Genetic consequences of reintroduction in two elusive European felids

Dissertation

zur Erlangung des Doktorgrades

der Naturwissenschaften

vorgelegt beim Fachbereich 15 Biowissenschaften

der Johann Wolfgang Goethe -Universität

in Frankfurt am Main

von

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aus Vermont, USA

Frankfurt am Main, 2021

vom Fachbereich 15 Biowissenschaften der Johann Wolfgang Goethe - Universität als Dissertation angenommen.

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Datum der Disputation: 18 October 2021

The research was conducted at the

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Abstract

Genetic and genomic tools have provided researchers with the opportunity to address fundamental questions regarding the reintroduction of species into their historical range with greater precision than ever before. Reintroduction has been employed as a conservation method to return locally extinct species to their native range for decades. However, it remains unknown how genetic factors may impact population establishment and persistence at the population and metapopulation level in the short- and long-term. Genetic methods are capable of producing datasets from many individuals, even when only low quality DNA can be collected. These methods offer an avenue to investigate unanswered questions in reintroduction biology, which is vital to provide evidence based management strategies for future projects. The Eurasian lynx (*Lynx lynx*) and European wildcat (*Felis silvestris*) are elusive carnivores native to Eurasia and have been the subject of multiple reintroduction attempts into their native range. During the 19th and 20th century, the Eurasian lynx was extirpated from West and Central Europe due to increasing habitat fragmentation and persecution. Similarly, the European wildcat was the subject of human persecution, residing in a few refugia in West and Central Europe. After legal protection in the 1950s, subsequent reintroduction projects of both species began in the 1970s and 1980s and continue to the present. Despite this large focus on species conservation, little attention has been given to the consequences these reintroductions have on the genetic composition of the reintroduced populations and if the populations have a chance of persisting in the long term. These species have not yet benefited from the large range of genetic and genomic techniques currently available to non-model organisms, leaving many fundamental aspects of their reintroduction poorly understood. In my dissertation, I investigate demography, population structure, genetic diversity and inbreeding at the population and metapopulation level in both species. In the introduction, which lays the foundation for the subsequent chapters of this PHD, I provide background on reintroduction, its role in conservation and the genetic consequences on populations, especially populations of apex and mesocarnivores. In Publication I, I investigated the reemergence of the European wildcat in a low mountain region in Germany using fine-scale spatial analysis. I found that the reintroduced population has persisted and merged with an expanding natural population. The reintroduced population showed no genetic differentiation from the natural population suggesting there is a good chance this population has retained sufficient genetic diversity despite reintroduction. In Publication II, I tracked population development and genetic diversity over 15 years in a reintroduced lynx population to determine the genetic ramifications on a temporal scale. I found slow genetic erosion after a period of outbreeding, which fits in line with other reintroduced taxa sharing similar demographic histories. I also found the

number of genetic founders to be a fraction of the total released individuals, indicating that reintroduced populations of elusive carnivores may have fewer founder individuals than previously thought. In Publication III, I sampled all surviving lynx reintroductions in West and Central Europe as well as 11 natural populations to compare levels of genetic diversity and inbreeding across the species distribution. I found that all reintroduced populations have lower genetic variability and higher inbreeding than natural populations, which urgently requires further translocations to mitigate possible negative consequences. These translocations could stem from other reintroduced populations or from surrounding natural populations. The results contribute to a growing body of evidence indicating that inbreeding is likely to be more prevalent in wild populations than previously understood. Finally, in the discussion I explore how genetic methods can be applied to post-reintroduction monitoring of felid species to illuminate questions relating to genetic composition after release. The methods employed in these studies and in future work will be highly dependent on the research questions posed. Additionally, I investigate the drivers of the observed genetic patterns including founder size, source population, environmental factors, and population growth. I found that genetic diversity loss patterns across these two felid species are not clearly defined, however, management actions can be taken to mitigate the negative effects of reintroductions. These management actions include further translocation, introducing a sufficient number of released individuals and situating reintroductions adjacent to natural populations. All of these actions can minimize genetic drift and inbreeding, two factors which negatively impact small populations. This thesis further supports mounting evidence that genetic considerations should be assessed before releasing individuals, which allows for incorporation of scientific evidence into the planning process thereby increasing the overall success of reintroduction projects. Ultimately, the resources developed during this dissertation provide a solid baseline and foundation for future work regarding the consequences of reintroductions. This is especially important as an increasing number of species are at risk of extinction and reintroductions of both the European wildcat and Eurasian lynx, as well as many others, are planned in the coming years.

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1. General Introduction

Rapid climate change and unprecedented biodiversity loss are the most pressing issues facing the current generation (Díaz et al. 2019) and estimates of global and regional species extinction rates are continually increasing (IPBES 2019). Historically, during periods of climatic change, species would gradually adapt to new environments, experience range shifts to find suitable habitat, or they would go extinct (Lawler et al. 2013). However, in the modern context, species face a wider range of barriers associated with changing climatic conditions (Urban 2015). Climatic change is occurring at unprecedented rates meaning adaptation may not occur fast enough to keep up with the changing environment. Given increasing temperatures, many species are predicted to experience severe range contractions, exacerbated by anthropogenic pressures (Hoegh-Guldberg et al. 2008). Further, when a range shift does occur, it generally involves dispersal into or across heavily human dominated landscapes, a new challenge compared to past climate shift events (Hoegh-Guldberg et al. 2008; Lambers 2015; Lawler et al. 2013).

Dispersal or colonization in areas of high human density may not be possible given that human activity drives some of the most destructive practices contributing to the current levels of biodiversity loss (Newbold et al. 2015; Segan et al. 2016). Habitat loss and fragmentation arguably play the largest roles in the demise of species persistence in the modern era (Segan et al. 2016). The link between habitat loss and fragmentation and the loss of biodiversity is two-fold. First, species dependent on a specific habitat are likely to be lost when the habitat is significantly altered (Fahrig 2003). It is important to note that there is a time-lag between habitat loss and species loss (Brooks et al. 1999). Second, habitat fragmentation creates small populations that can be partially or completely isolated, increasing the risk of extinction (Merriam and Wegner 1992). Other factors contributing to anthropogenic drivers of species extinction include overexploitation, pollution, persecution, and competition induced by biological invasions (Peres 2001; Rosser and Mainka 2002; Beissinger 2000). These pressures rarely act in isolation; a growing amount of research investigates how these stressors interact synergistically and can worsen the outlook for already vulnerable populations (e.g., Mantyka-pringle et al. 2012; Maulvault et al. 2018, Betts et al. 2019). All of these factors combined create conditions where many species have already been lost or are at risk of experiencing drastic declines or extinction in the near future (Urban 2015).

The loss of biodiversity is concerning given the complex network of ecosystem services each species provides. Maintaining species diversity is also crucial to continued ecosystem functioning, which provide many services humans rely on (Weiskopf et al. 2020; Cardinale et al. 2002). Among

other things, biodiversity supports food security, provides livelihood security and important resources; plays an important role in regulating infectious diseases; has social, cultural, and spiritual importance; is essential for climate change adaptation; and can reduce the impact of natural disasters (Science for Environment Policy 2015). Some have argued that species loss may not correlate to noticeable changes in ecosystem functioning if multiple species are present that fill similar ecological niches (Naeem et al. 2007). Species near the top of food webs, like apex and mesopredators, are generally not redundant and their loss can have significant impacts downward through the ecosystem (Estes et al. 2011; Paine 1980). Even if we concede that not all biodiversity loss will contribute to a loss in ecosystem functioning, we know that in cases where a species provides a unique service with possible cascading and cryptic effects, impacts on the ecosystem will certainly be noticeable, especially in the long term (Cardinale et al. 2012). Additionally, preserving biodiversity on a whole, even in cases where a role is redundant, can aid in preserving cryptic functions that are difficult to quantify and maintain ecosystem functioning over a longer temporal scale (Cardinale et al. 2012; Philpott et al. 2012, Hooper et al. 2005). Therefore, given the vast array of services humans receive indirectly from maintaining biodiversity, mitigating human-mediated pressures and preserving and restoring species, communities, and ecosystems should be a priority.

Despite the large incentives, increasing number of publications, and sizeable efforts to stop biodiversity loss, current conservation may not be adequate to prevent continued ecosystem degradation (Hoegh-Guldberg et al. 2008). This is especially true of apex predators, as these species have already become locally extinct over the past century despite their function in a variety of processes from regulating invasive species to nutrients cycling (Schmitz et al. 2010; Strong and Frank 2010; Ripple et al. 2014; Prugh et al. 2009). This disproportionate loss of many apex predators is likely due to their increased vulnerability to extinction (Duffy 2003). Apex consumers are more sensitive to habitat loss, specifically fragmentation due to their large ranges and low densities (Noss et al. 1996). Additionally, these predators are disproportionately affected by human persecution in both aquatic and terrestrial ecosystems (Hayward and Somers 2009; Strong and Frank 2010). When apex predators are lost, mesopredators can become the subsequent targets of human persecution and, in some cases, could lead to the local extinction of the mesopredator populations as well (Larsson et al. 2019; Piechocki 1990b). As many of these apex and mesopredators provide functionally unique roles in their respective ecosystems and have become locally extinct, resource and conservation managers must consider the best strategies moving forward.

In cases where a species has already become locally extinct, practitioners must contemplate translocating individuals to sites where the species does not presently occur to form new populations, aiming to restore the ecosystem to a state before human interference (Hoegh-Guldberg et al. 2008). This practice of reintroduction, deliberately releasing organisms into the wild either from captivity or captured and translocated from other populations, is becoming a highly utilized conservation tool (Taylor et al. 2017). Apex predators, mainly carnivores, are well represented among reintroduced populations globally. Possibly owing to their visual and emotional appeal, key role in top-down ecosystem functioning, and severe declines due to anthropogenic change, these species have been selected for a large number of reintroduction projects (Jule et al. 2008; Polak and Saltz 2011). However, the outcomes of reintroduction projects vary with many failing post release due to the lack of a theoretical framework based on research (Armstrong and Seddon 2008; Griffith et al. 1989). Understanding how reintroduced populations establish in their new environment and investigating the components contributing to successfully bringing a species back to its native range is crucial to the conservation of many species. To date, the majority of literature looking at reintroductions do not (i) target questions relevant for management, (ii) compare outcomes from different management strategies, or (iii) provide frameworks that can be used before species are reintroduced (Taylor et al. 2017; Seddon 1999). Therefore, we need scientific evidence to fill in these knowledge gaps to best maximize the success of reintroduction in practice.

In this introduction, I first review current thinking in reintroduction literature and summarize research to date relating to each aspect of reintroduction biology. I then present the Eurasian lynx (*Lynx lynx* Linnaeus, 1758) and European wildcat (*Felis silvestris* Schreber, 1777), two candidate species to evaluate questions relating to reintroduction, as they have been the subject of multiple conservation attempts. Finally, I present the aims of this thesis, which examine constructing demographic histories of reintroductions, comparing reintroductions to natural populations, evaluating different reintroduction outcomes to identify patterns in genetic diversity loss, and providing a framework for continued a priori research in the future.

1.1 The role of reintroduction in conservation

Reintroduction is a form of conservation translocation aimed at releasing a species from either captive or wild sources into its native range to ultimately create a self-sustaining population (full list of terms found in Table 1; Corlett 2016). Reintroductions date back to the early 1900s and gained more widespread use and acceptance in the 1970s and 1980s. These early attempts are marked with little planning and almost no post-reintroduction monitoring (Seddon et al. 2007). Since the 1980s, given the number of species that have gone locally extinct and the rise in conservation awareness and management, the number of reintroductions has increased rapidly (Figure 1; Seddon and Armstrong 2016). From this rise, the scientific field of reintroduction biology emerged with aims to integrate conservation policy with theories from the fields of ecology, demography, taxonomy, and more recently, genetics (Lauber et al. 2011). Reintroduction biology is therefore, at its core, an applied science, providing evidence-based information to aid in providing better management strategies. It is also increasingly considered under the umbrella of ecosystem restoration and rewilding, which aims to restore species, communities, ecological systems to what they were before human impact.

Table 1. Major Terms mentioned in relation to reintroduction biology and surrounding topics, briefly explaining their current definition highlighting the differences between each term (Corlett et al. 2016).

Umbrella Term	Specific Terms	Main Definition
Conservation Translocation	Reintroduction	Release within previous native range
	Reinforcement	Release into an existing population
	Assisted gene flow	Release within native range to assist adaptation
	Pleistocene reintroduction	Release within the Pleistocene range
	Conservation introduction	Release outside the native range
	Assisted colonization	To avoid extinction
	Assisted migration	To keep up with climate change
	Ecological replacement	To restore an ecological function
	Restocking	Mostly of harvested wild populations
Rewilding	Trophic rewilding	Introductions to restore top-down trophic interactions
	Pleistocene rewilding	Restoring to a pre-human Pleistocene baseline
	Ecological rewilding	Allowing natural processes to regain dominance
	Passive rewilding	Little or no human interference

Despite these lofty aims, there remains a disparity between field conservationists and scientists. This gap leads to discrepancies between practical conservation work and the theoretical background that should support any effort in preserving and restoring species, communities, or ecosystems. This ‘research-implementation gap’ (Knight et al. 2008) has been identified in many

areas of conservation biology, however, reintroduction biology is one of the most striking examples (Armstrong and Seddon 2008). This gap stems from reintroduction biology being a relatively young field driven primarily by practical applications and suffering from the lack of a theoretical framework based on research questions (Armstrong and Seddon 2008). In turn, this gap manifests in extremely low success rates of reintroductions across all taxa (Griffith et al. 1989; Fischer and Lindenmayer 2000). Several studies have attempted to quantify the overall success rate of reintroductions spanning flora and fauna, finding success rates of 26% for animals and 35% for plants (Fischer and Lindenmayer 2000; Godefroid et al. 2011). However, the reported rates are mainly based on evidence found in published articles suggesting the true success rates are even lower, given that may failed projects will never be published (Fischer and Lindenmayer 2000; Miller et al. 2014).

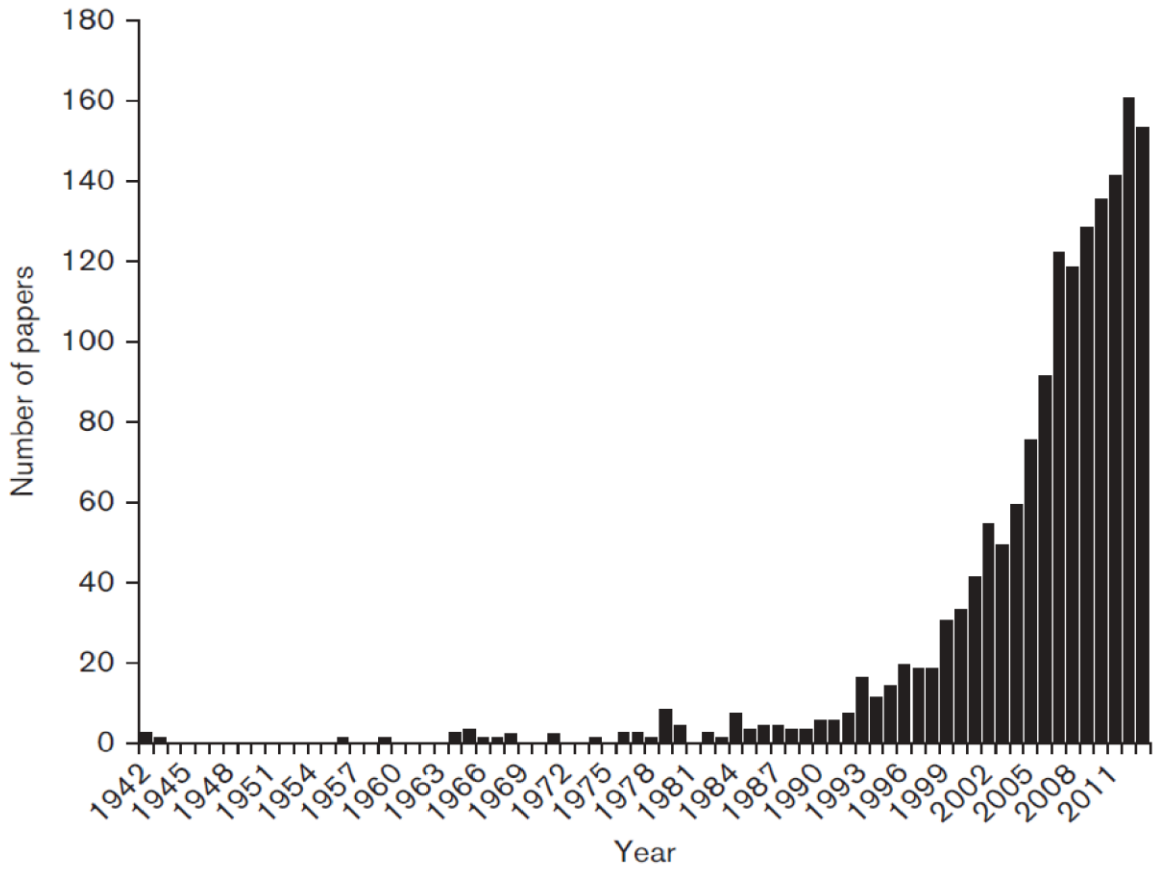

Figure 1. Number of published articles in peer reviewed journals referring to reintroduction from 1942 to 2014 (Seddon and Armstrong, 2016).

Such low success rates indicate that there are overarching issues with reintroductions which prevent released individuals from establishing viable populations in the receiving habitat (Seddon et al. 2007). Many factors contribute to the short- and long-term success of reintroduction programs and adequately quantifying these effects is a key goal in reintroduction biology. Successful reintroductions require released individuals to both establish and persist in the target habitat. Establishment refers to survival and reproduction of released individuals (Seddon et al. 2014), and persistence refers to the increase in numbers and density of reintroduced species in the recipient habitat in the long-term (Armstrong and Seddon 2008). Multiple factors can impinge on these phases requiring proper planning and management strategies.

The political, societal and cultural landscape tends to be an undervalued component to reintroductions. Carnivore reintroductions, more so than other species, tend to be controversial (Clark et al. 2002; Lüchtrath and Schraml 2015; Wilson 2004). The controversial nature arises from the negative bias of historical and cultural attitudes (Boitani and Linnell 2015). An additional source of tension arises from the possible conflict between humans and carnivores, not only in possible 'face-to-face' interaction, but also the perceived and real effects on human livelihoods (Breitenmoser 2000). There has been a great shift in public perception since the mid-20th century, moving towards a positive perspective on conservation and reintroduction of carnivores (Kellert et al. 1996). However, this general trend is not equally distributed, those more likely to be affected by human-wildlife conflict, like hunters and farmers, are understandably more reluctant to hold a positive view of reintroduction (Breitenmoser et al. 2001). For many populations of carnivores, illegal killings are the major threat to population expansion (von Arx et al. 2004). It is therefore essential to involve numerous stakeholders in the process of reintroductions to increase public acceptance and consequent success (Ovenden et al. 2019). Habitat and ecosystem considerations including the impact reintroduction will have on the ecosystem and how the current environment supports or hinders the establishment and persistence of a population in the long term. If the original factors leading to extirpation have not been corrected, or at least mitigated, the quality of the receiving habitat can directly affect the survival of released individuals (Sarrazin and Barbault 1996; Moorhouse et al. 2009). There has been a substantial push for reintroduction programs to consider the broader ecosystem questions, however, because there are multiple interconnected networks at play, these outcomes are difficult to foresee before reintroduction is carried out (Seddon and Armstrong 2016).

Increasingly, genetic factors are recognized as playing a significant role in the ability for a reintroduced population to persist in the short- and long-term (Frankham 2009). As the genetic

makeup of a population contributes to the risk of its extinction, it is important to assess the genetic components of any reintroduction strategy. Despite major advances in genetic techniques and the application of these techniques across a variety of taxa under controlled conditions, it can be difficult to obtain data for wild populations, especially in carnivores, which remain at low densities and pose difficulties to being physically captured (Mumma et al. 2015). Therefore, exploring the genetic consequences of reintroduction programs, especially, in carnivores, will give us better insight into how wild populations react to being reintroduced into their native habitats. Only with a full understanding of how reintroductions impact the population on a genetic level can we determine methods for better management and focus on areas of uncertainty in the field. In the following section, I examine in greater detail the genetic considerations of species reintroduction.

1.2 Genetic Considerations in Reintroduction Programs

The genetic considerations of reintroducing species have been increasingly recognized to play a pivotal role in the success or failure of reintroduction programs (Frankham 2009). This increased awareness resulted from the advancement of molecular genetic techniques and the increased ability and ease to sequence non-model organisms. These advancements have allowed for investigation into small populations at a genetic level and a better understanding of why small populations are at a higher risk of extinction. Most reintroduction programs have exceedingly small founding sizes; 50% of reintroduction programs have released less than 30 individuals, and 72% have released less than 75 individuals (Griffith et al. 1989). In a review of published articles, only 14 studies relating to the reintroduction of 200 or more individuals have been published (Fischer and Lindenmayer 2000). Therefore, when discussing the genetic considerations in small populations, we are inherently discussing reintroduced populations, as these constitute some of the smallest populations, particularly at their founding.

Small populations are more likely to experience extinction given the loss of genetic diversity due to inbreeding and genetic drift, which are unavoidable if no gene flow is present (Frankham 2009). The loss of genetic diversity in small populations is inversely proportional to the population's effective population size (Nei et al. 1975). This loss can have impacts on fitness as functional genetic diversity can influence a population's ability to adapt to changing environments (Lande and Shannon 1996). While genetic drift occurs in all populations, the effects are magnified in small populations, especially as a result of the founder effect. The founder effect is an extreme example of genetic drift, where only a small fraction of individuals split off to form a new population. This mimics what occurs in a reintroduction and allele frequencies will likely change given that the founding individuals may not represent the full spectrum of genetic diversity found in the original

population (Figure 2). This is especially true in already threatened populations as genetic diversity may already be lower than historical values. Additionally, the prevailing method among conservation managers is to use animals from locally adapted populations as sources for reintroductions or reinforcements instead of mixing sources or lineages. This is done to avoid outbreeding depression (Weeks et al. 2011) and restore intraspecific biodiversity comparable to the assumed historical state.

