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SHORT COMMUNICATION

The effect of reintroductions on the genetic variability in Eurasian lynx populations: the cases of Bohemian–Bavarian and Vosges–Palatinian populations

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Abstract Over the past ∼40 years, several attempts were made to reintroduce Eurasian lynx to suitable habitat within their former distribution range in Western Europe. In general, limited numbers of individuals have been released to establish new populations. To evaluate the effects of reintroductions on the genetic status of lynx populations we used 12 microsatellite loci to study lynx populations in the Bohemian–Bavarian and Vosges–Palatinian forests. Compared with autochthonous lynx populations, these two reintroduced populations displayed reduced genetic diversity, particularly the Vosges–Palatinian population. Our genetic data provide further evidence to support the status of 'endangered' and 'critically endangered' for the Bohemian–Bavarian and Vosges–Palatinian populations, respectively. Regarding conservation management, we highlight the need to limit poaching, and advocate additional translocations to bolster genetic variability.

Keywords Lynx · Microsatellites · Population history · Reintroduction

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Introduction

The Eurasian lynx (*Lynx lynx* Linnaeus, 1758) is the largest European felid. Historically it existed throughout most of continental and Scandinavian Europe—a distribution that was substantially reduced by direct persecution as well as loss of habitat and prey (Breitenmoser 1998). Presently, populations exist in Western Europe with too few individuals to be considered self-sustaining; all of which were established through reintroduction efforts since the 1970s (Arx et al. 2009).

Due to their complexity (logistical, socio-economic, political), reintroductions usually involve the translocation of only a small number of individuals, not all of which will survive to become founders (e.g. Vandel et al. 2006). Thus, genetic variation in reintroduced populations may be reduced compared with the source population. Subsequent loss of variation through genetic drift, compounded by inbreeding, is to be expected in such small populations (e.g. Fickel et al. 2005). Poaching of lynx—a manifestation of the conflict between humans and lynx (e.g. Andrén et al. 2006; Breitenmoser and Breitenmoser-Würsten 2008; Breitenmoser et al. 2010; Lüchtrath and Schraml 2015)—puts further strain on these genetically impoverished

populations. A reduction in fitness due to low genetic variation and/or inbreeding has not yet been documented for wild lynx, but has been shown for captive lynx (Laikre 1999) that suffer problems similar to reintroduced populations (low diversity, few founders, inbreeding).

To date, only one study has published the genetic assessment of a reintroduced lynx population (Dinaric population: Sindičić et al. 2013). The authors reported low genetic diversity and significant inbreeding, and summarized their findings as "grim". This study contradicts a previous 'demographic assessment' of the same population—i.e. from the viewpoint of population size and distribution over time—which had reported this reintroduction as "successful" (Arx et al. 2009). Such assessment discrepancies illustrate the need for an evaluation of the genetic status of reintroduced lynx populations in order to set appropriate genetics-based conservation goals (Frankham et al. 2010), and to provide an additional metric to monitor and to compare the development of reintroduced populations.

In light of this, we evaluated the genetic status of two reintroduced Eurasian lynx populations (Bohemian–Bavarian and Vosges–Palatinian; see Fig. 1a), and compared this with data for reintroduced and autochthonous European lynx populations of different demographic status. In this manner, we aimed to provide further genetic data to improve our understanding of the effect of establishing small, isolated populations of large carnivores by reintroduction.

Methods

The Bohemian–Bavarian (BB) population was founded by 'unofficial' introduction of 5–10 lynx of mostly unknown origin to Bavaria in the early 1970s (it included 3 + lynx from Slovakia; Festetics 1980; Wölfl et al. 2001), and later supplemented with individuals released on the Czech side of the BB Forest in 1982–1989 (18 lynx from Slovakia; Červený and Bufka 1996). The Vosges–Palatinian (VP) population was founded by 21 lynx released between 1983 and 1993, originating mostly from what are now Slovakia and the Czech Republic (Vandel et al. 2006).

