Conservation Genetics
https://doi.org/10.1007/s10592-022-01494-y

RESEARCH ARTICLE

Ex situ versus *in situ* Eurasian lynx populations: implications for successful breeding and genetic rescue

Jarmila Krojerová-Prokešová[1,2] · Barbora Gajdárová[1,3] · Tobias Erik Reiners[4] · Petra Bolechová[5,6] · Oddmund Kleven[7] · Petr Koubek[1,8] · Carsten Nowak[4,9] · Jānis Ozoliņš[10] · Branislav Tám[11,12] · Inna Voloshina[13] · Peter Vallo[1]

Received: 21 March 2022 / Accepted: 5 December 2022

Abstract

The main aim of *ex situ* programmes in conservation is to provide a suitable source of individuals for future reintroductions or reinforcement of existing populations. A fundamental prerequisite is creating and maintaining healthy and sustainable captive populations that show high levels of phenotypic and genetic similarity to their wild counterparts. The Eurasian lynx (*Lynx lynx*) is a model of a locally extinct species that has been subject to long-term captive breeding and of past and ongoing reintroduction efforts. To test for genetic suitability of *ex situ* population, a comparative genetic evaluation including *in situ* populations was undertaken. The assignment analysis of 97 captive lynx from 45 European zoos, wildlife parks and private breeds was performed using 124 lynx from different wild Eurasian populations belonging to three evolutionary lineages: the Carpathian, the Northern, and the Siberian lynx. The results showed a high proportion of Siberian lynx (51%) in the European captive lynx population. Remaining captive animals were assigned to either the Carpathian (28%), or the Northern lynx lineage (13%). Admixture between lineages was rather low (8%). Notably, no or very low difference in genetic diversity was detected between the wild and captive lynx populations. Our results support the potential of the captive population to provide genetically suitable individuals for genetic rescue programmes. The transfer of genes between isolated populations, including those in captivity, should become an important management tool to preserve genetic variability and prevent inbreeding depression in native and reintroduced populations of this iconic predator.

Keywords Captive breeding · Genetic variability · Inbreeding · Large carnivores · *Lynx lynx* · Reintroduction

✉ Jarmila Krojerová-Prokešová
krojerova@ivb.cz

[1] Institute of Vertebrate Biology of the Czech Academy of Sciences, Květná 8, 603 65 Brno, Czech Republic

[2] Department of Zoology, Fisheries, Hydrobiology and Apiculture, Faculty of AgriSciences, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

[3] Department of Botany and Zoology, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

[4] Center for Wildlife Genetics, Senckenberg Research Institute and Natural History Museum Frankfurt, Clamecystrasse 12, 63571 Gelnhausen, Germany

[5] Liberec Zoo, Lidové sady 425/1, 460 01 Liberec, Czech Republic

[6] Department of Ethology and Companion Animal Science, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká, 1176, 165 21 Prague 6 – Suchdol, Czech Republic

[7] Norwegian Institute for Nature Research (NINA), Høgskoleringen 9, NO-7034 Trondheim, Norway

[8] Department of Forest Protection and Game Management, Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Kamýcká, 1176, 165 21 Prague 6 – Suchdol, Czech Republic

[9] LOEWE Centre for Translational Biodiversity Genomics (LOEWE-TBG), Senckenberganlage 25, 60325 Frankfurt a.M, Germany

[10] The Latvian State Forest Research Institute SILAVA, Rīgas iela 111, Salaspils LV-2169, Latvia

[11] Zoological Department, National Zoological Garden Bojnice, Zámok a okolie 6, 97201 Bojnice, Slovakia

[12] Department of Small Animal Science, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia

[13] Lazovsky State Nature Reserve, Centralnaya St. 56, Lazo, Primorsky Krai 692980, Russia

Published online: 26 December 2022

Introduction

While captive breeding is an important tool in species conservation (Witzenberger and Hochkirch 2011), many reintroduction programmes based on captive animals failed due to a variety of reasons (Jule et al. 2008; Robert 2009). To be successful in the long-term, captive breeding programmes must focus on creating and maintaining healthy sustainable populations with high levels of phenotypic and genetic similarity to their wild counterparts (Frankham 2008; Pelletier et al. 2009; Robert 2009; Goncalves da Silva et al. 2010). Inbreeding and the loss of genetic diversity have been recognized as major problems in *ex situ* conservation since the 1970s (Ralls, Brugger and Ballou 1979; Bouman 1977), leading to the implementation of captive breeding schemes (Pelletier et al. 2009).

Genetic management of endangered species in zoos is traditionally based only on studbook data to minimize mean kinship and inbreeding (Pelletier et al. 2009; Galla et al. 2021). However, the calculations of inbreeding coefficients in studbooks assume that the founders are unrelated and non-inbred and that individuals of unknown origin do not have a high level of relatedness, which may therefore potentially underestimate the true degree of inbreeding (Ruiz-López et al. 2009; Goncalves da Silva et al. 2010). Since the emergence of molecular tools in biodiversity research, genetic analyses have been used to assess the accuracy of studbook data (Boakes et al. 2007). Several studies, such as waldrapp ibises (Geronticus eremita; Signer et al. 1994), Arabian oryx (Oryx leucoryx; Marshall et al. 1999), and Przewalski's horses (Equus przewalskii; Bowling et al. 2003) have documented that pedigree data in studbooks are often erroneous and can thus not serve alone as basis for accurate estimations of inbreeding and genetic diversity in captive breeding programmes and should be combined with molecular data.

One example of a locally extinct species that has been subject to long-term captive breeding and reintroduction efforts is the Eurasian lynx (*Lynx lynx*). Being one of the most widely distributed felids, ranging from Western Europe to the Far East of Asia (Nowell and Jackson 1996), the status of this species varies greatly within its large distribution range. Substantial differentiation has been found in size, demographic history, spatial distribution, and local adaptations throughout its range, leading to the description of several evolutionary lineages (frequently designated as subspecies) with differing conservation status. There is no broad consensus on the number of recognised lineages of Eurasian lynx and their geographical distributions, especially in Russia (Rueness et al. 2014; Lucena-Perez et al. 2020). According to the last taxonomic revision of Kitchener et al. (2017) that is supported also by recent (mito) genomic studies (Lucena-Peréz et al. 2020; Mengüllüoğlu et al. 2021; Mueller et al. 2022), six lineages of the Eurasian lynx are proposed as evolutionary significant units within its native distribution range: (1) the Northern lynx (*L. l. lynx*) in Scandinavia, Finland, Baltic States, Belarus, European part of Russia west to the Yenisei river; (2) the Carpathian lynx (*L. l. carpathicus*) in East and Central Europe; (3) the Balkan lynx (*L. l. balcanicus*) in the Balkans; (4) the Caucasus lynx (*L. l. dinniki*) in the Caucasus, Asia Minor, Iran and Iraq; (5) the Turkestan lynx (*L. l. isabellinus*) in Central Asia including the Himalayas and Tibet; and (6) the Siberian lynx (*L. l. wrangeli*) in Russia east of the Yenisei river to China (Fig. 1a).

Even though Eurasian lynx occupied the whole of Europe except for the Iberian Peninsula in the past, its European distribution range declined considerably until the mid-20th century due to human persecution (Kratochvíl 1968). At present, successfully reintroduced populations, established mostly in the 1970s and 1980s within West and Central Europe, are extremely fragmented and isolated (Fig. 1b) and potentially threatened by the loss of genetic diversity due to founder effect, isolation and stochasticity (Kaczensky et al. 2013; Mueller et al. 2022). In the long-term these threats can lead to the decrease of individual fitness (inbreeding depression) and finally to population extinction (Ralls et al. 1988; Ballou 1997; Newman and Pilson 1997; Saccheri et al. 1998). Some signs of inbreeding depression have been discussed in the Alpine (Ryser-Degiorgis et al. 2004) and the Dinaric population (Skrbinšek et al. 2019). Natural or human mediated connectivity among reintroduced populations or genetic rescue in the form of translocation of new individuals is the one way to save and to ensure long-term sustainability of these re-established populations (Sindičić et al. 2013; Bull et al. 2016; Gajdárová et al. 2021; Mueller et al. 2022).

Nevertheless, a low level of genetic variation in source material used for translocations brings two potential risks: the first is that reproduction between kin related individuals can lead to reduced vigour, reproductive output and survival (inbreeding depression); the second is a lack of adequate genetic variation to enable long-term survival and adaptation in the face of environmental change (IUCN 2013). A well-managed genetically health captive population thus can provide a suitable reservoir of genetic material for next reintroductions into new stepping-stone locations and/or for genetic rescue of these existing wild populations.

