Iberian Lynx Ex-situ Conservation Seminar Series

-Book of Proceedings-

Sevilla & Doñana (Spain), Sept-Nov 2006

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Fundación Biodiversidad

PROGRAMA DE CONSERVACIÓN EX-SITU
LINCE IBÉRICO

Conservación
Ex Situ
del Lince Ibérico

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The present "Book of Proceedings" is a product of a seminar series on Iberian Lynx Ex-situ Conservation, which took place in Sevilla and Doñana, Southwest Spain, between September and November, 2006. The objective of the Seminar Series was to review current knowledge on Iberian lynx biology as it relates to conservation breeding efforts and to share knowledge and experiences with experts on related fields of wild cat biology and conservation. The opening session counted with introductory speeches from representatives of international (IUCN- CatSG), national (Ministry of the Environment of Spain and Fundación Biodiversidad), and regional (Andalusian Environmental Counsil) administrations, followed by overview presentations on Iberian lynx in-situ and ex-situ conservation efforts. The seminar was structured in four sessions, each lasting two days. The first session covered Veterinary and Health Issues, the second versed on Genetics, Behavior and Husbandry Aspects, the third session discussed Reproductive Physiology Aspects, and the final session reviewed Reintroduction Aspects. Following are the abstracts from the talks that were presented at the four sessions.

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Introduction to the Iberian Lynx Seminar Series

INTRODUCTION TO THE IBERIAN LYNX SEMINAR SERIES: OVERVIEW OF THE VARIOUS DISCIPLINES INVOLVED IN THE EX-SITU CONSERVATION PROGRAM

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(*FIND ORIGINAL SPANISH VERSION IN APPENDIX I)*

Endangered species breeding programs are costly conservation tools that require careful planning to guarantee their integration into the overall species conservation strategy. The first responsibility of any ex-situ conservation program is to not interfere with ongoing in-situ conservation efforts. Government support, funding, and the infrastructures required to carry out the program must all be secured and in place prior to the launching of the program. Program planning should include both short- and long-term goals, together with objectives and actions for genetic and demographic management, health aspects, reproductive physiology, and the various husbandry aspects that could affect the captive population. Following is a summarized description of the Iberian Lynx Ex-Situ Conservation Program, and its progress to date.

Program Organization and Goals

The Iberian Lynx Ex-Situ Conservation program is conceived as a multidisciplinary effort, integrated within Spain's National Strategy for Conservation of the Iberian Lynx, with the cooperation of regional, national, and international organizations. In 2003, the National Ministry of Environment signed a "Memorandum of Understanding" with Andalusia's Regional Ministry of Environment to promote "…A single program of coordinated actions to implement the National Strategy for Conservation of the Iberian Lynx in Andalusia". As of July 2003, all actions proposed for the Conservation Breeding Program are subject to approval by a Bilateral Commission formed by representatives of both national and regional governments.

According the Bilateral Commission mandate, the organizational structure of the Iberian Lynx Conservation Breeding Program involves an executive director and a multidisciplinary Species Committee of an advisory nature. The Species Committee, which includes representatives from 15 national and international organizations, is responsible for task planning. Its mission is to implement the actions contained in the Iberian Lynx Captive Breeding Action Plan, approved by the National Ministry of Environment and Andalusia's

Regional Ministry of Environment in 2004. The Committee receives input from experts in husbandry, reproduction, genetics, small population management, health and veterinary issues, and in-situ conservation.

The Species Committee follows the model of European breeding programs (EEPs), and all the animals incorporated into the program are included in the Iberian Lynx studbook. Optimal pairings are established using the program PM2000, which also helps determine other specific objectives.

The two main goals of the Ex-situ Conservation Program are:

1. Ensure the conservation of the genetic material of the species

2. Create new populations of Iberian Lynx, in the medium to long term, through reintroduction programs

Managing the Genetics and Demographics of the Conservation Breeding Program

The best genetic management of endangered captive populations is achieved through rapid population growth until the established number of individuals required to maintain genetic variability for the species in question is reached. Afterwards, population size should be stabilized. In order to achieve this approach, the production of new individuals must be properly paced to meet both reintroduction and breeding program needs, since the latter will gradually require replacements of older individuals past their reproductive prime. Genetic and population management must be accompanied by proper husbandry, which involves stimulating natural behaviors in captive born individuals from early developmental periods in order to improve their potential for survival in the wild.

Based on recommendations provided by the IUCN Conservation Breeding Specialist Group, in consonance with the Iberian Lynx In-situ Conservation managers, the actual situation of this species will allow for the conservation of 85% of the current genetic variability for a period of 30 years (Lacy and Vargas, 2004). Captive populations that maintain less than 85% genetic variability are considered dangerously inbred, and therefore genetically unacceptable.

In order to achieve the established genetic goals, four cubs must be incorporated each year for five consecutive years. Thus, 20 cubs/juveniles are needed within a five year period. Maintenance of genetic diversity over 30 years will, therefore, require a group of 60 (30.30) breeders (comprised by the original founders plus individuals born in the breeding program). As a basic strategy for maintaining genetic variability, it is important

to achieve rapid population growth over the first 10 years of the program, until it reaches its "capacity phase", established at 60 breeding individuals. Efforts should also be made to ensure equal representation of founders, who should all provide a similar number of offspring to the program.

Captive Husbandry

Captive husbandry is based on multidisciplinary input from a variety of animal care fields such as nutrition, behavior, genetics, physiology, and veterinary medicine, together with the systematic use of the scientific method. Over the past two decades, a great deal of knowledge and experience has been gained in the management of wild felids in captivity. The American Zoo and Aquarium Association's (AZA) Felid Taxon Advisory Group (TAG) has compiled a Husbandry Manual for Small Felids with useful information on health, reproduction, nutrition, facilites, etc. (Mellen & Wildt, 1998). Many European zoos have broad ranging experience in breeding wild cats in general and lynxes in particular. These documents and experiences have been and continue to be a very useful reference to the Iberian Lynx Captive Breeding Program.

One of the Program's key husbandry challenges is to strike a balance between fostering natural behaviors in captivity (hunting, territoriality, social interactions, etc.) and creating a stress-free environment where animals are more prone to mate. In order to get important information about the animals (such as their weight or determining whether or not the females are pregnant), certain training techniques are being used. These techniques are designed to avoid using invasive methods, which would stress the animals, and they also serve as a way to strengthen the bond between the animals and their keepers.

The animals' behaviour is also being carefully observed by a round-the-clock video vigilance system, which provides a great deal of information on the species that could not be easily learned through observations in the wild. Based on the experience acquired at "El Acebuche" Center and at the Jerez Zoo, together with information obtained from programs established at European and American zoos, El Acebuche Breeding Center has developed a "Standard Operational Procedures" manual that details the various protocols that are applied to its breeding population (for more information, see: **http://www.lynxexsitu.es/documentos/manejo/pfcc.pdf**). Detailed protocols based on experiences at the "pilot facilities" will help smooth the way towards unified practices across ex-situ lynx breeding centers as new centers open.

Health Issues

The health considerations involved in captive breeding, reintroduction, and translocation programs are a source of great concern to conservation biologists. There have been various cases in which captive-bred animals reintroduced into the wild have transmitted

infectious diseases to wild populations. Wild-caught individuals have also occasionally infected captive populations with potentially lethal diseases. It is generally felt that most of the programs affected were lacking sufficient information on: 1) Disease distribution and risk in captive populations; 2) Disease incidence, distribution, and risk in wild populations; 3) Quarantine systems to prevent disease transmission; and 4) A system to adequately track and detect pathogens.

Since relatively little is known about the diseases affecting lynx, actions to improve our knowledge of the main diseases affecting the species are imperative. The Iberian Lynx Conservation Breeding Program has a Veterinary Advisory Team dedicated to diverse aspects of veterinary and research management, as well as protocol development. To tackle the understanding of the various diseases that potentially affect the species, the Program's main lines of action involve the establishment of preventive disease protocols for the captive populations, and research on general veterinary science.

Projects are now underway on the incidence and prevalence of infectious pathogens in captive and wild lynx populations, determination of normal vs. pathological blood values, and research on potential renal dysfunction. The results of research, protocol developments, and standardization efforts, coupled with dissemination and sharing of knowledge and experience among veterinarians working in the program are all contributing to more consistent diagnosis and treatment. For further information, see http://www.lynxexsitu.es/aaveterinaros/aaveterinarios.htm)

Reproductive Physiology

Reproductive physiology studies increase the success rate of any captive breeding program, and are important in helping with the conservation of wild, captive felids. The Iberian Lynx Conservation Program established a Biological Resources Bank for conservation of biomaterial gathered from wild and captive populations. In order to conserve the maximum possible genetic diversity, samples of male and female gametes, as well as different cells or tissues, are being kept. These samples can be easily transported and stored, and used in the future to exchange genetic materials between individuals from the captive breeding program, between the wild populations, between captive populations and, when advisable between individuals from the wild populations. The conservation of gametes allows us to extend future options without the limitations of space, or the risk of disease transmission. Also, the cryopreservation of gametes and embryos allows for the opportunity of prolonging the possibilities of reproduction for individual animals after their death. The preservation of somatic cells (or undifferentiated germ cells) could give individuals who have died before reaching sexual maturity a reproductive opportunity, or extend the reproductive potential of other individuals.
The Iberian Lynx Biological Resource Bank is presently being maintained at two locations; the Museum of Natural Sciences in Madrid and the Miguel Hernández University in Elche,

Alicante. Although the Museum of Natural Sciences specialises principally on reproductive samples and the University MH specialises in multipotential somatic cells, both banks preserve tissue, blood, serum, and other biological materials. The storage of these samples means that materials will be available for future analysis whenever needed.
Another important outcome of Iberian Lynx reproductive physiology studies is the development of non-invasive techniques that aid in captive population management. Over the past two years, work carried out to define Iberian lynx male and female hormonal profiles has helped us gain a clearer perspective on the length of breeding periods, and the potential use of hormonal metabolites in feces as a non-invasive gestation predictor. While not the best diagnostic tool, it has proven extremely useful to better understand the year-round reproductive activity of male and female Iberian lynx. Another gestation diagnostic technique, based upon analyses of relaxin in urine, is now being researched at the Institute for Zoo and Wildlife Research in Berlin. The non-invasive techniques described above provide a great deal of information, while not disturbing the animals under study.

Reintroduction

The greater the number of captive-bred lynxes produced and trained to maximize their potential for post-release survival, the lower the number of wild lynxes that must be captured to establish new populations, or to reinforce the existing ones. Reintroduction and translocation each pose advantages and disadvantages. A comparative study is required to determine which option –or combination of options- is most appropriate for the conservation of the Iberian Lynx.

Prior to any reintroduction/translocation a detailed viability study is required (see IUCN "Guidelines for Reintroductions"). 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 the habitat is currently prepared to support a viable population of the species.

All reintroductions and translocations must be performed using the scientific method. Such conservation techniques require an interdisciplinary approach, with input from experts in ecology, veterinary medicine, physiology, and behavioral sciences, as well as support from socio-political and information sciences. All stages of program development and implementation must have well defined protocols that document objectives, methodology, responsibilities, as well as the accountability of the organizations and individuals involved.

Communication, awareness and training

Awareness, education, and scientific training are essential to all conservation breeding programs. 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 programs, 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 of the need for habitat conservation to guarantee survival of the species in the wild is one of the Program´s primary objectives. The message is: breeding and keeping lynxes in captivity, with no hope of returning ever returning them to the wild or of recovering the natural population would be a pointless exercise. The Breeding Program encourages, cooperates with and supports media interest in the Iberian Lynx, while taking every opportunity to remind the public of the primary importance of in-situ conservation work.

We also share on-line information on the Program's web page (http://www.lynxexsitu.es), featuring monthly newsletters, pictures and videos of all captive lynxes, along with general interest and scientific articles, and descriptions of the Center's protocols and working methods. An English language version is currently in the works, to further expand the scope of communication and awareness efforts. The web page also contains an area accessible only managers, researchers, and technical personnel working directly with the Program, for restricted data base access and other information exchange.

As part of its training efforts, the Acebuche Center Breeding Program organizes on-site internships for recent college graduates interested in acquiring first hand knowledge within an endangered species conservation program. The Program further organizes talks and seminars, such as this one, on Ex-Situ Conservation of the Iberian Lynx.

The purpose of this seminar is to provide training to technical personnel, researchers, and managers directly involved in Iberian Lynx conservation, and also to offer an educational opportunity to university graduates considering working in the conservation of endangered species in Spain. It is also a vehicle for information exchange among specialist working on national and international wild felid conservation programs.

ACKNOWLEDGMENTS

Sincere thanks to the Fundación Biodiversidad for making the "Ex-Situ Conservation of the Iberian Lynx" seminar possible. The Iberian Lynx Ex-situ Conservation Program is presently possible thanks to the collaborative agreement signed between Spain's National Ministry of Environment and the Regional Ministry of Environment of the Andalusian Government. Over 26 different institutions collaborate in the program, including the International Union for the Conservation of Nature (Cat Specialist Group and Conservation Breeding Specialist Group), the Jerez Zoo, the National Scientific Research Council (Doñana Biological Station, the National Museum of Natural Sciences,

and Almeria Arid Zones Station), the Seville Diagnostic Analysis Center, the Autonomous University of Barcelona, the Miguel Hernández University, the Complutense University of Madrid, the University of Huelva, the University of Cordoba, the University of Zurich - Vet Clinical Laboratory (Switzerland), the Smithsonian Institution (USA), the Terra Natura Foundation, the Fuengirola Zoo, the European Association of Zoos and Aquariums, the Iberian Association of Zoos and Aquariums, the SEO/Birdlife and Doñana National Park volunteer programs, the Andalusian School of Biologists, and the Life-Nature Project for Iberian Lynx Conservation in Andalusia.

Thank you!

Veterinary Aspects

Applied to Iberian Lynx Conservation Efforts

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Iberian Lynx Health Program

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(FIND ORIGINAL SPANISH VERSION IN APPENDIX I)

Animals living in declining populations, such as the Iberian Lynx, are particularly susceptible to stochastic processes, including some diseases that can decimate populations, and lead them to extinction. Health programs are therefore needed for both in situ and ex situ management of these populations.

The Iberian lynx health management program was developed around the three health objectives established in the Iberian Lynx Captive Breeding Action Plan: Keep the captive population healthy, investigate health risks affecting the overall lynx conservation program, and avoid disease transmission between wild and captive populations. Five actions were planned to achieve these goals: Form a lynx health advisory group, standardize health care protocols, design emergency plans, develop a health study on Lynx and associated fauna and evaluate the safe and effective use of drugs and vaccines.

The Lynx Health Advisory Group (GAAS) is a multidisciplinary team of veterinarians with clinical experience in domestic animals, zoo medicine, wild fauna, and research, who volunteer their services to the Program. The GAAS has developed anesthesia and management protocols for live animals, and also necropsy protocols to provide biological material for scientific use.

Between November 2003 and August 2006, 63 anesthesias were performed on 40 animals,

24 of them from the breeding program. Normally, a combination of ketamine and medethomidine was used (tilethamine with zolazapam in 10 examinations of males for electro-ejaculation). When necessary, anesthesia was prolonged with isofluorane. The purposes of anesthesia varied: quarantine check-ups, periodical check-ups, radio collar fitting, follow-up screening, other therapeutic purposes, and animal transfer.

A total of 22 necropsies were performed during the same period. The majority of wild animals examined had been killed by vehicles (10 cases). No cause of death could be determined in 5 other necropsies performed on bone remains. One of the animals died of tuberculosis and another tested positive for CDV (Canine Distemper Virus)after PCR testing of blood and feces. Of 5 captive animal deaths: 1 was due to a fight between cubs, 1 was a premature still-born cub, and 1 a premature cub that survived less than 24 hours, 1 young female due to acute clostridium induced endotoximia, and 1 cub due to interstitial pneumonia and septicemia.

A preventive medicine plan for the lynx breeding program population reduces the risk of disease development or entry. Quarantines, periodical fecal analysis (parasitological and microbiological), worming, use of dead vaccines, check-ups, diet controls, facility reviews and 24 hour video surveillance have proven to be the most effective measures. The medical problems observed in the captive population that will be discussed in this presentation include: Vomiting that affected several animals, one for a prolonged period, an osteomielitic abscess on the extremity of one animal, an apical abscess on a cub, one case of humid dermatitis and two cases of anemia.
There currently are 23 captive Iberian lynxes, all in good health.

The systematic collection and processing of biological samples by participating institutions, and cooperative multidisciplinary work are helping expand the knowledge base on the specie's health, which is essential to improve both ex situ and in situ management.

All information gathered from animals tested and procedures performed is registered in a biomedical data base created for the species. The data base enables us to cross reference all health information on the animals, and is a fundamental tool in the management, research and conservation of the Iberian lynx.

Hematological reference values and critical difference of selected parameters for the Iberian Lynx using a flow cytometer laser analyzer (Advia 120).

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Determination of basal-line hematological and biochemical values for an animal population is important for biological studies and for the interpretation of laboratory data. Reference values are derived from an observed distribution of measurements of the parameter in an appropriate group of animals and containing the central 95% of the distribution. Another way to assess if an analytical result is normal for an individual is with the Critical difference (Dk) or Reference change value (RCV) in which the patient serves as its own reference using comparison of analytical results from samples obtained serially at appropriated intervals. The objective of this study is to describe the reference values for the analytical parameters obtained by the laser blood cell analyzer ADVIA 120 and introduce the use of the critical difference on repeated measumements.

Blood samples from 34 clinically healthy Iberian lynx (1 to 7 years old), 13 male and 18 females were studied. Complete blood counts were made using the laser blood cell analyzer ADVIA 120 (Bayer diagnostics, SA.) within 18 hours from extraction. The present of Cytauxzoon felis were studied by PCR techniques by another laboratory and by routine blood smears stained with Diff-Quick®.

All hematological parameters obtained by the ADVIA 120 followed a normal distribution but MCH, MCHC, RDW, HDW, % and Abs LUCS, % and Abs Basos, Lob-index, reticulocyte count, reticulocyte % , PLT clumps count and PLT clumps %. A T-student and U- Mann Whitney test was used to check for statistically significant differences between females vs males and Cytauxzoon sp positive vs negative animals. A nested ANOVA test was used to determine the variance within animals (S^2 within), between animals (S^2 between) and analytical variance (S^2 analytical). The following formula was used to determine the critical difference of selected parameters

$dk= 2x\ (2x(S^2 within+S^2 analytical)^{1/2}$

The index of individuality was calculated as follow $II= (S^2 within + S^2 analytical)^{1/2} / S2between^{½}$. If II was lower than 0.6 the specific interval of individuals is better than population one based on reference values.

No statistically significant differences were by sex or Cytauxzoon were observed for any

of the studied parameters. Table 1 shows the mean, DS, minimum, maximum value and percentiles 2.5, 50, and 97.5 from the 34 Iberian lynx. The reference values obtained in this paper are similar to that described for the same specie and other Felidae. The critical difference and Individuality index can be used in conjunction with the reference values in the study of repeated data, however larger numbers of animals are needed to be studied in order to know if the critical difference performs as expected.

Keywords: Iberian lynx, reference values, critical difference, ADVIA 120

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Table 1 shows the mean, Standard Deviation (SD), minimum, maximum value and percentiles 2.5, 50, and 97,5 from the 34 Iberian lynx.

	N	Mean	SD.	Minimun	Maximun	Percentiles (2.5; 50 and 97.5)		
WBCB(x10E03 cells/µL)	34	11.06	6.11	3.53	30.59	3.53	10.00	30.59
RBC(x10E06 cells/µL)	34	8.58	1.26	4.40	10.47	4.40	8.67	10.47
measHGB(g/dL)	34	12.70	1.60	8.00	15.40	8.00	12.95	15.40
HCT(%)	34	41.19	6.32	20.80	51.60	20.80	42.05	51.60
MCV(fL)	34	48.02	2.52	43.80	55.50	43.80	48.30	55.50
MCH(pg)	34	15.01	2.76	11.40	29.60	11.40	14.40	29.60
MCHC(g/dL)	34	31.31	5.81	26.00	62.60	26.00	30.45	62.60
CHCM(g/dL)	34	28.37	1.68	24.60	31.40	24.60	28.45	31.40
RDW(%)	34	16.12	1.86	14.70	25.10	14.70	15.60	25.10
HDW(g/dL)	34	2.01	0.69	1.59	5.67	1.59	1.84	5.67
%NEUT(%)	34	68.10	17.46	29.00	91.60	29.00	71.25	91.60
%LYM(%)	34	25.17	15.62	5.80	62.10	5.80	21.45	62.10
%MONO(%)	34	2.55	0.99	0.90	4.80	0.90	2.40	4.80
%EOS(%)	34	3.74	3.12	0.00	12.20	0.00	2.75	12.20
%LUC(%)	34	0.22	0.29	0.00	1.70	0.00	0.20	1.70
%BASO(%)	34	0.22	0.23	0.00	0.90	0.00	0.10	0.90
Abs_neuts(x10E03 cells/µL)	34	8.25	6.24	1.56	28.03	1.56	6.80	28.03
Abs_lymphs(x10E03 cells/µL)	34	2.17	1.33	1.00	8.33	1.00	1.79	8.33
Abs_monos(x10E03 cells/µL)	34	0.26	0.13	0.05	0.58	0.05	0.26	0.58
Abs_eos(x10E03 cells/µL)	34	0.34	0.28	0.00	1.02	0.00	0.23	1.02
Abs_lucs(x10E03 cells/µL)	34	0.03	0.04	0.00	0.21	0.00	0.01	0.21
Abs_basos(x10E03 cells/µL)	34	0.02	0.02	0.00	0.09	0.00	0.02	0.09
lob_Index([No Units])	34	0.37	0.91	0.00	2.75	0.00	0.00	2.75
MPXI([No Units])	34	12.90	6.99	-2.50	37.20	-2.50	12.80	37.20
#Retic(x10E09 cells/L)	34	36.07	41.37	12.10	195.20	12.10	23.00	195.20
%Retic(%)	34	0.42	0.44	0.10	2.00	0.10	0.30	2.00
PLT(x10E03 cells/µL)	34	347.68	122.11	86.00	702.00	86.00	336.50	702.00
MPV(fL)	34	13.47	3.20	10.10	25.70	10.10	12.80	25.70
PDW(%)	34	66.84	6.54	55.60	85.80	55.60	65.25	85.80
MPC(g/dL)	34	21.76	1.97	16.70	24.20	16.70	22.00	24.20
PCDW(g/dL)	34	4.90	0.42	4.00	5.70	4.00	4.95	5.70
MPM(pg)	34	2.51	0.32	2.05	3.36	2.05	2.46	3.36
PMDW(pg)	34	1.12	0.13	0.90	1.47	0.90	1.12	1.47
# Plt Clumps	34	976.32	1502.89	202.00	9011.00	202.00	578.00	9011.00
% Plt Clumps([No Units])	34	14.67	21.18	3.01	123.90	3.01	8.65	123.90

Biochemistry Reference Values for Iberian Lynx (*Lynx pardinus*)

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(FIND ORIGINAL SPANISH VERSION IN APPENDIX I)

Introduction

In recent years, particularly since the launch of the Iberian Lynx (Lynx pardinus) Conservation and Captive Breeding Plan, a variety of studies have been performed to expand the knowledge base on this emblematic species. However, few have involved evaluations of physiological and pathological alterations based on blood parameters (Beltran et al., 1991).

The objectives of this paper are to (a), establish reference values for 20 biochemical blood parameters and (b) determine the statistically significant differences of these based on selected variables (age, sex, origin and wild/captive status).

Material and Methods

Since 2004, the CAD has analyzed 31 plasma samples obtained from 31 Iberian Lynx (Lynx pardinus), taken from the populations living in Sierra Morena (15 individuals) and the Acebuche Captive Breeding Center (2 individuals). Eighteen samples (8 male, 10 female) were taken from captive individuals, and 13 (8 male, 5 female) from animals recently captured in the wild.

The biochemistry analysis was performed using the RA115000 – CLIMA MC 15 (Manufactured by RAL S.A. Spain. EC). The parameters determined via colorimetric reactions are: Glucose, cholesterol, triglycerides, uric acid, total proteins, albumin and phosphorous; quantified via kinetic reactions were: gamma glutamyl transferase (GGT) alkaline phosphatase (ALP) lactate dehydrogenase (LDH), urea, creatinine, aspartate aminotransferase (AST/GOT), alanine aminotransferase (ALT/GPT), creatine kinase (CK), pancreatic amylase, lipase and cholinesterase. Iron (FE) and calcium (CA) were determined using differential colorimetric techniques.

To establish the reference values, of the 48 samples analyzed, only samples from apparently healthy individuals (as determined by health care personnel) were used.

The samples analyzed were taken from lynxes born between 2000 and 2006. Although 30% of the animals were born in 2004, the majority of the serums (n=20) were obtained the following year. The sex ratio and age cohorts were very similar (1:1) in all samples.

Statistical analysis was performed using Windows SPSS, version 13.0. To determine parameter distribution normality, the Kolmogorov-Smirnov test was used. Parametric tests were also used to establish the mean differences among blood parameters and variables such as age, sex, animal origin and wild/captive state. Levene´s test was used to check homeostasis of the study variables, and when applicable, ANOVA testing was done. When variances were unequal, Welch´s robust test of mean equality was used. The significant minimum value set for all tests was 0,05.

Results and Discussion

Analysis of the 20 study parameters (Table 1) reveals findings similar to those reported by authors of previous feline species studies (Fuller and coll., 1985; Weaver and coll., 1995). The different parameter means observed in some cases could be due to extrinsic factors (sample size, individual management, habitat, sample collection) or intrinsic factors (stress, drugs, individual idiosyncrasies) (Marco and coll., 2000).

Statistically significant mean differences (± SD) were found in male / female albumin and CK serum levels (P = 0.017 y 0.030 respectively). Females had higher albumin serum levels than males (4.21 ± 0.77 vs. 3.54 ± 0.69), unlike results reported by Marco et al., 2000 in wildcats (Felis silvestris). However, for CK (UI/L) the reverse was true: males (940.27 ± 677.28, n= 15) had much higher concentrations than females (472.69 ± 300.7, n= 17). Similar studies conducted on bobcats (Felis rufus) have found higher ALP and glucose levels in females (Fuller and colls., 1985; Tocan and colls., 1985).

In age-based comparisons, young individuals had significantly higher levels of ALP and LHD (P = 0.00 and 0.04 respectively) while adults had higher concentrations of GGT and creatinine (P = 0.03 and 0.02 respectively). Beltrán and colleagues (1991) also found higher ALP levels in young Iberian Lynxes, as did Weaver et al., (1995), in Canada Lynxes. Higher ALP values in young individuals are directly linked to osteblastic growth. Higher creatinine concentrations in adults, also reported by Beltrán et al., (1991) and Meyer and colls., (1992), describe age-based serum creatinine correlation.

Individuals from Doñana National Park have higher triglyceride concentrations than Sierra Morena born cats (P < 0.05) (39.62 ± 21.54 vs. 24.13 ± 10.95) GGT (9.07 ± 4.76 vs. 4.47

± 1.73) and GOT (113.5 ± 120.3 vs. 49.67 ± 37.5).

Comparison between wild living (n=13) vs. captive (n = 18) population samples revealed significant differences over several parameters. Captive lynx values were higher in iron, calcium, ALP and creatinine (P<0.05). This can be explained by the differences between captive and wild living lynx diets.

Wild living lynx had higher GOT (131 ± 118.87 vs. 40.29 ± 22.28) and GPT (80.77 ± 41.75 vs. 49.12 ± 33.37) levels. These high enzyme values can be attributed to physiological manifestations of capture related stress (Marco et al., 2000).

Values	N	Mean	SD	Range	
Glucose (mg/dl)	10	132,9	69,2	39	250
Cholesterol (mg/dl)	30	206,1	103,2	80	488
Triglyceride (mg/dl)	31	31,4	18,1	8	82
Uric Acid (mg/dl)	31	0,4	0,4	0	1,6
Total proteins (gr/dl)	31	8,2	1,4	5,3	10,8
Albumin (gr/dl)	31	3,9	0,8	2,3	6,2
Phosphorous (mg/dl)	31	7,4	1,9	3,9	11,3
FE (mcg/ml)	28	73,4	36,0	13	134
CA (mg/dl)	31	8,5	2,0	5,1	12,9
GGT (UI/L)	30	6,6	4,1	2	17
ALP (UI/L)	31	120,5	68,7	28	286
LDH (UI/L)	16	415,6	533,0	78	1843
Urea (mg/dl)	31	64,5	27,9	10	151
Creatinine (mg/dl)	31	1,7	0,7	0,6	3,3
GOT (UI/L)	30	79,6	90,6	19	385
GPT (UI/L)	30	62,8	39,9	20	138
Pancratic amylase (UI/L)	31	875,2	253,9	404	1359
Lipase (UI/L)	29	11,8	2,8	8	21
CK (UI/L)	28	723,2	578,3	4	2691
Cholinesterase (UI/L)	27	6461,8	2461,6	2034	14209

Table 1. Biochemical Parameters of 31 Iberian Lynx Samples

Pathology of the Iberian lynx: up to date on membranous glomerulonephritis, lymphoid depletion, and lymphocyte subpopulations

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ABSTRACT
From the necropsies and samples of Iberian lynxes collected since the year 1998, certain histopathological lesions were notable because of their frequency and presence even in apparently healthy animals killed in car accidents. These lesions were mainly glomerulonephritis and lymphoid depletion with different grades of severity. A deeper study of these aspects was required in order to discover the pathogenesis and possible clinical repercussion.

Glomerulonephritis in the Iberian lynx.

To study the glomerulonefritis right and left kidney samples from 26 Iberian lynxes necropsied between the years 1998 and 2005 were fixed in formalin, processed for histopathology, paraffin embedded and stained with hematoxilin-eosin, PAS (Periodic Acid Schiff reagent), Masson's trichromic, Congo Red and silver (methenamine silver) stains. The animals came from the wild (n=21) or from captivity (n=5), with ages that ranged from 44 days to 17 years, 14 were females and 12 males. The main cause of death was by car accidents, followed by squamous cell carcinomas, tuberculosis, traumatisms and fights. The animals had resulted negative for infectious agents usually related to membranous glomerulonephritis with PCR and/or serologic analysis with the exception of one animal that was positive for FeLV in serology, and two that were FIP positive in serology without histological signs of the disease.

The same samples were used for immunohistochemical detection of laminin, fibronectin and type IV collagen in the glomerular capillary basement membranes and IgM, IgG, and IgA for immune complex determination. Ten of the kidney samples were selected and processed for the ultrastructural study. Three representative cases were chosen for immunogold labbeling against IgM.

Urine analyses from 10 of the necropsied Iberian lynxes were available and revised for this study, while blood and urine samples from live animals in Doñana National Park and Sierra Morena were included since October, 2004.

The histopathological, immunohistochemical and ultrastructural studies revealed the presence of membranous glomerulonephritis (MGN) in all of the animals, the severity increased with age, with deposits compatible with immune complexes. Gomerulosclerosis was considered an end-stage of the lesion. The urine analyses of the 10 necropsied Iberian lynxes revealed a high prevalence of hypostenuria and proteinuria. These findings were also common in the analyses from live animals. Proteinuria is the basic analytical finding when there is glomerular damage and the hypostenuria suggests filtration impairment[1,2]. Proteinuria together with other altered parameters suggests the existence of a compensated chronic renal disease.

Membranous glomerulonephritis is a common finding in cats. It is frequently related to infectious agents, specially FeLV, FIV, FeCoV, and *Mycoplasma gateae*. It can also be associated to neoplasms, intoxications (methyl mercury), familial diseases, autoimmune pathologies, or it is idiopathic (most of the cases)[3]. All of the Iberian lynxes presented some degree of membranous glomerulonephritis regardless of their age or sex, though the severeness did increase with age. It is not probable that it would be related with infectious agents since all but one of the animals were negative for PCR and serological detection. It is known that these animals possess a strong endogamy[4], therefore we could suspect of some sort of familial cause for this membranous glomerulonephritis. The presence of immune complexes confirms the immune origin of this pathology and could suggest an autoimmune affection. Further studies are required to confirm the origin of this glomerulopathy. Nevertheless, these findings should be considered if medical treatment or anaesthesia is needed. Part of this study has been presented in several meetings[5] and a paper is in process.

Depletion in lymphoid tissues.

The first purpose of the study of the peripheral lymphoid tissues was to evaluate the histopathological status of the these tissues and thymus of Iberian lynxes necropsied between the years 1998-2003. Seventeen animals including females (n=8) and males (n=9), age range from 10 months to 16 years, with different causes of death were histopathologically and immunohistochemically (anti-CD3, CD79, MAC387, CD68) studied. Feline immunosuppressive virus laboratorial tests were negative. Five individuals presented neoplasia and/or tuberculosis. All animals presented some degree of both B and T cells depletion in peripheral lymphoid tissues and follicular hyalinosis in the center of depleted follicles. A viral origin of the lymphoid depletion is postulated although other causes (inbreeding, stress, toxic) are not ruled out. This study has been published in 2006 by Peña et al[6]. The next step was to evaluate if these histopathological findings had

similar counterparts in circulating blood.

Cell immune response and lymphocyte subsets in the Iberian lynx.

We present values for the cell lymphoproliferative response and lymphocyte subsets of the Iberian lynx (*Lynx pardinus*). 39 blood samples were collected between November 2004 and July 2006. Samples were obtained from 30 animals of the Doñana and Sierra Morena lynx populations. 18 of the animals were captive and 12 were wild. *In vitro* lymphoproliferative response to concanavalin A and immunophenotype analysis of peripheral blood mononuclear cell subsets (CD4+, CD8+, CD5+ and B cells) was carried out.

Mean values were analyzed attending to both state of the animals and population. Analysis of mean lymphocyte numbers in lynx revealed no significant differences between captive or wild animals, as well as between the Doñana and Sierra Morena populations. Lymphoproliferation assays were positive in 68% of captured animals and in 76% of wild animals, while no differences of lymphoproliferative capability were observed attending to the origin of the animals.
Our study indicated that the immunological status of the Iberian lynx is similar both in wild and captive animals. The Sierra Morena and Doñana populations of lynx also showed a similar immunological status , A detailed study of the data obtained will permit to establish reference levels for the T cell function in the Iberian lynx

According to the bibliography reviewed, this is the first work that analyzes T cell functionality and lymphocytic subpopulations in blood using flow cytometry in any lynx species, therefore our results have been evaluated considering the parameters published for the domestic cat[15]. These results confirm the previous histopathological findings because they point out deficiencies (of more or less importance depending on the animal studied) in T (CD4+ and CD8+) and B cell representation in blood, especially in the CD4+ subpopulation. These results should be confirmed in the future by including a higher number of cases, though the study is highly representative of the existing Iberian lynx population (less than 200 animals)[7,8]. The loss of the effectiveness of the immune system could increase the vulnerability of the critically endangered Iberian lynx to pathogens.

Splenic follicular sclerosing vasculitis.

We also considered the follicular hyalinosis found in some depleted follicles. Folicular hyalinosis can be a response to systemic inflammations, septicemias or circulating immune complexes[1]. The severity of the follicular hyalinosis found in the lymphoid tissues of almost every dead Iberian lynx, encouraged us to focus in this particular lesion.

The following aim was to study the localization, distribution, severity and

immunohistochemical and ultrastructural features of these deposits, investigating its origin and possible relation with the lymphoid depletion. These studies have been presented in a recent meeting[9] and the full paper is being prepared.

A histological and immunohistochemical study was carried out in formalin fixed samples embedded in paraffin of peripheral lymphoid tissues (spleen, lymph nodes, mucosa associated lymphoid tissue) obtained from the necropsies of Iberian lynxes found dead or that had died in captivity (n=14) (1998-2003) with lymphoid depletion (B and T cells). A 44 day old Iberian lynx cub, born and accidentally killed in captivity was included. All the animals included in this study were negative for various infectious agents including imunosupressing viruses. The grade of hialinosis/tissue/animal was established and the immunohistochemical expression of actine, VIII factor, type IV collagen, laminin, fibronectin, type I collagen, type III collagen, type VIII collagen, and feline IgA, IgM and IgG was evaluated. The ultrastructural study was done from samples fixed in formalin (n=10). The white pulp was selected along with the follicular arteriole and hyaline substance. Immunogold labelling was done for IgM in 3 samples of spleen previously embedded in Lowicril® resin.

The hyaline deposits were only detected in the periphery lymphoid tissues studied. The most affected organ was the spleen. The diverse techniques used demonstrated the vascular basement membrane origin of the hyaline, located in follicular capillaries. The centrofolicular arterioles showed thickened basement membranes. A progressive collagenization was observed determining two phases: hyalinosis and vascular sclerosis. In the initial phase of hyalinosis, the deposist expresses type IV collagen mostly. Fibronectin, type III collagen and type VIII collagen are intensely expressed in the sclerotic vessels. There was variable expression of the immunoglobulins that were analyzed. The electronic microscopy revealed the existence of linear electron dense bodies (compatible with immune complexes) in the endothelial basement membranes of the follicular arterioles (n=9/10), but not in the capillaries. Ultrastructurally, the hyaline substance corresponded with tissue rests or sclerotic capillaries. Degeneration of endothelial cells and muscular cells of the arterial wall were observed along with the surrounding lymphoid tissue. The immunogold labelling confirmed the presence of IgM in the vascular lesions analyzed.

There are few publications of follicular hyalinosis in any animal species. Vascular hyalinosis due to immune complexes has been described in humans[10] and in dogs[11]. The domestic cat frequently presents centrofollicular hyalinosis in the spleen frequently associated to feline panleukopenia[1]. Our study indicates the existence of a sclerosing vascular pathology affecting lymphoid tissues of the Iberian lynx, with thickening of vascular basement membranes and progressive collagenization. The defect of vascular tissues could be the origin of the before mentioned lymphoid depletion by causing the degeneration and death of lymphoid cells along with the decrease of circulating leukocyte populations. The findings of immune complexes in arterioles along with the membranous

glomerulonephritis described in these animals seem to indicate the existence of an immune complex disease of unknown etiology (autoimmune-genetic, infectious or toxic). Our results, along with the fact that the splenic vascular lesions were observed in the 44 day old cub born and raised in captivity, indicate that the most probable etiology is autoimmune, related to the endogamy described in this species. Future studies are required to confirm or rule out this hypothesis.

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Prevalence of selected feline pathogens in the Eurasian and Iberian Lynx

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In order to support the ex-situ conservation programme of Iberian lynx (Lynx pardinus) we have studied the prevalence of selected feline pathogens in this endangered species. Since December 2003 we have tested 46 free-ranging and captive Iberian lynxes. By applying established molecular and serological methods, a total of 71 whole blood samples, 127 faecal samples and 64 serum samples were analyzed. Furthermore, to address the question of reservoir and transmission of such feline pathogens in the European wild felids, as comparison we analyzed 177 Eurasian lynxes (Lynx lynx) from Switzerland and Sweden as well as 32 free-ranging European wildcats (Felix silvestris silvestris) from Switzerland and France.

Cytauxzoon felis and other piroplasms

C. felis is a piroplasm of the family of Theileridae that infects erythrocytes and macrophages of domestic cats and wild felids (bobcats, Florida panthers, Texas cougars, lions and Palla's cats). It is transmitted by ticks of the genus Dermacentor. Whereas in the domestic cat infection with C. felis causes anorexia, lethargy, jaundice, fever, dehydration and in most cases death, so far no signs of pathogenicity could be detected in infected wild felids[1-5]. Of the 46 lynxes tested by PCR[6], 16 (34.8%) were positive for C. felis. The specificity of the PCR test was confirmed by sequencing of the amplified product and comparison with known sequences of C. felis in the GenBank. Animals tested at different time points remained positive over a period of at least two years. In addition, nine out of 35 (25.7%) of the Swiss Eurasian lynxes and 10 out of 32 (31.3%) of the European wildcats tested positive for C. felis. As the animals did not show any clinical signs of cytauxzoonosis, we hypothesize that they may be resistent to the disease and constitute the natural reservoir for this piroplasm.

