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SHORT COMMUNICATION

Genetic structure of the Eurasian lynx population in north-eastern Poland and the Baltic states

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Abstract We analyzed the genotypes of Eurasian lynx (*Lynx lynx*) from three populations in the westernmost part of the species main range. One population was situated at the distribution edge (NE Poland) and the two other (Latvia and Estonia) were located within the main, contiguous range of the species. The aim was to determine if the genetic composition varied among these populations and if there was evidence of isolation among them. Based on microsatellite allele frequencies, we found the allelic richness in Polish lynx to be lower than that in lynx from Latvia and Estonia. We also found significant differentiation among the lynx populations, with the NE Poland population forming a distinct genetic group relative to the two other populations ($R_{ST} = 0.15$ and 0.22, $P < 0.0001$). We suggest that genetic differentiation among lynx populations is the result of habitat insularisation that limits gene flow. This finding emphasizes the necessity to consider the lynx genetic differentiation in conservation planning of this species in Poland.

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Introduction

Large mammalian predators are highly mobile animals, whose long distance movements are expected to ensure effective gene flow among subpopulations (Slough and Mowat 1996, Schwartz et al. 2002). However, the results of recent studies on Canada lynx (*Lynx canadensis*) and the Scandinavian population of Eurasian lynx (*Lynx lynx*) suggest that the populations of these felids may undergo genetic differentiation resulting either from geographical distribution of populations or social and ecological constraints that hamper the genetic exchange among populations (Rueness et al. 2003a, b, Schwartz et al. 2003).

The Eurasian lynx is particularly sensitive to habitat fragmentation because of its aversion to traverse large open areas (Schmidt 1998). Lynx populations in the westernmost part of the species natural range (i.e. excluding the reintroduced populations) are highly fragmented across several forest patches in Poland, Lithuania and Belarus (Von Arx et al. 2004). Moreover, there is a large gap between these most western patches (considered here as the western limit of lynx range) and the remaining, more contiguous range that stretches north-east across Latvia and Estonia (regarded here as a core area and referred to as Baltic states) and further to the east. We therefore hypothesized that the Eurasian lynx living at the western periphery of its geographical range in a fragmented habitat have limited opportunities for genetic exchange with populations in the core area. Thus, the aims of this study were: 1) to determine the level of genetic variability of the lynx

population in the most peripheral western patch of the species' distribution (north-eastern Poland, henceforth NE Poland) in comparison to lynx in the core area (Latvia and Estonia) and 2) to elucidate lynx population genetic structure across Poland, Latvia and Estonia.

Materials and methods

We collected 64 Eurasian lynx samples consisting of 19 samples from NE Poland (Białowieża and Knyszyn Primeval Forests, tissue of dead animals and blood of live-trapped lynx, between 1995 and 2006), 20 samples from Latvia and 25 samples from Estonia (tissue of lynx shot legally in 2003 and 2004). DNA was extracted using the DNeasy kit (Qiagen, Germany) following the manufacturer's instructions (final DNA-elution in 80 µl). One of each primer pair for six microsatellite loci (FCA08, FCA43, FCA77, FCA78; Menotti-Raymond et al. 1999 and LC109 and LC110; Carmichael et al. 2000) was 5′-labelled with fluorescent dye (6-FAM or HEX) and used for genotyping. Four additional loci (FCA35, FCA45, FCA90 and LCA120) could not be amplified in numerous samples and had to be excluded. PCR conditions were: 95°C 5 min, 35× (95°C 20 s, 55°C 20 s, 72°C 45 s), 72°C 30 min. For locus FCA43, the annealing temperature was 50°C. Amplification products were analyzed on an A3130*xl* automated sequencer (Applied Biosystems). We calculated observed (H_O) and expected heterozygosity (H_E), number of alleles, average number of alleles per locus, as well as potential deviations from Hardy-Weinberg-equilibrium using the program CERVUS (v.3.03, Marshall et al. 1998). Allelic richness (k_{ar}) was estimated based on the rarefaction method using the software HP-RARE (v. from 6/6/2006, Kalinowski 2004). Tests for genotypic disequilibrium between loci, analysis of molecular variance (AMOVA) and estimation of *F*- and *R*-statistics (Wright 1951, Slatkin 1995) were performed using the software package ARLEQUIN (v.3.1, Excoffier et al. 2005).