The preservation of genetic variation is of special importance in captive populations (Lacy 1993; Gautschi et al. 2003). However, captive populations are usually derived from a small number of individuals and genetic variability may be lost not only due to founder effect, but also as a consequence of inbreeding and genetic drift during subsequent generations (Richards 2000). Until the 21st century

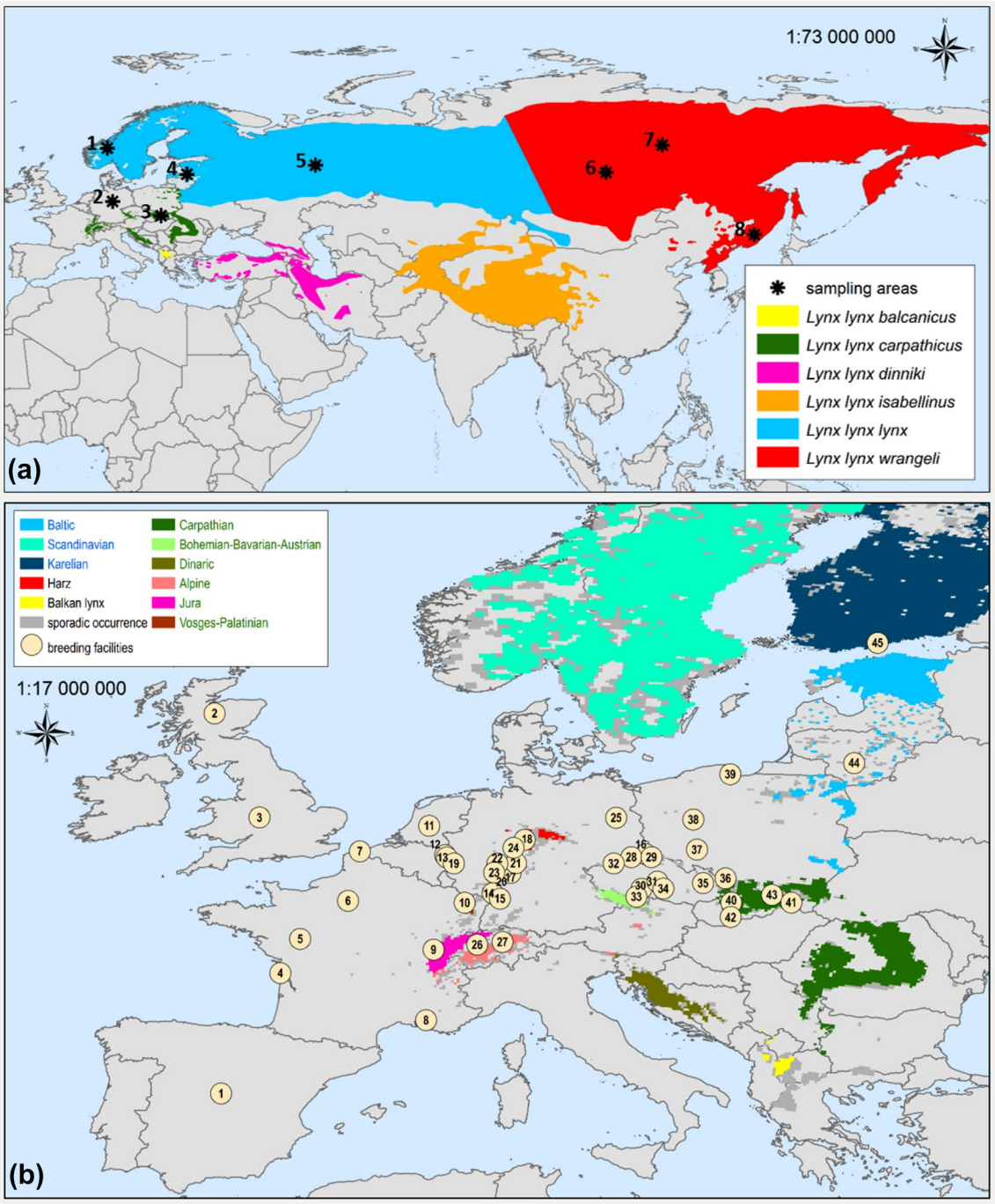

Fig. 1 **a** Distribution ranges of Eurasian lynx evolutionary lineages according to Kitchener et al. (2017). Sampling within wild populations is indicated by stars (1 Scandinavian, 2 Harz, 3 Carpathian, 4 Baltic, 5 Kirov, 6 Irkutsk, 7 Sacha, 8 Primorsky Krai). **b** Locations of breeding facilities included in this study, their full names are given in Table S1. Distribution ranges of particular European lynx populations according to IUCN Red List Mapping 2012–2016 (including corrections LCIE et al. 2020) are displayed as a background, their pertinence to the Carpathian and the Northern lineage is indicated in a legend by green and blue type colour, respectively

the European Association of Zoos and Aquariums (EAZA) kept no studbook for the Eurasian lynx (von Arx et al. 2004). However, the necessity for better management of this species became evident with an increasing number of lynx from zoos used in recent reintroduction programmes (e.g. in Harz Mountains, Germany, or in Kampinoski NP, Poland). The knowledge of the origin of captive individuals used in breeding programmes or subsequent reintroductions is necessary not only due to different conservation status of evolutionary lineages but also crucial to maintaining potential local

adaptations existing between these lineages. Success of (re-) introductions can be also threatened by the release of genetically distant animals (outbreeding depression; Turček 1951; Leimu and Fischer 2010). Moreover, all guidelines for the reintroduction of animals (e.g. IUCN 1987, 1998, and 2013) reject the use of hybrids between recognized evolutionary lineages in rescue programmes. Thus, a genetic screening of the captive population is necessary to ensure its proper management (von Arx et al. 2004).

The EAZA decided to establish a studbook for the Eurasian lynx in 2002. The studbook showed a large proportion of animals as of unknown taxonomic origin, reported as "generic". Moreover, the assignment of the animals to the evolutionary lineage by the zoos themselves is often based on incomplete life-history and phenotype. Further, records suggested that a large number of animals might be subspecific hybrids. Altogether 27 of 318 individuals (8.5%) kept in 129 institutions in 2002 were assumed to be admixed (von Arx et al. 2004). According to the European studbook report, the captive Eurasian lynx population currently consists of about 399 animals kept in 132 (110 of them registered within EAZA) zoos and breeding centres in Europe (Versteege et al. 2017). Two lineages are managed within the European studbook, the Northern lynx (164 individuals in 50 institutions) and the Carpathian lynx (124 individuals in 50 institutions). However, ancestry is still not known for all individuals and many individuals are still listed only as generic (76 lynxes in 32 institutions).

The aim of the current study was to: (1) examine the evolutionary lineage status of lynx bred in captivity in European institutions, (2) evaluate genetic variability of the captive Eurasian lynx populations in Europe with regards to particular *in situ* populations, (3) analyse extent of individual inbreeding of captive lynx, and (4) formulate recommendations for the genetic management of the captive Eurasian lynx population in Europe. To meet these objectives, we also sampled *in situ* lynx populations representing evolutionary lineages bred in captivity including a reintroduced Harz population (Germany) as an example of the population founded by captive individuals of supposed admixed origin. The results of the study were further discussed with regard to the importance of *ex situ* lynx population as a suitable reservoir of genetic material for further reintroduction and/or genetic rescue programmes in order to create a viable and interconnected lynx metapopulation across Europe.

Materials and methods

Sampling and DNA extraction

Those EAZA institutions known to breed Eurasian lynx were contacted by the keeper of the European studbook (ESB) Lars Versteege to ask for their participation in the study. We also obtained samples from zoos and wildlife parks non-registered within EAZA, including those German wildlife parks from which the population reintroduced to the Harz Mountains was founded. In total, we obtained hair (53), blood (18) and scat (27) samples collected during 2012–2019 in 45 zoos and private wildlife parks within Europe, 31 of them registered within EAZA (Fig. 1, Table S1). The collection of hair and scat samples does not pose a severe stress or harm to lynx, and blood samples were only taken from individuals anaesthetized for other purposes.

The dataset of 97 captive individuals (51 registered within ESB, Table S3) was complemented with tissue samples from legally culled wild lynxes, carcasses, museum and non-invasive samples from different wild populations. The samples were collected throughout the native distribution range of Eurasian lynx. We obtained 48 samples from the Carpathian population (Slovakia and the Czech Republic), 15 samples from the Baltic population (Latvia), 20 samples from the Scandinavian population (Norway), and 18 samples from Russia (2 Kirov, 8 Irkutsk and Sacha (hereafter: Irkutsk), 8 Primorsky Krai). Except for wild populations, we further included 23 samples from the reintroduced Harz population. The final dataset consisted of 221 individuals of wild and captive lynx.

Samples were fixed using silica gel, 96% ethanol or frozen. The Genomic DNA Mini kit Tissue (Geneaid Biotech Ltd., New Taipei City, Taiwan) was used to isolate DNA from hair, blood, and tissue samples, QIAamp (Fast) DNA Stool Mini kit (Qiagen) was used to isolate DNA from scats according to the manufacturers' protocols. We used dedicated laboratories for DNA extraction and PCR setup for non-invasive samples and enforced strict rules and procedures to prevent contamination.

Amplification of microsatellite loci and genotyping

Microsatellite analyses were performed using 15 nuclear loci and a sex specific marker (Amelogenin). Details about the markers and PCR conditions used are given in Krojerová-Prokešová et al. (2019). The samples were analysed on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems). DNA fragment sizes were scored by the same person using GENEMAPPER 3.7 (Applied Biosystems).

PCR amplification for hair, scat and museum samples was repeated according to the quality and quantity of extracted DNA following the multiple-tubes approach (Taberlet et al. 1996; Adams and Waits 2007), with a minimum of three positive PCRs for homozygotes and two for heterozygotes.

