species. And although there have been many reintroduction attempts (Figure 1), the best estimate of the number of wild breeding adults in 2007 is only about 20% of the proposed downlisting objective.

Programme partners have learned that to establish a moderately sized and stable ferret population requires relatively large (in excess of 4050 hectares) and high quality (high density) complexes of prairie dog colonies (even larger complexes in less densely occupied white-tailed and Gunnison's prairie dog habitat). Complexes such as that now found at Conata Basin. From a historical perspective, comparatively huge prairie dog complexes were common throughout the plains of North America and colonies were much more contiguous in distribution. Some complexes occupying tens of thousands of hectares were documented at the turn of the 20th century. However, in our contemporary western landscape, few large complexes remain, especially within the range of the black-tailed prairie dog. And further west, where the largest remaining complexes of both white-tailed and Gunnison's prairie dogs are found, periodic epizootics of sylvatic plague hold back ferret recovery potential.

Moreover, what 2004 taught us is that our most basic measure of prairie dog/ferret habitat (total area of occupied prairie dog habitat), is itself a highly dynamic and somewhat ineffective gauge of habitat quality and stability. In response to extreme drought conditions and limited forage at Conata Basin, prairie dogs were forced to disperse over a larger area, and the area of occupied prairie dog colonies swelled from about 6880 hectares to over 9300 hectares (Livieri and Perry, 2005). Yet, both prairie dog and ferret densities were stretched thin and reproduction of both suffered (Livieri and Perry, 2005). Consequently, prairie dogs, which unlike cattle are not constrained by fences and can more effectively spread foraging activity over drought-impacted lands, again became the focus of a long-standing debate and a convenient scapegoat for poor range conditions: impacts more reasonably attributed to the effects of cattle grazing and the drought itself. Habitat conditions substantially improved in 2005 with increased rainfall, yet the battle over prairie dogs and ferret recovery in Conata Basin goes on and significant threats to the recovery programme's premier reintroduction site persist.

Enormous resources have been vested in ferret recovery over the past two decades and with great success. And many programme partners believe we possess both the expertise and technical ability to fully recover this species. Moreover, compared to the vastness of lands potentially available for ferret recovery, the amount of habitat actually needed to meet population objectives represents a tiny, tiny fraction of the western landscape. In an upcoming revision of the Black-footed Ferret Recovery Plan, a delisting target of 3000 breeding adults is being considered. On average, adult ferrets occupy some 80 hectares of habitat. Consequently, only about 243,000 hectares of stable prairie dog habitat across the western states would be needed to meet such a recovery objective. Put in perspective, that is about one percent of the estimated 153 million hectares of "potential" prairie dog habitat that remains in the U.S. (Ernst, Clark and Gober, 2006).

The Forest Service's National Grassland units alone support more than 1.4 million hectares of public lands from Montana and North Dakota, south to Texas and New Mexico. The Forest Service manages more plague-free, black-tailed prairie dog habitat than any other federal agency. And yet, less than 1% of the National Grassland land base is currently involved in an active ferret recovery effort: Conata Basin, which as noted above is under considerable threat. Perhaps no other agency has more ability (and hence responsibility) to foster ferret recovery in the wild today than the Forest Service. But instead of moving recovery efforts forward, as outlined in an original 2002 Forest Plan revision for the entire northern Great Plains, the Forest Service has apparently succumbed to local political will and has largely reversed some favorable prairie dog and ferret management decisions contained in that plan. Indeed, in a pending Land Use Management Amendment for grassland units in Nebraska and South Dakota, covering over 283000 hectares of public land, the Forest Service could artificially cap the amount of occupied prairie dog habitat to only 3%. The multiple-use concept of federal management and associated commitment for the conservation of declining and endangered prairie species appears grossly out of balance on Forest Service lands today.

It is important to understand that ferret presence does not foreclose other land uses. Ferrets are not sensitive to human activities and disturbance, and as long as base habitat conditions are maintained (i.e. sufficient prairie dog numbers), ferret populations can exist in harmony with livestock grazing, oil and gas development, hunting, other recreation, etc. Grazing is in fact of integral importance in managing prairie wildlife communities, including prairie dogs and ferrets. However, ferret recovery cannot be achieved by attempts to force-fit the species into small parcels of habitat grudgingly conceded as acceptable to other land use interest groups. Nor can we realistically recover the ferret, if the islands of prescribed recovery habitat are themselves compromised whenever drought or other factors overwhelm our willingness to maintain priority management for prairie dogs over other forms of temporal competition (i.e., grazing). For example, after chronic plague episodes compromised a ferret reintroduction effort in north central Montana, the U.S. Bureau of Land Management overturned the management of habitats set aside for prairie dogs and ferrets. Recreational shooting of prairie dogs was again allowed in those areas even as prairie dog populations struggle to recover. Without long range commitments to prairie dog conservation and expanded efforts to block-up lands for even larger prairie dog complexes, regardless of their present status, the eventual recovery of the ferret becomes increasingly problematic.

And so, given current world events and societal pressures, just how much of a national priority is the recovery of species like the black-footed ferret? To many partners directly involved in ferret recovery, we not only see the value of restoring and safeguarding this beautiful, interesting and highly specialized carnivore itself, but the biological imperative of maintaining a good distribution of intact prairie/steppe ecosystems, lands to sustain natural biodiversity and populations of prairie dogs, ferrets, swift foxes, ferruginous hawks, burrowing owls, mountain plovers and a wide array of other wildlife species dependant on declining native grasslands. This is, after all, one of the fundamental tenets of the Endangered Species Act itself.

And what lessons have we really learned over the past 22 years since the last wild ferrets from Meeteetse, Wyoming were taken into captivity? The black-footed ferret can indeed be fully recovered. But it will require a recommitment of principles and resources to secure habitats, and the cooperation and support of those who would share lands with ferrets. To build on the notable successes of the ferret programme, the USFWS and its many involved partners must reexamine and amend basic administrative and management approaches to recovery. They need to become more proactive, responsive, and flexible in development of reintroduction projects while eliminating the fear of impacts on adjoining, non participating land owners. Although substantial progress has been made in this area over the past two years, political challenges remain. A series of National Wildlife Refuges are needed to conserve substantial segments of highly diverse and threatened prairie ecosystems in support of endangered and declining grassland species. Vast USFWS refuge holdings (over 38 million hectares) have been established for the protection of wetland habitats and associated wildlife values, game species and the recovery of certain endangered species. However, no National Wildlife Refuge lands exist today that could potentially support large prairie dog complexes. Also, ferret recovery should be spread over all states within the historical range of the species, recovery efforts should be focused on federal public lands (wherever possible); smaller "nursery" recovery sites (habitats perhaps too small to support self-sustaining ferret populations, but may persist with periodic augmentation) should be developed in plague-free areas, and more effective assistance/incentive programmes are needed to promote management of larger prairie dog complexes on both private and Indian Tribal lands.

Do we have that kind of resolve and can we overcome the prevailing sentiments of "not in my backyard"? Are we willing to actually dedicate the resources necessary to fulfill mandated ferret recovery responsibilities? Unfortunately, the answer to these questions at the present time, and especially under the direction and management of the 2000-2008 Administration, appears to be "no". And so, the roller coaster ride continues and as a nation the U.S. is up against the very socio-economic and political issues that caused the original decline of the black-footed ferret, factors which will ultimately determine the fate of this and other declining and endangered species, and will test our country's will to preserve our native wildlife heritage. It would be an unforgivable indictment for such a progressive and value-based society as ours to squander the significant recovery gains made to date and let the black-footed ferret slip back into oblivion or be denied a secure, wild future across the western plains of the U.S., Canada and Mexico (Figure 3).

Finally, what are the major lessons from ferret recovery efforts that can be applied to other imperiled species, such as Iberian lynx *(Lynx pardinus)*? First, for species reduced to such critically low numbers that complex captive breeding programmes are required, much of the available recovery resources and attention are necessarily focused on captive breeding itself. However, such concentrated effort often comes at the expense of important, long range work needed to restore native habitats and/or prey necessary for eventual wild recovery. In tragically backwards rationale, U.S. politicians and federal land managers over the past few years have used the success of the ferret captive breeding programme as justification to postpone or avoid essential land use decisions needed to promote ferret habitat enhancement. Prevention of extinction is not species recovery and the restoration of stable wild populations and biodiversity requires much greater, long-standing commitments to reduce those factors that cause and/or sustain species decline, and for development of adequate reintroduction refugia. Moreover, the ability to restore viable populations of locally extirpated species becomes increasingly difficult as native habitats are progressively compromised by other, often incompatible, land use practices. Concomitant decisions for both captive breeding and aggressive habitat/prey conservation programmes are therefore essential in the earliest possible stages of recovery project development.

Second, the conservation and management of endangered species is often fraught with controversy and disagreements between a variety of government, academic, and private advocates and adversaries. Most involved parties would agree that the early stages of development of a ferret recovery programme were heavily encumbered by individual disputes and agency parochialism. It is extremely important then to develop a collaborative and

Photo: Mike Lockhart

Figure 3. Biologist Jesus Pacheco, University of Mexico, releases as captive reared and preconditioned black-footed ferret at a reintroduction site in northern Chihuahua, Mexico.

Figura 3. El biólogo Jesús Pacheco, de la Universidad de México, suelta un turón de patas negras criado en cautividad y "entrenado" para su reintroducción, en un espacio de reintroducción en el norte de Chihuahua, México.

transparent management framework that has the support of the highest levels of national, state and provincial governments. Although the USFWS has ultimate authority in the U.S. for decisions related to endangered species recovery, management advice and direction for ferret recovery is largely provided by a Black-footed Ferret Recovery Implementation Team (BFFRIT). The BFFRIT is comprised of 27 involved state and federal agencies, Indian Tribes and conservation organizations, and is further divided into four subcommittees with responsibilities for policy direction, field conservation, captive breeding, and education and outreach. Although overall recovery efforts can be, and are, affected by political influences (especially under the constraints of the 2000-2008 U.S. Administration), the BFFRIT provides guidance on all elements of ferret recovery and has been instrumental in reducing intra-programme conflict. In administering the BFFRIT, the USFWS encourages broad and innovative approaches to implementation of captive breeding and reintroduction projects, solicits partner views on proposed research and ferret allocations (for reintroduction and research purposes) and fully responds to all comment and input provided by BFFRIT members in making final decisions. The development of such an open and collaborative management process resulted in correspondingly rapid progress toward ferret recovery and continues to be an effective vehicle for promoting the conservation of prairie habitats for ferrets and a host of other declining native grassland species.

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Losers live in the past. Winners learn from the past and enjoy working in the present toward the future.

Denis Waitley

Planning of veterinary supervision for translocation programmes of wild felids

Planificación de la supervisión veterinaria en programas de translocación de felinos silvestres

Marie-Pierre Ryser-Degiorgis

Resumen

Los riesgos de enfermedad en los proyectos de translocación y reintroducción se pueden resumir principalmente en dos tipos de escenarios, que en ambos casos afectan a los animales a translocar, a otros animales silvestres, al ganado doméstico, a mascotas y a seres humanos. Estos dos escenarios son: 1) la introducción por los animales reubicados de un patógeno en el entorno de destino y 2) la transmisión de un patógeno que sea nuevo para los animales a reubicar durante el proceso de translocación o procedente del entorno de destino. Para cuantificar y minimizar los riesgos sanitarios que son inherentes a la translocación de animales se necesitan medidas veterinarias apropiadas. Las evaluaciones veterinarias deben realizarse tanto a nivel individual como a nivel del ecosistema. La evaluación de los riesgos de enfermedad o para la salud consisten en la aplicación del sentido común para valorar si la translocación de los animales pueda implicar riesgos significativos relacionados con la salud, a sabiendas de que es imposible trabajar sin riesgo alguno. Para identificar los posibles riesgos para la salud hay que tener en cuenta tres aspectos: 1) la susceptibilidad a la enfermedad y posible papel portador de la especie a reubicar; 2) la presencia de patógenos y otros problemas potenciales para la salud en el entorno de origen, y 3) la presencia de patógenos (o de compuestos tóxicos) en el entorno receptor.