We obtained 130 non-invasively collected samples (scat) from the Bavarian portion of the BB population, 23 tissue samples from the Vosges portion of the VP population, and 117 tissue and/or blood samples from seven other Eurasian lynx populations (reintroduced: Croatia $n = 8$, Slovenia $n = 12$; autochthonous: Slovakia $n = 6$, Estonia $n = 34$, Latvia $n = 29$, Poland $n = 18$, Russia $n = 10$; Fig. 1a). DNA was extracted using a commercial kit (GEN-IAL GmbH, Troisdorf, Germany).

Fig. 1 a A map indicating the number and origin of lynx in study; larger occurrences are indicated (following IUCN red list data 2016): *light grey* for autochthonous populations, *dark grey* for reintroduced populations, *blue* and *green* for the Bohemian–Bavarian and Vosges–Palatinian populations, respectively. **b** An unrooted neighbour-joining tree based on the proportion of shared alleles $(1-P_S)$ between multilocus microsatellite genotypes (following Bowcock et al. 1994). **c** The total number of alleles plotted against the total number of samples, by locality. The Bohemian–Bavarian and Vosges–Palatinian populations are highlighted in *blue* and *green*, respectively. The *open blue circle* represents the Bohemian–Bavarian population, excluding one admixed individual (see text)

We genotyped samples at 12 microsatellite loci designed for domestic cat (Menotti-Raymond et al. 1999, 2003) or Canadian lynx (Carmichael et al. 2000): F115, FCA441, FCA506, LCA110, FCA718, FCA006, FCA008, FCA082, FCA0097, FCA105, FCA229, FCA1023, following protocols in Schmidt et al. (2009).

Amplification success rate for scat samples (Bavarian) was 17.12 % (2–4 PCR replicates per sample). To avoid misleading results by allelic dropouts and false alleles, we followed the Maximum Likelihood approach (Miller et al. 2002) by genotyping each sample in parallel, not allowing for any allele mismatch between parallels. If a mismatch occurred, we discarded that sample and analysed two new parallels from a new DNA extraction of that sample. Genotypes were scored only if no mismatch occurred, otherwise the sample was discarded (we did not extract the sample a third time). In the end, thirty of the 130 scat samples were successfully genotyped at a sufficient number of loci (success rate = 88.9 %) to proceed with the identification of individuals (using CERVUS v.3.0, Kalinowski et al. 2007). Genotypes of scat samples were assigned to the same individual based on genotype similarity. Despite the conservative approach described above, some discrepancies between genotypes assigned to the same individual existed: (i) one case of large allele dropout, and (ii) two cases of allele-size shift (2 bp) due to stuttering. While we detected 18 unique individuals among the 30 scat samples, we proceeded with genetic analyses for only 12 of these lynx to reduce the amount of missing data in the dataset (only one missing locus permitted per sample).