Assignment of captive lynxes to evolutionary lineages

A multiple assignment approach was used to detect genetic sub-structuring in Eurasian lynx and to identify lineages and population origin of captive individuals (Gajdárová et al. 2021). Factorial correspondence analysis (FCA) was performed in GENETIX v.4.05.2 (Belkhir et al. 1996–2004) and the relationships between individual multi-locus genotypes were visualized in 2D-space. A Bayesian clustering procedure implemented in STRUCTURE v.2.3.4 (Pritchard et al. 2000) was run with 10 independent simulations for each value of K from 1 to 10, with 1 000 000 permutations and an initial burn-in of 100 000 generations. In all simulations, an admixture ancestry model without using sampling locations as prior information and a correlated allele frequency model were used. The K value was estimated by Evanno's calculation (Evanno et al. 2005), which is based on the second order rate of change in the log probability of the data between successive values of K (ΔK), and by the estimators accounting for uneven sampling and hierarchical structure (Puechmaille 2016), both evaluated using the online application StructureSelector (Li and Liu 2018). The results of independent runs for each K were combined and displayed graphically using the same online application, integrating CLUMPAK (Kopelman et al. 2015). Further, we applied the frequency-based method of Paetkau et al. (1995) within the software GeneClass2 (Piry et al. 2004). Genotypes of all individuals from reference populations/evolutionary lineages were used within one file while genotypes of captive animals were input separately. Probability of assignment was performed by simulating 100 000 individuals with the Monte Carlo resampling method (Paetkau et al. 2004) and setting the type I errors to 0.05 (Piry et al. 2004). Finally, for investigation of the origin of captive animals, we applied a machine-learning approach in the package *assignPOP* v.1.1.4 (Chen et al. 2018) in R 3.6.3 (R Core Team 2020). Software *assignPOP* was developed to overcome issues associated with non-independence and imbalance of datasets (Chen et al. 2018). The *assignPOP* approach included data evaluation where all individuals from reference populations/lineages were randomly divided into training sets and the assignment accuracies were estimated via Monte-Carlo cross-validation based on the following parameters: proportion of individuals in training set: 0.5, 0.7, and 0.9; proportion of loci in training set: 0.1, 0.25, 0.5, and 1; loci sample method: F_{st}; iterations: 100; and model: LDA (Linear Discriminant Analysis). Based on the simulation results we performed assignment test of captive animals using criterion Kaiser-Guttman and the model LDA. The output was visualized using *ggplot2* functions in R (Wickham 2016).

Nonetheless, detecting admixture signals between closely related lineages sharing a recent common ancestry is not trivial and it is important to establish reliable *q*-thresholds to identify pure individuals (Caniglia et al. 2020). We assigned as "pure" individuals (with no or negligible admixture ancestry between lineages/populations) those with more than 80% of genotype assigned to one lineage/population (relevant for STRUCTURE, GeneClass2 and *assignPOP*). The same threshold $q > 0.8$ was previously used for detection of admixture between closely related lineages (e.g. for wildcat/domestic cat or wolf/dog admixture; Steyer et al. 2018, Dziech 2021). Using FCA the assignment was done manually based on the vicinity to samples with known ancestry in 2D space. A multiple assignment approach was further applied to all individuals and only those which were identified as "pure" by at least three of four methods were confirmed to be pure individuals.

Genetic diversity and inbreeding

Based on the assignment results of captive individuals, these were allocated to particular lineages and/or populations if the assignment fulfilled above mentioned criteria and original population was indisputable. For these groups of captive individuals, we calculated basic population genetic measures and compared the values to their wild conspecifics. The number of alleles (N_A), the allelic richness (AR), the observed (H_O) and the expected (H_E) heterozygosity were estimated for each locus and population using FSTAT v.2.9.3.2 (Goudet 2001). The number of effective alleles (n_e) and private alleles (P_A) were stated based on allele frequencies calculated in Excel MS Toolkit v.3.1 (Park 2001). Departure from the Hardy-Weinberg equilibrium (HWE) was tested using the exact probability test in GENEPOP v3.4 (Raymond and Rousset 1995). *P*-values for multiple testing were corrected using the Bonferroni correction (Rice 1989). GENEPOP was used to calculate Weir and Cockerham's (1984) estimator of inbreeding coefficients (F_{IS}). Nevertheless, in some cases (e.g. small population size), inbreeding may be undetectable using F_{IS} (Keller and Waller 2002). Hence individual inbreeding coefficients (F) were estimated using TrioML (Wang 2007) implemented in the software package COANCESTRY v.1.0 (Wang 2011) for all captive animals as well as for both, the wild and the captive populations. The pairwise index of genetic differentiation (F_{ST}) based on Weir and Cockerham (1984) was calculated in the *hierfstat* package (Goudet 2005) in R 3.6.3 (R Core Team 2020). Confidence intervals of F_{ST} values (95% CI) were estimated using 999 bootstrap replicates in the same R package to evaluate the significance of F_{ST} values. To determine effective population size (N_e) we employed the linkage disequilibrium (LDN_e) method implemented in NEESTIMATOR 2.01 (Do et al. 2014) that has shown to be reasonably precise and unbiased at small sample sizes (Waples 2006).

Results

Genotyping success and assignment of captive lynx samples

Lynx DNA was successfully amplified in 86 out of 97 samples of captive individuals (88% genotyping success). All 15 loci plus Amelogenin were successfully genotyped in 79 individuals. Three individuals were genotyped at 15 loci, one individual at 14 loci, two at 13 loci and one at 12 loci. Genotyping failed for 10 hair and 1 scat samples, likely due to poor quality of DNA (e.g. hairs collected with adhesive tape, old scat). Following a Bonferroni adjustment for multiple testing, no linkage disequilibrium was found between any pair of loci in any population. All analysed loci were polymorphic, even though a few proved monomorphic in particular populations. We did not detect any significant deviations from Hardy–Weinberg expectations, thus all loci were included in the following analyses.

Pairwise F_{ST} values confirmed genetic differentiation among populations from the wild (F_{ST}=0.122–0.335; Table S2). Pairwise F_{ST} values between Carpathian lynx and Northern/Siberian lynx were high, F_{ST}=0.185/0.187, respectively. Genetic differentiation between Northern and Siberian lynx was moderate (F_{ST}=0.136). Sufficient structuring of the wild reference dataset enabled the assignment of captive animals using all four methodological approaches to particular lineages (Table S3a) and for the majority also to particular populations (Table S3b).

Factorial correspondence analysis clearly separated genetic lineage of the Carpathian lynx. Further, there was no such clear border between the two other lineages, the Northern and the Siberian lynx. In this case, we observed a geographic pattern related to gradual genetic differentiation from the Baltic region to Far East Russia, compatible with an isolation by distance scenario (Fig. 2). But the difference in genetic variability between the Northern and Siberian lineages was more visible when samples assigned to the Carpathian cluster were excluded from the FCA (Fig. S1). The position of the Harz population between Northern and Siberian lineages confirmed its proposed admixed origin.

Bayesian clustering analysis for all 210 captive and wild lynx performed in STRUCTURE confirmed the maximum estimated value of likelihood for two clusters (K = 2). Similarly, the ΔK distribution (Evanno et al. 2005) showed the highest peak at K = 2 and a somewhat smaller at K = 5 (Fig. S2A, B). The number of clusters according to Puechmaille (2016) for uneven sampling was estimated to be K = 6 (Fig. S3). This clustering corresponded to six comparative populations, namely (i) Carpathian, (ii) Scandinavian, (iii) Baltic, (iv) Irkutsk, (v) Primorian as well as the reintroduced Harz population (vi) (Fig. 3).

Both FCA and Bayesian clustering suggested an unclear origin of two individuals sampled in the Kirov region. One lynx was assigned to the Baltic population, the second lynx was assigned to the Irkutsk population using FCA, while STRUCTURE identified it as a hybrid between both mentioned populations. Therefore, we decided to exclude these two samples from all comparisons of genetic diversity given below.

Assignment of captive lynxes to evolutionary lineages

The final assignment of captive individuals to particular lineages made with Bayesian clustering and FCA was further supported by two other methodological approaches implemented in GeneClass2 and *assignPOP* (Table S3, Fig. S4–S7). Using multiple assignment approach, we assessed

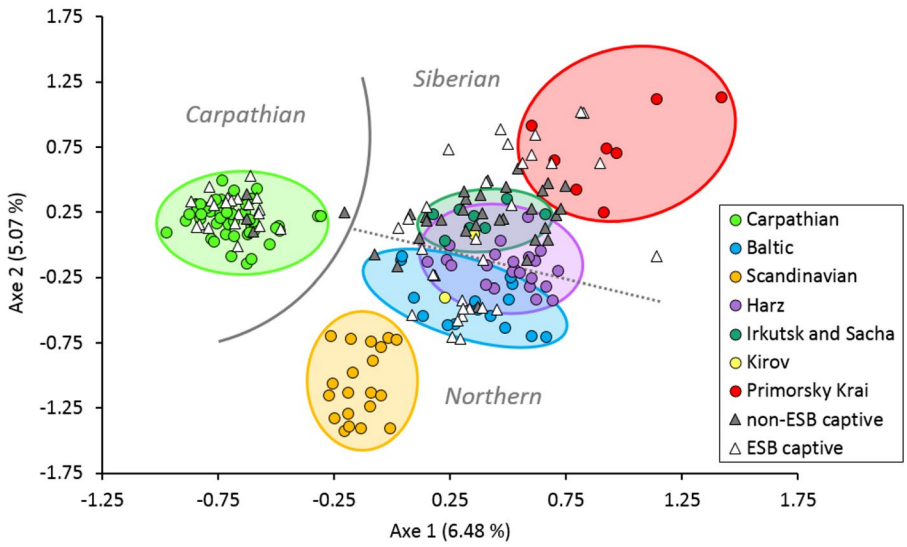

Fig. 2 Microsatellite-based genetic separation of wild and captive lynxes from 42 zoos and wildlife parks using factorial correspondence analysis (FCA) (N = 210). Approximate borders between recognized lineages are illustrated by lines

