## Cat Project of the Month - February 2006

The IUCN/SSC Cat Specialist Group's website (www.catsg.org) presents each month a different cat conservation project. Members of the Cat Specialist Group are encouraged to submit a short description of interesting projects For application use this **standardised form** (an editable word document)

# Genetic diversity and relatedness in the lynx population in Białowieża Primeval Forest – environmental and social circumstances



The ecology and spatial organization of Eurasian lynx population has been studied in Białowieża Forest since 1991. This project is to study the effect of forest fragmentation on genetic diversity of lynx population and the relationship between kinship and social behaviour in lynx.

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Photo: H. Yamaguchi

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A lynx kitten taken at kill by a photo-trap (Photo T. Podgórski)

The population of the Eurasian lynx *Lynx lynx* inhabiting the Białowieża Primeval Forest (BPF), Poland, lives in a specific situation. The forest is highly isolated and the lynx population has been estimated at 40-60 individuals only (Jędrzejewski et al. 1996). The isolation of the Białowieża Forest is not complete – some lynx have been proved to travel between neighbouring forests (Schmidt 1998) – however, it is not known if the range and the rate of these movements are sufficient for maintenance of adequate genetic diversity of this population. In fact, it was recently found that the geographic range of lynx in North-East Poland got shrank significantly during the last decade (Jędrzejewski & Schmidt 2001) and the reasons for that are not clear. An indirect indication of the effect of isolation may be a fact that the lynx inhabiting NE Poland (including BPF) are morphologically very uniform in terms of the fur and spot pattern (Schmidt and Kowalczyk unpublished data). This is especially striking when compared to other populations living in more continuous habitat, where a great variability of pelage forms is found (Bagrade 1999).

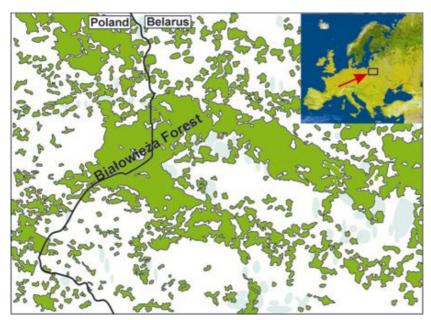
Genetic structure of the population is also known to be related with its social organization (Storz 1999). However, the relationship between genetic structure of the population and its social organization has not been studied in solitary cats, so far. We predict that in case of partial isolation of the lynx population their social interactions may play an important role in shaping its genetic structure by either moderating or deepening the inbreeding. We are going to test it by establishing the kinship relations between the radio-tracked individuals and following their fate in the population.

## Objectives:

- (1) to determine the degree of genetic diversity of the Białowieża and other fragmented lynx populations in north-east Poland in comparison with lynx from continuous range of the species (e.g. Baltic countries),
- (2) to learn if the isolation of the Białowieża Forest is advanced enough to decrease the genetic variability of the lynx population and affect its survival,
- (3) to test if the social organization of the population (dispersal, home range overlapping) may constitute a mechanism of inbreeding avoidance in the lynx population.



The Białowieża Primeval Forest is a lowland mixed forest characterised by a complex age and species structure (Photo K. Schmidt).



A map showing the localisation of the Białowieża Forest on the background of distribution of other forests on a borderland between Poland and Belarus and its placement in Europe (inset).

## Study area

The Białowieża Forest is on the westernmost limit of the natural range of the Eurasian lynx. Earlier research showed that during last century the lynx population was twice nearly extirpated by hunting, which demonstrates its great vulnerability to exploitation by humans (Jędrzejewski et al. 1996). It is probably enhanced as a result of a fragmented character of lynx habitat, which occurs over large areas of Poland, Belarus and Lithuania. Their numbers in BPF are presently limited by poaching (Jedrzejewski et al. 1996) and availability of roe deer, which is the main prey of lynx (Okarma et al. 1997). The mean home ranges of resident adults (240 km2 in males and 130 km2 in females) cover nearly 1/3 of Polish side of the BPF area (Schmidt et al. 1997).

#### Methods

There are two methods used in this study – radio-telemetry and DNA analysis. The lynx are caught with snare traps (Breitenmoser 1989) equipped with a radio-alarm system set at fresh kills made by them. After sedation with xylazine and ketamine mixture (Seal & Kreeger 1987) the VHF radio-collars (Wagener, Germany) are placed on the neck of the cat and samples of blood and hair for genetic analyses are collected. The results of radio-tracking combined with analysis of DNA should allow us to determine the influence of genetic relatedness on social interactions among individuals.

A sedated female lynx kitten. Please note a drop-off device mounted into the collar We use the survival blankets for cold prevention. We also recommend using them once for each animal as a prevention of transmitting pathogenes and parasites (Photo K. Schmidt).