Iberian lynxes were also tested for the presence of other piroplasms, i.e. Babesia spp.

and Theileria spp. All tests were negative.

Feline haemotropic mycoplasmas

Haemotropic mycoplasmas (aka haemoplasmas, formerly Haemobartonella) are cell wall-less bacteria, which parasitize feline red blood cells. Transmission occurs by lice, ev. ticks, flies and mosquitos[7-10]. So far, 3 different species have been identified in the domestic cat with different pathogenicity: Mycoplasma haemofelis, which causes haemolytic anaemia, anorexia, depression and fever[11,12]; 'Candidatus Mycoplasma haemominutum', which causes mild clinical signs but no anaemia[13]; and the in Switzerland recently discovered 'Candidatus Mycoplasma turicensis' (closely related to Mycoplasma coccoides) that can also cause anaemia and depression[14]. More severe disease may occur in case of co-infection with pathogens that compromise the immune system, such as feline Leukaemia virus (FeLV)[15]. In wild animals, M. haemofelis was found in two anaemic captive tigers, nonetheless the presence of other pathogens was not excluded[16].

In the 35 Iberian lynxes tested by real-time PCR[14,17], we found 7 (20%) positive for M. haemofelis, 9 (26%) for 'C.M. haemominutum', 3 (9%) for 'C.M. turicensis'. Of the 36 Eurasian lynxes tested, 4 (11%) were positive for M. haemofelis, 14 (39%) for C.M. haemominutum and 2 (6%) for C.M. turicensis. In the 31 European wildcats the percentages of PCR positive animals were 3, 19 and 35% respectively. Three Iberian lynxes and 3 Eurasian lynxes were co-infected with both M. haemofelis and 'C.M. haemominutum'; 1 Iberian lynx, 1 Eurasian lynx and 4 European wildcats were co-infected with 'C.M. haemominutum' and 'C.M. turicensis'; furthermore 1 Iberian lynx and 1 European wildcat were concurrently infected with all three species. Remarkably, no animal was co-infected with M. haemofelis and 'C.M. turicensis' in the absence of 'C.M. haemominutum' infection[17]. Haemoplasma infections were found to be associated with wild felid species and non-captive state. No association was found with lower PCV values or clinical signs. The pathogenic potential of feline haemoplasma infections in wild felids is still unclear. Phylogenetic analyses show no clear-cut geographical grouping of isolates, although some clustering within wild felid species was observed.

Canine Distemper Virus (CDV)

CDV belongs to the genus Morbillivirus, family Paramyxoviridae (same Genus as measle virus, RPV, PPR, Hendra and Nipah). It is an enveloped ss(-)RNA virus with a diameter of 120-150 nm. It shows a wide host spectrum: Canidae, Mustelidae, Procyonidae, Hyaenidae, Ursidae, Viverridae and Felidae are susceptible to infection. Transmission of the virus is by direct contact and through aerosol. Replication takes place in the lymphoid cells. A strong immune response leads to control of disease. Persistent infection may lead to immune suppression, respiratory and clinical signs of the gastrointestinal

and central nervous system. Predisposing factors for disease are concurrent illnesses and/or immune suppression[18,19].

In the wild, a CDV outbreak in 1994 in a seronegative lion population in Serengeti (Africa) showed a high mortality rate (30%)[20]. Tigers and panthers were found to be seropositive[21], the Canada lynx (Lynx canadensis)[22,23] as well. Dogs, stone martens and possibly foxes may act as infection source in these cases.

In 46 Iberian lynxes tested, one (2.2%) animal found dead tested positive in whole blood and faeces by real-time PCR[20]. Eight out of 42 Iberian lynxes (19.1%) were seropositive by IFA[24]. The CDV seroprevalence was also assessed in Eurasian lynxes from Switzerland (24.3%) and from Sweden (0.97%). From previous observations we know that a positive titre can occur in absence of clinical signs (emaciation, neurological signs and abnormal lameness).

Other feline viral pathogens

To assess the animal's infectious status, samples from Iberian lynxes were also tested for the presence of: feline corona viruses (FCoV) by both IFA[25] and real-time RTPCR[26]; viruses of the feline respiratory disease complex (feline herpes (FHV) and calici (FCV) viruses) by both IFA[27] and real-time (RT-)PCR[28,29]; feline Panleukopenia virus (FPV) by real-time PCR[30] and IFA[27]; feline Leukaemia virus (FeLV) by determining FeLV provirus load by real-time PCR[31] and the p27 antigen by sandwich ELISA[32]; and feline immunodeficiency virus (FIV) by real-time PCR[33] or ELISA[34]. During the last months we also started checking faecal samples for the presence of Rotavirus. The results are compiled in Table 1.

One road-killed animal, male, about one year old, was found to be FeLV provirus positive. Viral RNA was detected only in regional lymph node and salivary gland but not in whole blood or bone marrow. Sequencing of LTR and part of ENV genes revealed it to be a FeLV subtype A (97% identity with Rickard strain). So far this is the first documented case of FeLV infection in European lynxes.

Other feline pathogens:

Furthermore, we tested the Iberian lynxes for: Bartonella henselae, the main agent of cat scratch disease (CDS). In the Iberian lynx we found a prevalence of 22% by real-time PCR[35]; Anaplasma phagocytophilum, a tick-borne pathogen that infects humans, ruminants, dogs, horses and red foxes (granulocytic anaplasmosis, formerly ehrlichiosis) and was recently described to be present also in domestic cats[36]. So far none of the Iberian lynxes tested for A. phagocytophilum was positive by real-time PCR[37] or by IFA (antigen from VMRD, Inc. Pullman, Washington, USA). None of the lynxes tested was found to be positive by real-time PCR[38] for Chlamydophila felis, a pathogen commonly associated with feline ocular disease.

Table 1: Feline Viruses in the Iberian lynx

Viral pathogen	Whole blood Positive/tested (%)	Faeces Positive/tested (%)	Serum Positive/tested (%)*
FCoV	0/46 (0)	0/45 (0)	12/45 (26.6)
FCV	0/46 (0)	n.p.	8/30 (26.7)
FPV	4/46 (8.7)	5/45 (11.1)	6/30 (20)
FHV	1/46 (2.2)	n.p.	10/30 (33.3)
FeLV	1/46 (2.2)	n.p.	0/46 (0)
FIV	0/46 (0)	n.p.	0/45 (0)
Rotavirus	n.p.	0/25 (0)	n.p.

*= only unvaccinated animals included in table; n.p. = not performed

In conclusion, European wild felids may be infected with feline pathogens such as Cytauxzoon felis, FCoV, FeLV, FPV, FHV, FCV, CDV, A. phagocytophilum, B. henselae and haemotropic mycoplasmas. The pathogenicity of these infectious agents is still unclear. These infections might not be a problem for free-living populations since wild felids usually have only little social contacts: disease prevalence is expected to be low, with no impact on the population dynamic. However, problems may arise when animals are kept together in captivity, where transmission of pathogens is enhanced due to confinement of many individuals. Furthermore, concurrent infections may act synergistically, and stress factors might favour the appearance of clinical symptoms. Therefore, vaccination may be considered.

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Pathogens and Pollutants in Wild and Domestic Mammals in the Iberian Lynx Distribution Areas in Spain

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Aim of the study

Endangered populations are highly sensitive to diseases due to their low genetic diversity. Other more abundant wild or domestic species that share habitat with them can act as reservoir of diseases that could lead to epizooties. This is one of the causes that can lead an endangered species to extinction. The Iberian lynx (*Lynx pardinus*) is the most endangered felid in the world and diseases could be a threat for its survival. The aim of this study, included into the "Iberian lynx sanitary survey" (Consejería de Medio Ambiente, Junta de Andalucía), was to survey wild and domestic mammals for several infectious, parasitic and toxic agents in the areas where the lynx is still present.

Material and methods

We surveyed 293 animals from June 2004 to June 2006 in Sierra Morena (Northern Andalucía) and Doñana (Southern Andalucía). They were 96 free-ranging (24 Iberian lynxes, 35 red foxes *Vulpes vulpes*, 24 mongooses *Herpestes ichneumon*, 11 genets *Genetta genetta*, 2 badger *Meles meles*, 1 polecat *Mustela putorius*) and 83 domestic (54 cats *Felis catus*, 29 dogs *Canis familiaris*) carnivores; and 114 wild ungulates (56 wild boars *Sus scrofa*, 52 red deers *Cervus elaphus*, 6 fallow deers *Dama dama*). Animals were surveyed: (1) Dead (mostly road-killed or hunted): they were necropsied to detect lesions and samples were taken from different organs for serology, histopathology, culture, PCR, parasitolgy, and toxicology; or (2) Alive (carnivores): they were anaesthetized before sampling (with the exception of dogs) and entire blood, sera and plasma samples; oro-pharingeal, conjuntival and rectal swabs; oropharingeal smears for IF; and faeces and urine were taken. Cats were either feral or owned but free-ranging. Dogs were hunting or keeper dogs.

Results

a) Carnivores

(1) Histopathology: the most common lesions found in cats were: lymphadenitis and lymphoid hyperplasia (42%); enteritis (24%); parasitic pneumonia (19%); other pneumonias (5%); or viral infection of upper respiratory tract (5%). Foxes presented tuberculosis (25%) and signs of canine distemper (25%). Lesions compatible with filarial parasitation were a common finding among mongooses. No lesions of concern where found in other small carnivores.

(2) Infectious diseases (culture, serology, PCR): *Salmonella* sp. was obtained from 12/52 (4/24 foxes, 4/11 mongooses, 1/4 genets, 2/8 lynxes, 1/2 badger, 0/1 polecat, 2/23 cats) organ pool cultures (23%). In these pools, neither *Mycobacterium* spp. (0/37) nor *Yersinia* sp. (0/2, only lynxes) culture was obtained. No *Leptospira* spp. culture was obtained from urine or kidney samples.

Antibodies in sera against the following pathogens were found: Parvovirus (cat 17%; dog 100%); CDV (cat 11%; dog 64.5%; fox 70%); FeLV (cat 18%); CHV (lynx 12.5%; mongoose 14.5%, fox 10%); *Erlichia canis* (dog 18%; fox 20%); *L. icterohemorragiae* (lynx 33%; cat 12.5%; mongoose 28.5%; dog 45.5%; fox 50%); and *L. canalicola* (lynx 16.5%; dog 27%). No antibodies were found against FIV, FCV, FHV, FCoV, *Chlamydophila felis* or *Mycobacterium bovis*.

The presence of pathogens in the swabs was demonstrated by PCR and/or direct ELISA: CPV in rectum (cat 17%; dog 36%); FPV in rectum (cat 5.5%); CDV in the oropharynx and rectum (dog 18% in both cases); *M. bovis* in the orofarinx and CHV in oropharynx and rectum (fox 10% in both cases).

Cytauxzoon felis was not detected by means of PCR from 48 entire blood samples (31 cats, 12 lynxes, 5 others).

(3) Helminthology: *Ancylostoma tubaeforme* was found to be very frequent in cats (53%, 11.75 per cat). Other helminths such as *Toxocara cati* (40%) were also frequent.

(4) Toxicology: Zinc, lead, cadmium, and copper were detected from liver, bone and muscle samples, and organochlorine pesticides and polychlorinated biphenyls from plasma and liver samples from foxes, lynxes, mongooses and genets. However, the concentrations were below those indicating chronic intoxication.

b) Wild ungulates

(1) Bovine tuberculosis: macroscopic lesions in lymphatic nodes compatible with BTb (caseification or calcification) were detected in 19% of red deers (another 73% showed lymphadenitis or lymphoid hyperplasia); 100% of fallow deer; and 52 % of wild boars (18% with lymphadenitis or lymphoid hyperplasia). These results,

together with histopathology and acid-fast staining (carried out only in suspicious animals), and serology, showed an overall prevalence of 22% in red deer, 50% in fallow deer, and 55% in wild boar. Differences were found in the prevalence of wild boars depending on the origin of the sample: animals sampled in hunting states from Sierra Morena showed a prevalence of 100%; 58% in Doñana National Park; and 0% in Coto de Almonte.
(2)
(3) Other serologyc analysis: Brucellosis: 0% of deers, 6% of wild boars; Paratuberculosis: 0% in all the species; Aujeszky's disease: 26% of wild boars.

Conclusions

These preliminary results showed that other abundant, sympatric wild or domestic carnivore species hosted pathogens that could be a threat for lynxes. It is of concern the high prevalence of canine distemper found in dogs and foxes, or the presence of FPV and FeLV in cats from the areas, specially when, at the light of the results, lynxes seem to be naïve to these agents.

Lynxes presented antibodies against canine pathogens such as CHV or *L. canalicola*. Although probably these agents are probably not a threat for them, these results indicates that lynxes may acquire diseases throughout interspecies relationships.

A. tubaeforme, which was reported as very prevalent and abundant in lynxes, may have domestic cat as reservoir.

Pollutants do not seem to be a threat for lynx nor other wild carnivores in the areas studied.

Wild ungulates presented a high BTb prevalence, mainly in the areas where their densities are high. They may be a source of infection for lynxes, although further studies are needed.

Seroprevalence of Aujeszky's disease showed that the virus is present in the lynx areas. However, PCR analysis of wild boars tissue samples are still not available.

Causes of Mortality and Diseases in Free-Ranging Eurasian Lynx from Switzerland – an Update

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Introduction

Eurasian lynx (*Lynx lynx*) was reintroduced in Switzerland in the 1970s. Nowadays, the population is estimated to almost 100 lynx and is considered as stable (Breitenmoser & Breitenmoser-Würsten 2004). For the past 20 years, Swiss lynx have been submitted to a health monitoring programme including the necropsy of all dead animals according to a standard protocol, and the physical exam and blood sampling of animals caught alive (Ryser-Degiorgis, 2006). Causes of mortality and diseases observed in Swiss free-ranging lynx have been reported in previous reports (Schmidt-Posthaus et al. 2002, Ryser-Degiorgis et al. in press). In this paper, we present an update of the knowledge on diseases affecting lynx in Switzerland. Additional data from serological and PCR studies are presented by Meli et al.

Mortality 2006

From January to August 2006, six fatalities were recorded. Four male lynx, two juveniles and two adults from the Jura population, were killed in a traffic accident between mid-January and February. One of the adult males killed by car was affected by a severe purulent pneumonia secondary to an infestation with *Aerulostrongylus* sp. He also presented a severe gastritis of unknown aetiology and an abnormality of the right kidney. This organ was half the normal size, round, but showed a normal internal structure. It was considered as a congenital malformation without clinical significance.

Two animals from the Alpine population died of a disease. A subadult female was caught alive close to houses and euthanized due to severe debilitation. The lynx was cachectic and had very pale mucous membranes. The fur around the anus and on the heels was soiled with diarrhoea. At necropsy, there was an accumulation of black pigment on the abdominal serosa and mesenterium (pseudomelanosis). The stomach was filled with a large hair bezoar of 20 cm length. Histologically, there was a severe alveolar lung oedema. In the heart, multifocal myocardial fibrosis partly associated with muscle cell degeneration, arteriosclerosis and lymphoplasmocytic infiltration were present. In the

liver, there was randomly distributed fibrosis and moderate plasma cell infiltration. Multifocal mononuclear inflammation associated with fibrosis and muscle cell degeneration was also present in all samples of skeletal muscle (neck, legs, abdominal wall, lumbal area). Lung alveoles and foci of inflammation in the heart, liver and skeletal muscles contained numerous macrophages filled with hemosiderin.

Blood analysis revealed anaemia, leucocytosis (neutrophilia and lymphopenia), hypoglycaemia, uraemia, and low serum albumin value. The animal was negative for Feline Leukaemia virus, Feline Coronavirus, Feline Immunodeficiency virus, but positive for *Bartonella henselae* and *Cytauxzoon* sp. Bacteriological examination of the internal organs was negative.
A tentative diagnosis of chronic inflammatory myopathy associated with similar histologic lesions in the myocard and liver parenchyma was made, and to date the etiology of the disease remains unknown.

A female caught as an orphan at the age of about six months in November 2005 was kept alone in captivity. In March 2006, the animal was anesthetized for a pre-release check. No abnormalities were observed, except for an obstipated colon, and a leucocytosis (neutrophilia and lymphopenia) that were attributed to stress. Colon was massaged by hand, and according to the animal keepers of the station, in the following days the lynx excreted a large amount of normal faeces. Faeces were then regularly found. At the end of April, the lynx was suddenly apathetic and anorectic. It had a shaggy fur, pale mucous membranes, and tail and hind legs were soiled with diarrhoea. Blood analysis did not reveal abnormal values. Saliva and anal swabs were taken for bacteriological, parasitological and virological examination. The lynx was negative for *Salmonella*, Feline Parvovirus, Coronavirus, Leukemia virus and Immunodeficiency virus. Parasitological examination revealed an infestation with *Isospora* sp., *Capillaria* sp. and *Toxocara* sp. that are intestinal parasites commonly found in Swiss lynx (Ryser-Degiorgis et al., in press). The lynx received a symptomatic treatment, anti-parasitic drugs and antibiotics. The animal seemed to recover but five days later the animal keeper observed tenesmus, and the lynx died during the night.

Necropsy revealed acute purulent peritonitis due to two perforations of the rectum within the pelvis cavity. The colon was obstipated with hard faeces. Postmortal radiological examination indicated a severe pelvis malformation with narrowing and asymmetry of the pelvis cavity. This was seen as the cause of obstipation and tenesmus that lead to intestine perforation through sharp faeces content. Another very similar case was recorded in 1998. The pelvis bone of this animal was kept at the Museum of Natural History in Berne and could be examined retrospectively: Interestingly, the bone presented a mild but obvious asymmetry. Thus, pelvis bone malformation might be a cause of disease and eventually death in juvenile lynx in the wild. However, no representative data are available on the mortality of juvenile lynx in Switzerland.

Furthermore, bacteriological examination revealed a septicaemia with *Yersinia pseudotuberculosis*. In domestic cats, *Y. pseudotuberculosis* is known to cause symptoms such as anorexia, apathethic behaviour, pale mucous membranes and diarrhoea with subsequent obstipation. Focal necroses of the intestinal wall are typical findings. In septicaemic courses, liver and spleen are swollen and present multiple white nodules. Therapy is usually unsuccessful (Weiss 2005). The absence of typical macroscopical lesions in spleen and liver in the lynx indicated a recent diffusion of the bacteria into these organs. Infection with *Y. pseudotuberculosis* occurs orally through the consumption of infected prey (rodents) or of contaminated food. The source of infection of the lynx is unknown, but infection must have occurred in captivity. Chronic pseudotuberculosis was observed in free-ranging Swiss lynx in the past (P. Boujon, pers. comm.), indicating that this bacterial disease occasionally occurs in Swiss lynx under natural conditions.

Heart study

In 2003, an adult male died of a circulatory failure attributed to a cardiomyopathy (Ryser-Degiorgis et al. 2004). Main histological lesions in the heart were extensive myocard fibrosis and severe arteriosclerosis. Since then, similar lesions were observed in several asymptomatic lynx (Ryser-Degiorgis et al. in press). Therefore, we started a retrospective histopathological study of the heart of free-ranging Swiss lynx.

From 1999 to 2006, heart samples of 38 lynx were available. In most cases, only small pieces from the left chamber wall were present, but in a number of lynx, the heart septum and the right chamber wall had also been sampled for histology. All slides were stained with haematoxylin-eosin (HE) and van Gieson (VG), and analysed by light microscopy in a blind study. For comparison, HE-stained slides of heart samples from 21 free-ranging lynx from Sweden were analysed in the same manner. For statistical analysis, lesions of the left chamber wall were considered. Myocardial fibrosis (MF) and arteriosclerosis (AS) were both graded from 0 (absent) to 3 (severe), and both scores were added. Mild lesions were defined as either mild fibrosis or mild arteriosclerosis; if both lesions were present, there were particularly mild (total score 0.5-1.5). When both features were clearly present but mild, or when only one of them was present but in a moderate to severe form (total score 2-3), lesions were considered as moderate. If both MF and AS were present in a moderate to severe form (total score 4-6), lesions were defined as severe. For 19 Swiss and 17 Swedish lynx, data were available on both heart weight and body weight. A heart index was calculated by dividing the heart weight (kg) through the body weight (kg), and multiplying the result by 100. Data management and descriptive statistics were conducted in Microsoft Excel 2000. All statistical calculations were performed with NCSS 2001 Statistical Software. Statistical significance of differences was analysed using Chi-Square Test, two-tailed Fisher's Exact Test, or Mann-Whitney Test.

Histopathological findings consisted in prominent AS of coronary arteries associated with varying degree of perivascular dissecting fibrosis and/or apparently randomly distributed foci of interstitial myocardial fibrosis (MF). The left chamber wall and the septum were generally more severely affected than the right chamber wall. Lesions were more prominent in the inner half of the myocardium. AS seemed more severe in the area of papillary muscles.

Overall, 25/38 (65.8%) Swiss lynx were affected. Prevalence in juveniles (33.3%) was significantly lower ($P<0.005$) than in subadults and adults (95.0%). In contrast, 5/21 (23.8%) Swedish lynx presented heart lesions, with a prevalence of 26.3% in subadult and adults. Difference of prevalence between Switzerland and Sweden was highly significant ($P<0.005$), especially if considering the subadult/adult age classes only. In both countries, males were more commonly affected than females. The number of animals analysed per country was low for a statistical analysis, but if both countries were pooled, the difference between sexes was statistically significant ($P<0.005$).

Lynx presented heart lesions independently of their cause of death, disease status and nutrition status. Lesions in affected Swedish lynx were milder than in Swiss lynx. In Swiss lynx, lesions increased in severity with age, and were also more severe in males than females. These differences were not significant, however, the number of lynx was insufficient for reliable statistical analysis. Heart index appeared to increase with severity of lesions but differences were not statistically significant, even if lynx of both countries were pooled.

Preliminary data indicate that AS and MF are common heart lesions in Eurasian lynx. Subadult and adult males seem to be more commonly affected. Prevalence is much higher and lesions more severe in Swiss than Swedish lynx. However, these results might be biased due to the insufficient amount of data, and more lynx of both countries have to be investigated to draw conclusions.

MF is a characteristic feature of cardiomyopathy in both cats and dogs. In contrast, associated AS is rare in cats but common in dogs. Further investigations are necessary to evaluate the sanitary relevance of the observed heart lesions, and to assess whether the observed loss of genetic variability of reintroduced Swiss lynx (Breitenmoser-Würsten and Obexer-Ruf 2003) might be the cause of the very high prevalence of these cardiac lesions in Swiss lynx.

Conclusion

Several congenital malformations have already been observed in Swiss lynx (Ryser-Degiorgis et al. 2004). In 2006, two new malformations were observed. The kidney

abnormality was an incidental finding but the pelvis asymmetry was directly implicated in the death of the lynx. Furthermore, preliminary data on microscopical heart lesions in Eurasian lynx indicate that features characteristic of cardiomyopathy and arteriosclerosis are particularly prevalent in Swiss lynx. The real prevalence and possible impact of such findings on the population is unknown. An abnormally high occurrence of congenital malformations and/or heart lesions could be due to inbreeding depression. Therefore, further health monitoring and studies on genetics in lynx from Switzerland and other countries are necessary to assess the importance of such findings for the long-term survival of the Swiss lynx population. Results could have management implications.

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Diseases of Free-Ranging Non-Domestic Felids

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In general, non-domestic felids are susceptible to the same types of diseases as domestic cats. This provides enormous resources to the researcher interested in diseases of non-domestic felids. In wild populations disease surveillance relies heavily on ante-mortem serological and parasitological testing. Carcasses, when encountered, are extremely useful for not only determining cause of death but also for correlating exposure to infectious agents with actual disease. Mortalities in wild populations of felids are often associated with trauma (conspecific or human induced) or a failure to thrive, which is common in juveniles and aged animals as well as animals forced to the periphery of suitable habitats. Infectious agents can have heightened significance in geographically isolated or genetically impoverished populations of felids and therefore are of constant concern to wildlife managers. Rather than cover all the potential consequences of diseases to which non-domestic felids are exposed, this review aims to concentrate on diseases with known pathological consequences.

Two bacterial infections have been associated with mortalities in free-ranging felids. Anthrax, caused by the bacterium *Bacillus anthracis,* has been associated with mortalities in southern African free-ranging and captive lions and cheetahs. Felids are exposed by feeding on an infected carcass and present with grossly evident cervical edema. Tuberculosis, most commonly caused by *Mycobacterium tuberculosis* is an important cause of morbidity and mortality in free-ranging felids, most notably lions living in Kruger National Park and the Iberian lynx.

Much of the information we have about viral infections in free-ranging felids is from serosurveys. However, most serological assays detect antibody response and not viral antigen. Therefore, seropositivity only implies exposure. Disease associated with viral infections in free-ranging populations is rare, but can have devastating effects on small isolated populations. One virus that has been associated with significant mortality in free-ranging felids is canine distemper virus (CDV). In 1994, a canine distemper epidemic within the Serengeti ecosystem crossed over from canids and was associated with a loss of approximately 30% of the lion population. Histologically, animals had an interstitial pneumonia and encephalitis with rare intracytoplasmic and intranuclear inclusions. However, serological data suggests that lions were exposed at other times without

significant mortality suggesting that co-pathogens may be important in determining mortality. Lentiviral (Feline immunodeficiency virus: FIV) infections are common in lions but also occur in a variety of other felids. Infection has been associated with alterations in lymphoid subsets, however characteristic disease and morphological lesions are not typically noted.

A recent disease of concern is avian influenza (Orthomyxoviridae). Multiple experimental studies have shown that domestic cats can become infected with influenza A viruses and develop disease. During the recent H5N1 influenza outbreak clouded leopards, leopard cats, and tigers in Thailand and domestic cats in Europe died after feeding on infected carcasses. Information from this outbreak also indicated that horizontal spread was possible. Limiting exposure of non-domestic felids to infected carcasses, if possible, is suggested to minimize the potential impact of this disease on both captive and free-ranging populations of felids.

There have been sporadic reports of mange in both Eurasian lynx and in cheetahs. Affected animals have hair loss and crusting dermatitis. Although *Sarcoptes scabeii* was implicated in both cases, *Notoedres* were also found in the Eurasian lynx. It should be noted that *Notoedres cattii* is morphologically very similar to Sarcoptes scabeii and it can be difficult to differentiate between the two parasites.

Hemoparasites are not uncommon in free-ranging felids and are usually incidental findings. Hemolysis and anemia have been noted in some felids associated with *Cytauxzoan felis* infection. In general metazoan parasites commonly infect free-ranging felids and are not associated with disease, except when heavy burdens are present. One exception may be *Cylicospirura* nematodes in mountain lions. Although typically these parasites are not associated with any disease, recent reports suggest that at least regionally some mountain lions have associated gastric or duodenal perforations.

Diseases of captive felids

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In general, non-domestic felids are susceptible to the same types of diseases as domestic cats. This provides enormous resources to the researcher or veterinarian interested in diseases of non-domestic felids. However, there are unique diseases and or unique manifestations of common diseases in some species of non-domestic felids. In the captive situation, degenerative and age related diseases are more common than infectious disease. This is generally owing to preventative medicine programs, regular diets and an absence of predators and human conflicts. The most common degenerative disease in captive felids is chronic renal disease. Causes of renal disease vary among felids but with a few exceptions are generally similar to those noted in domestic cats. Chronic arthritis and degenerative joint disease are common in aged individuals of all species while vertebral spondylosis is more common in larger felids. Neoplasia is also common in aged felids. The most common types of neoplasia include lymphoma, gastrointestinal adenocarcinomas, pulmonary bronchoalveolar carcinomas, biliary neoplasms and tumors of the reproductive system. In females, uterine leiomyomas are common. Uterine carcinomas and mammary gland carcinomas are also common, but the incidence is greater in cats on progestagen contraceptives. In addition to the common neoplasms, certain species have predilections for specific, more unusual, neoplasms including transitional cell carcinomas in fishing cats, pheochromocytomas in clouded leopards, and ovarian carcinomas in jaguars.

Veno-occlusive disease, characterized by scarring of the centrilobular and sublobular veins that results in progressive liver disease, is a disease noted in captive cheetahs and snow leopards. The pathogenesis of this disease is not known. Previous nutritional hypotheses have been disputed by recent data. Amyloidosis, the extracellular accumulation of insoluble proteins, is common in black-footed cats and cheetahs. In both species the amyloid has been characterized as type AA. In cheetahs, the development of amyloidosis is strongly associated with chronic inflammatory conditions, most commonly gastritis. However, in black-footed cats the association is not as clear and the disease may have a genetic basis.

Although degenerative diseases are more common, infectious diseases are still important causes of morbidity and mortality in captive populations. Infectious agents can cripple a

captive breeding program not only by causing disease in genetically valuable individuals but also by limiting movements and breeding of "positive" or exposed animals. While vaccinations are available for some diseases, the safety of modified live vaccines is always a concern as their use in some non-domestic species has resulted in disease. Killed vaccines are generally considered safer, however they are generally considered less efficacious. Common domestic feline upper respiratory tract (URT) infections are also common in non-domestic felids. Feline herpes virus, in addition to causing URT can cause more serious disease manifestations in Cheetahs and Pallas' cats. Papillomaviruses are ubiquitous and generally of no clinical consequence. However, in snow leopards, transformation to squamous cell carcinomas is not uncommon. While feline parvovirus infection is relatively rare, increasingly, canine parvoviruses are noted as causes of colitis in felids. Feline immunodeficiency virus (FIV) and Feline leukemia virus (FeLV) are also concerns in captive collections primarily because seropositivity limits the movement of animals between breeding facilities.

While general bacterial infections can cause morbidity and mortality in captive felids, there are only a few distinct disease syndromes associated with bacteria. *Helicobacter sp*. are spiral bacteria that colonize the stomachs of felids. Generally, infection is an incidental finding though gastritis is observed in an occasional individual. However, in cheetahs the majority (>95%) of captive cheetah develop gastritis. The severity of gastritis varies among cheetahs but in some animals is sufficient to have warranted euthanasia. *Salmonella* sp. has been shown in one study to be shed by >90% of the examined captive felids. Rarely *Salmonella* infection is associated with diarrhea.

In addition to their susceptibility to Herpes virus, Pallas' cats are also exquisitively sensitive to infection with *Toxoplasma gondii*. Neonates and aged individuals have succumbed to disseminated infections with necrotizing inflammatory responses to the cysts and free zoites. No other felid species is similarly sensitive to this common felid parasite.

Captivity can also impact basic physiological homeostasis and the effects of stress, if not mitigated, can have profound impacts on the immune system's ability to deal with infectious agents and on the development of certain degenerative disorders. Although there is increasing data on the effects of stress in some felids (most notably cheetahs and clouded leopards), there are still significant gaps in our understanding of how stress can affect disease development in captive felids and how to document or measure stress.

Diseases in the European Endangered Species Program (EEP) Cheetah Population

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Abstract

In 2004 the cheetah EEP population included 345 cheetahs within 75 institutions. 270 cheetahs in Europe originated from Southern Africa (RSA and Namibia). 75 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 groups were: a.) centralized data management b.) standardized disease description c.) comparative disease description and prevalence – USA / RSA d.) research on cheetah ataxia / myelopathy e.) In accordance with the Global Cheetah Conservation Plan. As of December 2004 the 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.

Disease Overview: non-CNS diseases

1. Gastritis

Gastritis was observed in 81% of the samples, ranging from mild to severe, mainly lymphoplasmacytic inflammation of the mucosa, at times associated with neutrophilic infiltration. Spiral bacteria consistent with *Helicobacter* spp. were detected in most cases, but there was no correlation between the severity of the gastritis and the amount of bacteria in stomach glands.

2. Glomerulosclerosis / Nephrosclerosis / Glomerulonephritis

The most prevalent renal disease was glomerulosclerosis, affecting 80% of the >1y old cheetahs with kidney samples. GS was moderate to severe in about 40% of the cheetahs older than 6y. Other renal pathological findings were pyelonephritis and/or papillary necrosis, presence of crystals in the tubular lumen, and amyloidosis.

3. Lesions of the spleen

Multiple splenic myelolipomas were present in 39 cheetahs (54% of the examined spleens). The youngest cheetah affected was 1y old. More than 50% of the cheetahs had lymphoid depletion.

4. Lesions in the liver

No veno-occlusive disease (VOD) could be observed in the 76 liver samples examined (cheetahs >1y). Only mild increases of collagen fibers and reticulin fibers were observed around the central veins and in the sinusoids

5. Amyloidosis

Mild to marked amyloid deposition was recorded in 17 cases (45% of cheetahs >1y) mostly in both kidneys and liver. In 3 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.

6. Viral diseases

- Coronavirus: 2 cheetahs with granulomatous lesions consistent with FIP were recorded. The viral etiology of these cases have still to be confirmed by IHC.
- Feline Herpesvirus: 2 adult cheetahs (6 and 7y) had chronic conjunctivitis with typical histological lesions associated with intranuclear inclusion bodies. Herpesvirus is frequently associated with mild clinical signs including sneezing and conjunctitis especially in cheetah cubs. Herpesvirus genom has been sequenced from one conjunctival swab; the gene sequence has >99% overlapping with Feline Herpesvirus-1 (Genebank entry).
- Parvovirus: 2 cases of panleucopenia were observed in one 7w old and one 1y old cheetah

7. Bacterial diseases

- 1x Bronchopneumonia with *Pasteurella* sp. infection (1y)
- *Clostridium perfringens* was isolated from colon content in several cheetahs. In 1 case a perforating enterocolitis was caused by *Clost. perfringens* type A.
- *Campylobacter* sp. and *Salmonella* sp. are regularly isolated in cases of diarrhoea in cubs and adult cheetahs. Some of these are food associated.
- 2 institutions had deaths related to *Hemobartonella felis* infection (= *Mycoplasma haemophilus*)

8. Parasitic infections

- Massive infestation with Ascarid worms (*Toxascaris leonina, Toxocara* sp.) is a frequent problem in young and adult cheetahs despite regular deworming (up to 6 times a year).
- Lungworms (*Aelurostrongylus abstrusus*) are frequently detected in feces. 2

adult cheetahs showed severe parasitic pneumonia at necropsy.

Disease Overview: CNS diseases

1. Myelopathy

Myelopathy is a degenerative disease of the spinal cord and represents a serious threat to the European cheetah population. To date more than 60 cases have been registered in Europa, as well as a few cases in Dubai. This disease accounts for 25% of all deaths in the European cheetah population and represents a limiting factor in the growth of the European captive population. The etiology of the disease is still unknown and several causes has been considered, including genetic, environmental / toxic, nutritional (esp. Cu) and viral factors. On-going studies regarding etiology, characterisation and epidemiology of the disease are on progress.

2. FSE

Up to date 9 cases of Feline Spongiform Encephalopathy have been diagnosed in cheetahs. With the exception of 1 cheetah born in France, all affected cheetahs were either born in the U.K. or imported from the U.K. Aggressive or anxious behaviour and progressive ataxia were the dominant clinical features. It is broadly accepted that FSE is the result of BSE infection in felids.

The “Peaugres-Syndrom”

This “syndrome” might be one of the first “true” genetic diseases in cheetahs. 27 cubs were born in 5 litters from 2 normal dams which were sisters (Fanny and Rina) and 1 unrelated normal male (Fota). From these 27 cubs, 26 died between 1 and 134d of age. The cubs were more or less affected and presented with various pathological lesions including poor hair coat, heart malformation (aortic aneurysma and heart hypertrophy), liver fibrosis, stunted growth, osteoporosis and CNS diseases (encephalitis). 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 copper transport proteins.

Evaluation of Disease Risks for Felids Ex Situ

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Minimizing the impact of infectious diseases is central to preventive health programs for captive felid populations. The very acts, however, of bringing wild felines into a captive setting or managing well-established collections of multiple feline species increase the possibility of effective contact time for transmission of an infectious agent. Increasing biosecurity diminishes the risk but cannot eliminate the possibility of animals becoming infected. The consequences of infection will vary from no apparent clinical signs to non-specific illnesses to mortalities. Animals may either clear the infection and pose no further risk to the population or shed the infectious agent for a prolonged period or intermittently. Understanding the pathogenesis of an infectious agent is key to predicting risk of transmission within a population. Understanding the limits of diagnostic tests to determine the prevalence within a population and assess risk of transmission is also critical. Predicting how many animals will actually become ill if exposed is frequently the most important outcome.

Too often scientists and veterinarians claim that there is not enough known about an infectious disease in a given species to create valid models. Decisions, however, will be made about how animals will be moved between facilities or reintroduced into a suitable habitat with or without a model. Assumptions have to be made frequently with very limited data. For wild felids, the assumptions are usually based on what is understood for the domestic cat. Often there is incomplete understanding of disease pathogenesis even in domestic cats and there is the possibility that a wild felid will not react the same way. A good example of this is high neonatal mortalities in Pallas' cats (Otocolobus manul) and even deaths in adults due to toxoplasmosis. Felids are the definitive host for Toxoplasma gondii and rarely experience clinical disease, but exposure to the protozoa is relatively low in wild Pallas' cats, at least in Mongolia, suggesting the species is a naïve host.

The recent, commercial availability of PCR assays for feline herpesvirus-1 (FHV-1) has raised concerns about the significance of a positive test. Some zoos have refused

to accept animals that are PCR positive despite the absence of a clinical history of upper respiratory or corneal disease. Part of this stems from confusion about what a positive PCR means in conjunction with understanding how FHV-1 interacts with its host. Asymptomatic cats may be PCR positive and cats with clinical signs may be negative. Similar apparent contradictions occur when comparing PCR results with virus isolation. Feline herpesvirus -1 PCR assays detect DNA, but this does not necessarily mean that a cat is infectious. There may not be enough competent viral particles to constitute an infectious dose for effective transmission. These are all variables that are not actually known for FHV-1 and which current PCR assays will not distinguish. Consequently, making decisions based on one positive FHV-1 PCR about translocation of a healthy non-domestic cat with no previous history of upper respiratory disease is probably ill-advised based on our current understanding of FHV-1 in domestic cats. A simple model was developed in Excel to address the probability that exposed felids in a captive setting would develop clinical signs of FHV-1 based on what is known for the domestic cats.

Feline herpesvirus-1 causes a latent infection in an estimated 80% of the cats infected. Reactivation of virus replication and subsequent shedding can occur during stressful events such as changes in housing, pregnancy/lactation, post-surgery and other illnesses. Less virus is probably shed during reactivation than during the primary infection, but it is important to remember that cats can shed virus without any clinical signs. It is estimated that 50% of infected cats never exhibit clinical signs. Conversely, upper respiratory disease in cats is not always caused by FHV-1.

With this information, it is possible to make informed assumptions about the risk of observing clinical FHV-1 in a collection. Probability of effective contact is dependent on the proportion of time an animal is shedding and can actually contact susceptible individuals, as well as activities that could lead to exposure. This latter includes inter-specific behaviors such as grooming, fighting and sharing water bowls but also can include the probability that a caretaker could act as a mechanical vector by transmitting the virus on their hands, clothes and cleaning tools. Once a naïve animal is exposed, what is the probability it will shed virus and expose others?

An assumption needs to be made about proportion of susceptible felines showing non-specific respiratory signs and proportion showing clinical signs directly attributable to the specific agent. These are both functions of susceptibility, virus strain and exposure dose. Is the risk related to exposure to a new virus or is the virus circulating in the population? The former potentially carries a higher risk that clinical disease will be seen so this risk is weighted more in the calculations. Exposure to a new virus includes introduction of a novel agent into the population as well as vaccine failures. Vaccine failures include failure to protect from development of clinical disease as well as causing the disease, which has happened in Pallas' cats that were vaccinated with modified-live virus (MLV) vaccines at several facilities in the United States.