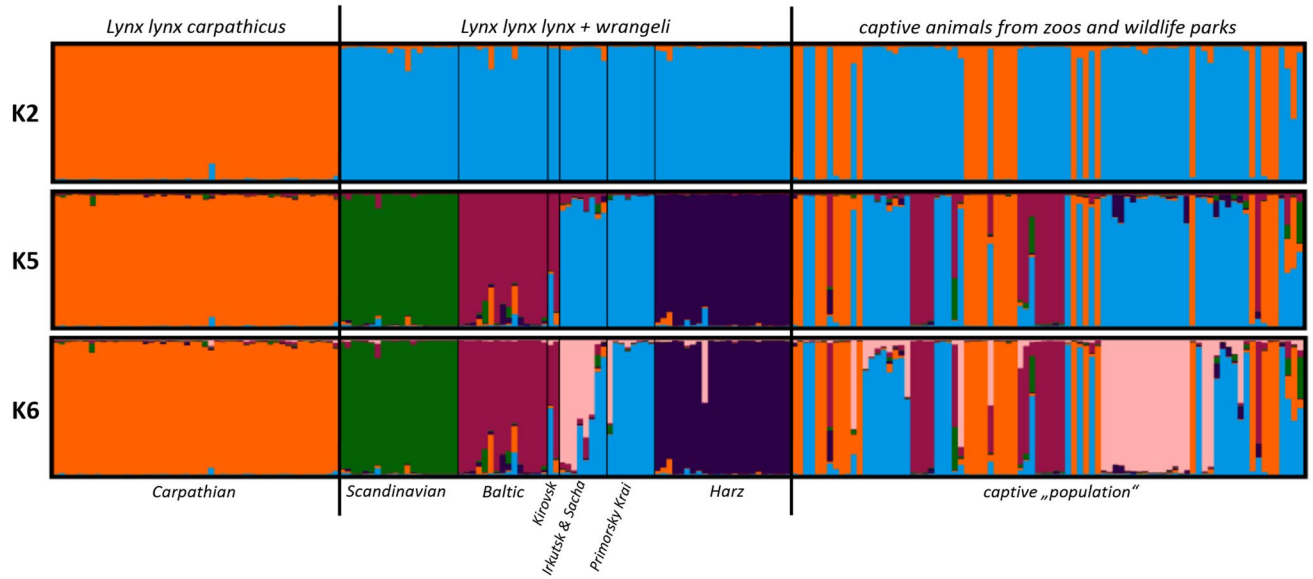

Fig. 3 Microsatellite-based genetic sub-structuring of captive and wild Eurasian lynx using Bayesian clustering in software STRUCTURE for K = 2, 5 and 6. Each column corresponds to one animal, the colour of each column corresponds to the probability of assignment to a certain cluster

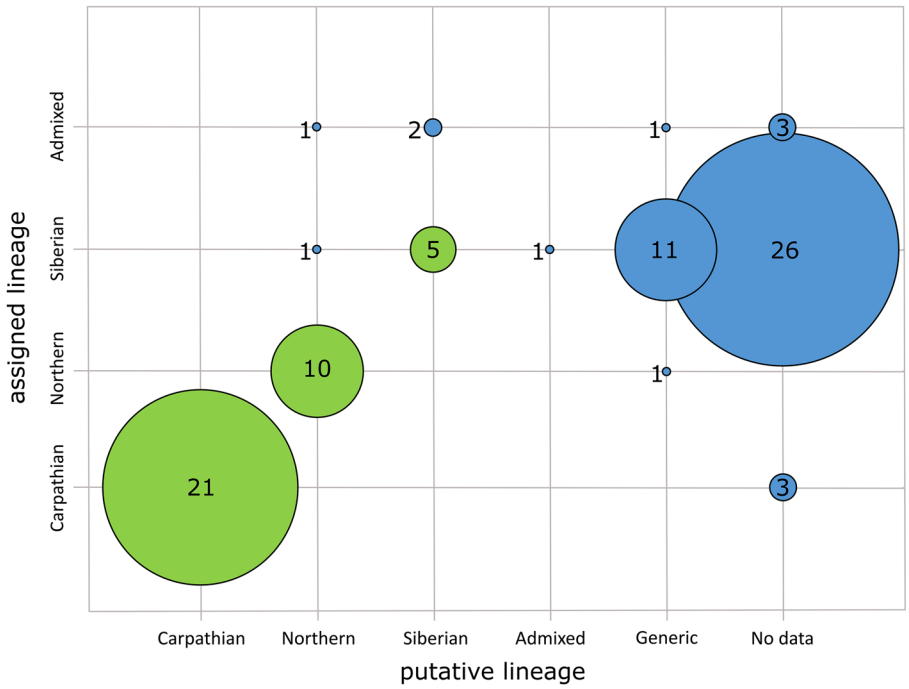

Fig. 4 The assignment of captive lynx to evolutionary lineage according to their putative origin stated by breeding facilities (the agreement is indicated by green, non-agreement or missing previous data are indicated by blue colour). The circles are displayed proportionally to the number of assigned individuals, which is given in or near the circle

the evolutionary lineage (Carpathian, Northern or Siberian lynx) of all genotyped captive animals. For 79 individuals (92%) at least three of four methodological approaches estimated the same lineage origin (mostly *assignPOP* (83%) supported different lineage origin (Table S3a)). The disagreement was detected for 7 admixed individuals.

From 86 captive individuals, 24 (28%) were assigned to the Carpathian lynx (Fig. 4). From these individuals, 21 were registered in the ESB and all were correctly recorded as Carpathian lynx.

Eleven captive lynxes (13%) were assigned to the Northern lynx. All these were registered within the ESB, ten out

of eleven correctly as Northern lynx, one individual was listed as generic.

The highest number, 44 captive individuals (51%), was assigned to the Siberian lynx. Only 17 of these individuals were registered in the ESB and only four with correctly identified lineage status. Eleven individuals were registered as generic individuals, one as a hybrid, and one incorrectly as a Northern lynx.

Seven individuals were detected to be admixed (8%). Only two were registered in the ESB, one as a generic lynx, and one as the Northern lynx. All of them, except for one, were detected to be admixed between Northern and Siberian lineage. Only one individual was detected to be admixed between Carpathian and Siberian lynx, and this came from private breeding.

Assignment to the particular population was confirmed by at least three of four methodological approaches for 67 out of 79 non-admixed individuals (85%). The discrepancies were detected within lineages (36%; Irkutsk/Primorian and Baltic/Scandinavian population) as well as between lineages (64%; mostly Irkutsk/Primorian/Baltic or Baltic/Irkutsk population) (Table S3b).

Surprisingly, the samples from wildlife parks in Germany, from which the founders of the Harz population came from, belonged all except for one to the Siberian lynx (Fig. 4, Table S3). Our results, however, revealed that, 20 years after foundation, the Harz population due to genetic drift forms a separate genetic cluster (Fig. 3).

Table 2 The level of inbreeding calculated using F_{IS} including its significance expressed by p-value and values of individual inbreeding F (± variance) calculated in COANCESTRY for particular lineage (in bold)/population

Lineage/population	Status	F_{IS}	p value	F
Carpathian	**Wild**	**0.039**	**0.0869**	**0.104 ± 0.018**
	Captive	**− 0.023**	**0.7346**	**0.092 ± 0.014**
Northern	**Wild**	**0.178**	**0.0002**	**0.159 ± 0.014**
	captive	**0.031**	**0.3013**	**0.062 ± 0.009**
Baltic	Wild	0.107	0.0056	0.174 ± 0.016
Baltic	Captive	0.034	0.2893	0.121 ± 0.013
Scandinavian	Wild	0.011	0.422	0.170 ± 0.013
Siberian	**Wild**	**0.179**	**0.0002**	**0.190 ± 0.017**
	captive	**0.135**	**0.0002**	**0.165 ± 0.044**
Irkutsk	Wild	0.181	0.0015	0.222 ± 0.015
Irkutsk	Captive	0.018	0.2694	0.102 ± 0.016
Primorian	Wild	0.041	0.2328	0.138 ± 0.015
Primorian	Captive	0.173	0.0004	0.201 ± 0.062
Harz	Reintroduced	− 0.031	0.8002	0.057 ± 0.006

Genetic variability and inbreeding

The comparison of genetic variability and inbreeding between *in situ* and *ex situ* populations was based on the assignment results for captive lynx to lineage and/or populations, with only clearly assigned ("pure") individuals being used for subsequent analyses. This approach enabled the comparison with respect to further *ex situ* genetic

Table 1 Population genetic measures given for captive and wild lineages (in bold) and Eurasian lynx populations (N—number of individual genotypes, N_A—number of alleles, A_r – allelic richness, n_e—effective number of alleles, P_A—number of private alleles between captive and wild counterparts, H_E—expected heterozygosity, H_O—observed heterozygosity, LDN_e—effective population size inferred via linkage disequilibrium method with estimated 95% Jacknife confidence intervals

