Ryser-Degiorgis, M.-P., Ryser, A., Obexer-Ruf, G., Breitenmoser-Wuersten, Ch., Breitenmoser, U., and Lang, J. Emergence of congenital malformations in free-ranging Lynx from Switzerland: first evidence of inbreeding depression? European Association of Zoo- and Wildlife Veterinarians (EAZWY). 307-311. 2004.

Keywords: 8CH/Alps/disease/genetic diversity/genetics/inbreeding/Jura/lynx/Lynx lynx/malformation/Malme/observation/veterinary

Abstract: The Eurasian lynx vanished from Switzerland during the 19th century, and free-ranging lynx from the Carpathian mountains were reintroduced in the 1970s. All together, 14-16 animals were released in the Alps and 8-10 in the Jura Mountains, and two distinct populations arose from these releases. Nowadays, the lynx populations are estimated to 50-70 resident animals in the Jura and 70-80 in the northwestern Alps. As they are still isolated and have developed out of a few individuals, small genetic diversity can be expected in both populations. In recent years, several congenital malformations were observed in free-ranging lynx from Switzerland. We report here these observations and discuss the possibility of a correlation between the emergence of congenital defects and loss of genetic diversity as a consequence of inbreeding.

EMERGENCE OF CONGENITAL MALFORMATIONS IN FREE-RANGING LYNX (*Lynx lynx*) FROM SWITZERLAND: FIRST EVIDENCE OF INBREEDING DEPRESSION?

M.-P. RYSER-DEGIORGIS[1], A. RYSER[2], G. OBEXER-RUF[3], U. BREITENMOSER[4], J. LANG[5] and CH. BREITENMOSER–WÜRSTEN[2]

Affiliation:
1. Centre for Fish and Wildlife Health, Institute of Animal Pathology, University of Berne, Postfach, CH-3001 Berne, Switzerland
2. KORA, Berne, Switzerland
3. Institute of Animal Genetics, Nutrition and Housing, University of Berne, Switzerland
4. Institute of Veterinary Virology, University of Berne, Switzerland
5. Small Animal Clinic, University of Berne, Switzerland

Abstract
The Eurasian lynx (*Lynx lynx*) vanished from Switzerland during the 19th century and was reintroduced in the 1970ies. Re-introductions are known to create artificially a bottleneck situation, which can lead to a reduction in genetic variability of the newly developing population. In recent years, several congenital malformations were observed in free-ranging lynx from Switzerland. Furthermore, genetic analyses revealed a remarkably reduced genetic variability. In this paper we report these observations and discuss the possibility of a correlation between the emergence of inborn defects and loss of genetic diversity. On the basis of the actual knowledge, the link between congenital defects and inbreeding can neither be confirmed nor refuted, and our goal is not to be alarming but vigilant.

Key words: congenital, Eurasian lynx, genetics, inbreeding, *Lynx lynx*, malformation, Switzerland

Introduction
One of the goals of conservation is to predict and try to advert the genetic deterioration of species, to preserve species' potential for adaptation to both short- and long-term environmental variation, and thereby reduce their chances of extinction (4). Heterozygosity is important for the conservation of species because all populations carry recessive alleles and a proportion of these alleles are lethal when homozygous and a larger proportion are deleterious. The increasing homozygosity from breeding between relatives usually results in reduced fertility, survivorship, disease resistance and growth rates, and is termed inbreeding depression. For example, in the endangered Florida panther (*Felis concolor coryi*), a remarkable reduction in genetic diversity was reported in correlation with skeletal, reproductive and congenital abnormalities (17).
The Eurasian lynx (*Lynx lynx*) vanished from Switzerland during the 19th century and was reintroduced in the 1970ies. The animals used for reintroduction were free-ranging lynx from the Carpathian mountains. The number of animals released was very low: in the Swiss Alps and Jura Mountains, only eight lynx were officially released. Further reintroductions raised the number to 14-16 in the Alps and 8-10 in the Jura Mountains, and two distinct populations arose from these releases (2). Nowadays, the lynx populations are estimated to 50-70 resident animals in the Jura and 70-80 in the northwestern Alps (23). As they are still isolated and have developed out of a few individuals, small genetic diversity can be expected in both populations. Re-introductions are indeed known to create artificially a bottleneck situation. This can lead to a reduction in genetic variability of the newly developing population. In this

context, a genetic study on the re-introduced lynx populations in Switzerland was designed (3).
In recent years, several congenital malformations were observed in free-ranging lynx from Switzerland. We report here these observations and discuss the possibility of a correlation between the emergence of congenital defects and loss of genetic diversity as a consequence of inbreeding.