Vaccines are used to protect against circulating viruses that have the potential to cause significant morbidity and mortality. It is beyond the scope of this paper to discuss vaccinations in non-domestic felids, but there are several key points to remember. The efficacy and safety of vaccines used in non-domestic felids are tested in domestic cats for which the vaccines were developed. There are many different manufacturers of vaccines for cats so it is difficult to compare vaccination protocols between different facilities with non-domestic felids about what is safe. Efficacy is usually just assumed. Killed and sub-unit vaccines are usually quite safe but some argue that MLV and recombinant vaccines afford better protection. It is important to remember, however, that neither killed nor MLV vaccines for FHV-1 prevents infection. They are considered prophylactic vaccines designed to minimize expression of clinical signs.

The final variable to be considered for predicting the number of clinical cases of FHV-1 within a captive population is the size of the population. The following values were assigned for determining the risk that clinical FHV-1 would be seen in a collection with PCR positive animals. These values favor the probability that the virus is shed in the population.

1) Ability of exposed cats to shed virus: 95%

2) Efficiency of transmission
   Proportion of time animal is shedding: 50%

   Proportion of activities: 80%

   Proportion of time: 90%

3) Cats exhibiting non-specific upper respiratory signs: 10%

4) Clinical signs caused by FHV-1: 50%

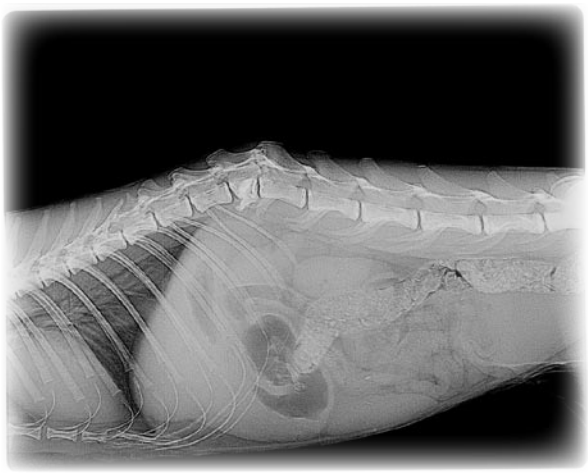
Results are shown below in Table 1 and demonstrate that few clinical cases would be seen. A study to determine the prevalence of positive PCR reactions in randomly sampled, healthy, non-domestic felids would be beneficial to approximate exposure rates in zoological settings. Approximation must be emphasized as rates of FVV-1 reactivation are not known so a latently infected cat could test negative even with repeat sampling. Recently, a collection of 10 healthy animals, four different species, was tested and 2 cats of different species were PCR positive. This would suggest that FHV-1 is probably common in zoo collections and not causing a major health problem in most settings. More work, however, needs to be done.

Table 1. Number of cats showing signs of clinical FHV-1 by size of collection.

Exposed	10	20	40	50
0%	0	0	0	0
10%	0	0	0	0
20%	0	0	1	1
30%	0	0	1	1
40%	0	0	1	1
50%	0	1	1	1
60%	0	1	1	2
70%	0	1	2	2
80%	0	1	2	2
90%	0	1	2	2

There are intangibles that are difficult to quantify when trying to predict risk of disease expression. These include changes in the animals' environment, diet and caretakers as well as co-infections with other infectious agents. None of these were considered here. More importantly, however, is the risk of user bias. Individuals involved in the breeding and recovery of threatened and endangered species unwittingly may have a hidden agenda. It is important that the individual assessing disease risk have no vested interest in the outcome.

Keywords: disease risks, captive wild felids, feline herpes virus-1



Husbandry and Management of Captive Populations:

Genetics, Demographics, Ethology, and Natural Reproduction.

Session II

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Genetic and Demographic Management of Conservation Breeding Programs Oriented Towards Reintroduction

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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 genetic diversity as possible. This is because genetic diversity represents the evolutionary potential captured within the population and because there is a correlation between genetic diversity (heterozygosity) and population fitness. Populations in captivity are often small, lack gene flow between subpopulations (different enclosures or captive colonies) without human intervention and live under unnatural conditions. 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.

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 faster population growth can be obtained in captivity. Apart from providing a safe environment, the more controlled conditions in captivity allow for pro-active genetic and demographic management in order to minimise the rate of loss of gene diversity.

In general principle, genetic diversity can best be maintained in captive populations by maximising the number of founders (without compromising the wild source population), maximising the growth rate in the growth stage of the population and basing the pairings of the animals, especially during the capacity stage of the programme, on their mean kinship values.

How many founders and how many Iberian lynx?

From a genetic point of view, the smaller a population at the carrying capacity stage of

a breeding programme, the more gene diversity is lost. Although bigger is better, both space and financial and human resources are always limited. How big is big enough? How much gene diversity is enough? Current genetic theory indicates that the minimum viable population size needed to prevent the loss of gene diversity is an effective population (Ne) of 500-5000, which for wild populations often corresponds to a true population size (N) of about 5000-50000 individuals. Even when taking into consideration that the Ne/N ratio for captive breeding programmes under proper genetic and demographic management, often lays in the vicinity of 0.3, this still implies a required true population size of several thousand individuals which is a practical impossibility in terms of space, finances and resources for the vast majority of programmes. 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 founders after 100 years of breeding in captivity an acceptable compromise between a modest loss of gene diversity and being ample to accommodate 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.

Lacy and Vargas (2004) employed the software programme PM2000 (developed by J.P. Pollak, B. Lacy, and J. Ballou) in order to determine the goals for the captive Iberian lynx population. 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: growth rate 21.5%, generation time 5.25 years, Ne/N ration 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, and the number of individuals required at carrying capacity (500) is exceeding the availability of space and resources for the programme. 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, as well as one extra founder every two years for the whole duration of the programme in the form of animals entering rescue centres.

Maximising growth rate in the growth stage of the captive population

As far as breeding from the founders is concerned, more is better! The more offspring a founder produces, the more of its genetic variation will have been passed on to the next generation. Given about 100,000 loci per vertebrate genome and the typical of recombination in vertebrate genomes, it has been estimated that 12 offspring (that survive and themselves breed) per founder are sufficient to provide 99% probability that all alleles of a founder are transmitted to at least one offspring. 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. The growth rate during the foundation phase strongly

influences the rate at which genetic diversity is lost in the future of the captive breeding programme. As long as carrying capacity in captivity has not yet been reached, founders should not be prevented from breeding. It is preferable to try and correct unevenness in founder representation during the capacity stage of the programme, rather than to compromise retention alleles the founding stage.

Capacity stage of the programme – breeding according to mean kinship value and limiting inbreeding

From a genetic point of view, what you would ideally like to do in an ex situ programme is to "stop" selection such that the gene diversity that is available for reintroduction is the same as that collected from the wild. However, in real life some founders will be more prolific then 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. In order to correct for such inequalities the technique that is currently employed by the zoo and aquarium community is to base the pairings of the animals on their mean kinship value. The mean kinship is a measure of the relatedness of an individual to every other individual in the population. It is calculated as the average of the coefficients of kinship of an animal with every other animal in the population. Priority for breeding is given to individuals with low mean kinship values (and few relatives). Furthermore, efforts should be made to combine individuals with similar mean kinship values, if not resulting offspring would be half important and half not important. Such offspring are also going to be related to many individuals in the population which will make it harder to find breeding opportunities for them 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 reduces the amount of gene diversity retained, it generally also leads to inbreeding depression (reduced fitness). The level of inbreeding depression significantly influences the extinction risk of a population. 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.

Choosing individuals to reintroduce

It is obvious that reintroduced individuals have to help improve the genetic and demographic health of the wild population. What is often forgotten however is that the removal of the animals destined for reintroduction may 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 genetic

diversity of the wild population (i.e. have few relatives in the reintroduced population), but are genetically overrepresented in the captive population. 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.

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Iberian lynx Population Genetics: Doñana, Sierra Morena and Ex-situ Program

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The contribution of genetics to conservation can be viewed as two-fold. Firstly, it provides effective tools for the unambiguous identification of species, gender and individuals thus contributing the knowledge of the distribution, demographic status, and general biology of our species. Secondly, and most importantly, it provides a description of patterns found in nature (diversity, inbreeding, structure...) and an insight on the evolutionary and demographic processes that generated them (selection, genetic drift, bottlenecks, gene flow...). Only with this information can conservation units be defined, short- and long-term viability evaluated and appropriate management strategies designed.

The application of molecular genetics to Iberian lynx has yielded a battery of effective tools to help conservation. Species-specific diagnostic assays applied to non-invasively collected samples have been used as a test for presence of the species in particular localities and has thus helped in the delimitation of current species distribution. Hypervariable molecular markers can be used as genetic tags to distinguish individuals, and when applied to scats provide a non-invasive way to estimate population sizes and monitor individuals and populations. Furthermore, these tags contain information about the ancestry of the animal and can thus be used to address questions on parentage and close kinship, allowing for the monitoring of reproduction and the resolution of pedigree ambiguities in wild and captive populations.

Beyond their use as diagnostic tools, molecular markers can be used to describe genetic patterns in nature and to investigate their associated evolutionary and demographic processes. We have used a set mitochondrial DNA sequences and a set of 21 microsatellite markers to describe contemporary genetic variation in Iberian lynx on a set of 95 samples (69 from Doñana and 26 from Sierra Morena). This study updates our previous results with smaller sample sizes (Johnson et al. 2004). A high level of genetic differentiation is observed between the two extant Doñana and Sierra Morena populations. Mitochondrial diversity is extremely low with only two haplotypes observed which differ in one single position. Nuclear microsatellite diversity levels seem globally low when compared to other felid species, and differ dramatically between populations, with Doñana showing about 30% lower diversity than Sierra Morena, both for heterozyosity and allelic diversity. A signal for a recent demographic bottleneck is evident in the data for both populations.

Observed genetic patterns are consistent with the predominance of genetic drift in recent times affecting both populations, but more intensely Doñana, where the magnitude and rate of accumulation of inbreeding could severely affect population viability. Risks of inbreeding depression and loss of genetic diversity, indirect evidences for historical connection and absence of evidence for adaptive divergence between populations call for the genetic management of the species as a single unit and for management actions involving translocation into Doñana of wild or captive-born individuals with ancestry in Sierra Morena population.

Finally, in order to meet its objective of preserving the most of the extant wild genetic diversity the Iberian lynx *Ex-situ* Conservation Program needs a proper genetic management that takes into account the observed genetic patterns in the wild. Management strategies are most commonly based on minimizing global mean kinship in the population, as calculated from the pedigree under the assumption of equally unrelated founders. In the case of Iberian lynx, the observed population genetic structure and inbreeding makes this assumption rather unrealistic. One possible way of incorporating the pattern of variation in the wild to the genetic management of the captive population through mean kinship minimization is to input estimates of relatedness among founders. Genetic markers can provide relatedness estimates for pairs of individuals based on the proportion of shared alleles relative to the average proportion in the population. When each population is analyzed separately relatedness will estimate recent common ancestry, allowing the identification of close direct relatives. On the other hand, if the hypothetical ancestral (unknown) population could be used as baseline reference for relatedness estimates, high relatedness between all Doñana individuals and low relatedness between pairs of individuals from different populations would be found, reflecting the inbreeding within and genetic differentiation between populations accumulated since their putative time of isolation. The consideration of founder relatedness in the genetic management will affect the optimal composition, objectives and needs of the *Ex-situ* Iberian lynx Program.

Johnson, W. E., J. A. Godoy, F. Palomares, M. Delibes, M. Fernandes, E. Revilla, and S. J. O'Brien. 2004. Phylogenetic and phylogeographic analysis of Iberian lynx populations. Journal of Heredity **95**:19-28.

Population and Conservation Genetics of Two Re-Introduced Lynx (Lynx lynx) Populations in Switzerland – A Molecular Evaluation 30 Years After Translocation

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Lynx went extinct in most of Central and Western Europe at the end of the 19th century. In the 1970s, re-introduction programs started in the Alps and in adjacent mountain ranges of Switzerland, Slovenia, Italy, Austria and France. For all projects, the founder individuals came from the same source population, the Carpathian Mountains of Slovakia. Some of the animals released were closely related (siblings, parent-offspring). Because of the clandestine manner of some of the releases in Switzerland, the whole re-introduction remained obscure, but all together, there were not more than 14-16 animals released in the Swiss Alps and 8-10 in the Jura Mts, respectively. The two populations in Switzerland are still relatively small and isolated. They consist today of not more than 40 - 60 reproducing individuals.

From this situation, the following questions arise: Do the re-introduced populations have nowadays a reduced genetic variability compared to the Slovakian source population and other autochthonous populations in Europe due to the severe bottleneck created by the reintroduction? What genetic signatures did the bottleneck leave? Do the two geographically separated populations of Switzerland differ genetically today?

To address these questions, genetic analyses were performed using microsatellites, which were developed in domestic cats, Canada lynx and Sumatra tigers. Currently, preliminary results based on 22 microsatellites and 530 samples from 13 populations are available. These samples have been provided by more than 20 colleagues from 18 countries.

The 8 autochthonous lynx populations from Europe showed heterozygosity values of 0.48-0.69 with 2.7-5.7 alleles per locus. Some of these populations went through a bottleneck in the 20th century or are very small today. The 5 re-introduced populations have a reduction in genetic variability of various extent with heterozygosity values of 0.44-0.62 and 2.9-3.5 alleles per locus. In both Swiss populations, the loss of allele diversity was more severe than the loss of heterozygosity, and rare alleles have become less frequent compared to the source population. The populations in the Alps, in the Jura Mts and in the Dinaric range have experienced an important genetic drift and are today distinct from the source population (F_{ST} 0.10-0.21), whereas the populations in the Vosges Mts and in

the Bavarian-Bohemian forest remained closer to the Carpathian lynx from Slovakia (F_{ST} 0.03 and 0.05, respectively). These two populations were founded with more individuals that were released over a higher number of years and about ten years later. Additionally, the two Swiss populations have drifted away from each other and are nowadays very distinct (FST = 0.25).

There seems to be very limited gene flow between the two Swiss populations. Only in 1995, 20 years after the releases, we have been able to detect an animal from the Alps in the Jura Mts. The male or his offspring have produced several litters since. The heterozygosity has increased by 2% and the number of alleles per locus from 3.3 to 3.5.

AZA ZOOS And In Situ Support: A New Partnership

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Since 2001, changes in the interpretation of the U.S. Endangered Species Act have prompted North American zoos belonging to the Association of Zoos and Aquariums (AZA) and other collections of captive animals to significantly increase their level of support for conservation of endangered felids in nature when they are involved in international transfer of regulated felids. While not all endangered felids have benefited equally, seven species of large and seven species of small cats have received significant levels of financial support from AZA zoos engaging in international trade involving these species. In the case of cheetahs and tigers, the sums provided by AZA zoos have exceeded US$ 10,000 per taxa per year, amounts of in situ support unheard of 10 years ago.

Management of Captive Snow Leopards: Links to In Situ Conservation

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STUDBOOKS – BASIC TOOLS FOR BREEDING PROGRAM-MES

Studbooks are tools in managing small and endangered populations and their importance is reflected by their rapid increase: in 1960 there were nine official international studbooks, while their number according to the latest published volume of International Zoo Yearbook *(Fisken & Miller 2005)*, had increased to 146. This development can be interpreted as a growing awareness of the urgency of captive breeding programmes to help conservation projects.

Although the first studbooks provided little more than registers of individuals to reduce inbreeding, they are today sophisticated tools of population management and can be used to produce Master Plans to preserve the genetic health of our *ex situ* populations. The international studbook for snow leopards was established at Helsinki Zoo in 1976 *(Blomqvist 1978)* and the species has since been focus of intensive management.

FROM STUDBOOKS TO JOINTLY RUN BREEDING PROG-RAMMES

Joint breeding programmes were initiated in our continent in 1985. There are three levels of management in Europe, of which the most intensive is called *EEP* where each programme has its own *Co-ordinator*, assisted by a *Species Committee*, elected among the participating zoos. The snow leopard was included in the programme in 1987. Today 151 taxa are managed on an EEP-level, while 140 species are included in European studbooks.

A similar development has taken part in other continents, in North America a breeding programme for snow leopards, a *SSP*, was established in 1983. Today there are breeding programmes for the species not only in Europe and North America, but also in Japan and SE-Asia (Australia and New Zealand). In addition to that, North America has also established a regional studbook for their snow leopards in 1995.

DEVELOPMENT OF THE CAPTIVE POPULATION

The population started to develop rapidly in the late 1980s and continued to do so until the mid 1990s when it had reached a level of almost 600 individuals. As there were clear indications that the population was expanding so fast that facilities were running out of space, restricted breeding in both main breeding programmes, the SSP and EEP, were applied. Due to these, the jointly managed population was reduced to 500 animals of which slightly less than 200 are located in the EEP.

Keeping endangered populations numerically sound by increasing longevity is not sufficient to ensure their survival. The quality of each population is as important as the quantity, and genetic degeneration and unfavourable selection must be avoided. Small captive populations are basically vulnerable to three serious breeding problems;

* Inbreeding
* Loss of genetic variability
* Unnatural selection

As pedigrees were formerly used to calculate the *Inbreeding* for existing animals or for the potential offspring of planned matings, they are today also employed to assess *Founder Representation* in the populations: or how large shares of the present population genome can be traced back to each founder individual in the wild-caught stock. *Mean Kinship* values *(MK)* explain the average relatedness betwen a particular individual and all the other individuals in the population. Care should therefore be taken NOT to breed animals with low *MKs* to those with high *MKs*, otherwise rare genes will be linked with common genes and cannot be increased in frequency without also increasing common lineagues.

Once such analyses are completed, they are translated into simple facts and figures which, along with basic biological information form a good picture of population status and of the biological constraints under which the co-ordinators operate. These facts and their synthesis provide the basis for understanding the importance of each individual in the population and which recommendations are the most appropriate for that particular specimen.

MAIN THREATS OF THE WILD SNOW LEOPARD

The snow leopard is classified as *Endangered (EN)* throughout its range by the *IUCN* and listed in *Appendix I* or "in urgent need for protection" by *CITES*. The most recent assessment was done 2002 and is based on the estimate that the effective population size is below 2.500 mature breeding animals with a declining trend and with no subpopulation exceeding 250 breeding animals. Although legal protection is offered, implementation

and enforecemnt varies in the vast distribution area which reaches over 12 different countries and includes several different cultures, languagues and religions. The demand for snow leopard products, including bone and pelts for a quickly expanding Chinese market, is increasing. Retaliation against livestock losses is also more frequent today than ever before. In general, the threats are very similar to threats other large Felids face today and they can still be summarized as;

* Illegal trade in snow leopards and their derivates
* Conflicts cause by humans and animals
* Habitat distruction
* Loss of prey – competition with livestock for grazing

LINKS TO *IN SITU* CONSERVATION

The concern zoo-people originally had for the well-being of snow leopards in captivity, encouraged us to arrange a series of international symposiums focusing mainly on improved management in captivity. The first *International Snow Leopard Symposiums* was arranged in Helsinki back in 1978. Since then a lot of improvements have been made which also can be seen in increased survival rates of captive-bred cubs, a longer life-span and in an expanding captive stock. As management improved and the population started to flourish, it was decided that future Symposiums should emphasis the animal's plight in the wild instead of in captive conditions. The following symposiums were consequently arranged in native snow leopard countries and provided excellent forums for proposals regarding the species' preservation. Most of the credit for this goes to *The International Snow Leopard Trust (SLT),* which was instrumental in creation and expansion of parks and reserves in snow leopard countries.

Many zoos exhibiting snow leopards are eager to participate in *in situ* conservation and they were therefore lucky to have an established organisation like the *SLT* from the beginning.

The *SLT* has been working with local communities to prevent the disappearance of the snow leopard for soon a quarter of a century. With the support of partners from the zoo world, conservation programmes are today running in China, Mongolia, India, Kyrgyzstan and in Pakistan. The *SLT* works not only to protect the snow leopard, but on protecting the whole mountain ecosystem in Central Asia by supporting;

* scientific research to understand the snow leopard and to define its main threats,
* public education through spreading information,
* public education among local people towards a better understanding.

Zoos support the activities of SLT by raising funds to support:

* habitat protection and field conservation efforts,
* public education,
* developing technologies that can be applied to field conservation.

The SLT is based in Seattle and much of its funds are collected among North American zoos although an increasing number are also coming from EAZA.

In the German speaking countries, *NABU (Naturschutzbund Deutschland)* – the German Society for Nature Conservation is a well-known organisation. Since 1999, NABU has taken an active role in protecting Kyrgzstan's free-ranging snow leopard population. In co-operation with the Kirghiz government and local experts, NABU has developed an effective concept against illegal hunting of both snow leopards and its prey species in Kyrgyzstan. A group of rangers, *"Gruppa Bars"*, has been established with special authorities. The rangers have had great success in their struggle against poaching and trade. Six years ago, another module was added to the project when a Kirghiz ecological tourist company started to travel around in the country to communicate with local inhabitants in order to establish ecotourism which would improve the economic situation in the countryside. A snow leopard film designed to encourage the awareness of the unique nature and the necessity to protect the snow leopard has furthermore been produced which is shown in local villages. If a species will have a chance of a long-term survival, the local inhabitants must play an active role and also benefit from ongoing conservation activities. Without local support, all activities are doomed. NABU's activities and support are presented in many continental zoos which collect funds to support the *in situ* conservation work.

Though many of the breeding programmes in Europe and North America, started as pure breeding programmes, a lot of them have inspired local actions via creative education programmes, and they are today moving toward a much more active and holistic approach to conservation.

Husbandry and Studbook Management of Captive Eurasian lynx

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In 2002 the recommendation of the EAZA Felid TAG (Taxon Advisory Group) to start a European StudBook (ESB) for Eurasian lynx was acknowledged by Lars Versteege, at that time working for the National Foundation for Research in Zoological Gardens in Amsterdam. The reasons to start this studbook were, because almost 50% of the lynxes in captivity were of unknown origin, because many reintroductions were and had been taking place, because of their relatively easy husbandry many lynxes were bred and because many studies were taking place. 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 the studbook investigation many discrepancies and problems were identified. The level of inbreeding was very high. Secondly, the number of lynxes of unknown origin was very high. Thirdly, the number of lynxes of mixed origin which had been determined was also very high. And lastly, still many lynxes were kept in suboptimal conditions.

Recommendations were sent out to avoid further inbreeding of the population, to improve the enclosure and the husbandry for this species and to convince zoos to participate in the genetical studies on determination of subspecies.

Especially the subspecies problem is an important one for the programme, since it is very difficult to manage the programme 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 the lynxes that are listed for reintroduction. The IUCN SSC Cat Specialist Group is very much involved in genetical sampling of lynxes and for some subspecies has already set up a databank. Zoos keeping lynxes should cooperate as much as possible by providing samples of known subspecies. This way, the ESB would serve as a catalyst for the EAZA institutions which will provide genetic samples from their lynxes.

Introduction

The EAZA Felid TAG (Taxon Advisory Group) had for some time already recommended to start a European StudBook (ESB) for Eurasian lynx. In 2002 the recommendation was

acknowledged by Lars Versteege, at that time working for the National Foundation for Research in Zoological Gardens in Amsterdam. From that time on all lynxes in EAZA zoos were to be managed in the breeding programme.

Status in the wild

Until the mid-1900 the habitat of the Eurasian lynx ranged throughout many countries of Europe, Asia and Russia. Their numbers were drastically reduced as a result of hunting and trapping for their coat and habitat destruction. In the second half of the twentieth century, legal protection helped the Eurasian lynx to recover in the Nordic countries. Also re-introduction programmes were established in certain areas of central and Western Europe. Through these programmes the lynx has been released back into forested areas of Switzerland, Germany and Italy, where they had become extinct.

Distribution

The Eurasian lynx is widespread throughout the expansive forests of northern Europe and Asia. Russia is the heartland of its range, which extends into China and south to the northern flank of the Himalayas. In earlier years, the Lynx was distributed throughout the whole of Europe, Asia and Russia. Now it is only spread in the places that are marked in figure 1. (http://dialspace.dial.pipex.com/town/plaza/abf90/bco/eurolynx.htm, 2000).

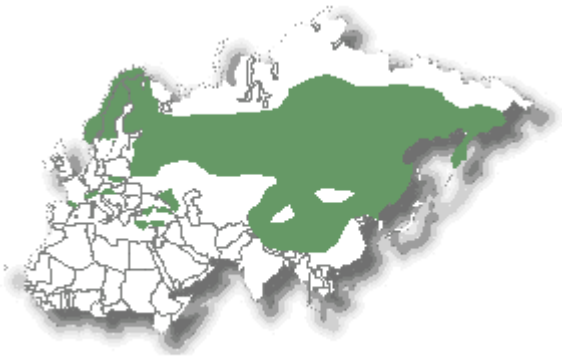

fig. 1. Distribution of the Eurasian Lynx

Throughout this range different subpopulations exist though not all of them have been recognised. The Northern lynx (*L. l. lynx*) has its range from North-West Europe to Western Russia. In the Carpathian Mountains and Greece, the Carpathian lynx (*L. l. carpathicus*) has its range. The Caucasian lynx (*L. l. dinniki*) lives in the Caucasian mountains, Iran

and Turkey. The Turkestan lynx (*L. l. isabellinus*)is spread from Kashmir to Mongolia. The Irkutsk lynx (*L. l. kozlovi*) has its range in Central Siberia, and the Siberian lynx (*L. l. wrangeli*) in Eastern Siberia. The existence of another Siberian lynx subspecies (*L. l. wardi*) is still under debate.

Justification for a European Studbook (ESB)

Why should a studbook be established for such a common species? Eurasian lynxes are not listed on the IUCN red list, like its "brother", the Iberian lynx is. It is a commonly held, popular species in zoos and almost 50% of the zoos which are a member of EAZA (European Association of Zoos and Aquaria) keep this species. Still the establishment of a studbook was prioritised by the EAZA Felid TAG (Taxon Advisory Group). This priority was based on the following facts:

- **Subspecies**

  Because almost 50% of the total captive population was of unknown origin or subspecific status and there is a lack of information

- **Reintroductions**

  Because reintroductions are taking place

- **Simple husbandry**

  Because Eurasian lynx are relatively easy to keep and breed, management needs to take place to oversee the genetic sustainability of the population

- **Studies**

  Because many different studies on Eurasian lynxes are ongoing

Getting started with the European studbook (ESB)

First of all, the zoos which keep Eurasian lynxes in their collection needed to be identified. The EAZA TAG Survey, held in 2002, which included the survey held for felids supplied the information on lynx holding institutions. From this point, all lynx holding institutions were contacted and were asked to send their historical report for this species. All EAZA institutions which are a member of ISIS (International Species Information System) should have their data current in the database of ISIS and this way a lot of information is quite easily available. The historical information received from all the zoos was double checked with the information received from ISIS and the information from the EAZA members which were not a member of ISIS was added. Through thorough investigations, many discrepancies were solved, but still a lot of information remained unknown.

Analysis of the European studbook (ESB)

After a year of studbook research a comparison was made between the data received from the EAZA TAG Survey and the data received through the studbook research. The discrepancies between both results were quite significant.

	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: probable lynx subspecies held in EAZA institutions

The most important conclusion from this analysis is that the registration of Eurasian lynxes in many institutions is very poor. Many institutions register their lynxes as a general species without subspecific identification. Also, many institutions automatically register them as the nominate subspecies. Apparent was the number of Carpathian lynxes which were identified after studbook investigation (from 7 listed in the EAZA TAG Survey to 31 after studbook investigation). Also the number of number of identified mixed origin lynxes is apparent.

Problems of the European studbook (ESB)

During the year of research, the following problems were identified:

- The level of inbreeding was very high.
- The number of lynxes of unknown origin was very high.
- The number of lynxes of mixed origin which have been determined was also very high.
- Still many lynxes were kept in suboptimal conditions.

Due to the fact that the determination of different subspecies is still unclear and many assumptions have been made without the proper scientific studies e.g. genetic evidence by DNA testing, it is very difficult to identify and manage scientifically justified “pure” breeding populations. In the studbook, all subspecies were regarded as such and placed in different populations but the scientific evidence for this decision was lacking and not all institutions agreed with this decision. Without evidence for this assumption it is still hard to convince all lynx holding institutions.

As lynxes are readily available for zoos, there have never been recommendations for housing and husbandry. In history lynxes were kept in small cages, and still many zoos have not invested in good, large and naturalistic enclosures. Slowly, zoos are improving their facilities and also ask advice on how to ideally design a new lynx enclosure.

Recommendations

Apparent from the problems faced in this studbook, several recommendations are made to improve this situation.

- **Avoid inbreeding (consult studbook)**

  In any breeding programme, the number of inbreeding should be addressed immediately. As inbreeding threatens the whole captive population by limiting the gene diversity, this should be reduced as much as possible. All institutions which have related breeding pairs should cease breeding and consult the studbook (which will be available shortly) or the studbook keeper for advice.

- **Improve enclosure and husbandry**

  All zoos are recommended to improve their facilities for lynxes. Since there is a "demand" for pure Carpathian lynxes, zoos wanting to start with this subspecies are stimulated to design and build a naturalistic enclosure.

- **Research needed to identify the different subspecies**

  As far as the studbook is concerned, all lynxes from different origin should be treated as subspecies until further scientific research proves otherwise. This way, the possible loss of important bloodlines might be prevented.

  Off course, for the breeding programme it is imperative that genetic evidence on the different origin lynxes will shed some light on the situation. Only through this research the number of subspecies can be determined which then will reflect upon the breeding programme.

Discussion Subspecies

The outcome of the genetic research on the different origin lynxes is in several ways very important for the studbook.

- **Management**

  On 31-12-02, a total of 318 individuals in 129 zoos were determined in seven different populations (see table 1). If through the genetic research it would appear that two subspecies currently can be recognised, many institutions will be confronted

with holding, unknown origin lynxes or hybrids and would be recommended to seize breeding. Secondly, to set up two viable, sustainable populations will also be difficult as the amount of holding facilities is limited.

- **Reintroductions**

  The IUCN/SSC Guidelines for Reintroductions, which are endorsed by EAZA, clearly 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 means that only with genetic evidence, reintroduction projects would be scientifically acknowledged!

Proposal

This means that the proper way to proceed would be to set up a scientific genetic study through which all lynxes, kept in the European region, can be genetically identified. As a scientific study of this multitude will create an enormous workload, and a lot of experience is required, it is important to involve people and organisations with experience in this field.

- **IUCN/SSC Cat Specialist Group**

  The IUCN/SSC Cat Specialist Group already has been involved with scientific studies regarding lynx genetics. They already have set up a genetic databank for some of the possible subspecies. For the ESB it would therefore be a great opportunity to work together with this organisation. The ESB would firstly function as source from which would be determined which lynxes need to be investigated firstly. Through the research, which has done to establish the studbook, many issues regarding origin have already been solved.

  Secondly, the ESB would serve as a catalyst for the EAZA institutions which will provide genetic samples from their lynxes.

- **Collect samples from lynx of know origin**

  To further develop the gene bank for Eurasian lynxes. Firstly the number of samples from known origin animals needs to be increased. With enough samples from animals from the same origin, a genetic "footprint" can be made of each origin. This way the genetic differences between the different origin lynxes will be apparent and with this a scientific recommendation can be given regarding the validity of the different subspecies. Finally the taxonomic debate regarding Eurasian lynx subspecies will reach the next phase using the scientific evidence.

- **Research on animals of unknown origin and mixed origin**

The genetic footprints will serve as base from which the genetic research will start investigating the unknown origin and mixed origin lynxes.

Husbandry of the Iberian Lynx in Captivity

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Abstract

One of the keys to the husbandry of captive Iberian lynxes is to achieve a balance between promoting natural behaviors in captive individuals (hunting, territoriality, social interactions, etc.) and promoting a stress-free environment more conducive to natural reproduction. In order to obtain relevant information for animal husbandry (animal weights, potential gestation status, etc.) we are fine-tuning various techniques that will prevent the use of invasive methods and also serve to strengthen the trust between captive lynxes and their keepers. Great care must be taken in application of these techniques to avoid ´domestication´ of the captive individuals.

Husbandry techniques are generally based on multidisciplinary input and knowledge acquired on the species´ life history, behavior, nutrition, veterinary and health aspects, ethology, genetics, reproductive physiology, and ecology, together with the systematic use of the scientific method. The content of this chapter is based on experience acquired with the lynxes held at El Acebuche Breeding Center, in Doñana National Park, and at the Jerez Zoo, as well as on information from other felid programs established at North American and European zoos.

The behavior of the lynxes in the conservation breeding program is closely monitored using a remote video-survey system that allows round-the-clock observation. This system is providing us with valuable knowledge on the species in areas that could not easily be studied in nature. In this chapter we will provide details on Iberian lynx captive husbandry, paying special attention to specific aspects such as genetic management, incorporation of new founders to the program, nutrition, husbandry of breeders during breeding season, husbandry of pregnant females, parturition, cub development, as well as preventive medicine within the captive breeding program.

As new breeding centers become part of the Iberian lynx captive breeding program, it is important that husbandry criteria follow unified protocols based on the experience acquired and the adaptive management methods applied at the El Acebuche Breeding

Center and the Jerez Zoo.

1. Genetic Management in the Iberian Lynx Conservation Breeding Program

The Husbandry Committee follows the organization of EEP and SSP programs, including an interdisciplinary approach that involves the development of husbandry protocols, the maintenance of a studbook, and placing special care of the genetic and demographic management of the captive population. Selection of individuals for breeding is based on the genetic priorities established in PM 2000. Exchanges of individuals among centers will be based on the results of the PM 2000 program, and on the advice of a subcommittee of experts.

The Ex-situ Conservation Program has two main goals:

1. To ensure the conservation of as much genetic diversity as possible for the species
2. Create new populations of the Iberian Lynx through reintroduction programs

Given the current status of the Iberian Lynx, the basic conditions required to conserve 85% of genetic variability could be fostered over a period of 30 years (Lacy and Vargas, 2004). Captive populations conserving less than 85% genetic variability are considered dangerously inbred, and would be genetically unacceptable.

In order to achieve the genetic goals stipulated, 4 cubs must be added each year over five consecutive years. Thus, 20 cubs/juveniles are needed within a 5 year period. To guarantee maintenance of genetic diversity over 30 years, 60 (30.30) breeders are needed (comprised initially by the founders plus individuals born within the breeding program). As a basic strategy for maintaining genetic variability, it is important to achieve rapid population growth over the first 10 years of the program, until it reaches its “capacity phase”, established at 60 breeding individuals. Efforts should also be made to ensure equal representation of founders, who should all provide a similar number of offspring to the program.

2. Procedure for the Incorporation of New Individuals

Efforts are made to reduce the inevitable stress caused by transfer of animals into a new environment. All lynxes brought into the captive breeding program are placed under a two-month quarantine period, with the first check-up performed two weeks after arrival. This 2 week period serves the double purpose of providing time for adaptation to new surroundings, and allowing time for the animal to show any potential signs of disease if it were to be incubating any agent upon its capture. A second check-up is performed a

month later. If tests reveal no infectious diseases, the animal is incorporated into the breeding program.

Individuals scheduled for transfer within the network of breeding centers are not anesthetized, but prior to transfer additional coprological and PCR analyses for the main diseases that could be detected from fecal analyses. All lynxes in the program undergo annual health and reproductive screenings.

Because the arrival of a new lynx can affect the behavior of resident lynxes, greater precautions and closer observation are required during the initial period. While adapting to their new territory, new arrivals will seek isolation and protection in the area furthest from daily keeper activity; thus, hiding places and refuges are important features in the enclosures. The adaptation period is highly variable between individuals. Initially, the preferred refuge is usually the furthest den, from which a few new Acebuche arrivals did not emerge during daylight hours for up to 5-6 months. Eventually, all animals regain confidence and adjust to the captive situation, showing different degrees of adaptation. We are presently beginning a project to test the effects of moving lynxes between enclosures at different facilities on potential stress and adaptation. This will later help to refine husbandry for animal exchange between programs.

3. Nutrition

An important aspect of maintaining animals in captive breeding programs is to provide a balanced nutrition since diet deficiencies could compromise reproductive capacity. The Iberian Lynx Program nutritionist, Helena Marqués (ConZOOlting), conducted a study on captive lynx nutritional and energy requirements, which helped the Center determine the best diets for daily maintenance and for extra nutritional needs during gestation, lactation, and cub development.

Wild rabbits account for 80-90% of lynx diet in the wild, with rodents, birds, and ungulates serving as alternative prey, though in certain instances they could even feed on carrion. In order to meet both nutritional and behavioral needs, the diet pf captive animals diet should mirror the diet of their wild counterparts to the extent possible.

Except for pregnant and lactating females, all Program lynxes are fed 6 days per week, with one additional day of fasting. The main diet is live rabbit, as similar in appearance to their wild prey as possible. Diet is normally live rabbit three times per week, dead rabbit twice per week and, on alternate weeks, one day of either fowl or ungulate. All wild ungulate meat must be certified as tuberculosis free. To compensate for any vitamin and mineral deficiencies arising from consumption of dead prey, a multi-vitamin supplement is given in food two times per week. We provide “Mazuri Whole Prey”, because it is specific for carnivores and it best covers both maintenance and reproduction needs. Any medication required is always incorporated to food, in either liquid or powdered form (eg., crushed tablets), with preference given to drugs allowing a once per day dosages.

3. Husbandry Database

Husbandry information is recorded daily in paper and electronic files and it includes information on feeding (live/dead), food type, weight, and other relevant observations such as: samples collected that day (scats or urine), change of enclosure, various behaviors, administration of supplements or medication, symptoms indicating animal is approaching estrous, etc. The information is stored in duplicate in an Access database. (See files for the "El Acebuche" Standard Operation Procedures Manual in: http://www.lynxexsitu.es/manejo/documentos_manejo.htm)

4. Preparation for Breeding Season

4.1. Hormonal Profile Monitoring/Follow up - Fecal and Urine Samples

In collaboration with doctors Katey Pelican and David Wildt, from the National Zoo Conservation and Research Center, Front Royal, USA, and Teresa Abaígar, from the Estación de Zonas Áridas, Almería, hormonal profiles of male and female Iberian lynx have been determined over three breeding periods (see section 3 of the Proceedings).

This information is very important for distinguishing general patterns of Iberian lynx reproductive physiology; for example, females have ovarian activity between January and May, while males appear to maintain testosterone levels year-round. Hormonal information is equally important for identifying patterns and differences in female gestation.

Working with the Institute for Zoo and Wildlife Research in Berlin, in collaboration with Dr. Katarina Jewgenow, a test to diagnose gestation based on potential levels of relaxin in urine levels is now being fine tuned. A comparative study of the reproductive physiology of the Iberian lynx and its closest relative, the Eurasian lynx, is also being conducted.

4.2.Reproductive Check-up

During the Fall, prior to each breeding period, transrectal ultrasounds are performed on females to determine their reproductive status. In collaboration with doctors Frank Goeritz and Katarina Jewgenow, from the Institute for Zoo and Wildlife Research in Berlin, each October adult and subadult females in the program are anesthetized for ultrasound examination of their ovaries (presence or absence of follicles, corpus luteums, and any potentially abnormal structure). The screening also includes an evaluation of the uterus, kidneys, adrenals and other internal structures. This examination is oriented towards detecting any potential reproductive health problems in females and to help solve them whenever possible.

n early November, all males older than two years of age are also subject to reproductive examinations. In collaboration with Dr. Eduardo Roldán and his team at the National Museum of Natural Sciences, electroejaculations are performed and sperm is frozen and stored at the Museum´s Genome Resources Bank in Madrid. The information obtained through electroejaculation is extremely useful for evaluating individual male reproductive health. As well as for comparisons within males to determine if their sperm quality varies from year to year. These studies are essential for gaining a better understanding of the reproductive physiology of the species.