La documentación científica correcta es casi tan importante como la evaluación de riesgos para la salud. Cada uno de los ejemplares, tanto vivos como muertos, es una fuente valiosa de información para el presente y para el futuro. Por consiguiente, se debe poner mucho énfasis en el muestreo amplio y en la recopilación de información.

Las decisiones acerca de si hay que proceder o no con las traslocaciones de animales silvestres pueden estar determinadas por los resultados de la evaluación de riesgos para la salud, los recursos, los aspectos logísticos, sociopolíticos y por cuestiones de conservación. Teniendo en cuenta que los recursos suelen ser limitados, es necesario establecer prioridades. Además, es imprescindible determinar cuáles son los resultados necesarios para decidir si un animal puede ser reubicado o no. Una vez finalizada la evaluación de riesgos para la salud y determinadas las prioridades, se pueden proponer los protocolos correspondientes. Es necesario un enfoque multidisciplinario durante las fases de planificación e implantación. Asimismo, es imprescindible que todo proyecto sea considerado como un proceso flexible: hay que intentar reducir al mínimo las pérdidas y los problemas, pero en caso de que ocurriesen, hay que aprender de ellos y adaptar los procedimientos debidamente.

Palabras clave
Evaluación de riesgos para la salud, enfermedad, documentación, patógeno, prioridades, protocolos, reubicación

Abstract
Appropriate veterinary measures are required to quantify and minimise the health risks that are inherent in the translocation of animals. Veterinary considerations should be addressed both at the individual level and at the ecosystem level. Disease risks in translocation and reintroduction projects can be basically summarized in two main scenarios, both involving the animals being translocated, other wildlife, domestic livestock, pets, and humans: 1) introduction of a pathogen into the destination environment by the animals being translocated and 2) transmission of a pathogen that is new to the animals being translocated during the translocation process or from the destination environment. Disease or health risk assessment (HRA) is the application of common sense to evaluate whether or not important health-related risks are associated to the translocation of animals, acknowledging that it is impossible to work without any risk. To identify potentially associated health hazards, three points need to be considered: 1) disease susceptibility and potential carrier role of the species to be translocated; 2) presence of pathogens and other potential health problems in the source environment, and 3) presence of pathogens and other potential health problems (including toxic compounds) in the destination environment.
Adequate scientific documentation is almost as important as HRA. Every single individual, alive or dead, is a valuable source of information, for the present and for the future. Thus, emphasis should always be placed on extensive sampling and information collection. Decisions on whether or not to proceed with wild animal translocations may be determined by the results of HRA, resources, sociopolitical aspects, logistics and conservation issues. In this regard, and since resources are usually limited, priorities have to be set. Amongst others, it is essential to establish clear criteria to decide what are the minimum standards to render an individual acceptable for translocation –i.e., what are the key agents to be tested for– before deciding whether an animal can be translocated or not. As a next step, suitable protocols can be proposed. A multidisciplinary approach is required both during the planning and the implementation stages. Also, it is essential to consider these projects as adaptive processes, i.e., to learn from results and adjust the methodology accordingly.

Keywords
Health risk assessment, disease, documentation, pathogen, priorities, protocols, translocation

Planning of veterinary supervision for translocation programmes of wild felids

Marie-Pierre Ryser-Degiorgis

Introduction

Translocations are defined as the intentional movement of animals and their release into the wild in an attempt to reintroduce a species in an area which was once part of its historical range, to supplement an existing low density native population or to introduce a species outside of its recorded distribution (IUCN, 1998). Translocations are thus an important tool in wildlife conservation and management (Figure 1).

The success of translocation projects depends on several factors. Besides non biological and biological considerations, such as local support together with the choice of a suitable habitat with sufficient and appropriate food resources in the release area, health aspects play a crucial role in wildlife translocation and reintroduction strategies. Indeed, the veterinary implications of projects involving the movement of wild animals, even over relatively short distances, are sometimes unexpected (Woodford and Kock, 1991).

Appropriate veterinary measures are required to quantify and minimise the health risks pertaining to the translocations of animals, and to ensure the health and wellbeing of released stock throughout the length of the Programme (IUCN/SSC, 1998; Woodford and Rossiter, 1993; Kock et al., 2007). Disease risks in a translocation project can be basically summarized in two main scenarios: 1) Introduction of a pathogen into the destination environment by the animals being translocated. Relocation of wild animals never consists of the movement

Photo: Andreas Ryser

Figure 1. Release of a translocated Eurasian lynx.

Figura 1. Suelta de un lince eurasiático en un proyecto de translocación.

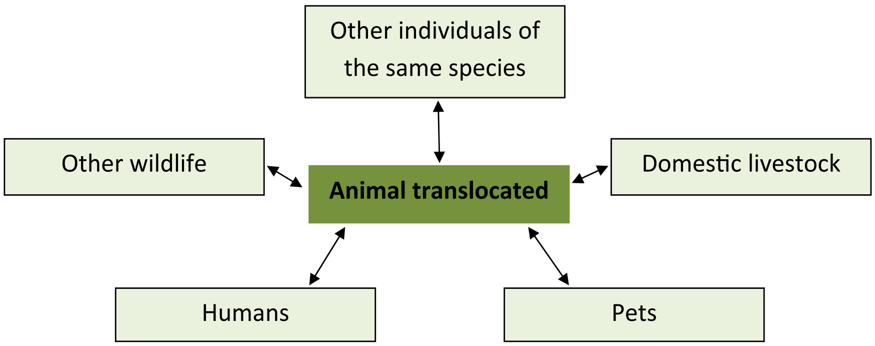

Figure 2. Intra- and interspecific interactions to be considered for health risk assessment.

Figura 2. Interacciones intra e interespecíficas que deben tenerse en cuenta en una evaluación de riesgos sanitarios.

of a single species, rather, it always entails relocation of a "biological package" consisting of the animal itself and its passenger organisms such as parasites, viruses, bacteria and fungi (Davidson and Nettles, 1992). 2) Transmission of a pathogen new to the animals being translocated during the translocation process or from the destination environment. In both cases, pathogen transmission has to be considered between the animals being translocated and other wildlife, domestic livestock, pets and humans (Figure 2). All of them can be healthy carriers and/or potential victims of pathogens. Thus, for a health risk assessment, it is necessary not only to consider diseases affecting the species of concern, but also the infectious agents that the translocated animals could potentially pass on to other animal populations or even to humans.

Veterinary considerations should be addressed both at the individual and at the ecosystem level. In the first case, the aim is to maximize the survival of each single individual to be translocated, which is essential for the success of the project. Safe and effective anaesthesia, stress management, wound treatment, and individual health screening are crucial at this level. Clinically healthy animals are not necessarily pathogen-free (Cunningham, 1996) and thus, capture, transport and change of environment –which are always a source of enormous stress– may affect animals in various ways; e.g., in predisposing them for the development of a disease (Woodford, 2001; Teixeira et al., 2007). Furthermore, animals can get wounded during capture or transport and may require medical treatment. And last but not least, animal welfare considerations have to be taken into account (IUCN, 1998; Woodford, 2001). At the ecosystem level, the aim is the prevention of the movement of pathogens through the movement of animals, since the movement of pathogens to new environments may have important effects on wildlife, agriculture or public health, and may affect the translocation effort itself (Leighton, 2002). This implies detailed disease risk assessment in both the source and destination environments, as well as individual health screening procedures.

A thorough planning of the veterinary supervision is a necessity. However, not only health concerns, but also further factors such as biological, political and scientific considerations will influence the veterinary protocols (Figure 3; Jiménez, this book). A multidisciplinary approach is therefore required both during the planning and the implementation stages (Woodford & Kock, 1991). All involved staff must be consulted for decision making, and potentially critical situations that may occur during the project have to be discussed prior to taking action.

Health risk assessment

Disease or health risk assessment is the rigorous application of common sense to evaluate whether or not important health-related risks are associated with a proposed activity, such as the translocation of animals (Leighton, 2002). Health risk assessment requires a detailed translocation plan, and the subsequent identification of associated health hazards, both in the source and destination ecosystems. For this purpose, a comprehensive list of potential health hazards has to be provided. This includes three important steps (Figure 3).

Disease susceptibility and eventual carrier role of the species to be translocated

All existing information on the species needs to be gathered, including published and unpublished reports or personal observations. Known causes of morbidity and mortality, potential sources of infection, performed

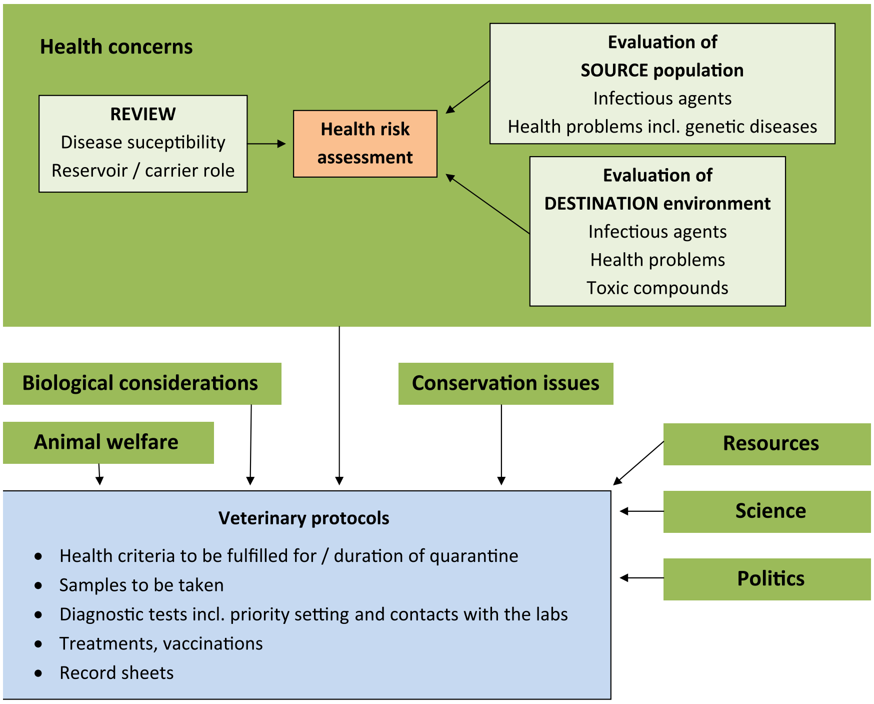

Figure 3. Factors to be considered for the planning of the veterinary supervision of a reintroduction/translocation project and when proposing protocols.

Figura 3. Factores a considerar durante la planificación para la supervisión veterinaria de proyectos de reintroducción/translocación, así como durante la propuesta de protocolos.

serological surveys, epidemiological relationships with other species, persistence of relevant infectious agents in the environment, their pathogenicity and routes of transmission are all important information to be considered. The compiled data must be organized in a database and presented in a detailed written review. In this way, the knowledge on the species at the time of project planning is well documented and available for all concerned participants, facilitating discussions regarding veterinary procedures.
In this context, it should be noted that if no diseases are recorded for the species in question, it does not mean that this species is not susceptible to diseases (Cunningham, 1996).

Presence of pathogens and other potential health problems in the source environment.
Knowledge regarding the health status of the source population(s) (captive or free-living) is essential to evaluate the risk of "pathogen translocation" and of "translocation" of non infectious health problems such as genetic defects. Therefore, data need to be collected on the infectious agents and diseases present in the source ecosystem, i.e., all available information concerning causes of death, infectious and non-infectious diseases, and carrier status of the concerned population(s) –post-mortem investigations, epidemiological surveys, etc.– must be compiled and taken into account during the planning process.

Presence of pathogens in the destination environment
The presence of pathogens that could entail a risk for animals being translocated (according to the abovementioned review) has to be assessed in the release area. Such pathogens might be present in other wildlife species, or in domestic stock and pets that live within the surrounding area. Furthermore, the presence of infectious agents that may be introduced by translocated animals has to be investigated, both in the species of concern (in case of re-stocking) and in the associated fauna. In this second scenario, pathogens that are already present in the area do not necessarily need to be considered, while those that are absent would require more attention. In addition, it is important to assess the presence of toxic substances in the environment (e.g. pollutants, poison distributed for control of pest species, etc.) that could affect the animals targeted for translocation.