Material and Methods

Animals

Both Swiss lynx populations have been studied in the frame of several research projects: 1983-1985 in the northwestern Alps, 1988-1997 in the Jura Mountains, and again 1997-2000 in the northwestern Alps. In addition, a translocation project was started in 2001 and since then nine lynx were moved from the northwestern Alps and the Jura Mountains to eastern Switzerland (18, 20). All together, about 100 lynx were captured and radio-collared, and 11 of them were submitted to clinical examination by a veterinarian at time of capture.
Furthermore, each autumn 1-2 orphans were caught in villages while looking for easy prey like chickens. Most of them were brought to the wildlife rescue centre of Landshut, close to the city of Berne, where they were usually submitted to a veterinary checkup. If the animals were healthy, they were forwarded to interested zoological gardens.
Lynx is a protected species in Switzerland and it is compulsory to report any lynx mortality. Since 1987, lynx carcasses found by means of telemetry or reported to the state game wardens have been sent to the Centre for Fish and Wildlife Health (FIWI), University of Berne, for post-mortem examination. Complete necropsies were performed on 98 lynx that died from 1987 to 2004.

Genetic analysis

We used microsatellite markers isolated from domestic cats (14, 15, 16) and Canada lynx (6) to measure genetic variability. To assess the impact of a bottleneck we compared the results of the re-introduced populations with the autochthonous source population from the Carpathian Mountains of Slovakia. Currently, results of 17 markers are available to present preliminary conclusions.

Results

Case reports

1) In November 1992, a juvenile male was killed by a hunting dog, which was very unusual as lynx are normally able to escape such attacks. Besides the fatal injuries, necropsy revealed multiple skeletal deformities: scoliosis, kyphosis and fusion of two vertebrae in the thoracal area; deformity of the ribs (with indications of old factures); and pronounced symmetrical deformity of the anterior extremities at the level of the elbow that prevented a normal extension of the limbs.
2) In 1995, a juvenile male was found alive in a private garden in the village of Gstaad in the canton of Berne. It was brought to the wildlife rescue centre of Landshut. According to the animal keepers, the lynx ate and behaved normally for five days. He then suddenly stopped eating and was found dead one day later. At necropsy, the animal appeared very emaciated and dehydrated, and a congenital diaphragmatic hernia with following displacement of a liver lobe into the pericardial sac was diagnosed (peritoneo-pericardial hernia).
3) In 1998, an adult male was caught in the canton of Berne and equipped with a radio-collar. At capture, the biologists noticed a prognathism of the lower jaw. The animal died two years later for reasons independent of this malformation.
4) In the frame of the translocation project, an adult male from the canton of Vaud was caught in a box trap in early spring 2001 but not translocated. At clinical examination, it appeared that this animal was monorchid.
5) Another adult male (“Vino”) from the canton of Fribourg was caught the same year and translocated. At clinical examination, this animal presented a discrete heart murmur. Since this lynx seemed rather old but was in a good general condition, and no congenital abnormalities had ever been reported, neither from lynx from Switzerland nor from any other

Eurasian lynx population, he was considered as healthy and was translocated. During the two following years, intensive radio-tracking revealed no abnormality, neither of feeding nor of social behaviour. However, at re-capture in April 2003, the biologists noticed a very enlarged abdomen and an abnormally slow response to anaesthetics. About one month later, the lynx almost stopped to move and caught small prey only; he was found dead two weeks later. At necropsy, the main findings were an accumulation of fluid in all body cavities as well as in the subcutaneous tissues, and a highly fibrotic liver consequently to a circulatory failure. A subaortic stenosis, arteriosclerosis and severe fibrosis were found in the heart.