4.3. Weighing and X-Rays

Ideally, each individual should be weighed at least once a week, although this is not always possible in practice. Some animals are less cooperative during handling than others, and while training could be used to improve certain behaviors and management procedures, avoidance of domestication is an important concern. Therefore, the techniques used vary depending upon the individual, and the least intrusive method is always selected. Some animals are accustomed to entering a metal tunnel equipped with a scale, and they can easily be weighed this way, with the keeper close by to record the scale reading. In other cases, the scale can be baited with meat, and placed at a selected spot in the enclosure. In the case of particularly elusive animals, keeper presence is minimized and

a remote reading can be taken using a tripod mounted video surveillance camera focused on the scale. The scale surface is cleaned after each use.

The X-ray procedure is similar to the one used for weighing. For this, a specially constructed crate with a transparent plexiglass front panel is placed in the transfer chute, to slightly restrict animal movement. The portable X-ray machine is placed on one side of the chute, lined up with the opening, and the plate holder on the other. A keeper positions the animal, and the technician takes the X-ray. All personnel involved in X-ray procedures wear protective clothing, lead aprons, etc. The X-ray provides the definitive pregnancy diagnosis, and, if image quality allows, it can even help determine the exact number of cubs (See Management during Gestation, in this same chapter).

5. Husbandry during Reproduction

5.1. Pre-copulation

All potentially intrusive work at the facilities, such as new camera installation, protective netting, carpentry, plumbing, clearing, fumigation, outside nest box installation and inside nest box repairs, should be finished before each breeding season begins.

Optimal breeding pairs are determined based on the advice of the Program Geneticist, Dr. J.A. Godoy, and with the help of PM2000, a computer software that aids in the selection of best pairings. Other important factors include pair compatibility and logistic limitations. In this initial stages of the program is important to increase numbers in the captive population as soon as possible, thus, behavioral compatibility between individuals is a critical determining factor. Other aspects are taken into account include

the establishment of "mixed" couples (breeding members of the Sierra Morena population with the Doñana population). Female / male compatibility is tested before the female enters estrous by gradually increasing the degree of contact between the individuals. In the initial stage, contact is through a mesh screen, and one of the individuals (alternating males and females) is given access to the corridor connecting the enclosures. If signs of compatibility such as friendly greetings, (like head rubbing, etc.) are observed, the guillotine separating the enclosures is raised, but only after precautions are taken to enable rapid intervention in case of confrontation. All encounters are video recorded for later behavior analysis.

At the Acebuche Breeding Center, males normally begin "calling" the females in mid-December, and females become more vocally active about 2-4 weeks later, just prior to entering estrous.

5.2. Copulatory Behavior

When in estrous, females vocalize very frequently and males keep them under constant watch. Both sexes continuously mark their environment until the female finally indicates her readiness. Short (about 1½ - 2 min) and frequent (every 2½ hours) copulations take place over about 2 days, after which the male loses interest. Like most felids, the lynx is an induced ovulator, i.e., females require the mechanical stimulation of intromission for ovulation to occur. Provided the individuals are compatible, and that the male is not needed to breed with another female, the pair is kept in the same enclosure until two or three weeks prior to the estimated date of parturition.

5.3. Gestation

The husbandry for potentially gestating females rests basically on keeping their enclosures as tranquil as possible, stimulating natural exercise and hunting behaviors, closely monitoring their behavior via camera surveillance, and weighing them twice a week. The enclosure is carefully examined to eliminate any areas that the female might prefer over of the inside and outside nest boxes/den zones, since these are equipped for closer monitoring of parturition and cub development. If the male has been left in the same enclosure, it is moved approximately two weeks before estimated parturition date. Routine maintenance is limited to elimination of food remains. The tranquil environment is to help females feel safe in both inside and outside nesting and den zones, which are set up for 24-hour close circuit TV (CCTV) observation. X-rays are taken 55-60 days after copulation to confirm gestation and to determine the number of cubs, if possible. Half-way through the estimated gestation period, (30-37 days), females are allowed to feed 7, rather than 6 days a week. Food is then supplied ad libitum.

The average gestation period of female Iberian lynxes that have had litters at the El Acebuche Breeding Center is between 63-65 days (counting from the day of the first copulation observed). In Spring 2006, there was one premature dysthocic parturition at 56 days. Of the 2 offspring, 1 cub was stillborn, and the second died after a few hours.

Table 1. Iberian Lynx mated at Acebuche Breeding Center in 2006.

Female	Male	Date of copulation	Nº copulations	Gestation	Parturition date	Days gestation	Nº cubs	Comments
Saliega	Garfio	19-I/20-I	43 (19 c/ Garfio y 15 c/ Cromo)	YES	23-III	63	2	Both cubs by Garfio (64 day gestation in 2005)
Saliega	Cromo	22-I/23-I						
Adelfa*	Cromo	19-I/20-I	19	NO	--	--	--	Possible false pregnancy
Aura	Garfio	30-I/1-II	26	NO	--	--	--	Possible false pregnancy
Esperanza	JUB	9-II/11-II	25	YES	15-IV	65	2	Cares for 1 cub, abandons other
Aliaga	Cromo	15-II/16-II	13	YES	12-IV	56*	2	Premature dysthocic parturition No surviving cubs

* = subadult females

5.4. Parturition

The following protocols are applied to all Breeding Center females approaching estimated parturition dates:

-- Two days prior the estimated whelping date: minimum human activity in and near their enclosures.

-- Observation for signs of: Nervousness/agitation, loss of appetite, changes in resting periods and other behavior, etc.

-- Round the clock video observation.

-- Double check all hand rearing materials: Space, incubator, heaters, milk and other feeding utensils.

- Upon onset of labor, observe stages to determine if parturition will be normal (euthocic)

or abnormal (dysthocic; i.e., requiring human intervention. See El Acebuche Breeding Center Procedural Manual). http://www.lynxexsitu.es/manejo/documentos_manejo.htm)

-- If whelping is normal, but female exhibits no maternal instinct (abandonment, cannibalism, etc.), or, if one of the cubs is lethargic /fails to suckle, intervene as specified by the protocol.

6. Husbandry of Females with Cubs

During first three days after parturition, the breeding enclosures are only entered for providing live rabbits. All activity in and around the enclosure is kept to a minimum for 4 weeks. In the four weeks after birth, female food intake rises to almost 2Kg daily (two or three 600 – 700 g. rabbits)

Under ideal circumstances (mother with good maternal instincts and healthy cubs), cubs should be handled as little as possible. Human interference can be avoided through use of CCTV cameras, which provide for careful monitoring of cub behavior and development. After 2-3 weeks, periodical weighing of cubs should commence.

Cases where Cub/Mother Separation is recommended

1.- Cannibalism Normal behavior in cases of still born or unviable cubs Intervene if a female starts cannibalizing an apparently healthy cub to avoid aggression on the rest of the litter.

2.- Cub Abandonment If the female ignores newborn cubs for more than 2 hours after birth, and cubs show signs of lethargy, we consider it abandonment. Immediate intervention is required to avoid cub death from hypothermia.

3.- If the female is caring for cubs, but observation shows that one cub is having problems during the suckling period (lethargy, not suckling, etc.) or if an individual cub is clearly shunted aside by litter members, we intervene.

Patience is required, and care should be taken to avoid interference with the female, unless absolutely necessary.

7. Hand Rearing

Ideally, Iberian Lynx cubs should never be hand-reared. Hand-rearing is an emergency course of action taken only when all others have failed. The conservation breeding program strives to provide the environment and husbandry measures that a female lynx should need for normal gestation, parturition, and cub-rearing, since we believe that the

best care possible is that of their own mother. This is especially true also considering that the program is geared towards future reintroductions.

If problems arise and hand rearing is unavoidable, specific details on hand rearing Iberian lynx can be found in the "Guidelines for Hand Rearing Iberian Lynx Cubs" http://www.lynxexsitu.es/aaveterinaros/manuales.htm).

8. Management Techniques to Prevent or Counteract Confrontations

Lynxes are highly territorial, and frequently admit no territorial incursions by members of their own species, especially those of the same sex. In the wild, territorial disputes do not usually lead to physical confrontation, due to the high cost of losing a fight. While this is also generally true for captive lynxes, precautions must be taken to offset confrontation should it occur. Managers must be well informed and supplied with proven methods and means to break up fights. Selection of the methods to be used depends on the known characteristics of the individuals involved in the fight: (age, sex, personality, degree of dominance, etc.)

8.1. Adult Lynxes

Fights between compatible adult lynxes of different sexes are most likely to happen when animals are first given contact to each other as part of the preliminary socialization during breeding season. Since fights are also likely to occur in relation to feeding time, as a preventive measure males and females sharing the same enclosure are fed separately. Noise distraction (whistles and shouting), squirting with a water hose, and, specially, non-toxic fire extinguishers, can all be good methods to break up fights. If a fight occurs beyond hose or extinguisher range, for example in the inside nesting dens, the back side of the den area must be entered as soon as possible. Radio contact with the CCTV monitor room should be used to verify effectiveness of noise deterrence measures, which

should start with loud banging on entry gate then range up to whistle use. If these measures fail, carefully open the sliding metal door, making sure that enclosure gate is closed, and use water for deterrent as needed.

8.2. Cubs

Since fights are most likely to occur when cubs are 30 - 65 days old, extreme precautions are taken during this period. 24-hour observation shifts are set up and two people are assigned to night shift observation if deemed necessary. As with adults, water is the main deterrent used to break up fights. If a fight occurs outside hose range, keepers must quickly enter the enclosure. Since the female will presumably try to defend her cubs, keepers must take extra personal safety precautions.

When there is high risk of a fight between two cubs, or immediately after a fight, they are separated, and the corridor that separates the large enclosures is temporarily set up as cub enclosure (approx. size = 12 m2). The corridor top is covered with plastic and dried heather, and the walls are lined with raffia. The zone is then enriched with hollow tree trunks and nesting boxes. In a corner located near the box, an infrared lamp is installed outside the mesh to provide a heat source. Hot water bottles covered by soft cotton socks are provided and regularly replaced for the cub to use as a ”warm body”. Since it is important for the cub separated in the corridor to maintain constant visual and olfactory contact with the mother, a window is opened in the raffia lining the mesh between corridor and enclosure. A rotation system is used so that all cubs have opportunities to nurse and spend time with the mother.

9. Health Care Management

A breeding center should make every attempt to keep necessary health management procedures as non-invasive and stress free as possible. Preventive medicine is extremely important to keep diseases from entering and spreading. Once a disease enters the facility, eradication can be sometimes difficult and, in certain cases, impossible.

Any procedures requiring animal capture and anesthesia must be fully justified and the pros and cons weighed carefully, since such procedures could bring associated risks (wounds, problems with anesthesia, etc.) in addition a temporary dent in the animal/keeper trust dynamic.

Part of the healthcare management includes non-invasive analysis of fecal samples, on which parasitological analyses, cultures, urine and other analyses (such as for FCoV and FPV) are performed.

9.1. Internal Coproparasitological and Antiparisitic Screening

Coproparasitological studies are performed on all individuals every three months. The method used is a smear and flotation. Analysis can be done at the Center´s laboratory or

sent to an outside lab if necessary.

Coproparasitological studies of El Acebuche lynxes frequently reveal Eimeriidae cysts from the live rabbits they are fed, which are therefore not problematic. Endoparasite related problems are very uncommon in captive lynxes. Quarterly coprological screening enables us to monitor parasitemia levels and treat if necessary.

Toxascaris and Toxacara are the nematodes most frequently found in felines. The eggs are highly resistant and difficult to eliminate completely from the environment. Reinfections are common, due to eggs contaminating food, or to ingestion of secondary hosts (rodents). Once infestation occurs, complete elimination is extremely difficult, and prophylactic treatment with antihelmintics is needed every six months. 20-50mg/kg of phenbendazol (Panacur) can mixed in food for 3 - 5 days. The antihelmintic should be changed frequently to avoid resistance build up. Antiparasitic products effective against both nematodes and cestodes (Drontal cats, Vitaminthe) can be used. After treatment, three coproparasitological analyses (a minimum of one every 24 hours) are done to check for effectiveness. Should cestode eggs be detected and further treatment required, a one time dose (5 mg/kg) of pziquantel (Droncit) can be given. Prophylactic medication for dirofilaria is currently deemed unnecessary.

9.2. External Parasites and Antiparasitics

Lynxes can be affected by fleas, ticks and ear mites. While ectoparasite treatment should ideally be done simultaneously on animals and environment, long term ectoparasite control in facilities like the Breeding Center is next to impossible.

Topical ectoparasite control products (Frontline, Stronghold, etc.) can be sprayed on or administered via pipette to animals that admit handling or are trained to enter the corridors. Outside areas and corridors are sprinkled with long lasting low toxicity insecticides to create a barrier against ectoparasite entry. Antiparasitic products with different active ingredients (pyrethrine, diazinon, etc.) should be rotated to prevent resistance build up. Treatment should be intensified between March - October. Treatment is more effective when accompanied by periodical brush and vegetation clearing of perimeters, corridors and handling cages, where ectoparasites multiply. Backpack sprayers are used to treat the area every 6-8 weeks, depending on the season / parasite level. Ticks are used to check treatment effectiveness before and after each application.

9.3. Vaccinations

For captive wild felid vaccination, triple-action dead vaccines are the general recommendation. The Iberian Lynx Breeding Program currently uses the Fort Dodge Fevaxyn-i-CHP, a dead virus vaccine for feline panleukopenia (FPV), feline herpes virus (FHV) and feline calcivirus (FCV). In the past, another dead vaccine that inoculates against Feline leukemia(FeLV) and Chlamydiophila in addition to FPV, FHV and FCV, has been used

on occasions. Domestic cats have registered a few cases of sarcoma development in the inoculation zone, possibly associated with the FeLV vaccine drug excipient; vaccination for Chlamydiophila is generally not recommended unless the disease poses a serious risk.

Cubs receive their first triple action vaccination at 4 weeks, a booster at 8 weeks, another booster at 12 weeks, and again at 1 year. After this, vaccination is every two years, with the exception of breeding females, who are revaccinated prior to breeding season. Vaccinations are usually subcutaneous, and require animal capture in squeeze cages or are performed during the annual health screening under full anesthesia. Dart vaccination has proven less effective (e.g., full dosis might not be injected or dart lodged in a low absorption zone of body).

9.4. Urine Analysis

High protein levels in urine are found in both captive and wild Iberian lynx. While this is generally considered a sign of renal disease, none has been observed in the animals. Periodical urine analyses are being performed to gain a better understanding. During other routine collection procedures (scent attractors for photo tracking) urine is collected in sterile recipients for strip analysis at the Breeding Center. This permits monitoring, not only of abnormal protein and leukocyte levels, but also of urine density, and of any blood /crystals in found in urine.

9.5. Fecal Cultures

Salmonella, Shigella and Campylobacter are the bacteria most frequently responsible for feline intestinal disorders. Twice yearly non-invasive feces analysis are used to screen Center lynxes.

9.6. Routine Check-ups

A complete health/reproductive screening is performed yearly is recommended for all individuals. These are scheduled prior to breeding season, or to coincide with other examinations or procedures (The animal must be anesthetized for check-ups. Those trained to enter squeeze cages receive hypodermic injections; darts are used for all others. Whenever possible, animals should be trained to enter handling tunnels or squeeze boxes, to facilitate safe and effective anesthesia and other health maintenance chores.

Prior to anesthesia, all drugs, instruments and or materials required for emergency anesthesia procedures should on hand (see Iberian Lynx Clinical Handbook: http://www.lynxexsitu.es/aaveterinaros/manuales.htm). If the animal has a transponder, it should be checked, and if not found, another microchip should be implanted. Animals should be weighed. Special attention should be assigned to mouth: gum health, broken teeth and tar accumulated. The examination should include claws, foot pads, eyes, ears, genitals,

and mammary glands. Requisite samples should be taken for diagnostic testing, with some blood, serum and feces samples set aside for later analysis.

For more information on the management, infrastructures and emergency procedures used in the Iberian Lynx Ex-Situ Conservation Program, see: www.lynxexsitu.es

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Abnormal Behaviors in Captive Felids

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(FIND ORIGINAL SPANISH VERSION IN APPENDIX II)

This presentation will describe the principal anomalies observed in captive feline behavior, followed by a discussion of causes and suggested management strategies for prevention or correction. The term "abnormal behavior" means (1) behaviors observed only in captive animals --i.e., not seen in animals in the wild-- that are indicative of a lack of well being or (2) behavior that does occur in the wild, but is manifested much more intensely and frequently in captivity, due to stress or lack of well-being.

Abnormal behaviors in captive felines are usually due to stress caused by inadequate enclosures, handling, or a stimulus poor environment that inhibits expression of the normal species behaviors and leads to what is known as "behavioral restriction".

Stress is defined as the body's response to a threatening situation. This response includes both physiological and behavioral changes that, to a great extent are due to an increase in hypothalamus synthesis and release of CRH -Corticotropin Releasing Hormone. Whether a situation is stressful or not, depends on the animal's perception of it. Generally, a stress response is elicited in situations perceived by the animal as unexpected, or beyond its control.

Behavioral restriction occurs when the stimulus affecting the animal impedes expression of certain behaviors that are important in and of themselves, in other words, behaviors for which strong motivation exists, regardless of the animal's environment. Considerable evidence has been obtained in studies on a variety of species indicating that animals are strongly motivated to perform foraging, or food hunting behaviors even when their nutritional needs are covered. Consequently, an environment that does not allow the animal to exhibit such behavior, will lead to "behavioral restriction". It is highly probable that other behaviors besides foraging are equally important, although less evidence has been documented.

The two factors responsible for the behavior abnormalities exhibited by captive felines -stress and stimulus poor environment- are closely related, since behavioral restriction frequently triggers stress.

The most common behavioral alteration in captive felines is stereotypia, which is defined as repetitive, invariant behavior patterns with no obvious goal or function. Stereotypia

has not been observed in wild animals living free, but is frequent in wild animals living in captivity, and in domestic animals. There are two main types of stereotypia: Those caused by a pathological process –organic based stereotypia- and those caused by improper environment –environmental stereotypia-. These latter are the most frequent. Environmental stereotypes develop when an individual predisposed to stereotypic behavior is confined in an environment where it cannot perform a behavior for which there is an important motivation, or when the environment itself causes situations of conflict or stress.

When the cause is the environment, the predisposition to perform stereotypic behaviors varies by species and also among individuals of a same species. Reactions differ due to genetic factors, and to the animal's early life experiences. Stereotypic behavior is more common in carnivores and primates than in herbivores. It has been suggested that species that would normally cover large daily ranges in the wild are more prone to stereotypic behavior when in captivity than species that cover a smaller daily range.

Within the same species, individuals that suffered stress in early stages of development are more likely to exhibit stereotypic behavior in adulthood. Studies have found that animals weaned prematurely are more prone to stereotypias as adults than those weaned at a normal age. This could also explain the apparently contradictory fact that, in some species, captive born individuals develop more stereotypic behaviors than those captured in the wild.

Among domestic animals, genetic differences have also been cited as the explanation for differences in stereotypic behavior levels. However, such differences are manifested only in inadequate environments. If the environment is satisfactory, no individual exhibits stereotypia.

In captive felines, the most common environmentally induced stereotypia is pacing.

Pacing, and other stereotypias are important indicators of an animal's lack of well-being. Furthermore, some findings indicate that animals held in environments that cause stereotypia development may also suffer reproductive dysfunction.

It is important to note that, while stereotypias are a clear indication of a lack well-being, the opposite cannot be assumed, i.e., the fact that no stereotypic behavior is exhibited, cannot be interpreted as proof of animal well-being. Development of stereotypias due to unsatisfactory environment differs by species, and also by individual members of the same species.

Other behavioral changes observed in captive felines involve lower activity in general, and reduced responses to internal and external stimuli. This apathetic behavior is also manifested in lower food intake and changes in grooming habits. Such abnormal behaviors can be a passive stress management strategy.

In the case of the domestic cat, it is a potentially serious problem, since the lower food intake described can lead to hepatic lipidosis, an accumulation of fatty acids in the hepatocyte cytoplasm that can eventually cause liver failure and death. While hepatic lipidosis can occur due to any factor triggering lower food intake, in a high percentage of cases –at least in the domestic cat- drops in food intake are normally due to stress, and have no organic cause.

Stress also inhibits reproductive behavior, particularly maternal behaviors. Stress increases the activity of neuronal circuits that negatively affect a female's maternal response to cubs, therefore females suffering from stress show a higher incidence of cub rejection and aggression.

There are several management strategies to help prevent or control stress induced behavioral alterations or behavioral restriction. Of these, the best known is environmental enrichment, which consists of providing the animal a more stimulating environment in which it can perform behaviors similar to those it would exhibit in its natural habitat. Environmental enrichment can be divided into the categories of social –commonly used for gregarious species- and non-social. This latter can be subdivided into the following categories: food, sensory, structural and cognitive enrichment.

Food based environmental enrichment is the one most frequently used with felines. It consists of providing food in ways that stimulate natural foraging/hunting behavior, rather than simply providing quick and easy access to food. One way to do this is to hide small portions of food in several areas of the enclosure. This has proven to be an effective technique for reducing stereotypic behavior and increasing general activity levels, and also for reducing the level of cortisol in plasma – or metabolites in feces-, which would appear to indicate that environmental enrichment also reduces the physiological stress response.

Another type of environmental enrichment commonly used with felines is structural enrichment, which consists of providing the animal with an environment that enables it to hide or climb, to cite two examples.

It is important to remember that environmental enrichment practices should always be based on the natural history of the species, since, as mentioned earlier, the aim is to achieve behaviors as similar as possible to those of the species in the wild. After implementation, the effects of the enrichment program should be tested, using behavioral and physiological indicators. Examples of behavioral indicators include the total time of animal inactivity, or time employed in stereotypic behaviors. Physiological indicators include cortisol in feces, to measure for chronic stress.

Occasionally stress problems can be palliated through the use of antipsychotic drugs, including long acting neuroleptics (LAN). LAN are tranquilizers that act on the dopamine system, with effects lasting 2-3 weeks, depending on the species being treated, and the drug used. LAN can help ease the animal's adaptation to a new environment and reduce aggressive behaviors. However, while potentially of interest, very few studies have been

performed on LAN therapy usefulness and safety in felines.

Other molecules –like fluoxetine and buspirone- have proven highly effective on domestic cats, and occasionally on wild felids. Fluoxetine markedly reduces aggressive behavior, and buspirone is an ansiolytic. The lecture will include presentation of one case where these drugs were successfully used to treat wild captive felines, with discussion of active mechanisms and limitations.

Antipsychotic drugs should be used only occasionally, and never routinely to mask or counteract possible deficiencies in the animal's management or enclosure.

Finally, the facial pheromones of cats, which have an ansiolytic effect, have been synthesized and are now commercially available. If, as it appears, they have the same effect on other felines, pheromones could be a useful tool in the captive management of some wild feline species.

Sibling Aggression in Eurasian Lynx (*Lynx lynx*)

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Early sibling aggression is common in bird species (Mock, Parker 1997). It occurs during the first days after hatching and can lead to the death of nestlings (obligate or facultative siblicide). This can be a way of brood size modification according to available resources (Mock, Parker, 1997; Drummond, 2001). In mammals early sibling has been described and intensively studied in spotted hyenas (*Crocuta crocuta*) (Frank et al., 1991). Hyena cubs start fights within the first minutes of birth and birth order is an important factor; the first-born attacks the later-born. In contrast to many other mammals these newborns have fully erupted incisors and canines and they are capable of well-coordinated behavior at birth.

Unlike hyenas, lynx kittens are born blind with closed external ear canals; eyes open on postnatal day 12-13 (Stehlik, 1980). Average litter size is two (it varies from one to three, sometimes four) (Naidenko, Erofeeva, 2004).

The specific fights between lynx littermates occur at the age 36-64 day more then in 50% of litters with two-three kittens (n=29). At this age seemingly spontaneous attacks of one kitten to a sib are observed. These fights differ from other types of aggression in the lack of ritualized behavioral elements. They may last for several hours after which aggression usually ceases. These fights may lead to kittens being wounded and to intervention by the mother. Four kittens died during these fights (10 % of kittens which fought with each other). Females usually try to stop the fights but sometimes they are not successful.

These fights result in changes in physical development and social behaviour of sibs that may have an effect for their future life-history. Fight lead to the changes of sibling relations increasing an asymmetry in playful contacts and siblings' play partner preferences. These fights were accompanied by the appearance of a hierarchical structure in the litters, where the losers had the lowest rank.

Duration of time after a fight was significantly related to the frequency of social play (the first observation of play after a fight compared to time to other observations after $F=4.18$; $df=1$; $p<0.05$). The frequency of play decreased significantly for some time after fights. Observed and expected play frequencies differed significantly in two of the 11 kittens (18%) before fights. In contrast, during the first two weeks after the fights, differences

between chance and observed frequencies were registered for six kittens (55%; χ^2-test: χ^2=5.87; p<0.05).

Of the identified aggressors, 11 were female (of 22 females in group A), and three were male (of 17 males in group A) (χ^2-test: χ^2=3.00, *df*=1; p=0.083). Females attacked males (six cases) as often as they attacked other females (five cases). The probability of fights was approximately the same for litters of primeparous (50%; n=10) and multiparous females (mothers) (53%; n=19) as well as the probability of a lethal outcome (10% and 16 %, respectively).

Fights were observed more often in litters with a smaller daily gain in body mass (<20 g; five of six litters) than in litters with a larger daily gain (>30 g; one of seven litters) (χ^2-test: χ^2=26.9, *df*=1, *p*<0.001). Such way, these fights may be related to the lack of food for the kittens because they seem related with the kittens' growth rate. However these fights are not related with the availability of food for their mothers (Sokolov et al., 1994). Probably, it is because kittens switch to the solid food during this period.

Growth rates of winners and losers (g/day) did not differ significantly at the day of fight (day 0), 10 or 30 days later. We estimated the changes in growth rate of kittens 10 and 30 days after a fight in comparison to their growth rate on the day of the fight. Values were significantly different 30 days after fights. Whereas winners increased their daily body mass gain on average by 7 g/day, losers decreased it by an average of 13 g/day.

The mechanism of this phenomenon is not clear yet. Our preliminary data allow us to say that this phenomenon is androgen-independent (we measured testosterone, androstenedione and DHEA). In other side the probability of these fights may be related with the adrenal glands activity and level of cortisol in kittens' blood plasma.

Such way, lynx sibling aggression is a phenomenon which may effect on the survival of lynx kittens during the early stage of their ontogeny. Although this phenomenon was never observed directly in nature the high mortality of lynx kittens in wild allow us to speculate about the role of siblicide.

Husbandry training of wild felids in captivity

Michelle Templeton Skurski
Disney's Animal Kingdom

A husbandry training program is considered an integral component of progressive animal husbandry programs in zoos today. Over the past decade there has been great strides made in the management of captive felids. The introduction of animal training utilizing a positive approach has greatly enhanced the lives of captive felids and changed the way they are managed in a zoo situation. Implementation of a husbandry training program can provide captive felids with choices and behavioral opportunities in their environments. The result is healthier animals, better educational experiences, and enhanced research opportunities. Training felids leads to a wide variety of animal management options, from shifting and crating, to safer, less stressful medical procedures such as voluntary blood draws, injections and examinations. Simply put, implementing a husbandry training program into the animals' daily lives may enhance the welfare of felids.

The purpose of this presentation is to offer ideas of the types of husbandry and medical behaviors that can be trained with felids. Details will be provided about a process that can be used to set goals for a training program, such as considerations for animal health, animal management, research, education and facility design. Disney's Animal Kingdom's felid training program will be used as an example. Using images and video, I will illustrate how specific behaviors were trained and the use of the trained behaviors implemented. This presentation will also give direction on considerations when starting a felid training program, such as, safety of both keepers and animals during training sessions, how the facility is designed, the importance of building a relationship with the animal being worked, reinforcement selection for some species, motivation issues, and what behaviors to start training first. Resources for additional training information will also be provided.

Reproductive Physiology & Biological Resources Bank

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Contributions of Reproductive Science to Wild Felid Conservation

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Abstract

Innovative and advanced technologies have allowed impressive advancements in the reproductive sciences, including overcoming infertility and increasing offspring numbers. Incentives for this progress largely were derived from commercial efforts directed at enhancing livestock production and addressing human health issues. For example in the cattle industry, artificial insemination allowed the wide scale distribution of 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 couples to overcome infertility and to 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 reproduction in livestock and humans, the management and conservation of wildlife species has more complex ideas and logistics. For endangered species maintained in *ex situ* collections, the aim is not only to produce more young, but offspring of known provenance and appropriate genotype that will preserve the integrity of a species, subspecies or a population. This often requires the breeding of two individuals of the appropriate genotype, but which may be sexually incompatible or physically separated by long distances. It also may be advantageous to preserve the genes of a valuable individual for long durations to be periodically infused back into the collection to maximize genetic vigor. For the same reason, there can be advantages to linking individuals or populations maintained in breeding centers to counterparts living in nature. Dealing with these issues is one concern of wildlife managers.

Reproductive technologies have long been considered a means of assisting in the genetic management and propagation of endangered species. So far, assisted breeding 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 the practical applicability for

offspring production. Thus, the limiting factor is the lack of basic knowledge about thousands of unstudied species, the foundation that is essential to allowing reproduction to be enhanced. There now is excellent evidence that reproductive technologies are most useful as tools for studying how different species reproduce, especially defining novel and unique mechanisms. In this paper, we review the status and relevance of various reproductive technologies that are useful or have potential for wild felid in terms of improved breeding, management and conservation. From our more than 25 years of experience, 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.

The Role of Teratospermia in Felid Reproduction and Fertility

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Teratospermia (production of >60% morphologically abnormal sperm/ejaculate) is relatively common among various species in the family Felidae, which is comprised of 37 species. Over 2 decades of research in this area have produced a significant understanding of the phenotypic expression, its impacts on sperm function and etiology. There is good evidence suggesting that a reduction in genetic diversity contributes to this phenomenon. Results to date, demonstrate that spermatozoa from teratospermic donors are compromised in the ability to undergo capacitation and the acrosome reaction, penetrate the zona-pellucida, fertilize conspecific oocytes and survive cryopreservation. Recent studies also reveal abnormalities in chromatin integrity in sperm from teratospermic donors, which interestingly fails to impact fertilization or embryo development after intracytoplasmic sperm injection. Through planned inbreeding studies, we now have established that teratospermic cats also produce more sperm by virtue of more sperm producing tissue, more germ cells per Sertoli cell, and reduced germ cell loss during spermatogenesis. Overall, it now is clear that gain in sperm quantity is achieved at the expense of sperm quality, suggesting an extensive disruption of normal testicular function in teratospermic donors. Preliminary studies on testicular gene expression in teratospermic cats have also revealed abnormal expression patterns. These findings have markedly increased our understanding of testis biology in the teratospermic donor and reaffirm the value of cats, including wild species, as models for studying novel regulatory mechanisms controlling spermatogenesis and spermiogenesis.

A Genetic Resource Bank and Development of Assisted Reproductive Techniques for the Iberian Lynx

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The best strategy for biodiversity conservation is the preservation of the natural habitat. However, implementing this statregy is not always possible or viable. It is 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 and to allow interchange of genetic materials between individuals of threatened populations and to allow for the opportunity to reproduce to individuals that die before reaching reproductive age. With these objetives, our group has developed work since 1995 on three species of endangered ungulates, has organized since 2003 a germplasm and somatic tissue bank for endangered Iberian species and has started in 2005 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 centered, in a first stage, on the Iberian lynx and on the 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, we also rely 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.

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 is collected and banked. Such somatic cells may be used in the future with the help of assisted reproductive techniques. We have implemented a system to collect, transport and process in the laboratory samples from dead animals and biopsies from live individuals. Tissue samples are transported or sent by courier in culture medium in a refrigerated styro-foam containers. Tissue sub-samples are both cryopreserved and processed and incubated in D-MEM with 10% bovine serum, L-glutamine, antibiotics and antifungal agents at 37ºC under 5% CO_2 in air. Cells grow out of explants until confluency (with a maximum of 1-3 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. Cryopreserved cells mantain viability and can grow in culture after several months of storage in liquid nitrogen. Occasional problems arise due to contamination of tissues (perhaps due to incomplete cleaning during collection) or to lack of viability during incubation due to a long delay between death of the animal and collection of tissues. Using this methodology, somatic tissues and cells have been cryopreserved from Iberian lynxes, Eurasian lynxes and bobcats.

Cryopreservation of epididymal cat spermatozoa as a model for wild felids

The success of 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 phyllogenetically related model species that allow the provision of sufficient number of samples for the development of adequate methods of sperm evaluation and protocols of sperm cryopreservation. We use the dometic cat as species model for endangerd felids. Since December 2003, we have collected epididymal spermatozoa from over 300 subadult and adult cats and about half of the samples have been found to be suitable

for cryopreservation. Samples have been selected based on the presence of at least 40% motile sperm and a total of $20x10^6$ sperm/ml. Variations were observed throughout the year both in the availability and quality of the samples. Mean values observed have been: 55% motile sperm, $24x10^6$ total spermatozoa, 80% sperm with normal morphology and 75% sperm with intact acrosomes.

For cat sperm cryopreservation, we have tested 4 diluents (Tes-Tris, PDV-62, Biladyl, 18.5% raffinose, all with 20% egg yolk and 4% glycerol), 2 cooling rates (0.5ºC/min and 0.125ºC/min), 2 packaging methods (pellets and straws) and timing of glycerol addition (1 and 2 steps). 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. We found no significant differences between diluents, cooling rates or addition of glycerol in 1 or 2 steps, although data showed a trend for better results with slow cooling and 2-step glycerol addition. Cryosurvival was significantly better in straws than in pellets.

Semen collection from Iberian lynxes and related species

Between September 2004 and August 2006 we had access to biomaterials from various Iberian lynx males. We have obtained epididymal spermatozoa from 2 out of 6 dead males (5 of them killed by cars). Most of them were subadult dispersing males that died before reproducing. We have also collected semen by electroejaculation on 10 occasions from 6 captive males. In two cases no sperm were recovered and in another one quality was very low. Average values of semen parameters were similar to those found by us in Eurasian lynxes and bobcats sampled at Spanish zoos and 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 refrigeration of Iberian lynx spermatozoa. Two diluents were evaluated: Tes-Tris (TEST) and Biladyl (A+B fractions), both with 20% egg yolk and 4% glycerol. After cooling from 22ºC to 5ºC during 2 h, results were similar between TEST and Bildayl A+B with regards to proportion of motile sperm and the proportion of spermatozoa with intact acrosomes. Future work should reveal whether they are equally good for sperm freezing.

In vitro maturation, fertilization and culture of oocytes

Oocytes recoverd post-mortem or after ovariectomies, as well as those collect 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 techniques of in vitro maturation and fertilization, as well as those necessary for oocyte and embryo cryopreservation, it is necessary to resort to cat oocytes

as models. With the collaboration of various veterinary clinics, we have collected cat ovaries which were transported to the laboratory, where they were sliced to recover immature oocytes. Oocytes have been matured in vitro, fertilized with fresh epididymal spermatozoa, and cultured up to the blastocyst stage.

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%). Additional studies are under way to examine further the developmental potential of embryos obtained from epididymal cat spermatozoa stored using various cyropreservation protocols.

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 those that die before having a reproductive opportunity. Xenotransplantation of testis grafts is successful even when done between distant species. 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. 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.

Preservation of Iberian lynx female germline

To allow for a maximum preservation of genetic diversity, female germline of Iberian lynxes should 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. 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.

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 valuble 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.

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Iberian Lynx Cell Bank. Biological Reserves and their Applications to the Conservation of the Species

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The Iberian Lynx Cell Bank was created several years ago as a support for the species' conservation programme. This bank is co-directed by Miguel Ángel Simón, from the Environmental Council of the Andalusian Government, and Trinidad León from the Bioengineering Institute, Miguel Hernández University. It began as part of the first somatic cell bank for Spain's most endangered animals. This bank was supported from the beginning by the Environmental Council of the Andalusian Government and by the General Directorate on Biodiversity of the National Ministry of Environment. We later extended the reserve and at present the cell bank is composed of somatic cells, tissues, germ cells, gonads as well as other biological samples such as blood, urine, hair or faecal samples.

The aim of animal conservation is to maintain or, if possible, increase biodiversity. The ideal approach to conserve the species is by in-situ strategies that pursue the preservation of animals in their natural habitats, this being always the main objective. Nevertheless, in spite of the 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. Biological reserves serve as an insurance policy in small populations. Collecting the necessary biological materials is crucial before further biodiversity is lost. The samples taken can be divided into two categories: cells from which we can obtain, at least potentially, individuals, and materials allowing studies that can help preserve the species within their habitat, which includes cells/tissues as well as other biological samples.

The aim is to create biological reserves representing an interface between in-situ and ex-situ conservation programs, and thus support global conservation.

Cell Bank and Reproductive Technologies

The cell reserve is mainly focused on obtaining individuals. For this we need to consider all possible kinds of cells, not only mature germ cells but also immature and somatic cells. This is because with regards to the reproductive biotechnologies we can distinguish between modern reproductive biotechnologies and assisted reproductive techniques. By using modern reproductive biotechnologies we can potentially obtain individuals from somatic cells such as fibroblasts from skin, etc. Assisted reproductive techniques include artificial insemination, in vitro fertilization, embryo transfer and gamete/embryo micromanipulation. Both types of biotechnologies allow more individuals to be obtained from selected parents assuring genetic diversity.

However, reproductive biotechnologies usually are species-specific and for many endangered animals there is insufficient knowledge about their basic reproduction as well as the processing and cryopreservation of their gametes and gonads, which is the first step in creating a germ cell bank. Unfortunately, in cryobiology the optimal protocol that would assure the survival of 100% of frozen-thawed cells/tissues/embryos does not exist for either gametes/gonads or somatic cells/tissues. This is due to, among other reasons, that cells, tissues, gametes, gonads and embryos have different ages, stages of development or they come from different sources. Thus for each species we must consider more than one approach and develop different protocols.

Advances in modern reproductive biotechnologies have been performed, but they must be more effective and require perfecting the technique. However, when future progress in the field of nuclear transfer is obtained and this technique becomes functional, we must have a somatic cell reserve reflecting the widest biodiversity as possible. In order to try to improve the efficiency of this technique we search for stem cells, independently of their differentiation potential: uni, multi or pluripotential because all types of stem cells have a greater plasticity than the 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 had been made.

Other Cells and Biological Reserves

The majority of the cells cultured present no signs of stem cells and thus these cells together with other biological reserves such as blood, urine, hear or faeces can provide the material to develop different studies to improve the Iberian lynx preservation within its habitat. Such samples will serve to repeat analyses if necessary or to start new research/study. To make this aim possible each component is processed considering its present and future utilisation.

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Comparative Endocrinology of Domestic and Non Domestic Felids

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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 nondomestic felids as well. The latter has been possible largely through the development of noninvasive 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 nondomestic cats, and describes how the rapidly growing endocrine database is aiding ex situ management efforts.

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 felids, urinary analysis of reproductive steroids is not a viable option because steroids are excreted almost exclusively (>90%) in feces. Extracting steroid metabolites from feces generally involves boiling, vortexing or shaking samples in combinations of organic (e.g., ethanol, methanol) and aqueous solvents. Estradiol-17b is excreted in its native form and as conjugates; therefore, both estradiol-specific and broad spectrum (e.g., total estrogens or estrogen conjugates) immunoassays are effective for monitoring follicular dynamics. By contrast, progesterone is highly metabolized before excretion, so luteal activity is best defined using group-specific antisera that crossreact with varied pregnane metabolites.