The pathogenic potential of infectious organisms can basically be classified into three categories: 1) pathogenic, for those known to produce disease; 2) non-pathogenic, for those studied well enough to determine that they never produce illness, and 3) unknown, for those where there is insufficient information to evaluate their pathogenicity. However, risk assessments are not absolutely predictable, especially since biological factors in the release areas might favour "exotic" pathogens normally considered harmless, thereby producing unpredictable disease syndromes (Davidson and Nettles, 1992).

Since an assessment of the health risk of each identified hazard is almost never feasible, it is necessary to select a small number of hazards that appear to have the greatest potential to pose important health risks. Risk must be then completely and rigorously estimated for each selected health hazard, i.e., the probability that the hazardous event will occur and the magnitude of the consequences that may result if such event does occur (Leighton, 2002). It is important to note that it is impossible to work without taking any risks. The aim is to minimize them as far as possible, but one has to keep in mind that a certain risk will always remain.

Scientific documentation and sample archives

Disease risk assessment is of central importance in the veterinary supervision of translocation projects. Yet, especially when dealing with an endangered species, adequate scientific documentation of the project implementation –including individual veterinary records– is crucial. Conservation projects imply a significant amount of manpower and financial investment. Every single individual, alive or dead, is a truly valuable source of information, for the present and for the future. Thus, even if disease risk assessment reveals that there are no diseases of concern in the frame of the translocation project, emphasis should still be placed on extensive sampling and information collection.

On the one hand, all procedures, results from physical exams, complementary diagnostic tests and laboratory analysis need to be recorded in detail. Data on pathogens that are apparently not influencing the health status of the animals should, as far as possible, also be collected in order to learn about the species and about the pathogens.

On the other hand, biological samples should be stored for eventual retrospective studies. For example, an apparently emerging pathogen that was not considered at the time of translocation might be detected in the release area several years after translocation. If appropriate samples of the translocated animals have been stored, it will be possible to use them for a retrospective analysis in order to determine whether the translocated animals were already infected with this apparently new pathogen at the time of translocation.

Priority setting: decision-making vs. documentation

Within the frame of any project, priority-setting is key, since human and financial resources are often limited. This has to be done for every single aspect of the project, including the health criteria for the selection of animals for the translocation programme, the length of the quarantine period, and the individual health screening protocols (Figure 3). Decisions whether or not to proceed with wild animal translocations may be determined by the results of health risk analysis, but they also may be influenced by a variety of other factors such as political and/or conservation issues. Health risk analysis informs decision makers regarding potential health risks and provides them with options to reduce risk if it is decided to proceed with the translocation (Leighton, 2002). For example, in case of a highly endangered species, it might be very difficult to capture animals for translocation that are free of any pathogen that could represent a risk for another, widely distributed species in the destination environment. If this risk appears to be rather low, the conservation goals of animal translocation might be of higher priority than the avoidance of introducing pathogens into the destination environment.

Furthermore, it is fundamental to differentiate between optimal and minimal –or essential– requirements. It is always interesting to perform testing for numerous infectious agents in all animals. However, it is necessary to establish clear criteria to decide which are the minimum standards to render an individual acceptable for translocation. The selected key agents should have first priority, and the tests should be done as soon as possible. All other infectious agents will have second priority, i.e., the needed samples will be taken, but only analyzed according to the laboratory capacity and the financial resources. No simple guidelines can cover all situations. Each species, each geographical area, each project is a case in itself and must be evaluated separately, taking into account all biological, ecological, geographical and epidemiological circumstances (Woodford and Rossiter, 1993).

Table 1. Check list for veterinary procedures in the frame of translocations projects.

Tabla 1. Lista de comprobación de los procedimientos veterinarios en el marco de los proyectos de translocación.

• Disease risk assessment	
• Health criteria for inclusion into the translocation programme	
• Options if the health criteria are not fulfilled*	
• Considerations of the legal and veterinary restrictions on translocation of wildlife	
• List of duties of the field veterinarian(s)	
• List of material:	
	Immobilization
	Physical exam
	Pharmacy
	Record sheets (physical exams, anaesthesia, quarantine, transport, post-mortem)
	Individual record sheets and boxes for sample shipment
• Telephone list:	
	Project veterinarians
	Laboratories
	Animal hospital
	Quarantine station
	Field biologists
	Project leader
	Responsible authorities
• Sampling:	
	Required samples (e.g. blood, faeces, swabs)
	Sampling methods
	Collaboration with laboratories
	Priority list for analysis
	Preservation
	Shipment
• Capture (in the wild or in captivity):	
	Anaesthesia protocol
	Health screening (physical exam, sampling)
	Complementary diagnostic tests (radiography, ultrasound, etc.)
	Treatment (e.g. against parasites), vaccination
	Identification
• Transport (from capture site to quarantine station, and from quarantine station to release site):	
	Design of transport boxes
	Vehicles
• Heating/cooling of vehicle according to the temperature:	
	Anaesthesia / tranquillization
	Animal monitoring
	Paperwork (e.g. official health certificates, transport documentation)
• Quarantine:	
	Duration**
	Location
	Isolation of animals (other species, humans)
	Design of facilities (size of enclosures, structure)
	Diet (type of food, sources, frequency)
	Hygiene (incl. disinfection after release)
	Observation (e.g. camera surveillance)
	Use of tranquillizers
	Medical records
• Release:	
	Procedure for re-captures in the enclosure
	Pre-release physical exam
	Sampling
	Treatments, re-vaccination
• Post-release monitoring:	
	Health screening and sampling protocol for captures
	post-mortem protocol

* Release in the source area, treatment, euthanasia, collaboration with a veterinary clinic or a rehabilitation center.
** Minimal requirements, considering both the goals of the quarantine and the fact that it is a stressful situation for animals coming from the wild.

* Suelta en el area de origen, tratamiento, eutanasia, colaboración con clínicas veterinarias o centros de rescate de fauna.
** Requerimientos mínimos, consi–derando tanto los objetivos de la cuarentena como el hecho de que es una situación estresante para los animales que provienen de la naturaleza.

Photo: Andreas Ryser

Figure 4. Examination under field conditions of a Eurasian lynx caught for translocation. At first, vital signs are recorded by the veterinarian of the capture team.

Figura 4. Examen en condiciones de campo de un lince euroasiático capturado para su translocación. En primera instancia, el veterinario del equipo de captura debe registrar las constantes vitales del animal.

Protocol proposals

Once a health risk assessment has been performed and priorities have been set, suitable protocols can be proposed. Reduction of health risks identified in the risk assessment may be achieved by changing some of the translocation procedures, such as choice of source and destination environments, methods of capture, transportation, quarantine and release of animals, and veterinary procedures such as immobilization protocol, disease testing, therapeutic treatments and vaccinations (Leighton, 2002).

Clear procedures are essential to avoid confusion, destructive emotions and stress situations in case of problems. Procedures should therefore be proposed in advance for each potential worst-case scenario. Furthermore, the project has to be considered as an adaptive process: protocols should be regularly re-evaluated in order to improve them if necessary (see Shenk, 2001). Overall, veterinary planning must take into account a number of points, which are summarized in Table 1. A comprehensive checklist for health risk analysis and protocol development is provided by the Office International des Epizooties and the Canadian Cooperative Wildlife Health Centre (Anonymous).

The following aspects are of particular concern in wild cats. At physical examinations (Figure 4), particular attention should be given to claws, footpads, teeth, and gums. Split claws and abraded pads are hard to observe in conscious animals. Dental problems, such as fractured teeth with pulp exposure, are common problems in captive non-domestic cats and severe cases could be life-threatening due to a potentially secondary systemic disease (Roelke et al., 1991; Blomqvist et al., 1999; Ryser-Degiorgis et al., 2002; Ryser-Degiorgis, this book). Once in quarantine, newly arrived individuals might refuse to eat as a result of the stress caused by the changes in their environment (Roelke et al., 1991; Blomqvist et al., 1999; Ryser-Degiorgis et al., 2002). Freshly killed, whole animals with the abdominal cavity opened can provide an effective feeding stimulus to some cats (Blomqvist et al., 1999). Although a minimal duration of 30 days is generally recommended for the quarantine of non domestic cats (Blomqvist et al., 1999, Woodford, 2001), animal husbandry issues (e.g., stress in captivity) may require that the quarantine period be shortened (Woodford, 2001; Ryser-Degiorgis, unpubl. obs.). There are thorough overviews of common pathogens to be included in the health screening protocols of non domestic cats, and recommended vaccinations and anti-parasitic treatments (Blomqvist et al., 1999; Woodford 2001). Further general information on diseases of non-domestic felids is reported by Terio et al. (this book) and Munson et al. (in press). For transport, animal welfare is a prime consideration. It is not necessary to provide food and water on short journeys (Blomqvist et al., 1999). Crates and boxes must be large enough to meet the relevant regulations and strong enough to contain the species concerned. Recommendations for the design of transport boxes for wild cats are presented in Blomqvist et al. (1999). A crated felid should be left in quiet, dimly lit surroundings and the attention of curious bystanders kept to a minimum (Blomqvist et al., 1999; Ryser-Degiorgis, 2002). Excited cats overheat very easily in confined spaces.

Post-release veterinary monitoring

For a complete, long-term evaluation of the health situation in particular and of the success of the translocation project in general, veterinary data must also be recorded *after* release (health screening at captures, reproductive success, causes of mortality, archiving of samples; e.g., Wild et al., 1999; Shenk, 2001; Wild et al., 2006). Losses of single individuals in small populations can have a significant impact on future population characteristics. Furthermore, even if epidemics are considered improbable, they still can play an important role in the long-term viability of a population (Ballou, 1993). Valuable information can be gained from blood samples, faecal samples, and post-mortem material (Blomqvist et al., 1999).

Figure 5. Eurasian lynx in a quarantine enclosure. Small, healed superficial wounds that resulted from the capture in a box trap are visible on the forehead and nose of the animal.

Figura 5. Lince euroasiático en instalaciones de cuarentena. El animal presenta pequeñas heridas superficiales en la frente y la nariz, que resultaron del proceso de captura en jaula trampa.

Learning from experiences

Many reintroduction projects have been poorly documented (von Arx et al., this book). In order to learn from experiences and to allow for a long-term approach to conservation, documentation is essential (Breitenmoser et al., 2001). Hence, all recorded data should be maintained in a database and presented in written reports, ideally in form of internationally available publications. Information such as anaesthesia protocols that are considered safe and efficient, reference data for the species (e.g. haematology, biochemistry), evaluation of capture methods (stress, injuries; Figure 5), observed infectious agents (serology, PCR, parasitology), diseases, behavior in quarantine (including problems in the enclosures), and encountered difficulties throughout the process, represent important information to be recorded and shared.

Acknowledgements

Many thanks to Astrid Vargas, Fernando Martínez and Andreas Ryser for valuable comments on the early version of the manuscript.

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Photo: Alexander Sliwa

Seek simplicity – and, having found it, suspect it.
James Conant

Photo: Ignacio Jiménez

Non-biological aspects to be considered in endangered species recovery programmes

Aspectos no biológicos en programas de recuperación de especies amenazadas

Ignacio Jiménez

Resumen

El propósito de este capítulo es resaltar la importancia de los factores de tipo no biológico en cualquier programa de conservación de especies amenazadas, con énfasis en aquéllos que tienen un componente *ex situ*. A la hora de manejar este tipo de programas, se debe tener en cuenta que la supervivencia final de la población que tratamos de conservar se ve últimamente influenciada por cómo la sociedad en general percibe y prioriza el problema de su conservación y por cómo nos organizamos los profesionales de la conservación para evitar la extinción de una especie.