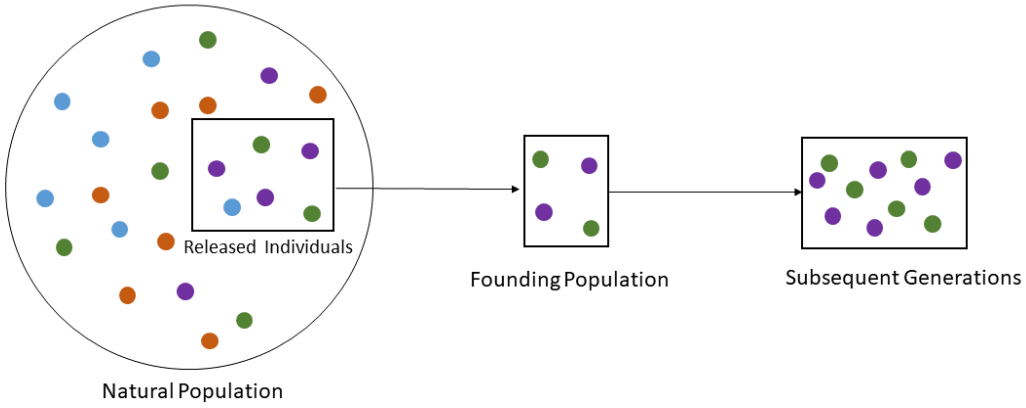

Figure 2. Example of founder effect within a reintroduced population, specifically how founder effect can influence genetic and phenotypic variation in the resulting population after subsequent generations.

Outbreeding depression is the reduction of fitness that results from crossing genetically distant individuals (Frankham 2010). Introduced alleles can cause an impaired adaptation through a genotypic combination that is not well suited for the local environmental conditions (Huff et al. 2011). The most well-known example is the Alpine ibex (*Capra ibex* Linnaeus, 1758) population in the Tatra Mountains that went extinct after the introduction of individuals from several different subspecies causing mating and birth time of hybrids to shift adversely (Turcek and Hickey 1951). Given this extreme example, the risk of outbreeding depression is taken very seriously when planning and executing reintroduction, especially with regards to mammal populations where the resources used for reintroduction are much higher. However, more recent evidence points towards the risk of outbreeding depression being lower than previously thought, in some cases populations from mixed sources can be just as successful and maintain higher genetic diversity (Frankham et al. 2011).

The other major consideration in small populations is the higher likelihood of inbreeding, which is particularly abundant in several large carnivore populations, particularly felid species (Buk et al. 2018; Grauer et al. 2017; Abascal et al. 2016). Inbreeding becomes more likely with decreasing

population size and when inbreeding is present there are considerable consequences for the future genetic makeup of a population. Inbreeding also contributes to decreased genetic diversity and can consequently cause long-term reduced fitness (Jamieson 2011). In populations of reintroduced species, especially mammals, inbreeding is unavoidable and therefore must be minimized through active management (Frankham 2009, 2010). Further, these genetic considerations (genetic drift and inbreeding) can interact synergistically with demographic considerations including environmental variation and catastrophic events, which makes small populations even more vulnerable to extinction.

Therefore, the goals of reintroduction are generally to create a self-sustaining population, which has mitigated to the extent possible the detrimental genetic consequences of small population size. While theoretical knowledge is extremely helpful to provide context to the observed phenomena, reintroduction biology remains a crisis discipline based on integrating science and action. Setting realistic goals that meet genetic, demographic, political, and social constraints is difficult to achieve. Nonetheless, a few practical genetic goals in reintroduction programs have been derived and are aimed at minimizing the key deleterious genetic effects. The first goal is the long-term preservation of genetic diversity. Soule (1986) suggested a 10% loss over 200 years represents an acceptable level of genetic diversity loss where the evolutionary potential will not be compromised when beginning a captive breeding program. This can be extended to reintroduced populations as this also constitutes the formation of a new population from an existing one. Second, reaching genetic diversity levels comparable to healthy extant populations can be an indicator that a reintroduction project has sufficient genetic diversity, however, few studies draw comparison to source or extant populations (IUCN 2013). In fact, a review of reintroduction literature found that only 4% of studies address issues at a metapopulation level (Taylor et al. 2017). Last, a reintroduction program should aim to prevent the accumulation of inbreeding given the deleterious effects this can have on the population. Monitoring genetic diversity can not only reveal levels of outbreeding and inbreeding but can also provide detailed insight into population dynamics, demography, genetic status, and trends. Post-release monitoring of reintroduced populations is becoming more common (Armstrong and Seddon 2008), likely due to advances in sequencing technology and the ability to sequence DNA from non-invasive material (e.g., urine, scat, hair), which makes tracking carnivore movement and populations easier (Steyer et al. 2016).

1.3 The European Wildcat

The wildcat (*Felis silvestris* Schreber, 1777) is a felid with a large native range including Asia and Europe with 2 recognized subspecies (Kitchener et al. 2017, Figure 3). Although there is still discussion on the exact number of subspecies, the European wildcat (hereafter referred to as wildcat) has long been accepted as a distinct subspecies (Ottoni et al. 2017). In historical times, the habitat of the wildcat was extensive, spanning from Europe, including the Iberian Peninsula, into central Asia. Males are larger than females weighing 3−6.5 kg and measuring on average 0.91 m compared to females at 2.3−4.9 kg and 0.83 m (Piechocki 1990b). The primary habitat is forested land, specifically broad leafed and mixed forest, which provides shelter in the form of den cavities and access to high densities of small mammals (Sarmento et al. 2006). The European wildcat preys primarily on rodents, however, other small mammals including lagomorphs may contribute substantially to their diet as well (Sarmento 1996). Home ranges differ based on sex; males have larger territories ranging significantly across the distribution from 4 to 25 km^2 (Anile et al. 2018). Females, in contrast have smaller home ranges, anywhere from 1.63 to 6.24 km^2 has been observed (Anile et al. 2018). Reproduction usually begins after two years, with mating occurring from January to March producing litters during spring and summer, with an average of 3 to 4 kittens in each litter (Piechocki 1990b).

Figure 3. Map showing present-day distribution of *Felis silvestris* with the range of each subspecies (Kitchener et al. 2017). *Felis silvestris silvestris* remains in isolated regions within Europe due to heavy persecution in the 1900s. The border between subspecies remains speculative.

1.3.1 Brief History of European Wildcat between 1800−1950

The decline in wildcat populations across Europe began at the turn of the 19th century. Before this, wildcat populations had likely experienced a period of expansion give the decline of apex predators like wolves and lynx (Prugh et al. 2009; Ritchie and Johnson 2009; Ripple et al. 2013; Ripple et al. 2014). However, hunters and forest owners turned to smaller carnivores such as the wildcat after larger carnivores experienced severe declines (Piechocki 1990a). Following the proclamation of a trophy price for hunted wildcats in 1781, populations suffered from massive persecution and experienced intense range contraction, resulting in a strong population bottleneck between 1920−1930 in Central Europe (Piechocki 1990a). The wildcat's habitat was severely restricted into a few refugial areas across Central Europe, mainly dense forest where the species could avoid detection (Eckert et al. 2010; Piechoki 1990b). In some areas of Europe, the wildcat became extinct. In Germany, the wildcat was largely restricted to small populations in the Palatinate Forest, Eifel, and Harz Mountains with further refugia in areas like Solling and Hainich suspected (Piechoki 1990b). These substantial declines in population and further isolation of populations put the wildcat at risk for increased genetic drift, inbreeding and hybridization with feral domestic cats (Eckert et al. 2010).

1.3.2 Natural Recovery

The wildcat benefited greatly from strict legal protection in the 1930s, leading to a complete hunting ban (Haltenorth 1957). This allowed for small, refugial populations to begin recovering. Current populations of wildcat across Europe are split into five genetically distinct geographic groups (Mattucci et al. 2016). One hypothesis for the distinction between these five groups is isolation in glacial refugia during late Pleistocene (Mattucci et al. 2016). However, a recent study shows that the Central German cluster is likely a result of recent genetic drift due to persecution and isolation in refugia (von Thaden et al. in prep). Therefore, while there is clear population structuring occurring in natural populations across the species distribution, the causes of these patterns remain unclear. The recent reemergence of wildcat across Germany has largely been attributed to the expansion from refugial populations in West and Central Germany (Steyer et al. 2016). This form of natural reemergence is a trend currently seen across multiple carnivore species across Europe (Chapron et al. 2014). The current German populations can be split into two distinct clusters, West and Central, and low levels of hybridization with domestic cat are seen across the German range (Steyer et al. 2016). The Western lineage showed higher levels of gene-flow, possibly due to its connection to larger populations in France and Belgium, while the central

populations reside at the edge of the species distribution and are not connected to other populations (Steyer et al. 2016).

1.3.3 Reintroduction of the Wildcat in Germany

Despite natural reemergence at the turn of the century, earlier conservation projects focused on returning this small carnivore to its native range through active reintroduction. Beginning in 1984, a captive breeding program emerged, which took individuals from various sources, many from Eastern Europe, and bred them in captivity, releasing offspring into three locations in Germany (Worel 2009, Figure 4). The reintroductions ended in 2011 and while the actual released number of individuals remains unknown, it was estimated that over 600 individuals were released (Worel 2009). Little systematic, well documented field, or genetic monitoring was ever carried out to determine if animals established a population or persisted in the region.

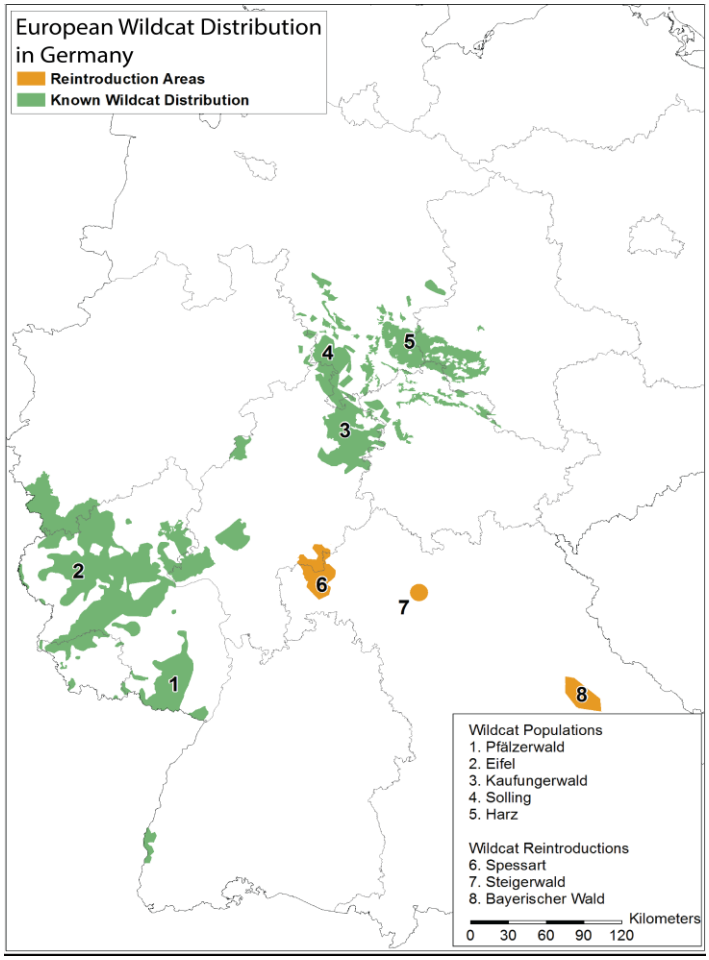

Figure 4. Map of known wildcat distribution in Germany (green), as documented by Birlenbach and Klar (2008). Additionally, the three reintroduction areas are shown (orange). Figure adapted from Birlenbach and Klar (2008).

1.4 The Eurasian Lynx

Belonging to the genus *Lynx*, which consists of four species across the northern hemisphere, the Eurasian lynx (*Lynx lynx* Linnaeus, 1758) (hereafter referred to as lynx) is the largest and most expansive of the species. Historically, it could be found across Europe into Russia and Scandinavia and as far south as southern France (Kaczensky et al. 2012; Breitenmoser 2000). Females weigh around 16−20 kilograms, while males weigh on average 26 kilograms, with some reaching maximum weights around 30kg (Breitenmoser 2000). The lynx mainly resides in forested habitats preying upon ungulates as they have a strong preference for larger prey (Molinari-Jobin et al. 2007). However, hares, rodents, mustelids, and birds also contribute to their overall diet (Andersen et al. 2007; Odden et al. 2006). Lynx territories range from 100−1000 km² and are dependent upon, among other things, the density of available prey (Herfindal et al. 1999). Males tend to inhabit larger territories which can be shared with one or two females (Herfindal et al. 1999; Breitenmoser-Würsten et al. 2007b). Mating takes place in late winter (February - April) and sexual maturity is reached at approximately 2 years of age. After a gestation period of 63-75 days an average of two or three young are born, although litter sizes of up to five are possible (Breitenmoser-Würsten et al. 2007a; Anders and Middelhoff 2016). Juvenile lynx tend to leave around ten months of age to establish their own territory (Zimmermann et al. 2005).

1.4.1 Brief History of Eurasian Lynx between 1800−1980

Over the last four centuries the lynx has experienced a severe decline across its European range. By 1850, no lynx were present in Germany and very few in neighboring countries (Linnell et al. 2001). Multiple factors led to the species' expatriation including limited prey abundance, increased urbanization and habitat fragmentation (Linnell et al. 2001, Breitenmoser 2000). Additionally, there was a high level of hunting pressure exerted on the lynx, as it was a predator of game species and livestock; some countries even had bounty programs in place (Basille et al. 2009). While little is known about the exact dates of expatriation in each country, a 1968 census by Kratochvíl (1968), determined that outside of Scandinavia, Baltic countries and the Carpathian Mountains, the lynx was nowhere to be found (Figure 5). A few documents also provide insight into the population history in certain regions. There are documents of the last lynx shot in the Harz from 1818 and other specimens collected from the Swiss Alps and Swabian-Jura dating to 1910 and 1846. Between 1800 and 1960 the lynx showed a 48% decline in the total range of the species coupled with significant losses in numbers of individuals as well (Deinet et al. 2013) (Figure 5).

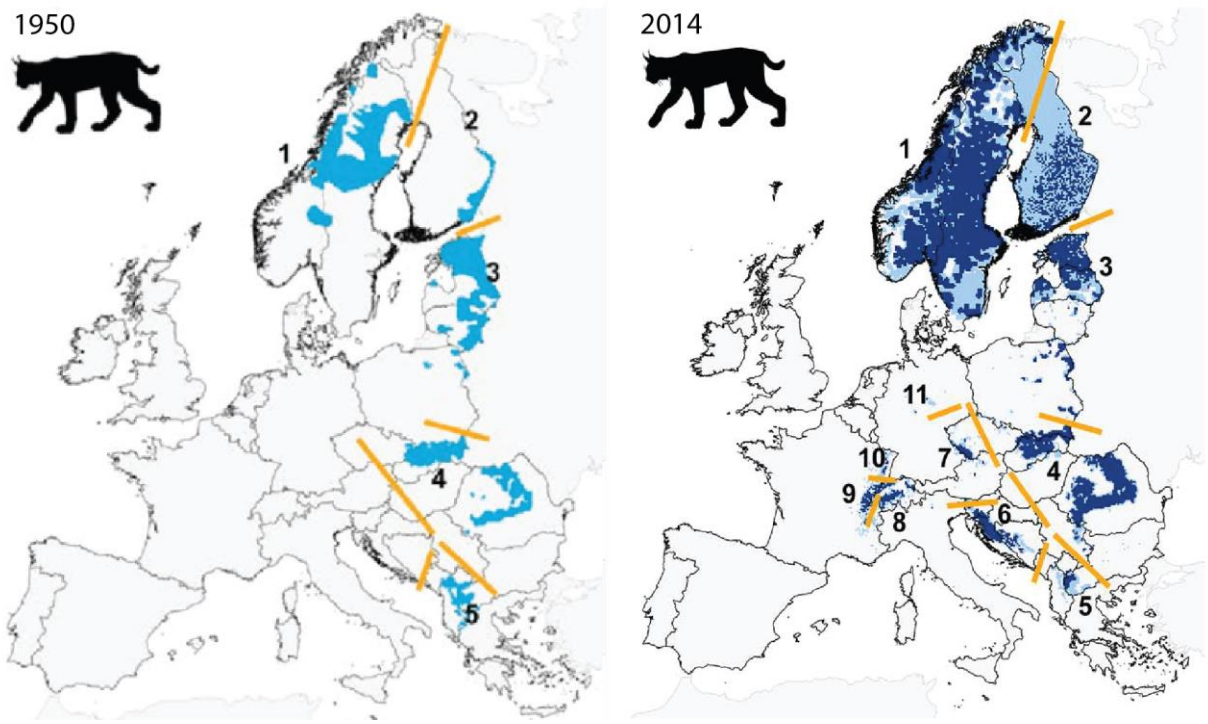

Figure 5. Map of Eurasian lynx distribution in the 1950s compared to distribution as of 2014 (Chapron et al. 2014). 1: Scandinavian population, 2: Karelian population likely connected to the larger Kirov population in Russia, 3: Baltic population, 4: Carpathian population, and 5: Balkan population. The reintroduced population visible on the right consist of 6: Dinaric, 7: Bohemian Bavarian Austrian, 8: Swiss Alpine and NE-CH, 9: Swiss Jura, 10: Pfälzerwald, and 11: Harz.

During the late 20th century, a change in legislation as well as public perception paved the way for both natural and human mediated carnivore return (Deinet et al. 2013). The active conservation action such as the legal protection of the species and its habitat likely contributed widely to their comeback. In addition, conservation measures including reintroduction and continued translocation have brought the lynx back to many regions where a natural recolonization would have been unlikely. The lynx was listed on CITES (Appendix II) in 1975, protected under the Bern Convention (Appendix III) in 1988, and EU Habitats and Species Directive (Annexes II and IV) in 2001 and is therefore strictly protected in all EU member states except Estonia, where it is included on Appendix V (Kaczensky et al. 2012). The political development within Europe, specifically within the European Union, created new, promising opportunities for large carnivore conservation on a European-wide scale.

The combined legal action protecting the habitats of lynx and the lynx itself from hunting in excess has led to a 37% increase in occupied area in the second half of the 20th century (Deinet et al. 2013). These increases in range and abundance in the lynx appear to be associated with specific countries and regions. The countries with the most pronounced recoveries were Austria,

Germany, and France, all in the Western European region. In this region the range increase is a clear result of the reintroductions in areas from which the lynx had previously been extirpated. In Eastern Europe, the remaining wild populations appear to be declining, however, our understanding of this population is incomplete as investigation into these populations remains limited.

1.4.2 Remaining natural populations

Natural lynx populations, meaning populations that are extant and not reintroduced, can be divided into 7 populations representing 12 countries (Table 2). Carpathian, Baltic, Scandinavian, and Balkan populations are assumed to be bottlenecked populations with decreasing trends between 1996 and 2001 (von Arx et al. 2009; Breitenmoser 2000). Populations spanning Russia and parts of Asia are reported as stable (Rueness et al. 2014; Tang et al. 2019). However, this remains unclear as limited surveys of lynx in Asia have taken place and there has been evidence of extensive harvesting in these populations (up to 4000 skins exported annually; Matyushkin et al. 2003).

Table S2. List of natural populations, which region and population the samples belong to (European populations defined by the European Commission, asian populations defined by geographical region in Lucena-Perez et al. (2020)). Additionally, the current status and approxiamte population size as estimated by the European Commission and the IUCN, and any additional population history comments that could have impacted the genetic composition of the population.

Region	Population	Population Size	Status
Carpathian	Carpathian	~2,400	Stable
Poland	Baltic	~1,500	Small Decrease
Latvia	Baltic	~1,500	Small Decrease
Estonia	Baltic	~1,500	Small Decrease
Finland	Karelian	~2,500	Stable
Norway	Scandinavian	~1,800	Decreasing
Kirov	Western Russia	~30,000	Stable
Ural	Western Russia	~30,000	Stable
Tuva	Eastern Russia	~30,000	Stable
Mongolia	Mongolia	~10,000	Stable
Yakutia	Eastern Russia	~30,000	Stable
Primorsky	Eastern Russia	~30,000	Stable

1.4.3 Return of the Lynx in West and Central Europe

Over the last 50 years, 17 reintroduction attempts of lynx have been carried out in Central Europe (Linnell et al. 2009, Idelberger et al. 2021, in press; Molinari et al. 2021 in press). Many of these reintroduction attempts failed post-release due to the lack of planning behind the releases (von Arx et al. 2009). While major strides have been made in the political and cultural sphere to create a favorable environment for the lynx return, persecution and low acceptance of the general public, as well as infrastructure development are still major threats throughout Europe and likely led to the failure of some projects (Breitenmoser 2000). There are six surviving reintroduced populations remaining. Each population history has left an impact on the genetic make-up and status which will continue to shape the population demography for decades to come. A brief summary of the reintroduction projects can be found below, with a more detailed description in Publication III, Appendix I.

In the 1970s, lynx were translocated from the Slovakian part of the Western Carpathians to four different reintroduction sites. Two sites were in Switzerland: one site was in the Swiss Alpine region (ALP) (Breitenmoser et al. 1998) and the last site was in the Swiss Jura Mountains (JURA) (Breitenmoser et al. 2007; Breitenmoser and Baettig 1992). Another project began in the Bavarian National Park, Germany, later supplemented by releases in the neighboring Šumava National Park in the Czech Republic (BBA) (Červený and Bufka 1996). Last, a project in the Dinaric Mounatins in Slovenia (DIN) (Čop 1987; Figure 5). The number of released individuals varied in each project. The Swiss reintroductions had 10-12 individuals each, however, they were released over 5 sites in each respective region. The original release in the Bavarian Forest of 5-10 individuals was later supplemented at another site with 17 individuals. Finally, the Dinaric release consisted of 6 individuals. No genetic information is available on founding individuals from any of these early projects, however, two known sibling pairs were released in Slovenia. In 2001, individuals from both the Swiss Alpine and Swiss Jura were translocated to create a secondary population in Northeastern Switzerland (NE-CH) (Robin and Nigg 2005). Around the same time, between 2000 and 2006, a reintroduction of 24 captive-bred individuals originating from zoos and wildlife parks was conducted in the Harz Mountains in Germany (HARZ) (Anders and Sacher 2005).