For all analyses, sampling locations (countries) were defined as populations rather than using the occurrence-based definitions (Arx et al. 2004). None of the loci were at linkage disequilibrium in any of the populations tested (GENEPOP v.3.4, Raymond and Rousset 1995). We found no deviations from HWE; such deviations are potentially due to presence of null alleles, allelic dropout and/or false allele scoring due to stuttering (MICRO-CHECKER v.2.2.3; Oosterhout et al. 2004). F_{IS} was
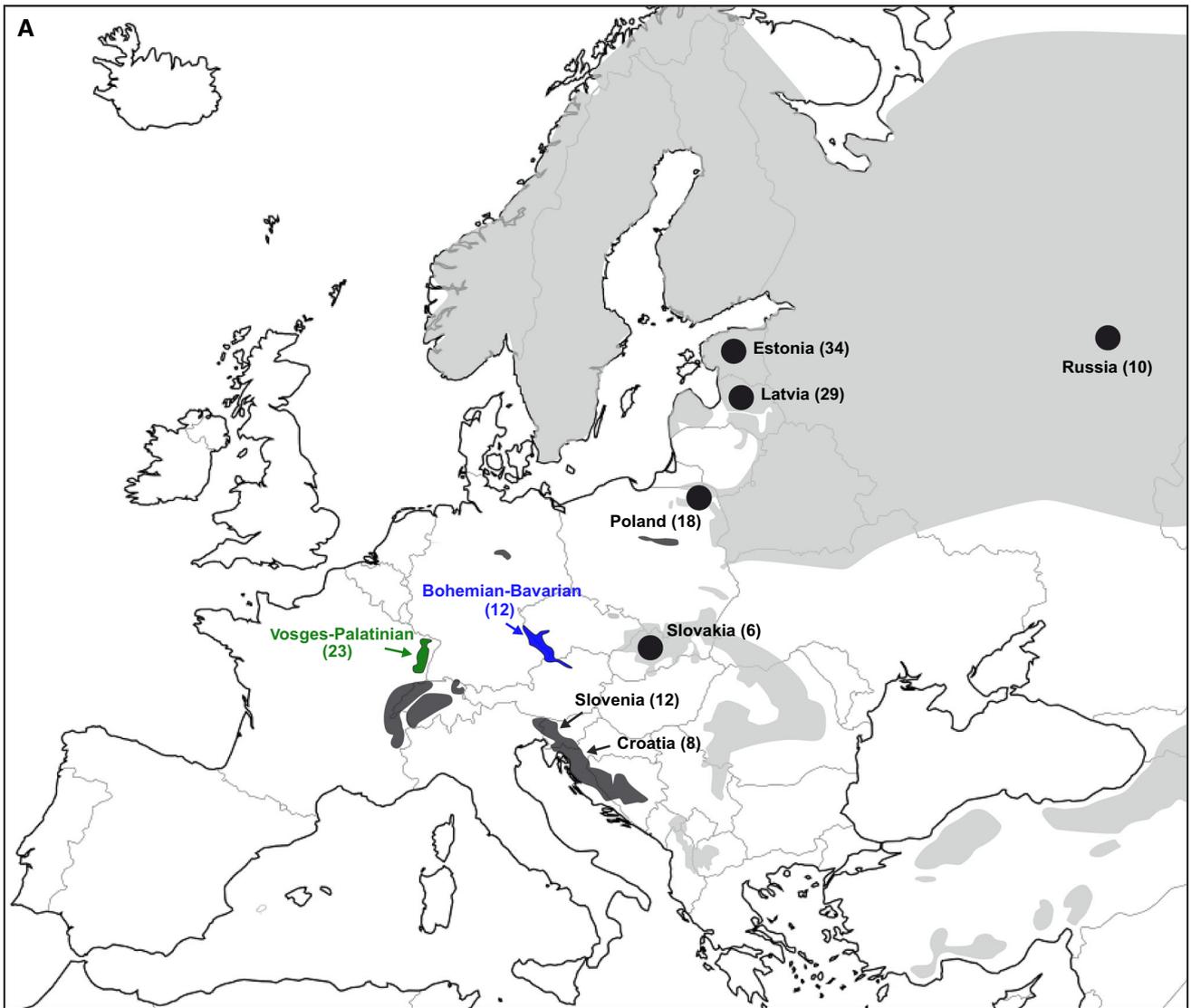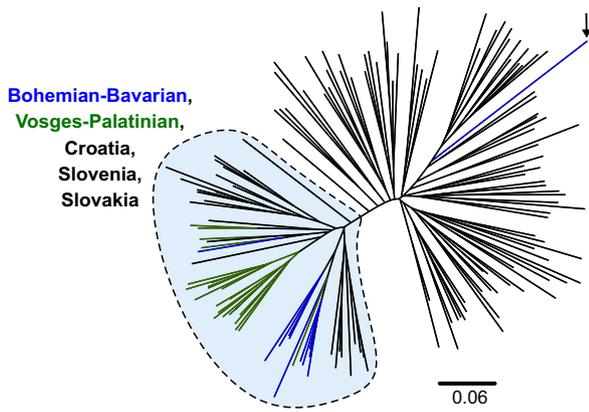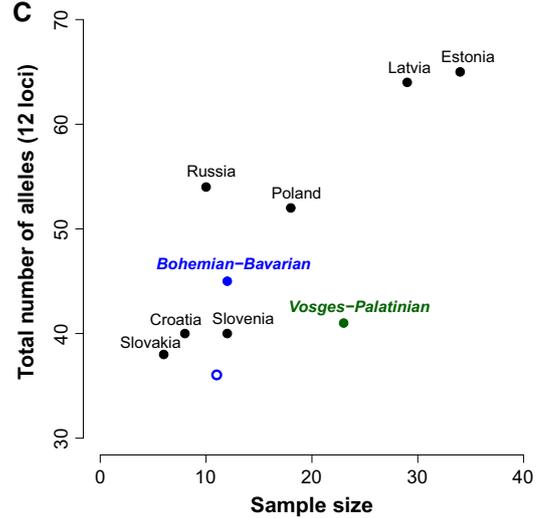
calculated for each population using FSTAT v.2.9.3.2 (Goudet 2002). Several analyses were carried out in the statistical programming environment R (http://www.cran.r-project.org/): *Memgene* v.1.0 (Galpern et al. 2014) to determine the proportion of shared alleles among samples, calculated as $1-P_S$, where P_S is the proportion of shared alleles (following Bowcock et al. 1994); *PopGenReport* v.2.1 (Adamack and Gruber 2014) for determining allelic richness (A_R) accounting for differences in sample size and genotyping success; and *PopGenKit* v.1.0 (Paquette 2012) for calculating observed (H_O) and expected heterozygosities (H_E).

