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Original Investigation

Hair snaring and molecular genetic identification for reconstructing the spatial structure of Eurasian lynx populations

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ABSTRACT

Non-invasive genetic sampling (NGS) is being increasingly applied in wildlife monitoring and population genetic research. This study was designed to evaluate the use of NGS for reconstructing the spatial structure of populations of large felids. We developed a procedure for reliably genotyping individuals of Eurasian lynx (*Lynx lynx*) from samples obtained through a hair-trapping scheme based on a network of lynx scent-marking sites. The spatial locations of the identified genotypes were matched with the home ranges distribution of radio-tracked individuals, thus cross-checking the accuracy of the two methods. We analyzed DNA extracted from 170 hair samples and 11 blood samples from live-trapped lynx collected in 2004–2009 in the Białowieża Primeval Forest, Poland. We obtained PCR products in 96 (67%) hair samples; 82 (85%) of them were reliably genotyped at 12 autosomal microsatellite loci following a multiple-tubes protocol and stringent quality-controls of the data set. The sample included 29 distinct genotypes; 18 were found only in hair samples, five were determined only in live-trapped animals, and six in both hair and blood samples. Based on linkage disequilibrium we estimated an effective population size $N_e = 20.3$ (90% CI = 15–28). The total population size estimated with CAPWIRE was $N_c = 32$ (95% CI = 25–37) in close agreement with the observed number of genotypes. The genotypes obtained from hair samples were re-sampled on average 3.9 times and 50% of them were recorded for more than one year. The spatial distribution of six hair-genotypes was consistent with their home ranges obtained by radio-tracking in the same period. The distribution ranges of hair-trapped genotypes overlapped on average in 86.4% (mode 100%) with home ranges of the corresponding individuals. Hair-trapping and molecular identification is a reliable method for reconstructing the spatial organization of lynx population. It is likely to be also efficiently used in other rare and endangered species of felids in combination with data from other monitoring techniques, such as radio- and snow-tracking and photo-trapping.

Introduction

Although non-invasive samples, such as scats or hair, are considered valuable sources of DNA (Waits and Paetkau 2005), most of research concerning wildlife population genetics is still based on higher quality tissues or blood samples (e.g. Eizirik et al. 2001; Pilot et al. 2006; Nikolov et al. 2009). As long as tissues can be collected without threatening the conservation status of the species and without interfering with the dynamics of the studied populations, bioptic samples warrant providing sufficient material, and are used to generate genotypes that are mostly error-free (Paetkau 2003). On the other hand, elusive, rare and endangered species are often protected by strict conservation rules that preclude obtaining good quality samples, while demographic and genetic monitoring of their populations is recommended to improve the design and efficiency of conservation measures (Breitenmoser et al. 2000; Delibes et al. 2000; Nichols and Williams 2006). In these cases, non-invasive genetic sampling (NGS) might be the only practical way to generate the needed information (Schwartz et al. 2007).

NGS, eventually integrated with other monitoring tools like camera-trapping (Woods et al. 1999; Davison et al. 2002; Rowcliffe and Carbone 2008), is particularly useful in long-term monitoring projects, because demographic and genetic information can be obtained through sampling schemes replicated in time, without the need to capture or even to observe the animals (Taberlet et al. 1999). Hair can be trapped and scat can be collected within well planned randomized sampling schemes (Boulanger et al. 2006), individual capture-recapture histories can be reconstructed and analyzed using a variety of population genetic and demographic approaches (De Barba et al. 2010; Gervasi et al. 2010). Sampling schemes should

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be integrated with reliable laboratory protocols, which guarantee correct individual genetic identifications, avoiding the risks of generating false genotypes in consequence of human errors, false alleles (FA) and allelic drop-out (ADO, Pompanon et al. 2005). Errors in NGS can be identified through complex multiple-tubes and quality-control procedures. For these reasons, NGS projects should be carefully planned (Taberlet et al. 1999), and the results of only a few long-term NGS monitoring projects have been reported, so far (Boulanger et al. 2004; Fabbri et al. 2007; De Barba et al. 2010).

NGS has been successfully used in various carnivore species, including felids, with an array of hair-trapping methods (Woods et al. 1999; Mowat and Paetkau 2002; Palomares et al. 2002; Weaver et al. 2005; McKelvey et al. 2006; Schmidt and Kowalczyk 2006; Sawaya et al. 2011). Those sampling protocols were aimed at detecting presence of the species, estimating the population size and analyzing the population genetic diversity. However, no study has been designed for more detailed research on the spatio-temporal distribution of individuals. The hair-snaring methods proved to work well in a number of felids, including the North American (*Lynx canadensis*: McDaniel et al., 2000) and Eurasian (*Lynx lynx*: Schmidt and Kowalczyk, 2006) lynx species, but the potential of this method for solving ecological questions has not yet been addressed. Although some interesting insights into the lynx's population genetic structure and ecological processes were recently achieved using invasively (but opportunistically) obtained samples (e.g. Rueness et al. 2003; Schwartz et al. 2003; Janečka et al. 2006; Schmidt et al. 2009), hair-sampling procedures may open up new research possibilities, especially in studies of protected populations.