Lineage/population	Status	N	N_A	A_r	n_e	P_A	H_E	H_O	LDN_e	95% CI of LDN_e
Carpathian	**Wild**	**48**	**4.20**	**2.25**	**2.41**	**5**	**0.585**	**0.563**	**97.7**	**51.8–364.8**
	Captive	**24**	**4.00**	**2.24**	**2.40**	**8**	**0.582**	**0.596**	**29.2**	**19.1–51.9**
Northern	**Wild**	**35**	**6.07**	**2.50**	**2.82**	**33**	**0.645**	**0.531**	**9.2**	**7.0–11.7**
	Captive	**11**	**4.60**	**2.35**	**2.46**	**9**	**0.593**	**0.576**	**11.0**	**7.8–16.1**
Baltic	Wild	15	5.27	2.52	2.94	27 (16*)	0.660	0.591	44.1	24.5–143.9
Baltic	Captive	10	4.27	2.33	2.41	12 (9*)	0.586	0.567	8.3	5.7–12.2
Scandinavian	Wild	20	3.93	2.07	1.97	8	0.492	0.487	27.6	15.4–72.6
Siberian	**Wild**	**16**	**6.87**	**2.88**	**4.39**	**21**	**0.772**	**0.638**	**15.6**	**12.7–19.4**
	Captive	**44**	**7.33**	**2.86**	**4.24**	**20**	**0.764**	**0.662**	**19.6**	**17.1–22.7**
Irkutsk	Wild	8	4.33	2.58	3.27	8 (3*)	0.694	0.575	2.0	1.7–2.5
Irkutsk	Captive	22	5.73	2.65	3.39	30 (16*)	0.705	0.693	9.8	8.1–11.9
Primorian	Wild	8	5.33	2.77	3.67	26 (19*)	0.728	0.700	105.8	29.8–Inf
Primorian	Captive	10	5.47	2.76	3.65	28 (16*)	0.726	0.606	9.9	7.6–13.2
Harz	Reintroduced	23	3.67	2.16	2.20	1	0.546	0.562	9.6	7.0–13.1

*The values in brackets indicate the number of private alleles for the population if the comparison between all populations within particular lineage is done

management of particular lineages as well as from the point of view of their potential for genetic rescue programmes.

Carpathian lynx

Genetic diversity values were similar between captive and wild Carpathian lynx (H_e = 0.582 and 0.585, respectively) and only a few private alleles were found in low frequencies in both *in situ* and *ex situ* populations (Table 1). However, the effective population size was lower for the captive than for the wild population (Table 1). We did not detect significant inbreeding (F_{IS}) within the wild or the captive population (Table 2). Similarly, the level of individual inbreeding F was comparable between both populations (Fig. S9). Higher individual inbreeding coefficients ($F \geq 0.25$) were detected for four captive individuals (Table S4). The F_{ST} (Table S3c) indicates no significant genetic difference among wild and captive counterparts.

Northern lynx

Even though the Northern lynx also includes the population in Scandinavia, no captive lynx were assigned to this population (Table S3b). As the Scandinavian population is quite well differentiated from the Baltic one (Figs. 2 and 3), we excluded it from further analysis and compared genetic variability only between the wild and the captive populations of the Baltic origin (comparative values are given in Table 1).

The wild Baltic population showed slightly higher values of all genetic diversity measures than the captive one. The F_{ST} indicates only low genetic differentiation between wild and captive counterparts (Tables S3c). We also detected a higher number of private alleles for this population than for the captive animals. F_{IS} was high in the wild Baltic population (0.107) but not significant in the captive Baltic population (Table 2). Only one individual showed an individual inbreeding coefficient of $F \geq 0.25$ (Table S4). The estimated effective population size of the wild population was slightly smaller than that of the captive one but both are based on small sample size and should be taken with caution (Table 1).

Siberian lynx

Again, at the lineage level, the genetic variability was comparable between the wild and the captive Siberian lynx and was the highest in comparison to other lineages (Table 1). However, a high number of private alleles was detected within the wild (21) as well as the captive (20) population, which supports the idea that some of the captive individuals could have originated from other Russian regions within the large distribution range of the Siberian lynx that were not sampled within this study.

Pairwise genetic diversity values at the population level were comparable between natives and captives (Table 1). We detected significant inbreeding within the wild Irkutsk population and within the captive Primorian population, which influenced the inbreeding values detected for the lineage (Table 2). As a consequence of higher inbreeding detected, the effective population size of the Irkutsk wild population was low. In contrast, the effective population size of the Primorian wild population was the highest of all populations included (N_e=105.8). However, again the values of N_e play just indicative role due to small sample size. Within captive Siberian lynx almost one quarter of lynx had individual inbreeding higher than $F \geq 0.25$ (Table S4). Again, the F_{ST} indicates only low genetic differentiation between wild and captive population (Tables S3c).

Harz population

The reintroduced Harz population founded by captive animals showed slightly lower genetic variability than wild and captive populations, except for the wild Scandinavian one (Table 1). The admixed origin (between the Northern and the Siberian lynx) of this population was confirmed by FCA (Fig. 2) and partially also by Bayesian clustering (Fig. S8, K3b). We analysed in detail the alleles detected in the Harz population with comparison to all three lynx lineages. We detected the majority of alleles common with all of them (58%) or with the Northern and the Siberian lynx (23%). Five alleles were shared exclusively with the Siberian lynx, two with the Northern lynx. No allele was shared only with the Carpathian lynx. One allele was unique to the Harz population. No significant inbreeding was detected in the Harz population; however, the effective population size was estimated to be less than 10 individuals.

Discussion

Assignment of captive Eurasian lynxes to evolutionary lineages

The identification of taxonomic conservation units is one of the most fundamental tasks for conservation (O'Brien 2007) and is necessary for breeding management of captive populations if different lineages are bred within the same geographical region. This is also the case for the Eurasian lynx.

Using four assignment methods we were able to assess lineage status of all successfully genotyped captive individuals. In the majority of cases, at least three of four approaches resulted in the concordant lineage assignment of captive lynxes (Table S3a). The Carpathian lynx clade

was especially well-supported and clearly separated from two other lynx lineages. The assignment of lynxes to the Northern or the Siberian lineage was also unambiguous. Lower genetic differentiation between the two latter lineages together with small sample size in some populations affected population-level but not lineage-level assignments. The most discrepancies were detected between the Baltic and the Irkutsk population (the lowest genetic differentiation, F_{ST}=0.122) or between the Baltic, the Irkutsk and the Primorian population. The high number of private alleles recorded in both the captive as well as the wild Baltic, Irkutsk and Primorian populations, indicates for future the necessity to use a much larger comparative dataset of genotypes within the large Russian distribution range of the Eurasian lynx.

However, microsatellite genotyping allowed us to identify the evolutionary lineage of all 51 individuals registered within the ESB. From these individuals, 38 had previously been provisionally assigned to the lineage. This assignment was correct for all except for three individuals: ID1564, ID2063, ID2791. Two of these individuals were incorrectly assigned to the Northern lynx instead of the Siberian lynx. In one case the individual was proposed to be admixed, but genetic analysis did not prove admixed origin and assigned the animal to the Siberian lynx using all four methods. Further, we assessed lineage status of 12 individuals registered within ESB as generic. Eleven of them were assigned to the Siberian lynx and one to the Northern lynx. Only one lynx registered in ESB was detected to be admixed (between the Northern and the Siberian lynx).

We also revealed the lineage of 35 lynx bred in European zoos, wildlife parks and private breeds not registered within the ESB. Except for three, the lineage status of these individuals was unknown. The majority of them (27) were assigned to the Siberian lynx, three to the Carpathian lynx and five were detected to be of admixed origin. From three individuals with previously assigned lineage status, only one was classified correctly. Most of these non-ESB individuals were sampled within those German wildlife parks from which founders of the Harz population originated. All those individuals except for one belonged to the Siberian lynx. This fact does not support the assumption about the release of captive individuals of admixed origin (von Arx et al. 2009). Currently existing admixture may thus be the results of post-release matings between founders. The position of Harz samples within 2-dimensional space in FCA output (Fig. 2) shows its relationship to the Irkutsk as well as to the Baltic population. This agrees with the fact that founders came from German wildlife parks (Siberian lynx origin confirmed by this study) and from Swedish zoos (Mueller et al. 2020). Even though mtDNA analysis (haplotype H4; Gugolz et al. 2008; Ratkiewicz et al. 2014) suggested that the Harz population (39th Standing Committee meeting of Bern Convention, file T-PVS (2019)7, Strassbourg; Bonn Lynx Expert Group 2021), our findings did not support this prediction. However, due to the initial founder effect and consequent genetic drift the Harz population is now clearly distinguishable from all other populations and started to form a separate cluster from K = 3 in the Bayesian clustering analysis (Fig. S8).

Genetic differentiation between captive and wild populations

The majority of papers on *ex situ* conservation genetics have focused solely only the captive population. Comparisons with wild populations are scarce, although it is exactly this comparison that is needed in order to evaluate whether the goals of breeding programmes for endangered species are really being met. This data would simplify the work of studbook coordinators by providing more detailed knowledge on the genetic variability of the breeding stock (Witzenberger and Hochkirch 2011).