Cualquier programa de recuperación que tenga cierta relevancia pública involucra a un conjunto numeroso y diverso de actores con identidades, perspectivas, demandas y recursos claramente diferenciados. Resulta crucial tener una visión lo más completa posible de este contexto social para gestionarlo eficientemente, evitando bloqueos interinstitucionales y conflictos destructivos. Aunque el conflicto tiende a ser visto como un proceso negativo, si se maneja adecuadamente, puede convertirse en una fuerza creativa de mejora constante de un programa. Para lograr esto, se propone: 1) incentivar el desarrollo de relaciones colaborativas frente a otras más competitivas entre proyectos e instituciones; 2) incluir a profesionales en gestión de conflictos dentro de los programas de conservación, y 3) fomentar el amplio reparto de recursos no distributivos. Igualmente, se destaca la importancia que los aspectos organizativos pueden tener sobre la recuperación de una especie amenazada. En este sentido, se propone: 1) desarrollar procesos de planificación colaborativa; 2) incentivar la creación de equipos de trabajo efectivos adecuadamente liderados y con capacidad de trabajo sobre el terreno; 3) evitar que las estructuras de control institucional detengan el desempeño de acciones concretas y necesarias sobre el terreno, y 4) identificar y minimizar procesos de desplazamiento de objetivos. Finalmente, se hace una llamada a la múltiple y frecuente evaluación de los programas de recuperación de especies amenazadas para asegurar su mejora constante y sistemática.

Palabras clave

Contexto social, gestión de conflictos, aspectos organizativos, desplazamiento de objetivos, equipos de conservación, evaluación de programas

Abstract

This chapter seeks to highlight the key influence of non-biological issues in any recovery programme, with an emphasis on *ex situ* conservation. When managing a recovery programme, we need to be aware that the final survival of any population of concern will be ultimately determined by how society perceives and prioritises its conservation, and by how professional conservationists organize themselves to avoid its extinction. Any conservation programme of public relevance involves a complex arrangement of stakeholders with distinct identities, perspectives, demands and resources. It is key to achieve a clear picture of this social context in order to manage it effectively, and to avoid inter-institutional gridlocks and destructive conflict. Even though conflict tends to be perceived as a negative process, when properly managed it can become a creative force, encouraging programme improvement. To achieve this, I propose to: 1) include professionals with experience in conflict management within conservation programmes; 2) promote the establishment of collaborative instead of competitive relationships between projects; and 3) encourage the open exchange of non-distributive values. Organizational issues can exert a significant influence on species recovery. In this regard, I propose to: 1) develop collaborative planning processes; 2) establish on the ground teams with effective leadership and strong capabilities; 3) avoid institutional arrangements focused on process control, which end up hindering actual implementation of necessary actions, and 4) identify and defuse goal displacement processes. There is a final call for multiple and regular evaluation of recovery programmes to promote constant and systematic improvement.

Keywords

Social context, conflict management, organizational issues, goal displacement, conservation teams, programme evaluation

Non-biological aspects to be considered in endangered species recovery programmes

Ignacio Jiménez

Integrated approaches for *ex situ* conservation?

Most readers would not be surprised when hearing that endangered species recovery is a complex challenge consisting of biological, ecological and health issues, compounded by social, organizational, political, economical or even psychological factors. This has been said in enough places and occasions to astonish any seasoned conservationist. What is most surprising is the wide gap that still remains between accepting the previous proposition and really bringing it into our daily practice. We repeat this and similar phrases, nodding sympathetically when someone proclaims these kind of statements in each congress or professional meeting and, afterwards, it seems like we almost forget about it. It sounds like a concept that it is always nice to say, but we don't really need to turn it into actual actions.

One might even find a certain contradiction in the title of this book, which calls for an interdisciplinary approach to *ex situ* conservation and then invests most of its space discussing biological aspects focused on husbandry, genetic management, veterinary aspects and reproductive physiology. Is this really a multidisciplinary approach to *ex situ* conservation? I have no doubt about it. Does the width and detail of subjects included in the book adequately represent the challenges that will determine the final success or any recovery programme? Can we face *ex situ* conservation armed with this powerful multidisciplinary biological tool-kit? Would it be enough to bring species from the brink of extinction? I'm afraid not, and I would assume that many readers would agree with me. Then, why do we keep acting, meeting and publishing like if it was so?

I believe that the answer to this question does not lie in a deliberate effort to ignore pressing and uncomfortable non biological issues, but in our professional training. Through several years of reading books, attending meetings and workshops, we have been taught –in a very indirect subtle way, never explicitly– that conservation is mostly a biological challenge. When a certain group of subjects are repeatedly taught, discussed and written, they become the *only subjects that exist.* Such process is best described through what the economist and political scientist Herbet Simon (1983) called *bounded rationality:* the ability of the human mind to perceive and comprehend our environment through certain filters and approaches that allow us to selectively reject and ignore facts and views that are alien to our personality and education. Thus, it looks like we, conservationists, refuse to explicitly and systematically incorporate most social issues in our professional algorithms. This rejection is carried on even if we hear and read about the importance of such issues or while we might be actually dealing with them most of our time.

I think of my present professional situation: I must coordinate two *ex situ* conservation programmes for locally endangered mammals in Argentina: one for the giant anteater and the other for the pampas deer. My original training is that of a zoologist. However, I spend around 80% of my time managing these programmes on social tasks: negotiating with government agents for animal donations; convincing other colleagues and institutions that they should join the processes or, at least, not try to block them; designing collaborative recovery plans with other stakeholders that would have a strong scientific, political and social support; talking to land-owners, forestry and agricultural businessmen to convince them that destroying critical habitat for those species should be part of their concerns or, at least, interests. Very seldom do I carry out some deer census on the field or supervise the capture, handling and transport of anteaters; these being the few clearly biological moments of my work.

It is clear to me that my biological-scientific training has been –and still is– extremely useful to guide my decisions and to help me convince other stakeholders. But it is also true that most of the time I see myself navigating through and trying to manage problems that are essentially non biological and will eventually determine if we achieve our conservation goals. And I do not see myself as an exception among professionals managing *ex situ* conservation programmes.

As an example, the Captive Breeding Programme for the Iberian lynx was blocked for years by interpersonal and interinstitutional conflict. As a result of such conflict, a measurable biological result was obtained: no Iberian lynx were born in captivity. Only when this history of destructive conflict was properly managed through the establishment of a consensus policy and a widely respected captive breeding team, we started witnessing the history of biological success so well described in this book. The lesson is simple: social and political problems can be behind the final failure or, at least, significant delay of many recovery programmes.

Hence, my purpose with this chapter is to join my voice to many others (Clark et al., 1994; Stephens and Maxwell, 1996; Clark, 1997; Clark and Wallace, 1998; Wallace et al., 2002, Groom et al., 2006; Robinson, 2006) to highlight one more time the key importance of social issues on any conservation programme, this being *in situ* or *ex situ*. To do so, I'll present some themes and recommendations that might guide professionals involved in these programmes. Whenever possible, and in order to fit the chapter within the spirit of this book, I will use examples from the Iberian lynx conservation process, of which I have been an external but passionate witness for more than 10 years.

I would like to send a simple message through this chapter. To specialists with a background in natural sciences who are involved in *ex situ*/*in situ* conservation programmes: be aware of your conceptual and academic biases –your bounded rationality– and be sensitive to the need for calling social scientists and professionals to bring their knowledge and expertise into the conservation challenge. To conservation professionals involved in actual management of *ex situ* and *in situ* populations: open your mind to a wide transdisciplinary understanding of the challenge at hand and try to set up interdisciplinary teams that can comprehend and handle it in its myriad of biological and social aspects.

Endangered species recovery as a complex social endeavour

Figure 1 presents groups of factors that determine the final success or failure of *ex situ*/ *in situ* conservation programmes. At the end, success will depend of the final balance between reproduction and mortality. These two processes are directly influenced by the specie's own biology and some proximate biological threats affecting it, both in a potential reintroduction site as well as in captivity. Most of these factors have been discussed throughout this book. But reality tends to be more complex. There are social factors that affect and are also affected by the previous biological issues and wield an enormous effect on the recovery or extinction of an endangered species or population.

Some biological traits determine how society responds towards a certain species. Thus, large carnivores, like wolves, big cats or bears, tend to elicit passionate stands in favor or against their conservation, while many insects, fishes or plants receive a lukewarm response from public opinion (Kellert, 1997). On the other hand, the arrangement and interplay of stakeholders who are interested, affected or opposed to the conservation programme can affect its overall economical, political or legal support. Conservation programmes do not exist in a social vacuum. They always compete with other social interests for public support, these having to do with health, education, trade, entertainment or cultural issues, to name a few. As a result of this, the whole programme can flourish within its social context, get gridlocked in the middle of rampant conflict or dwindle from general public indifference.

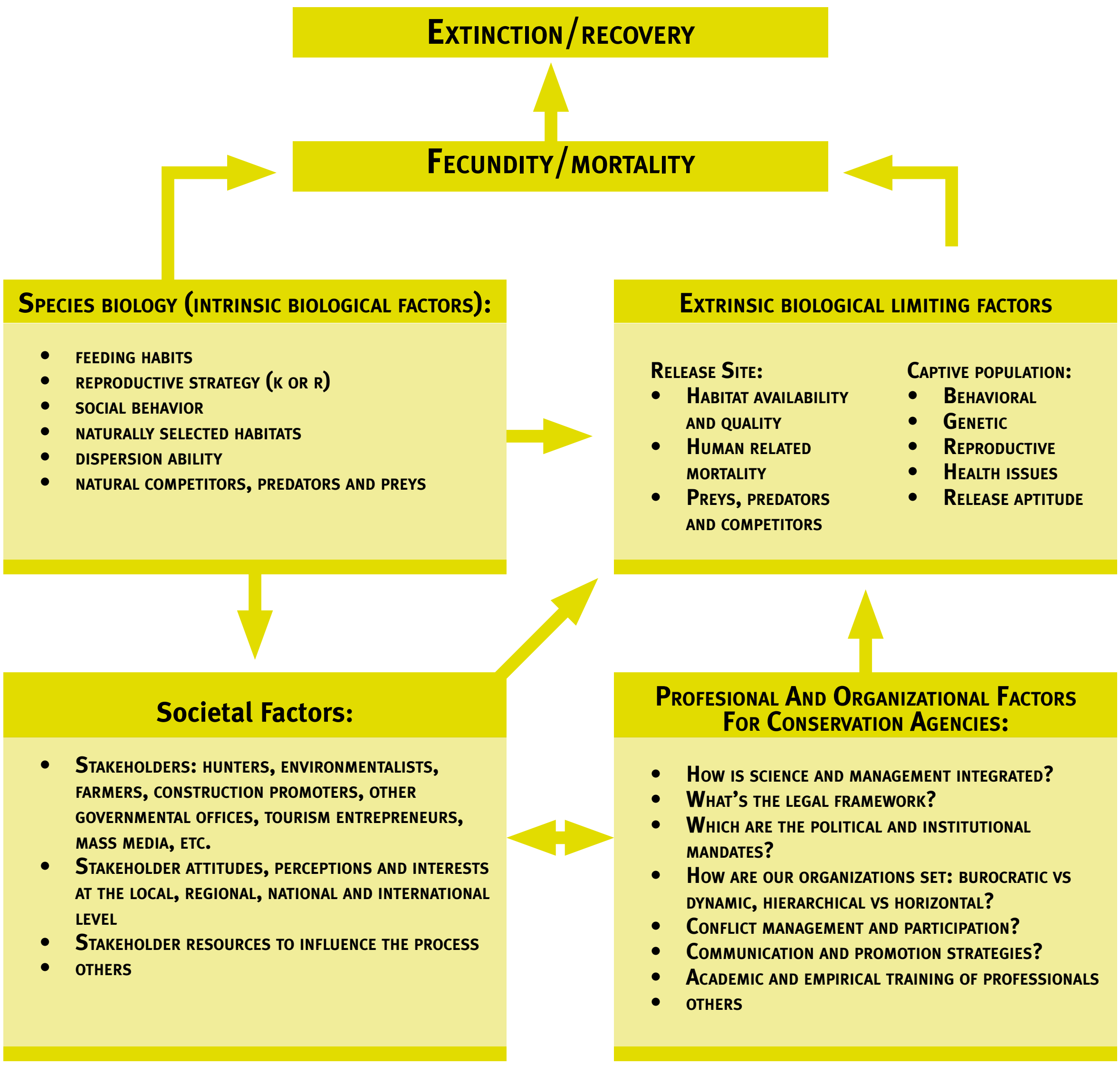

Figure 1. Factors affecting population recovery in *ex situ/in situ* conservation programmes.