6) During the fall 2002, a juvenile female was caught on a farm close to the village of Spiez in the canton of Berne and was brought to the wildlife rescue centre of Landshut. At clinical examination, the animal was emaciated but otherwise healthy. During the following days, it ate and behaved normally. Five days after its arrival to the station, the animal keeper found it laying on the ground in agony. Vomit was found everywhere in the enclosure. The animal was hypothermic and dehydrated, and showed an elevated respiratory rate. Attempts to treat it failed. Necropsy revealed a very large esophageal hiatus with no sign of any old or recent trauma, thus indicating a congenital malformation. According to the clinical symptoms, it was assumed that a part of the stomach had been temporarily displaced into the mediastinum (hiatal hernia).

7) Furthermore, a young adult female from the Bernese Alps translocated in 2001 has not given birth to any kitten so far, although intensive radio-tracking showed a normal behaviour and evidence of meetings with the adult male "Vino" during the breeding season for the two past years. According to the knowledge gathered up to now, this lack of reproduction appears as very unusual and we suspect either this female or the male to be infertile.

Genetic analysis

The heterozygosity value was higher in the autochthonous source population than in the re-introduced populations in the Jura Mountains and in the Alps (0.63 vs 0.54 and 0.42). The same was true for the mean number of alleles per locus (4.65 vs 3.47 and 2.87). In the Jura Mountains, 27% and in the Alps 39% of the alleles are missing today compared to the source population. The proportion of rare alleles (having a share of less than 20%) dropped from 56% to 38%.

Discussion

Intensive monitoring of the Swiss lynx populations during the past 20 years by means of captures, radio-tracking and post-mortem examinations hadn't revealed any congenital abnormalities until 1992. Since then, one lynx from the Jura mountains and five from the northwestern Swiss Alps were found to present inborn defects: one juvenile suffered of skeletal deformities, two died of the consequences of a diaphragmatic hernia, one adult male is cryptorchid, one presented a deformity of the lower jaw, and another one a heart defect. Furthermore, there are indications that an adult female (or a male) from the Alps might be infertile.

Cryptorchidism can have different etiologies, including genetic factors. Low genetic diversity has indeed been shown to be responsible for a high prevalence of cryptorchidism within the Florida panther population (13, 17). The incidence of cryptochidism in this puma subspecies has increased dramatically from 0% of pre-1975 birth to 80% of the males born after 1989 and is associated with documented consanguineous matings. However, other factors can result in cryptorchidism, including prolonged breech labor, navel infections during testicular descent, exposure to the fetus to an increased maternal estrogen concentration, or antiandrogenic chemicals, or maternal vitamin A deficiency during fetal development (review in 7). The prevalence of cryptorchidism in non-inbred domestic animals varies by species and breed from 1-2% (review in 13). In apparently non-inbred mountain lions (*F. concolor*), Barone et al. (1) found a prevalence of cryptorchidism of 3.7%.

Subaortic stenosis is one of the most common congenital heart disease in dogs but the condition is uncommon in the cat. Subaortic stenosis was shown to be hereditary in Newfoundland dogs and is likely to be hereditary in other breeds (10). However, the etiology of this condition is not fully understood.