1.5 Objectives and Aims

Given the scope of the previous sections where I have introduced current scientific knowledge and general concepts, this thesis aims to determine the genetic consequences of reintroduction in two elusive European felid species that were returned into their native habitats in West and Central Europe. I also aimed to develop a better understanding of the status of these reintroductions in relation to natural and source populations. In order to give a more comprehensive and comparative look across studies, I have organized the main research questions as follows:

1. How can genetic methods be best applied to monitor felid reintroduction success?

2. What is the degree of genetic diversity loss and inbreeding in felid reintroductions compared to natural populations? Does this loss mainly occur directly after reintroduction as a result of founder effect, or as a continuous trend?

3. What factors contribute to observed patterns in genetic diversity loss and inbreeding?

4. How can we translate the results of genetic assessment to conservation action and monitoring, closing the 'research-implementation' gap and improving the success of future felid reintroductions in Europe and elsewhere?

I aim to discuss my results as they relate to overall observed patterns, with the goal to answer the questions laid out above. In addition, I discuss the result in regard to applied conservation management. This applied aspect is extremely timely in European felids given ongoing plans for forming viable metapopulations through reintroduction of additional populations in the lynx as well as local recovery of wildcat through captive breeding and reintroduction.

2. Discussion

Reintroduction biology aims to facilitate an evidence-based approach to the conservation practice of releasing locally extinct species into their native range (Taylor et al. 2017). Given that the outcomes of such projects are highly variable and there remains a 'research-implementation' gap, studies targeting areas of uncertainty relating to reintroduction outcomes are critical for maximizing future success. Despite the consensus that genetic components have a significant impact on the short- and long-term success of reintroductions (Groombridge et al. 2012), we lack sufficient understanding of the impact reintroduction has on a population's genetic composition. Multiple investigations of the potential impact reintroduction can have in small, isolated populations have been carried out (Frankham 2009; Hayward and Somers 2009). However, how these possible outcomes manifest in the wild remains limited given the logistical effort and cost associated with monitoring populations in the long-term and the range of genetic and genomic techniques available (Groombridge et al. 2012). More difficult yet is obtaining comparison to natural or historical baselines to draw broader conclusions about the status of reintroduced populations. This broader perspective and comparison can indicate the population's adaptive potential, which is an important consideration in the long-term. Therefore, leveraging study systems where long-term monitoring data as well as samples from natural or source populations are available will benefit the overall understanding of the impact genetic components play in population establishment and persistence.

In my PhD, I examined the genetic consequences of reintroduction in two elusive felids in the West and Central European range. Here, I highlight the major findings of these publications and how they relate to current knowledge within the study system. I then take a step back and look at how these publications contribute to the current knowledge in the field regarding the genetic consequences of reintroduction. Finally, I discuss how the genetic consequences identified in this study can enhance our knowledge of factors contributing to reintroduction success and bridging the gap between conservation practitioners and scientists.

2.1 Genetic Assessment of Reintroduced Felids in Europe

In this thesis, I wanted to determine if genetic methods can be used to expand post-release monitoring of reintroduced populations, particularly several years or decades after reintroduction. Genetic methods such as mtDNA, microsatellites, SNP genotyping, and GBS sequencing can be used to investigate genetic diversity, potential inbreeding, and population structuring (Schwartz et al. 2007). I will first explore the techniques used in each publication, their contribution to our understanding on genetic monitoring of reintroduced populations and discuss methodological considerations for felid reintroduction monitoring, including how to best apply these methods.

2.1.1 Fine Scale Assessment in the European Wildcat

In Publication I, we used a combination of microsatellite and SNP genotyping alongside mtDNA haplotypes on a fine spatial scale to describe the population genetic structuring within a wildcat population in a low mountain region in Germany. We found evidence of recent demographic growth representing one continuous population. Analysis of genetic diversity and population structuring showed no significant differences between individuals originating from natural and reintroduced regions. However, mtDNA haplotype evidence showed that genetic traces of past reintroduction, consisting of approximately 600 individuals over 24 years, was still present within the region. This reintroduced population showed signs of expansion into the surrounding regions, mixing with a natural population at the northern edge of its distribution as evidence by fine-scale spatial structuring resulting from sPCA analysis. We found that expansion into new territories is driven by male dispersal, as females carrying a unique mtDNA haplotype associated with reintroduction are only found in the known reintroduction area. On a broader scale, this case highlights the utility of employing several genetic methods to determine reintroduction persistence, even when field data is not available. It was necessary to use a combination of genetic methods, which is likely the case for all reintroduction programs as each method gives insight into a different area. Even years post-reintroduction, genetic methods can offer previously unknown insight into the success of a particular reintroduction, which is applicable across a wide range of reintroductions whose success remains unknown (Armstrong and Seddon 2008).

2.1.2 Temporal Assessment in the Eurasian Lynx

In Publication II, we tracked population development and genetic diversity over time in a reintroduced lynx population in central Europe. This population is the only reintroduced lynx population where monitoring occurred since founding, making it a candidate to determine the genetic consequences of reintroduction in felids. We utilized mtDNA haplotypes and microsatellite analysis in conjunction with demographic monitoring methods, like camera-trap and telemetry

evidence to reconstruct the demographic history since first release in this population. We found that the population underwent a demographic bottleneck following reintroduction, as we found evidence of 7 genetic founders of the 24 released individuals. This was followed by subsequent demographic and spatial expansion in the decade following. These demographic trends were contrasted by the genetic assessment, which found elevated levels of observed and expected heterozygosity in the years directly after reintroduction, followed by a slow decline in genetic diversity over time. We also found that the population growth is dependent on relatively few well-established highly reproductive individuals suggesting that further genetic erosion will occur as few individuals currently contribute to the gene pool.

Multiple studies have suggested that felids are difficult to reintroduce due to high spatial requirements and low population growth rates (Noss et al. 1996; Buk et al. 2018; Abascal et al. 2016), but this study is one of few that shows how populations are formed in practice. Here, the genetic monitoring was imperative to quantifying the loss of genetic diversity, gaining insight into the effective population size, observing the distribution of breeding success, assisting in census monitoring and identifying potential migrants. All these factors influence the success of a reintroduction. Without the genetic information, the population would have appeared stable and growing, indicating that further monitoring and action was not needed. Only when we include the number of alleles as calculated on a temporal scale along with pedigree information can we see that genetic diversity is in fact declining despite demographic increase. Further, this study illustrates that in the years post-release, the population is likely to experience fluctuations in the overall genetic diversity and consistent temporal monitoring is key to observe changes and better predict outcomes. Therefore, genetic methods are best applied when they are integrated as soon as possible into the effort for reintroduction: from the founding population into routine monitoring, especially in the decades following release.

2.1.3 Comparative Genomic Assessment in the Eurasian Lynx

In Publication III, we sampled surviving reintroduced lynx populations and 11 natural populations from across Europe and Asia to assess the current genetic status of reintroductions with the ability of comparison. Comparison of reintroduced populations to natural populations has been recognized as providing important baseline data critical to determining reintroduction success, however, comparative studies remain difficult to achieve mainly due to funding and resources for long-term studies (Monks et al. 2012). While all reintroduced lynx populations are routinely monitored in the frame of national programs, each lab utilizes different methods making comparison difficult. Therefore, we sampled all populations and utilized GBS sequencing to

produce 13,525 genome-wide SNPs for investigation, which allowed for comparison across all populations. This density of SNP markers allowed for an in-depth analysis, which is not possible with other genetic techniques. The next generation sequencing method provided a more robust consideration of current population status, as recent genetic trends, most importantly, current inbreeding can be disentangled from past events. We found genetic diversity loss in all populations of reintroduced lynx to differing degrees of severity. Reintroduced populations showed in some cases alarming rates of recent inbreeding, the worst of which occur in populations with the lowest number of released individuals. We also found that the source population for five of the reintroductions shows genetic impoverishment and signs of recent inbreeding, questioning if this population can provide sufficient genetic diversity for a reintroduction. This comparative analysis allowed for clear baselines regarding levels of genetic diversity and inbreeding, which can provide the basis for accurate allocation of conservation resources to populations that are most at risk to experience the negative consequences of small population size and inbreeding.

2.1.4 Methodological Considerations

We used three different approaches to examine the genetic consequences of reintroduction across populations and species. These examples showed the versatility of genetic markers to look at recent demographic histories of reintroduced populations and show the applicability of genetic methods to monitor and evaluate reintroductions, in the short- and long-term. The ability to mitigate the negative genetic consequences associated with reintroduction relies on our ability to detect them, and therefore the appropriate method must be chosen for the study system at hand. Genetic approaches using mtDNA, microsatellites and reduced SNP panels are most useful in post-release monitoring where non-invasive samples can be reliably used to discriminate individuals and build pedigrees, as shown in Publication I and II. Several studies have questioned if microsatellites are accurate predictors of overall genome-wide diversity, particularly when it comes to predicting inbreeding, which is a key source of negative genetic consequences (Väli et al. 2008; Slate et al. 2004; Hedrick 2001). I compared the genetic diversity measures of 13 individuals calculated from microsatellites in Publication II and the diversity measures calculated with 13,525 genome-wide SNP sites from Publication III to look for evidence of this pattern in our study system. I found that genetic diversity calculated from microsatellites is higher than when calculated across genome-wide SNP sites in the lynx (Figure 6). Similar trends are identified in the wildcat (unpublished data) and fits in line with these previous studies, suggesting that while overall trends are similar in both markers, GBS methods can likely provide better estimates of overall genome-wide diversity. However, the utility of microsatellite methods given their high

mutation rates and simple Mendelian mode of inheritance should not be overlooked. They are candidate markers for looking at fine population structure, mating systems and pedigrees (Abdelkrim et al. 2018). Additionally, it represents a cost-effective way to gather data on a broad scale from many different sample types which would otherwise be missed when considering genomic methods with higher sample requirements.

However, as shown in Publication III, the use of next generation sequencing techniques added valuable insight that would otherwise be missed. The analysis in Publication III adds to a small but growing list of publications that provide evidence of comparative analysis using GBS methods providing higher resolution investigation into population structuring, genetic diversity and inbreeding in reintroduced populations, which is important for embedding scientific evidence into reintroduction practice (Humble et al. 2020; Grossen et al. 2018). Additionally, tying this genetic diversity to functional traits will be extremely important to predicting phenotypic consequences in species with low genome-wide diversity and future studies should focus on quantifying this in the lynx study system.

In sum, genetic methods are key to determine reintroduction outcomes and should be integrated into future reintroductions of felid species to evaluate the status of released populations. This is especially important, as current reintroductions of lynx and wildcat are being planned and executed. The results of the publications here provide evidence that genetics must be integrated from project conception to enhance our understanding of population dynamics. Each genetic and genomic method has a unique functionality and correctly identifying where each can be applied, and where we can build upon these is vital for conservation planning in the future. The publications included in this thesis argue that next generation sequencing is a powerful tool for setting clear baselines and targets, but SNP panels and microsatellites provide invaluable data for specific population monitoring.

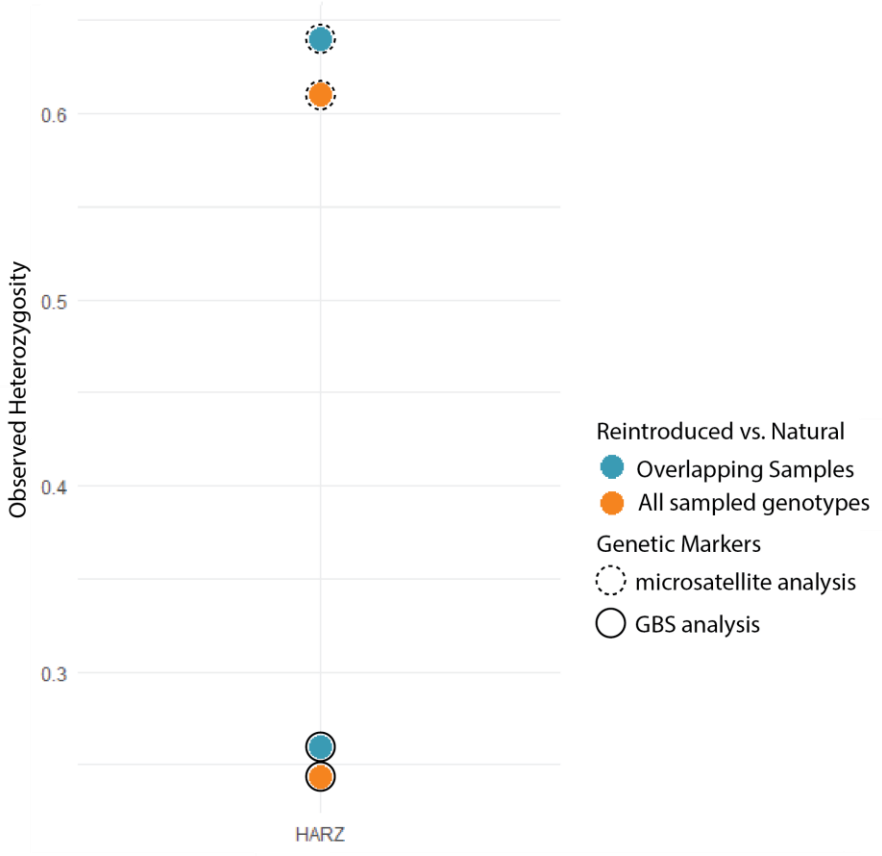

Figure 6. Comparison between observed heterozygosity using 19 microsatellite markers and 13,525 SNP loci. Measures were calculated across 8 overlapping samples used in both methods and across the entire sample set (141 samples with microsatellites and 13 samples with GBS methods).

2.2 Patterns in genetic diversity

In the introduction, I outlined a three major goals of reintroduction projects on a genetic level to avoid the detrimental effects of small population size. Briefly, these were to minimize loss of genetic diversity (10% in 200 years), maintain levels of diversity similar to natural populations, and minimize inbreeding to the best extent possible. However, reintroduction biology is far from an exact science and therefore we must explore how conservation management plays out in practice to determine if we can reach these goals. Therefore, one of the major aims of this thesis was to disentangle patterns in genetic diversity within reintroduced populations of felids and determine factors contributing to these patterns. Using the publications presented here, I will now examine patterns found in the two study systems and their causes.

In the publications presented here, there was no clear trajectory of genetic diversity following reintroduction. In the lynx, temporally declining genetic diversity in the resulting population was

identified (Publication II). When comparing different reintroductions of the lynx (Publication III), the trend of genomic erosion was present across all reintroductions to differing levels of severity. In the wildcat, there was no significant difference in levels of genetic diversity between the reintroduction area and surrounding natural population (Publication I). Several factors influencing the preservation of genetic diversity in reintroduced populations have been discussed, namely (i) levels of diversity in the source population prior to translocation, (ii) connection to wild populations, (iii) rate of population growth, (iv) features of the receiving environment and (v) the number of founders (Groombridge et al. 2012, Frankham 2009, 2010). In the following, I elaborate on these factors, highlighting the main findings in light of the results from the two study systems.

i) Levels of diversity in the source population prior to translocation

Levels of diversity in the source population have a direct impact on the genetic composition of the reintroduced population as the source contains the maximum number of alleles that can be passed to the resulting population. In many cases, especially in reintroductions involving small population sizes, founder effect and genetic drift further reduce the number of possible alleles that can be retained (Frankham 2009). Therefore, the genetic composition in source populations can be an indicator for potential reduction or enhancement of genetic diversity.

In Publication II, the source population for the Harz National Park lynx reintroduction was a variety of zoo individuals assumed to be from different lineages, namely Carpathian, European and Asian lineages (confirmed in Publication III). This variety in source population created a large pool of alleles that had the potential for being passed on to subsequent generations. Analysis in Publication III confirmed that the mixing of different lineages has led to higher levels of diversity compared not only to all other reintroductions, but to some natural populations as well. Importantly, the natural populations which showed lower heterozygosity had experienced severe bottlenecks during the 20th century (Hellborg et al. 2002). While this mixing of different lineages has achieved genetic diversity comparable to natural populations, the project received considerable criticism as there was potential for releasing hybrids between subspecies (von Arx et al. 2009). The partial Siberian ancestry found in the Harz reintroduction in Publication III confirmed these suspicions.

The five reintroductions originating from the Carpathian lineage had lower expected and observed heterozygosity values than the Harz population. It is important to note that there was significant variation with these five reintroduced populations, suggesting that source population is only one of many contributing factors to genetic diversity in reintroduced populations. However, upon

further investigation, the Carpathian source population had one of the lowest observed heterozygosity values of sampled natural populations. This suggests that lower levels of diversity were present in the ancestral Carpathian population prior to translocation, which have impacted genetic diversity in the reintroduced populations. Lower levels of genetic diversity would match what is already known regarding the lynx in the Carpathian Mountains. This lineage has faced long-term isolation from other natural populations resulting in a unique mitogenome and haplotype in the region (Rueness et al. 2014; Lucena-Perez et al. 2020). Additionally, the population faced severe bottlenecks in the early 1900s due to human persecution (Kratochvíl J. 1968). Therefore, the lower genetic diversity measures seen in these five reintroductions could be partially explained by low diversity in the source population (Figure 7). Other studies have documented the results of genetic diversity loss sourced from populations with already impoverished diversity levels (Taylor and Jamieson 2007). In these cases, there was little to no loss of genetic diversity, not because of optimal reintroduction parameters, but rather due to a lack of genetic diversity in the source population (Groombridge et al. 2012). This emphasizes the importance of comparative analysis and the inclusion of historical data where possible to elucidate the nuances in genetic patterns seen in reintroduced populations.

The reintroduction of wildcat in the Spessart, like the Harz lynx population, was founded from captive bred individuals mainly from Eastern Europe (Büttner and Worel 1990). One captive breeding center in Wiesenfelden along with 30 different zoos participated in providing animals for release (Hartman-Furter 2008). In Publication I, I found that levels of genetic diversity were comparable to natural populations in the adjacent regions. Given findings from other studies, where reintroduction can achieve or even gain genetic diversity where source populations are already impoverished (Groombridge et al. 2012), one must question if this is the case for the wildcat.

The source population for this reintroduction mainly derived from wild-caught individuals originating from Eastern Europe (Worel 2009), which were then bred in captivity and released into different regions in Bavaria, Germany, mainly the Spessart Mountains. This was confirmed in Steyer et al. (2016) and in Publication I by the presence of Haplotype 23, which is only found in parts of Eastern Europe. I used genotype information from 59 samples from Eastern Europe at four microsatellite loci from Mattucci et al. (2016) that overlapped with loci in Publication I to look at possible genetic diversity loss between the source population and the current reintroduction region (Figure 7). There is a considerable reduction in genetic diversity between the source and reintroduction region at these four loci, however, further investigation would be needed to confirm

this hypothesis. Additionally, given that the Eastern European wildcat population constitutes the most genetically diverse group, we do not suspect that the source population was genetically impoverished at the time of reintroduction. We must also consider that the impact the captive breeding program may have had on genetic diversity before reintroduction.

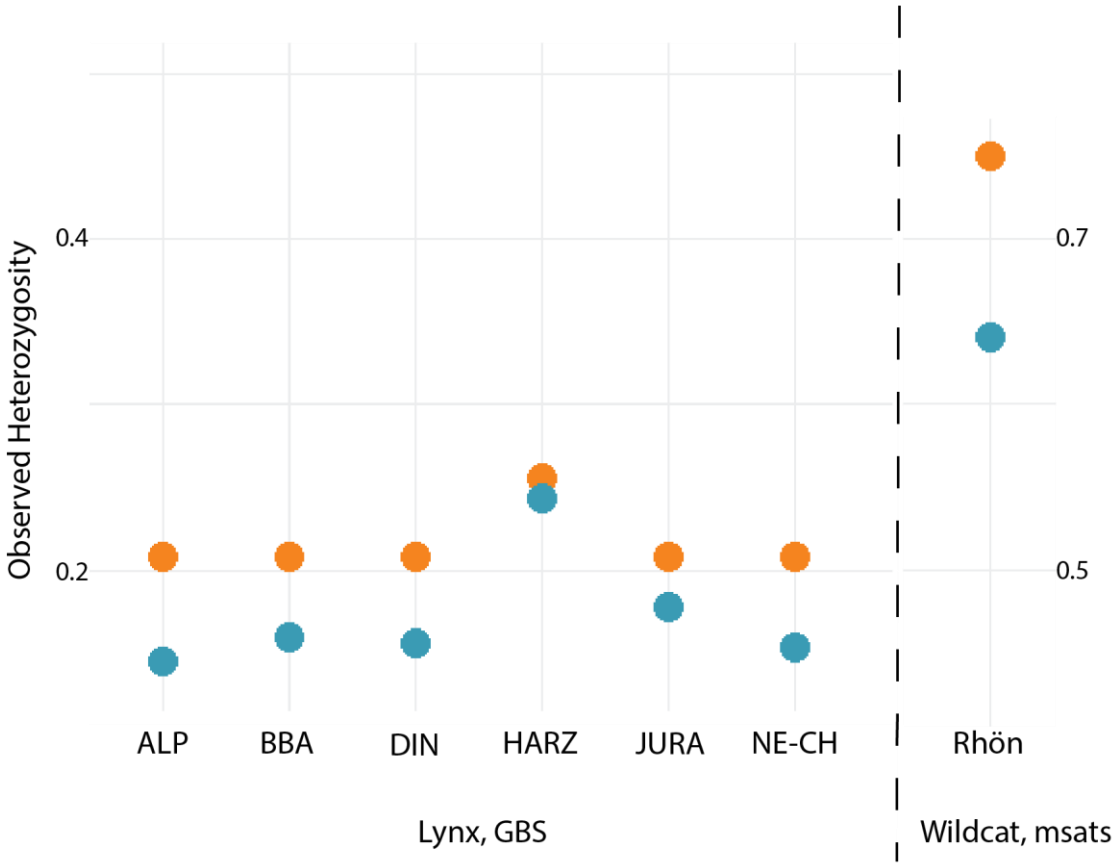

Figure 7. Comparison of observed heterozygosity in source populations (orange) compared to reintroduced populations (blue) across 13,525 SNP loci for the Eurasian lynx and 4 overlapping microsatellite loci used in Publication I and Mattuci et al. (2016).