Results and discussion

In order to place measures of genetic diversity of the reintroduced BB and VP populations in the context of their source of origin, we examined their genetic relationships to other populations using an individual-based multilocus approach (Bowcock et al. 1994). The unrooted neighbour-joining tree (Fig. 1b) shows that most individuals from the two target populations were more closely related to lynx from their known (for VP) or suspected (for BB) origins than to lynx from the other reference populations. For both BB and VP, individuals were found to cluster largely within distinct clades, while this was not observed for the Dinaric population (Croatia and Slovenia) and its founder, the Carpathian population (represented here by Slovakia): lynx from these populations were not distinguishable from each other in this analysis (black branches in highlighted portion of Fig. 1b).

One BB individual was genetically similar to lynx from Poland (indicated by the arrow in Fig. 1b). Two independent scat samples of this individual were genotyped, and these differed by only one locus (missing data). It is thus unlikely that the genetic similarity of this lynx to those in Poland stems from genotyping error(s) of the non-invasively collected samples. An assignment analysis using structure (Supplementary Material) suggests that this individual is admixed, with a high proportion ($\sim 60 \%$) of Polish ancestry. This implies successful interbreeding of local wild (BB) lynx with Polish lynx from an unknown source. The putative Polish ancestor(s) may represent natural long distance disperser(s), escapee(s) from an enclosure in the Bavarian Forest National Park (four of which escaped over the past 25 years), or undocumented/unofficial release(s) in or near the BB population.

Measures of genetic diversity (A_R, H_O, H_E, and F_{IS}) for lynx populations other than BB or VP (Table 1) corresponded well to those published elsewhere (Ratkiewicz et al. 2014; Sindičić et al. 2013; Schmidt et al. 2009; Rueness et al. 2003): (1) the autochthonous Baltic,

Table 1 Number of Eurasian lynx and genetic diversity measures

Location	N	A_R	H_O	H_E	F_{IS}
Bohemia-Bavaria (BB)	12	3.26	0.472	0.473	0.051
Vosges–Palatinian (VP)	23	2.79	0.454	0.474	0.068
Other reintroduced populations					
Croatia	8	3.33	0.510	0.511	0.068
Slovenia	12	3.04	0.490	0.511	0.088
Autochthonous populations					
Slovakia	6	3.16	0.639	0.523	−0.133
Estonia	34	4.16	0.667	0.662	0.009
Latvia	29	4.28	0.652	0.682	0.062
Poland	18	3.71	0.616	0.601	0.004
Russia	10	4.37	0.717	0.700	0.029