Reproductive ovarian steroid cycle patterns now have been published for over two thirds of the 36 nondomestic 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), with fecal steroid analyses being used in over three quarters of the studies. Surges of fecal estrogens distinguish estrous from interestrus periods, and in general females cycle at 2- to 4-week intervals with estrus lasting 3-10 days. In many species, variable periods of follicular inactivity not associated with season are observed (e.g., cheetah, ocelot, fishing cat). In domestic and nondomestic felids, progestin concentrations during pregnant and nonpregnant luteal phases are quantitatively similar, with a length that is about one third to one half that of pregnancy. Although typically considered reflex ovulators, spontaneous ovulations are known to occur in domestic cats and a number of nondomestic species, including the lion, clouded leopard, leopard, Pallas' cat, fishing cat and margay. In cheetahs, periods of alternating estrous cyclicity in some group-housed females are due to social suppression. Cheetahs in the wild are solitary, yet many facilities house females in groups because intra-species aggression is relatively low. Separation of pairs showing evidence of reproductive suppression resulted in a reinitiation of normal cyclic ovarian activity. Reproduction is at least somewhat seasonal in many non-domestic felids like the tiger, clouded leopard, Pallas' cat and snow leopard. Conversely, follicular activity in captive lions, leopards, pumas, margays, ocelots and margays and fishing cats is not influenced by season.

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. 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, tiger, domestic cat). The etiology of excessive estrogen production after gonadotropin treatment appears to be due, in part, to the development of ancillary ovarian follicles. 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. It is in this species that pregnancy success after AI is among the best for felids.

In recent years there has been a growing demand to develop methods to assess stress in zoo animals, and 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. Indeed a lack of stimuli can lead to boredom, with equally detrimental consequences. For felids, failure to reproduce has long been attributed to stress ensuing from suboptimal housing and husbandry. 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 and in domestic cats and leopard cats after translocation. Conversely, elevated urinary cortisol concentrations in leopard cats were reduced after barren cages were enriched with branches and hiding places. 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. Appropriate assays need to be broad spectrum to crossreact with the variety of corticoid metabolites found in cat feces, none of which are native cortisol or corticosterone.

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, cheetah), translocation and introduction to a male (cheetah). Cheetahs identified by keepers as being more 'nervous' also had higher fecal corticoid concentrations than 'calm' individuals. In clouded leopards, comparisons across temperament categories indicated that 'nervous' animals had higher corticoid concentrations than 'calm' individuals. 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. In a study of Brazilian small cats, females were subjected to three enclosure conditions over successive time periods: Phase I - large, enriched enclosures for 3 months; Phase II - small, empty enclosures for 6 months; Phase

III – the same small enclosures enriched with branches and nest boxes for 6 months. 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.

In summary, 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 noninvasive 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. We also need a quick and reliable single sample test for diagnosing pregnancy, preferably one that is noninvasive. 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.

Hormone Profiles in the Iberian Lynx: Application to In Situ and Ex Situ Conservation

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The Iberian lynx is the most endangered of the world's felid species. The current population is estimated at fewer than 200 animals, which are living in two fragmented populations: Sierra Morena (Jaén) and Doñana National Park (Huelva). In 2004, an Iberian lynx captive breeding program was initiated in Doñana National Park (Huelva), Spain both as a hedge against extinction and to extend our understanding of species biology. In March 2005, three Iberian lynx cubs were born from a three-year-old dam, the first successful birth ever in captivity.

An endocrine evaluation study was conducted to begin to understand the hormonal control of reproduction in the Iberian lynx. Daily fecal samples were collected from a total of six peri-pubertal and adult females (1.5 to 14 years old (y) at study onset) and four males (1.5 to 3 y) from April 2004 through June 2006 for the purpose of characterizing normative seasonal and reproductive endocrine profiles. All of the evaluated females were wild-caught, four from the Sierra Morena Mountains and two from Doñana National Park. Whole fecal samples (2004-2005) or steroid hormone extracts (2005-2006) were shipped frozen from Spain to the National Zoo's Conservation & Research Center, and metabolites of estrogen and progesterone (female) or testosterone (male) were quantified using enzyme immunoassays. From 2004-2005, steroid hormones were extracted from lyophilized feces, while in 2005-2006, wet feces were used. Data were analyzed for the dry feces and wet feces separately using mixed model repeated measures analysis. Hormonal metabolite concentrations greater than twice the standard deviation from baseline were considered elevated.

There were marked seasonal changes in estrogen metabolites in all females. In 2004-2006, in the presumed early breeding season (January through August), estrogen metabolite levels increased above baseline from 14 January through 31 January. The end of the breeding season was marked by a fall in estrogen concentrations to baseline from 11

May to 22 August and remained at nadir until January of the following year. In 2004, the mean estrogen metabolite concentrations were lower ($p < 0.05$) in the non-breeding (mean ± SEM; 1.2 ± 0.2 ¤g/g dry feces) compared to the breeding (7.9 ± 2.7¤¤g/g dry feces) season, a pattern that also held for the 2005 breeding season (10.2 ± 4.3 ¤g/g dry feces). Similarly, the 2005-2006 data showed a similar significant increase ($p < 0.05$) in mean fecal estrogen metabolites during the breeding season (4.1 ± 1.4 ¤g/g wet feces) compared to the non-breeding season (0.4 ± 0.2 ¤g/g wet feces). Compared to other felids species studied in our laboratories (e.g., cheetah, tiger, clouded leopard, domestic cat, fishing cat among others), estrogen metabolite concentration in the female Iberian lynx was ~10 to 100-fold greater during the breeding season (with levels being in ¤g versus ng concentrations for other species).

In contrast to the dramatic seasonality seen in the estrogens, progesterone metabolites showed a more subtle seasonal pattern that did not conform to the known breeding season in this species. Progesterone metabolites remained elevated ($p < 0.05$) from February through October (range in mean monthly concentrations: 2004-2005, 25.0-54.4 ¤g/g dry feces/month; 2005-2006, 6.4-11.1 ¤g/g wet feces/month) in all females, then decreased ($p < 0.05$) in November through January (2004-2005, 17.2-21.9 ¤g/g dry feces/month; 2005-2006, 4.5-5.4 ¤g/g wet feces/month) before rising again to seasonal levels.

Male Iberian lynx exhibited subtle seasonal changes in testosterone levels with elevations in testosterone occurring from January to May (range in mean monthly concentrations: 2004-2005, 46.1-77.5 ¤g/g dry feces/month ; 2005-2006, 31.8–45.9 ¤g/g wet feces/month). Males then showed generally lower ($p < 0.05$), though variable, testosterone metabolite levels from June to December (2004-2005, 38.0–52.6 ¤g/g dry feces/month; 2005-2006, 27.0–36.4 ¤g/g wet feces/month).

In 2005 and 2006, signs of estrus (vocalization, conspecific sexual interest) were observed from 17 January, and breeding was observed in all females from 19 January through 15 February. During the 2005 breeding season, all four adult females copulated (ages 3-15 y). Of these, one female (25% pregnancy rate) that copulated multiple times on 24 January gave birth to three cubs on 28 March 2005 (gestation interval = 64 days). In 2006, five females (ages 2, 2, 4, 4, and 5 y), copulated during the breeding season and three (60%; ages 2, 4 and 5 y) became pregnant. The 2 y female had still-born cubs at day 56 of pregnancy. The remaining two females had full-term pregnancies (63 and 65 days) and viable litters with a total of 3 surviving cubs.

Of the 9 copulation events in the Iberian lynx, 7 females showed initial seasonal elevations in estradiol immediately prior to copulation, however, levels were ~ 1/10 the levels seen in the breeding season (range in peak estradiol elevation: 0.6–3.85 ¤g/g feces). The remaining two females did not show significant elevations in fecal estrogen metabolite levels prior to breeding.

Mean estrogen and progesterone metabolite concentrations during pregnancy (overall mean concentrations: 6.4 ± 4.1 ¤g/g feces and 10.5 ± 4.6 ¤g/g feces respectively) did not

differ ($p > 0.05$) over time from females that copulated but were not pregnant (5.0 ± 1.5 ¤g/g feces and 19.2 ± 5.4 ¤g/g feces). In fact, elevations in estrogen and progesterone metabolite concentrations during pregnancy appeared to be more attributable to a seasonal change rather than pregnancy-associated, since neither parturition nor lactation altered ($p > 0.05$) the seasonal trajectory for either hormone.

In 2006, the two copulating peri-pubertal (2 y) females showed altered hormone profiles compared to the three copulating adults (3-5 y). Estrogen profiles showed time and age effects, with 2 y females showing lower ($p < 0.05$) estrogen concentrations in the late (April – June) breeding season compared to the adults. In addition, overall mean progesterone metabolite levels were lower ($p < 0.05$) in 2 y females (2.5 ± 1.6 ¤g/g wet feces) compared to adults (7.9 ± 3.6 ¤g/g wet feces). One aged (16 y) female failed to show regular seasonal elevations in estrogen or progesterone metabolites in 2006, did not display behavioral estrus and did not copulate.

Results demonstrate that lynx successfully breed in captivity with ~50% pregnancy rates in the first three years since the establishment of the program. The female Iberian lynx is a seasonal breeder, with reproductive activity reflected primarily in fecal estrogen metabolite profiles with marked elevations in estrogens occurring from January - August. Males of this species show only mild seasonal alterations in testosterone levels and are probably producing semen throughout the year. The female of this species excretes extraordinarily high quantities of estrogen in the feces, much elevated compared to other felid species studied to date. Contrary to estrogens, progesterone metabolites (at least using standard felid assays) are a poor indicator of reproductive status in the Iberian lynx as they (1) show only minor seasonal fluctuations that do not correlate with the known breeding season; and (2) fail to show clear elevations following copulation or during pregnancy. Overall, these milestone births combined with an emerging database on endocrine profiles and reproductive behaviors provides encouragement that such a scholarly and applied approach will be helpful in achieving successful captive breeding in this critically endangered felid.

A Comparative Analysis of the Endocrine Patterns of the Eurasian and the Iberian Lynx in Captivity

Análisis comparativo de los patrones dereproducción del lince boreal y del lince ibérico en cautividad.

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Monitoring sexual hormones is especially important in efficient ex-situ breeding programs of endangered mammals. Thus, the aim of the presented study was (i) to identify relevant fecal steroid hormone metabolites in captive Eurasian lynx, (ii) the biological validation of fecal hormone measurements in both sexes, and (iii) finally a comparative metabolite analysis between Eurasian and Iberian lynxes. The radiometabolism study was performed for testosterone (i.v. injection of [^{3}H]testosterone in a male) and for progesterone (i.v. injection of [^{3}H]progesterone in a female). Fecal and urine samples were subjected to HPLC analysis to identify radiolabelled metabolites and immunoreactive hormones.

In males, the majority of testosterone metabolites are substances with a higher polarity than testosterone. Only minor proportion of radioactivity co-eluted with authentic testosterone and dihydrotestosterone. The testosterone immune assay measured substantial amount of immunoreactivity, correspondeding to two radioactive peaks. The seasonality of Eurasian lynx reproduction was confirmed by a seasonal pattern of testosterone excretion, with high testosterone levels during breeding season in March/ April. Preliminary results on testosterone measurements in Iberian lynx feces confirmed the suitability of the applied testosterone immune test in this highly endangered species. HPLC separation of Iberian lynx feces extracts revealed a similar metabolite pattern determined by EIA that were typical for Eurasian lynx fecal extracts.

In females, the seasonal fecal hormone profiles showed two peculiarities: (i) a significant positive correlation between fecal gestagen and estradiol metabolites and (ii) elevated concentrations of gestagen metabolites during lactation. Therefore a pregnancy diagnosis based on fecal steroid hormone metabolite seemed to be unattainable. In Eurasian lynx, majority of radiolabelled metabolites were substances with a high polarity. The first of the two major polar HPLC peaks was hydrolyseable, and seemed to vanish towards the end of pregnancy. The elution position of the major immunoreactive metabolites coincided with both polar radiolabelled peaks. Minor peaks were found at the positions of authentic progesterone, DHP, pregnenolone and pregnandiol. The HPLC profile of Iberian

lynx feces showed the same elution pattern suggesting similar gestagen metabolism in both lynx species.

Since the present results don´t facilitate pregnancy diagnosis in the two lynx species, other analytical options, like urinary relaxin, are under current investigations.

Determining Stress and Reproductive Status of Reintroduced Canada Lynx

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Canada lynx (Lynx canadensis) are declining throughout much of their former range, and were recently listed as a threatened species in the U.S. In response to this decline, the state of Colorado initiated a large-scale effort to reintroduce lynx in 1999. Initially, the success of this effort seemed tenuous due to the lack of successful reproduction during the first 4 years of the reintroduction. However, since 2003, researchers have found a significant number of healthy kittens. Despite extensive post-release monitoring, scientists still debate which factors contributed to the initial inhibition, and subsequent recovery, of reproduction. The lack of information about the reproductive physiology of lynx further hinders our ability to understand patterns of reproduction in this population.

In order to determine how an individual's physiology is impacted by the reintroduction process, we have initiated a three-fold study of lynx physiology using fecal hormone analysis. First, we are intensively monitoring captive individuals to establish a detailed understanding of hormone expression in lynx. Second, we are obtaining fecal samples from lynx in naturally-occurring populations across North America in order to describe patterns of hormone expression in the wild and identify differences between captive and wild lynx. Finally, fecal samples are being collected throughout the Colorado reintroduction effort in conjunction with ecological and behavioral data. This allows us to document physiological changes that occur during the process and identify factors that correlate with these physiological changes.

Preliminary Data

Reproductive physiology of captive males

We found that males display pronounced seasonal variation in fecal androgen expression. Although such strong seasonality is not common among felids, other lynx species show similar patterns. Since lynx inhabit temperate-to-arctic environments, it should be very adaptive to time breeding so that kittens are raised when environmental conditions are the most favorable. It is interesting to note that this pattern of seasonality was shown by all males, regardless of the latitude at which they were housed.

Reproductive physiology of captive females

To date, there has only been one successful pregnancy in this study, and the mother abandoned the kittens within a few days of parturition. In this case, fecal progestogens did not increase as dramatically as expected during the pregnancy. We have tested five different progesterone antibodies, and while some show a slight increase in fecal progestogens towards the end of the pregnancy, none show the characteristic elevation seen in other mammal species. We are still working to determine whether this is a unique characteristic of lynx reproductive physiology, or if this pattern is driven by their metabolism of steroid hormones.

Stress and reproduction in reintroduced lynx

The preliminary data strongly suggest that certain stages of the reintroduction process are stressful for lynx. Fecal corticoids increase following translocation to Colorado, physical exams, and periods of intense human activity around the holding pens. Further evidence that this initial translocation and holding period is stressful is that pregnancies failed when females were transported while pregnant. Following release, fecal corticoids in at least one male decreased markedly and fecal androgens increased; this also suggests that the holding period alters normative patterns of hormone expression.

The initial data also indicate interesting differences between groups of lynx. Females have much higher fecal corticoid concentrations than males. However, it is difficult to say whether this is due to gender differences in normative patterns of corticoid expression or metabolism, or if it is indicative of an actual gender difference in sensitivity to stress. Also, males that were initially trapped and handled by biologists had higher fecal androgen concentrations and more pronounced stress responses (as measured by fecal corticoids) than males that were trapped and handled by trappers. These differences persisted for at least 2 months, which suggests that it is important to consider handling practices at all stages of the reintroduction effort.

Applying Knowledge of Reproductive Physiology to Help In Situ and Ex Situ Populations of Wild Felids: The Clouded Leopard as a Model

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Abstract

Significant strides have been made since the 1970s in rare felid reproduction through the systematic study of basic reproductive mechanisms including gamete (sperm and oocyte) biology, nutritional influences on reproduction, stress-hormone responses to captive management regimes, and endocrinology of the male and female. This basic research forms the foundation for improving survival of endangered felids through integrated science-based conservation projects that improve genetic management and propagation of captive populations while at the same time improving our understanding of the health, reproductive capacity and survival of wild populations in increasingly altered landscapes. One example of how studies in reproductive physiology have formed the foundation for conservation of a species *in situ* and *ex situ* is the clouded leopard.

1. Using Science to Conserve an Endangered Felid: The Thailand Clouded Leopard Conservation Project

The world population of captive clouded leopards is in a crisis. The population is not self-sustaining primarily due to male aggression with females frequently being killed by males. As a result, only 2.5% of females and 0% of males have reproduced with more than one mate in the North American Clouded Leopard SSP. The situation in zoos in range countries like Thailand is even more dramatic. A survey of clouded leopards in Thailand zoos in 2003 found that only 3 of the 27 leopards in the zoos were born in captivity. At the same time, the population in the wild is under increasing pressure due to wide-scale environmental degradation and poaching. Listed as Vulnerable in the IUCN Red Data Book, as an Appendix 1 species under CITES and Endangered under the United States Endangered Species Act, the species is estimated to be in decline throughout its historic

range in Southeast Asia.

The Smithsonian's National Zoological Park has been breeding clouded leopards and studying their reproductive physiology for over 25 years. Specifically, three major 'breakthrough' discoveries have helped improve captive breeding in this species. First, anecdotal evidence from experienced clouded leopard breeders indicated that pairing males as juveniles to juvenile or adult females reduces the risk of aggressive attacks on females. Second, studies in South America and Asia indicated that nutritional status plays a significant role in felid reproduction and health. Some feline diets used in zoos around the world are high in fat content and provide reduced and/or imbalanced levels of protein, vitamins and minerals. These diets have been associated with health and reproductive problems in felids, including metabolic bone disease, renal (kidney) disease, reduced sperm production, poor sperm viability, depressed reproductive cycling and high cub mortality. Finally, stress associated with inappropriate housing facilities has been found to significantly impact breeding success. Recent research on clouded leopards conducted at North American zoos has determined that small enclosures, limited vertical height, lack of hiding spaces and proximity to other species, particularly potential large predators (such as tigers and bears), correlates strongly with high stress hormone levels in the clouded leopard. Chronic increases in stress hormone concentrations, in turn, are associated with poor reproductive performance in a number of species and have been associated with aggressive and lethal attacks by male clouded leopards on females. Ideal housing for the clouded leopard, therefore, is a large enclosure with ample height and hiding that is isolated from visual contact with other large carnivore species (tigers and bears).

In light of this new information on felid management and reproduction, Dr. JoGayle Howard of the Smithsonian's National Zoological Park and Rick Schwartz, Director of the Nashville Zoo initiated a collaborative project with the Thailand Zoological Parks Organization, WildAid, the Thailand Department of National Parks, Wildlife and Plant Conservation and the Clouded Leopard Species Survival Plan to develop a clouded leopard conservation program *in situ* and *ex situ*. The goal of the program was to create a science-based clouded leopard program in Thailand that included improving husbandry and breeding success in the *ex situ* (captive) population in Thai zoos and determining the status of the *in situ* (wild) population.

1.1. Building a Science-Based *Ex Situ* Breeding Program

The majority of the Asian cat species in Thailand zoos are genetically valuable, wild-born animals that have been confiscated and/or donated to the zoos. Health and reproduction, however, frequently are compromised due to poor husbandry, imbalanced

diets and inadequate enclosures. In 2002, an improved clouded leopard breeding program was established at the Khao Kheow Open Zoo in Chonburi, Thailand. Based on the reproduction and management research breakthroughs for clouded leopards in North America, this program emphasized: 1) providing experienced clouded leopard breeders year-round to manage the program in Thailand; 2) pairing juvenile clouded leopard males where possible; 3) providing a balanced diet; 4) improving management of the animals to minimize stress; and 5) integrating research into the breeding program to increase our foundation of knowledge in this difficult species.

Since May 2002, animals have managed full-time by an experienced clouded leopard keeper and moved from enclosures in Thailand zoos known to induce stress (small size, lack of hiding places, adjacent to large carnivores such as tigers and bears) to large, vegetation-rich enclosures with nest boxes. Finally, nutritionally poor diets were altered to include whole prey and vitamin/mineral supplementation.

To document the impact of management changes on stress and reproduction, detailed data were collected on animal behavior, enclosure size, carnivore proximity and enclosure enrichment. Daily fecal samples were collected from 10 adult females and 7 adult males before and after the improvements in diet and enclosure, and then samples were shipped to the U.S.A. for assessment of fecal reproductive and stress hormones. Results show that 60% (6 of 10) of the female clouded leopards in the program showed markedly improved reproductive status within six months of diet improvements and moves to low-stress enclosures. Males showed much more subtle changes in hormone levels compared to females, with 6 of 7 adult males showing mild increases in testosterone within six months of the management improvements. The ultimate test of the improvements, however, was the success of the breeding program. In all, 19 clouded leopard cubs have

been born (16 surviving) to 6 successful male-female pairs since 2002. These results document the importance of good diet and management on reproductive success in captive carnivores.

In addition to the breeding success, though, this program also has provided an important opportunity to study clouded leopard introductions and determine what constitutes a successful pairing and what leads to pairing failure. The presence of the on-site manager and complete access to consortium-supported animals has provided an unprecedented opportunity to investigate various pairing strategies in clouded leopards. Previous research in North American clouded leopards has been limited in scope since most North American institutions have a minimal capacity for a large clouded leopard collection and lack the skilled staff necessary to attempt pairing this difficult species. Thus, no research to date has been able to compare different pairing techniques or successful versus unsuccessful pairs in any meaningful way. To improve our understanding of clouded leopard introductions, we are using clouded leopards at the Khao Kheow Open Zoo to compare the impact of different pairing strategies for successful and unsuccessful clouded leopard pairs using a combination of behavioral observations, personality assessments and stress and reproductive fecal hormone assessments. Four categories of pairings are being compared: 1) successful pairings involving juvenile (< 12 months of age) males (n = 3 pairs); 2) successful pairings involving adult males (n = 3); 3) unsuccessful pairings involving juvenile males (n = 3); and 4) unsuccessful pairings involving adult males (n = 6). For the first time, changes in hormones and behavior can be compared between clouded leopards undergoing different pairing protocols. At the same time, changes in behavior associated with stages of the introduction process can be correlated with changes in stress hormones and reproduction hormones. Already it is clear that pairing juvenile males whether to adult or juvenile females requires less time and is successful more often than when adult males are used. As a result, this technique has been used whenever possible in Thailand, but many males were adult (> 12 months old) when they joined the consortium and, therefore, necessitated the introduction of adult pairs.

Ultimately, important questions will be answered about the pairing process in this species including: 1) does pairing induce stress responses in clouded leopards; 2) is one method of pairing more stressful than the other; 3) do alterations in stress and reproductive hormones correlate with particular behaviors during the pairing process; 4) are the different pairing methods associated with differences in behavioral or endocrine parameters; 5) what are the behavioral and hormonal correlates of pair failure; 6) does personality influence the success or failure of pairings in this species? Overall, this information will be critical to understanding the biological foundation of successful pairings in this complex and difficult carnivore species.

1.2. Using Science to Build *Ex Situ/In Situ* Linkages

Since the inception of the Thailand clouded leopard project, a focus has been to link *ex situ* and *in situ* conservation efforts. As is the case in many countries, there has historically been little communication between the zoo community and the National Parks in Thailand. Therefore, one major focus of the Thailand Clouded Leopard Conservation Project has been to use science to integrate the two branches of the project. This has occurred on two levels. First, National Park rangers from the Thailand Department of National Parks, Wildlife and Plant Conservation were brought to the Khao Kheow Zoo as part of a carnivore monitoring workshop taught by ecologists from the National Zoo. At the Khao Kheow Zoo, they were able to see and touch some of the animals they were being trained to monitor, sometimes for the first time. They took imprints of the animal's paws to use in identifying pug marks in the field, studied the zoos large collection of skeletons for reference to field samples and practiced radio-collaring animals. In addition, research was undertaken in collaboration with geneticist, Lori Eggert, as part of the breeding program at the zoo to validate field techniques using the clouded leopards. Specifically, research studies were able to: 1) determine the impact of radio-collaring on clouded leopard physiology and behavior using fecal stress and reproductive hormone monitoring; 2) develop a fecal DNA species identification reference library and sexing marker; and 3) establish the timing of fecal hormone and DNA degradation under field conditions in Thailand. Once fully validated, these techniques will be used to assess and monitor wild carnivore populations as part of the broader *in situ* conservation project.

1.3. *In Situ* Field Survey Project: Science and Training

Southeast Asia is one of the fastest growing regions of the world and as a result this part of the world is experiencing wide-scale deforestation, industrialization, agriculturization and pollution. Felid conservation programs must take into account a number of potential contributors to species decline. Research conducted over the past 25 years in *ex situ* and *in situ* carnivore populations, highlights a number of potential contributors to poor reproductive and immune function and, thus, species decline *in situ*. These include: 1) inbreeding associated with population isolation and decline; 2) stress associated with environmental degradation and human encroachment; 3) environmental contamination leading to endocrine disruption; and 4) nutritional deprivation due to food chain disruptions. All of these represent important areas that reproductive physiologists could contribute to understanding species decline and extinction. In addition, conservation of gene diversity through gamete cryopreservation and genome resource banking provides a proven method to transfer genes between isolated populations and across generations to manage gene diversity in endangered species as a metapopulation.

Before any detailed study of felid population health and reproduction can be undertaken, however, there must be an understanding of the basic ecology and parameters of the

population. Almost nothing is known about the *in situ* status of most carnivores in Thailand. Thus, conservation plans for saving endangered cats *in situ* must start by building a foundation of knowledge on their status and distribution. This is certainly true of the clouded leopard whose status, distribution and density across remaining habitat was completely unknown at the start of the Thailand conservation project. In part, the lack of available information is due to a lack of training on the part of the National Park rangers to monitor their own wildlife populations. Thus, to conserve clouded leopards *in situ*, the Smithsonian's National Zoological Park (Dr. Peter Leimgruber and Dr. JoGayle Howard) has been working in partnership with the Thailand Department of National Parks, Wildlife and Plant Conservation and the non-profit organization WildAid to assess the numbers of clouded leopards and other carnivores living in Khao Yai National Park, the closest National Park to Bangkok. A major component to this Carnivore Conservation Project is the training of Thai forest rangers to monitor wild carnivores (including clouded leopards) and poaching in the park. The project now employs six permanent Thai park rangers, a Thai graduate student (Kanda Damrongchainarong), and a USA graduate student (Kate Jenks) who collect data on the status, distribution and threats to wild carnivores. The carnivore monitoring team also uses their observations to inform the park about poaching and illegal wildlife activity in Khao Yai National Park.

Currently, there are three main components to the *in situ* cat project: 1) a regional habitat analysis of Thailand using satellite imagery and GIS to identify areas with the greatest potential for felid and carnivore conservation; 2) training the Thailand forestry rangers in field techniques for monitoring wildlife; and 3) a field survey of cat populations using motion-detection cameras to identify species and animal densities, transect surveys of animal sign and GPS (global positioning system) units to identify exact location of each field camera. One major advantage of the *in situ* cat project at Khao Yai National Park is that information is being obtained on all cat species, as well as all carnivore species in this large nature reserve. Cameras also continue to photograph poachers and document the presence of illegal wildlife activity in the park. Once this initial ecological assessment is completed, and the monitoring team is established in the park, the next step will be to use the radio-collaring, fecal DNA and fecal steroid hormone field-collection techniques developed at the Khao Kheow Zoo to begin to investigate the reproductive and health status of the clouded leopards and other carnivores *in situ*.

2. Conclusions

Reproduction is fundamental to species survival *in situ* and *ex situ*. A great deal of knowledge has been generated on the fundamental mechanisms of reproduction in endangered felids over the past 25 years. As felids around the world face increasing population pressures, it will be imperative to build science-based conservation programs in range countries that maximize reproductive potential both *in situ* and *ex situ*, while

at the same time building strong science programs that further our understanding of this challenging taxa.

Reintroduction of Wild Carnivores:

Applicable Experiences to the Iberian Lynx

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Black-footed Ferret Reintroduction in the Great Plains of North America

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Historically, the black-footed ferret (ferret; *Mustela nigripes*) was distributed over a vast portion of the Great Plains and steppe ecoregions of central North America, from southern Canada, through 12 central/western states in the U.S., to northern Mexico. The black-footed ferret is a highly specialized and obligate carnivore, dependant on prairie dog species (*Cynomys* spp.) for most of its life history requirements. The ferret became critically endangered following decades of native grassland conversion to agricultural cropland and extensive prairie dog poisoning campaigns waged by ranching interests over much of the 1900s -- actions which eliminated over 95 percent of the occupied range of the black-tailed prairie dog (*Cynomys ludovicianus*), the most common of prairie dog species. Widely considered extinct by the late 1970s, the ferret has a rich history of rediscovery, ecological research, wild extirpation, captive breeding, and reintroduction. And since 1987, when only 18 known surviving individual ferrets remained, remarkable progress has been made in its recovery. Captive breeding objectives have been fully achieved, and excess ferrets have been available for wild reintroduction since 1991. A total of 10 separate reintroduction projects have been initiated within six states in the U.S. and at a single site in northern Chihuahua, Mexico. And following the final releases of captive-reared ferrets by December of 2006, over 2300 ferrets will have been reintroduced into the wild. Reintroduction success varies by site and is affected by overall habitat quality and the presence an exotic disease, sylvatic plague (bacterium *Yersina pestis*); a disease devastating to both prairie dog and ferret populations. Still, viable wild populations of ferrets have been established and many program biologists now believe we possess the expertise and technical ability to fully recover the ferret. However, prairie dog persecution continues, and other substantial socio-political factors may limit overall recovery potential. This paper highlights black-footed ferret recovery successes and remaining obstacles in hopes that many of the lessons learned will aid in recovering other imperiled species.

European Mink Conservation Breeding Program and Re-Introduction Operation in Estonia

Tiit Maran & Madis Põdra.

The European mink, *Mustela lutreola,* is a highly endangered mammal in Europe. It originally ranged across almost the whole continental Europe. Today it has still survived in the form of few fragmented populations in the north-east of Spain, south-west of France, in the Romanian Danube delta, in the Ukraine and also in a few regions of Russia. The remnant populations are declining everywhere. The extinction of the species is caused by a set of factors, whose content varies across time and space. From the global perspective, the most important agents of extinction are habitat loss, over-exploitation and the imapct of an invasive alien species – the American mink, *Mustela vison*. As the removal of the American mink at the continental scale in Europe is most likely not a feasible option, a realistic goal for the conservation of the species would be an effectively managed captive population of the European mink and establishment of island populations for the species in areas inaccessible for the alien mink. The term 'island populations' is used in the broader sense here and includes also suitable habitat patches on the mainland.

The conservation goals for the European mink in Estonia have been formulated as: (1) conservation management of a captive population and long term maintenance the heterozygisity of the species in captivity, (2) establishment of viable island populations in two Estonian islands - Hiiumaa and Saaremaa.

The captive population has been managed in the framework of the European mink EEP program since the early 1990s. It originates from 22 founders and a little more than 90% of its pedigree is known. The genetics and demography of the overall captive population have been managed with the help of Population Management 2000 software. An overall achievable aim for captive management in case the current population mangement scheme is not changed the maintainance of 83% of gene diversity for 20 years. With special management it could be increased to the level of 85% of gene diversity for 50 years. Such special management includes the provision of additional 30 additional founders and increasing of the generation length (from 1.7 to 2).

Although genetic and demographic management is of high importance for conservation breeding, we emphasise the importance of understanding the animal's behavioral requirements, as these may seriously jeopardize the success of conservation breeding.

The main complication faced in the captive management of the European mink is inadequate breeding behavior in males and to a lesser extent in females. The abnormal breeding behavior results from the management scheme used so far but the actual factor causing it is still not fully understood.

The release of the European mink in Hiiumaa Island started in 2000 after successful eradication of the alien American mink there. Almost 300 animals have been released in total since then. The main problem faced so far is the high mortality of the released animals during the adaptation period (50 – 70%) and in winter (20%). This results in a too small number of animals surviving until the next breeding season – the surviving animals do not reach the critical number of animals needed to start breeding in the wild. Several re-introduction techinques have been tested since the beginning of the release, shifting from the hard release approach to the soft release approach. The following operations have been tested during the release process: various ways of preconditioning of the animals, release in different seasons, release of pregnant females, conceiving of re-trapped females and releasing them back into the wild, release of animals with nest boxes, release of litter, release of animals of different age, release of litter from enclosures in riparian habitats at the time of weaning. The failures and successes with these release methods will be discussed during the presentation.

The issues critical for the release seem to be as follows: (1) identification of the best time period for release, (2) number of animals relased, (3) repeated releases, (4) preparation of the animals for release, (3) selection of locations for the release.

At the beginning of the release operation, much attention was paid to genetic considerations, that is, to how to ensure the best genetic quality of the captive population simultaneously with the release of genetically important animals. Given the high level of mortality, the genetic considerations were postponed to be handled once the wild population has been established in the island and increasing of the survival rate of the released animals became the most critical issue.

Since the beginning of the release, the number of animals surviving the winter has been continuously increasing, reaching around 20 – 30 in 2006. In 2006, the first signs of success were observed: tracks of the animals were observed in most of the streams and rivers in Hiiumaa Island, including ditches in human settlements; three wild born litters were discovered and two wild born animals were live trapped.

The release will be continued in 2007 in Hiiumaa Island and the plan is to start in 2008 with the release operation in another island – Saaremaa.

Participatory Planning in Large Mammal Restoration: The Esteros de Iberá Case in Argentina

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(FIND ORIGINAL SPANISH VERSION IN APPENDIX III)

Abstract

Like all Biodiversity conservation efforts, the reintroduction of endangered mammals is a complex challenge calling for proper understanding and management of the biological, social, political and organizational issues involved. This chapter discusses the tremendous importance of the planning process, a delicate and crucial step in any reintroduction program. The case of an initiative to restore large mammal populations in the region of Iberá (Argentina) will be described, followed by a discussion of general planning principles applicable to other introduction programs. The large mammal restoration program for Iberá involved several stages and methods of participatory decision making. In an initial stage, we worked with a small, informal and diverse group of professionals to openly and creatively discuss program priorities. This approach helped us flesh out two initial recovery programs (the giant anteater and the pampas deer) and identify another program for a highly charismatic species –the jaguar- that would not be launched until the first two programs had gained sufficient ground. The giant anteater reintroduction planning process was done in two stages: One with the participation of technical and scientific experts, and another with that of government authorities. Different tools and focuses were employed in management of each. The product of the planning process was a plan for recovery of the species within the Iberá region, with tremendous backing from the technical and scientific community, and the explicit support of the competent government authorities. The planning process also laid the foundations for the program's scientific and institutional advisory committees. At the start of the pampas deer conservation program, which required the use of private ranch and farm property, it was decided that the minimum requirements had not been met for commencement of a formal planning process that would effectively involve all relevant stakeholders. Several principles that could prove useful to other programs were applied in the Iberá experience. First, planning should be seen as a key action and convergence point for the numerous decisions involved the reintroduction process. Second, as much care should be taken with the planning process as with its proposed product. Third, the context of each individual case will determine the planning process required. Fourth, the influence

and willingness of the individual/institutional stakeholder must be considered during planning process design. Fifth, a wide range of perspectives and knowledge of disciplines should be incorporated in the planning process, to facilitate examination of problems and solutions from a variety of angles. And last, a new professional approach to planning is needed to effectively address the challenges posed by the combination of biological and social complexity, and context specificity.

Scientific Planning for the Translocation of Iberian lynx in Doñana´s National Park

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(FIND ORIGINAL SPANISH VERSION IN APPENDIX III)

The Iberian lynx (Lynx pardinus) is the most highly endangered felid species in the world. The surviving population now stands at approximately 150-200 individuals, all on the Iberian Peninsula. One of the best known surviving lynx populations lives in and around Doñana National Park, in the best and most highly protected natural setting left of their historic ranges. The majority of the 40 member population live outside Park boundaries. Spain has the tremendous –global- responsibility of conserving this species, even as Doñana and surrounding areas managers are assigned the delicate task of preventing the extinction of an emblematic lynx population, whose fate may serve to forecast that of the rest of the species.

The Doñana lynx population is now at critically low levels. Most of the populations living inside the Park have either disappeared completely, or the number of members has dropped considerably. While 15-20 years ago, up to 10 potentially reproductive females lived inside the park, we estimate there are now 3. This major reduction in source nuclei drastically changes the species´ conservation status in Doñana. The disappearance of one more lynx range inside the national park could lead to extinction of the Doñana lynx population within the next 10 years.

Recovery of the reproductive source areas inside the national park, helped along by the translocation of a few lynxes, along with recovery and increased carrying capacity of the source areas inside the park, would reduce the probability of extinction of the entire Doñana area lynx metapopulation to below 5% in the next 100 years.

Reintroduction of Captive-Bred Wildcats in Germany

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In the course of a long-term experimental study in Switzerland, a species-specific enclosure for European Wildcats was developed in which animals encounter all the structures and stimuli relevant to their behaviours as regards all functional cycles. In this enclosure, the wildcats do not develop any behavioural disturbances and are able to perform their natural behaviour. They show a rhythm of activity similar to the one of their conspecifics in the wild, and an electronic feeding device, specifically tailored to wildcats, enables them to express very nearly the whole range of their natural hunting behaviour. The cats' well-being is linked to the species-specific structures. These must be available in the right arrangement within the enclosure, with the keeper's appropriate behaviour as the second and equally important factor. The results of this study as well as the feeding technique have been applied in several wildlife parks in Switzerland and Germany.

Since 1993 the offspring of the Swiss enclosure cats have been provided for the wildcat reintroduction project in Bavaria. This project by the Bund Naturschutz in Bayern has been underway since 1984 under the direction of Guenther Worel. Up to now, more than 500 wildcats have been released in three different areas in Bavaria. For several years there was no way of getting any information as to the fate of the Swiss cats after their release, because no direct monitoring was being done at that time. No further radio-tracking study had been planned by the Bund Naturschutz in Bayern after the one realized in the very first years of the reintroduction.

In 1999 we could radio-track eleven wildcats in the course of a pilot study. Eight of these cats had been bred in the species-specific enclosures in Switzerland, whereas three cats came from zoos. All cats equipped with collar transmitters were released in the Spessart, a hilly and wooded region in Bavaria. Besides testing the tracking equipment our interest was focused on the behaviour and survival of the cats.

Radio-tracking is a standard method used in wildlife research. Usually, wild animals are captured, equipped with radio collars and released immediately after recovery from anaesthesia. Normally, there is no possibility of observing the animals' reactions to their collars. In our pilot study, the impact of radio collars on the behaviour of the wildcats in enclosures was observed prior to their release. Four different types of radio collars were used. Behavioural data were collected by direct observation of two females with collars of 32 g and of two males with collars of 52 g. These animals were observed in the same

enclosure first without their collars on and again when they were wearing the collars.

Surprisingly the cats showed almost no behavioural reaction to the collars, not even on the first day. There was no difference in the number of behaviours shown during the day, nor any difference either in the frequency and duration of comfort behaviour (including scratching oneself and shaking one's head) or in resting position. One male showed a higher frequency of the behaviour "shaking oneself" with the collar. The cats showed a slightly lower percentage of locomotion with their collars on, but this was assumed to be an effect of the anaesthesia, which was necessary for their collars to be fitted. Two out of three collars provided with an elastic section were taken off in the enclosure within three weeks, but none in the wild following release. One cat was seriously injured by a collar and had to be captured and treated medically after two weeks in the wild.

We conclude that the wildcats' behaviour is not impaired by the radio collars, but that not all types of collars available for cats are in fact suitable for cats considering the risk of injury.

After release the cats were tracked for three to five weeks. After that time they were tracked only sporadically. The eleven wildcats were released from two sites in different seasons. Five ten-to-eleven months old cats bred in the species-specific enclosures in Switzerland were released in June. After leaving their cage in the forest the cats roved about for three to twenty days before they settled down. One male cat wandered about more than 60 km (linear distance between points of localization) until it settled down at a distance of 19 km (aerial distance) from the release site. All of them survived until at least the middle of August and there was proof for two of them of being alive in Mai 2000, eleven months after their release. In addition, we have obtained evidence in the field indicating that they had no problems with catching enough prey.