These three publications illustrate the complexity in disentangling how the diversity present in the source population impacts the trajectory of genetic diversity in reintroduced populations. Mixing of lineages may create temporary increases in genetic diversity, however, it appears difficult to achieve levels of genetic diversity comparable to the source population when considering population persistence in the short- to mid-term. If reintroduction aims to maintain 90% of the genetic variation from the source population (Soule 1986), the studies presented here show the importance of sampling the source and other natural populations. This quantification of baseline

values can be used for temporal comparison, especially in the absence of gene flow from surrounding populations. Having these clear reference points from project inception makes subsequent management decisions easier because they are based on scientific evidence and not intuition.

ii) Connection to free-living populations

Another aspect contributing to overall trends in genetic diversity loss or gain in reintroduced populations is the proximity to surrounding autochthonous populations. This can influence the genetic diversity outcomes of reintroduction by possible gene-flow and natural supplementation from other populations. As the main goal of reintroduction is to create self-sustaining populations (Armstrong and Seddon 2008), gene-flow from adjacent populations is preferred to continued human-mediated translocations. In Publication III, we found evidence that there may be connection between certain lynx populations, namely the BBA, Dinaric, and Carpathian, through Treemix analysis. This fits to our knowledge of 5 documented cases of long-distance dispersal in the lynx (Gajdárová et al. 2021). In the BBA and Dinaric reintroductions, there were the lowest levels of genetic drift observed and minimal genetic differentiation from the source population as evidenced by F_{ST} values, hinting at the possibility that infrequent migrations are occurring. This also falls in line with results showing that the Swiss reintroductions have experienced the largest signatures of genetic drift. These populations are excluded from possible migrants given the extremely long distances to the closest populations. While this influenced population structuring, no correlation between possible gene-flow events and genetic diversity (Ho and He) was found.

In Publication I, using the wildcat, we found that the reintroduction and the adjacent natural population have converged. Given the lack of population structure and signs of genetic differentiation between the reintroduced region and northern refugia, it can be concluded that this population currently acts as one connected metapopulation. This may also contribute to the similarity in genetic diversity values and low population differentiation observed. This trend is likely driven by male dispersal through the landscape, in line with other documented cases of dispersal in both the wildcat and lynx (Samelius et al. 2012; Daniels et al. 2001).

The publications included in this thesis provide examples that proximity to larger, natural populations can be beneficial for reducing genetic differentiation. From a theoretical perspective, this statement is not revolutionary, however, its application in practice and the consideration of how a reintroduction will fit into metapopulations is rarely considered (Taylor et al. 2017). Therefore, having publications that clearly show that migration from surrounding areas helps buoy

genetic diversity and genetic drift is vital. In Publication III, we saw the contrast between genetic drift in Swiss versus BBA lynx reintroductions, illustrating it is vital to push conservation action to at least consider stepping stone populations, maintenance of corridors, or in extreme cases, translocation between reintroductions. Metapopulations need to be considered as a principal question before reintroduction begins, and when reintroduction has already occurred, there should be conservation emphasis on connecting isolated populations.

iii) Rate of population growth

The rate of population growth post-reintroduction plays a role in the genetic trajectory of a population, as smaller populations are more likely to experience the negative effects of genetic drift and inbreeding (Frankham 2005). Little to no loss of genetic diversity after reintroduction has been observed in some populations of rapidly expanding reintroduced mammals, most notably the reintroduction of wolves into Yellowstone (vonHoldt et al. 2008; Wisely et al. 2008). In these examples, populations expanded rapidly over approximately 10 years and temporal monitoring revealed no decrease in genetic diversity estimated from microsatellite analysis.

In the case of the wildcat, where rapid expansion through demographic estimates was confirmed in Publication I, temporal estimates of genetic diversity did not fluctuate significantly similar to the aforementioned results in Yellowstone wolves. Several factors including lower spatial requirements and ability to persist in human-dominated landscapes could have contributed to this rapid demographic increase (Jerosch et al. 2017; Steyer et al. 2016). However, one important consideration is the baseline for genetic diversity. In the above-mentioned papers, there was no reference to the source population or other natural populations. There may be a loss of genetic diversity compared to the source population in the wildcat (Figure 7), but not to the adjacent natural populations (Publication I) despite a lack of temporal loss in genetic diversity since reintroduction. However, given the lack of information regarding the sources of all released individuals, a concrete conclusion can likely not be drawn. However, these external reference points to other natural populations clearly provide invaluable data needed to place the reintroduction in context.

The lynx falls on the other end of the spectrum as this species is known to have low population growth rates making it a more challenging candidate for reintroduction (Noss et al. 1996). Despite reintroduced populations currently reporting over 100 individuals, these population numbers are not enough to overcome high levels of recent inbreeding within the populations (Publication III). Estimates of runs of homozygosity across SNP markers revealed that all populations of

reintroduced lynx suffer from recent inbreeding. Recent inbreeding has been linked to functional traits that can impact fitness (Xue et al. 2015; Robinson et al. 2019), which can in turn lead to inbreeding depression. Additionally, we found elevated rates of inbreeding in some natural populations that experienced bottlenecks in the 20th century (Publication III). Despite demographic recovery, signatures of these past bottlenecks are still visible. This suggests that populations of lynx take a long time to recover from such significant range contractions and persecution, which could be in part be due to slow population growth rates. Other reasons could stem from their high reliance on one source of prey, large spatial requirements, sensitivity to environmental change, and low population densities (Noss et al. 1996).

iv) Features of the receiving environment

Features of the receiving environment also leave an impact on the genetic patterns seen within reintroduced populations. In cases where the original threats leading to local extinction have not been mitigated, the reintroduced population is likely to face a similar setting that led to extinction in the first place. Two major environmental factors contributing to genetic patterns include: quality and availability of suitable habitat and levels of human induced mortality. In both the lynx and wildcat, persecution was a major reason for original decline in historical populations (Kratochvíl J. 1968; Piechocki 1990b). At the point of reintroduction, however, conservation attitudes had changed significantly and, in general, the public is more accepting of these species. The wildcat faces few hurdles in the receiving environment. This species has lower spatial requirements (Anile et al. 2018) and it has recently been shown to persist in human dominated landscapes (Jerosch et al. 2017; Jerosch et al. 2018). Human induced mortality is due to incidences of traffic mortality rather than active persecution (Klar et al. 2009). This creates a generally favorable environment for reintroduction, which increases the likelihood of success in reintroduction programs.

Again, the lynx poses as a contrast to this system. As described above, high spatial and prey requirements leaves the lynx more vulnerable to habitat fragmentation. Some studies have questioned the ability to provide adequate habitat in the human dominated European landscape for such large-scale reintroductions (Kramer-Schadt et al. 2005). While spatial requirements may not influence a reintroduced population in the short-term, it certainly impacts the carrying capacity in the long-term (Steenweg et al. 2016). Currently, the six lynx reintroductions remain isolated and plans to connect these populations through stepping stones may be limited by the amount of available habitat. Additionally, the lynx still faces legal and illegal killing across all reintroduced populations. The lowest rates of human induced mortality occur in the central German Harz population, with only 1 documented case (Publication II), and the largest in the southwest BBA

population with up to 25% of the population killed each year because of poaching (Heurich et al. 2018). This is a major concern limiting demographic growth in multiple populations and can impact the genetic composition of the population by reducing the population size over time. In sum, environmental factors offer explanations and a greater context to why we observe different outcomes across populations and species. Given mounting environmental change, it is only more likely that these will play a larger role in reintroduction consideration in the coming years (Roberts 1988).

v) *Number of founder individuals*

The last aspect I will consider is how the number of founders influences genetic patterns within reintroduced populations. The number of individuals has time and time again been identified as one of the most important factors impacting the genetic composition of resulting population (Griffith et al. 1989; Armstrong and Seddon 2008; Frankham 2009; Groombridge et al. 2012). The link between number of individuals and genetic composition is obvious: the founding individuals represent the maximum number of alleles present in the population.

The reintroduction literature pronounces the importance of released individual numbers. In a review of studies across a variety of taxa, rates of translocation success significantly increase if at least 100 individuals are released (Fischer and Lindenmayer 2000). However, what is less often quantified is the number of genetic founders, especially in reintroductions that began prior to the widespread use of genetic techniques in non-model organisms. In Publication II, we show that the number of genetic founders can be inferred from the resulting population through a combination of field monitoring and genetic methods to reconstruct pedigrees. This likely has implications mainly for other carnivore reintroductions, where non-invasive genetic monitoring can fill in important gaps in knowledge. My results showed that likely 7 of 24 released lynx individuals in the Harz population contributed to the genetic pool in the first generations. This had clear impacts on the population's genetic composition, as multiple inbreeding events were detected and pedigree analysis suggests that the current territorial, highly reproductive females are related to one another, posing a risk to future generations.

This trend was generally reflected when looking across all lynx reintroductions. In Publication III, we identified the highest inbreeding levels in populations with the lowest number of released individuals. Considering that released individuals do not reflect the number of genetic founders, we can clearly identify that populations with fewer released individuals, namely the Swiss Alpine and Dinaric, are the populations in the most critical state. The Dinaric population is functionally

extinct, reliant on further translocation of individuals from natural populations (Sindičić et al. 2013). The Swiss Alpine population experienced decline in the last decades and it could be suspected to meet a similar fate if no connection to other populations is forged. Reintroduced populations with over 20 released individuals fared the best, with lower rates of recent inbreeding, however, this does not mean they are exempt from experiencing the negative consequences of inbreeding. This generally fits to studies modelling allele loss in populations with moderate population growth after reintroduction (Tracy et al. 2011), suggesting that future management decisions should aim for at least a 20 individuals.

In the wildcat, approximately 600 individuals were released (Worel 2009). This represents one of the largest reintroductions of a carnivore into its historical range (Fischer and Lindenmayer 2000). This reintroduction is now connected to an adjacent natural population and reached genetic diversity levels similar to natural populations not currently under active management. This suggests that the population has retained sufficient genetic diversity to persist in the landscape. The convergence with the adjacent population supplemented the number of released individuals with additional gene flow, and therefore, there is no way to tell in this case the number of individuals contributing to the genetic composition of the founding population.

The publications included here strongly suggest that the number of founder individuals is directly linked to the genetic consequences observed. In cases where founding numbers likely do not reach double digits, there are negative consequences, specifically with regard to inbreeding, which can hinder a population's potential for long-term survival. While inbreeding in these populations range in severity, the signatures will likely persist for a long time. In the case where founding individuals were numerous, lower rates of genetic diversity loss was observed.

2.3 Conclusions: Turning practice into science?

Increasing the chances of success in reintroduction projects relies on an increased understanding of the genetic consequences faced by reintroduced populations and defining concrete actions to minimize any detrimental effects. The research presented here spans across two elusive Palearctic felid species, regional to continental spatial scales, and a variety of genetic methods. The ultimate aim was to assess how genetic factors might impact felid reintroduction and how genetics can be used to advance felid reintroduction monitoring. I have shown the feasibility of using genetic methods to undertake a post-reintroduction evaluation in these two elusive species. I employed genetic methods to reconstruct the demographic histories of reintroduced populations, quantitatively defined genetic diversity, and utilized the power of comparison across multiple populations. These methods illustrate that non-invasive sampling can be an informative addition to field monitoring and demographic surveys. In the past, scientific evidence relating to reintroduction relied heavily on snapshot demographic data to confirm establishment of a population in the area where it was reintroduced. However, this type of analysis provides little evidence regarding the adaptive potential of a population in the long-term. Therefore, the combination of genetic approaches can help to quantify the outlook of populations in the mid- to long-term, specifically in cases where the demographic data has left uncertainties.

The utilization of multiple genetic techniques allowed for an exploration of the advantages and drawbacks genetic methods pose. Particularly, microsatellites are well suited for non-invasive monitoring given the low quality sample requirements and ability to process a large amount of samples. Genomic methods on the other hand, offer possibilities not yet standard in reintroduction biology and hold substantial potential. This potential lies in the ability to obtain more accurate estimates of genetic diversity and inbreeding, two factors which influence reintroductions tremendously. It is only logical that accurate estimates allow for informed decisions and the possibility to develop scientifically informed strategies for future reintroductions. For example, in future lynx reintroductions, the evidence provided here advocates for the release of at least 20 individuals and translocation to mimic gene flow where connection with natural populations is not possible. This would likely mitigate genetic drift and inbreeding currently observed in all reintroduced populations. Additionally, if genetic baselines can be incorporated into routine conservation management through genomic analysis, we have a better chance to carry out early intervention, which can increase the chances of success. A routine incorporation of genetic techniques, including modern genome-wide tools, is one important step towards integrating scientific evidence into reintroduction application.

In my investigation, I found that genetic patterns are highly dependent on the study species and specific population histories. For example, despite felids sharing similar life-history traits, there are clearly many differences that can lead to contrasting reintroduction outcomes between the European wildcat and Eurasian lynx. On top of that, the reintroduction histories, including source population, number of individuals released, and features in the receiving environment are variable not only across species, but across populations of the same species. Therefore, while reintroduction biology has pushed for the definition of standardized markers for establishment, persistence, and success (Armstrong and Seddon 2008; Taylor et al. 2017), the results presented here illustrate that universal markers may not be useful to define. For example, the European wildcat likely experienced genetic diversity loss as a result of reintroduction and captive breeding (Figure 7), yet it has successfully persisted and formed a metapopulation with adjacent populations. In contrast, the lynx population has also experienced considerable genetic diversity loss and multiple populations are currently experiencing the negative consequences of reintroduction, namely inbreeding. Despite the contrasting outcomes in these elusive felids, the results suggest that loss of genetic diversity may not be avoidable. However, loss does not imply failure. The number of released individuals and the source population are two critical factors, where a rigorous quantification of genetic diversity in a pre-release state of early reintroduction planning could greatly benefit conservation efforts and enhance potential success. Therefore, instead of universal standards, genomic baselines carried out before reintroduction begins are likely a better way to gauge individual project success in the short- and long-term given the differences observed among study systems.

In sum, the practice of reintroduction has a history of being just that: a practice. Practitioners are often faced with the difficult task of making decisions based on intuition as there is a lack of scientific evidence to sufficiently inform decisions. As current and future projects reintroducing both the wildcat and lynx are planned in the coming years across Europe, it is vital that we base our decisions on available empirical evidence. This thesis constitutes an important step in verifying the validity of genetic methods in reintroduction monitoring and understanding how genetic diversity loss and inbreeding affects reintroduction outcomes. Additionally, it provides a large-scale status quo of a continent-wide reintroduction effort, which can serve future research and decision making for the years to come, hopefully achieving one step towards integrating science into the practice of reintroduction.

Publications

Publication I

Overlooked or recolonized? Revealing the origin of wildcat reappearance after presumed long-term absence

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Erklärung zu den Autorenanteilen

Mueller, Sarah Ashley; Reiners, Tobias E.; Steyer, Katharina; Thaden, Alina von; Tiesmeyer, Annika; Nowak, Carsten (2020): Revealing the origin of wildcat reappearance after presumed long-term absence. In: Eur J Wildl Res 66 (6), S. 1–8. DOI: 10.1007/s10344-020-01433-7.

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Was hat der Promovierende bzw. was haben die Koautoren beigetragen?[*3]

(1) Entwicklung und Planung

SAM: 50%, CN: 20%, TER: 15%, KS: 10%

(2) zur Durchführung der einzelnen Untersuchungen und Experimente

Sample Collection: TER: 40%, KS: 60%

Sample Selection: SAM:100%

Microsatellite lab work: TER: 25%, KS: 25%, AT: 25%, SAM: 25%

SNP genotyping lab work: SAM: 80%, AVT: 20%

(3) zur Erstellung der Datensammlung und Abbildungen

Data Collection: SAM: 50%, KS: 20%, AT: 20%, AVT: 10%

Figures: SAM: 100%

(4) zur Analyse und Interpretation der Daten

SAM: 80%, TER: 10%, CN: 10%

(5) zum Verfassen des Manuskripts

SAM: 70%, CN: 10%, TER: 5%, AVT: 5%, KS: 5%, AT: 5%

Datum/Ort: 22.06.2021, Frankfurt a.M.

Unterschrift Promovend: _____

Zustimmende Bestätigungen der oben genannten Angaben

Unterschrift Betreuer: _____ Datum/Ort: _____

Ggfs. Unterschrift *corresponding author* SAM: _____ Datum/Ort: 22.06.2021, Frankfurt a.M.

European Journal of Wildlife Research (2020) 66: 94
https://doi.org/10.1007/s10344-020-01433-7

ORIGINAL ARTICLE

Revealing the origin of wildcat reappearance after presumed long-term absence

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Received: 24 August 2018 / Revised: 12 October 2020 / Accepted: 21 October 2020 / Published online: 29 October 2020

Abstract

Following severe population decline and local extinction due to massive habitat destruction and persecution, wildcats have recently reappeared in several parts of Germany's low mountain region. It remains unknown how this reemergence occurred, specifically if local populations have been overlooked at low densities or if the species has successfully spread across the highly fragmented anthropogenic landscape. In the central German Rhön Mountains, for instance, wildcats were believed to be extinct during most of the twentieth century, however, the species was recently detected and subsequent genetic monitoring found the presence of a sizeable population. In this study, we used microsatellite and SNP genotypes from 146 wildcat individuals from 2008 to 2017 across a ~ 15,000 km^2 area in the central German low mountain region to understand the population re-establishment of wildcats in the region. Bayesian clustering and subsequent analyses revealed that animals in the Rhön Mountains appear to be a mix from the two adjacent populations in the North and South of the area, suggesting a recent range expansion from two different directions. Both populations meet in the Rhön Biosphere Reserve, leading to an admixture of the northern, autochthonous, and the southern reintroduced wildcat population. While we cannot completely exclude the possibility of undetected population persistence, the high genetic homogeneity in the central German wildcat population and the lack of any signatures of past population decline in the Rhön favor a scenario of natural expansion. Our findings thus suggest that wildcats are well capable of rapid range expansion across richly structured landscape mosaics consisting of open land, settlements, and forest patches and document the potential of massive non-invasive genetic sampling when aiming to reconstruct the complex population and range dynamics of wildlife.

Keywords Felidae · Non-invasive sampling · Dispersal capacity · Recolonization · Reintroduction

Introduction

Many populations of large- and medium-sized carnivores are currently re-expanding their ranges across the densely populated and anthropogenically modified landscapes of central Europe (Chapron et al. 2014). These altered landscapes exhibit high levels of habitat fragmentation, which is the disruption of continuous stretches of suitable habitat (Schadt et al. 2002). Habitat fragmentation can negatively affect the viability of populations and species. However, the extent to which species are affected varies significantly (Haddad et al. 2015). A species' reaction to cultural landscapes depends heavily on the size of suitable habitat, the distance between habitat patches, and the individual species' life history traits (Caruso et al. 2016). Carnivores, for instance, are at a heightened risk of being negatively impacted in fragmented landscapes due to their relatively large home ranges and low population densities (Crooks 2002; Noss et al. 1996; Woodroffe 1998). Therefore, investigating how carnivores successfully disperse and establish viable populations in altered landscapes is important to understand the potential for long-term existence of wildlife in human-dominated landscapes (Riley et al. 2003).

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10344-020-01433-7.

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The European wildcat is a flagship species for nature conservation in Germany, as it primarily relies on highly structured natural deciduous and mixed forests (Driscoll and Nowell 2010). This reliance on connected forests has made it a pillar for the preservation of natural habitats. Recently, the wildcat has made a reemergence across the central German low mountain region (Steyer et al. 2016), providing a relevant example to understand dispersal dynamics in a highly fragmented landscape.

Historically, European wildcat populations were heavily depleted due to human persecution until the early twentieth century. In Germany, the species was restricted to few refugial areas within the low mountain region, such as the Pfälzerwald, Eifel, and the Harz Mountains (Eckert et al. 2010). After hunting pressure was reduced, distance between refugial areas, habitat fragmentation, and increasing anthropogenic development left local wildcat populations geographically isolated (Hille et al. 2000; Pierpaoli et al. 2003). Considering the possible negative effects of isolated populations, including inbreeding and genetic drift, efforts to connect these distinct wildcat populations have become a conservation priority (Klar et al. 2012; Mattucci et al. 2016). Given the concern for wildcat population connectivity, genetic monitoring relying on hair trapping was established across known wildcat territories (Klar et al. 2008; Simon and Hupe 2008; Steyer et al. 2013). The results of increased monitoring showed strong evidence for the recent recovery of wildcat populations in various areas (Steyer et al. 2013; Steyer et al. 2016). Most recently, the species had been documented in areas distant from the known source regions, such as in the Bayerischer Wald in the Southeast and the Lüneburger Heide in Northern Germany (unpublished data). These new detections of the species in areas outside long-standing refugia and reintroduction sites suggest that the wildcat is capable of rapid dispersal and establishment across anthropogenically modified landscapes (scenario 1). However, it remains unknown if the species may have persisted in small, overlooked populations that are now increasing locally (scenario 2). Revealing the origin of wildcat reemergence is of considerable importance, especially considering the first scenario, expansion from refugia and reintroduction areas, which would imply that effective dispersal and population establishment of mobile mammals such as wildcats may be less obstructed by habitat fragmentation and barriers within anthropogenic landscapes than previously assumed (Klar et al. 2008; Jerosch et al. 2018; Balkenhol and Waits 2009).