We estimated population differentiation with use of both R_{ST} (Slatkin 1995) and F_{ST} (Wright 1951) fixation indices, as suggested by Balloux and Lugon-Moulin (2002). The R_{ST}-value is considered to provide more relevant biological information when applied to microsatellites as it also accounts for allele sizes and not only for different alleles as does the F_{ST}-value. F_{ST} may greatly underestimate differentiation in highly structured populations (Balloux and Lugon-Moulin 2002), however we used it to allow comparison with other studies.

Population structure and assignment of individuals to populations were inferred based on microsatellite allele genotypes using the Bayesian clustering approach implemented in STRUCTURE (v.2.1, Pritchard et al. 2000). Initially, we tested our data using both models of allele frequency distributions, correlated and independent. Although the correlated model is better suited to detect a subtle population structure, it is somewhat biased towards overestimating K. Based on the pair-wise population F_{ST} and R_{ST} estimates we went for the conservative approach and applied the independent allele frequencies distribution model with K-values from 1 to 5. The required allele frequency distribution parameter λ was estimated for $K = 1$, as suggested by Pritchard et al. (2004). Its value of 0.74 was subsequently used in all other K-likelihood estimations. To determine the appropriate burn-in and run lengths for accurate parameter estimates of P and Q, we set $K = 1$ and watched for the likelihoods to converge under various burn-in and run lengths. The final burn-in and run lengths of Markov chains were then both 10^6. We ran five independent runs for each K and its associated parameter set to verify the consistency of estimates across runs. For presentation of the assignments, we have chosen a single run as the remaining runs were identical. A factorial correspondence analysis (Greenacre and Degos 1977, Clausen 1998) was carried out to examine the relationship between lynx populations and allele frequencies using the software package GENETIX (v.4.05.2, Belkhir et al. 1996–2004) under a 3D by populations option.

Results

Microsatellite genotyping rendered 64 individual genotypes. The mean number of alleles per locus in the whole sample was 6.7 and the mean H_E was 0.68. The allelic richness was lowest in the lynx from NE Poland compared to the Latvian and Estonian samples (Table 1), however the difference was only significant between NE Poland and Latvia (Wilcoxon signed ranks test, $Z = 2.20$, $P = 0.028$; NE Poland–Estonia: $Z = 0.943$, $P = 0.34$). Surprisingly, the Estonian lynx had a lower allelic richness than the Latvian sample ($Z = -1.99$, $P = 0.046$). The mean H_E in the NE Poland lynx population was lower than that in Latvia, while it was higher than the one found in Estonian lynx (Table 1), although the differences were not significant (Mann-Whitney *U*-test, $U = 10.0$, $P = 0.20$, $U = 15.0$, $P = 0.631$, respectively). There was no inbreeding in any of the three populations investigated, as demonstrated by F_{IS} values (Table 1).

The analysis of molecular variance (AMOVA) accounting for allele size differences showed that 14.6% of variation was attributed to among-population variation. Only 1.2% of variation was allocated among individuals within populations and the remaining 84.2% were distributed among individuals within the whole sample. The

Table 1 Genetic characterization of the Eurasian lynx populations and mean proportions of membership of each pre-defined population (based on sampling location) assigned with STRUCTURE software to each of the determined clusters

Sampled population	n	N_A (SD)	k_{ar} (SD)	H_O (SD)	H_E (SD)	F_{IS} P	Mean proportion to belong to	
							Cluster 1	Cluster 2
NE Poland	19	4.33 (0.52)	3.44 (0.48)	0.649 (0.140)	0.621 (0.105)	−0.0216 (0.539)	0.76	0.24
Latvia	20	5.83 (0.75)	4.63 (0.63)	0.662 (0.114)	0.695 (0.085)	0.0685 (0.284)	0.38	0.62
Estonia	25	5.00 (1.26)	3.91 (1.01)	0.613 (0.223)	0.594 (0.203)	0.0033 (0.468)	0.28	0.72

n—number of individuals, N_A—average number of alleles per locus, k_{ar}—allelic richness, H_O—observed mean heterozygosity, H_E—expected mean heterozygosity, F_{IS}—inbreeding coefficient, K—presumed number of genotypic clusters (populations), *SD*—standard deviation, P—probability value for the tested Null-hypothesis that there is significant inbreeding. The Null-hypothesis was rejected in all three populations