In this paper we are going a step farther compared to previous research as we aimed at providing a detailed field and laboratory protocol for studying population spatial structure with NGS data in felids. In particular we aimed at testing: (1) the reliability of the hair-sampling protocol developed by Schmidt and Kowalczyk (2006) for the Eurasian lynx for obtaining good quality DNA; (2) the feasibility of individual lynx identification by microsatellite genotyping of hair samples and following their relocations over time and space; and (3) the consistency of spatial distribution of individuals genotyped from hairs with those monitored via radio-tracking.

Material and methods

Study area

The study was conducted in 2004–2009 in the Białowieża Primeval Forest (BPF, 52°30′–53°00′N, 23°30′–24°15′E), Poland, located on the Polish-Belarussian border. The BPF is a temperate mixed lowland forest, characterized by a high percentage of natural stands (Faliński 1986). The entire forest area is 1500 km^2 and its Polish side is 600 km^2. Most of the Polish side of the BPF (500 km^2) is managed by state forestry, while the rest is protected as the Białowieża National Park (BNP, 100 km^2) with a 50 km^2 zone under strict protection. The area is flat and easily accessible for vehicles by a dense network of dirt roads that usually follow a regular grid of square forest compartments (1 km × 1 km) (Fig. 1). The average temperature during the study was −3.9 °C in the winter (December–March) and 19.1 °C in the summer (June–September). Snow cover persisted for an average of 96 days per year from November to March. The lynx population in the Polish part of BPF has been estimated by snow- and radio-tracking at 29 individuals in 1994 (Jędrzejewski et al. 1996). However, the population apparently decreased by about 30–35% in 2003–2006 (Schmidt 2008).

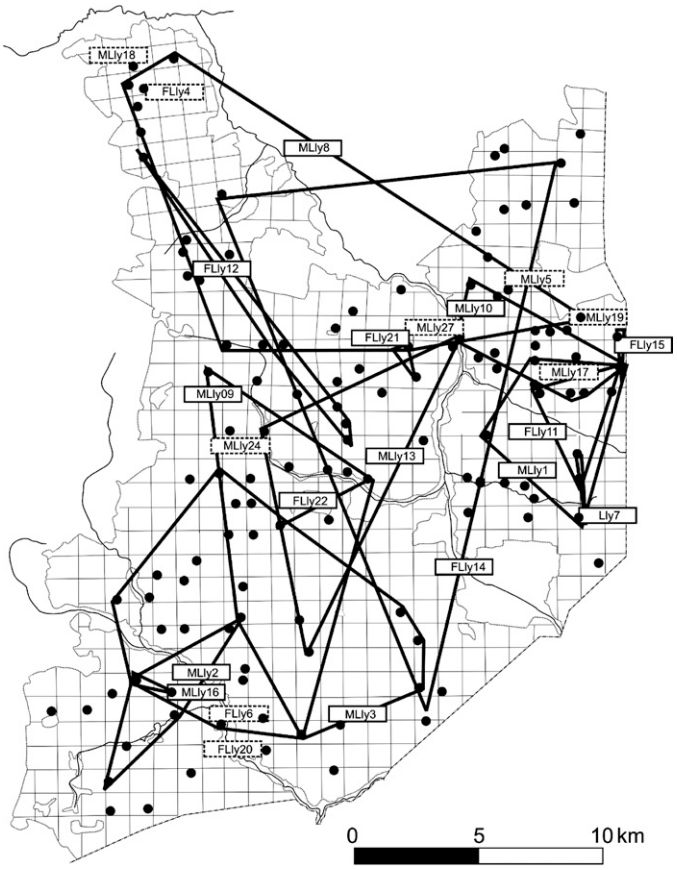

Fig. 1. Distribution of lynx individuals genotyped based on non-invasive hair sampling in the Białowieża Primeval Forest, Poland. Polygons denote ranges of particular individuals (M, males; F, females), solid rectangles refer to genotypes recorded more than two times and dashed rectangles are those recorded only once; points denote the hair-trapping sites. The square grid represents the forest area with a network of compartments (partly corresponding with road system) used to design the hair-trapping composition.

Sample collection

The hair-trapping system was based on a network of 153 lynx scent-marking sites identified by snow-tracking (Schmidt and Kowalczyk 2006). We placed hair-traps (8 cm × 8 cm pieces of carpet supplied with 10 nails) directly on the scent-marked objects spaced at 1–2 km intervals and distributed along forest roads in the entire Polish part of BPF (Fig. 1). To evoke cheek-rubbing in lynx we applied a mixture of beaver castoreum and catnip oil to each trap (see Schmidt and Kowalczyk 2006 for details on the sampling and baiting protocols applied for the Eurasian lynx in BPF and efficiency of hair collection). We conducted the surveys in 2004–2006, in different periods of the year in sessions lasting for 7–13 weeks with 36–113 (mean = 85.2 ± 29.0) scent-stations per session. Majority of samples were collected in winter months (December–March). As the lynx home ranges in BPF were 133 and 250 km^2 for females and males, respectively (Schmidt et al. 1997), there were approximately 30 traps within an average lynx home range. We attempted to check and re-bait hair-traps every 10–14 days though the actual range of intervals was 1–23 days. Hairs were dried at temperature 30–40 °C and stored in paper envelopes at room temperature in plastic boxes with silica gel for 2–5 years before analysis (following Wasser et al., 1997; Roon et al. 2005).

Additionally, blood samples were collected from 11 (5 males, 6 females) live-trapped lynx. The lynx were captured during winter using foot-snare traps (Breitenmoser 1989) set at fresh ungulate kills and with wooden box-traps (Kolbe et al. 2003). Snare