Most established breeding programmes implement genetic and demographic management with the expressed goal of establishing a captive population that will maintain at least 90% of its original heterozygosity for 200 years (Ebenhard 1995). However, conservation of genetic diversity in a captive population is a difficult task. These populations are usually established by a very small number of founders leading to severe founder effects (Hedrick 2005). Additionally, the growth of the population is usually restricted due to limited availability of breeding facilities (Hedrick 2005). Small populations are prone to inbreeding, which causes reduction of heterozygosity, and genetic drift, resulting in the loss of allelic diversity (Quattro and Vrijenhoek 1989; Vrijenhoek 1994). Allelic richness generally tends to be more sensitive to bottlenecks than heterozygosity. For example, for Atlantic salmon, four to five generations of captive breeding reduced allelic richness, while heterozygosity did not decline until up to 12 generations (Säisä et al. 2003; Kraaijeveld-Smit et al. 2006). However, our results did not support either a decrease of heterozygosity or the loss of allelic richness in captive lynx population (Table 1). Beside the differences detected among wild populations, which are similar to those detected on genome-wide data (Lucena-Perez et al. 2020), our results indicated that similar levels of genetic diversity persist in both captive and wild populations. Although we detected some private alleles unique to the *ex situ* populations (as well as to the wild populations), the low values of F_{ST} (Table S2c) indicate no or only very low genetic differentiation between wild and captive counterparts regarding lineage/population as only F_{ST} values greater than 0.15 can be considered as significant (Frankham et al. 2002).

Our findings could be biased by the limited number of comparative samples for the Northern and the Siberian lynx. Nevertheless, for the Carpathian lynx with much better

sampling we detected almost the same values of genetic variability (heterozygosity as well as allelic richness) as for the wild Carpathian population. We did not detect significant inbreeding in both populations and the value of effective population size was for the Carpathian captive population the highest among all captive ones even though the N_e estimates for other populations could be biased due to small sample size (Wang et al. 2016). The results confirmed that especially the Carpathian captive population adequately represents the gene pool of their wild counterparts and may represent a suitable reservoir for future lynx reintroductions or reinforcements. There can be several reasons for such a well-preserved gene pool. Firstly, the Carpathian lynx are bred mostly within areas of its natural occurrence, which may have affected the accuracy and the amount of information about the founders that were initially available. Secondly, to a certain extent, there is probably a constant influx of new individuals from the wild in the form of orphaned young or injured adult individuals which have been rescued (Versteege et al. 2017). Continued introduction of genes from the wild may slow genetic depletion as well as the rate of genetic adaptation to captivity (Gilligan and Frankham 2003).

Many reintroduced populations established in Western and Central Europe during the last century now suffer due to low number of founders, isolation, and inbreeding (Breitenmoser-Würsten and Obexer-Ruff 2003; Bull et al. 2016; Mueller et al. 2020). Their vulnerability was very recently confirmed also by genome-wide study (Mueller et al. 2022). Several reinforcement projects have already been started e.g. in the Dinaric Mountains and Palatinate Forest (https://www.lifelynx.eu/ and https://snu.rlp.de/de/projekte/luchs/, respectively) and new animals were captured in the Slovak and Romanian Carpathians and released in these areas. However, the wild Carpathian population is also isolated, and some preliminary results indicated that the gene flow between Western and Eastern Carpathians might be interrupted (Skrbinšek et al. 2019), so the animals from the wild should probably not be used in high numbers. This underlines the importance of the *ex situ* Carpathian lynx population as a possible genetic reservoir and separate source of suitable individuals for human-mediated conservation efforts preventing further genetic erosion of the re-established European lynx populations mentioned above.

While neutral genetic markers such as microsatellites or many SNPs can provide very valuable information about diversity measures, they are usually not able to uncover changes in fitness-related traits because they are mostly situated in non-coding DNA regions (Waples 1998; Hedrick 1999; Lucena-Perez et al. 2020). There are particular difficulties unique to captive-born animals, which include loss of socially learned skills (e.g. hunting), conditioning to humans, experience feeding on livestock, inappropriate social behaviours (e.g. mating and dominance) and other factors associated with adaptation to captivity (Soorae and Price 1997). Therefore, before considering translocation of individuals, not only the genetic similarity and suitability between the captive and the possible target populations in the wild must be fulfilled, but also all these fitness traits should be considered and evaluated. In addition, the new high-throughput sequencing methods for the whole-genome mapping will enable more comprehensive insight into the genome of animals in captivity and the degree of genetic change and potential loss of functional adaptations compared to their wild counterparts. However, this approach requires samples with higher DNA quality than what was the case for many of the non-invasive samples used in this study.

Implications for conservation management

Our results confirmed the importance of molecular data to manage the ESB correctly and to identify errors that may disrupt genetic integrity of conservation units according to the three lynx evolutionary lineages present in breeds. Even though this study rejected concerns about substantial presence of admixed individuals within captive lynx, we detected a few cases of incorrect lineage assignment in the ESB. This highlights an urgent requirement to verify the lineage status of those captive lynx registered in the ESB and not included in this study. Our findings also revealed that many European captive lynxes, previously not assigned to a particular lineage, belong to geographically remote Siberian lynx. This lineage is present also in many private collections. Based on these facts, the use of captive lynx in reinforcement and genetic rescue programs without previous rigorous genetic testing cannot be recommended (Kutal et al. 2021). Further, the fact that many private breeding centres maintain a lineage different from those present in the wild populations of that region, emphasizes the need for better control by state administrative bodies to prevent accidental escapes of these captive animals into the wild, in order to protect wild lynx populations as significant evolutionary units.

On the other hand, our results support the strong potential of the captive population to provide genetically suitable individuals for genetic rescue, especially for the Carpathian lynx. Using neutral genetic markers, we did not detect any significant difference between wild and captive Carpathian lynx in regard to genetic structure, inbreeding and diversity, making the captive population an apparently suitable pool of individuals for future reintroductions and spare source of suitable individuals for human-mediated conservation efforts preventing further genetic erosion of re-established European lynx populations. However, the suitability of individuals for release has to be supported by breeding under special conditions to increase their ability to survive in the wild and to prevent their habituation.

Especially for felids, the suitability of captive animals for reintroduction projects appears to be significantly higher than for other carnivores (Jule et al. 2008) and the successful example of lynx reintroduction and steady population growth in the Harz Mountains underlines the suitability of captive-raised animals for reintroduction projects (Mueller et al. 2020). The transfer of genes between isolated populations, including captive ones, should become an important management tool for this iconic predator to preserve genetic variability of native and reintroduced populations and to minimize risk of inbreeding depression. These findings are highly important in the light of the ongoing and planned European reintroduction efforts and the lynx conservation vision of establishing a connected lynx metapopulation within Central and Western Europe (Bonn Lynx Expert Group 2021).

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10592-022-01494-y.

Acknowledgements The study was financially supported by the Ministry of the Environment of the Czech Republic (projects of Liberec Zoo: 60453/ENV/12-2239/630/12 and 49730/ENV/13-2154/630/13), by INTER-EXCELLENCE - INTER-COST (LTC20021), by EEA grants (project MGSII-40), and by Institutional Research Plan (RVO: 68081766). We would like to thank previous ESB keeper Lars Versteege and zoo's and other breeding facilities, who participated in this study, for providing samples of lynx in their care. Further, we thank to PLA Administration Beskydy, ČSOP Salamandr, Friends of the Earth Czech Republic, Ole Anders, Beňadik Machciník, Lilli Middelhoff, and Jerguš Tesák for their valuable help with sample collection, and to Anna Bryjová for technical support related to fragmentation analysis. Also, we would like to thank to Rory Putman for revision of English and to the editor and anonymous reviewers for valuable comments and suggestions to the manuscript.

Author contributions JK-P conducted the statistical analysis and prepared the manuscript. JK-P with the help of PV and PB designed the study. All co-authors contributed samples, JK-P with the help of BG performed laboratory analyses. All authors were involved in revision and editing the final manuscript.

Data availability Individuals genotypes associated with this study are available within the supplementary material of this article.

Declarations

Conflict of interest The authors declare that they no conflict of interest.