R-statistics calculated among all three sampling populations indicated a moderate, significant genetic structuring ($R_{ST} = 0.145$, $P < 0.0001$). In pair-wise population comparisons, the differentiation between pairs of populations ranged from small (R_{ST}[Latvia *vs*. Estonia] = 0.052, $P = 0.009$) over R_{ST}[Poland *vs*. Latvia] = 0.15, $P < 0.00001$ to large (R_{ST}[Poland *vs*. Estonia] = 0.216, $P < 0.0001$). As expected, the overall F_{ST} value was lower than the corresponding R_{ST} value, although still significant and likewise indicating a moderate differentiation among all the populations ($F_{ST} = 0.098$, $P < 0.0001$). The population pair-wise F_{ST}'s were all highly significant ($P < 0.0001$) but lower than the pair-wise R_{ST}'s and showed moderate differentiation between the NE Poland and both the Latvian and the Estonian population ($F_{ST} = 0.104$ and $F_{ST} = 0.151$, respectively), while the least differentiation was seen between Latvian and Estonian lynx ($F_{ST} = 0.041$).

The STRUCTURE analysis yielded following values of posterior probabilities for the number of populations (K): ln Pr(X|K) = − 1044.0; −1034.6; −1092.8; −1130.8 and −1157.1, for K = 1–5, respectively. As the probability was highest for K = 2, we assumed that two clusters were most likely, with cluster 1 corresponding to the NE Poland and cluster 2 to the combined sample of Latvia and Estonia (Table 1, Fig. 1). The mean estimated membership percentage of NE Polish lynx belonging to cluster 1 was 76%, whereas Latvian and Estonian lynx were assigned to cluster 2 with a mean posterior probability of 62 and 72%, respectively. On the other hand, only 42% of individuals from NE Polish population and 13% from the Latvian–Estonian lynx were assigned within 90% confidence intervals to their respective clusters.

The results of the factorial correspondence analysis provided two factorial components of which component 1 explained 68% of variation among the geographically defined lynx populations and component 2 the remaining 32% (Fig. 2). The NE Poland population was clearly separated from the Latvian and Estonian ones that formed a single common cluster.

Discussion

As presumed from the spatial distribution of Eurasian lynx in Latvia, Estonia and Poland, the allelic richness was lowest in the western-most (peripheral) patch—NE Poland, which is consistent with a suggestion by Schwartz et al. (2003) for Canada lynx, whereby gene flow is probably not strong enough to offset some loss of genetic variation caused by drift at the periphery of the lynx's geographical range. On the other hand, it is conspicuous that the heterozygosity of the NE Poland population was not lower than in the other populations and was also slightly higher than it

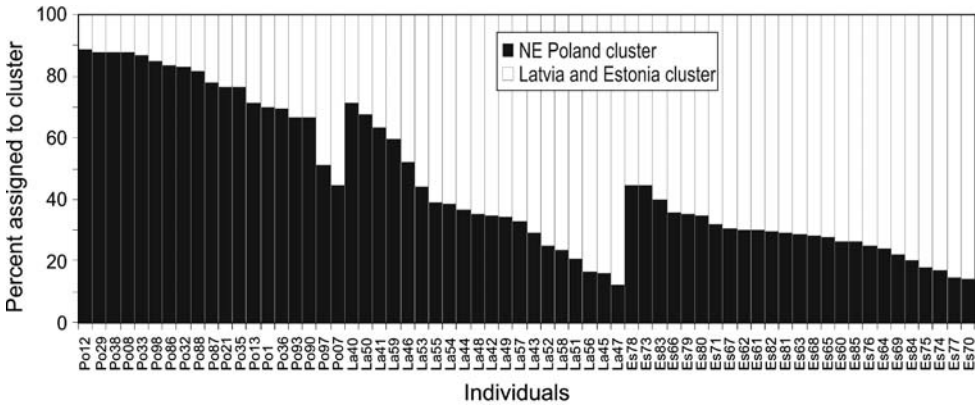

Fig. 1 Genotypic assignment of Eurasian lynx individuals, sampled in three predefined populations (NE Poland, Latvia and Estonia), to population clusters determined with the software STRUCTURE. The data fit a two-cluster model. The bars represent individuals sorted by membership probability of belonging to one of the clusters, which are reflected by black or white colour