Here, we aim to disentangle the above-mentioned recolonization scenarios by investigating the fine-scale genetic population structure of wildcats in a region where the species was recently detected, namely the Rhön Biosphere Reserve (RBR). The RBR is located on the southeastern edge of published wildcat distribution in the central low mountain region (Fig. 1) (Klar et al. 2009). The RBR is a biodiversity-rich landscape, dominated by meadows and open land, surrounded by multiple large highways (A7, A71, A4) (Jedicke 2013). The RBR is home to a variety of protected species, including the wildcat. Wildcats in the RBR were thought to be extinct during most of the twentieth century until first genetic detections of wildcats occurred between 2007 and 2009 (Birlenbach et al. 2009). Beginning in 2009, under the frame of various monitoring projects (Table S1), the RBR and surroundings were heavily searched for evidence of wildcat, resulting in the detection of a substantial presence of wildcat in this region (Reiners et al. 2014; Thein 2008). Scenario 1 would imply wildcats expanded from adjacent populations into the RBR. Given the regional history, there are two possible source populations; (i) the refugial population in the north and (ii) the reintroduced population in the adjacent Spessart Mountains (Fig. 1). The known wildcat distribution to the north of the RBR was documented since before 2009 as a known source of a stable wildcat population (Birlenbach et al. 2009). The wildcat reintroduction in the Spessart region was launched in 1984 under the frame of a long-term reintroduction project. Approximately 600 wildcats were released until 2008 (Büttner and Worel 1990). Additionally, in 2005, six wildcats were reintroduced in the Neuwirthauser Forest, which is at the southernmost part of the RBR. It remains unknown if this reintroduction was successful and contributed to the reemergence in the RBR or if the current population resembles dispersal of individuals from the north. Scenario 2 would indicate a local increase from a small, overlooked relict population within the Rhön Mountains, which would likely result in population sub-structuring, due to the loss of rare alleles in small isolated populations, indicating the presence that a relict population survived and is now expanding in the low mountain region (Excoffier et al. 2009).

The present study aims to disentangle the two proposed scenarios to shed light on the origin of wildcats in this low mountain region. For this, data was obtained from a large-scale genetic monitoring program conducted over the past 10 years in Germany. From this monitoring program, we genotyped a subset of samples with SNP and microsatellite markers to reveal the genetic makeup in the RBR. We compared the genetic structure of wildcats in the RBR with both adjacent source and reintroduced populations (Steyer et al. 2016) to test the two scenarios explaining the emergence of the species in the RBR given above.

Materials and methods

Study site

The study area comprised the Rhön Mountains, including the RBR and surroundings (Fig. 1). The reserve spans over 2433 km^2, which was expanded in 2014 to include the southern Rhön with the NF. The Rhön is a low mountain range ranging from 250 to 950 m, with approximately 40% forested land.

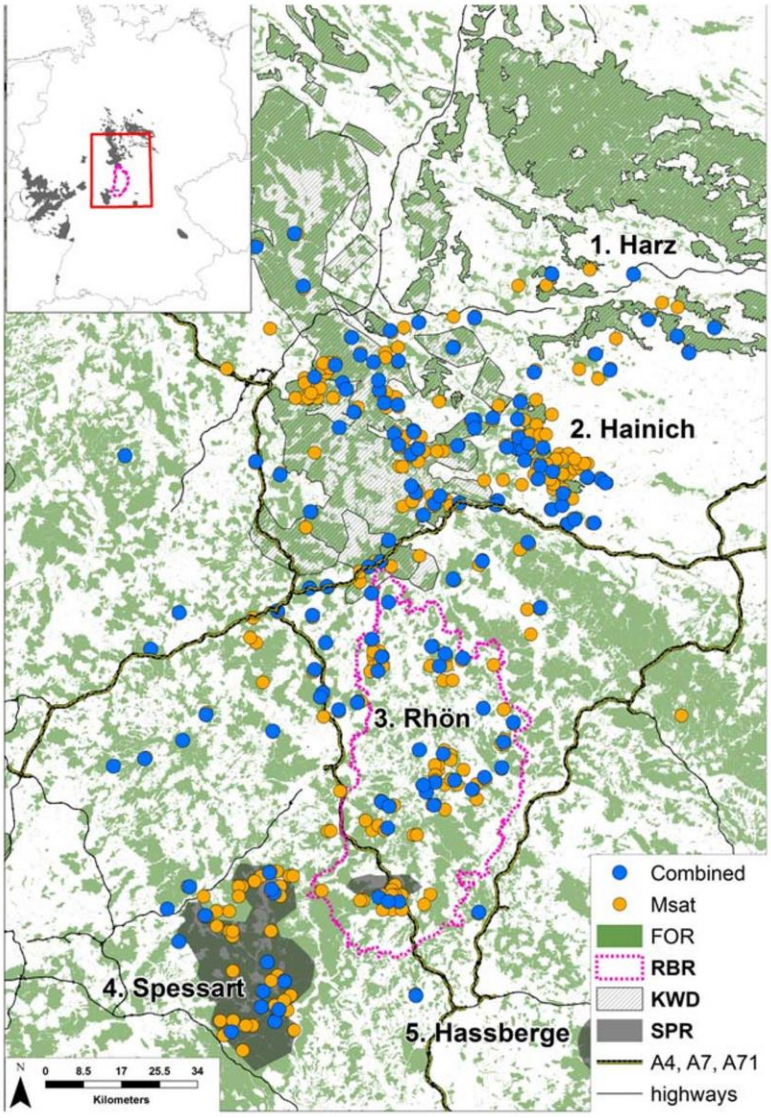

Fig. 1 Map of 146 wildcat samples used for combined SNP and microsatellite approach (blue) and 439 wildcat samples analyzed only with msats (orange) from 2004 to 2017 used for analysis showing the known wildcat distribution (KWD), Rhön Biosphere Reserve (RBR), and Spessart reintroduction (SPR) as well as the forested area based on ATKIS (FOR). Map of the study area is shown in the red box within the inlet

Within the study area, the closest potential source populations were included; mainly the region surrounding the National Park Hainich in the north, and the Spessart Mountains, bordering the Rhön Mountains to the southwest. The Hainich Mountains range from 225 to 494 m and are the most expansive broad-leaved forest, the primary habitat of the wildcat, in Germany. The Spessart Mountains include one of Germany's most forested areas, peaking at 586 m. In total, our study consisted of approximately 15,000 km^2 of variable habitats.

Sample collection and laboratory methodologies

Multiple opportunistic and standardized monitoring projects collected samples from 2004 to 2017. Opportunistic wildcat roadkill samples were collected throughout the study period. In addition, multiple monitoring projects began in 2008, most notably Rhoen Natur e.V., BUND Wildkatzensprung, and Biosphärenreservat Rhön projects, which resulted in intense monitoring within the RBR during this time (for a complete list, see supplementary Table S1). In this study, we combined microsatellite and SNP genotyping methods. We took 119 wildcat microsatellite genotypes from 2004 to 2013 from a German-wide study on wildcat population structure (Steyer et al. 2016) and added 49 additional genotypes from 2014 to 2017 to be further analyzed using a wildcat specific SNPtype™ marker panel (von Thaden et al. 2020). Microsatellite genotypes were considered for SNP genotyping if they had a minimum of 11 loci and showed amplification success rates of 80%. Additionally, this selection excluded potential hybrids as there are low rates of hybridization across

the study area (Steyer et al. 2018). The samples were then selected to create an even distribution in location, time, and sample type. Of the selected genotypes, 64 samples originated from tissue of dead found wildcats, and the remaining 104 samples are hair samples from various monitoring projects.

We used the primers LF4 (Eckert et al. 2010) and H16498 (Kocher et al. 1989) to sequence a 110 bp fragment of the mitochondrial control region following the protocol in Steyer et al. (2016). All samples were analyzed using 14 microsatellite markers and a zink finger sex marker (Hartmann et al. 2013). For non-invasively collected samples, the multiple tube approach using three replicates per sample was applied (Hartmann et al. 2013). Individualization was carried out through a custom R script and duplicated individuals were removed before further analysis (see Steyer et al. 2016 for further details). The microsatellite genotypes utilized from the Steyer et al. (2016) study were compared with the samples collected between 2014 and 2017 to look for batch effects. As none was found, the sample sets were combined.

The SNP genotyping of wildcat samples (n = 168) was performed on a EP1 platform (Fluidigm Corp., USA) using microfluidic 96.96 Dynamic Arrays™. Detailed methods and wildcat-specific SNPtype™ Assays are presented in von Thaden et al. (2017) and von Thaden et al. (2020). The SNPtype™ marker panel encompasses 84 loci selected for individual and population identification, 10 loci for hybrid detection, and 2 SRY-linked loci. All SNP experiments included four no template controls (NTC) per array and non-invasively collected hair samples were triplicated to detect potential errors. From 168 samples, seven samples did not show sufficient (> 70%) amplification success to be scored and used in downstream analysis. Subsequent individualization resulted in 146 individuals to be analyzed using the combined SNP and microsatellite data.

Data analyses

First, we tested the combined SNP and microsatellite data set for isolation by distance (IBD) effects. We performed an IBD analysis as implemented in GeneAlEx 6.5 (Peakall and Smouse 2012) to account for genetic differentiation solely based on geographical position of individuals. The program STRUCTURE v2.3.4 (Pritchard et al. 2000) was used to evaluate population genetic structure. After 100,000 steps of burn-in, 200,000 MCMC steps were performed with admixture model and correlated allele frequencies using a range of K 1–10 with 10 iterations. We used Structure Harvester (Earl and vonHoldt 2012) with the Evanno method (Evanno et al. 2005) to determine the most likely number of population clusters. The replicates were consolidated with the software CLUMPP (Jakobsson and Rosenberg 2007) using the GREEDY algorithm.

Further review of the spatial structure was carried out with the ADEGENET package in R (v.3.4.2) using a spatial principal component analysis (sPCA) (Jombart 2008). No requirements of the data to meet Hardy-Weinberg expectations or linkage equilibrium are needed for this method. In addition to the genetic data, sPCA also uses spatial information and is particularly suitable for the analysis of weak genetic structures (Storfer et al. 2007). sPCA relies on Moran's I (Moran 1948, 1950) to identify spatial patterns within the genetic structure of the sampled individuals. The method distinguishes between global scores, which indicate gradients in allele frequencies, and local scores, indicating differences in neighboring samples.

Standard genetic diversity indices including expected and observed heterozygosity, allelic richness, and population pairwise F_{ST} values were calculated for the combined SNP and microsatellite genotypes using Arlequin version 3.5.2.2 (Excoffier and Lischer 2010).

Results

Of the 146 genotyped individuals, mtDNA haplotypes were successfully determined in 134 individuals and corresponded to SNG-HP-FS03/-04/-06/-22/-23 (Steyer et al. 2016). Haplotype SNG-HP-FS23 was found in eight individuals solely in the southern part of the study region (Fig. 2b). The other four haplotypes appeared in all parts of the study area.

Analysis of genetic structure based on combined SNP and microsatellite genotypes revealed no evidence of isolation by distance (IBD; Mantel test: r = 0.011, p = 0.48). Bayesian clustering implemented in STRUCTURE indicated K = 2 as the most likely number of clusters within the study area. Separation of these clusters could not be attributed to a geographical pattern, with individuals clearly showing representation from both clusters (Fig. 3).

Clustering with sPCA resulted in a significant global structure, indicating correlation between the genetic and geographic distances (p = 0.01). However, no significant local structure was found (p = 0.14). A plot of lagged scores from the first principal component suggested the global structure is linked to a north-south genetic border, with a transitional area within the RBR (Fig. 2a). Subsequent principal components show weaker genetic structure although the eigenvalues suggest the first principal component explains most of the genetic variance found in wildcat individuals from the study area (Fig. S1).

Standard measures of genetic diversity were carried out based on the two clusters defined by the sPCA (Table 1). When looking at two clusters, observed (0.41, 0.43) and expected (0.42, 0.43) heterozygosity values from the north and south, respectively, were highly similar. Allelic richness was also identical (2.45, 2.45) along with a comparable fixation

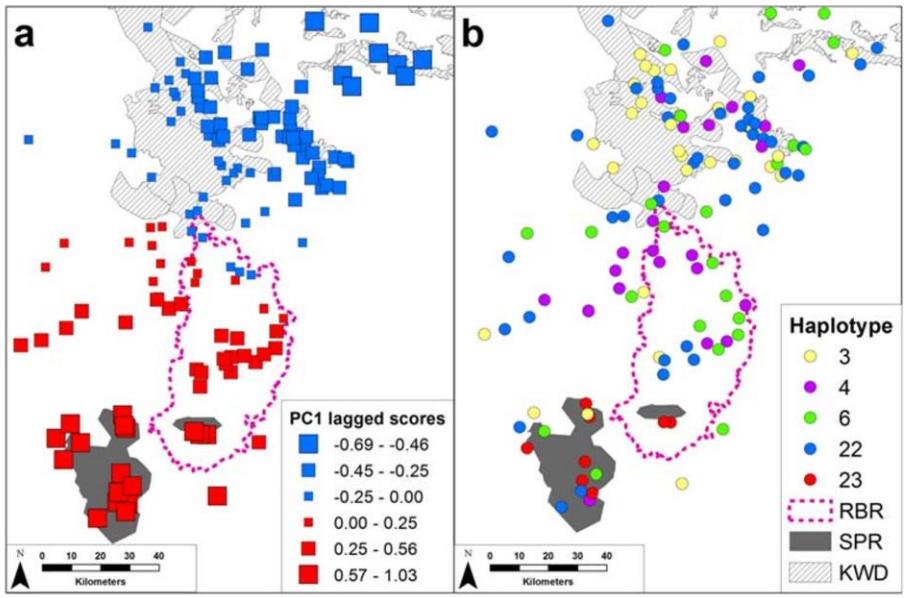

Fig. 2 Map showing the spatial genetic structure as assessed by sPCA analysis and haplotype map. **a** Individual scores from the first principal component: large blue squares indicated a highly positive score, large red squares indicated highly negative scores. **b** Map of individuals within the study area with the haplotype 23 (red) and all other haplotypes (3, 4, 6, 22)

index (0.047, 0.011). The RBR, which comprised much of the south cluster, showed no local genetic signature such as higher allelic richness or specific clustering in the region. We also carried out pairwise F_{ST} analysis based on the two clusters, which were not significantly differentiated from each other (Table 2). We also compared our dataset with a larger, microsatellite-only sample set of 439 individuals originating from a previous study (Steyer et al. 2016), which revealed some sub-structuring in the south, specifically between the reintroduced region and the RBR (Fig. S2).

Discussion

In recent decades, wildcat populations across low mountain regions in Germany showed signs of expansion, despite a considerably fragmented landscape (Hartmann et al. 2013; Würstlin et al. 2016). While the recolonization process has been well documented (Canters et al. 2005; Nussberger et al. 2018; Streif et al. 2017), few attempts have been made to distinguish between active range expansion and locally growing populations. We assessed regional population structure of wildcat based on available samples from various monitoring projects in the region (Table S1). We investigated population sub-structuring and fine-scale genetic diversity to shed light on two possible scenarios for the rapid appearance of wildcats within this low mountain region.

The number of wildcat individuals found within the study region between 2009 and 2014 (Fig. 1) indicates the presence of a viable wildcat population within the RBR. In part, this high number of wildcat detections can be attributed to the intense monitoring activities by multiple concurrent projects (Table S1). However, it appears unlikely that the marked increase of wildcat evidence since 2009 can be explained solely by increased monitoring activities. To our knowledge, local experts have continuously looked for wildcat presence in the region, making it unlikely that a population remained undetected for decades in the Rhön (Franz Müller, pers. comm.). Roadkill monitoring has occurred since 2004 rather opportunistically, involving local authorities, conservationists, and hunters. In addition, the overall observed trend of recent wildcat expansion across various regions within Germany (Steyer

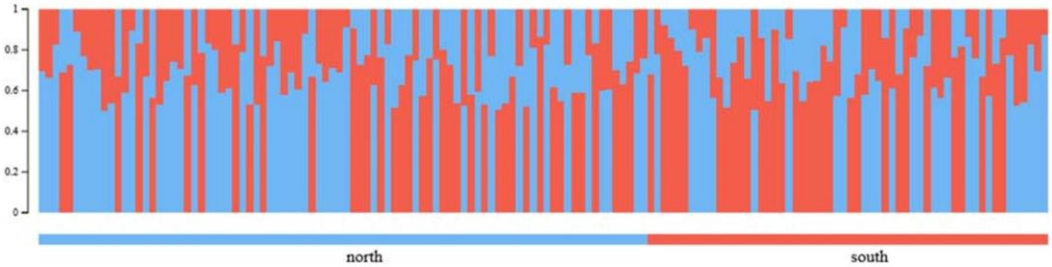

Fig. 3 STRUCTURE results from $K = 2$, which was suggested by Structure Harvester as the most likely value from K 1 to 10. Each bar represents a single individual. The coloration corresponds to the estimated proportions of posterior probability assignments of each sample to each cluster

Table 1 Genetic diversity of the wildcats sampled in the study region divided based on sPCA groups. Number of individuals (n), allelic richness (AR), observed heterozygosity (H_o), expected heterozygosity (H_e), fixation index (F), number of individuals carrying the haplotype 23 within the population (Hap 23)

Pop	n	AR	H_o	H_e	F	Hap 23
North	88	2.45	0.41	0.42	0.047	0
South	58	2.45	0.43	0.43	0.011	8

et al. 2016; Streif et al. 2017; Würstlin et al. 2016) supports the observed pattern of population increase within the study area.

The past reintroduction of wildcats in the Spessart allows for tracking possible wildcat expansion in this region through a unique mtDNA haplotype (Steyer et al. 2016). While haplotypes SNG-HP-FS03/-04/-06/-22 are the most common haplotypes in German wildcat populations (Steyer et al. 2016), SNG-HP-FS23 is restricted to the Spessart Mountains (Fig. 2b). While this gives clear indication that reintroduced wildcats have successfully established in the Spessart region, the distribution of this haplotype also documents a lack of substantial spread into the RBR. Our findings of the haplotype SNG-HP-FS23 being largely restricted to the Spessart reintroduction area suggests that the expansion into the Rhön area is mainly driven by male dispersal from the Southern reintroduction area and confirms the general observation of male-dominated dispersal in carnivores (Støen et al. 2005).

Our results from population structure analysis indicate a highly admixed population that comprises the northern refugial and the reintroduced populations. The sPCA results indicate that wildcats in the northern area of the RBR are genetically indistinguishable from northern refugia, which derive from the central German wildcat population described in Steyer et al. (2016) (Fig. 2a). In addition, the data suggests that wildcats from the reintroduction are moving into the RBR. Interestingly, STRUCTURE results show a highly admixed population with no clear geographic boundaries (Fig. 3). This may be explained by overall weak substructuring ($F_{ST} < 0.05$) (Hubisz et al. 2009; Stift et al. 2019). The F_{ST} measures also showed no significant deviation between the northern and southern clusters defined by the sPCA. This hints at a highly admixed population throughout the study region.

Table 2 Pairwise F_{ST} values based sPCA groups identified in Table 1. F_{ST} values are represented below the diagonal and corresponding p values above the diagonal, where ns is not significant

	North	South
North	-	ns
South	0.00193	-

We found no evidence of an overlooked population within the RBR, as no private alleles were discovered in the area and we did not find any genetic sub-structure separating the Rhön from adjacent regions. Thus, the most probable explanation for the observed genetic pattern is a recolonization from both (northern and southern) directions. In case of overall low substructure within the central German wildcat population, which has been found previously (Steyer et al. 2016) and is confirmed in this study, a scenario of local population size increase together with significant dispersal from adjacent areas appears feasible. Still, the lack of evidence for wildcat appearance prior to 2008 makes the first option more likely and both scenarios ultimately require substantial dispersal from adjacent regions, suggesting a significant permeability of the landscape for wildcats.

Our results imply that wildcats can disperse through human-dominated landscapes. Land use within the newly recolonized RBR comprises 41% forested land (Jedicke 2013), with the remaining being settlement, open meadows, or arable land. Our study suggests that wildcats can, within a few years or few generations, recolonize a region with a substantial proportion of open land. This reemergence implies the wildcat is not significantly isolated due to road infrastructure, and can establish home ranges in primarily agricultural habitats, which has been supported by recent studies showing similar results (Jerosch et al. 2017; Jerosch et al. 2018; Klar et al. 2009; Würstlin et al. 2016). The presence of green bridges, built in 2011, potentially facilitates the exchange between individuals on either side of the A7 highway, as has been shown in other regions (Pir et al. 2011). The main factor determining habitat choice in wildcats is thought to be distance to forest (Sarmento et al. 2006). Jerosch et al. (2018) highlight structural heterogeneity in open landscapes as a determining factor allowing wildcats to persist in human-dominated landscapes. Therefore, the presence of rich-structured mosaics of open land and forest patches might have provided suitable conditions for successful recolonization in the RBR.

Our findings confirm a recolonization process of the wildcat in the Rhön Mountains, which has important implications for current wildcat conservation strategies. The results mainly suggest that connecting suitable habitat through stepping stones of rich-structured patches, rather than continuous forest, may be sufficient for wildcat dispersal. Therefore, it is important to conserve landscapes of rich-structured mosaics of open land and forest patches, similar to the Rhön, as habitat fragmentation continues to occur. As a flagship species, efforts to create heterogeneous landscapes will help other forest-dwelling species to migrate between patches.

Acknowledgments We are deeply grateful to all volunteers who collected hair samples from lurestick traps across the study range. Special thanks go to Ulrike Schade, Doris Pokorny (Biosphärenreservat Rhön, Verwaltungsstelle Bayern), Eckard Jedicke and Matthias Metzger (Rhönnatur e.V.), Wolfgang Fremuth and Michael Brombacher (FZS),

and Kai Frobel (BN). All DNA sequencing and fragment length analyses in this study were performed in the laboratory center of the Senckenberg BiK-F funded by the LOEWE initiative (Landes-Offensive zur Entwicklung Wissenschaftlich-ökonomischer Exzellenz) of the Federal State of Hessen. We highly appreciate the laboratory assistance provided by Mascha Siemund, Susanne Carl, and Berardino Cocchiararo (Senckenberg).