At the end of September, six additional animals marked by collar transmitters were released from a different site in the Spessart. They had been bred in the wildcat-specific enclosure in Switzerland and in two different zoos respectively, the zoo-bred animals having undergone a training of several months' duration in the wildcat station in Bavaria, prior to release. Their ages ranged from five and a half months up to about one and a half years. Three of them were killed crossing roads during the first two weeks after their release. We assume that mainly seasonal effects led to this high mortality. With dusk setting in at an earlier time in autumn, we registered dislocations of the cats already in the earlier evening when traffic is much heavier, and, therefore, the danger for a cat to get killed much higher than at later hours in summer.

Our three-years' study planned to follow the pilot phase has not been realized up to now. Extensive data on the behaviour of the animals after release, on population density and on the survival and reproduction of part of the population should have been collected. These data would allow us to measure the success, which is indispensable for a reintroduction project.

The Colorado Lynx Reintroduction Program

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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. 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. 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 key question to be asked when considering the re-establishment of any species is, "What is different now from when they disappeared?" For lynx, the causative factor(s) of their extirpation may never be known. Many of the hypothesized factors, however, have changed substantially since the early and mid-1900's. For example, widespread predator poisoning no longer occurs; conservation of wildlife habitat is now given much stronger consideration in public land management decisions; trapping and hunting are more strictly regulated and regulations enforced; and in some areas, at least, the passage of time has allowed the landscape to recover from abuses of the past, perhaps to a state that is more conducive to lynx survival. It must be acknowledged, however, that there may be other detrimental factors operating now that did not exist previously. In particular, increased human density and development have occurred in some areas and exotic diseases such as plague have been introduced in Colorado.

The uncertainty surrounding the cause of the extirpation of lynx and the effects of current conditions in Colorado on lynx makes it impossible to predict with confidence whether Colorado can sustain viable population(s) of lynx. In order to perform the best test of this question the CDOW led a cooperative effort to reintroduce wild-trapped lynx from Canada and Alaska into southwestern Colorado beginning in 1997. It was hoped the effort would clarify whether or not Colorado is or is not suitable for sustaining viable lynx populations, provided the fate of the released animals could be determined.

The goal of the Colorado lynx reintroduction program is to establish a 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 be equal to or greater than mortality.

The first lynx were released in Colorado in February 1999. From 1999-2006, 218 lynx were released in southwestern Colorado. Each lynx was released with dual satellite and VHF radio transmitters to allow intensive monitoring of animals after release. Locations of each lynx were collected through aerial-tracking (n = 8680) or satellite-tracking (n = 18, 963) to document survival, movement patterns, reproduction, and habitat-use. Most lynx remained near the core release area in southwestern Colorado. From 1999-2006, there were 80 mortalities of released adult lynx. Approximately 31.3% were human-induced which were attributed to collisions with vehicles or gunshot. Malnutrition and disease/illness accounted for 21.3% of the deaths while 32.5% of the deaths were from unknown causes. Reproductive females had the smallest 90% utilization distribution home ranges ($\bar{x}$ = 75.2 km^2, SE = 15.9 km^2), followed by attending males ($\bar{x}$ = 102.5 km^2, SE = 39.7 km^2) and non-reproductive animals ($\bar{x}$ = 653.8 km^2, SE = 145.4 km^2).

Reproduction was first documented during the 2003 reproduction season with 6 dens and 16 kittens found. A second successful breeding season was documented in 2004 with 30 kittens found at 11 dens and an addition 9 kittens found after denning season. In 2005, 46 kittens were found at 16 dens with an additional den located but not visited for safety reasons. Four dens with 11 kittens were found in 2006. Lynx CO04F07, 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.

From snow-tracking, the primary winter prey species (n = 426) were snowshoe hare (*Lepus americanus*, annual $\bar{x}$ = 75.1%, SE = 5.17) and red squirrel (*Tamiasciurus hudsonicus*, annual $\bar{x}$ = 15.3%, SE = 3.09); other mammals and birds formed a minor part of the winter diet. Mature Engelmann spruce (*Picea engelmannii*)-subalpine fir (*Abies lasiocarpa*) forest stands with 42-65% canopy cover and 15-20% conifer understory cover were the most commonly used areas in southwestern Colorado. Little difference in aspect (slight preference for north-facing slopes), slope ($\bar{x}$ = 15.7°) or elevation ($\bar{x}$ = 3173 m) were detected for long beds, travel and kill sites (n = 1841). Den sites (n = 37) however, were located at higher elevations ($\bar{x}$ = 3354 m, SE = 31 m) on steeper ($\bar{x}$ = 30°, SE = 2°) and more commonly north-facing slopes with a dense understory of coarse woody debris.

A study comparing snowshoe hare densities among mature stands of Engelmann spruce/subalpine fir, lodgepole pine (*Pinus contorta*) and Ponderosa pine (*Pinus ponderosa*) was completed in 2004 with highest hare densities found in Engelmann spruce/subalpine fir stands and no hares found in Ponderosa pine stands. A study to evaluate snowshoe hare

densities, demography and seasonal movement patterns among small and medium tree sized lodgepole pine stands and mature spruce/fir stands was initiated in 2005 and will continue through 2009.

Results to date have demonstrated that CDOW 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.

Restoration of Bobcats to Cumberland Island, Georgia, USA: Lessons Learned and Evidence for the Role of Bobcats as Keystone Predators

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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. In this paper, we summarize our methods of capturing and handling bobcats and results of post-release monitoring of movements, survival, and reproduction. We use this as an opportunity to (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 that provide evidence for bobcats initiating a top-down trophic cascade on the Cumberland Island forested ecosystem.

We captured bobcats from the coastal plain of Georgia in the hope that these bobcats would have gene complexes adapted to the environment on Cumberland Island. Captured bobcats were temporarily held in a facility on the mainland so that groups of 4-6 individuals could be translocated and released on the island in fall 1988 and 1989. All reintroduced bobcats were vaccinated for three common feline diseases and fitted with radio-collars. Post-release monitoring included radio-telemetry to monitor movements, spatial organization, and survival, re-capture to assess body condition and replace radio-collars, prey surveys to assess food abundance, and scat collection to assess prey use. We analyzed white-tailed deer (*Odocoileus virginianus*) harvest data from 1980-1997 to

assess changes in the deer population (body weights, age-sex structure, and abundance) because deer were a dominant prey species during our post-release monitoring of 1988-1991. Also, because browsing by deer suppressed oak (*Quercus* spp.) regeneration prior to the bobcat reintroduction, we investigated whether declines in deer abundance resulted in increased numbers and size of seedlings and root sprouts of these tree species.

We reintroduced three males and 11 females in fall 1988 and 12 males and six females in fall 1989. One female released in 1988 returned to the mainland, another died from physical injuries, and one male released in 1989 swam into the Atlantic Ocean and presumably drowned. However, 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). We documented reproduction of 10 kittens in 1989 (4 litters), one litter of two kittens in 1990, and one litter of two kittens in 1991.

Bobcat prey abundance varied spatially and seasonally, but we detected no effect of season or year on diet composition during 1989-90. Marsh rabbits (*Sylvilagus palustris*), white-tailed deer, and hispid cotton rats (*Sigmodon hispidus*) were the only prey species identified in bobcat scats in all seasons and we considered these species principal prey. Only the presence of marsh rabbits and cotton rats in bobcat diets were correlated with abundance, which suggested bobcats exhibited a functional response to these prey species. Diet species diversity and richness were negatively correlated with marsh rabbit abundance, which agrees with predictions of a diet optimization model in which alternate prey species (raccoon [*Procyon lotor*], feral hogs [*Sus scrofa*], and cotton deermice [*Peromyscus gossypinus*]) increased with a decrease in abundance of a preferred 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.

Female bobcats reintroduced during the first year of the reintroduction exhibited little change in the location or size of their home range (95% fixed kernel utilization distribution), but failed to exclude newcomers reintroduced in 1989 from either their home range or core areas (50% fixed kernel utilization distribution). 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 each home range overlap was equivalent to each female sharing her home range with >2 other females. Overlap of core areas was equivalent to each female sharing her core area with nearly one other female.

Mean eviscerated body weights of harvested white-tailed deer increased 5.0–7.6 kg for males and 2.0–4.9 kg for females between the pre-reintroduction years (1984-1989) and post-reintroduction years (1990-1997). 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. Population estimates indicated hunter success rates declined following the bobcat reintroduction, even though the number of hunters

prior to the reintroduction ($\bar{x}$ = 270 hunters/year) differed little from after the reintroduction of bobcats ($\bar{x}$ = 278 hunters/year).

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).

We were able to successfully capture and translocate approximately 15 bobcats per year, although we were not able to capture enough bobcats to control the sex ratio of the population of reintroduced bobcats; at the conclusion of the translocations in 1989 the reintroduced population consisted of 14 males and 15 females, although it was female-biased (11females, 3 males) after the translocations in 1988. This introduced potential confounding effects when interpreting results of our post-release monitoring of food habits and spatial organization. We strongly recommend that future reintroduction projects establish a means of conducting 'soft' releases of animals whereby animals are held in captivity at the release site and allowed to leave captivity following a waiting period. Even holding bobcats overnight might have prevented the disorientation of the released bobcat that swam into the Atlantic Ocean and presumably drowned.

A dramatic 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. 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%). 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. Also, there was no evidence the frequent occurrence of deer in the diet was because of a lack of food availability because bobcat survival was high, recaptured bobcats exhibited weight gains, and bobcats maintained normal home range sizes.

Results of the analysis of spatial organization of bobcats were consistent with the hypothesis that bobcats maintain home ranges via a system of land tenure established by prior rights. 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. 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.

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 indicated 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. However 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. We believe the results of this study provide strong justification for post-release monitoring of a reintroduced species to be able to understand why a reintroduction project succeeds or fails. Moreover, the role of predators in ecosystems in poorly understood, especially vertebrate predators, and restoration projects of predator populations should consider monitoring trophic level characteristics of ecosystems. If such a monitoring program were developed to test theories of community population dynamics, there would be much potential to better understand food webs of terrestrial ecosystems and trophic level inter-relationships.

Key words: Bobcat, *Lynx rufus*, Cumberland Island, food habits, Georgia, keystone predator, prey availability, prey use, reintroduction, reproduction, restoration, spatial organization, survival translocation, trophic cascade.

LIFE-Nature project (life 06/nat/e/209) for the reintroduction of the Iberian lynx in Andalusia. (Proyecto Life Conservación y Reintroducción de Lince Ibérico en Andalucía (life 06/nat/e/209))

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1.- INTRODUCTION

Thanks to proper implementation of LIFE Project "Recovery of Iberian Lynx Populations in Andalusia" (LIFE 02/NAT/E/8609), and recognition of the fact that endangered species conservation strategies require medium and long term efforts covering numerous fronts, Andalusia´s Regional Ministry of Environment feels we are now in a good position to present a new Life Project for conservation of the Iberian lynx in Andalusia

Other encouraging circumstances such as: the high marks assigned by the European Commission during the current program's technical evaluation; the fact that the Commission has declared the Iberian lynx a European priority on several occasions; and, the complete remodeling now underway of funding mechanisms for the conservation of nature, particularly those of Red Natura 2000, indicate that the time is right for presentation of a new project, to follow-up on former project actions and pursue new ones. The new project will guarantee medium term funding for species conservation.

Due to the above, at the Iberian Lynx Working Group meeting held January 28, 2005, the

Regional Ministry of Environment announced its decision to present a new Project, and extended an invitation to potential partners. The announcement was recorded in the minutes, as no. 7 of agreements reached: "Agreed to consider a Life 2005 proposal by Andalusia, and if approved, to support candidacy in writing".

At the April 27, 2005 meeting of the current Project LIFE Progress Committee, the Regional Ministry of Environment repeated its intention to present a new Project, and presented general ideas for the project

2.- OBJECTIVES

This LIFE Project pursues two objectives: continuance of conservation and population monitoring now underway, and experimentation and reintroduction efforts to reinforce existing populations. The bases for this 5 year project are:

Guarantee long term maintenance of the 2 sole surviving wild lynx populations, each small and isolated from other surviving pop. (continue monitoring and management).

Long term maintenance of these two populations does not guarantee long term species conservation.

The physical isolation of the groups suggests probable genetic differences between them.

2. Thus, to guarantee maximum genetic maintenance, an exchange of individuals between groups is highly advisable (reinforcement).

The limited range of the species, and its high dependency on fluctuating rabbit populations for prey, are major risk factors that should be cut short via founding of new populations (reintroduction).Individuals for reintroduction should come from the captive breeding program now underway, not from wild lynx capture.

The reasons cited were assessed and approved by international feline conservation experts UICN/SSC Cat Specialist Group (com. pers. Urs Breitenmoser), and received legislative and institutional support expressed in:

Action Plan for the Conservation of the Iberian Lynx (Lynx pardinus) in Europe (Council of Europe. Nature and environment, No. 111. 2000).).

Strategy for Conservation of the Iberian Lynx (Lynx pardinus). National Commission for the Protection of Nature. 1999.

Conclusions of International Seminars held in Andujar (2002) and Córdoba (2004)

Draft Plan for the Recovery of the Iberian Lynx in Andalusia.

3.- PROJECT CONTEXT

In addition to the philosophy and strategies cited above, the project is structured around and based on the "Recovery Strategy for the Iberian Lynx", presented in the II International Seminar on the Conservation of the Iberian Lynx (Córdoba, Dec. 2004)

Conservation activities and ongoing population monitoring

Continuation of monitoring activities, habitat quality improvement efforts and agreements, etc. implemented under the previous LIFE program.

These measures are geared to consolidate current populations, increase the number of good breeding grounds, bring the two Andujar lynx population nuclei in contact and expand species home range to surrounding areas.

The actions approved for development and consideration under the respective agreements underwent a detailed evaluation, and a timeline of the actions required to recover rabbit populations was drafted.

Build up current lynx populations

Launch a new program to build up the Doñana population via translocation of individuals from the Andujar population. This dual purpose action will: recover historical ranges (most in Doñana National Park), and slow down the loss of genetic variability. This part will be implemented in Project year 2, thus providing a margin for optimum preparation of the individuals selected for reintroduction.

The methodology will be: capture and translocation of (mostly juvenile) lynx from Andujar, to large holding facilities located on historical wild lynx ranges inside Doñana National Park.

This intermediate step permits process monitoring, gives the individuals time to adapt to their new environment, while also lowering mortality and dispersion rates, and limiting interaction with individuals from the Doñana population.

In parallel, habitat improvements (rabbit re-population, brush clearing, construction of rabbit refuges, fencing for supplementary feeding, etc.) will be made inside the facilities scheduled to receive the Sierra Morena population individuals and throughout the ranges scheduled for recovery.

Reintroduction

Reintroduction could well mean a qualitative leap forward in Iberian lynx conservation. As mentioned earlier, maintenance of the two remnant populations will not guarantee long term species conservation.

Given the current situation, with the captive breeding program underway, the next

step is to commence a reintroduction program coordinated between in situ and ex situ conservation programs.

Potential sites for reintroduction of the Iberian lynx in Andalusia have already been identified under Action A5 of the current LIFE project. For this, Multi-Criteria Analysis and Biomapper tools were used and expert advice provided by the Doñana Biological Station (E.B.D.) and the UICN/SSC Cat Specialist Group (Urs Breitenmoser).

In actions preceeding the new LIFE program (Preparatory Actions; Drafting of Management and/or Action Plans), two actions specific to reintroduction will be included: Analysis and Selection of Reintroduction Areas, and Socio-economic Impact Analysis of Acceptance and Interest in Reintroduction within the Pre-selected Areas.

All population reinforcement and reintroduction work will be performed following I.U.C.N. guidelines. An Advisory Committee of national and international experts will be formed.

The Regional Ministry of Environment has received 2004-2005 rabbit census data for the areas identified for lynx reinsertion. The data was gathered as part of prior sampling done to support the work envisioned for the new project, and will be used for final selection of reintroduction zones.

The chronological structure of the project will be:

Year 1: Evaluation of the 4 pre-selected areas (Guadalmellato, Arenoso, Guarrizas and Villanueva del Río). Detailed census of rabbit populations, potential threats, socio-economic status, etc. Evaluation of specific habitat improvement measures required in each zone.

Year 2: Final selection of best of 4 the pre-selected areas. Habitat improvement and infrastructure construction in area selected. The runner-up area will be targeted for agreements for moderate action and held in reserve (back-up, should the no. 1 choice be rejected due to unavoidable circumstances).

Year 3: Continue habitat improvement in reintroduction area.

Year 4: Reintroduction, continue habitat improvement in area.

Year 5: Monitor reintroductions performed; continue with reintroduction program.

Other actions

Transversal actions throughout the project will include monitoring of current populations (radio tracking), and public awareness campaigns targeting the areas affected by reintroduction, and society in general.

3.- OUTLINE OF NEW LIFE PROJECT

The following is a summary of the new LIFE Project.

A: Preparatory actions, drafting of management plans and/or actions (year 1).

Purpose: guarantee compliance with IUCN Guidelines for Reintroductions. Evaluation and selection of best reintroduction site, (year 1) based on 2 criteria: Biological requirements of species, and socio-economic aspects of reintroduction location.

Training action for personnel assigned to reinsertion, including contact with international experts in the field.

B: Purchase / lease of lands and/or rights

These actions will concentrate on obtaining hunting rights (mainly for rabbit) to reduce hunting pressure within the species´ current range. Similar agreements entered earlier (previous LIFE project) will be maintained. New leases will be taken out in the reintroduction area.

One strict rule applied across the entire action is refusal to lease small game hunting without proof that hunting cutbacks would cause loss of income. Priority is assigned to collaborative agreements whereby hunting impact is lowered through habitat improvement. Lease/buy measures will be restricted to priority zones (those with confirmed large rabbit populations or high interest bordering properties), and to situations in which the best alternative for conservation is to curtail small game hunting.

Another measure is pasture leasing, to lower the impact of domestic hoof stock and

expand the range of lynx populations in "dehesa" pasturelands of Cordoba. These actions also contemplate payment for any lynx related damages.

C. One off Biotope Management Tasks

These one time actions will focus on infrastructure building and habitat improvement of the areas destined for population reinforcement and reintroduction.

They involve fencing off large areas to benefit the reinforcement of the Doñana population and that of the action A reintroduction site. The fenced area will include nest boxes, and also small supplementary feeding posts, to guarantee prey availability.

Major improvements of forestry and farm access roads and highways around Doñana are planned, to lower species road kill mortality rates in current ranges and reintroduction sites.

D. Periodical Biotope Management

These are environmental restoration and quality improvement actions. Due to the lynx´s dietary dependence on rabbit, most of the actions are for wild rabbit population recovery and improvement. Measures include: Fences for rabbit breeding, brushland clearance, seeding and fertilization of pastureland, installation of watering points and recovery of natural watering areas, construction of rabbit breeding zones, rabbit repopulation, pine grove pruning, predator controls, partridge repopulation, fencing to prevent entry of ungulates.

Some of the actions, such as supplementary feeding points and nest box installation, specifically target the Iberian lynx. They are also part of a program to eradicate the use of illegal hunting methods affecting the lynx (snares and traps).

All actions are aimed at guaranteeing continuation of the excellent results achieved for the species under the previous LIFE program.

Measures for reintroduction areas are designed to ensure the best possible habitat for the individuals reintroduced.

A cost estimate has been done on the actions required within each of the individual properties to be involved in the collaborative agreement for this new project.

E. Public Awareness Campaign and Communication of Results

The public awareness actions are designed to reach out beyond traditional stakeholder sectors.

Actions will include: campaigns targeting the sectors directly involved in lynx conservation.

Other stakeholder sectors targeted:

Education: grade schools, secondary schools, activities such as drawing/sketching competitions, games, etc.

Government & Administrative Bodies: judges, civil guard, game authorities, Min. of Environment officials, municipalities, agencies, etc.

Rural areas: hunters, ranchers, farmers, property managers and owners, etc.

Other Sectors: business community, associations, the media, local population of El Rocío, etc.

The following organizations will collaborate in performance of these actions: Ecologistas en Acción –Andalucía, SECEM, APROCA, ATECA, FAC, etc.

Actions will include distribution of: T-shirts, caps, brochures, stickers, pins, posters, etc. Short informative films targeting key sectors.

Actions will cover populations living within area of influence of current species habitat and those destined for reintroduction programs. Population specific campaigns are planned to achieve maximum effect within each target community/stakeholder group.

F. Project Operations

These are to ensure proper coordination among the numerous partners, collaborators and bodies (Min. of Environment, European Commission, NGOs, Public Administrations, etc.) The Doñana Biological Station will be in charge of scientific monitoring to guarantee the quality of all actions and the best possible use of project resources, particularly as regards the lynx and rabbit populations, and effects of related vegetation management on the surrounding area.

As prescribed by LIFE regulations, a Financial Audit will be performed on project expenditures.

Partners participating in single management actions, land leasing and others, include WWF-Adena, Fundación CBD-Hábitat, Regional Ministry of Transport and Public Works, Regional Ministry of Agriculture and Fisheries and the Regional Ministry of Agriculture and Environment of Extremadura.

Seville, November 17, 2006

Lessons from the Reintroduction of the Eurasian Lynx in Central and West Europe

Urs Breitenmoser, Manuela von Arx & Christine Breitenmoser-Würsten

Three of the four species of the genus *Lynx* have been targets of reintroduction programmes: Canada lynx *L. canadensis* (2 projects in New York and Colorado, USA), bobcat *L. rufus* (1 project in Georgia, USA) and Eurasian lynx *L. lynx* (projects in 11 different regions in Germany, Switzerland, Italy, Slovenia, Austria, Czech Republic, France, and Poland). In the North American projects, a considerable number of wild caught animals were immediately released at the reintroduction site: 83 and 218 Canada lynx in the Adirondacks (NY, 1988–90) and in Colorado (1999–2006), respectively, and 32 bobcats in Cumberland Island (GE, 1988/89). The bobcat reintroduction was a success, whereas the outcome of the two Canada lynx projects is still uncertain. Reintroduction of Eurasian lynx in Europe started as early as 1970 and continued to present. In all attempts, only a small number of animals, ranging from 2 to 31 animals, were released compared to the North American projects. All together, we know of 162 lynx released in 15 sites. 57 percent of the animals were wild caught in the Carpathian Mountains of Slovakia, 40 percent 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. Many of the programmes were started from groups of enthusiastic naturalists in the 1970s, before reintroductions came into the focus of scientists and before the IUCN Re-introduction Guidelines were available. But even some of the recent projects failed to use adequate scientific methods to monitor the fate of the released animals and the success of the programme. The comparison of the different project however allows some general conclusion, which might provide some lessons for the recovery of the Iberian lynx. *Reversal of threats leading to the extinction*: The Guidelines mention as the first requirement of any reintroduction the removal of (historic) threats leading to the extinction of the species. This precondition was fulfilled in all reintroduction projects for the Eurasian lynx in regard to the ecological conditions: Habitat (forests) and prey population (roe deer) have recovered throughout Central and West Europe in the 20th century, also the fragmentation of the landscape has increased. One threat was however only partly reversed – the negative attitude of people. Although the general public welcomes the return of the lynx, important target groups such as hunters and sheep breeders strongly oppose. *Number and origin of animals*: Although an assessment of carnivore reintroduction in general demonstrates that success is a function of the number of animals released and that wild caught animals have a higher survival than captive born candidates, this pattern is not true for the Eurasian lynx. Some of the

most successful programmes – such as the reintroduction in Slovenia – originated from only six released lynx. Captive born animals seem to adapt quite well to living in the nature and catching wild prey, although there might be a somewhat higher dropout rate. *Choice of release site*: The selection of the release site seems to be important, but rather from a strategic aspect than in regard to local conditions. The area of reintroduction should on one hand be "closed", so that animals dispersing from the release site do not risk loosing the contact with conspecifics, but on the other hand, the connection to neighbouring nuclei must be granted in regard to the long-term development of the (meta-) population. *Information and public involvement*: All reintroduction programmes of Eurasian lynx were controversial, and there is no correlation between short-term success and public information or involvement of stakeholders. Nevertheless, public relation seems to be important for the long-term acceptance of the returning predator. The recovery of lynx in Switzerland still suffers from the fact that all releases in the 1970s were done in a very clandestine way. *Monitoring and follow-up programme*: Monitoring *per se* is no guarantee and not even a requirement for a successful reintroduction, but it is an ultimate requirement for the control of the programme. Regardless to 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 success or failure; knowledge on individual fates is indispensable for a stern 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.

Appendix

(Original versions in spanish)

Aspectos Generales de Todas las Disciplinas que Integran el Programa de Conservación Ex-Situ del Lince Ibérico

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La cría para la conservación de especies amenazadas es una herramienta de conservación costosa que requiere una cuidadosa planificación para asegurar su integración en la estrategia global de recuperación de la especie. Una vez establecida la necesidad de iniciar un programa de conservación ex-situ, es primordial evitar poner en peligro las actividades de conservación in-situ. Un programa de cría sólo debería comenzar cuando el apoyo administrativo necesario, los recursos económicos y las infraestructuras adecuadas para llevarlo a cabo estén consolidadas. Se considera importante una planificación del programa basada en metas a corto y largo plazo, en la que se incluyan objetivos y acciones concernientes al manejo genético y demográfico en cautividad, y que a su vez atienda a los aspectos sanitarios, reproductivos y etológicos de la población cautiva. A continuación, se resumen diversos aspectos que conforman el Programa de Conservación Ex-situ del Lince Ibérico, actualizando información sobre los últimos avances en el Programa.

Organización

El Programa de Conservación Ex-situ del Lince Ibérico se plantea como un esfuerzo multidisciplinar integrado dentro de la Estrategia Nacional para la Conservación del Lince Ibérico en el que colaboran entidades autonómicas, nacionales e internacionales. La primera fase del programa de cría se desarrolla en la actualidad en el Centro de Cría de El Acebuche, situado en el Parque Nacional Doñana y en el zoobotánico de Jerez, Cádiz. La siguiente fase comenzará con la inauguración del Centro de Cría de La Aliseda, Jaén, que tendrá lugar en otoño de 2006. La responsabilidad de la coordinación del Programa Ex-situ corre a cargo de la Dirección General para la Biodiversidad, en colaboración estrecha con la Junta de Andalucía. La planificación de las diversas tareas se realiza a través de un *Comité de Cría* en el que participan representantes de 15 instituciones nacionales e internacionales. El *Comité* es el encargado de impulsar las Acciones contenidas en el Plan de Acción para la Cría en Cautividad del Lince Ibérico (MMA-CMA, 2004), y cuenta con expertos en reproducción, manejo de animales en cautividad, genética y demografía de pequeñas poblaciones, aspectos sanitarios, etología y conservación in-situ. Desde el año 2003, la ejecución de las acciones que se proponen para la realización del programa ex-situ en Andalucía ha de estar avalada por la Comisión Bilateral, según lo establecido en el *"Convenio de Colaboración entre el Ministerio de Medio Ambiente y la Consejería*

de Medio Ambiente de la Junta de Andalucía para el desarrollo de un único programa coordinado de actuaciones para la aplicación de la Estrategia Nacional a la Conservación del Lince en Andalucía" (julio, 2003).

Metas del Programa

Para alcanzar las metas establecidas en el Plan de Acción para la Cría en Cautividad del Lince Ibérico (MMA-CMA, 2004) es necesario establecer una organización bien definida que se integre adecuadamente con el resto de las actuaciones para la recuperación del lince ibérico. Según lo aprobado por la Comisión Bilateral, el programa de Cría para la Conservación del lince ibérico tiene una dirección científico/técnica de carácter ejecutivo y un comité multidisciplinar de carácter asesor. El Comité de Cría sigue el modelo de los programas de cría europeos (EEPs), y todos los ejemplares incorporados al programa se han de incluir en el s*tudbook* ("libro de reproductores") del lince ibérico. Los cruces entre ejemplares se realizarán atendiendo a las prioridades genéticas establecidas por el programa PM 2000. El intercambio de ejemplares entre centros lo determinará la dirección del programa, basándose en los resultados del programa PM 2000, y según el asesoramiento un subcomité de expertos.

Las metas principales del Programa de Conservación Ex-situ son dos:

1. Asegurar la conservación del material genético de la especie
2. Crear, a medio y largo plazo, nuevas poblaciones de lince ibérico a través de programas de reintroducción

Gestión Genética Y Demográfica Del Programa De Cría

El manejo genético óptimo de una población cautiva se logra aumentando rápidamente su tamaño hasta un límite que estará determinado por el número de ejemplares que se considere idóneo para mantener la variabilidad genética estipulada para la especie en cuestión. Una vez alcanzado este límite, la máxima eficacia se consigue estabilizando el tamaño de la población. Para ello es preciso acompasar la producción de ejemplares con las necesidades de los programas de reintroducción, y con las del propio programa de cría, que requiere sustituir a los individuos post-reproductores. El manejo genético y demográfico ha de ir acompañado de un buen manejo etológico con el fin estimular las conductas naturales en los individuos cautivos procurando que, desde un principio, todos los ejemplares nacidos en cautividad tengan el potencial de sobrevivir en la naturaleza.

Dada la situación actual del lince ibérico, se podrían fomentar las condiciones básicas para conservar un 85% de la variabilidad genética actual durante un periodo de 30 años (Lacy y Vargas, 2004). Una población cautiva con una variabilidad genética por debajo

del 85% se considera peligrosamente endogámica y no sería aceptable desde el punto de vista genético.

Para alcanzar los objetivos genéticos estipulados se deberían incorporar 4 cachorros por año durante cinco años consecutivos. Es decir, habrá que incorporar 20 cachorros/ juveniles en el plazo de 5 años. Para asegurar el mantenimiento de la diversidad genética en un plazo de 30 años se deberá contar con un grupo de 60 reproductores (constituido en principio por los propios fundadores más ejemplares

nacidos en el programa de cría). Como estrategia básica para el mantenimiento de la variabilidad genética se considera importante conseguir un crecimiento poblacional rápido durante los 10 primeros años del programa, hasta alcanzar la fase de capacidad de carga; es decir, 60 (30.30) ejemplares reproductores. Asimismo, como parte del mencionado manejo, se procurará igualar la representación de los fundadores de modo que todos aporten un número similar de crías al programa.

Manejo de los Linces Mantenidos en Cautividad

El manejo de vida silvestre mantenida en cautividad se basa en la aportación de conocimientos multidisciplinarios en los campos de cuidados animales, nutrición, veterinaria, genética, fisiología, junto al uso sistemático del método científico. A lo largo de las dos últimas décadas se han adquirido conocimientos y experiencia importantes en el manejo de felinos silvestres mantenidos en cautividad. El TAG (Grupo Asesor del Taxon) de Félidos de la Asociación de Zoológicos Americanos (AZA) ha elaborado una Guía de Manejo de Felinos en la que se recopila información importante relativa a aspectos sanitarios, reproducción, nutrición, instalaciones, etc. (Mellen & Wildt, 1998). Asimismo, muchos zoológicos europeos tienen amplia experiencia en cría de felinos en general y de linces en particular. Estos documentos y experiencias son de gran utilidad para la ejecución del programa de cría en cautividad para el lince ibérico.

Una de las claves del manejo del programa de cría para la conservación del lince ibérico consiste en lograr un equilibrio entre el fomento de las conductas naturales de la especie (caza, territorialidad, interacciones sociales, etc.) y la creación de un entorno libre de estrés en el que los animales sean más proclives a aparearse. Para obtener información importante para el manejo (peso de los ejemplares, estado potencial de gestación, etc.) se están poniendo a punto técnicas de entrenamiento que eviten el uso de métodos invasivos y a su vez ayuden a aumentar el vínculo entre los animales y sus cuidadores.

Los comportamientos de los ejemplares del Programa de Cría se siguen con un sistema de video-vigilancia que permite observarlos sin causarles molestias durante las 24 horas del día. Esto está permitiendo aprender muchas cosas sobre la especie que no se podrían estudiar fácilmente en la naturaleza. Utilizando como base las experiencias obtenidas

hasta la fecha con los linces ibéricos mantenidos en El Acebuche y en el Zoo de Jerez, junto a información obtenida a partir de programas establecidos por zoológicos europeos y americanos, el Centro de Cría de El Acebuche, en el Parque Nacional de Doñana, ha elaborado un Programa de Funcionamiento en el que se detallan los protocolos de manejo de los ejemplares reproductores que allí residen (para más información ver http://www.lynxexsitu.es/documentos/manejo/pfcc.pdf). Se pretende que, conforme aumente el número de centros que alberguen linces destinados a la cría en cautividad, se unifiquen los procedimientos de manejo que hayan sido aplicados con éxito en los centros piloto de El Acebuche y del Zoo de Jerez.

Aspectos Sanitarios

Las consideraciones sanitarias que hay que contemplar tanto en programas de cría en cautividad como en reintroducciones y translocaciones de fauna silvestre han suscitado gran preocupación en la comunidad de biólogos de conservación. Se han documentado numerosos casos de transmisión de enfermedades infecciosas a poblaciones silvestres, a partir de especies amenazadas criadas en cautividad con el fin de ser reintroducidas.

Igualmente, existen casos de introducción de enfermedades letales de la población silvestre a la cautiva. Se considera que en la mayoría de estos programas no existe suficiente información sobre: 1) La distribución y riesgos de enfermedades en poblaciones cautivas; 2) La incidencia, distribución y riesgos de enfermedades en poblaciones silvestres; 3) Sistemas de cuarentena que ayuden a prevenir de modo efectivo la transmisión de enfermedades; y 4) un sistema de detección y seguimiento que ayude a identificar agentes patógenos sin error alguno.

Poco se conoce sobre las enfermedades que afectan al lince, y ha sido por tanto imperativo iniciar acciones encaminadas a mejorar el conocimiento de las principales enfermedades que pueden afectar a la especie. El Programa de Cría para la Conservación del Lince Ibérico cuenta con un equipo asesor de aspectos sanitarios encargado de varios aspectos de manejo veterinario e investigación, así como del desarrollo de protocolos. Dentro de la investigación sobre aspectos sanitarios que afectan al lince ibérico, las líneas principales de trabajo incluyen el establecimiento de un sistema de medicina preventiva que evite el desarrollo de enfermedades en la población cautiva y la investigación científica en varios aspectos veterinarios. En la actualidad, se están llevando a cabo proyectos sobre la incidencia y prevalencia de distintas patologías infecciosas tanto en cautividad como en la naturaleza, sobre establecimiento de valores sanguíneos normales y patológicos para lince ibérico, así como sobre problemas renales. El desarrollo y unificación de protocolos, así como los resultados obtenidos de las diversas investigaciones están ayudando a mejorar la consistencia en los diagnósticos y tratamientos, y a diseminar el conocimiento y las experiencias de los distintos veterinarios trabajando en el programa (para más información, consultar http://www.lynxexsitu.es/aaveterinaros/aaveterinarios.htm)

Fisiología Reproductora

Los estudios sobre fisiología reproductora son necesarios para aumentar las posibilidades de éxito en programas de cría en cautividad y para ayudar en la conservación de felinos silvestres. Como parte del programa de conservación del lince ibérico se ha considerado importante establecer un Banco de Recursos Biológicos para conservar biomateriales de individuos pertenecientes al programa de cría en cautividad y de aquellos que se encuentran en poblaciones naturales. Con el fin de conservar el máximo de diversidad biológica se preservan muestras de germoplasma masculino y femenino, así como células o tejidos, que podrán utilizarse en el espacio y en el tiempo para realizar intercambios de material genético entre individuos del programa de cría en cautividad, entre poblaciones naturales y el programa de cría en cautividad y, cuando sea aconsejable, entre individuos de poblaciones naturales. La conservación de gametos permitirá extender las opciones reproductivas de los individuos evitando limitaciones de espacio, o previniendo posibles transmisiones de enfermedades. Asimismo, la criopreservación de los gametos o embriones dará la oportunidad de prolongar las posibilidades reproductivas de los individuos más allá de su muerte. La preservación de células somáticas (o de células germinales no diferenciadas) podrá servir para dar una oportunidad reproductiva a individuos que han muerto antes de llegar a la madurez sexual, o para extender el potencial reproductivo de ciertos individuos.

En la actualidad, el Banco de Recursos Biológicos del Lince Ibérico se halla en dos ubicaciones: el Museo de Ciencias Naturales de Madrid y Universidad Miguel Hernández de Elche. Se procura mantener un duplicado de muestras de cada animal en cada sede. Aunque el banco del Museo de Ciencias se especializa principalmente en germoplasma y el de la Universidad MH en células somáticas, en ambos se conservan tejidos, sangre, suero u otros materiales biológicos que, en caso de necesidad, permitirán realizar análisis de prevalencia de enfermedades, o análisis genéticos de diverso tipo. El almacenamiento de estas muestras permitirá disponer de material para análisis futuros que se identifiquen oportunamente.

Otro aspecto importante de la fisiología reproductora del lince ibérico es el desarrollo de técnicas no invasivas que ayuden con el manejo reproductor en cautividad. Durante los dos últimos años se ha trabajado en identificar los perfiles hormonales de machos y hembras de lince ibérico, lo que nos ha ayudado a definir más claramente la longitud del periodo reproductor y el posible potencial del estudio de metabolitos hormonales en heces para la detección de la gestación en linces. Como esta última técnica no es óptima como diagnóstico, aunque si muy útil para llevar a cabo diversos estudios, se está trabajando en la puesta a punto de de una técnica para diagnosticar gestación determinando relaxina en orina. Estas técnicas permiten obtener gran cantidad de

información sin causar la menor molestia a los individuos objetos de estudio.

Reintroducción

Con una buena producción de linces cautivos adecuadamente entrenados para maximizar sus probabilidades de supervivencia, evitaríamos la necesidad de extraer muchos individuos del campo para establecer nuevas poblaciones o reforzar las ya existentes en el caso que esto se considerase necesario. Tanto las reintroducciones como las translocaciones tienen ventajas y desventajas, por lo que se recomienda hacer un estudio comparativo para ver qué vía – o qué combinación de opciones— resultaría más apropiada para la conservación del lince ibérico.

Antes de realizar cualquier reintroducción/translocación hay que hacer un detallado estudio de su posible viabilidad (consultar los "Criterios para Reintroducciones" de la Unión Internacional para la Conservación de la Naturaleza). Uno de los requisitos fundamentales es la realización de Estudios de viabilidad del hábitat que demuestren que han desaparecido las causas que motivaron la extinción y que este sea de suficiente calidad para establecer una población viable; es decir un área de tamaño adecuado para mantener una población de la especie en cuestión y donde esté garantizado que las razones que causaron la extinción local de la especie ya no estén ejerciendo su efecto.

Es importante resaltar que las reintroducciones y translocaciones han de llevarse a cabo de modo científico. Estas técnicas de conservación son multidisciplinarias y han de recibir el consejo y apoyo de ciencias como la ecología, veterinaria, etología, fisiología, al igual que la sociopolítica y las ciencias de la información. Tanto durante la fase de desarrollo como la de ejecución del programa, han de existir protocolos detallados que documenten objetivos, procedimientos y responsabilidades (individuales y de organizaciones). Si los resultados de la evaluación indican que la reintroducción del lince ibérico no es recomendable en el área propuesta, habrá que determinar qué elementos son los que fallan y qué medidas hay que impartir para solventarlos.

Comunicación, sensibilización y formación

La sensibilización, educación y la formación científica deberían ser metas integrantes de cualquier programa de cría para la conservación. Los programas de educación y sensibilización deberían dirigirse hacia conseguir un cambio de las actitudes que contribuyen a la destrucción de especies y de hábitats. Uno de los puntos fuertes de los programas de cría para la conservación es que atraen la atención del público, particularmente si el animal es carismático y atractivo. Este es el caso del lince ibérico. Uno de los objetivos importantes del programa de cría es concienciar a la sociedad sobre la importancia de conservar hábitat para asegurar el futuro de esta especie. Criar y mantener linces en cautividad sin la esperanza de poder devolverlos un día a la naturaleza

sería como mantener un muerto viviente sin esperanza de recuperación. Aprovechando la atención mediática que recibe el lince ibérico, el programa de cría aporta apoyo y reconocimiento constante a la importancia primordial del trabajo de conservación in-situ.