Figura 1. Factores que afectan a la recuperación de una población en programas de conservación *ex situ* e *in situ*.

At the same time, while external social actors can have a key effect on the conservation programme, the way we, as conservationists, interact with other people and organizations involved in the same pursuit –allegedly to save the species from extinction– will have significant effect in the Programme's and, ultimately, the specie's fate. These interactions can be expressed in psychological, scientific, economic, communicational or organizational terms. It is no small paradox that some of the worst and most destructive conflicts blocking and threatening conservation programmes happen amongst conservationists; the previous example about the Iberian Lynx Captive Breeding Programme being a good example of this point.

As an example of social complexity surrounding a recovery programme we could check the stakeholders involved in the Iberian lynx reintroduction programme lead by the Andalusian Environmental Agency (Simón et al., this book). This institution holds the legal mandate and authority to conserve lynxes within its territory, and it can muster significant technical, financial and political resources to this task. The European Union stands out as an essential funding agency of such programme, which bestows a significant power to influence, if not veto, the whole process.

There are also two other agencies or ministries of the Andalusian government –Public Infrastructures and Agriculture– with less legal authority on the specie's conservation and, arguably, less interest on it, but much more clout over the general political process for the whole region. Other groups should be added to this list: the Spanish Central Government, represented by the Ministry of Rural, Marine and Natural Environment, is funding some components of the Programme and the neighboring government of Extremadura is an official partner of the Programme. Meanwhile, the government of Portugal and other regional governments in Spain have expressed their interest to gain access to the "Andalusian" lynxes to start their own *ex situ* programmes (Sarmento et al., this book; Vargas et al., this book).

But this is not all. The Programme also includes as official partners four environmental NGOs and three hunting associations, each with different access to public institutions and political centers. Around them there are several scientific advisors influencing the process, like the internationally renowned Doñana's Biological Station (CSIC) and the IUCN Cat Specialist Group, plus the always-vigilant gaze of the public opinion and the mass media. Any of these groups, by themselves or in alliance with others, has enough clout to promote, deter or divert major conservation actions and, eventually, the whole reintroduction programme. Each of these institutions and the many individual actors working for and within them bring into the overall conservation process a complex set of beliefs, demands, expectations, attitudes and approaches; many of which point towards interpersonal or interinstitutional conflict. Could anyone doubt that we are talking about a highly complex social endeavor?

Within this framework, it becomes especially relevant to analyze the social context surrounding an *ex situ*/*in situ* conservation programme. This implies identifying and understanding individuals and institutions whose very distinct interests are involved or affected by such programmes, and who can mobilize resources in order to arrive to results that favor those interests. Which are these interests? Following Lasswell (1971) we can propose that both individuals and groups are always looking to augment their share of the eight following basic "values": respect, rectitude, power, enlightenment, wealth, well-being, skill and affection. The key issue is that these values are not equally shared and sought by each person and institution. Even though we often assume that "we all look for the same things", this is not usually the case. For example, in the Iberian lynx reintroduction programme some institutions typically treasure and deploy power (i.e., Andalusian Environmental Agency), while others use wealth (i.e., European Union), enlightenment (i.e., Doñana Biological Station) or respect (IUCN Cat Specialist Group) as leverage. Even within these general groups there are interpersonal struggles, alliances and conflicts directed to trade and obtain any of the eight basic values. The startling fact is that many destructive conflicts are caused by people and institutions that claim the conservation of species as their main goal. Once we start seeing who gets or wants to get what from whom, we will be able to understand these conflicts and manage the social play of conservation in a much more efficient way. Achieving this does not require as much of a scientific-quantitative analysis of social process but a systematic and alert predisposition to study and understand human behavior. Any open-minded conservation professional armed with enough curiosity and patience should be able to achieve this.

Conflict as a potentially destructive or creative force

Throughout the previous paragraphs the term conflict has been used in several occasions. Conflict can be defined as the energy created by individuals and groups when they try to satisfy interests and objectives perceived as incompatible. Thus, conflict occurs when two or more players disagree over the distribution of material or symbolic values all of them related to the eight basic values described above, and start acting based on these perceived incompatibilities. Hence, convergence of multiple individuals and stakeholder groups with unique expectations, demands and identifications make conflict an inevitable ingredient of any conservation process receiving significant public attention.

The key issue is that, depending on how we manage any public conflict, it can either promote or harm actual conservation. People are most familiar with the negative aspects of conflict, which explains why many tend to avoid acknowledging its mere existence. The main problem with this approach is that it actually prevents us from seeing and promoting many positive aspects related to these situations. Thus, a conflict that is managed creatively tends to advance learning, team spirit, constant self-reflection, organizational adaptation and improvement. In 1999, after years of destructive conflict and interinstitutional gridlock surrounding captive breeding of Iberian lynxes, the Spanish Ministry of Environment called all relevant stakeholders to attend a participatory planning workshop in Madrid (see Vargas and Heredia, 2001). The explicit goal of such meeting was to agree on a National Captive Breeding Plan for the species and establish the basis for the Conservation

Figure 2. Reintroduced juvenile giant anteater in the Iberá region, Argentina.

Figura 2. Oso hormiguero gigante reintroducido en la región de Iberá, Argentina.

Figure 3. Building trust: biologist y gaucho talking about anteater reintroduction in Iberá.

Figura 3. Construyendo confianza: biólogo y gaucho hablando sobre la reintroducción del oso hormiguero gigante en Iberá.

Breeding Programme. The implicit goal, even if not evident to all attendants, was to try to manage destructive conflict in a more effective manner. To this purpose, an external and respected facilitator was hired, who used a collaborative decision making approach to create consensus from existent destructive dissension. In retrospect, one can identify that meeting as a turning point in the Iberian lynx conservation history and an excellent example of conflict and dissent used as a base from which to seek and build a more accepted public policy expressed through the resultant Captive Breeding Action Plan. In this line of thought, Lee (1993) identified bounded conflict as a major force for adaptation and improvement in conservation and other environmental processes in democratic societies.

Which specific actions can be pursued to advance creative conflict? First, we can follow the previous example with the Iberian lynx: call a professional with experience in conflict management. In another example, Johnson (2000) describes how, after many years of unproductive conflict between government biologists and landowners involved in the Alala or Hawaian crown recovery, they decided to hire a family therapist to work in relationship-building. This author describes how this unusual decision served to improve the way both groups communicated and worked together. It must be said that such progress did not suffice to avoid final extinction of this bird in the wild a few years later (Walters, 2006).

Second, it is important to turn possible competition into collaboration. When we started a reintroduction programme for the giant anteater in Northeastern Argentina (Figure 2) we soon found out that there was an Argentinean zoo that was already announcing a conservation project for the same species. This project was coordinated by a veterinarian experienced in zoo management, while we were field biologists trained in wildlife management. Zoos do not benefit from wide credibility in the Argentinean conservation community and many people tend to criticize them at ease. They were putting anteaters into a zoo, while we wanted to reintroduce animals in the wild. The setting was ripe for competitive and destructive conflict based on typically opposing world-views: biologists vs. veterinarians, wildlife managers vs. zoo people, *in situ* vs. *ex situ* conservation. Our decision was to turn this potential conflict into collaboration (Figure 3). Open, frank and respectful talks were initiated aimed to look for common ground. As a result of these, and in spite of remaining important differences and external pressures against collaboration, trust was built and both programmes have agreed to work together in the reintroduction of two captive anteaters previously included in the zoo project.

Third, another way to prevent and manage unproductive conflict is to build trust and collaboration through sharing non-distributive resources. When looking at the list of eight basic values sought by people, it might be noted that some of these values like power, wealth and, somehow, rectitude tend to show a distributive nature. This means that whatever you give of these values you tend to loose it for yourself. When a governmental agency delegates authority to another institution it tends to loose its own power on this matter. A similar fact happens with money. One could even say that when one admits that a moral rival might be right (i.e., giving her the value of rectitude), at the same time one could be debilitating one's own moral position. Here is the foundation of competitive and destructive conflict: "I cannot help the other, because whatever I give her I end up loosing it for myself". Under this light we are condemned to rivalry entangled within a mesh of interdependent relationships.

The trick is that our cultural tradition is too fixed on distributive interpersonal exchanges. When looking to the other values sought by people, including potential rivals or allies, we notice that they do not have a distributive nature. Any person can share respect, skills, information and affection with another without finding its share of them diminished. On the contrary, the fact that I offer respect to another person or institution –even if major disagreements interpose between us may actually increase my own respect. Here is a pathway to building trust and creative collaboration: one can freely share these values with actual or potential rivals and turn them into allies or, at least, into respectful and civilized neighbors.

Managing organizational issues

Most issues described up to this point deal with the way we relate with other conservationists and society in general. But there are also issues related to the way we organize ourselves to achieve our goals as conservationists that are critical to the success or failure of such enterprise (see the box on the bottom right of Figure 1). We could assume that most organizational shortcomings and failures stem from three general problems: 1) lack of a clear and shared vision and direction; 2) lack of action, also known as the *implementation gap*, and 3) inaccurate assumptions and inadequate knowledge.

Many recovery conservation programmes are built without a clear and shared idea of their ultimate goals and the approaches and methods needed to achieve them. In some cases an *ex situ* conservation programme is proposed when there is no clear evidence of how it would benefit the target population or species. Animals are bred and/or released without a clearly identified conservation need for these actions. In other cases, different actors and organizations hold divergent or antagonistic views of the challenge at hand, its desired result and the means to achieve it. Clark (1997) uses the conservation story of the black-footed ferret captive breeding and reintroduction programme as an example of this interinstitutional lack of agreement.

Open, frequent and effective communication amongst all relevant stakeholders serves as a general solution for this major problem. This should include arranging and managing regular collaborative planning instances that can help to: 1) establish a clear and shared vision of the common task; 2) call for multiple resources for *ex situ*/*in situ* conservation actions; 3) establish effective organizational structures, and 4) set the bases for continuous learning through monitoring, evaluation and widespread information exchange.

Plans can be agreed, and a clear and shared vision can be built, but that does not assure proper implementation of effective actions directed to change the status of our target population. Clark (1997) described this organizational problem as the implementation gap: actions may be identified and agreed upon but they are not actually carried on. A classical solution proposed for these situations is to call for increased funding. Proper funding is certainly a key issue but it does not assure adequate implementation, unless there is an organizational structure that is designed and adapted for its effective and efficient management. Several organizational arrangements can improve actual performance.

First, it is important to find a right balance between high control and low executive levels. In any organization there are high decision levels (i.e., chiefs, directors or political appointees) that are in charge of outlining general policies and monitoring their compliance. Below them there are usually teams or individual professionals who are in charge of implementing these policies. The former offer a sense of general direction and tend to insert any programme within a larger policy frame, while the latter take care of on-site executive matters. Implementation gaps tend to appear when high levels of control override executive groups thwarting their timely and efficient functioning, this being a typical result of bureaucratic organizations. Clark et al. (1994) described and analysed several conservation processes where this happened.

Second, in order to avoid implementation gaps in recovery programmes it is key to identify, train and empower programme leaders and active teams who are in charge of executive matters. What kind of leader do we want to have in such position? It could be summarized to: someone who wakes up in the morning reflecting about how to reestablish the species in accordance to other groups and people. What kind of person we do not want in such position? Someone who wakes up without reflecting about how to recover the species, who promotes a business as usual attitude and who is just thinking about how to keep control of the process or wanting to save the population excluding other interested parties. In order to promote effective implementation, conservation teams would benefit from showing the following traits: motivated, professional, empowered, focused on the task, ready to learn, interdisciplinary and open-minded.