Funding Open Access funding enabled and organized by Projekt DEAL. Funding for this project comes from the Frankfurt Zoological Society (FZS), the Allianz Umweltstiftung, the Biosphärenreservat Rhön, Rhoen Natur e.V., Friends of the Earth Germany (BUND), Bund Naturschutz in Bayern e.V. (BN), and Federal Agency for Nature Conservation (BfN; Bundesprogramm Biologische Vielfalt – project "Wildkatzensprung"). SM is funded by the DAAD (German Academic Exchange Service), CREOi (Conservation, Research and Education Opportunities International), and the University of California Santa Cruz. AVT received funding from the Karl und Marie Schack-Stiftung. CN received funding from the grant SAW-2011-SGN-3 from the Leibniz Association.

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Supplementary Material

Overlooked or recolonized? Revealing the origin of wildcat reappearance after presumed long-term absence

European Journal of Wildlife Resources

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Table S1. List of projects during the study period. Year, name, number of confirmed samples from roadkill (Tissue) and hairtraps (Hair), *n* individuals are indicated. Fo

Year	Projects	Type of Activity	Tissue	Hair	Individuals	# hairtraps	location	time frame
2004	TLUG	Roadkill Assessment	2	0	2	0	na	na
2005	TLUG	Roadkill Assessment	3	0	3	0	na	na
2006	TLUG Franz Müller	Roadkill Assessment	7	0	7	0	na	na
2007	TLUG Franz Müller Auswilderung Spessart (AS)	Roadkill Assessment Hair trapping	1	7	5	11 (AS)	Spessart	Jan.-April
2008	TLUG Franz Müller BUND Thuringia	Roadkill Assessment Hair trapping	11	1	12			
2009	TLUG Franz Müller BUND Thuringia Rhön Natur e.v. (RN)	Roadkill Assessment Hair trapping	25	8	25	504 (RN)	Rhön	year long
2010	TLUG Franz Müller BUND Thuringia Rhön Natur e.v. (RN) HLSV	Roadkill Assessment Hair trapping	23	86	64	504 (RN)	Rhön, highway A44/A7	year long
2011	TLUG Franz Müller BUND Wildkatzensprung (WKS) Rhoen Natur e.v. (RN) BUND Thuringia Hessen Forst FENA	Roadkill Assessment Hair trapping	24	5	25	100 (WKS) 504 (RN)	Rhön, Spessart	year long
2012	TLUG Franz Müller BUND Wildkatzensprung (WKS) Rhön Natur e.v. (RN) Hessen Mobil Senckenberg project (SN)	Roadkill Assessment Hair trapping	21	258	127	100 (WKS) 504(RN) ~50 (SN)	Spessart, Hainich	year long
2013	TLUG Franz Müller BUND Wildkatzensprung (WKS) Institut für Tierökologie und Naturbildung BUND Thuringia Biosphärenreservat Rhön (BRR)	Roadkill Assessment Hair trapping	26	100	77	100(WKS) 161 (BRR)	Spessart, Hainich, Rhön	year long
2014	Franz Müller BUND Wildkatzensprung (WKS) Biosphärenreservat Rhön (BRR) Forstamt Schlüchtern	Roadkill Assessment Hair trapping	3	152	89	150 (WKS) 162 (BRR)	Spessart, Hainich, Rhön	year long
2015	Franz Müller BUND Wildkatzensprung (WKS) Biosphärenreservat Rhön (BRR) Forstamt Schlüchtern	Roadkill Assessment Hair trapping	3	100	120	150 (WKS) 135 (BRR)	Spessart, Hainich, Rhön	year long
2016	Franz Müller BUND Wildkatzensprung (WKS) Biosphärenreservat Rhön (BRR) Forstamt Schlüchtern	Roadkill Assessment Hair trapping	3	56	50	150 (WKS) 60 (BRR)	Spessart, Hainich, Rhön	year long
2017	Franz Müller BUND Wildkatzensprung (WKS)	Roadkill Assessment Hair trapping	3	30	12	50 (WKS)	Spessart	Jan.-Apr.

Fig. S1. Lagged scores from the second PC axis (a). sPCA eigenvalues showing the significant axes of the spatial analysis (b).

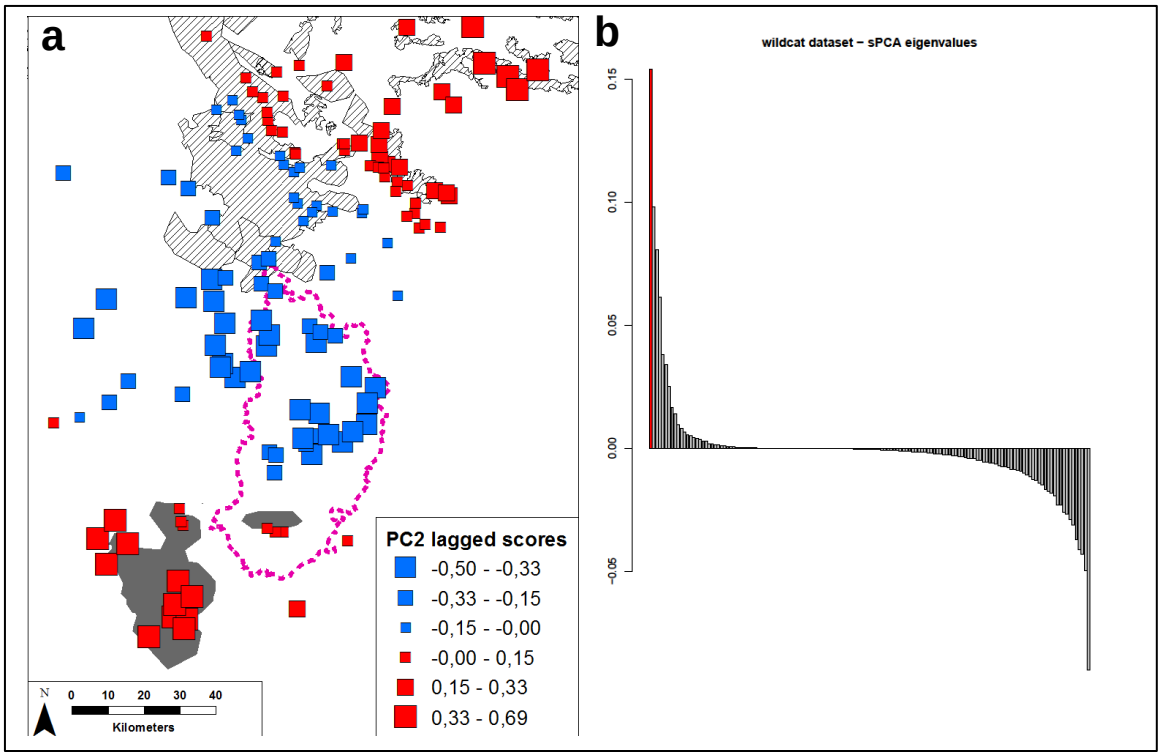
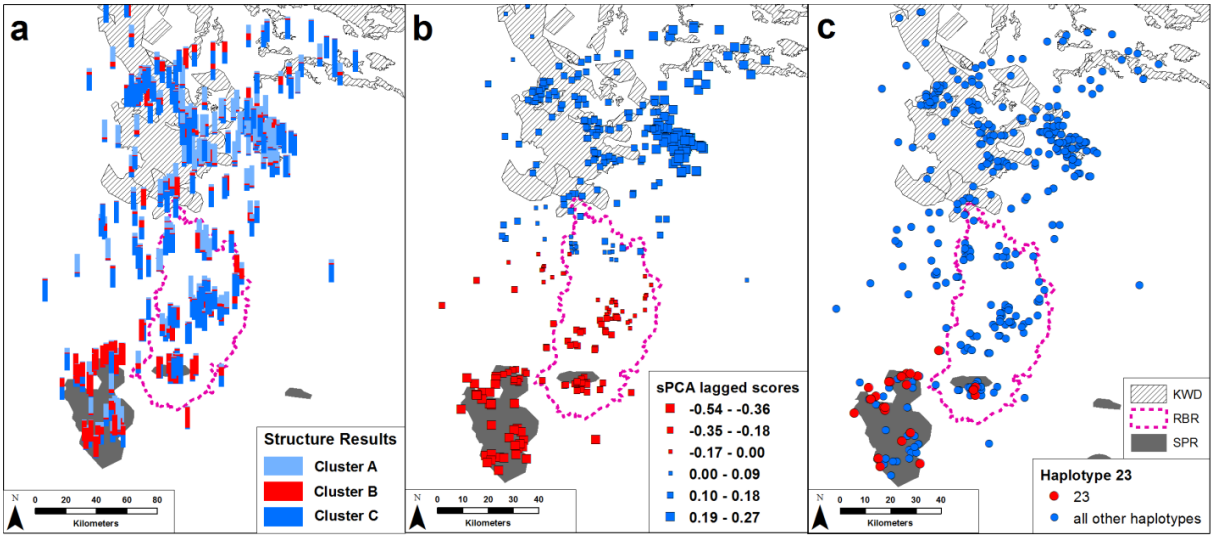


Fig. S2 Map showing results from an extended dataset of 439 wildcat individuals using 14 microsatellite markers. Here we show the STRUCTURE results with three clusters, the spatial genetic structure as assessed by sPCA and haplotype map highlighting the reintroduced haplotype SNG-HP-FS23. (a) The most likely number of clusters, *K*3, indicated by STRUCTURE results. (b) Individual scores from the first principal component: large blue squares indicated highly positive scores; large red squares indicated highly negative scores. (c) Map of individuals within the study area with the haplotype SNG-HP-FS 23 (red) and all other haplotypes (blue).



Publication II

The rise of a large carnivore population in Central Europe: Genetic evaluation of lynx reintroduction in the Harz Mountains

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Erklärung zu den Autorenanteilen

Mueller, Sarah Ashley*; Reiners, Tobias Erik*; Middelhoff, Tomma Lilli; Anders, Ole; Kasperkiewicz, Anna; Nowak, Carsten (2020): The rise of a large carnivore population in Central Europe: genetic evaluation of lynx reintroduction in the Harz Mountains. In: Conserv Genet 21 (3), S. 577–587. DOI: 10.1007/s10592-020-01270-w.

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Was hat der Promovierende bzw. was haben die Koautoren beigetragen?

(1) Entwicklung und Planung

SAM: 50%, TER: 40%, CN: 10%

(2) zur Durchführung der einzelnen Untersuchungen und Experimente

Sample collection: OA/TLM: 100%

Sample selection: SAM: 30%, TER: 30%, OA/TLM: 40%

Microsatellite/mtDNA lab work: SAM: 20%, AK: 10%, TER: 70%

(3) zur Erstellung der Datensammlung und Abbildungen

Monitoring activities: OA/TLM: 100%

Genetic data: SAM: 60%, TER: 40%

Figures: SAM: 60%, TER: 30%, OA/TLM: 10%

(4) zur Analyse und Interpretation der Daten

Population Assignment/Diversity measures: SAM: 100%

Temporal Analysis: SAM:70%, TER:30%

Parentage and Pedigree: SAM: 50%, TER: 25%, OA/TLM: 25%

(5) zum Verfassen des Manuskripts

SAM: 75%, TER: 10%, CN: 10%, OA/TLM: 5%

Datum/Ort: 22.06.2021, Frankfurt a.M.

Unterschrift Promovend: _____

Zustimmende Bestätigungen der oben genannten Angaben

Unterschrift Betreuer: _____ Datum/Ort: Frankfurt, 25.06.2021

Ggfs. Unterschrift *corresponding author* SAM: _____ Datum/Ort: 22.06.2021, Frankfurt a.M.

Conservation Genetics (2020) 21:577–587
https://doi.org/10.1007/s10592-020-01270-w

RESEARCH ARTICLE

The rise of a large carnivore population in Central Europe: genetic evaluation of lynx reintroduction in the Harz Mountains

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Received: 13 August 2019 / Accepted: 24 March 2020 / Published online: 7 April 2020

Abstract

Large carnivores have made a successful comeback across human-dominated landscapes in Central Europe. The Eurasian lynx, for instance, has been actively reintroduced in different regions. Genetic diversity is quickly eroding in these isolated, small populations, questioning the long-term success of lynx reintroductions. To track population development and genetic diversity in a reintroduced lynx population, we used microsatellite analysis and mtDNA haplotyping based on 379 samples collected during the initial 15 year period of lynx reintroduction in the Harz mountains National Park, Germany. The Harz lynx population shows higher genetic diversity relative to other lynx reintroductions, due to initial cross-breeding of divergent captive source lineages and a comparably high founder size. While the population shows significant population growth and spread into adjacent regions, genetic diversity is continiously declining. Expected heterozygosity values dropped from 0.63 after reintroduction (2006/2007) to 0.55 within a 10 year period. Despite this, the Harz lynx population is currently a viable component to an envisioned lynx metapopulation spanning across Central Europe. The ongoing genetic erosion in the Harz population along with a lack of geneflow from adjacent populations indicates that such connectivity is urgently needed to ensure long-term population persistence.

Keywords Reintroduction · Lynx lynx · Genetic diversity · Large carnivore · Inbreeding

Introduction

Large carnivores, such as wolves and lynx, are currently expanding their ranges across Central and Western Europe (Chapron et al. 2014). This process of de-extinction is generally considered beneficial to restore biodiversity as native top-level predators have overall-positive effects on ecosystem function and health (Ripple et al. 2014; Schmitz et al. 2010). In contrast to wolves, Eurasian lynx (*Lynx lynx*) have been actively reintroduced in Central Europe in the frame of several reintroduction projects. Between 1971 and 2018, 16 reintroductions of lynx have been attempted across Central Europe (Linnell et al. 2009). These reintroductions were widely unsuccessful; establishment occurred in only five areas, while the long-term fate remains unknown for multiple reintroductions due to lack of sufficient information post release (Linnell et al. 2009).

The low success rates in lynx reintroductions stems from extremely small founder sizes, which leads to inbreeding and low genetic diversity. In fact, all genetically investigated reintroduced populations show inbreeding

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Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10592-020-01270-w) contains supplementary material, which is available to authorized users.

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and loss of genetic diversity (Breitenmoser-Würsten and Obexer-Ruff 2003; Bull et al. 2016). Additionally, most reintroduction attempts used individuals from one source population. Another major driver of this low success rate is reintroduced populations remain isolated from other Eurasian lynx populations (Kramer-Schadt et al. 2004, 2005). This isolation is a major threat to the long-term viability of reintroduced lynx populations (Molinari-Jobin et al. 2010). While there is considerable data on the genetic structure of autochtonous lynx populations in Europe including phylogeographic assessments (Gugolz et al. 2008; Ratkiewicz et al. 2012; Sindičić et al. 2013a; Rodríguez-Varela et al. 2016), Europe-wide population characterization (Rueness et al. 2014; Ratkiewicz et al. 2014; Schmidt et al. 2011), fine-scale population structure (Sindičić et al. 2013a; Bagrade et al. 2016; Schmidt et al. 2016; Holmala et al. 2018) and non-invasive genetic monitoring (Davoli et al. 2013; Krojerová-Prokešová et al. 2018; Hollerbach et al. 2018), such genetic assessment of reintroduced lynx populations is sparse (Bull et al. 2016).

One reason for the absence of scientific data regarding reintroduction projects is obtaining samples for standardized genetic population monitoring is notoriously difficult for this species (Schmidt and Kowalczyk 2006; de Barba et al. 2010; La Haye et al. 2017). Hair sampling was successfully carried out by Schmidt et al. (2016) however, this approach did not prove successful in regions with less information on individual movements and marking sites. This is somewhat unfortunate, as genetic factors play an important role in the long term-viability and reintroduction success in small isolated populations. Thus, measuring and evaluating genetic diversity over time is vital to develop optimized strategies for long-term population management (Boitani et al. 2015).

Here we present a multiple-year genetic assessment of a reintroduced lynx population in the Harz Mountains (HM) in Central Germany. Official reintroduction of the Eurasian lynx in the HM started in 2000. The long term success of this reintroduction was initially regarded with skepticism (von Arx et al. 2009; Kramer-Schadt et al. 2005). In contrast to former reintroductions, where mostly wild caught lynx from Carpathian origin were used, lynx released in the HM originated from zoos and wildlife parks.

In this study we aim to describe the (i) population spread, (ii) genetic structure and diversity through time, (iii) pedigree of the wild population since reintroduction and (iv) effect of founder size on genetic diversity. We discuss these issues in respect of the captive origin of this population in contrast to other reintroduced lynx populations with founders of wild-caught origin. Understanding the success of lynx reintroductions originating from captivity has important implications for the design of future reintroductions, ultimately with the goal of creating a viable connected lynx metapopulation throughout Europe.

Study area

The study region consists of the Harz lynx population (HLP) range comprising parts of four federal states; Lower Saxony (NI), Saxony-Anhalt (ST), Thuringia (TH) and Hesse (HE). Additionally single dispersing individuals have been sampled in North Rhine-Westphalia (NW) and Bavaria (BY) (Fig. 1). The core of the current distribution is the HM where the reintroductions occurred. The HM is a low-mountain region in central Germany ranging up to 1141 m. Approximately ten percent of the 2200 km^2 area is protected under the status of a National Park (IUCN, category II). Lynx were absent in the region for more than 200 years until reintroduction occurred (Anders pers. com). Between 2000 and 2006, 24 lynx originating from German and Swedish zoos and wildlife parks were released within the National Park (9 males, 15 females) (Table S1 and Fig. 2). Additionally, at least ten lynx escaped from wildlife parks or were illegally released. Four of those animals were recaptured due to their habituation to humans. The first evidence of reproduction in the wild was reported in 2002 (Anders and Sacher 2005).

Methods

Monitoring activities

The HLP is monitored through the collection of proven and unproven lynx indications and camera traps. The intensity of monitoring has increased over time due to the greater availability of resources for such activities (Anders 2013; Anders and Sacher 2005; Anders and Middelhoff 2016a, b). Staff of the Harz National Park were responsible for the monitoring in NI and ST. Governmental agencies monitor the lynx in TH, HE, NW, and BY. Lynx reproduction occurred in HE between 2010 and 2015; NW and BY show evidence of single individuals of Harz origin (Anders pers. com). All lynx observations are classified using the Status and Conservation of the Alpine Lynx Population (SCALP) framework (Molinari-Jobin 2003) as adopted by the German monitoring authorities in 2009 (Kaczensky et al. 2009; Reinhardt et al. 2015). We considered three SCALP classes of records: C1 records (confirmed data e.g. georeferenced pictures, dead lynx and genetic detections) to assess lynx distribution over time. C2 (confirmed data, e.g. prey remains confirmed by experts) and C3 (unconfirmed data, e.g. sightings) were recorded but not considered in the dataset as the rate of false positives can lead to biased conclusions (Molinari-Jobin et al. 2012).

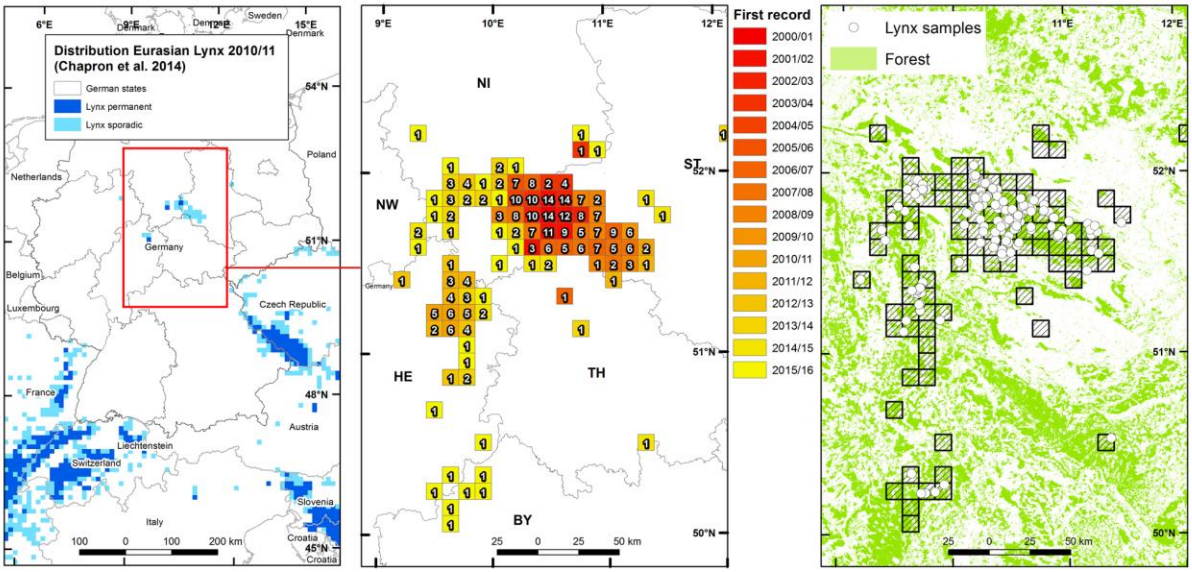

Fig. 1 **a** Lynx distribution according to Chapron et al. (2014) with additional data from the HLP added according to the monitoring year 2010/2011. Dark blue indicates permanent occurrence and light blue indicates sporadic occurrence. **b** The occurance of C1 evidence of lynx within the study area between 2000 and 2016. The color represents the year of first appearance. The number indicates the number of years the species was detected. **c** Map of the 295 genetically confirmed lynx samples

Telemetry was implemented in the HM in 2008. Seventeen lynx were equipped with collars until 2016. Systematic camera trapping was tested in 2012 and routinely implemented in 2014 (Anders and Middelhoff 2016a, b, Port unpubl.). To estimate lynx spatial spread, we used 10 km × 10 km grid cells (EEA reference Grid) and overlaid all cells with C1 evidence from 2000 to 2016. Evidence of known migratory individuals was excluded from the map.