Diaphragmatic hernias in veterinary medicine are mostly traumatic in origin, but developmental and heredity etiologies have been identified in several species (11, 22, 24). Hiatal hernias occur in a variety of dog and cat breeds; however, males and Chinese shar-pei dogs appear to be predisposed to this condition. These congenital hernias are usually the consequence of a lax or stretched phrenicoesophagal ligament (8). Congenital pericardioperitoneal diaphragmatic hernias are not uncommon in domestic cats, and long-haired breeds seemed to be predisposed to this condition (25). This malformation is believed to be the result of a teratogen, genetic defect, or prenatal injury (9).
Skeletal abnormalities can have diverse origins, e.g. nutritional imbalance, metabolism or endocrine dysfunction, and genetic defect. In humans many constitutional disorders of the bones occur as sporadic cases and represent new mutations or the expression of rare recessive traits; the same is true in animal populations. Abnormal metaphyseal modelling, scoliosis, fusion of vertebrae and prognathism are all malformations described in cats and/or dogs that might be inherited (5, 12).
Small populations are susceptible to genetic deterioration through genetic drift, which may lead to the loss of genetic variability and the expression of deleterious, recessive alleles. Our genetic analyses revealed a remarkably reduced genetic variability, especially in the Alpine population. Interestingly, except the first case, all animals observed with congenital malformations so far belonged to the Alpine population.
The congenital defects recently observed in lynx from Switzerland were so far all isolated cases and might be just accidental findings without any hereditary origin. The reported prevalence is very low and might be considered as normal in comparison with observation in domestic animal populations. However, the emergence of such defects in the past years is worrying. Indeed, a retrospective study performed in Sweden on more than 500 lynx necropsies revealed only one case of skeletal disorder (a scoliosis); congenital malformations of the diaphragma, heart, genitals or any other organ were not observed (Ryser-Degiorgis et al., in prep.). To our knowledge, data from other Eurasian lynx populations are not available, making further comparisons impossible.
Furthermore, prevalence of congenital malformations in Swiss lynx might indeed be fully underestimated: All three adult lynx were radio-collared, and two of three juveniles were found in villages as orphans looking for food; these five animals might not have been found in other circumstances. In field studies about wildlife diseases, animals that die due to human activities (e.g. traffic accidents, hunting) are more likely to be found than diseased animals that might hide in retired areas of their habitat. In a retrospective study performed on Eurasian lynx from Switzerland by Schmidt-Posthaus et al. (21), it was shown that the distributions of causes of mortality clearly differed between the radio-tagged animals and the animals found by chance and it was assumed that radio-tagged animals represent better the actual situation in the wild because these animals are found independent of the cause of death. Overall, 20% of the lynx died of infectious diseases; however, based upon the data from radio-tagged animals, 40% died due to infections. This figure might be true for congenital malformations as well. Telemetry studies of lynx populations are over in both the Jura Mountains and the northwestern Swiss Alps and detailed data won't be available in the future any more. If the prevalence of congenital malformation increases, it might stay undiscovered.
On the basis of the reported observations and of the actual knowledge, the link between congenital defects and inbreeding can neither be confirmed nor refuted. Our goal is not to be alarming but vigilant. Therefore, we suggest giving particular attention to the genital organs, the heart, the diaphragm and the skeleton during future clinical and post-mortem examinations of Eurasian lynx from Switzerland as well as from other countries. Furthermore, we advise to be cautious regarding genetic diversity in the frame of future reintroduction projects.

Acknowledgements
Many thanks go to all people who helped in any way to collect these data, in particular (in alphabetical order): Philipp Burri, Martin Janovsky, Cristina Köppel, Daniel Mayer, Nadia Robert, Michel Schmidt, Kuno von Wattenwil, Christian Willisch, and Fridolin Zimmermann.