Provided are the number of lynx (n), allelic richness (A_R), observed (H_O) and expected (H_E) heterozygosity, and the inbreeding coefficient (F_{IS})

A_R determined using rarefaction (sampling 8 random lynx per location, except for Slovakia)

Carpathian and Russian lynx populations had moderate-to-high diversity, and (2) the reintroduced Dinaric population had lower diversity. In this context, the BB and VP lynx clearly displayed the low genetic diversity characteristics of other reintroduced lynx populations (Table 1).

Although our BB samples all originated from the Bavarian side of the BB ecosystem, we believe that our results are valid for the population as a whole, given that there is no barrier to dispersal (Magg et al. 2015). The measures of diversity for this population (Table 1; Fig. 1c) are very similar to those for the Dinaric population (Croatia & Slovenia), which has been aptly characterized as "grim" (Sindičić et al. 2013). Lynx of the BB population fare no better, and their 'endangered' status (Arx et al. 2004) is merited. Suitable habitat connecting BB to neighbouring populations (Carpathian) is available and ought to be maintained and enhanced to promote the influx of diversity into the BB population (Magg et al. 2015).

The VP population had the lowest diversity of all our study areas (Table 1; Fig. 1c). The total number of alleles ($N_A = 41$) among 23 VP samples is only one more than among 8 Croatian samples ($N_A = 40$), which themselves exhibit low variability. The low diversity in VP can probably be attributed to the low number of reproducing founders (estimated to be 4 females and 6 males *at best*; Vandel et al. 2006); absence of connectivity to other populations means that there is no opportunity for immigrants to bolster diversity and to replace potential losses. We concur with the assessment that the VP population should be regarded as critically endangered (Chapron et al. 2014).

Given their genetic status, both populations (especially VP) would benefit from an influx of genetic diversity by means of further reintroductions. The impact on variability following the introduction of individuals is illustrated by the admixed BB lynx (above); when this lynx is omitted from analysis, the amount of variation observed in this population is notably reduced (open and closed blue circles in Fig. 1c). In a long-term context, previous studies have shown that few individuals (immigrants/translocations) can suffice to significantly bolster variability in a population (Keller et al. 2001; Pimm et al. 2006; but see also Kenney et al. 2014); this is fortunate, as large home ranges and limited suitable habitat restricts the number of lynx that can feasibly be released successfully in these areas. For the BB and VP populations, we advise a small number of lynx originating from a portion of the Carpathian population other than Slovakia, which directly or indirectly already contributed most/all of their founding individuals. However, to determine which of these other Carpathian populations would be most suitable to supplement BB and VP requires further study. For BB, additional measures (including releases) to reinstate and maintain connectivity to neighbouring populations would also be highly beneficial (Müller et al. 2014; Magg et al. 2015).

A major obstacle in establishing and maintaining sustainable lynx populations in Europe is poaching (e.g. Breitenmoser 1998; Andrén et al. 2006; Breitenmoser and Breitenmoser-Würsten 2008; Breitenmoser et al. 2010). Lynx are protected under the EU Habitats Directive (Council Directive 92/43/EEC) since 1992, and their illegal killing is a criminal offense that may carry up to 5 years of imprisonment. However, enforcing the law is very challenging because perpetrators essentially need to be 'caught in the act' for conviction, for which government resources are sorely lacking or entirely missing. For this reason, dialogue and education of stakeholders (e.g. hunters, wildlife and forest managers, conservationists) is advocated, to identify and mitigate colliding interests and, ultimately, to reduce illegal killing of lynx (e.g. Breitenmoser et al. 2010; Lüchtrath and Schraml 2015). While law enforcement in cases of poaching needs to be enhanced, addressing the social dimension of hunters' opposition to lynx is also a priority. For both BB and VP, illegal killing represents the greatest threat to lynx, reducing their numbers and restricting their range to protected areas and their vicinity (Müller et al. 2014; Chapron et al. 2014; Wölfl et al. 2001). Low genetic diversity in these populations revealed by our study may further increase the threat for their survival. As we have illustrated above (Fig. 1c), the removal of a single individual (e.g. by poaching) can greatly impact the genepool in these small reintroduced populations.