Otro objetivo del programa de comunicación es el compartir información a través de una página web (http://www.lynxexsitu.es/comunicacion), que incluye desde artículos científicos y de divulgación, hasta fotos y vídeos de todos los animales, así como protocolos y métodos de trabajo. Asimismo, el Programa elabora un boletín mensual que resume los aspectos más importantes que han acontecido durante cada mes. Aunque la página existe actualmente sólo en español, está siendo traducida al inglés, lo que nos ayudará con las tareas de comunicación y sensibilización a nivel internacional. La página web cuenta también con áreas accesibles únicamente a los investigadores, técnicos y gestores que trabajan directamente en el Programa, para permitir el intercambio de información, acceso a bases de datos, etc.

Como parte de las tareas de formación, el programa de cría organiza periodos de prácticas en el Centro de Cría de El Acebuche, que sirven para dar oportunidades a estudiantes recién graduados a que adquieran experiencia de primera mano con un programa de conservación de especies amenazadas. Asimismo, el Programa organiza charlas y jornadas, como las que nos ocupan, que ayudan con las labores de formación.

La finalidad de las jornadas que nos ocupan es la formación de técnicos, investigadores y gestores trabajando directamente en la conservación del lince ibérico y formación de estudiantes que hayan acabado recientemente la carrera y que en un futuro podrían trabajar en la conservación de especies amenazadas en España. Asimismo, las Jornadas propuestas servirán para fomentar el intercambio de información entre técnicos, gestores e investigadores que trabajan en los programas de conservación in-situ y ex-situ del lince ibérico.

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Programa Sanitario del Lince Ibérico

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Las poblaciones de animales reducidas, como las de lince ibérico, son particularmente susceptibles a procesos estocásticos, incluyendo brotes de enfermedades que pueden llevar a causar drásticos descensos de sus efectivos o incluso la extinción. En la gestión de estas poblaciones, tanto in situ como ex situ es necesario el establecimiento de programas sanitarios.

El programa sanitario del lince ibérico se ha ido desarrollando a partir de los tres objetivos en aspectos sanitarios planteados en el Plan de Acción para la Cría en Cautividad del lince ibérico: mantener la población cautiva en un estado sanitario óptimo, investigar los riesgos asociados a todo el conjunto del programa de conservación del lince, y evitar la transmisión de enfermedades entre la población silvestre y cautiva. Para alcanzar estos objetivos se plantea el desarrollo de cinco acciones: desarrollar un grupo asesor de aspectos sanitarios del lince, estandarizar protocolos sanitarios, desarrollar planes de emergencia, desarrollar un estudio sanitario del lince y fauna asociada, y evaluar el uso seguro y eficaz de vacunas y farmacéuticos.

El grupo asesor de aspectos sanitarios del lince (GAAS) es de carácter multidisciplinar, constituido por veterinarios con experiencia en clínica de animales domésticos, medicina de zoo y fauna salvaje, e investigación. Los colaboradores del grupo adquieren un compromiso de trabajo voluntario y productivo con el programa. El GAAS ha desarrollado protocolos de anestesia y manejo de animales vivos, y protocolos de necropsia, que permiten además el aprovechamiento científico del material biológico.

Entre noviembre del 2003 y agosto del 2006 se han realizado 63 anestesias sobre un total de 40 animales, 24 de ellos del programa de cría. Normalmente se ha empleado la combinación de ketamina con medetomidina (tiletamina con zolazepam en 10 exámenes de machos para electroeyaculación) y cuando ha sido necesario se ha prolongado la anestesia con isofluorane. Las anestesias han tenido diversas finalidades: controles en cuarentena, controles periódicos, colocaciones de radiocollares, exámenes complementarios, terapeútica y traslados.
En ese mismo periodo se han realizado un total de 22 necropsias. La mayoría de las muertes en animales de vida libre han sido por atropello (10 casos). No se ha podido determinar la causa de muerte en 5 casos, todos ellos restos óseos. Un animal ha muerto

por tuberculosis y otro que se encontró muerto resultó positivo a CDV (moquillo) en una PCR de sangre y heces. De las cinco bajas que ha habido en cautividad: un caso de muerte por la pelea entre dos cachorros, un cachorro prematuro que nació muerto y otro que murió antes de 24 horas, una hembra joven que murió por una endotoxemia aguda por clostridios y un cachorro que murió por una neumonía intersticial y una septicemia.

El establecimiento de un plan de medicina preventiva en la población de linces del programa de cría permite reducir el riesgo de desarrollo o entrada de enfermedades. Cuarentenas, controles fecales periódicos (parasitológicos y microbiológicos), desparasitaciones, vacunas inactivadas, chequeos, control de la alimentación, revisión de instalaciones y una videovigilancia contínua son las medidas más relevantes. Entre los problemas médicos observados en la población cautiva y que se discutirán en la presentación destacan: un cuadro de vómitos que afectó a varios animales y a alguno de ellos de forma prolongada, un absceso con osteomielitis en la extremidad de un animal, un absceso apical en un cachorro, una dermatitis húmeda y un cuadro de anemia en dos animales. Actualmente se encuentran 23 linces ibéricos en cautividad en buen estado de salud.

La sistemática en la recogida y en el procesamiento de las muestras biológicas en diversas instituciones conveniadas y el trabajo cooperativo multidisciplinar está permitiendo ampliar los conocimientos en temas sanitarios de la especie, lo que es fundamental para mejorar su manejo tanto ex situ como in situ.
Toda la información recogida de los animales en análisis y procedimientos se va registrando en una base de datos biomédica que se ha elaborado para la especie. La base de datos permite relacionar toda la información sanitaria de los animales y es una herramienta fundamental aplicada a la gestión, investigación y conservación del lince ibérico.

Valores Bioquímicos de Referencia en Lince Ibérico (*Lynx pardinus*)

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Introducción

En los últimos años y especialmente, desde la puesta en marcha del Plan de Conservación y Cría en Cautividad del Lince ibérico (Lynx pardinus), han sido muchos y muy diversos los estudios realizados con el fin de profundizar en el conocimiento de esta especie tan emblemática. Sin embargo, han sido escasos los relacionados con la valoración de las alteraciones fisiológicas y patológicas a partir de parámetros sanguíneos (Beltran y cols., 1991).

Los objetivos de este trabajo son (a) establecer los valores de referencia de 20 parámetros bioquímicos sanguíneos y (b) determinar las diferencias estadísticamente significativas de dichos parámetros con respecto a determinadas variables (edad, sexo, procedencia y estado de libertad).

Material y métodos

Desde el año 2004, se han analizado en el Centro de Análisis y Diagnóstico de la Fauna Silvestre de Andalucía (CAD), 31 muestras de plasma obtenidas de 31 ejemplares de Lince ibérico (Lynx pardinus), procedentes de las poblaciones localizadas en Sierra Morena (15 ejemplares), Doñana (14 ejemplares) y el Centro de Cría en Cautividad del Acebuche (2 ejemplares). Dieciocho muestras (8 machos y 10 hembras) se obtuvieron de ejemplares en cautividad, y 13 (8 machos y 5 hembras) de animales recién capturados.

El análisis bioquímico se ha llevado a cabo mediante el analizador RA115000 – CLIMA MC 15 (Manufacture by RAL S.A. Spain. CE). Los parámetros determinados mediante reacciones colorimétricas son: glucosa, colesterol, triglicéridos, ácido úrico, proteínas totales, albúmina y fósforo; se cuantificaron mediante reacciones cinéticas: gamma glutamil transferasa (GGT), fosfatasa alcalina (ALP), lactato deshidrogenasa (LDH), urea, creatinina, aspartato aminotransferasa (AST/GOT), alanina aminotransferasa (ALT/GPT), creatina kinasa (CK), amilasa pancreática, lipasa y colinesterasa; el hierro (FE) y el calcio (CA) se determinaron mediante técnicas colorimétricas diferenciales.

Para establecer los valores de referencia, del total de muestras analizadas (48) sólo se incluyeron muestras de individuos aparentemente sanos según el criterio del personal especializado encargado del control sanitario de los ejemplares.

Las muestras analizadas procedieron de linces nacidos entre los años 2000 y 2006. Aunque el 30% de los animales nacieron en el 2004, la mayoría de los sueros (n= 20) se obtuvieron durante el siguiente año. La sex ratio y la cohorte de edad fue muy similar (1:1) en el total de las muestras.

Se ha realizado un análisis estadístico con ayuda del programa informático SPSS versión 13.0 para Windows. Para determinar la normalidad en la distribución de los diferentes parámetros se ha empleado la prueba de bondad de ajuste de Kolmogorov-Smirnov. Así mismo se han utilizado pruebas paramétricas para establecer la diferencia de medias entre los parámetros sanguíneos y variables tales como la edad, sexo, procedencia del animal y estado de cautividad. Mediante el test de Levene, se comprobó la homoestacidad de las variables objeto de estudio aplicándole en su caso un análisis de varianza (ANOVA). Cuando las varianzas fueron desiguales se aplicó la prueba robusta de igualdad de medias de Welch. El valor significativo mínimo se estableció en 0,05 en todas las pruebas.

Resultados y discusión

El análisis de los 20 parámetros incluidos en el estudio (Tabla1) manifiestan valores similares a los descritos por otros autores en diferentes especies de felinos (Fuller y cols., 1985; Weaver y cols., 1995). Las diferencias observadas en las medias de algunos de los parámetros obtenidos podrían estar relacionadas con factores extrínsecos (tamaño de la muestra, manejo de los ejemplares, hábitat, recolección de la muestra) e intrínsecos (estrés, fármacos, idiosincrasia individual) (Marco y cols., 2000).

Se encontraron diferencias estadísticamente significativas entre medias (± SD) en machos y hembras en niveles séricos de albúmina y CK (P = 0.017 y 0.030 respectivamente). La concentración de albúmina en suero de hembras es superior a las halladas en los machos (4.21 ± 0.77 vs. 3.54 ± 0.69) resultados que difieren de los obtenidos por Marco y cols., 2000 en Gato silvestre (Felis silvestris). Sin embargo, estas diferencias se invierten en relación a la CK (UI/L), observándose que los machos (940.27 ± 677.28, n= 15) poseen concentraciones mucho más elevadas que las hembras (472.69 ± 300.7, n= 17). Diferentes estudios similares manifiestan niveles superiores de ALP y glucosa en hembras de Lince rojo (Felis rufus) (Fuller y cols., 1985; Tocan y cols., 1985).

Values	N	Media	SD	Rango	
Glucosa (mg/dl)	10	132,9	69,2	39	250
Colesterol (mg/dl)	30	206,1	103,2	80	488
Triglicérido (mg/dl)	31	31,4	18,1	8	82
Ácido Úrico (mg/dl)	31	0,4	0,4	0	1,6
Proteínas totales (gr/dl)	31	8,2	1,4	5,3	10,8
Albúmina (gr/dl)	31	3,9	0,8	2,3	6,2
Fósforo (mg/dl)	31	7,4	1,9	3,9	11,3
FE (mcg/ml)	28	73,4	36,0	13	134
CA (mg/dl)	31	8,5	2,0	5,1	12,9
GGT (UI/L)	30	6,6	4,1	2	17
ALP (UI/L)	31	120,5	68,7	28	286
LDH (UI/L)	16	415,6	533,0	78	1843
Urea (mg/dl)	31	64,5	27,9	10	151
Creatinina (mg/dl)	31	1,7	0,7	0,6	3,3
GOT (UI/L)	30	79,6	90,6	19	385
GPT (UI/L)	30	62,8	39,9	20	138
Amilasa pancreática (UI/L)	31	875,2	253,9	404	1359
Lipasa (UI/L)	29	11,8	2,8	8	21
CK (UI/L)	28	723,2	578,3	4	2691
Colinesterasa (UI/L)	27	6461,8	2461,6	2034	14209

Tabla1. Parámetros bioquímicos de 31 muestras de Lince ibérico

La comparación entre edades, resulta niveles significativamente superiores en individuos jóvenes para la ALP y la LHD (P = 0.00 y 0.04 respectivamente) siendo los adultos los que mantienen mayores concentraciones de GGT y creatinina (P = 0.03 y 0.02 respectivamente). Niveles superiores de ALP en ejemplares jóvenes fueron igualmente descritos por Beltrán y cols. (1991) y Weaver y cols., (1995) en Lince ibérico y Lince canadiense respectivamente. Los valores superiores de ALP en individuos jóvenes, están directamente relacionados con la actividad osteoblástica asociada al crecimiento. Concentraciones superiores de creatinina en animales adultos fueron igualmente encontradas por Beltrán y cols., (1991). Meyer y cols., (1992) describen la correlación existente entre la edad y los niveles de creatinina en suero.

Por otro lado, los ejemplares procedentes del Parque Nacional de Doñana contienen concentraciones superiores a los nacidos en Sierra Morena (P < 0.05) para los triglicéridos (39.62 ± 21.54 vs. 24.13 ± 10.95) GGT (9.07 ± 4.76 vs. 4.47 ± 1.73) y GOT (113.5 ± 120.3 vs. 49.67 ± 37.5).

Finalmente, los resultados manifiestan diferencias significativas en diversos parámetros al comparar los animales muestreados en libertad (n = 13) con los mantenidos en cautividad (n = 18). Así, el hierro, calcio, ALP y creatinina (P<0.05) muestran valores superiores en

animales mantenidos en cautividad. El tipo alimentación de los ejemplares sometidos a condiciones de cautividad puede dilucidar la diferencia en estos valores.
Sin embargo, los linces muestreados en libertad manifiestan niveles superiores de GOT (131 ± 118.87 vs 40.29 ± 22.28) y GPT (80.77 ± 41.75 vs 49.12 ± 33.37). Valores altos en estas enzimas son atribuibles al estrés fisiológico de la captura (Marco y cols., 2000).

Manejo del Lince Ibérico en Cautividad

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Resumen.
Una de las claves del manejo del Programa de Cría para la Conservación del Lince Ibérico consiste en lograr un equilibrio entre el fomento de las conductas naturales de la especie (caza, territorialidad, interacciones sociales, etc.) y la creación de un entorno libre de estrés en el que los animales sean más proclives a aparearse. Para la obtención de información relevante para el manejo (peso, estado potencial de gestación, etc.) se están poniendo a punto técnicas de entrenamiento que eviten el uso de métodos invasivos y que sirvan, a su vez, para aumentar el vínculo entre los animales y sus cuidadores. En el desarrollo de las técnicas de manejo habrá que tener cuenta en todo momento la necesidad de no incurrir en la domesticación.

En general, las técnicas de manejo están basadas en la aportación de conocimientos multidisciplinarios en las áreas de cuidados animales, nutrición, veterinaria, genética, fisiología, etología y ecología de la especie, junto al uso sistemático del método científico. Este capítulo se ha elaborado utilizando como base las experiencias obtenidas hasta la fecha con los linces ibéricos mantenidos en El Acebuche y en el Zoobotánico de Jerez, junto a la información procedente de programas establecidos por zoológicos europeos y americanos.

Los comportamientos de los ejemplares del Programa de Cría se siguen con un sistema de video-vigilancia que permite observarlos sin causarles molestias durante las 24 horas del día. Esto está permitiendo aprender muchas cosas sobre la especie que no se podrían estudiar fácilmente en la naturaleza. En este capítulo se detallará información sobre el manejo del lince ibérico en cautividad, profundizando en varios aspectos como la nutrición, incorporación de nuevos ejemplares al programa, manejo de reproductores durante el periodo de cópulas, manejo de hembras gestantes, partos,

y desarrollo de cachorros, así como el manejo sanitario de la población cautiva.

Se pretende que, conforme aumente el número de centros que alberguen linces destinados a la cría en cautividad, se unifiquen los procedimientos de manejo que hayan sido aplicados con éxito en los centros piloto de El Acebuche y del Zoobotánico de Jerez.

1. Manejo Genético del Programa de Cría para la Conservación del Lince Ibérico

El Programa de Cría para la conservación del lince ibérico sigue el modelo de los programas de cría europeos (EEPs), y todos los ejemplares incorporados al programa se han de incluir en el s*tudbook* ("libro de reproductores") desarrollado para esta especie. Los cruces entre ejemplares se realizarán atendiendo a las prioridades genéticas establecidas por el programa PM 2000. El intercambio de ejemplares entre centros lo determinará la dirección del programa, basándose en los resultados del programa PM 2000, y según el asesoramiento un subcomité de expertos.

Las metas principales del Programa de Conservación Ex-situ son dos:

3. Asegurar la conservación del material genético de la especie
4. Crear, a medio y largo plazo, nuevas poblaciones de lince ibérico a través de programas de reintroducción

Dada la situación actual del lince ibérico, se podrían fomentar las condiciones básicas para conservar un 85% de la variabilidad genética actual durante un periodo de 30 años (Lacy y Vargas, 2004). Una población cautiva con una variabilidad genética por debajo del 85% se considera peligrosamente endogámica y no sería aceptable desde el punto de vista genético.

Para alcanzar los objetivos genéticos estipulados se deberían incorporar 4 cachorros por año durante cinco años consecutivos. Es decir, habrá que incorporar 20 cachorros/juveniles en el plazo de 5 años. Para asegurar el mantenimiento de la diversidad genética en un plazo de 30 años se deberá contar con un grupo de 60 reproductores (constituido en principio por los propios fundadores más ejemplares nacidos en el programa de cría). Como estrategia básica para el mantenimiento de la variabilidad genética se considera importante conseguir un crecimiento poblacional rápido durante los 10 primeros años del programa, hasta alcanzar la fase de capacidad de carga; es decir, 60 (30.30) ejemplares reproductores. Asimismo, como parte del mencionado manejo, se procurará igualar la representación de los fundadores de modo que todos aporten un número similar de crías al programa.

2. Incorporación de nuevos ejemplares

Hay que procurar reducir al máximo el inevitable estrés que experimentan los animales cuando se incorporan a un entorno nuevo. La cuarentena de los nuevos ejemplares que se incorporan al programa de cría en cautividad dura un total de dos meses. La primera revisión sanitaria se efectuará a las dos semanas de la llegada del ejemplar. La finalidad de esta espera de dos semanas es tener al animal más aclimatado al nuevo entorno y dar un margen de tiempo preliminar para determinar si está incubando alguna enfermedad. La segunda revisión sanitaria se efectuará tras haber transcurrido un mes de la primera. Si los resultados de las analíticas indican que el animal no padece ninguna enfermedad infectocontagiosa, se procederá a su incorporación al programa de cría. Para aquellos ejemplares que van a ser trasladados entre centros del propio programa de conservación ex-situ, no será necesario efectuar la anestesia del animal. Se llevarán a cabo análisis coprológicos y PCRs de las principales enfermedades que puedan ser detectadas a partir de heces. Este procedimiento antes de cualquier traslado es una medida de seguridad adicional ya que los linces del programa son objeto de una revisión anual.

Una vez que el ejemplar se traslada a las instalaciones donde habitan otros linces en jaulones adyacentes, conviene agilizar en lo posible su proceso de adaptación. La llegada de un nuevo ejemplar bien podría también tener consecuencias en el comportamiento del resto de linces residentes, por lo que se extremaran las precauciones y las medidas de vigilancia.

Durante los primeros días, el animal recién llegado, tras haber pasado la correspondiente cuarentena, estará sometido a estrecha vigilancia en un área de manejo debidamente acondicionada, proporcionándole el refugio y la intimidad necesarios. Al salir al campeo por vez primera, los ejemplares recién llegados buscarán aislamiento y protección en la zona más alejada del trasiego diario de los cuidadores. Este refugio suele ser la paridera interior, en la que algunos animales han llegado a pasar prácticamente todas las horas de luz durante sus primeros 5 o incluso 6 meses de estancia en El Acebuche. El macho *Garfio*, incorporado al Centro el 31/12/04, tardó seis meses en mostrarse abiertamente frente a sus cuidadores. En otros casos, como el del macho *JUB*, transcurridos 6 meses desde su incorporación se estimó oportuno cerrarle el acceso a la paridera interior para inducirle a cazar en los campeos y a habituarse al trabajo diario de sus cuidadores.

3. Nutrición

Uno de los requisitos más importantes para el desarrollo de un Programa de cría en cautividad es que tanto machos como hembras estén en perfecto estado nutricional. Una dieta deficiente podría llegar a comprometer la capacidad reproductora de los ejemplares. Con el fin de proporcionar una dieta equilibrada a los linces del Programa de Cría en Cautividad, el Centro de Cría de El Acebuche en colaboración con la nutróloga del Programa, Helena Marqués (de la empresa ConZOOlting) ha llevado a cabo un estudio sobre los requerimientos energéticos y nutricionales de los linces cautivos. Además de la dieta específica de mantenimiento, este estudio contempla ajustes nutricionales para los periodos de gestación, lactancia y crecimiento de cachorros en cautividad.

La alimentación de los linces en libertad está basada en el conejo de campo en un 80 o 90%. Pueden depredar sobre otras especies, como roedores, aves, ungulados e incluso alimentarse con carroña. En cautividad, siempre que sea posible, se procurará ajustar su alimentación a la dieta que toman en la naturaleza con el fin de cubrir todas sus necesidades, tanto nutricionales como de comportamiento.

Todos los animales residentes, excepto en situaciones concretas como la gestación o lactancia, normalmente se alimentan 6 días a la semana, ayunando el día restante. La alimentación, en su mayor parte, es a base de conejo, procurando ofrecerlos con pelajes oscuros, similares a los de los conejos silvestres. Normalmente se alimentan de conejo vivo tres veces por semana, conejo muerto (2 veces por semana) y ave o ungulado un día por semana, alternando las semanas entre aves y ungulados. No se empleará carne de ungulado silvestre sin control sanitario por el riesgo que supone la tuberculosis en estos animales. Para cubrir las posibles deficiencias vitamínico-minerales que puede ocasionar en los linces la ingesta de presa muerta, se les administra con la comida un suplemento multivitamínico dos veces por semana. Se emplea el complejo vitamínico para carnívoros "*Mazuri Whole Prey*", por ser el tipo de vitamina que proporciona el mejor aporte de cara a la reproducción y mantenimiento.

En caso de ser necesario administrar algún medicamento, siempre se procurará hacerlo junto con la comida, ya sea en solución o en forma de polvo (por ejemplo comprimidos pulverizados). Conviene utilizar aquellos fármacos y posologías que admitan una administración al día.

3. Fichas de toma de datos y base de datos de manejo

Diariamente se anota en fichas individualizadas para cada animal (ver fichas en el Programa de Funcionamiento: http://www.lynxexsitu.es/manejo/documentos_

manejo.htm) toda la información pertinente, como la comida ofrecida, su peso, la clase de alimento y su presentación, así como las observaciones más relevantes: cambios de instalación, comportamientos, síntomas de celo, montas, administración de suplementos o medicamentos, recogida de muestras, tipo de muestra, etc. Toda esta información se mantiene en una base de datos (en access) que se guarda por duplicado.

4. Preparación de machos y hembras para el periodo reproductor

4.1. Seguimiento/monitorización de perfiles hormonales a partir de muestras de heces y orina

En colaboración con los doctores Katey Pelican y David Wildt, del Centro de Conservación e Investigación del Zoo Nacional, Front Royal, USA, y Teresa Abaígar, de la Estación de Zonas Áridas, Almería, se han determinado los perfiles hormonales de machos y hembras de lince ibérico a lo largo de tres temporadas de cría. Esta información es muy importante para distinguir patrones generales sobre la fisiología reproductora del lince ibérico; p.ej., las hembras de lince presentan actividad ovárica entre los meses de enero y mayo mientras que los machos parecen mantener niveles de testosterona a lo largo de todo el año. La información hormonal es igualmente importante para establecer patrones y diferencias en la gestación de las hembras de esta especie.

Junto al Instituto de Zoología y Vida Silvestre de Berlín, en colaboración con Katarina Jewgenow, se está intentado poner a punto una prueba de detección de gestación a partir de mediciones de relaxina en orina. Asimismo, se está llevando a cabo un estudio comparativo fisiología reproductora del lince ibérico y de su pariente próximo, el lince boreal.

4.2.Revisión/Chequeo Reproductor

Durante el otoño anterior a que de comienzo cada periodo reproductor se procede a realizar una ecografía transrectal que nos ayude a determinar el estado reproductor de las hembras. En colaboración con los doctores Frank Goeritz y Katarina Jewgenow del Instituto de Zoología y Vida Silvestre de Berlín, cada octubre se anestesia a las hembras adultas y subadultas del programa para examinar a fondo el estado de los ovarios (si hay o no folículos, cuerpos lúteos, etc.), útero, riñones, adrenales y otras estructuras internas. Esta revisión, que se acompaña de una revisión sanitaria completa, es muy útil para detectar cualquier problema potencial en la salud reproductora de las hembras

e intentar solventarlo si es posible.

A principios de cada mes de noviembre se procede a realizar una revisión reproductora de los machos de más de dos años. En colaboración con el equipo de Eduardo Roldán, del Museo de Ciencias Naturales de Madrid, se procede a realizar electroeyaculaciones y congelación de esperma para el Banco de Germoplasma del Lince Ibérico que se mantiene en dicha institución. La información que se obtiene de las electroeyaculaciones es particularmente útil para evaluar la salud reproductora de cada macho, ayudando a establecer comparaciones entre distintos machos y a determinar si cada macho sufre variaciones en su calidad espermática de un año a otro. Estos estudios son esenciales para comenzar a entender la fisiología reproductora de esta especie.

4.3. Pesajes y radiografías

Idealmente el pesaje de cada uno de los ejemplares se debería realizar, al menos, una vez a la semana, aunque en la realidad esto no siempre es posible. Algunos animales son menos proclives que otros a la hora de colaborar en el manejo, y aunque a base de entrenamiento sería factible conseguirlo, conviene tener en cuenta la necesidad de evitar la domesticación. La técnica a emplear, por tanto, variará en función de las características de cada ejemplar, buscando siempre la menos intrusiva. Para aquellos habituados a entrar en el túnel metálico, el pesaje se realiza situando la báscula en su interior. El pesaje, en este caso, se lleva a cabo en presencia del cuidador, quien anotará la lectura directa del peso. En los demás casos se podrá colocar la báscula, cebada con carne cuando sea necesario, en el lugar que se estime oportuno. Si el animal es especialmente receloso, conviene evitar la presencia de personal, realizando en este caso una lectura remota del panel de la báscula mediante la instalación en trípode de una cámara de video-vigilancia debidamente orientada. La superficie de la báscula se limpia después de cada uso, eliminando los restos de suciedad y comida.

Para la obtención de placas de rayos-X se utiliza la misma técnica que para el pesaje. En este caso se introduce el cajón con el panel frontal de metacrilato transparente, construido al efecto, en el túnel de comunicación, con el objeto de limitar ligeramente los movimientos del animal. En uno de los lados del túnel se coloca el aparato portátil de rayos-X, alineado con el hueco practicado en el túnel, y en el otro el chasis portaplacas. Un cuidador, protegido con un delantal de radiología, se encarga de situar al animal en la posición correcta, mientras que el técnico, protegido de la misma manera, se encarga de disparar el aparato.

La información obtenida a partir de las radiografías es un diagnóstico definitivo de la gestación que, en aquellos casos en que la calidad de la placa lo permite, ayuda a determinar el número exacto de cachorros que trae la hembra y, por tanto, detectar si hay posibles problemas durante el parto (ver Manejo durante la gestación, en este mismo capítulo).

5. Manejo durante la reproducción

5.1. Pre-cópulas

Antes del comienzo de cada temporada, todos los trabajos más intrusivos como la instalación de nuevas cámaras, redes de protección, trabajos de albañilería, fontanería, desbroces, deinsectaciones, instalación de parideras exteriores, acondicionamiento de parideras interiores, etc. deben estar terminados.

Si bien en el futuro los cruces entre ejemplares, o el establecimiento de parejas, vendrán dictados por los programas informáticos diseñados para mantener el máximo grado de variabilidad genética, además de otros factores como la compatibilidad o las meras limitaciones logísticas, en esta fase inicial del Programa de Cría se pretende aumentar cuanto antes la población cautiva por lo que, sin dejar de valorar otros aspectos (p.ej., cruce entre ejemplares de Sierra Morena y Doñana) se considerará la compatibilidad entre ejemplares como el factor más importante a la hora de determinar los cruces. Antes de que una hembra entre en celo se comprueba la compatibilidad con el macho, aumentando paulatinamente el grado de aproximación entre los ejemplares. En la fase inicial, el contacto se realiza a través de la malla de separación, dejando que uno de los ejemplares (normalmente el macho) acceda al pasillo de comunicación entre campeos. Si se observan signos de compatibilidad (saludos amistosos como el choque de cabezas, etc.) se abre la guillotina de comunicación entre ambas instalaciones, no sin antes disponer los medios necesarios para actuar en caso de enfrentamiento. En los reencuentros entre animales que ya hayan demostrado compatibilidad con anterioridad se sigue el mismo procedimiento. Tanto los encuentros a través de la malla de separación como la unión de los ejemplares se graban con el sistema de video-vigilancia para realizar un análisis meticuloso de las conductas.

En general, los machos del centro de cría de El Acebuche comienzan a maullar a mediados de diciembre y las hembras responden con maullidos de celo aproximadamente un mes más tarde.

5.2. Cópulas

Durante la época de celo las hembras vocalizan casi continuamente, el macho no las pierde de vista ni un instante y maúlla delimitando su territorio. Ambos marcan continuamente cada elemento de su entorno y finalmente la hembra se muestra receptiva. Las cópulas, que son breves y frecuentes, suelen durar un par de días, transcurridos los cuales el macho pierde el interés. El lince, como la mayoría de felinos, es una especie de ovulación inducida; es decir que necesita el estímulo mecánico de la penetración para que aquella se produzca. Las cópulas son breves y frecuentes. Una vez la hembra se muestra receptive, el macho se mantiene a pocos metros de ella, copulando con regularidad cada 2-3 horas durante un periodo de 2 a 2 días y medio. Siempre y cuando los ejemplares sean compatibles y no sea necesario utilizar el macho para cubrir a otra hembra, los animales se mantienen juntos hasta unas dos o tres semanas antes de la fecha estimada del parto.

5.3. Gestación

El manejo de las hembras potencialmente gestantes consiste principalmente en mantener en el recinto de reproductores un ambiente lo más tranquilo posible, estimular el ejercicio y las conductas naturales de caza, en realizar un seguimiento lo más exhaustivo posible de sus conductas y en pesarlas un par de veces a la semana. La instalación se revisa en profundidad para intentar que no queden lugares querenciosos para el parto que pudieran resultar inadecuados para el correcto seguimiento de la hembra; se pretende que éstas utilicen las parideras para tener un mayor control durante el parto y el desarrollo de los cachorros. Aproximadamente unas dos semanas antes de la fecha estimada del parto se retira el macho del campeo de la hembra (en aquellos casos en que todavía estuvieran compartiendo instalación) y se suspende el trasiego rutinario. En estos casos el mantenimiento en parideras se limita a vigilar por si se dejaran restos de comida que puedan pudrirse. Se pretende que las hembras consideren seguras tanto la habitación paridera (paridera interior) como los habitáculos naturalizados dentro de los campeos (paridera exterior), entre otras razones por ser las zonas que están vigiladas por las cámaras de forma continua y sin interferencias. Entre los días 50 y 55 desde las primeras cópulas observadas se tomarán placas de rayos-X para confirmar el embarazo y estimar el número de cachorros. Mediado el tiempo de la gestación potencial (entre 30 y 37 días) a las hembras se les suprimirá el día de ayuno. A partir de entonces se proporcionará comida ad libitum.

El tiempo medio de gestación de las hembras de lince ibérico que han alumbrado cachorros en el centro de cría de El Acebuche es de 63-65 días (contados a partir de la primera cópula). En la primavera de 2006 se produjo un parto prematuro y distócico a los 56 días de gestación; uno de los cachorros nació muerto y el otro murió a las pocas horas de nacimiento.

Tabla 1. Cruces entre linces ibéricos del Centro de Cría de El Acebuche durante la temporada del 2006

Hembra	Macho	Fecha cópulas	Nº cópulas	Gestación	Fecha Parto	Nº de días gest.	Nº cachorros	Comentarios
Saliega	Garfio	19-I/20-I	43 (19 c/ Garfio y 15 c/ Cromo)	SI	23-III	63	2	Ambos cachorros de Garfio. En 2005, gestación de 64 días
Saliega	Cromo	22-I/23-I						
Adelfa*	Cromo	19-I/20-I	19	NO	--	--	--	Posible pseudogestación
Aura	Garfio	30-I/1-II	26	NO	--	--	--	Posible pseudogestación
Esperanza	JUB	9-II/11-II	25	SI	15-IV	65	2	Atiende bien a un cachorro y abandona al otro

* = hembras subadultas

5.4. Parto

Cuando se acerca la fecha estimada de parto de una de las hembras del centro de cría se establece el siguiente protocolo:

- Minimizar las actividades en torno a la zona de reproductores dos días antes de la fecha estimada de parto.
- Vigilar posibles signos: nerviosismo, inapetencia, horas de descanso, cambios de comportamiento, etc.
- Videovigilancia intensiva.
- Tener a punto todos los materiales necesarios para la cría artificial: espacio adecuado, incubadora, leche y útiles para la alimentación, calentadores, etc.
- Una vez comience el parto intentar identificar las características del parto y sus fases con el objeto de discernir si es un parto normal (parto eutócico) o un parto distócico

que requiera intervención (ver programa de funcionamiento del centro de cría de El Acebuche: http://www.lynxexsitu.es/manejo/documentos_manejo.htm)

— Si el parto se desarrolla normalmente pero la hembra no muestra un buen instinto maternal (abandono, canibalismo, etc.) o algún cachorro parece letárgico o no mama, habrá que intervenir consecuentemente.

6. Manejo de hembras con cachorros

Durante los primeros tres días después del parto, la presencia y las faenas del personal en el recinto de reproductores se reducen al mínimo imprescindible (alimentación), manteniendo a lo largo de las cuatro primeras semanas la máxima tranquilidad posible. En este período, la alimentación de la hembra se incrementa hasta llegar casi a los 2kg diarios (2 o 3 conejos de 600 o 700g).

En una situación ideal (hembra con buen instinto maternal y cachorros sanos) el manejo de cachorros debe ser mínimo. Conviene reducir al máximo la interferencia humana, utilizando las cámaras para realizar un cuidadoso seguimiento, tanto de las conductas como de los cambios físicos que experimentan. A partir de la 2ª o 3ª semana puede dar comienzo el registro periódico del peso.

Casos en los que se considera necesario retirar cachorros de la hembra

1.- Canibalismo. Esto es normal en el caso de que un cachorro nazca muerto o sin viabilidad. Intervenir si estimamos que la hembra comienza a canibalizar un cachorro aparentemente sano para evitar que canibalice a otros.

2.- Abandono de cachorros. En el caso de que la hembra no atienda a los neonatos durante más de 2 horas y que los cachorros comiencen a mostrar signos de letargia, se considera que se trata de un caso de "abandono". Hay que intervenir de inmediato para evitar que los cachorros mueran por hipotermia.

3.- Si la hembra está cuidando bien a sus cachorros pero se observa que en cualquier momento durante la lactancia alguno tiene problemas (**letargia, no mama**, etc.) o está **visiblemente rezagado** respecto a sus hermanos de camada, se intervendrá consecuentemente.

Es fundamental tener paciencia y no molestar a la hembra a no ser que sea estrictamente necesario.

7. Crianza artificial

Idealmente un lince ibérico nunca debería criarse artificialmente. En la mayoría de

los casos la cría artificial es una medida de emergencia que se adopta cuando todo lo demás ha fallado. Un centro de cría debe proporcionar el ambiente, la alimentación y el manejo necesarios para que una hembra lleve de forma normal la gestación, parto y cuidado de sus crías. Las crías recibirán así la mejor atención posible, la de su propia madre.

Si se hace necesario criar un cachorro a biberón, las técnicas específicas para criarlo a mano involucran un gran número de detalles que se puntualizar en el Manual de Crianza Artificial de Cachorros de Lince Ibérico: http://www.lynxexsitu.es/aaveterinaros/manuales.htm).

8. Manejo para prevenir o actuar en los enfrentamientos

El lince es un animal territorial, es decir, que defiende su territorio de las intromisiones de otros congéneres, fundamentalmente de su mismo sexo. En la naturaleza, por regla general, no suelen llegar al enfrentamiento físico, dado el alto coste que puede implicar salir malparado en una pelea. Las mismas razones se aplican a los linces en cautividad, a pesar de lo cual hay que prever la posibilidad de que se produzcan enfrentamientos. Por ello es necesario disponer con antelación los métodos y medios que hayan demostrado su eficacia en la desactivación de peleas. Estos métodos variarán en función de las características de los ejemplares implicados (edad, sexo, carácter, grado de mansedumbre, etc.).

8.1. Linces Adultos

Los conflictos entre dos linces adultos de distinto sexo, siempre que se trate de animales compatibles, son extremadamente raros y cuando ocurren suelen estar relacionados con la comida. Como medida preventiva, cuando macho y hembra compartan instalación, se les administra la comida por separado. Hasta la fecha tan sólo hemos registrado pequeñas escaramuzas sin consecuencias entre algunos ejemplares que comparten instalación rutinariamente. Los métodos utilizados hasta el momento para separar a dos ejemplares han consistido fundamentalmente en alarmas sonoras (silbatos y voces) y aplicación de agua, siendo éste último el método preferido puesto que no afecta al resto de los ejemplares en el mismo grado que los ruidos estrepitosos o las voces.

Para que la utilización del agua sea efectiva hay que contar con la presión suficiente para abarcar la mayor parte posible del área de campeo sin necesidad de entrar en él. Es necesario también poder regular la presión o el caudal para evitar dañar a los

ejemplares cuando el enfrentamiento se produzca cerca de la valla perimetral de los campeos. Si la pelea se produce en un área que quede fuera del alcance del agua, como las parideras interiores, hay que acceder con la mayor rapidez posible a la parte trasera de la paridera. En comunicación mediante radio con la sala de monitores para verificar el resultado de las medidas disuasorias, ensayar primero fuertes golpes en la puerta o el empleo del silbato.

Si estas medidas fallan, abrir con suma precaución la puerta corrediza de metal, cerciorándose antes de que la puerta de entrada al cercado está cerrada, y arrojar agua si fuera preciso.

8.2. Cachorros

Durante la época de peleas potenciales entre cachorros, entre los días 30 y 65, se extreman las medidas de vigilancia, estableciéndose a tal fin turnos de 24 horas, con dos personas durante la noche, según las circunstancias. Al igual que con los adultos, el lanzamiento de agua es el método principal de disuasión durante las peleas. Si la pelea se produce fuera del alcance de las mangueras, será preciso entrar en los campeos con rapidez. Es de suponer que la hembra intentará defender a sus cachorros, por lo que será necesario contar con la debida protección. Aún en fase experimental, el Centro de Cría ha modificado un antiguo "hide" que se utilizó hace algunos años para la toma de fotografías en el interior de los campeos.

Cuando el riesgo de enfrentamiento entre dos cachorros se considere muy alto, o inmediatamente después de una pelea, se les separará utilizando como área de amortiguación la mitad de los pasillos de separación entre campeos. Estos pasillos se acondicionan cubriendo el techo con plástico y brezo, y las paredes con rafia. Se enriquecen con algunos elementos como troncos huecos y cajones-paridera. En una de las esquinas, cercana al cajón y por fuera de la malla, se instala una lámpara calorífica de infrarrojo de forma que los animales puedan acercarse o alejarse a su voluntad de la fuente de calor. También se proporcionan botellas de agua caliente cubiertas con tela de algodón para dar la opción al cachorro de aproximarse a un "cuerpo caliente". Es importante que el cachorro en el pasillo mantenga contacto visual y olfativo con su madre en todo momento, para lo cual se despeja una ventana en la rafia que cubre la malla de separación entre pasillo y campeo. Si la camada cuenta con varios ejemplares, se aplica un sistema de rotación entre los cachorros de forma que todos puedan mamar y pasar tiempo con la madre.