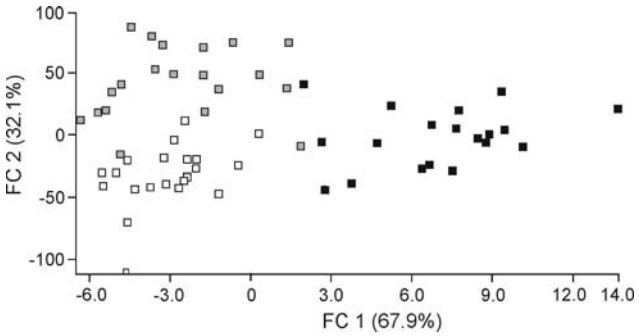

Fig. 2 Factorial Correspondence Analysis of genotype distributions in Eurasian lynx populations in Eastern Europe (NE Poland—*black squares*, Latvia—*grey squares*, Estonia—*white squares*), based on allele frequencies of six microsatellite loci. FC—factorial component

was reported for Scandinavian lynx ($H_E = 0.51$; Hellborg et al. 2002). Such inconsistency may occur in small populations that went through bottlenecks of short durations (Allendorf 1986). Indeed, this corresponds to the history of that population as it recovered after it almost became extinct twice—at the end of the 19th Century and during the 1960s (Jędrzejewski et al. 1996). The genotypes of immigrating individuals could then have caused the current variability, which is additionally supported by a lack of inbreeding in that population.

A close-to-extinction event has also been suggested as contributing to low genetic diversity in the Scandinavian lynx population (Hellborg et al. 2002). This effect could have influenced the Estonian population as well, because it has surprisingly low allelic richness and the lowest heterozygosity values despite being located within the core range. The Estonian lynx population severely declined due to past persecution and is still heavily harvested (Lõhmus 2002). However, hunting alone is not sufficient to explain the differences in genetic variability among the populations of Eurasian lynx, because the Latvian population, while having a similar history, has the highest allelic richness and heterozygosity. This in turn, could have resulted from admixture between the two distinguished clusters as suggested by the STRUCTURE analysis for the Latvian population.

The lowest allelic richness of the Polish lynx population remarkably coincides with their reduced morphological variability compared to Latvian and Estonian populations (Schmidt et al. unpublished data). The former appear to have only one pelage pattern (largely unspotted), contrasting the animals from two Baltic states that have a great variety of coat patterns: from completely unspotted to clearly spotted through various transitional forms.

The results of both the estimation of the fixation indices (F_{ST} and R_{ST}), and the STRUCTURE and the factorial correspondence analyses indicated a significant genetic differentiation between lynx from NE Poland and lynx from the two Baltic states. The inferred composition of our sample, consisting of a NE Poland cluster on one hand and a combined Latvian and Estonian cluster on the other seems very likely as the two Baltic populations inhabit a contiguous habitat and the fixation indices were the lowest between them. The stronger differentiation between Polish and Baltic lynx may be due to the existence of an actual barrier that is composed of extensive agricultural lands in Lithuania and parts of Belarus with only sparse forests scattered in between. A similar differentiation among lynx populations (though more widely spaced from each other) was suggested to exist without obvious geographical limitations affecting gene flow in Scandinavia (Rueness et al. 2003a). The genetic structure found in Eurasian lynx was surprising considering that these predators are capable of covering distances equal to those separating the Polish and the Latvian lynx populations during a single dispersal event (~450 km; Andersen et al. 2005).

We propose that the genetic differentiation observed in our study may be due to habitat insularization, however, social constraints such as territorial behavior and natal philopatry (Rueness et al. 2003a) may have also played a role in the current lynx genetic population structure.

Our study suggests that the Eurasian lynx in Poland is subjected to limitations of gene flow. It should, however, be a subject of future studies to determine if that is a recent phenomenon or a historic process (Matocq and Villablanca 2001). Therefore, conservation measures concerning this species involving translocations and reintroductions require a very thorough consideration for regional subdivision of population in these felids. If the NE Poland population is to be managed as an independent unit or a part of a larger population needs yet to be clarified.

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