References

Adams JR, Waits LP (2007) An efficient method for screening faecal DNA genotypes and detecting new individuals and hybrids in the red wolf (*Canis rufus*) experimental population area. Conserv Genet 8:123–131. https://doi.org/10.1007/s10592-006-9154-5

Ballou JD (1997) Ancestral inbreeding only minimally affects inbreeding depression in mammalian populations. J Hered 88:169–178. https://doi.org/10.1093/oxfordjournals.jhered.a023085

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (1996–2004) GENETIX 405, logiciel sous Windows TM pour la génétique des populations. Laboratoire Genome, Populations, Interactions, CNRS UMR 5000. Universite de Montpellier II, Montpellier

Boakes EH, Wang JL, Amos W (2007) An investigation of inbreeding depression and purging in captive pedigreed populations. Heredity 98:172–182. doi: https://doi.org/10.1038/sj.hdy.6800923

Bonn Lynx Expert Group (2021) Recommendations for the conservation of the eurasian lynx in Western and Central Europe. Conclusions from the workshop of the "Bonn Lynx Expert Group" in Bonn, Germany, 16–19 June 2019. Cat News Special Issue 14:78–86

Bouman J (1977) The future of Przewalski horses (*Equus przewalskii*) in captivity. Int Zoo Yb 17:62–68

Bowling AT, Zimmermann W, Ryder O, Penado C, Peto S, Chemnick L, Yasinetskaya N, Zharkikh T (2003) Genetic variation in Przewalski's horses, with special focus on the last wild caught mare, 231 Orlitza III. Cytogenet Genome Res 102:226–234

Breitenmoser-Würsten Ch, Obexer-Ruff G (2003) Population and conservation genetics of two re-introduced lynx (*Lynx lynx*) populations in Switzerland—a molecular evaluation 30 years after translocation. In: Proceedings of the 2nd Conference on the Status and Conservation of the Alpine Lynx Population (SCALP):7–9

Bull JK, Heurich M, Saveljev AP, Schmidt K, Fickel J, Förster DW (2016) The effect of reintroductions on the genetic variability in eurasian lynx populations: the cases of bohemian–bavarian and Vosges–Palatinian populations. Conserv Genet 17:1229–1234. https://doi.org/10.1007/s10592-016-0839-0

Caniglia R, Galaverni M, Velli E et al (2020) A standardized approach to empirically define reliable assignment thresholds and appropriate management categories in deeply introgressed populations. Sci Rep 10:2862. https://doi.org/10.1038/s41598-020-59521-2

Chen KY, Marschall EA, Sovic MG, Fries AC, Gibbs HL, Ludsin SA (2018) *assignPOP*: an R package for population assignment using genetic, non-genetic, or integrated data in a machine learning framework. Methods Ecol Evol 9:439–446. https://doi.org/10.1111/2041-210X.12897

Do C, Waples RS, Peel D, Macbeth GM, Tillett BJ, Ovenden JR (2014) NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (ne) from genetic data. Mol Ecol Resour 14:209–214. doi: https://doi.org/10.1111/1755-0998.12157

Dziech A (2021) Identification of Wolf-Dog Hybrids in Europe – An Overview of Genetic Studies. Front Ecol Evol 9:760160. https://doi.org/10.3389/fevo.2021.760160

Ebenhard T (1995) Conservation breeding as a tool for saving animal species from extinction. Trends Ecol Evol 10:438–443. https://doi.org/10.1016/S0169-5347(00)89176-4

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620. DOI: https://doi.org/10.1111/j.1365-294X.2005.02553.x

Frankham R (2008) Genetic adaptation to captivity in species conservation programs. Mol Ecol 17:325–333. DOI: https://doi.org/10.1111/j.1365-294X.2007.03399.x

Frankham R, Ballou JD, Briscoe DA (2002) Introduction to Conservation Genetics. Cambridge University Press, Cambridge

Gajdárová B, Belotti E, Bufka L, Duľa M, Kleven O, Kutal M, Ozoliņš J, Nowak C, Reiners TE, Tám B, Volfová J, Krojerová-Prokešová J (2021) Long-distance eurasian lynx dispersal – a prospect for connecting native and reintroduced populations in Central Europe. Conserv Genet. https://doi.org/10.1007/s10592-021-01363-0

Gautschi B, Müller JP, Schmid B, Shykoff JA (2003) Effective number of breeders and maintenance of genetic diversity in the captive

bearded vulture population. Heredity 91:9–16. https://doi.org/10.1038/sj.hdy.6800278
Gilligan DM, Frankham R (2003) Dynamics of genetic adaptation to captivity. Conserv Genet 4:189–197. Doi: https://doi.org/10.1023/A:1023391905158
Goncalves da Silva A, Lalonde DR, Quse V, Shoemaker A, Russello MA (2010) Genetic approaches refine ex situ Lowland Tapir (*Tapirus terrestris*) conservation. J Hered 101:581–590. https://doi.org/10.1093/jhered/esq055
Goudet J (2005) Hierfstat, a package for R to compute and test hierarchical F-statistics. Mol Ecol Notes 5:184–186. https://doi.org/10.1111/j.1471-8286.2004.00828.x. Latest version available at github
Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Updated from Goudet 1995
Gugolz D, Bernasconi MV, Breitenmoser-Würsten Ch, Wandeler P (2008) Historical DNA reveals the phylogenetic position of the extinct Alpine lynx. J Zool 275:201–208. https://doi.org/10.1111/j.1469-7998.2008.00428.x
Hedrick PW (1999) Perspective: highly variable loci and their interpretation in evolution and conservation. Evolution 53:313–318. DOI: https://doi.org/10.1111/j.1558-5646.1999.tb03767.x
Hedrick PW (2005) Genetics of populations. Jones and Bartlett Publishers, Sudbury
IUCN, the Commission on Environmental Policy (1987) The IUCN Position Statement on Translocation of living organisms. Introductions, Re-introductions and Re-stocking. Prepared by the Species Survival Commission in collaboration with the Commission on Ecology, and, Law and Administration. As approved by the 22nd Meeting of the IUCN Council, Gland, Switzerland, 4 September 1987, pp. 1–13
IUCN (1998) Guidelines for re-introductions. Prepared by the IUCN/SSC re-introduction specialist Group. IUCN, Gland, Switzerland and Cambridge, UK, pp 1–10
IUCN Red List Mapping 2012–2016 for the regional assessment of the Eurasian Lynx (*Lynx lynx*) in Europe. Large Carnivore Initiative for Europe IUCN/SSC Specialist Group et al (2020) shapefile corrected in May 2020
IUCN (2013) Guidelines for reintroductions and other Conservation Translocations. Version 1.0. Gland. IUCN Species Survival Commission, viiii + 57 pp, Switzerland
Jule KR, Leaver LA, Lea SEG (2008) The effects of captive experience on reintroduction survival in carnivores: a review and analysis. Biol Conserv 141:355–363. https://doi.org/10.1016/j.biocon.2007.11.007
Kaczensky P, Chapron G, von Arx M, Huber D, Andrén H, Linnell J (2013) Status, management and distribution of large carnivores – bear, lynx, wolf and wolverine – in Europe. Report to the EU Commission, Part 1 and Part 2
Keller LF, Waller DM (2002) Inbreeding effects in wild populations. Trends Ecol Evol 17:230–241
Kitchener AC, Breitenmoser-Würsten Ch, Eizirik E, Gentry A, Werdelin L, Wilting A, Yamaguchi N, Abramov AV, Christiansen P, Driscoll C, Duckworth JW, Johnson W, Luo S-J, Meijaard E, O'Donoghue P, Sanderson J, Seymour K, Bruford M, Groves C, Hoffmann M, Nowell K, Timmons Z, Tobe S (2017) A revised taxonomy of the Felidae. The final report of the cat classification Task Force of the IUCN/ SSC cat specialist Group. Cat News Special Issue 11:80
Kopelman NM, Mayzel J, Jakobsson M, Rosenberg NA, Mayrose I (2015) CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across K. Mol Ecol Resour 15(5):1179–1191. https://doi.org/10.1111/1755-0998.12387
Kraaijeveld-Smit FJL, Griffiths RA, Moore RD, Beebee TJC (2006) Captive breeding and the fitness of reintroduced species: a test of the responses to predators in a threatened amphibian. J Appl Ecol 43:360–365. https://doi.org/10.1111/j.1365-2664.2006.01137.x
Kratochvíl J (1968) Recent distribution of the lynx in Europe. Acta Scientarum Naturalium Academiae Scientiarum Bohemicae Brno 5/6:1–74
Krojerová-Prokešová J, Turbaková B, Jelenčič M, Bojda M, Kutal M, Skrbinšek T, Koubek P, Bryja J (2019) Genetic constraints of population expansion of the Carpathian lynx at the western edge of its native distribution range in Central Europe. Heredity 122(6):785–799. https://doi.org/10.1038/s41437-018-0167-x
Kutal M, Duľa M, Krojerová-Prokešová J, Belotti E, Volfová J, Bufka L (2021) Uncoordinated reintroductions of eurasian lynx might be a threat for the species recovery in Central Europe. Biodivers Conserv 30:3737–3740
Lacy RC (1993) Impacts of inbreeding in natural and captive populations of vertebrates: implications for conservation. Perspect Biol Med 36:480–496. Doi: https://doi.org/10.1353/pbm.1993.0081
Leimu R, Fischer M (2010) Between-population outbreeding affects plant defence. PLoS ONE 5(9):e12614. https://doi.org/10.1371/journal.pone.0012614
Li YL, Liu JX (2018) StructureSelector: a web-based software to select and visualize the optimal number of clusters using multiple methods. Mol Ecol Resour 18:176–177. https://doi.org/10.1111/1755-0998.12719
Lucena-Perez M, Marmesat E, Kleinman-Ruiz D, Martínez-Cruz B, Węcek K, Saveljev AP, Seryodkin IV, Okhlopkov I, Dvornikov MG, Ozolins J, Galsandorj N, Paunovic M, Ratkiewicz M, Schmidt K, Godoy JA (2020) Genomic patterns in the widespread eurasian lynx shaped by late quaternary climatic fluctuations and anthropogenic impacts. Mol Ecol 29:812–828. https://doi.org/10.1111/mec.15366
Marshall TC, Sunnucks P, Spalton JA, Greth A, Pemberton J (1999) Use of genetic data for conservation management: the case of the arabian oryx. Anim Conserv 2:269–278. https://doi.org/10.1111/j.1469-1795.1999.tb00073.x
Mengüllüoğlu D, Ambarlı H, Barlow A, Paijmans JLA, Sayar AO, Emir H, Kandemir İ, Hofer H, Fickel J, Förster DW (2021) Mitogenome phylogeny including data from additional subspecies provides new insights into the historical biogeography of the eurasian lynx *Lynx lynx*. Genes 12:1216. DOI: https://doi.org/10.3390/genes12081216
Mueller SA, Reiners TE, Middelhoff TL, Anders O, Kasperkiewicz A, Nowak C (2020) The rise of a large carnivore population in Central Europe: genetic evaluation of lynx reintroduction in the Harz Mountains. Conserv Genet 21:577–587. https://doi.org/10.1007/s10592-020-01270-w
Mueller SA, Prost S, Anders O, Breitenmoser-Würsten C, Kleven O, Klinga P, Konec M, Kopatz A, Krojerová-Prokešová J, Middelhoff TL, Obexer-Ruff G, Reiners TE, Schmidt K, Sindičič M, Skrbinšek T, Tám B, Saveljev AP, Naranbaatar G, Nowak C (2022) Genome-wide diversity loss in reintroduced eurasian lynx populations urges immediate conservation management. Biol Conserv 266:109442. https://doi.org/10.1016/j.biocon.2021.109442
Newman D, Pilson D (1997) Increased probability of extinction due to decreased genetic effective population size: experimental populations of *Clarkia pulchella*. Evolution 51(2):354–362. https://doi.org/10.1111/j.1558-5646.1997.tb02422.x
Nowell K, Jackson P (1996) Wild cats. Status survey and conservation action plan. IUCN, Gland
O'Brien J (2007) Animal Conservation Genetics – an overview with relevance to captive breeding programmes. EAZA News 57:26–35
Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in canadian polar bears. Mol Ecol 4:347–354. https://doi.org/10.1111/j.1365-294X.1995.tb00227.x