Third, conservation institutions should be aware of the need to prevent and avoid *goal displacement*. Goal displacement occurs when a person or organization starts acting in ways that harm their explicit goal but benefit a second and often unexpressed purpose, typically related to programme control, career advancement or position strengthening. The problem is widespread, and also very human, because we all want other things besides avoiding some specie's extinction. Examples abound: Clark (1997) and Reading and Miller (1994) use the concept to describe actions taken by the Wyoming Department of Game and Fish in order to control the black-footed ferret programme. Lieberknecht (2000) identifies "goal substitution" as the "the root of the policy problem" in the conservation of the Barton Springs Salamander in Texas. The author proposes that "participants, such as the federal government (...), the state government (...), and environmentalist groups (...), have substituted the purpose of power for their stated goal of salamander conservation". And, Naves (2005), while analysing brown bear conservation in Asturias, Spain, described decisions taken by the relevant governmental agency, which favoured programme control over implementation of needed research and management actions.

Programme evaluation: organizational learning for *ex situ* conservation

Conservation is a complex task inserted in a context of scientific, political and social uncertainty. There is a need to act early to prevent species extinction, even if we have not discerned all relevant facts. Still, when we are able to get a clear picture of what is happening and what needs to be done, the context becomes too dynamic and tends to change in fast and unpredicted ways by the time we start acting. Here lies a major organizational challenge: the need to take decisions and act in an environment of uncertainty and change, while trying to avoid that these decisions and subsequent actions are based on inadequate knowledge and wrong assumptions. In this regard, several authors have proposed adaptive management as the paradigm that should guide decision-making in conservation programmes and other complex natural resource challenges (Lee, 1993; Salafsky et al., 2001). Within this framework, programme evaluation takes a leading role.

Programme evaluation implies the continuous questioning and reflection on our assumptions, objectives and methods and, sometimes, even our final goals. A permanent questioning when managing a conservation programme should be: what are we assuming or doing that is wrong or, at least, clearly improbable , perhaps ineffective or even potentially harmful? Programme evaluation helps us frame and answer that question. Evaluation could be either internal or external and formal or informal (see Backhouse et al., 1996) (Figure 4). Any programme can benefit from a combination of these complementary approaches. Informal internal evaluation implies creating a working environment where all programme participants can openly share thoughts, worries and proposals related to the conservation task. It also involves encouraging reflection and constructive criticism while searching for and creating spaces and moments when most programme members can meet face to face.

External informal evaluation implies bringing frequent "fresh air" into the Programme. The key word is transparency: make your objectives, methods and protocols public so they can be reviewed and criticized by all relevant experts and, whenever possible and sensible, all possible stakeholders. Open your breeding and quarantine facilities, and show your release and monitoring methods to national and foreign experts. This will encourage a collective learning process that goes beyond the Programme's staff and turns conservation into a matter of truly public interest.

Internal formal evaluation means designing plans and strategies in order to monitor programme performance at several levels (i.e., goals, objectives and activities; see Margoluis and Salafsky, 1998). Such evaluation requires developing measurable goals and objectives, establishing performance indicators for each one of these

	Informal	Formal
Internal	Regular (weekly, monthly) coordination and evaluation meetings among team members	Regular (bi-annual, annual) evaluation and planning among team members and collaborators
External	*Ad hoc* evaluation, taking advantage of visits from experts in the subject	Programme evaluation by external experts (recommended every 5 years)

Figure 4. Types of evaluation in conservation programmes.

Figure 4. Tipos de evaluación en programas de conservación.

and, most important, assigning time and resources within the programme for systematic monitoring of their compliance. It also means taking programme-monitoring results seriously and being ready to change based on these findings.

Finally, external formal evaluation implies calling for an external agent to carry out a thorough review of your programmes' performance. Backhouse et al. (1994) describe the story and results of one such evaluation for the Eastern barred bandicoot recovery programme. This Australian marsupial was protected since the 1970s and a conservation programme was initiated in the 80s. By 1988 the species had lost 98% of its original distribution and abundance, with only 190 remaining animals alive. At that time it was obvious that the recovery programme was not working properly and a major formal evaluation was called for, which included foreign evaluators from the United States. The evaluation cited major weaknesses related to the Programmes' organization and operation: 1) causes of decline were poorly known; 2) there was an underestimation of the situation's urgency and a business as usual attitude towards the whole programme; 3) inadequate planning was used where clear goals, time frames and responsibilities were absent; 4) there was lack of good information about relevant social and organizational aspects; 5) absence of systematic monitoring and evaluation procedures directed to learning and programme improvements; 6) lack of effective leadership, and 7) poor communication among programme participants. As a result of this evaluation, there was a major programme reorganization, which was very much focused on improving programmatic and organizational aspects. Greatly as a result of this evaluation and reorganization process, by the end of the 1990s there were more than 1000 bandicoots established at six reintroduction sites, plus the original population and a captive breeding programme.

Each one of these evaluation approaches has its own advantages and caveats. Discussing them goes beyond the space and scope of this chapter. Nevertheless it must be highlighted that any recovery programme would benefit from using all four approaches at different times and under different circumstances throughout its history. In an environment where programme organization and performance can make the difference between a species survival or extinction it is peremptory to invest time, thoughts and resources in making constant assessments and improvements of our own actions.

Acknowledgements
I want to thank Astrid Vargas for inviting me to prepare this chapter and Javier Calzada and Paco Palomares for their reviews and criticism.

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Epilogue

EPÍLOGO

Pegado a la tierra, aplastado entre las ramas, desolado por el cansancio y quizás también por el miedo, el lince dejó pasar la noche sin moverse, tratando de dominar el hambre. De pronto cerró los ojos y se quedó dormido; como una piedra cae en el agua de un pozo oscuro, así le vino el sueño. Por los movimientos de la boca -que repetían la perfecta dentellada mortal en el cuello-, parecía que el lince estaba soñando. Soñaba con un inmenso espacio cubierto de encinares y repleto de conejos..

El lince, el cazador y los sueños
Julio Manuel de la Rosa

El lince ibérico de ayer a mañana

Escribió Antonio Machado: "El hoy es malo, pero el mañana... es mío/ Y es hoy aquel mañana de ayer". No lo decía el poeta en un contexto optimista, pero sus versos, a nosotros, nos permiten serlo. Hace apenas un par de lustros constatábamos la nefasta situación del lince ibérico. Cada día empeoraba un poquito. Paradójicamente, como a veces se atribuye a la política, para el lince también fue realidad aquello de "cuanto peor, mejor". Resultó necesario tocar fondo para empezar a alzarse, pero entre todos decidimos hacerlo y nos estamos levantando. Nuestro hoy no es peor, por tanto, que el mañana soñado ayer, y el programa de conservación *ex situ* tiene mucho que ver en ello.

Bastantes años atrás, en una reunión del Grupo de Trabajo del Lince (injustamente olvidado y, en la práctica, lamentablemente disuelto) que convocaba y presidía el Ministerio de Medio Ambiente, nos dimos cuenta de que, para evitar la inminente extinción del lince ibérico (y, más aún, para posibilitar su recuperación), era insuficiente reiterar las medidas genéricas habituales (i.e. respetar el hábitat, evitar la mortalidad reduciendo el furtivismo, incrementar la abundancia de conejos, etc.). Había que plantearse actuaciones activas, y urgentes, en espacios concretos. Necesitábamos para ello abandonar el oscurantismo y hacer la luz, pese a sus evidentes riesgos. En primer lugar, había que saber, y había que decir, donde había linces, de forma que pudieran optimizarse los esfuerzos y las inversiones. Y luego, donde se decidiera, había que tomar medidas drásticas, negociando con propietarios, repoblando con conejos, limitando las infraestructuras, erradicando lazos y cepos, etc. Y la sociedad debía saberlo. Pero todo eso aún era poco para lo que pretendíamos.

Recuerdo haber hablado en aquella época de la necesidad de medidas urgentes estructuradas como un taburete. Mencionaba tres patas y un asiento, una superficie horizontal uniéndolas. La primera y más urgente de las patas era trabajar para detener la hemorragia, para frenar el declive poblacional; inútil parecía diseñar el futuro de los linces si, allí donde todavía vivían, no se les garantizaba el presente. La segunda pata incluía confirmar la presencia de la especie en áreas propicias pero tal vez poco muestreadas, o donde pudieran quedar muy pocos ejemplares, así como garantizar, no sólo en esas áreas, la conservación de hábitat suficiente para permitir la existencia en un futuro próximo de otras poblaciones, además de las conocidas. La tercera pata eran las medidas "artificiales" tendentes a crear o, en su caso, a consolidar, esos nuevos núcleos poblacionales, desde la translocación de ejemplares silvestres de unas zonas a otras, a la producción de animales en cautividad para su posterior liberación. Recuerdo haber escrito a ese respecto: "Hay que aprender a criar linces en cautividad, como último recurso, y existe ya un buen plan sobre el modo de llevarlo a cabo. Alguien tiene que tomar el toro por los cuernos y decidirse a hacerlo, con todas sus consecuencias". Por fin, el asiento debía estar formado por las medidas horizontales imprescindibles, como la educación y la sensibilización.

Hemos avanzado mucho, aunque siempre sepa a poco. La mayor parte de esas propuestas están recogidas en la recientemente aprobaba Estrategia Nacional para la recuperación del lince, a la que parece sumarse de buena gana Portugal (convendría, por cierto, oficializar una estrategia ibérica). La conservación de las poblaciones conocidas ha sido abordada con éxito en Sierra Morena y algo menos en Doñana por la Junta de Andalucía, con enorme esfuerzo gracias al apoyo de un proyecto LIFE. Y en su día la Junta de Andalucía y el Ministerio de Medio Ambiente cogieron, efectivamente, el toro por los cuernos y el proyecto de conservación *ex situ*, como refiere este libro, es una brillante realidad. Pero lo que quiero enfatizar en el contexto del libro es que, contra todo pronóstico, la tabla que sujeta las patas de nuestro taburete, el engrudo que une a unos actores con otros, unas actuaciones con otras, el lubricante que suaviza y aminora las tensiones personales e institucionales, ha resultado ser la conservación *ex situ*. Lejos de limitarse a proporcionarnos los ejemplares precisos para los ensayos de recolonización de áreas perdidas, más allá de constituir ese salvavidas, o colchón protector, que debía permitirnos trabajar con más tranquilidad justo en el borde del abismo, el programa de cría en cautividad, con su directora al frente, se ha mostrado como el más enérgico y transparente propulsor de la conservación del lince, también *in situ*.

Es digno de aplauso (y unánimemente aplaudido), pero creo sinceramente que es una carga excesiva para el equipo de conservación *ex situ* y las personas que lo dirigen. Lo han hecho ellos porque era necesario y nadie lo hacía, porque no sabíamos o simplemente porque no podíamos. Gracias al éxito de la cría en cautividad y, sobre todo, gracias a las complejas redes (políticas, administrativas, científicas, informativas, humanas...), nacionales e internacionales, tejidas alrededor del programa, la disposición psicológica hacia la conservación del lince ibérico ha cambiado 180º. Hasta hace muy poco nos reuníamos con ánimo desesperado, con el único objetivo de evitar la extinción de la especie, que se antojaba casi inevitable. Desde hace muy poco tiempo, casi sin darnos cuenta, hemos pasado a discutir en las reuniones cómo vamos a conseguir que el lince vuelva a Portugal, y enseguida a las Comunidades Autónomas españolas que lo han perdido hace poco. Está de moda el término "Podemos", pues lo utilizó Obama y ganó por goleada. Con los altibajos inevitables en un mundo y un sistema económico que no invitan al optimismo, al menos en lo que se refiere a la conservación de la naturaleza, en el caso del lince hemos pasado a creer en nuestro propio trabajo.