References

Adamack AT, Gruber B (2014) PopGenReport: simplifying basic population genetic analyses in R. Methods Ecol Evol 5:384–387

Andrén H, Linnell JDC, Liberg O, Andersen R, Danell A, Karlsson J, Odden J, Moa PF, Ahlqvist P, Kvam T, Franzén R (2006) Survival rates and causes of mortality in Eurasian lynx (*Lynx lynx*) in multi-use landscapes. Biol Conserv 131:23–32

Bowcock AM, Ruiz-Linares A, Tomfohrde J, Minch E, Kidd JR, Cavalli-Sforza LL (1994) High resolution of human evolutionary trees with polymorphic microsatellites. Nature 368:455–457

Breitenmoser U (1998) Large predators in the Alps: the fall and rise of man's competitors. Biol Conserv 83:279–289

Breitenmoser U, Breitenmoser-Würsten C (2008) Der Luchs—Ein Großraubtier in der Kulturlandschaft. Salm Verlag, Wohlen/Bern

Breitenmoser U, Ryser A, Molinari-Jobin A, Zimmerman F, Haller H, Molinari P, Britenmoser-Würsten C (2010) The changing impact of predation as a source of conflict between hunters and reintroduced lynx in Switzerland. In: Macdonald DW, Loveridge AJ (eds) Biology and conservation of Felids. Oxford University Press, Oxford, pp 493–506

Carmichael LE, Clark W, Strobeck C (2000) Development and characterization of microsatellite loci from lynx (*Lynx canadensis*), and their use in other Felids. Mol Ecol 9:2197–2199

Červený J, Bufka L (1996) Lynx (*Lynx lynx*) in south-western Bohemia. In: Koubek P, Cerveny J (eds) Lynx in the Czech and Slovak Republics. Acta Hist Sci Nat, Brno, pp 16–33

Chapron G, Kaczensky P, Linnell JD, von Arx M, Huber D, Andrén H, López-Bao JV, Adamec M, Álvares F, Anders O, Balčiauskas L (2014) Recovery of large carnivores in Europe's modern human-dominated landscapes. Science 346:1517–1519

Festetics A (1980) Die Wiedereinbürgerung des Luchses in Europa. In: *Der Luchs in Europa—Verbreitung, Wiedereinbürgerung, Räuber-Beute-Beziehung*. 1. Internationale Luchs-Kolloquium, Murau/Steiermark, 1978. Kilda Verlag, Greven, pp 89–146

Fickel J, Schmidt A, Putze M, Spittler H, Ludwig A, Streich WJ, Pitra C (2005) Genetic structure of populations of European Brown hare: implications for management. J Wildl Manag 69:760–770

Frankham R, Ballou J, Briscoe D, McInnes KH (2010) Introduction to conservation genetics, 2nd edn. Cambridge University Press, Cambridge

Galpern P, Peres-Neto PR, Polfus J, Manseau M (2014) MEMGENE: spatial pattern detection in genetic distance data. Methods Ecol Evol 5:1116–1120

Goudet J (2002). FSTAT 2.9.3.2. Available at http://www2.unil.ch/popgen/softwares/fstat.htm

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Mol Ecol 16:1099–1106

Keller LF, Jeffery KJ, Arcese P, Beaumont MA, Hochachka WM, Smith JN, Bruford MW (2001) Immigration and the ephemerality of a natural population bottleneck: evidence from molecular markers. Proc Royal Soc Lond B 268:1387–1394

Kenney J, Allendorf FW, McDougal C, Smith JL (2014) How much gene flow is needed to avoid inbreeding depression in wild tiger populations. Proc Royal Soc Lond B 281:20133337

Laikre L (1999) Conservation genetics of Nordic carnivores: lessons from zoos. Hereditas 130:203–216