9. Manejo sanitario

En un centro de cría se debe procurar adaptar todo el manejo sanitario de los animales para que sea lo menos invasivo y estresante posible. Se extremaran las medidas de medicina preventiva para evitar tanto la entrada como el desarrollo de enfermedades, ya que una vez instauradas suelen ser mucho más complicadas de tratar y eliminar.

Toda captura y anestesia de animales deberá estar plenamente justificada. Estos procedimientos llevan un riesgo asociado importante (heridas, problemas anestésicos, etc.), además de la posible pérdida de confianza del animal hacia el personal, y hay que sopesar bien los posibles beneficios y perjuicios antes de realizarlos.

Parte del manejo sanitario incluye la realización de controles sanitarios no invasivos, tales como coproparasitológicos, coprocultivos, urianálisis y análisis de determinadas enfermedades en muestras de heces (FCoV, FPV).

8.1. Coproparasitológicos y antiparasitarios internos

Cada tres meses se realizan análisis coproparasitológicos de todos los ejemplares. Los coproparasitológicos, consistentes en una extensión fecal y en una flotación, se pueden realizar en el laboratorio del propio centro o son enviados a un laboratorio exterior.

En los coproparasitológicos de los linces del centro de El Acebuche es frecuente encontrar quistes de Eimeriidae que provienen de los conejos vivos que reciben de alimento, y que por tanto no se tratan. No hemos observado problemas asociados a endoparásitos en los linces del centro; se han detectado algunos huevos de nemátodos en un animal y algunos huevos de cestodos en otro, ambos sin síntomas, y en los que se consideró que no era necesario el tratamiento. Al realizar los coprológicos cada tres meses podemos ver si estas parasitemias se elevan y en su caso tratarlas.

En general los nemátodos más frecuentes en felinos son T*oxascaris y Toxacara*. Los huevos de *Toxascaris* y *Toxacara* son muy resistentes y difíciles de eliminar del medio. Son comunes las reinfecciones por la contaminación de la comida con huevos o por la ingesta de hospedadores secundarios (roedores). Una vez que se ha producido la infestación, como es muy difícil eliminarla, hay que realizar tratamientos profilácticos con *antihelmínticos* cada seis meses. Se puede emplear *fenbendazol* (*Panacur*) a razón de 20-50mg/kg durante 3 o 5 días mezclado con la comida. Para evitar la aparición de resistencias conviene ir alternando el *antihelmíntico*. Se pueden emplear productos

antiparasitarios que son combinaciones eficaces tanto contra nematodos como cestodos (*Drontal* gatos, *Vitaminthe*). Tras el tratamiento se realizan tres coproparasitológicos (uno cada 24 horas como mínimo) para comprobar la eficacia del antiparasitario. En caso de que se detecte huevos de cestodos y que sea necesario el tratamiento, se puede emplear *praziquantel* (*Droncit*) a razón de 5 mg/kg una sola vez. Actualmente no se considera necesaria la medicación profiláctica contra dirofilarias.

8.2. Parásitos externos y antiparasitarios externos

Los linces pueden verse afectados por pulgas, garrapatas y ácaros auriculares. Idealmente, el tratamiento contra ectoparásitos debe dirigirse simultáneamente sobre el animal y el ambiente. Resulta prácticamente imposible llevar un control total de ectoparásitos a largo plazo en unas instalaciones como las del centro de cría.

Para el control de ectoparásitos en aquellos animales que se puedan manipular o que estén entrenados a entrar en pasillos, se podrán emplear pulverizaciones o pipetas con insecticidas (tipo *Frontline, Stronghold*, etc.). El tratamiento de las zonas exteriores y pasillos se lleva a cabo rociando con un insecticida de baja toxicidad y de larga duración, con el fin de crear una barrera que evite la entrada de ectoparásitos. Los distintos productos antiparasitarios (piretrinas, diazinón, etc.) se irán alternando para evitar la aparición de resistencias. El tratamiento antiparasitario se realizará con mayor intensidad durante los meses de marzo a octubre. El tratamiento tiene mayor eficacia cuando va acompañado de un desbroce periódico de la vegetación de las jaulas de manejo, así como la perimetral del centro y de los pasillos, disminuyendo así las zonas idóneas para el desarrollo de ectoparásitos. Los tratamientos se aplican con mochilas de pulverización cada seis u ocho semanas, en función de la época del año y, por tanto, de la posible carga parasitaria. Para comprobar la eficacia del tratamiento se realiza un manteo de garrapatas antes y después de cada aplicación.

8.3. Vacunaciones

En general la recomendación para la vacunación de felinos exóticos es emplear vacunas inactivadas trivalentes. Actualmente, en el programa de cría del lince ibérico se emplea la vacuna *Fevaxyn-i-CHP* de FortDodge, vacuna inactivada contra el virus de la panleucopenia felina (FPV), el herpesvirus felino (FHV), el calicivirus felino (FCV). En alguna ocasión se ha utilizado una pentavalente inactivada que, a parte de los virus anteriormente mencionados, también incluye el virus de la leucemia felina (FeLV) y la *Chlamydiophila*. En gatos domésticos se han detectado, aunque en un número muy

bajo, el desarrollo de sarcomas en la zona de inoculación asociados posiblemente al excipiente de las vacunas de FeLV; por otro lado no se recomienda la vacunación contra *Chlamydiophila* salvo que represente realmente un riesgo.

La pauta en cachorros es administrar la primera vacuna trivalente a las 6-8 semanas, revacunar a las 12 y 16 semanas, y después al año. A partir de entonces las revacunaciones serán bianuales, excepto en el caso de las hembras con potencial reproductor, que se revacunaran antes de que de comienzo la temporada de cría. La vacuna normalmente se aplicará por vía subcutánea, con lo que suele ser necesaria la captura del animal en la jaula trampa-compresión o bien la anestesia completa del animal. También se puede aplicar la vacuna con dardo, aunque en este último caso puede ser menos efectiva (bien porque no se inyecte toda la dosis o lo haga en una zona con menor absorción).

8.4.Urianálisis

En la orina de muchos linces ibéricos, tanto de ejemplares en cautividad como silvestres, se detecta una elevada cantidad de proteínas. Aunque esto podría indicar la existencia de algún tipo de problema renal no se ha observado que los animales manifiesten síntoma alguno. Para poder conocer y entender mejor este hallazgo conviene ir analizando periódicamente muestras de orina. Aprovechando la recolección de orina para su uso como atrayentes en el foto trampeo, periódicamente se recoge orina en recipientes estériles y se analiza en el propio Centro mediante tiras de orina. De esta manera se pueden monitorizar y detectar ciertas anomalías, no solo la presencia de proteínas, sino también la presencia de sangre, leucocitos, cristales, cambios de densidad, etc.

8.5. Cultivos fecales

Salmonella, Shigella y Campylobacter son las bacterias que con mayor frecuencia se han aislado en desórdenes intestinales en felinos. Una forma de control no invasivo del estado sanitario de los linces es realizar cultivos fecales dos veces al año.

Nota: a lo largo del 2005 se detectó Campylobacter en los cultivos fecales de dos ejemplares del Centro de Cría que no mostraban ningún síntoma de enfermedad. No se han obtenido crecimientos de Shigella ni de Salmonella.

8.6. Chequeos sanitarios

Se estima necesario realizar un chequeo completo de todos los ejemplares cada dos

años. Conviene planificarlos durante los periodos no reproductivos, o coincidiendo con exámenes especiales (electroeyaculaciones o toma de muestras, etc.), cambios de instalación, etc. Los chequeos se realizan bajo anestesia, que se puede aplicar directamente con jeringa en la jaula trampa-compresión o con cerbatana. Es conveniente entrenar a los animales a entrar en los túneles de manejo o en la jaula trampa-compresión, lo que facilitará y hará más segura la anestesia.

Antes de anestesiar a un animal hay que asegurarse de que se dispone de todo el material y fármacos para poder actuar en caso de una emergencia anestésica (v. Manual Clínico del Lince Ibérico: http://www.lynxexsitu.es/aaveterinaros/manuales.htm). Durante el examen se comprueba el correcto funcionamiento del microchip, injertándolo en caso de que no lo tenga, y se toma el peso. Hay que poner especial atención en el examen de la boca para detectar el estado de la encía, fracturas de piezas dentarias o presencia de sarro. El examen debe incluir las uñas, cojinetes plantares, ojos, oídos, genitales y glándulas mamarias. Durante el examen se toman muestras para las diferentes pruebas diagnósticas y también se conservan muestras de sangre, suero y heces por si fuera necesario realizar análisis posteriores.

Para más información sobre detalles de manejo, infraestructura, protocolos de emergencia, etc., que se utilizan dentro del Programa de Conservación Ex-situ del Lince Ibérico, por favor consultar la página web: www.lynxexsitu.es

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papel de apoyo importante para que la conservación ex-situ del lince ibérico se integre adecuadamente dentro del objetivo cumbre de este programa: recuperar al lince en la naturaleza.

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Anomalías en el Comportamiento de los Felinos en Cautividad

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El objetivo de esta ponencia es describir las principales anomalías en el comportamiento de los felinos en cautividad, así como discutir sus causas y las posibles estrategias de manejo que permiten prevenirlas o corregirlas. Por "anomalía en el comportamiento" entendemos (1) conductas que aparecen sólo en animales en cautividad –es decir, que no se han descrito en animales en libertad- y que son indicativas de una falta de bienestar o (2) conductas que, pese a ocurrir también en libertad, ven modificada su frecuencia o intensidad de forma significativa en cautividad como consecuencia, una vez más, de una situación de estrés o falta de bienestar.

Las anomalías en el comportamiento de los felinos en cautividad son consecuencia del estrés causado por el alojamiento o el manejo, o de un ambiente pobre en estímulos que no permite expresar el comportamiento normal de la especie y da lugar a un fenómeno que se conoce con el nombre de "restricción de la conducta".

Tradicionalmente el estrés se define como una respuesta del organismo frente a una situación de amenaza. Esta respuesta incluye tanto cambios de conducta como fisiológicos; estos cambios son en buena medida consecuencia de un aumento en la síntesis y liberación por parte del hipotálamo de la CRH –hormona liberadora de corticotropina-. El hecho de que una determinada situación sea estresante depende de la percepción que el animal tiene de la misma. En general, aquellas situaciones que son percibidas como imprevisibles o que escapan al control del animal dan lugar a una respuesta de estrés.

La restricción de conducta aparece cuando el estímulo en el que se encuentra el animal no permite expresar determinadas conductas que son importantes por sí mismas o, dicho de otro modo, para las que existe una fuerte motivación independientemente del ambiente en el que se encuentra el animal. Existe una considerable evidencia obtenida a partir de estudios realizados con varias especies que indica que los animales están fuertemente motivados a mostrar conductas de "forrajeo" o búsqueda de alimento,

incluso cuando sus necesidades nutricionales están cubiertas. En consecuencia, un ambiente que no permita que un animal muestre dicha conducta dará lugar al fenómeno de "restricción de conducta". Aunque existe menos evidencia al respecto, es muy probable que otras conductas además de la búsqueda del alimento sean igualmente importantes.

Los dos factores responsables de las anomalías de comportamiento que muestran los felinos en cautividad –estrés y ambiente pobre en estímulos- están muy relacionados, puesto que la restricción de conducta desencadena a menudo una respuesta de estrés.

La alteración del comportamiento más frecuente en los felinos en cautividad son las estereotipias, que se definen como conductas repetitivas, invariables y sin función aparente. Las estereotipias no se han descrito en animales salvajes en libertad, pero son comunes en animales salvajes en cautividad y en animales domésticos. Existen dos tipos principales de estereotipias: las que resultan de un proceso patológico –estereotipias con causa orgánica- y las que están causadas por un ambiente inadecuado –estereotipias ambientales-. Estas últimas son con diferencia las más frecuentes. Las estereotipias ambientales se desarrollan cuando un individuo con predisposición a mostrar estereotipias se encuentra en un ambiente que impide la realización de conductas para las que existe una motivación importante o bien cuando el ambiente resulta en situaciones de estrés o conflicto frecuentes.

La predisposición a realizar estereotipias cuando el ambiente no es adecuado varía entre especies y también entre individuos de una misma especie, debido tanto a factores genéticos como a la experiencia temprana del animal. Así, las estereotipias son más frecuentes en carnívoros y en primates que en herbívoros. Se ha sugerido igualmente que las especies que en condiciones naturales utilizan una superficie mayor en sus desplazamientos diarios tienen más tendencia a mostrar estereotipias cuando se encuentran en cautividad que las especies que utilizan una superficie menor.

Dentro de una misma especie, el estrés en edades tempranas del desarrollo incrementaría la tendencia a realizar estereotipias en la edad adulta. Esto explicaría, por ejemplo, que los animales destetados de forma prematura realicen –según algunos estudios- más estereotipias cuando son adultos que aquéllos que se destetaron a una edad normal. Igualmente, esto podría explicar el hecho aparentemente contradictorio de que en algunas especies los individuos nacidos en cautividad desarrollan más estereotipias que los capturados en su medio ambiente natural.

Igualmente, en animales domésticos se han descrito diferencias genéticas en la

tendencia a realizar estereotipias. Estas diferencias se ponen de manifiesto sólo cuando el ambiente no es adecuado –si el ambiente es adecuado ningún individuo realiza estereotipias-.

En felinos en cautividad, la estereotipia ambiental más común es el desplazamiento siguiendo un circuito fijo dentro de la instalación ("pacing").

Las estereotipias son importantes porque indican una carencia de bienestar. Además, hay algunas evidencias que sugieren que los animales que se encuentran en ambientes que resultan en el desarrollo de estereotipias podrían ver alterada su función reproductiva.

Es importante tener en cuenta que si bien la realización de estereotipias indica una falta de bienestar, el hecho de que un animal no haga estereotipias no puede considerarse por sí mismo evidencia de que su bienestar es satisfactorio. Esto es debido, en parte, a las diferencias entre especies y entre individuos de una misma especie en su tendencia a realizar estereotipias cuando el ambiente no es satisfactorio.

Una segunda alteración del comportamiento que puede observarse en felinos en cautividad es una reducción de la actividad general acompañada de una disminución de la respuesta del animal a los estímulos externos. A menudo, los animales que muestran ste estado de apatía reducen la ingesta de alimento y la conducta de acicalamiento. Se ha sugerido que esta anomalía de la conducta podría ser lo que se conoce como una estrategia pasiva de afrontamiento de las situaciones de estrés.

Al menos en el gato doméstico, este problema es potencialmente grave puesto que la reducción del consumo de alimento antes descrito puede dar lugar a lipidosis hepática, es decir, a una acumulación de ácidos grasos en el citoplasma de los hepatocitos que puede ocasionar la muerte del animal por insuficiencia hepáticas. Es interesante recordar que aunque la lipidosis hepática puede aparecer como consecuencia de cualquier factor que reduzca el consumo de alimento, en un porcentaje muy elevado de casos –al menos en el gato doméstico- dicha reducción del consumo es consecuencia de una situación de estrés y no tiene, por lo tanto, causa orgánica.

En tercer lugar, el estrés inhibe la conducta reproductora, muy especialmente la maternal. Esto es debido a que el estrés aumenta la actividad de algunos circuitos neuronales que disminuyen la respuesta maternal de la hembra antes las crías. El resultado es que las hembras que sufren situaciones de estrés son susceptibles de mostrar una mayor incidencia de conductas de rechazo de las crías o de agresión hacia las mismas.

Existen varias estrategias de manejo para prevenir o controlar las alteraciones de

comportamiento secundarias al estrés o a la restricción de conducta. Sin duda, la más conocida de ellas es el enriquecimiento ambiental, que consiste en proporcionar al animal un ambiente más rico en estímulos que permita la manifestación de una conducta parecida a la que mostraría el animal en su hábitat natural. El enriquecimiento ambiental puede dividirse en varias categorías, tales como el enriquecimiento social –que es más común en especies gregarias- y el no social. Este último puede dividirse a su vez en enriquecimiento ambiental relacionado con la alimentación, sensorial, estructural y cognitivo.

El enriquecimiento ambiental relacionado con la alimentación es uno de los más utilizados en felinos y consiste en proporcionar la comida de forma que el animal vea estimulada su conducta de búsqueda, en lugar de obtener la comida de forma rápida y fácil. Esto puede conseguirse, por ejemplo, escondiendo pequeñas porciones de comida en varios lugares de la instalación. Se ha demostrado que esta técnica resulta en una disminución de las estereotipias y en un aumento de la actividad general de los animales, así como en una disminución de la concentración plasmática de cortisol –o de sus metabolitos en heces-, lo que indicaría que el enriquecimiento ambiental reduce también la respuesta fisiológica de estrés.

Otra forma de enriquecimiento ambiental comúnmente utilizada en felinos es el enriquecimiento estructural, que consiste en proporcionar al animal un ambiente que le permita esconderse o trepar, por ejemplo.

Es importante tener en cuenta que el enriquecimiento ambiental debe basarse siempre en la historia natural de la especie, puesto que –tal como hemos dicho anteriormente, su objetivo es conseguir que los animales en cautividad muestren una conducta lo más parecida posible a su comportamiento natural-. Una vez se ha implementado el programa de enriquecimiento, es interesante comprobar sus efectos utilizando, si es posible, indicadores de comportamiento y fisiológicos. Entre los indicadores de comportamiento se encuentran, por ejemplo, el tiempo total de inactividad o el tiempo dedicado a realizar estereotipias. Entre los indicadores fisiológicos, destaca la concentración de metabolitos de cortisol en heces, que es una medida de estrés crónico.

De forma puntual, los problemas de estrés pueden paliarse con la utilización de psicofármacos, incluyendo los denominados neurolépticos de larga duración (LAN). Los LAN son tranquilizantes mayores que actúan especialmente sobre el sistema de la dopamina y cuyos efectos se prolongan unas 2-3 semanas, dependiendo del fármaco y de la especie en la que se utilicen. Los LAN facilitan la adaptación del animal a un

ambiente nuevo y pueden reducir el comportamiento agresivo. No obstante, a pesar de que son potencialmente interesantes, es necesario insistir en el hecho de que existen muy pocos estudios acerca de su utilidad y seguridad en felinos.

Existen otras moléculas –tales como la fluoxetina y la buspirona- que han demostrado ser muy útiles en el gato doméstico y, ocasionalmente, en algunos felinos salvajes. La fluoxetina reduce marcadamente la conducta agresiva, mientras que la buspirona es un ansiolítico. En la ponencia se expondrá un caso en el que se utilizaron estos fármacos con éxito en felinos salvajes en cautividad y se discutirán sus mecanismos de acción y sus limitaciones.

Es necesario insistir en que la utilización de psicofármacos no debería convertirse en una rutina destinada a enmascarar o contrarrestar las posibles deficiencias en el manejo o las instalaciones de los animales, sino que deberían usarse sólo de forma esporádica.

Finalmente, las denominadas feromonas faciales del gato –que han sido sintetizadas y comercializadas, y tienen un efecto ansiolítico- parecen ser activas en otros felinos. Si este extremo se confirma, dichas feromonas podrían ser una herramienta útil en el manejo de algunas especies de felinos salvajes en cautividad.

Planificación Participativa para la Restauración de Grandes Mamíferos: el Caso de los Esteros de Iberá en Argentina

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Resumen

La reintroducción de mamíferos amenazados, al igual que cualquier otra tarea destinada a la conservación de la biodiversidad, es un desafío complejo que requiere la adecuada comprensión y gestión de aspectos biológicos junto con otros de tipo social, político u organizativo. El propósito de este capítulo es presentar a la planificación como un paso delicado y crucial en cualquier programa de reintroducción. Para ello se analiza un caso concreto basado en una iniciativa de restauración de poblaciones de grandes mamíferos en la región de los Esteros de Iberá (Argentina) para luego extraer algunos principios generalizables para otros programas de reintroducción. La planificación del programa de restauración de grandes mamíferos en Iberá ha utilizado diferentes fases y métodos de toma de decisiones participativas. En una primera fase se trabajó con un grupo pequeño, informal y diverso de profesionales para explorar de una manera abierta y creativa las prioridades de dicho programa. Esta aproximación permitió identificar dos programas iniciales (oso hormiguero gigante y venado de las pampas) y un programa basado en una especie altamente carismática –el jaguar—que debería quedar detenido hasta que los programas previos hayan creado un ambiente adecuado para su iniciación. El proceso de planificación del programa de reintroducción del oso hormiguero constó dos instancias de planificación: una en la que participaron expertos técnico-científicos y otra en la que participaron autoridades gubernamentales. Ambas fases fueron manejadas con enfoques y herramientas diferenciados. Como resultado de este proceso de planificación se obtuvo un Plan de recuperación para la especie en la región de Iberá, el cual contó con un gran respaldo técnico-científico y el apoyo explícito de las principales autoridades gubernamentales. El proceso de planificación sirvió para sentar las bases de los comités asesores del programa, uno científico y otro institucional. Al iniciar un programa de conservación para venados de las pampas en propiedades privadas de estancieros ganaderos se decidió que todavía no se cumplían los requisitos mínimos como para comenzar un proceso de planificación formal que pudiera incorporar eficientemente a todos los grupos relevantes. Varios principios generales pueden ser extraídos de la experiencia en Iberá. En primer lugar, la planificación debe ser

entendida como una acción clave donde convergen las diferentes decisiones del proceso de reintroducción. En segundo lugar, se debe cuidar tanto el proceso de planificación como el producto de ésta. Tercero, el contexto de cada caso va a determinar el proceso de planificación. Cuarto, se debe considerar la influencia y la predisposición de los actores afectados a la hora de diseñar el proceso de planificación. Quinto, se debe incorporar una amplia gama de perspectivas y conocimientos disciplinarios al proceso de planificación con el fin de que se pueda explorar el problema y sus soluciones desde múltiples ángulos. Y, por último, la combinación de complejidad biológica y social con alta contextualidad requiere un nuevo tipo de aproximación profesional hacia la planificación.

Aspectos Científicos en la Planificación de la Traslocación de Lince Ibérico en el Parque Nacional de Doñana

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El Lince Ibérico (Lynx pardinus) es la especie de félido más vulnerable a nivel mundial. Sus efectivos globales, limitados a la Península Ibérica, pueden apenas ser 150-200 ejemplares. La población mejor conocida de linces, y aquélla ubicada en la zona más y mejor protegida de toda su área de distribución, se encuentra en el Parque Nacional de Doñana y su entorno, donde sobreviven unos 40 ejemplares, la mayor parte de ellos habitando terrenos fuera del Parque Nacional. España tiene una enorme responsabilidad a nivel mundial en lo que respecta a la conservación de esta especie, y a los gestores de Doñana y su entorno les corresponde el no menos delicado papel de salvar de la extinción a una población que es todo un símbolo, y que de alguna manera anticipa el futuro que le espera al resto de la especie.

En la actualidad, la situación de la población de lince de Doñana es más crítica que nunca por el hecho de que los núcleos de población ubicados en el interior del Parque Nacional han desaparecido o se han reducido considerablemente. Mientras que hace 15-20 años, podía hasta haber 10 hembras potencialmente reproductoras en el interior del parque, en la actualidad solo puede que sean 3. Esta importante reducción de los núcleos fuente, cambia drásticamente el escenario de conservación de la especie en Doñana, donde la desaparición de un territorio más en el interior del parque nacional podría llevar a la extinción la población de linces de Doñana en los próximos 10 años.

La recuperación de los núcleos fuente del interior del parque nacional con la ayuda de traslocaciones de unos pocos linces, junto con la recuperación y el aumento de la capacidad de carga de esas áreas fuente ubicadas en el interior del parque nacional, disminuiría por debajo del 5% las probabilidades de extinción de toda la metapoblación de linces del área de Doñana en los próximos 100 años.

Proyecto LIFE Conservación y Reintroducción de Lince Ibérico en Andalucía (LIFE 06/NAT/E/209))

Miguel A. Simón & Rafael Cadenas
Consejería de Medio Ambiente-Junta de Andalucía
Dirección General de Gestión del Medio Natural

1.- INTRODUCCIÓN.

Gracias a un correcto desarrollo del Proyecto LIFE "Recuperación de las poblaciones de Lince ibérico en Andalucía" (LIFE 02/NAT/E/8609), a que una estrategia de conservación de una especie en peligro de extinción ha de abarcar múltiples frentes y ha de plantearse a medio o largo plazo, la Consejería de Medio Ambiente considera que nos encontramos en buena posición para la presentación de un nuevo Proyecto LIFE para la especie en Andalucía.

Si a estos aspectos le unimos que la valoración del desarrollo del actual proyecto, desde un punto de vista técnico, ha sido muy buena por la Comisión Europea, que ésta ha manifestado en varias ocasiones que esta especie es prioritaria a nivel europeo y a que nos encontramos inmersos en una profunda remodelación de los mecanismos financieros relacionadas con la conservación de la naturaleza y en especial los relativos a la Red Natura 2000, es casi obligado la presentación un nuevo proyecto que abarque un gran número de actuaciones, algunas de ellas nuevas, y un ámbito temporal de aplicación que garantice fondos para la conservación de la especie a medio plazo.

Por todo ello, la Consejería de Medio Ambiente en la reunión del Grupo de Trabajo del Lince de 28 de enero de 2005 expuso la intención de la presentación de un nuevo Proyecto y ofreció la entrada de organismos y entidades como socios, constando en el correspondiente acta, entre los acuerdos de la reunión, en su punto número 7 que "Se acuerda examinar la propuesta Life 2005 de Andalucía, para su consideración y en su caso, apoyarla por escrito en la candidatura".

Con posterioridad, en la Comisión de seguimiento del actual Proyecto LIFE de 27 de abril

de 2005, la Consejería de Medio Ambiente volvió a exponer la idea de la presentación de un nuevo Proyecto, y avanzó las ideas básicas del mismo.

2.- OBJETIVOS.

El Proyecto LIFE que se ha puesto en marcha persigue dos objetivos distintos, por un lado la continuar de manera coherente con las actividades de conservación y seguimiento de las poblaciones actuales y por otro lado experimentar e iniciar la reintroducción y reforzamiento de poblaciones. Su duración es de 5 años y las bases fundamentales de este proyecto son las siguientes:

Actualmente, sólo existe 2 poblaciones de reducido tamaño y aisladas entre si, siendo un objetivo imprescindible garantizar su mantenimiento a largo plazo (continuar seguimiento y manejo).

El mantenimiento a largo plazo de las dos poblaciones actuales no es suficiente para garantizar la conservación de la especie a largo plazo.

El aislamiento físico entre ambas hace pensar que deben existir diferencias a nivel genético entre ellas. Por lo tanto, para garantizar el mantenimiento de la máxima variabilidad genética sería muy conveniente hacer intercambio de individuos entre ellas (reforzamiento).

La reducida distribución actual de la especie, así como su estrecha dependencia de las fluctuantes poblaciones de conejo son un factor de riesgo muy importante que debería atajarse mediante la creación de nuevas poblaciones (reintroducción).

La reintroducción debería llevarse a cabo no extrayendo ejemplares silvestres, sino a partir del programa de cría en cautividad puesto en marcha actualmente.

Todas estas razones han sido evaluadas y aceptadas positivamente por expertos internacionales en conservación de felinos UICN/SSC Cat Specialist Group (com. pers. Urs Breitenmoser). Además, también se cuenta con el apoyo legal e institucional:

Plan de Acción para la conservación del lince ibérico (Lynx pardinus) en Europa (Consejo de Europa. Nature and environment, No. 111. 2000).

Estrategia para la Conservación del Lince Ibérico (Lynx pardinus). Comisión Nacional de

protección de la Naturaleza. 1999.

Las conclusiones de los Seminarios Internacionales de Andujar (2002) y Córdoba (2004).

Borrador de Plan de Recuperación del Lince ibérico en Andalucía.

3.- CONTEXTO DEL PROYECTO.

El proyecto está estructurado y basado, además de sobre la filosofía y estrategias indicadas anteriormente, en la "Estrategia de recuperación del Lince ibérico" presentada en el II Seminario Internacional de Conservación del Lince ibérico en Córdoba (Dic. 2004) definida por Breitenmoser. El desarrollo del mismo se basará en tres aspectos fundamentalmente:

Desarrollo de actividades de conservación y seguimiento de las poblaciones actuales.

Se continuará con las actividades de seguimiento, mejora de la calidad del hábitat, mantenimiento de convenios, etc., que se han aplicado en el anterior LIFE. Mediante estas medidas se pretende consolidar las poblaciones actuales, aumentar en lo posible el número de territorios reproductores, comunicar los dos núcleos de población de Andújar y expandir la distribución de la especie a las zonas periféricas.

Las actuaciones que han de desarrollarse y contemplarse en los respectivos convenios han sido evaluadas detalladamente, realizándose un análisis espacio temporal de las que son necesarias para la recuperación de las poblaciones de conejo.

Reforzamiento de poblaciones actuales de lince.

En este apartado se iniciará un programa de reforzamiento de la población de Doñana mediante la traslocación de individuos desde la población de Andujar. Esta acción persigue un doble objetivo, por un lado la recuperación de algunos territorios perdidos (principalmente en el Parque Nacional de Doñana) y por otro lado frenar la pérdida de variabilidad genética. Esta parte se iniciaría en el segundo año del proyecto y permitiría perfeccionar las técnicas de manejo de ejemplares como paso previo a la reintroducción.

La metodología consistiría en la captura y traslocación de ejemplares de Andujar, principalmente juveniles, que serán ubicadas en unas instalaciones localizadas en áreas de territorios históricos desocupados en el interior del Parque Nacional de Doñana.

De esta forma se consigue un control del proceso, se garantiza la adaptación de los ejemplares al nuevo entorno y se minimiza la mortalidad, la dispersión y as posibilidades de interacción con ejemplares de la población de Doñana.

En paralelo a estas actuaciones las mejoras de hábitat (truecas, repoblaciones de conejo, desbroces, construcción de refugios para conejo, cercados de alimentación suplementaria, etc.) se realizarán tanto en el interior de las instalaciones destinadas a acoger los ejemplares procedentes de la población de Sierra Morena como en la totalidad del territorio/s que pretendan recuperarse.

Reintroducción.

Este último apartado pretende ser un salto cualitativo en la conservación del lince ibérico. Como ya se ha comentado antes, el mantenimiento de los núcleos poblacionales actuales no es suficiente para asegurar la conservación de la especie a largo plazo.

En la situación actual y una vez iniciado el programa de cría en cautividad, el siguiente paso lógico y fundamental es el inicio de un programa de reintroducción coordinado entre la conservación in situ y ex situ.

En Andalucía ya se ha hecho una selección previa de áreas potenciales de reintroducción de lince ibérico, Acción A5 del LIFE actual, mediante Análisis Multicriterio y Biomapper y asesoramiento de la E.B.D. y del UICN/SSC Cat Specialist Group (Urs Breitenmoser).

En las acciones previas del nuevo LIFE (Acciones preparatorias, elaboración de planes de gestión y/o de planes de acción) se incluirán dos acciones específicas relacionadas con la reintroducción que serán el análisis y selección de áreas de reintroducción y análisis socioeconómico del impacto y de aceptación e interés en la reintroducción en las áreas preseleccionadas.

Todos los trabajos sobre el reforzamiento y la reintroducción se llevarán siguiendo las recomendaciones de la U.I.C.N. en la materia. De manera que se cuente con el apoyo de expertos nacionales e internacionales, para lo cual se constituirá una Comisión de Asesoramiento específica para este aspecto del Proyecto.

De otro lado, la Consejería de Medio Ambiente ya disponen de datos de conejo de 2004 y 2005 sobre algunas de estas zonas potenciales de distribución. Estos trabajos se

iniciaron como muestreos previos de apoyo a los trabajos que se contemplarán en el nuevo proyecto y servirán para la selección definitiva del área de reintroducción.

Cronológicamente, el proyecto se basaría en la siguiente estructura:

1º año: Evaluación de las 4 áreas preseleccionadas (Guadalmellato, Arenoso, Guarrizas y Villanueva del Río). Se evaluarán en detalle las poblaciones de conejo, posibles amenazas actuales, situación socioeconómica, etc. Además, también se realizará una evaluación de las medidas de mejora del hábitat específicas necesarias en cada zona.

2º año: De las cuatro áreas preseleccionas se escogerán la mejor, iniciándose las actuaciones de mejora de hábitat y comenzando la construcción de infraestructuras en el área seleccionada. De las otras 3 áreas, se seleccionara la mejor y en ella se establecerán convenios y un nivel de actuación moderado para mantenerla como reserva en caso de necesidad (desestimación del área seleccionada por causas de fuerza mayor).

3º año: Se continuara con los trabajos de mejora en el área de reintroducción.

4º año: Se llevara a cabo la reintroducción y se continuará con el programa de mejora en el área de reintroducción.

5º año: Seguimiento de la reintroducción y continuar con el programa de reintroducción.

Otras acciones.

De manera paralela y como actividades transversales para todo el proyecto se incluirán tanto el seguimiento de las poblaciones actuales (incluido el radiotraking), como las actividades de concienciación y divulgación dirigidas a los distintos sectores de la sociedad y de manera específica en las áreas de reintroducción.

3.- ESQUEMA DEL NUEVO PROYECTO LIFE

A continuación se muestra de manera resumida un esquema del nuevo Proyecto LIFE.

Acciones A.- Acciones preparatorias y elaboración de planes de gestión y/o acción (primer año).

Estas acciones van encaminadas a cumplir adecuadamente con las recomendaciones

de la UICN en materia de reintroducción. Se centraran en la evaluación y selección del lugar más adecuado para la reintroducción, esta selección se realizará siguiendo criterios de dos tipos (primer año de proyecto): requerimientos biológicos y criterios socioeconómicos del lugar de reintroducción.

Además, también se incluye una acción de formación para el personal destinado a la reintroducción que permitirá establecer los contactos oportunos con expertos a nivel mundial en estos trabajos.

Acciones B.- Compra arrendamiento de tierras y/o derechos.

Estas acciones están destinadas al arrendamiento de los derechos de caza (conejo principalmente) en las áreas de distribución actual de la especie (mantenimiento de arrendamientos del proyecto LIFE previo) como método para la reducción de la presión cinegética. Igualmente, también se harán arrendamientos en el lugar de reintroducción.

Como criterio irrenunciable, de aplicación a la totalidad de la acción, es no abonar arrendamiento de derechos de caza menor si no se demuestra que existe un lucro cesante, siendo prioritarios los convenios de colaboración en los que se reduzca la actividad cinegética del conejo mediante la ejecución de mejoras de hábitat. Esta medida solo será de aplicación en áreas prioritarias de la especie (con presencia comprobada o en fincas colindantes de alto interés) y cuando la mejor alternativa de conservación sea la eliminación de la caza menor.

Se incluye también el arrendamiento de pastos como medida para reducir la carga de ungulados domésticos en dehesas. Esta acción persigue la expansión de las poblaciones actuales de lince en áreas de dehesa (Córdoba). Dentro de estas acciones también se contempla el pago de posibles daños asociados al lince.

Acciones C.-Tareas únicas de gestión del biotopo.

Se trata de acciones que solamente se van a realizar en una ocasión a lo largo del proyecto y están encaminadas principalmente a la construcción infraestructuras y adecuación tanto de los lugares de reforzamiento como de los lugares de reintroducción.

Así pues, se contempla la construcción de cercados de amplias dimensiones tanto para el reforzamiento poblacional de Doñana como para el lugar de reintroducción seleccionado en las acciones A, dichos cercados contarán con pequeños instalaciones

de alimentación suplementaria que garanticen la disponibilidad de presas para los ejemplares manejados. Igualmente, también se instalaran parideras o cubiles en dichas instalaciones.

Con el objeto de reducir la mortalidad potencial de la especie, tanto en las áreas de distribución actual como en las de reintroducción se actuará intensamente en la adecuación de caminos forestales, agrícolas y carreteras de la comarca de Doñana.

Acciones D.- Gestión periódica del biotopo.

Se trata de acciones de restauración y mejora de la calidad del medio natural. Debido a la estrecha dependencia trófica que el lince tiene con el conejo, la mayor parte de estas acciones están encaminadas a la recuperación y mejora de las poblaciones de conejo silvestre. Entre estas medidas se encuentran las siguientes: construcción de cercados de cría de conejo, desbroces de matorral, siembras de pastizales, fertilización de pastos, instalación de bebederos y recuperación de puntos de agua, construcción de vivares para conejo, repoblaciones de conejo, aclareos de pinares, control de depredadores, repoblaciones de perdiz, cercados de exclusión de ungulados,

Algunas de estas actuaciones están encaminadas específicamente al lince ibérico, como son la construcción de cercados de alimentación suplementaria y la instalación de cubiles. Además, también se incluye un programa de vigilancia encaminado a erradicar el uso de artes ilegales de caza que puedan afectar al lince ibérico (lazos y cepos principalmente).

Mediante la ejecución de estas actuaciones se pretende continuar con los excelentes resultados obtenidos en el LIFE previo en las áreas de distribución actual de la especie. En el caso de las áreas de reintroducción, estas medidas pretenden garantizar una óptima calidad del hábitat para los ejemplares reintroducidos.

Se dispone de una estimación de todas las actuaciones que han de desarrollarse en todas y cada de las fincas que han de ser objeto de convenio de colaboración para este nuevo proyecto.

Acciones E.- Sensibilización del público y divulgación de resultados.

Se trata de acciones destinadas a la sensibilización del público a distintos niveles. Se incluirán acciones específicas de diseño de diferentes campañas de divulgación enfocadas directamente a los distintos sectores implicados en la conservación de la

especie.

Los diferentes sectores implicados serán los siguientes:

Sector Educativo: colegios, institutos, concursos de dibujo, juegos, etc.

Sector Administración: jueces, guardia civil, guardas de caza, agentes M.A., ayuntamientos, administraciones, etc.

Sector Medio Rural: Cazadores, ganaderos, agricultores, gestores, propietaros de fincas, etc.

Otros Sectores: empresarios, asociaciones, medios de comunicación, rocieros, etc..

Para la ejecución de estas acciones se contara con la colaboración de Ecologista en Acción –Andalucía, SECEM, APROCA, ATECA, FAC, etc. Dentro de estas acciones se contempla la edición de: camisetas, gorras, trípticos, pegatinas, pins, poster, etc. Además de la elaboración de cortometrajes de divulgación para cada uno de los sectores específicos de divulgación. Estas acciones se llevaran a cabo tanto en las áreas de distribución actual de la especie como en las áreas de reintroducción con programas específicos en cada caso.

Acciones F.- Funcionamiento del proyecto.

Estas acciones están orientadas principalmente a garantizar una adecuada coordinación entre los distintos socios, colaboradores y entidades (Ministerio de Medio Ambiente, Comisión Europea, ONG, Administraciones, etc.). También se incluye un seguimiento científico de la calidad de las actuaciones ejecutadas que garantice la optimización de los recursos invertidos en el proyecto, especialmente en cuanto a la población de linces, conejo y efectos de los manejos de la vegetación sobre este lagomorfo, que desarrollará la EBD. Finalmente, según las normas administrativas LIFE, se realizará una Auditoría Financiera de los gastos del proyecto.

En las acciones de gestión única, arrendamiento de tierras y periódicas participan los socios WWF-Adena, Fundación CBD-Hábitat, Consejería de Obras Públicas y Transportes, Consejería de Agricultura y Pesca y Consejería de Agricultura y Medio Ambiente de Extremadura.

Sevilla, a 17 de noviembre de 2006.

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