Pero decía que los responsables del programa de conservación *ex situ* no pueden seguir haciéndolo todo. Hay que adoptar su espíritu de trabajo, su voluntad de diálogo y, si posible fuera, incluso sus actitudes personales, pero deben sustituirlos los investigadores en los laboratorios, las Comunidades Autónomas (y Portugal) en el campo, y el Ministerio de Medio Ambiente, Rural y Marino en la coordinación que le corresponde. Mi sensación en estos momentos es que el armazón que sujeta a la recuperación del lince ibérico lo componen, básicamente, unas pocas personas concretas. Son cañas fuertes, pero pueden quebrarse, y si no se reparte el trabajo acabarán quebrándose algún día. Necesitamos una estructura administrativa fuerte, una coordinación eficiente, un diálogo permanente, transmitir a la sociedad una información clara... A fuer de sincero, creo que vienen tiempos buenos para el lince ibérico, pienso que va a ir a mejor. Pero van a ser, también, tiempos difíciles. Inevitablemente, habrá cosas que nos saldrán mal y otras que, aun saliendo bien, parecerán negativas a ojos de la gente (e.g. muchos linces morirán antes de que otros puedan establecerse de forma permanente en nuevas tierras). Esas dificultades deben pillarnos unidos y con una estructura fuerte, capaz de aguantar críticas, eventuales desánimos e incluso abandonos.

Hoy es el mañana de ayer, y siempre será así. Otro poeta, Ángel González, ha escrito: "Te llaman porvenir/ porque no vienes nunca", y enseguida: "¡Mañana!/ Y mañana será otro día tranquilo/ un día como hoy, jueves o martes,/ cualquier cosa y no eso/ que esperamos aún, todavía, siempre”. Efectivamente, nos cansaremos de esperar ese futuro feliz que anhelamos, el mañana de una Península Ibérica poblada por linces en gran parte de su extensión, sin mayores problemas. Nunca ha ocurrido y seguramente nunca ocurrirá. Si alguna vez hubo muchos linces, se cazaron por su piel y su carne, cuando hubo muchos conejos, se colocaron cepos y lazos que mataron a los linces, en las épocas en que el monte se limpiaba estaba demasiado limpio y usado… Conservar al lince ibérico será complicado siempre y exigirá trabajar duro. Pero hemos de hacerlo porque, aunque el hoy no sea tan malo como hace diez años, el incierto mañana sigue siendo nuestro.

Miguel Delibes de Castro

The Iberian lynx from yesterday to tomorrow

As the great Spanish poet Antonio Machado wrote, "Today is bad, but tomorrow… is mine/ And today is the tomorrow of that yesterday." Although the context in which he wrote this was not optimistic, his verses give us reasons for hope. We became aware of the disastrous situation of the Iberian lynx only a decade ago. It was getting a little worse every day. Paradoxically, as it sometimes happens in politics, only after hitting rock bottom did we start to go up again, but we decided to do it together and we are rising. Therefore, our today is not worse than yesterday's dream of tomorrow, and the *ex situ* conservation programme has a lot to do with this.

Quite a few years ago, the Spanish Ministry of the Environment organised and chaired a meeting of the Working Group on the Iberian lynx, which has been unjustly forgotten and was unfortunately terminated. On that occasion, we realised that, to avoid the imminent extinction of the Iberian lynx (and, what is more, for its recovery to be possible), it was not enough to reiterate the usual generic measures (i.e., conserve the species' habitat, avoid mortality by reducing poaching, increase rabbit abundance, and the like). Active and urgent measures were needed in specific areas. To achieve this, we had to come out of the darkness and shed some light on these issues, in spite of the obvious risks involved. First, we needed to know and say where the lynx was present to optimise efforts and resources. Also, strict measures had to be taken in targeted areas, negotiating with landowners, restocking rabbit populations, limiting infrastructures, eradicating traps and snares, and so on. And society had to hear about it. But this was still not enough to achieve our goal.

Back then, I remember saying that we needed urgent measures with the structure of a stool. I mentioned three legs and a seat, a horizontal surface bringing the legs together. The first and most urgent of the legs was to try to stop the haemorrhage, to slow down the population decline; it seemed useless to design the future of the lynx if we did not guarantee its presence in areas where it still occurred. The second leg involved confirming the presence of the species in favourable areas where little sampling had been done, or where very few individuals might still be left; we should also guarantee the conservation of enough habitat in those areas and others for other lynx populations apart from the known ones to be able to exist in the near future. The third leg referred to "artificial" measures to create or consolidate new populations; such measures ranged from the translocation of wild individuals to other areas to captive breeding of animals for later release. I wrote about this at the time, saying "We must learn to breed lynx in captivity as a last resort, and there is already a good plan on how to do it. Someone has to grab the bull by the horns and do it, with all its consequences.” Finally, the seat should be formed by the essential cross-cutting measures such as education and awareness-raising.

We have made great progress, although it always seems too little. Most of these proposals have been included in the recently adopted Spanish National Strategy for the Recovery of the Iberian Lynx, which Portugal seems to willingly adhere to (it would be good to set up an official Iberian Strategy, by the way). The conservation of the known populations of Iberian lynx has been dealt with successfully in Sierra Morena and somewhat less so in Doñana by the Regional Government of Andalucía, in a huge effort thanks to the support of a LIFE project. Indeed, the Regional Government of Andalucía and the Spanish Ministry of the Environment grabbed the bull by the horns at that point and the *ex situ* conservation programme that this book is about is now a brilliant reality. However, in the context of this book I wish to highlight that, against all odds, the board that holds the legs of our stool together, the glue that joins the various players and actions, the oil that eases and reduces personal and institutional tensions has turned out to be *ex situ* conservation. The captive breeding programme has done much more than just provide the specimens needed to try to recolonise lost areas and be a safety net to allow us to work with greater confidence on the edge of the abyss. Beyond all this, the captive breeding program, headed by its director, has proven to be the most energetic and transparent driving force of lynx conservation *in situ* as well.

This is worthy of praise (and unanimously praised), but I honestly consider that it is an excessive burden for the *ex situ* conservation team and the people who head it. They did it because it was necessary and nobody was doing it, because we did not know how to or we simply were not able to. Thanks to the success of captive breeding and especially thanks to the complex national and international networks (political, administrative, scientific, information, human networks and more) woven around the programme, the psychological attitude towards the conservation of the Iberian lynx has radically changed. Until very recently, our meetings were held in a desperate mood, with the only objective of preventing the extinction of the species, which was seen as almost inevitable. Almost without noticing, we have recently started to discuss how to ensure that the lynx returns to Portugal and the Spanish regions where the species recently disappeared. The expression “Yes we can” has become popular after Obama used it and won by a landslide. With the inevitable ups and downs in a world and an economic system that do not inspire optimism, at least for nature conservation, in the case of the lynx we now believe in our own work.

Yet, I was saying that the people in charge of the *ex situ* conservation programme cannot continue to do everything. Their working spirit, their willingness for dialogue and, if possible, even their personal attitude should be adopted by others, but these people should be replaced by researchers in laboratories, the Spanish regions (and Portugal) in the field, and the Spanish Ministry of the Environment and Rural and Marine Affairs in the coordination work that suits it. My feeling today is that the structure upholding the recovery of the Iberian lynx is basically composed of just a few people. They are strong poles, but might break and will eventually break if work is not shared by others. We need a strong administrative structure, efficient coordination, permanent dialogue, and to convey clear information to society… To be honest, I think good times are coming for the Iberian lynx. I think the situation will improve, but times will also be difficult. Inevitably, we will sometimes fail; even when good results are obtained, people will sometimes see them as being negative (e.g., many lynx will have to die before others can permanently settle in new areas). When these difficulties happen, we must be united and have a strong structure that can withstand criticism, possible discouragement and even resignations.

Today is yesterday’s tomorrow, and it will always be so. Another Spanish poet, Angel González, wrote “They call you the time to come because you never come,” and “Tomorrow!/ And tomorrow will be another quiet day/ a day like today, Thursday or Tuesday,/ anything but what we still, always wait for.” Indeed, we will get tired of waiting for this happy future that we long for, the tomorrow of an Iberian Peninsula with lynx in most of its areas without any problems. It has never happened and most likely never will. If there were ever many lynx, they were hunted for their pelts and their meat; when there were many rabbits, traps and snares were placed to kill the lynx; when the woodland was cleaned it was too clean and used... Conserving the Iberian lynx will always be complicated and will require hard work. But we must do it because although today is not as bad as ten years ago, the uncertain tomorrow is still ours.

Miguel Delibes de Castro

Agradecimientos

No ha sido fácil llegar hasta aquí, pero resultará sencillo darse cuenta del extenso trabajo recopilado en esta obra, en gran medida gracias al esfuerzo de un gran número de profesionales, administraciones, organismos y entidades que han apoyado y financiado gran parte de los proyectos aquí presentados. Autores de países tan diversos como Alemania, Argentina, Austria, Bélgica, Estados Unidos, Holanda, Inglaterra, Portugal, Rusia y Suiza, así como de diversos puntos de la geografía española, han aportado capítulos a esta obra. Estamos especialmente agradecidos a los 124 autores y coautores que, con su tiempo y esfuerzo, han preparado capítulos para el libro, compartiendo su trabajo y conocimientos para ayudar en la conservación del lince ibérico.

No queremos dejar de mencionar a la Fundación Biodiversidad representada por su presidente, Josep Puxeu, autor del prólogo de este libro, que supo ver la importancia de este trabajo y agradecer el apoyo incondicional y generoso que nos han brindado durante las diversas fases de preparación de esta publicación.

Elvira Valbuena y Juan Carlos Gauli merecen mención especial por su excepcional esfuerzo, profesionalidad, paciencia y dedicación durante las innumerables revisiones que tuvieron lugar a lo largo de la edición y maquetación de este libro. Joe Zammit-Lucia preparó, expresamente para esta publicación, las fotografías de las cubiertas y de las aperturas de cada sección donando gentilmente su tiempo y su trabajo. Fernando Martínez ayudó a revisar parte de los manuscritos iniciales, Eva Vázquez revisó las referencias de cada capítulo, Wendy Byrnes tradujo gran parte de los resúmenes y pies de las ilustraciones. Josie Dade, Jessica Reeves, Mariella Superina y Roberto Aguilar ayudaron a traducir algunos de los capítulos y Richard Smith (Ricky) leyó las versiones revisadas de los 32 capítulos escritos por autores no angloparlantes para comprobar que los textos editados fluyesen adecuadamente en inglés. Diversos profesionales donaron altruistamente su trabajo fotográfico para las aperturas de cada uno de los capítulos; gracias por ello a Antonio Sabater (ENFOQUE-10), Héctor Garrido (Estación Biológica de Doñana, CSIC), Alexander Sliwa (EAZA y Zoo de Colonia), José María Pérez de Ayala (MARM), Tanya Shenk (CDW), Marianne Hartmann (Tierstation Bockengut), Urs Breitenmoser (IUCN CatSG y KORA), Peter Oxford, Andoni Candela, Eduardo Abad (EFE), Antonio Rivas y Luis D. Klink (Programa de Conservación *Ex situ* del Lince Ibérico).

Gracias al compromiso de colaboración entre el Ministerio de Medio Ambiente y Medio Rural y Marino y la Consejería de Medio Ambiente de la Junta de Andalucía,

la Cría para la Conservación del Lince Ibérico es hoy una importante herramienta de apoyo para la recuperación del lince en la naturaleza. Actualmente, un total de 77 linces forman parte del Programa *Ex situ*, 36 de ellos son fundadores potenciales –representantes de la variabilidad genética de la especie– que han sido incorporados a los centros de cría gracias al destacable esfuerzo de los técnicos del Proyecto LIFE-Lince, coordinado por la Junta de Andalucía, y al apoyo de sus socios. Un total de 58 instituciones (administraciones, organizaciones, fundaciones y otras entidades) han colaborado en la Conservación *Ex situ* del Lince Ibérico durante los últimos cinco años. Entre ellas, un extenso número de profesionales han participado activamente en este Programa. Queremos destacar la labor de los tres "Migueles" –Miguel Ángel Simón (Junta de Andalucía), Miguel Delibes (Estación Biológica de Doñana, CSIC) y Miguel Aymerich (Ministerio de Medio Ambiente y Medio Rural y Marino)– que dieron el impulso para la firma del Convenio Bilateral de Colaboración entre el Estado Español y la Junta de Andalucía en el año 2003. Elvira Rodríguez (ex ministra de Medio Ambiente) y Fuensanta Coves (anterior Consejera de Medio Ambiente de la Junta de Andalucía) firmaron el convenio que resultó en el establecimiento del Programa de Conservación *Ex situ*. Cristina Narbona (ex ministra de Medio Ambiente) continuó su apoyo y propulsó el desarrollo de las Jornadas que organizó la Fundación Biodiversidad y que sirvieron de simiente para la creación de este libro. En los siguientes párrafos queremos destacar la participación de muchas de las personas que, con su trabajo dedicado y, en numerosas ocasiones altruista, han colaborado en la Conservación *Ex situ* del Lince Ibérico. Si alguno de ellos no se encontrase reflejado en estos agradecimientos, pedimos nuestras más sinceras disculpas por la omisión no intencionada.