Lüchtrath A, Schraml U (2015) The missing lynx-understanding hunters' opposition to large carnivores. Wildl Biol 2:110–119

Magg N, Müller J, Heibl C, Hackländer K, Wölfl S, Wölfl M, Bufka L, Červený J, Heurich M (2015) Habitat availability is not the factor limiting the distribution of the Bohemian–Bavarian lynx population. Oryx. doi:10.1017/S0030605315000411

Menotti-Raymond M, David VA, Lyons LA, Schäffer AA, Tomlin JF, Hutton MK, O'Brien SJ (1999) A genetic linkage map of microsatellites in the domestic cat (Feliscatus). Genomics 57:9–23

Menotti-Raymond M, David VA, Agarwala R, Schäffer AA, Stephens R, O'Brien SJ, Murphy WJ (2003) Radiation hybrid mapping of 304 novel microsatellites in the domestic cat genome. Cytogenet Genome Res 102:272–276

Miller CR, Joyce P, Waits LP (2002) Assessing allelic dropout and genotype reliability using maximum likelihood. Genetics 160:357–366

Müller J, Wölfl M, Wölfl S, Müller DW, Hothorn T, Heurich M (2014) Protected areas shape the spatial distribution of a European lynx population more than 20 years after reintroduction. Biol Conserv 177:210–217

Paquette SR (2012) PopGenKit: Useful functions for (batch) file conversion and data resampling in microsatellite datasets. R package version 1

Pimm SL, Dollar L, Bass OL (2006) The genetic rescue of the Florida panther. Anim Conserv 9:115–122

Ratkiewicz M, Matosiuk M, Saveljev AP, Sidorovich V, Ozolins J, Männil P, Balciauskas L, Kojola I, Okarma H, Kowalczyk R, Schmidt K (2014) Long-range gene flow and the effects of climatic and ecological factors on genetic structuring in a large, solitary carnivore: the Eurasian lynx. PLoS ONE 9:e115160

Raymond M, Rousset F (1995) GENEPOP. Version 3.1d: population genetics software for exact tests and ecumenism. J Hered 86:248–249

Rueness EK, Jorde PE, Hellborg L, Stenseth NC, Ellegren H, Jakobsen KS (2003) Cryptic population structure in a large, mobile mammalian predator: the Scandinavian lynx. Mol Ecol 12:2623–2633

Schmidt K, Kowalczyk R, Ozolins J, Mannil P, Fickel J (2009) Genetic structure of the Eurasian lynx population in north-eastern Poland and the Baltic states. Conserv Genet 10:497–501

Sindičić M, Polanc P, Gomerčić T, Jelenčić M, Huber Đ, Trontelj P, Skrbinšek T (2013) Genetic data confirm critical status of the reintroduced Dinaric population of Eurasian lynx. Conserv Genet 14:1009–1018

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Vandel J, Stahl P, Herrenschmidt V, Marboutin E (2006) Reintroduction of the lynx into the Vosges mountain massif: from animal survival and movements to population development. Biol Conserv 131:370–385

von Arx M, Breitenmoser-Würsten C, Zimmermann F, Breitenmoser U (2004) Status and conservation of the Eurasian lynx (Lynx lynx) in 2001. KORA, Strasburg

von Arx M, Breitenmoser-Würsten C, Breitenmoser U (2009) Lessons from the reintroduction of the Eurasian lynx in central and west Europe. In: Vargas A, Breitenmoser-Würsten C, Breitenmoser U (eds) Iberian lynx ex situ conservation: an interdisciplinary approach (Conservación Ex Situ del LinceIbérico: Un Enfoque Multidisciplinar). Fundación Biodiversidad in collaboration with IUCN Cat Specialist Group, Madrid, pp 405–409

Wölfl M, Bufka L, Červený J, Koubek P, Heurich M, Habel H, Huber T, Poost W (2001) Distribution and status of lynx in the border region between Czech Republic, Germany and Austria. Acta Theriol 46:181–194