Genetic sample collection

Between 2001 and 2016, 379 genetic samples were opportunistically collected alongside standard monitoring in the study area (Table S2). In total, 41 tissue, 66 blood, 118 hair, 45 scat, and 109 saliva samples were collected. Tissue and scat samples were transferred to 96% ethanol. Hair samples were stored wrapped in filter papers with silica gel. Saliva traces were sampled with cotton swabs from carcasses and stored dry at room temperature (Harms et al. 2015).

DNA extraction

DNA from blood and tissue was isolated using the QIAGEN Blood and Tissue Kit following the manufacturer's protocols. Tissue extracts were diluted to 10 ng/µl. Isolation of DNA from hair, saliva, and scat was carried out in a separate laboratory for noninvasive samples using the QIAGEN Investigator Kit and the QIAamp DNA Stool Kit, respectively. Hair and saliva were eluted twice with 40 µl each; elution volume of scat samples was 120 µl.

Mitochondrial DNA analysis

Two sequence fragments targeting the control region of mitochondrial DNA were used for species identification and haplotyping. Primers L16782 and H16922 (Gugolz et al. 2008) or primers Lynxfwd4 and Lynxrev5 (Buhrmester 2014) amplified a 180 bp fragment or a 248 bp fragment, respectively. The latter primer is lynx specific and designed for samples of low DNA concentration. Amplification was carried out by real-time PCR with a reaction volume of 10 µl. GENEIOUS 8.1 (Biomatters Limited) was used for sequence alignment and sequences were assigned to haplotypes described by Hellborg et al. 2002) and Gugolz et al. 2008)

Microsatellite genotyping

A microsatellite marker set of 19 loci and 2 sex markers were used (Table S3). The markers were derived from sets originally developed for domestic cat, *Felis catus* (Menotti-Raymond et al. 1999, 2005), Canadian lynx, *Lynx canadensis* (Carmichael et al. 2000) and Sumatran tiger, *Panthera tigris sumatrae* (Williamson et al. 2002). Each sample was run with a minimum of three replicates in 5 µl or 10 µl reaction volume using the Multiplex PCR

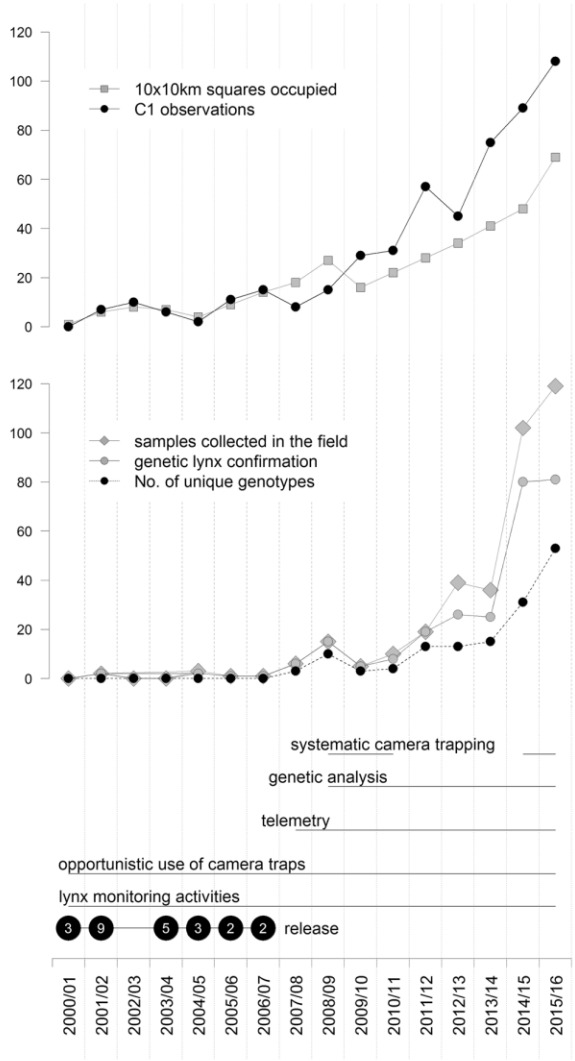

Fig. 2 Temporal spread of lynx observations over 16 monitoring years (May–April). The top panel refers to the spatial spread of the newly founded lynx population. C1 observations refers to the SCALP criteria of confirmed sighting in NI, ST, HE, TH, NW and BY. The study area was divided into 10 × 10 km squares and number of squares occupied in each monitoring each calculated. The middle panel shows the number of genetic samples collected during the study period, showing the relationship between number of samples collected and number of confirmed unique indviduals. The bottom panel shows the type and duration of monitoring activity, with the numbers reporting the released individuals between 2000 and 2006

Mastermix (QIAGEN), with a negative control. Fragment analysis was performed on a 3730xl DNA Analyzer (Applied Biosystems). Consensus genotypes were derived using a custom R script based on the algorithms used in GIMLET 3.3 (Valière 2002) with a maximum of three mismatching loci accepted to assign a sample to the same individual. The customized script also takes gender, haplotype, sampling date and location into account. Samples between 2000 and 2015 were first run with 14 loci (Table S3) and later on the 19 loci set; in these cases we used results from both sets to create consensus genotypes of 19 loci. Therefore, estimation of sample quality and genotyping errors was carried out on both the 14 loci and 19 loci set.

Amplification success, allelic dropout (ADO), and false allele (FA) calculations were carried out based on the total replicates for each individual and the corresponding consensus genotype. Samples with < 25% amplification across all replicates were excluded.

Population assignment

Population ancestry of sampled individuals was analyzed using *discriminant analysis of principal components* (DAPC) implemented in the adegenet package (Jombart 2008) using R and STRUCTURE (Pritchard et al. 2000). We included 27 individuals from zoos, 10 sampled founders and 105 genotypes originating from the HLP. DAPC assumed seven clusters and retained the first six PCA axes, estimated by the *optim.a.score* and *find.clusters* function, which predicts the optimal number of principal components and clusters, respectively.

STRUCTURE was executed using the admixture model with correlated allele frequencies. K ranged from one to eight using a burn-in of 500,000 runs, following 500,000 MCMC runs. STRUCTURE runs were repeated 10 times for each K and CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007) was used to match runs. The most likely number of clusters was investigated using the method described by Evanno et al. (2005) and implemented in STRUCTURE HARVESTER (Earl and vonHoldt 2012).

Genetic diversity through time and generations

Standard measures of genetic diversity including number of alleles (Na), observed (Ho) and unbiased expected heterozygosity (He) were calculated with GENALEX 6.5 (default settings) (Peakall and Smouse 2006) using the same groups defined for population assignment methods. In addition, each monitoring year (May 1st–April 30th) was considered as a distinct group in order to assess the development of the lynx population over time. Genetic diversity was also estimated for all sampled offspring born each monitoring year. Private alleles in all groups were evaluated using GENALEX 6.5 (Peakall and Smouse 2006).

Parentage analysis and analysis of pedigree

Parentage estimation was conducted with COLONY 2.0 (Jones and Wang 2010; Wang 2004) allowing inbreeding with long run length, full-likelihood method with high precision, update of allele frequency and complexity prior. We used polygamy as the mating system to not exclude rare instances of polyandrous litters (Lucena-Perez et al. 2018). We included the prior error rates for each locus and allelic frequencies. Parent–offspring associations were tested in a stepwise approach for each monitoring year to account for known changes to the population, including confirmed births, deaths, and recapture events. As no total population estimates were given from year to year, probability of parent to be sampled based on initial population size and ratio of known individuals to the whole population was set to 0.2 for mothers and fathers.

For each monitoring year, we determined the set of candidate parents based on known adults (> 3 years). For six individuals, age was not determined from traditional monitoring and were included as candidate parents since the first year these individuals were sampled. Information on known sibling relationships (e.g. juveniles photographed together), parent-offspring relationships (e.g. mother photographed with offspring) and exclusion of parent relationships (e.g. mismatched haplotypes or death) was included a priori.

No threshold was set for assigning a parent–offspring relationship as the output was compared with monitoring data to refine the relationships defined by COLONY 2.0. Parent pairs were compared for compatible territories and known sitings of parental individuals. When no candidate parents were assigned to offspring in a target year, we sampled the parental genotypes inferred by COLONY 2.0. These unsampled individuals were considered in the following years. The derived pedigree was used to calculate inbreeding, kinship and estimate the number of generations with the R package *pedigree* and *kinship2*. The pedigree of reproducing individuals was visualized using the pedigree tool provided by Progeny Genetics (https://www.progenygenetics.com/online-pedigree/).

Founder size

Five out of 24 released animals died early or were removed from the population shortly after reintroduction (Anders, pers. communication), leaving 19 potential founders. For 10 of them (eight females and two males) genetic samples were available (Table S1). To estimate the number of founder individuals in the HLP, results from the parentage analysis, occurrence of private alleles and mitochondrial haplotypes were considered. We also ran ML-Relate to determine the degree of relatedness between sampled founders.

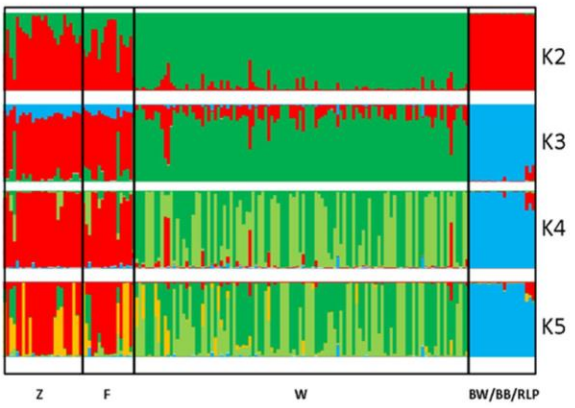

Fig. 3 STRUCTURE plots where each bar represents one individual. Most likely clusters K = 2 as indicated by STRUCTURE HARVESTER. Lynx from the Harz (W) were assigned to a distinct cluster separated from zoo (Z), founder (F), and individuals from Baden-Wurtenberg, Bohemian-Bavarian and Rhineland Pfalz reintroductions (BW/BB/RLP)

Results

Sample collection and species determination

Blood and tissue samples showed very high amplification and low ADO (0.08, 0.02) and FA (0.02, 0.02). Of the noninvasive samples, hairs showed the highest rate of assigment to lynx (83%), followed by scats (73%) and saliva traces from prey remains (53%). Hair samples showed the lowest ADO among the noninvasively collected samples (0.16), followed by scat samples (0.19). Lowest amplification (0.78) and highest ADO (0.29) were found in saliva traces. FA rates were lowest for saliva (0.03) with the highest rate (0.10) found in scat samples (Table S2). Genotypes for all individuals can be found in Table S6.

Haplotype frequencies

The group of 10 sampled founders showed five haplotypes: L1, L2, L4, L6, L7 (Table S1). Three of those haplotypes were identified in 96 successfully analysed wild individuals ($n = 105$), with L1 found in 3, L4 in 84, and L6 in 9 individuals (Table S6).

Population assignment

Bayesian assignment implemented in STRUCTURE separated Harz individuals from sampled founders, captive lynx, and the BBA population. STRUCTURE HARVESTER indicated K = 2 as the likely number of clusters. Lynx individuals from zoos and sampled founders formed one cluster, and the individuals from the BBA population formed another

distinct cluster. With a K = 4, the HLP formed two clusters (Fig. 3). These population subdivisions were largely confirmed by DAPC analysis (Fig. S1).

Genetic diversity through time

Between 2 and 14 alleles were found among the genotyped loci, a mean of 7.47 alleles per locus across all samples. When corrected for sample size, the HLP (3.32) showed lower average allele numbers than zoo (4.82) and founder (4.46) individuals. In contrast, observed and expected heterozygosity are comparable in zoo, founders, and the HLP, while considerably lower in BBA population (Table S4). Both the effective number of alleles and expected heterozygosity show a continuous decline over time (Fig. 4a, b). For observed heterozygosity, values initially rose and then show a similar decline. This pattern becomes stronger when considering generation sequence (only juveniles considered; Fig. 4a).

Parentage and cohorts

Analysis of relatedness using COLONY as well as additional data from field monitoring allowed to reconstruct a partial pedigree of the HLP, with the fifth generation of lynx confirmed in 2015/2016 (Fig. 5). COLONY estimated 16 sampled females and 9 sampled males as having reproduced in the HLP and the average assignment probability of parent pairs was 0.88 ± 0.22. Additional unsampled individuals were identified by COLONY as having contributed to the pedigree. We also considered known offspring and territories based on available field monitoring data to refine COLONY results. This resulted in a total of 43 individuals forming the final pedigree (Fig. 5) Twenty-two lynx were identified as influential breeding individuals, of which 5 have died or been taken out of the wild. Analysis of the partial pedigree revealed an average inbreeding rate of 0.01 ± 0.03 and mean kinship of 0.03 ± 0.02. Cases of two identified inbreeding events occurred in the first generations.

Founder size

Three reintroduced females (LL018, LL037, LL006) were confirmed to have offspring by camera traps during field monitoring and re-affirmed through COLONY. These confirmed females carried haplotypes L4 and L6. The seven remaining sampled founders were not assigned by COLONY to any sampled offspring. Two offspring of a putative fourth female (UF01 in the pedigree) were found to carry haplotype L1. At locus FCA026, eight alleles

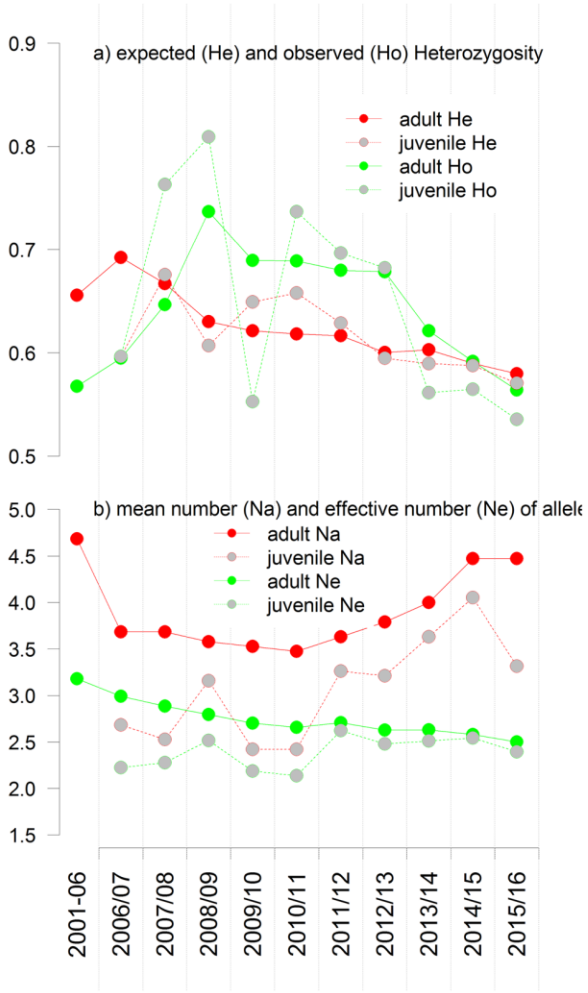

Fig. 4 Genetic diversity in the reintroduced Harz lynx population over time (2000–2016). **a** observed heterozygosity (Ho) and expected heterozygosity (He) by year. **b** number of alleles (Na) and number of effective alleles (Ne) for each year. In both, dashed lines show values from juveniles confirmed or estimated to have been born within the monitoring year. Solid lines represent values from the entire population. Note that the first unit on the x axis represents the time span of reintroduction over multiple years

were detected which are not present in the three confirmed founder females. Founders were also analysed in ML-Relate which found 2 sets of known siblings (Table S5). One set includes LL006, LL0036w, and LL0037w, which all orginate from Wildpark Neuhaus and share haplotype L4. LL0030m is a full sibling with LL0031w originating from Wildpark Edersee with haplotype L7. LL031w died in 2004, and the haplotype 7 has not been found in the HLP. LL006w and LL0037 are known to have reproduced and LL006w died in 2008.

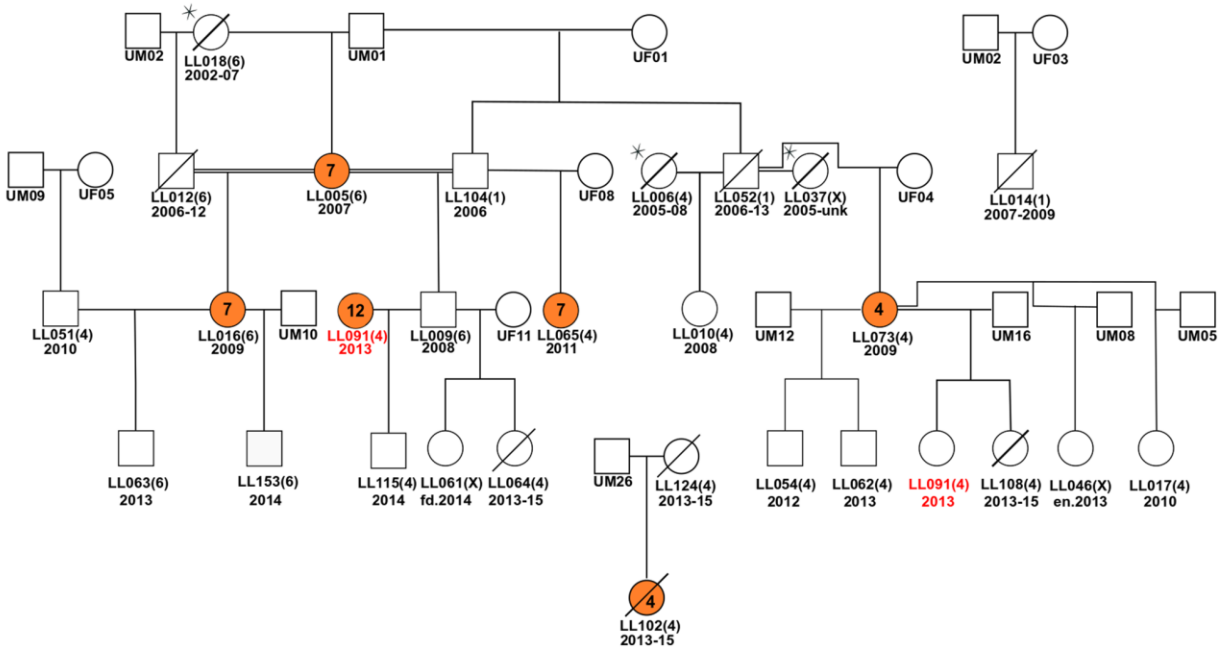

Fig. 5 Pedigree of all reproducing individuals in the Harz lynx population since the first reintroduction until 2016 based on COLONY results with aid from field monitoring and haplotype comparison. Individual identification number is followed by mitochondrial haplotype (in parentheses) and birth year below. When no birth year is known the first detection year (fd.) is provided. Orange represents territorial breeding females identified through COLONY and confirmed with monitoring data and number of confirmed offspring is located within. Unknown male and female individuals identified by COLONY are marked with a UM/UF

Discussion

The HLP has been monitored consecutively over the last 15 years using both traditional field methods and genetic analyses to track population size, demographic expansion and genetic diversity over time, with the ultimate goal to draw conclusions concerning the current status and likely future development of this reintroduced population.

The reintroduction of lynx from captivity in the HM initially received considerable concern. For instance, it was questioned if animals from captivity could survive in the wild and form a new population (Wotschikowsky et al. 2001). There was also some degree of uncertainty regarding the genetic origin of the released lynx, thus increasing the risk of inbred individuals (Laikre 1999), hybrids between subspecies (von Arx et al. 2009) or outbreeding depression (Huff et al. 2011) in the established population. In addition, there was apprehension about the successful dispersal across anthropogenic barriers to connect with other populations, which would maintain genetic diversity and ensure long-term viability (Kramer-Schadt et al. 2005). Our data show that despite the multiple concerns, lynx have spread over the past 15 years and form an expanding population with a decreasing level of genetic diversity. Given the number of failed reintroduction attempts across Europe, the reconstruction and scientific analysis of the population growth of the reintroduced HLP is of considerable importance to guide future reintroduction attempts and work towards a long-term viable lynx metapopulation spreading across Europe.

Population growth and expansion

Our results support that since the release between 2000 and 2006, the HLP has experienced a substantial increase in population size and spatial spread across the HM and its surroundings, which has been found in other studies (Anders et al. 2012, 2016; Anders and Middelhoff 2016a, b). We have genetically identified 105 wildborn individuals from the HLP (Fig. 2), which have spread up to 280 km Euclidean distance from the area of reintroduction. As sampling was opportunistic and not evenly spread through space and time, this number likely only represents a fraction of all individuals between 2000 and 2016.

Several factors have contributed to the steady increase in size and spatial spread of the HLP. First, there are presumably low rates of illegal killing, with only a single detected case. Illegal killing is among the dominant factors preventing the spatial growth of lynx populations in Europe (Müller et al. 2014; López et al. 2014; Heurich et al. 2018;

Červený et al. 2002). In the Bohemian forest, for instance, an estimated 62 lynx have been illegally hunted outside of the national park borders post-reintroduction (Müller et al. 2014). Second, there is a relatively consistent availability of roe deer (*Capreolus capreolus*) and other ungulate species in the HM region likely providing suitable conditions for the establishment and growth of a stable lynx population. Lynx are more sensitive to changes in habitats and prey abundance than other large carnivores, making both forest cover and availability of prey important factors in determining the likelihood of survival (Bagrade et al. 2016; Schmidt et al. 2011). This rapid demographic and spatial expansion confirms that reintroduction of captive born lynx is possible, which has important consequences for future reintroduction planning. As the current availability of wild caught lynx is highly restricted in Europe and poses a significant impediment for current reintroduction attempts (Krebühl, pers. comm.), we show here a potential alternative for the costly and laborious capture of animals in the wild.