Paetkau D, Slade R, Burden M, Estoup A (2004) Direct, real-time estimation of migration rate using assignment methods: a simulation-based exploration of accuracy and power. Mol Ecol 13:55–65. https://doi.org/10.1046/j.1365-294X.2004.02008.x

Park SDE (2001) The excel microsatellite toolkit (version 3.1) Animal Genomics Laboratory, University College, Dublin

Pelletier F, Reale D, Watters J, Boakes EH, Garant D (2009) Value of captive populations for quantitative genetics research. Trends Ecol Evol 24:263–270. https://doi.org/10.1016/j.tree.2008.11.013

Piry S, Alapetite A, Cornuet J-M, Paetkau D, Baudouin L, Estoup A (2004) GeneClass2: a Software for genetic assignment and first-generation migrant detection. J Hered 95:536–539. https://doi.org/10.1093/jhered/esh074

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959. doi: https://doi.org/10.1093/genetics/155.2.945

Puechmaille SJ (2016) The program structure does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. Mol Ecol Resour 16:608–627. https://doi.org/10.1111/1755-0998.12512

Quattro JM, Vrijenhoek RC (1989) Fitness differences in remnant populations of the endangered Sonoran topmin-now. Science 245:976–978. doi: https://doi.org/10.1126/science.2772650

R Core Team (2020) A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna

Ralls K, Brugger K, Ballou JD (1979) Inbreeding and juvenile mortality in small populations of ungulates. Science 206:1101–1103. DOI: https://doi.org/10.1126/science.493997

Ralls K, Ballou JD, Templeton AR (1988) Estimates of lethal equivalents and the cost of inbreeding in mammals. Conserv Biol 2:185–193. DOI:https://doi.org/10.1111/J.1523-1739.1988.TB00169.X

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(12):e115160. https://doi.org/10.1371/journal.pone.0115160

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248–249. https://doi.org/10.1093/oxfordjournals.jhered.a111573

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223–225. https://doi.org/10.2307/2409177

Richards CM (2000) Inbreeding depression and genetic rescue in a plant metapopulation. Am Nat 155:383–394. DOI: https://doi.org/10.1086/303324

Robert A (2009) Captive breeding genetics and reintroduction success. Biol Conserv 142:2915–2922. https://doi.org/10.1016/j.biocon.2009.07.016

Rueness EK, Naidenko S, Trosvik P, Stenseth NC (2014) Large-scale genetic structuring of a widely distributed carnivore – the eurasian lynx (*Lynx lynx*). PLoS ONE 9(4):e93675. https://doi.org/10.1371/journal.pone.0093675

Ruiz-López MJ, Roldán ERS, Espeso G, Gomendio M (2009) Pedigrees and microsatellites among endangered ungulates: what do they tell us? Mol Ecol 18:1352–1364. DOI: https://doi.org/10.1111/j.1365-294X.2009.04121.x

Ryser-Degiorgis M-P, Ryser A, Obexer-Ruff G, Breitenmoser-Würsten Ch, Breitenmoser U, Lang J (2004) 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

Saccheri I, Kuussaari M, Kankare M, Vikman P, Fortelius W, Hanski I (1998) Inbreeding and extinction in a butterfly metapopulation. Nature 392:491–494. https://doi.org/10.1038/33136

Säisä M, Koljonen M-L, Tähtinen J (2003) Genetic changes in Atlantic salmon stocks since historical times and the effective population size of a long-term captive breeding programme. Conserv Genet 4:613–627. DOI: https://doi.org/10.1023/A:1025680002296

Signer EN, Schmidt CR, Jeffreys AJ (1994) DNA variability and parentage testing in captive Waldrapp ibises. Mol Ecol 3:291–300. doi: https://doi.org/10.1111/j.1365-294x.1994.tb00069.x

Sindičić M, Polanc P, Gomerčić T, Jelenčič 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. DOI: https://doi.org/10.1007/s10592-013-0491-x

Skrbinšek T, Boljte B, Jelenčič M, Sindičić M, Paule L, Promberger B, Rigg R, Bartol M, Pop M, Kubala J, Slijepčević V, Molinari P, Molinari-Jobin A, Krofel M, Bizjan P, Hočevar L, Oliveira T, Potočnik H, Kos I, Kljun F, Stronen AV, Tám B, Črtalič J, Konec M (2019) Baseline (pre-reinforcement) genetic status of SE Alpine and Dinaric lynx population. Report of LIFE Lynx, p 24

Soorae PS, Stanley Price MR(1997) Successful re-introductions of large carnivores- what are the secrets? Paper presented at the 11th International Conference on Bear Research and Management, Graz, Austria

39th Standing Committee meeting of Bern Convention, file no, T-PVS (2019) 7 – Meeting conclusions of the Expert conference on the conservation of the lynx in West and Central Europe, Strassbourg, https://www.coe.int/en/web/bern-convention/-/39th-standing-committee-meeting

Steyer K, Tiesmeyer A, Muñoz-Fuentes V, Nowak C (2018) Low rates of hybridization between european wildcats and domestic cats in a human-dominated landscape. Ecol Evol 8:2290–2304. https://doi.org/10.1002/ece3.3650

Taberlet P, Griffin S, Goossens B, Questiau S, Manceau V, Escaravage N, Waits LP, Bouvet J (1996) Reliable genotyping of samples with very low DNA quantities using PCR. Nucleic Acids Res 26:3189–3194. doi: https://doi.org/10.1093/nar/24.16.3189

Turček FJ (1951) Effect of introductions on two game populations in Czechoslovakia. J Wildl Manage 15:113–114*)*

Versteege L, Lengger J, Fienieg E, Schad K (2017) Long-term management plan for the eurasian lynx (*Lynx lynx*) european studbook (ESB). Safaripark Beekse Bergen, Hilvarenbeek, the Netherlands

von Arx M, Breitenmoser-Würsten Ch, Zimmermann F, Breitenmoser U(2004) Status and conservation of the Eurasian lynx (*Lynx lynx*) in Europe in 2001. KORA Bericht Nr. 19e. KORA, Muri, Switzerland

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. Fundación Biodiversidad in collaboration with. IUCN Cat Specialist Group, Madrid, pp 403–409

Vrijenhoek RC (1994) Genetic diversity and fitness in small populations. In: Loescheke V, Tomiuk J, Jain SK (eds) Conservation Genetics. Birkhauser-Verlag, Switzerland, pp 37–53

Wang J (2007) Triadic IBD coefficients and applications to estimating pairwise relatedness. Genet Res (Camb) 89:135–153. doi:https://doi.org/10.1017/S0016672307008798

Wang J (2011) Coancestry: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. Mol Ecol Resour 11:141–145. doi:https://doi.org/10.1111/j.1755-0998.2010.02885.x

Wang J, Santiago E, Caballero A (2016) Prediction and estimation of effective population size. Heredity 117:193–206. https://doi.org/10.1038/hdy.2016.43

Waples RS (1998) Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. J Hered 89:438–450. https://doi.org/10.1093/jhered/89.5.438

Waples RS (2006) A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. Conserv Genet 7:167–184. https://doi.org/10.1007/s10592-005-9100-y

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. Evolution 38:1358–1370. https://doi.org/10.2307/2408641

Wickham H(2016) *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. ISBN 978-3-319-24277-4, https://ggplot2.tidyverse.org

Witzenberger KA, Hochkirch A (2011) Ex situ conservation genetics: a review of molecular studies on the genetic consequences of captive breeding programmes for endangered animal species. Biodivers Conserv 20:1843–1861. https://doi.org/10.1007/s10531-011-0074-4