References

1. Barone MA, Roelke ME, Howard JG, Brown JL, Anderson AE and Wildt DE. Reproductive characteristics of male Florida panthers: comparative studies from Florida, Texas, Colorado, Latin America, and North American Zoos. J Mammal 1994; 75(1) 150-162.
2. Breitenmoser U, Breitenmoser-Würsten Ch and Capt S. Re-introduction and present status of the Lynx (*Lynx lynx*) in Switzerland. Hystrix 1998; 10: 15-28.
3. Breitenmoser-Würsten, Ch and G. Obexer-Ruff. Population and conservation genetics of two re-introduced lynx (*Lynx lynx*) populations in Switzerland – a molecular evaluation 30 years after translocation. Proc 2nd conf on the status and conservation of the Alpine lynx population, Amden, Switzerland 2003; 28-31.
4. Burgman MA, Ferson S and Akçakaya HR. Risk assessment in conservation biology. 1st ed London. Chapman & Hall 1993; 217-236.
5. Byrne MJ and Byrne GM. Inheritance of 'overshot' malocclusion in German shorthaired pointers. Vet Rec 1992; 130(17): 375-6.
6. Carmicheal LE., Clark W and Strobeck C. Development and characterization of microsatellite loci from lynx (*Lynx canadiensis*), and their use in other felids. Mol Ecol 2000; 9: 2155-2234.
7. Dunbar MR, Cunningham MW, Wooding JB and Roth RP. Cryptorchidism and delayed testicular descent in Florida black bears. J Wildl Dis 1996; 32(4): 661-664.
8. Fossum TW, Hedlund CS, Hulse DA, Johnson, AL, Seim HB III, Willard MD and Carroll GL. Small animal surgery St. Louis, Missouri. Mosby-Year Book, Inc 1997; 250-254.
9. Fossum TW, Hedlund CS, Hulse DA, Johnson, AL, Seim HB III, Willard MD and Carroll GL. Small animal surgery St. Louis, Missouri. Mosby-Year Book, Inc 1997; 285-287.
10. Fossum TW, Hedlund CS, Hulse DA, Johnson, AL, Seim HB III, Willard MD and Carroll GL. Small animal surgery St. Louis, Missouri. Mosby-Year Book, Inc 1997; 588-591.
11. Fox RR and Crary DD. Hereditary diaphragmatic hernia in the rabbit. Genetics and Pathology. J Hered 1973; 64: 333-336.
12. Jezyk FP. Constitutional disorders of the skeleton in dogs and cats. In: Newton DC and Nunamaker DM. Textbook of small animal orthopedics. 1985, Chapter 57. HYPERLINK “http://cal.nbc.upenn.edu/saortho/chapter_57/57mast.htm”. 30-01-2004
13. Mansfield KG and Land ED. Cryptorchidism in Florida panthers: prevalence, features, and influence of genetic restoration. J Wildl Dis 2002; 38(4): 693-698.
14. Menotti-Raymond M and O'Brien SJ. Evolutionary conservation of ten microsatellite loci in four species of Felidae. J Hered 1995; 86: 319-322.
15. Menotti-Raymond M, David VA, Stephens JC, Lyons LA and O'Brien SJ. Genetic individualisation of domestic cats using feline STR loci for forensic applications. J Forensic Sci 1997; 42: 1039-1051.
16. Menotti-Raymond M, David VA, Lyons LA, Schaffer J., Hutton MK and O'Brien SJ. A genetic linkage map of microsatellites in the domestic cat (*Felis catus*). Genomics 1999; 57: 9-23.
17. Roelke ME, Martenson JS and O’Brien SJ. The consequences of demographic reduction and genetic depletion in the endangered Florida panther. Curr Biol 1993; 3: 340-350.
18. Ryser-Degiorgis M-P, Lutz H, Bauer K, Sage H, Ryser A, Zimmermann F, Breitenmoser-Würsten Ch, and Breitenmoser U. Veterinary supervision of lynx translocation within the Swiss Alps. Proc EAZWV and EWDA scientific meeting, Heidelberg, Germany 2002; 147-153.
19. Ryser-Degiorgis M-P, Bröjer C, Hård af Segerstad C, Bignert A, Bornstein S, Gavier-Widén D, Jansson D and Mörner T. Assessment of the health status of the free-ranging lynx population in Sweden. In prep.
20. Ryser A, von Wattenwyl K., Ryser-Degiorgis M-P, Willisch Ch, Zimmermann F and Breitenmoser U. Luchsumsiedlung Nordostschweiz 2001-2003, Schlussbericht Modul Luchs des Projektes LUNO. KORA-Bericht 2004; 22: in press.
21. Schmidt-Posthaus H, Breitenmoser-Würsten Ch, Posthaus H, Bacciarini L and Breitenmoser U. Causes of mortality in reintroduced Eurasian lynx in Switzerland. J Wildl Dis 2002; 38(1): 84-92.
22. Valentine BA, Cooper BJ, Dietze AE and Noden DM. Canine congenital diaphragmatic hernia. J Vet Intern Med 1988; 2(3): 109-112.
23. von Arx M, Breitenmoser-Würsten Ch and Breitenmoser U. Status and distribution of the Eurasian lynx (*Lynx lynx*) in Europe in 2001. KORA-Bericht 2004; 19: in press.
24. Wilson G and Muir W. Diaphragmatic hernias. In: Bojrab MJ (ed). Current techniques in small animal surgery. 2nd ed Philadelphia, Pennsylvania. Lea and Feiber 1983; 409-413.
25. Wotton PR and Gruffydd-Jones TJ. The cardio-vascular system. In: Chandler EA, Gaskell CJ and Gaskell RM (eds). Feline medicine and therapeutics. 2nd ed London. Blackwell Sciences Ltd 1994; 398.