Additional data on distribution of unmarked lynx individuals and their genetic identity is collected with a hair- trapping procedure (McDaniel et al. 2000, Mowat et al. 2001). The hair-trap is a piece of carpet with roofing nails sticking through it around the edge and nailed to a tree or other objects (McDaniel et al. 2000). The lynx are attracted with a scent lure to rub against the trap and leave their hair on the nails. Comparative material to test for general genetic diversity of the lynx population has been obtained from the harvested lynx in the Baltic countries (Latvia and Estonia). The genetic analyses based on microsatellites developed for domestic cats (Menotti-Raymond et al. 1999) and Canada lynx (Carmichael et al. 2000), are done in cooperation with the Institute for Zoo and Wildlife Research (IZW) in Berlin by Joerns Fickel.

A hair trap attached to a root scent-marked by lynx (Photo K. Schmidt).

In this project we focused on getting data on the question: how the population is limited from the genetic point of view. Therefore, we put a big effort to collect DNA samples from lynx in BPF and other populations. As the lynx in Poland is protected, the only way to obtain sufficient DNA samples was by means of non-invasive methods. However, the procedure described for Canada lynx (McDaniel 2000) didn't work in Białowieża, apparently as a result of lower densities in Eurasian lynx. To cope with this problem we developed a modification of collecting hairs from free-ranging lynx by setting hair-traps on the objects previously scent-marked by lynx (Schmidt and Kowalczyk 2006). The method proved to be successful and we collected over 260 hair samples during 2 years of the project in the BPF. Important sources of DNA were also dead animals acquired occasionally and individuals live-trapped for radio-tracking. This way we obtained 30 samples from NE Poland. For estimation of genetic variability of lynx population we received 46 samples from lynx harvested in Latvia (in cooperation with Janis Ozolins) and Estonia (in cooperation with Peep Mannil).



The preliminary genetic analyses conducted by Joerns Fickel were performed on 7 microsatellite loci developed for domestic cats and 3 loci for Canada lynx. The analysis of hairs retrieved from hair-traps resulted in 60% of samples giving a PCR product. This result is satisfactory and can be still improved during further work. We will use this material for identification of individuals and paternity assessment. The lynx identified from hairs will also enrich a sample for determination of genetic diversity of Białowieżan population. So far, only 10 individuals originating from the Białowieża forest were scored for 6 microsatellite loci and compared to the sample of 20 lynx from Latvia. Four of those loci were less polymorphic in lynx from the BPF than those from Latvia and two other were equal in both populations. Although this comparison should not be treated as binding, because it was based on a few animals and few loci, it seems to confirm our hypothesis and encourages for further study.

Female lynx rubbing its cheek against a hair-trap (Photo K. Schmidt).

Four lynx were equipped with radio-collars in Białowieża Forest at the beginning of the study. We attempted to search for them 5-6 days a week, which resulted in a total of approximately 1000 localisations. The collars were equipped with a drop-off device (a piece of leather strap mounted into the collar) that allowed lynx to detach it after certain time. An adult female was radio-tracked for 1.5 year, during which she gave birth to two litters with 3 and 2 kittens. We followed an adult male, who probably fathered (not yet confirmed genetically) at least one of her litters, for nearly 1 year. Their home ranges (196 and 236 km2, respectively) overlapped almost completely. These two individuals lost their collars owing to drop-off device. We also captured and radio-collared a 9-months old female kitten, a daughter of the radio-tracked female. After separation from the mother she established a home range partly overlapping with her mother's. Another female (<2 years old) was radio-tracked for only 5 months and was found dead for unknown reasons. We suspect she could have been illegally shot, as she was very efficiently hunting roe deer just before her death.

There are two main steps, we want to continue next. First is to intensify the analyses of genetic variation to confirm (or deny) the preliminary inference, by studying more microsatellite loci on more individuals (both by including a sample from Estonia and searching more lynx material in NE Poland – from hunters' trophies and hair-traps). Second is to continue live-trapping and radio-collaring lynx to obtain more data on relationship between social interactions and kinship in these cats. In case we find an indication that the Białowieżan lynx show relatively low genetic variability a further step will be an estimation of the males' fertility by analysing the sperm quality. A reference material is being already collected from other populations (Latvia: Janis Ozolins, Sweden: Henrik Andren) and a material from the Białowieża population will be obtained either from live-trapped lynx or dead individuals. The analysis will be done in collaboration with the IZW (Katarina Jewgenow).

Before all analyses are finished it is premature to predict what kind of conservation measures should be undertaken as a result of this research. However, we believe that the study will constitute a scientific basis for any conservation decisions concerning lynx population in Poland in the future. We predict that the project should provide a strong support for a need to restore and maintain their population in all suitable habitats of the previously occupied range by connecting the remaining forest patches through the net of ecological corridors. Conservation and strengthening of the lynx population at the edge of its main range is important for the species as a whole, as it may hinder a possible process of gradual shrinkage of the species range. The more so, the study will contribute to understanding the social mechanisms underlying the genetic structure of the population in solitary cats.



A radio-collared adult female lynx watching a radio-tracking person. Lynx do not appear very shy at accidental encounters, though they are rarely observed by people (T. Podgórski).

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