Colaboradores de los equipos de gestión y de conservación *in situ*: Miguel Angel Simón, Rafael Cadenas, Isabel Mateo, José Guirado, Charo Pintos, Fernando Ortega, José María Oliet, Rafael Arenas, María Navarro, Antonio Franco, Marina Martín, Rocío Espinosa y Cinta Castillo (de la Consejería de Medio Ambiente de la Junta de Andalucía); Miguel Aymerich, Juan Garay, Jesús Casas, José Jiménez, Borja Heredia, Luis Mariano González, Juanjo Areces, Nicolás Guzmán, José Luis Herranz, Inés G. Doncel, Basilio Rada, Alberto R. de Larramendi, Marta García, Josep Puxeu y Elena Espinosa (de los equipos de gestión del Ministerio de Medio Ambiente y Medio Rural y Marino entre los años 2004 y 2009); Juan Carlos Rubio, Francisco Quirós, Manolo Delgado, Laló Cobo y Teresa Agudo y demás compañeros del Espacio Natural de Doñana (CMA, Junta de Andalucía); Gema Ruiz, José María Gil-Sánchez, Guillermo López, Marcos López-Parra, Leonardo Fernández, José García, Silvia Saldaña, Pilar Delgado, Antonio Leiva, Javier Rodríguez, Eva Rojas, Rafa Arenas, Manolo Moral, Germán Garrote y el resto de compañeros del Equipo in situ LIFE-LINCE, así como los compañeros de la Fundación CBD Hábitat, WWF-España y Ecologistas en Acción; Miguel Delibes, José Antonio Godoy, Francisco Palomares, Eloy Revilla, Jacinto Román, Juan Carlos Rivilla, José Vicente López, Alejandro Rodríguez y Fernando Hiraldo (de la Estación Biológica de Doñana, CSIC). Javier Calzada y los miembros del Grupo Lince de la Sociedad Española para la Conservación y el Estudio de Mamíferos; Lourdes Anaya, Manuel Abascal, José Cornejo, Soledad Centenera, Ramón Martínez y compañeros de Tragsega; Ignacio Antón, Antonio Tomé, José Guarnizo, José Barrera, Coronada, Angel Campuzano (de Tragsa); Bosco Neches, Juanchu Hernani, Roberto García y Eva Rodríguez (de Egmasa); Guillermo Crespo, Pedro Muñoz, Javier Caldera y Maria Jesús Palacios (de la Junta de Extremadura); Antonio Aranda (de la Junta de Castilla-La Mancha); Lurdes Carvalho, Margarida Fernandes y Pedro Sarmento (del ICNB de Portugal); Eladio Fernández-Galiano y A. Olszanska (del Convenio de Berna); Manuela von Arx, Andreas Ryser,y Fridolin Zimmerman (de KORA, Suiza).

Colaboradores de los Centros de Cría del Lince Ibérico: Iñigo Sánchez, José María Aguilar, Miguel Ángel Quevedo, Luis Flores, Mariano Cuadrado, Maria José Coca y Verónica Cancelo (del Zoobotánico de Jerez); Mª José Pérez, Claribel León, Vanessa Lobato, Daniel Martín, Francisco Valdivia, Antonio Esteban, Joaquín Ramírez, Antonio Martínez, Marina Barquín, Leticia Coloma, Julia Sánchez, Olga Muñoz, Sebastian Ruiz e Ignacio Mallea (de La Olivilla); Rodrigo Serra,

Nuno Gaspar, Nuno Gonçalves, Olga Jiménez y Catarina Ferreira (de Silves, Portugal); Antonio Rivas, Fernando Martínez, Juana Bergara, Anastasio Vázquez, Antonio Juan Pardo, Eva Vázquez, Javier López, Maty Chaparro, Lourdes Anaya y Javier Yerga (de El Acebuche). Gracias también a Antonio Romero y a su equipo, a José Luis Escobar (de DCD informática), y a todos los que han trabajado anteriormente en alguno de los centros, incluyendo a Luis Díez Klink, José Manuel Rodríguez, David Rodríguez y Luna Poveda, y a los más de 80 voluntarios que, con su generoso trabajo, han contribuido significativamente al funcionamiento de los centros de cría del lince ibérico.

Colaboradores de Aspectos Genéticos, Comportamiento y Manejo: José Antonio Godoy (de la Estación Biológica de Doñana, CSIC); Jesús Fernández (del INIA); Robert Lacy y Kristin Leus (del Grupo Especialista de Cría para la Conservación de la UICN); Mireia Casas-Marce (de la Estación Biológica de Doñana, CSIC); Xavier Manteca (de la Universidad de Barcelona); Helena Marqués (de Conzoolting); Sergey Naidenko y Anastasia Antonevich (del A.N. Severtsov Institute of Ecology and Evolution de Moscú).

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Colaboradores de Aspectos Reproductivos: Eduardo Roldán, Montserrat Gomendio, Natalia Gañán, Raquel González, Cristina Crespo y Lucía Arregui (del Museo Nacional de Ciencias Naturales); Trinidad León-Quinto y equipo (del Instituto de Bioingeniería de la Universidad Miguel Hernández); Katarina Jewgenow, Frank Goeritz, Beate Braun, Martin Dehnhardt, Thomas Hildebrandt, Christian Voigt y equipo técnico del IZW de Berlín; Teresa Abáigar (de la Estación de Zonas Áridas de Almería); David Wildt, Janine Brown and Katey Pelican (del CRC, Smithsonian Institution, USA); Julián Garde (UCM); Adrián Sestelo (Fundación Bioandina).

Patrocinadores de pequeños y grandes aspectos del Programa: Fundación Biodiversidad (España); Fundación Terra Natura (España); Fundación Rainforest (España); Fundación Gypaetus (España); Fundación BBVA (España); Pole Position (España); Disvisión (España); Pfizer (España); Merial (España); Fort Dodge (España); Farmadiet (España); Zoo de Fuengirola (España); Coronel Tapiocca (España); Fundación del Zoo de Dierenrijk (Holanda); Asociación Europea de Zoos y Acuarios (EAZA); CatsCraze (España); “International Society for Endangered Cats” (ISEC, Canadá); Fauna & Flora Internacional; DCD informática.

Y también a los que nos quieren poco y critican nuestro trabajo, ya que ellos nos ayudan a reevaluar lo que hacemos y a superarnos cada día.

Hay dos personas que con su apoyo incondicional han contribuido a que el Programa *Ex situ* en general, y a que este libro en particular, hayan recibido toda la atención que merecían: a Mario y a Ricky, por su cariño y comprensión, y por todo el tiempo que les he "robado". Y a mi madre y hermanos, Bea, Marina, Javi, Laura y Nicky, por estar siempre ahí, apoyando desde la retaguardia (AV).

Esperamos que este libro sirva como herramienta de trabajo para conservar especies y como herramienta de ocio e inspiración para quienes desean disfrutar de las hermosas imágenes, versos y citas que acompañan a los textos. Nuestra intención es acercar al lector al lince ibérico combinando ciencia y arte. Como expresa el artista Joe Zammit-Lucia en su obra más reciente *"El arte puede ayudar a que las personas se involucren a través de sus emociones. Esto es vital porque si la gente no se implica emocionalmente en temas ambientales no tendremos ninguna posibilidad."* Con la ayuda de todos conseguiremos que el lince ibérico vuelva a ser una pieza funcional de su ecosistema, el monte mediterráneo. Como parte de este ecosistema, el lince es una especie clave ya que mantiene a raya a otros carnívoros, como los zorros y meloncillos, y paradójicamente, consigue de ese modo que donde viven linces haya más conejos, su presa principal. Además, es un símbolo del monte mediterráneo, un abanderado de la conservación en el mundo y también un paraguas que puede servir para conservar a muchas otras especies. Si logramos recuperar al lince, conseguiremos proteger una gran diversidad de plantas, animales, hongos y microorganismos que dependen de este rico ecosistema para su supervivencia. Invertir en el futuro del lince ibérico es, por tanto, invertir en la sostenibilidad de nuestro ecosistema más diverso y emblemático. Como recientemente apuntaba Miguel Delibes, *"No podemos dejar caer al lince. Los humanos necesitamos la naturaleza bien conservada para vivir. Defendiendo al lince defendemos la dignidad, las haciendas, e incluso la vida de millones de personas vivas o que vivirán".*

ACKNOWLEDGEMENTS

It was not easy to get here, but it should be simple to realize the extensive work compiled in this publication, largely due to the effort of a considerable number of professionals, administrations and organizations that have supported and financed many of the projects portrayed in the book. Authors from countries as diverse as Germany, Argentina, Austria, Belgium, United States, Holland, England, Portugal, Russia and Switzerland, as well as from many areas of the Spanish geography, have contributed chapters to this book. We are particularly indebted to the 124 authors and co-authors that, with their time and effort, have prepared chapters for this book, sharing their work and their knowledge to help conserve the Iberian lynx.

We have chosen not to repeat the list of names that appear in the Spanish version of the acknowledgements, yet, we would like to include the words that wrap up this final part of the publication.

We hope that this book will serve as a working tool for conserving species and as a tool for leisure and inspiration for those who want to enjoy the beautiful images, verses and quotes that complement the chapters. Our intention is to bring the reader closer to the Iberian lynx by combining science and art. As the artist Joe Zammit Lucia expresses it, *“Art can help engage people at an emotional level. That is vital because without emotional involvement in environmental issues we have no chance.”* With everyone´s engagement we will be able to achieve that the Iberian lynx becomes again a functional piece of its ecosystem, the Mediterranean forest. As a part of this ecosystem, the lynx is a keystone species, since it controls the presence of other carnivores, such as foxes and mongooses and, paradoxically, where lynxes thrive so do rabbits, its main prey base. In addition, the lynx is a symbol of the Iberian Mediterranean forest, a conservation flagship for the world, and an umbrella that can help preserve many other species. If we manage to recover the Iberian lynx, we will manage to protect a great diversity of plants, animals, fungi, and microorganisms that depend on this rich ecosystem for survival. To invest in the future of the Iberian lynx is to invest in the sustainability of our most diverse and emblematic ecosystem. As Miguel Delibes recently conveyed, *“We cannot let the lynx fall. To survive, humans need a well-conserved nature. Defending the lynx we are defending the dignity, the homesteads, and even the lives of millions of people that live in the present or that will live in the future.”*

Circles of Our Lives

Within the circles of our lives
we dance the circles of the years,
the circles of the seasons
within the circles of the years,
the cycles of the moon
within the circles of the seasons,
the circles of our reasons
within the cycles of the moon.
Again, again we come and go,
changed, changing. Hands
join, unjoin in love and fear,
grief and joy. The circles turn,
each giving into each, into all.
Only music keeps us here,
each by all the others held.
In the hold of hands and eyes
we turn in pairs, that joining
joining each to all again.
And then we turn aside, alone,
out of the sunlight gone
into the darker circles of return.

Wendell Berry

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Like the resource it seeks to protect,
wildlife conservation must be dynamic,
changing as conditions change, seeking
always to become more effective.

Rachel Carson
(1907-1964)

La ciencia se compone de errores,
que, a su vez, son los pasos
hacia la verdad.

Julio Cortázar
(1914-1984)

No vayas a creer lo que te cuentan
del mundo (ni siquiera esto
que te estoy contando)
ya te dije que el mundo es incontable.

Mario Benedetti
(1920-2009)

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