Declining genetic diversity over time

While standard measures of genetic diversity for the HLP are higher than for other reintroduced populations (e.g., He of 0.50 compared to 0.43 in BBA), they do not match levels seen in autochthonous populations (Supplementary Material 1). The HLP experienced a demographic bottleneck post-reintroduction, as a low number of released individuals reproduced (Fig. 5). This demographic bottleneck has been well documented in other lynx reintroductions across Central Europe (Schmidt et al. 2011; Sindičić et al. 2013b; Abascal et al. 2016). STRUCTURE and DAPC results show that the HLP now forms a cluster, which is separated from the founders and sampled zoo individuals (Fig. 3 and Fig. S1). In addition to this central HLP cluster, some individuals group in the vicinity of other released individuals, which have not been identified as founders in this study. As we are unfortunately missing genetic data on nine potential founders, it appears likely that we are not able to generate a complete picture of population establishment in this study. The genetic structure during the initial population founding phase, including those genetically distant individuals, might thus be explained by the genetic contribution of unsampled founder individuals. In 2008/2009 we saw observed heterozygosity (0.74), expected heterozygosity (0.61), and number of effective alleles (3.6) considerably elevated in the F1 generation (Fig. 4). Likely, this is a result of distant captive lineages mating in the wild. We currently see a decline across these measures of genetic diversity; after 7 years observed heterozygosity (0.56), expected heterozygosity (0.57), and number of effective alleles (2.92) all showed some degree of decline (Fig. 4). Interestingly, the overall number of alleles rose from 3.6 to 2009 to 4.5 in 2016. However, this can be attributed to a higher number of samples being collected due to more intensive genetic monitoring (Fig. 2). This downward trend suggests that genetic diversity will likely continue to decline in the future, likely resulting in similarly low values as currently observed in the other European lynx reintroduction areas, if no gene flow through some degree of population exchange happens. Notably, there is evidence of long distance dispersal of single males from the HM to a distance of up to 280 km, which documents the potential of lynx to disperse across fragmented anthropogenic landscapes in Central Europe. Such long distance dispersal has been documented previously in Central Europe (Zimmermann et al. 2005; Schmidt 1998). This evidence raises hope for the long-term conservation goal of connecting isolated lynx populations in Central Europe, ultimately leading to the formation of a viable metapopulation (Breitenmoser-Würsten et al. 2007). However, the next years will likely show if the population is capable of spreading further into more fragmented areas while sustaining territorial and reproducing females. Therefore, there is a heightened importance for continued genetic population monitoring to screen genetic diversity, inbreeding and gene flow.

Founder size and pedigree Pedigree reconstruction revealed a low founder size with reproductive success of the population contingent upon a small number of territorial, prolific breeders. Kramer-Schadt et al. (2005) argued that a minimum of ten females is necessary to establish a sustainable population. Our haplotype and COLONY results indicate that the founder size of the HLP consisted of a minimum of seven individuals (four females and three males). We cannot rule out the possibility that some of the ten undocumented escapes and illegal releases of lynx in the study area contributed to reproduction. While this is likely the highest number of founder individuals reached in a reintroduction (von Arx et al. 2009), seven founders are not sufficient to form a genetically sustainable population without considerable levels of inbreeding. This confirms that the release of a relatively high number of animals is vital to reach a moderate number of genetically significant founders. Our pedigree confirms two instances of inbreeding, which is likely an underestimate given the relatedness analysis finding one pair of full siblings within the founders of the HLP. This inbreeding rate is therefore a highly conservative estimate.

Finally, the pedigree shows that only 25 of the 115 genetically identified individuals, including founders, have certainly reproduced. These reproducing individuals tend to be well established territorial individuals from the 1st and 2nd generations (Fig. 5). It must be taken into consideration that several reproducing individuals have not been genetically identified, as shown by unknown individuals in the pedigree, so the number of well-established territorial individuals is likely underestimated. However, a

comparably low number of reproducing individuals has also been noticed in other lynx populations (Schmidt et al. 2016; Krojerová-Prokešová et al. 2018; Holmala et al. 2018).

Conclusions

Long term post-release monitoring over the past two decades has enabled a detailed reconstruction of the demographic history of the Eurasian lynx in the HM. Despite a low number of founders, the captive origin of released individuals, and highly divergent reproductive success within the population, we conclude that the population is currently growing despite the continuing loss of genetic diversity occuring in each subsequent generation. If the growth continues further, we believe that the HLP might become one of the cornerstones of the envisioned interconnected Central European metapopulation, which will ensure long-term establishment and survival of the lynx within the human-dominated Central European landscape. To ensure this natural exchange, it is vital to keep illegal killing low and facilitate the permeability of potential migration corridors (Kramer-Schadt et al. 2005). Additionally, the translocation of individuals between different reintroductions or from authochtonous populations to stabilize declining genetic diversity and mitigate genetic drift appears a necessary measure until a sufficient level of natural geneflow occurrs between the reintroduction areas.

We strongly urge for the continuation of an efficient genetic and demographic monitoring of the current HLP as well as adjacent reintroduced lynx populations. Detailed knowledge on the development of population status, inbreeding and genetic diversity is crucial for the implementation of optimized conservation strategies. This is particularly true for a species divided into small, isolated subpopulations such as the lynx in Central Europe. Despite the genetic depletion, the population growth of the species within a densely populated country such as Germany proves that, given an appropriate genetic long-term management, lynx may successfully establish and persist in anthropogenic landscapes.

Acknowledgements Open Access funding provided by Projekt DEAL. Funding and support for this project comes from Harz National Park and other conservation initiatives. We are deeply grateful to all students who aided in field and lab work. Special thanks go to Jenny Wertheimer and Verena Harms. SM is funded by the DAAD (German Academic Exchange Service), CREOi (Conservation, Research and Education Opportunities International) and the University of California Santa Cruz. All DNA sequencing and fragment length analyses in this study were performed in the laboratory center of the Senckenberg BiK-F funded by the LOEWE initiative (Landes-Offensive zur Entwicklung Wissenschaftlich-ökonomischer Exzellenz) of the Federal State of Hessen.

Compliance with ethical standards

Conflict of interest The authors are not aware of any conflicts of interest.

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Supplementary Material

The rise of a large carnivore population in Central Europe: Genetic evaluation of lynx reintroduction in the Harz Mountains

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List of Abbreviations

Harz Mountains (HM)

Harz lynx population (HLP)

Hesse (HE)

North Rhine-Westphalia (NW)

Bavaria (BY)

Saxony (NI)

Saxony-Anhalt (ST)

Thuringia (TH)

Status and Conservation of the Alpine Lynx Population (SCALP)

allelic dropout (ADO)

false allele (FA)

Discriminant analysis of principal components (DAPC)

Bohemian-Bavarian Austrian (BBA)

number of alleles (Na)

observed heterozygosity (Ho)

expected heterozygosity (He)

Supplementary Table 1

Table S1. List of reintroduced individuals in the Harz Mountains, including origin, release date, sex (unk.=unknown), haplotype, when the animal was recaught from the wild due to habituation, when the animal died if known, and if the animal reproduced

Individual ID	Origin	Release date	Sex	Haplotype	Recaught	Died	Reproduction
LL025m	Zoo Rostock	6/10/2003	m	6		4/7/2004	no
LL028m	Wildpark Edersee	8/11/2003	m	7	10/2/2003		no
LL029m	Zoo Rostock	6/10/2003	m	6		8/3/2003	no
LL032w	Wildpark Bayerischer Wald	6/3/2004	w	2	11/11/2004		no
LL035w	SW Berge/ Nordens Ark, Schweden	8/14/2001	w	1			no
LL008w	Skansen, Stockholm	6/18/2001	w	1		2/14/2005	possible
LL026w	Tiergarten Bernburg	6/27/2005	w	7			possible
LL030m	Wildpark Edersee	8/11/2003	m	7			possible
LL031w	Wildpark Edersee	6/18/2001	w	unk.		4/13/2004	possible
LL033m	Wildpark Bayerischer Wald	6/3/2004	m	2			possible
LL036w	Wildpark Neuhaus	6/20/2006	w	4			possible
LL043w	Wildpark Alte Fasanerie Hanau	9/27/2000	w	unk.			possible
LL006w	Wildpark Neuhaus	10/18/2006	w	4		11/3/2008	yes
LL018w	Zoo Osnabrück	8/11/2003	w	6	11/21/2007		yes
LL037w	Wildpark Neuhaus	5/11/2005	w	unk.			yes
n.a	Heimattiergarten Fürstenwalde	4/21/2004	w				possible
n.a	Bayerischer Wald	8/22/2000	m				possible
n.a	Wildpark Lüneburger Heide	10/10/2000	m				possible
n.a	Skansen, Stockholm	6/18/2001	w		1/8/2003	1/8/2003	possible
n.a	Skanes Djurpark/Schweden	6/18/2001	w				possible
n.a	Wildpark Schwarze Berge	8/14/2001	w				possible
n.a	Wildpark Lüneburger Heide	8/14/2001	w		6/25/2003	6/27/2003	possible
n.a	Zoo Osnabrück	8/14/2001	m				possible
n.a	Bayerischer Wald	8/14/2001	m				possible
Escaped Individuals							
n.a	Wildpark Christianental	12/1/2007	unk.				possible
n.a	Wildpark Christianental	12/1/2007	unk.				possible

Supplementary Table 2

Table S2. Type and number (n) of samples analysed for this study between 2000 and 2016. The number of samples assigned to unknown (n.a), Canis sp. (C), Felis sp. (F), or V. vulpes (V) as well as to lynx using mitochondrial DNA is also reported. The majority were identified as lynx although multiple saliva samples were also idnetified as fox. Total number of genotypes (genotypes) and the corresponding success (ampli) and error rates (ADO and FA) for the different sample types and two marker sets used in this study.

							14 markers				**19 markers**			
Type	**n**	**n.a**	**C**	**F**	**V**	***L. lynx***	**Genotypes**	**Ampli**	**ADO**	**FA**	**Genotypes**	**Ampli**	**ADO**	**FA**
Blood	66	1				65	65	0.90	0.08	0.019	57	0.84	0.08	0.02
Tissue	41					41	41	0.96	0.02	0.023	39	0.96	0.03	0.016
Hairs	118	9	1	3	7	98	73	0.90	0.16	0.025	47	0.93	0.16	0.018
Scat	45	4	1	2	5	33	24	0.86	0.19	0.043	12	0.86	0.2	0.012
Saliva	109	29	3		19	58	39	0.78	0.29	0.032	31	0.74	0.27	0.037
Total	**379**	**43**	**5**	**5**	**31**	**295**	**242**	**0.89**	**0.138**	**0.026**	**186**	**0.87**	**0.13**	**0.026**

Supplementary Table 3

Table S3. 19 microsatellite marker system used for this study, the asteriks marks the 12 overlapping microsatellites and sex marker used from the previous 14 marker set.

Locus	Primer F (5'-3')	Primer R (5'-3')	Size Range	Reference	GenBank Accession no.
FCA126*	GCCCCTGATACCCTGAATG	CTATCCTTGCTGGCTGAAGG	119-141	Menotti-Raymond et al. 1999	AF130532
FCA069*	AATCACTCATGCACGAATGC	AATTTAACGTTAGGCTTTTTGCC	97-107	Menotti-Raymond et al. 1999	AF130500
FCA718*	TGACAGCTCAGAGCCTAAAGC	GAGTGCACCCCTCCCATAC	210-238	Menotti-Raymond et al. 2003	---
FCA096*	CACGCCAAACTCTATGCTGA	CAATGTGCCGTCCAAGAAC	191-227	Menotti-Raymond et al. 1999	AF130519
FCA723*	TGAAGGCTAAGGCACGATAGA	CGGAAAGATACAGGAAGGGTA	243-317	Menotti-Raymond et al. 2005	AY988124
FCA082*	TCCCTTGGGACTAACCTGTG	AAGGTGTGAAGCTTCCGAAA	233-245	Menotti-Raymond et al. 1999	AF339955
FCA008 *	ACTGTAAATTTCTGAGCTGGCC	TGACAGACTGTTCTGGGTATGG	123-139	Menotti-Raymond et al. 1999	AF130476
FCA031*	GCCAGGGACCTTTAGTTAGATT	GCCCTTGGAACTATTAAAACCA	225-239	Menotti-Raymond et al. 1999	AF130484
FCA006*	GACTTCTGCCTTCTTGTGGC	CCCCTAATGTGACTACAGATAGGG	180-184	Menotti-Raymond et al. 1999	AF130475
FCA115*	CTCACACAAGTAACTCTTTG	CCTTCCAGATTAAGATGAGA	193-217	Menotti-Raymond et al. 1999	AY988109
FCA1018*	CATCACGGTCTCGGGAAC	CGTTGTTTCTTGTGTCGGG	183-191	Menotti-Raymond et al. 2003	AY434998
LCA110*	CCTTTGTCACTCACCA	CGGGGATCTTCTGCTC	93-105	Carmicheal et al. 2000	AF288056
HDZ700	TCCTCCTTCCAGGATGCCA	AGGATGGGGGAAAATCTCTC	133-149	Williamson et al. 2002	AF296747
FCA567	TCAGGGTTTTCCAGAGAAACA	TAGACACATACAGATGGGGTGC	92-106	Menotti-Raymond et al. 1999	AF130661
FCA293	GATGGCCCAAAAGCACAC	CCCACATCTTGTCAACAACG	176-204	Menotti-Raymond et al. 1999	AF130598

FCA026	GGAGCCCTTAGAGTCATGCA	TGTACACGCACCAAAAACAA	136-154	Menotti-Raymond et al. 1999	AF130482
FCA576	GTGCCATTGGATTTGACCTT	ATGGCCAGCTGCTTCATTAT	133-157	Menotti-Raymond et al. 1999	AF130665
FCA201	TCTGCAGGACCAGTCAGATG	AGCATACACAAATTGATGCTGG	90-169	Menotti-Raymond et al. 1999	AF130563
FCA005	CCTAAGGAAACAGTAATCCTGGC	TGGCAGGCATACCAGGAT	130-155	Menotti-Raymond et al. 1999	AF130474
F-zf*	AAGTTTACACAACCACCTGG	CACAGAATTTACACTTGTGCA	158,162	Pilgrim et al. 2005	AF253001
SRY	GAACGCATTCATGGTGTGGTC	GCCTGTAGTCTCTGTGCCTCC	161	Ciani et al. 2008	AB099654
Additional Loci from 14 marker system					
FCA478	TATATGTATGTGCGCGTGTACC	GATCGTGGTTTTTTGACACTTG	194-218	Menotti-Raymond et al. 1999	AF130632
FCA506	AATGACACCAAGCTGTTGTCC	AGAATGTTCTCTCCGCGTGT	232-258	Menotti-Raymond et al. 1999	AF130639

Supplementary Table 4

Table S4. Number of samples (N), number of alleles (Na), number of effective alleles (Ne), Alleleic richness corrected for samples size (Arc), private allelic richness rarefaction (pArc), observed heterozygosity (Ho), expected heterozygosity (He), fixation index (F) among 4 populations, and the same measures calculated per monitoring year within the Harz population. Bohemian-Bavarian Population is named BBA.

Pop	N	Na	Arc	pArc	Ho	He	F
Zoo	27	**6.1** ± 0.5	4.82	0.72	**0.62** ± 0.03	**0.70** ± 0.03	**0.14** ± 0.03
Founders	10	**4.7** ± 0.4	4.46	0.43	**0.57** ± 0.06	**0.62** ± 0.04	**0.09** ± 0.06
BBA	25	**3.3** ± 0.2	2.68	0.32	**0.43** ± 0.04	**0.43** ± 0.03	**0.01** ± 0.04
Harz	105	**5.0** ± 0.5	3.32	0.13	**0.61** ± 0.03	**0.59** ± 0.02	**0.00** ± 0.02
2001/06	10	**4.7** ± 0.4	3.84	0.67	**0.57** ± 0.06	**0.62** ± 0.04	**0.09** ± 0.06
2006/07	6	**3.7** ± 0.3	3.57	0.02	**0.59** ± 0.05	**0.63** ± 0.03	**0.04** ± 0.08
2007/08	7	**3.7** ± 0.3	3.42	0.00	**0.65** ± 0.04	**0.62** ± 0.03	**-0.06** ± 0.06
2008/09	14	**3.6** ± 0.3	3.14	0.00	**0.74** ± 0.03	**0.61** ± 0.03	**-0.22** ± 0.03
2009/10	10	**3.5** ± 0.3	3.12	0.00	**0.69** ± 0.04	**0.59** ± 0.03	**-0.17** ± 0.05
2010/11	11	**3.5** ± 0.2	3.08	0.01	**0.69** ± 0.04	**0.59** ± 0.03	**-0.17** ± 0.05
2011/12	19	**3.6** ± 0.3	3.05	0.02	**0.68** ± 0.03	**0.60** ± 0.03	**-0.14** ± 0.03
2012/13	26	**3.8** ± 0.4	2.97	0.04	**0.68** ± 0.04	**0.59** ± 0.03	**-0.15** ± 0.03
2013/14	33	**4.0** ± 0.4	3.00	0.06	**0.62** ± 0.04	**0.59** ± 0.03	**-0.04** ± 0.03
2014/15	48	**4.5** ± 0.4	2.97	0.09	**0.59** ± 0.04	**0.58** ± 0.03	**-0.01** ± 0.03
2015/16	56	**4.5** ± 0.4	2.92	0.09	**0.56** ± 0.03	**0.57** ± 0.03	**0.02** ± 0.03

Supplementary Table 5

Table S5. Results of kinship analysis from ML-Relate showing sibling relationships FS (full sibling, HS (half sibling), and (U) unrelated above, and the relatedness scores below. The half sibling indications do not fit with known history and origins of these individuals and is likely due to the poor quality of genotyping in LL043w_X. Full sibling suggestions matched origins and haplotype analysis.

	LL006w_4	**LL008w_1**	**LL018w_6**	**LL026w_7**	**LL030m_7**	**LL031w_X**	**LL033m_2**	**LL036w_4**	**LL037w_X**	**LL043w_X**
LL006w_4	--	U	U	U	U	U	U	**FS**	**FS**	U
LL008w_1	0	--	U	U	U	U	U	U	U	U
LL018w_6	0	0	--	U	U	U	U	U	U	U
LL026w_7	0	0	0	--	U	U	U	U	U	HS
LL030m_7	0	0	0	0	--	**FS**	U	U	U	U
LL031w_X	0	0	0	0	0.49	--	U	U	U	HS
LL033m_2	0	0.12	0	0	0.01	0	--	U	U	U
LL036w_4	0.71	0	0	0	0.01	0	0	--	**FS**	U
LL037w_X	0.42	0	0	0	0	0	0	0.76	--	U
LL043w_X	0	0	0.01	0.36	0	0.33	0	0	0	--

Supplementary Figure 1

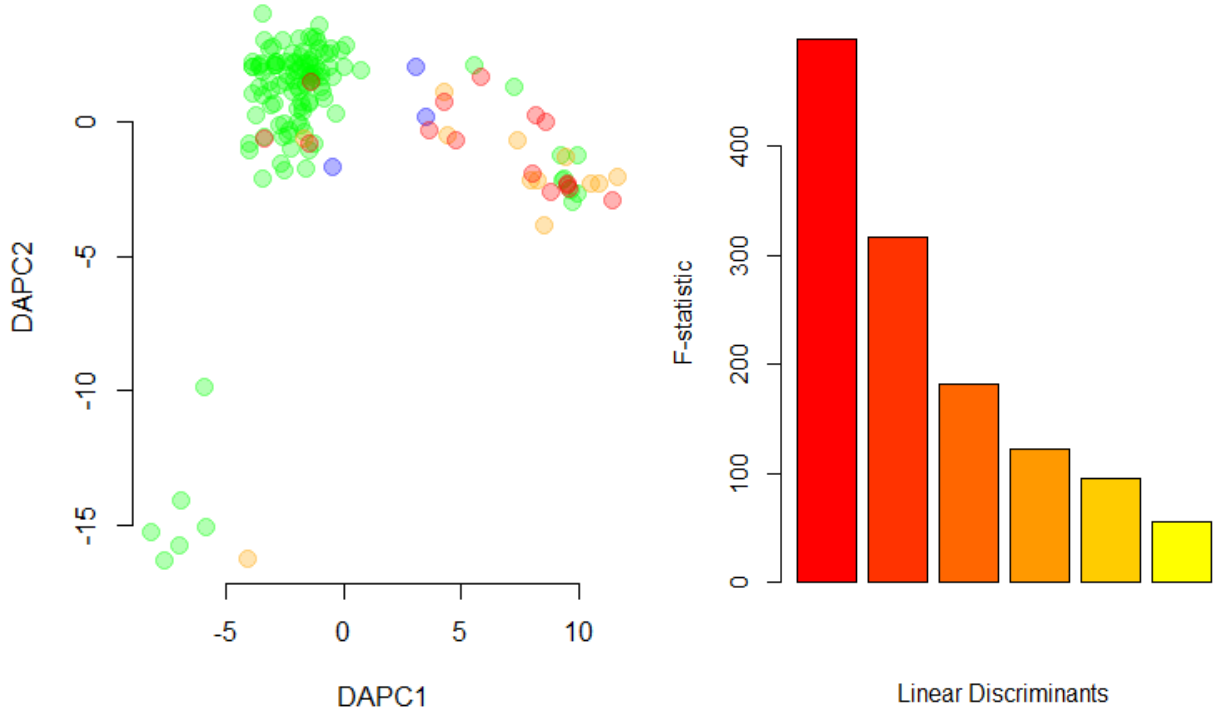

Figure S1. DPCA showing wild born (green), released individuals (orange), and known founders (blue) of the Harz lynx population and additional zoo individuals (red). On the right, the principal components, with their respective F-statistic.

Supplementary Material 1

Comparison to other European populations

Comparison to other European populations was preformed based on six loci and published data by Bull et al. (2016) are summarized in Supplementary Table S5. Comparison of diversity parameters of reintroduced lynx populations in relation to the number of used loci revealed differences between the results of six and 11 or more loci. Measures published by Bull et al. diverge in particular for Croatia and are higher in all cases but for the Vosges-Palatinate population. Results from genotyping within this study with 14 samples from the Bohemian-Bavarian population in turn match exactly those of Bull et al. with the Harz lynx population showing higher allelic richness and unbiased expected heterozygosity (Table S5). The identity of the alleles was uncertain due to different scoring but the diversity can nevertheless be compared as the same loci are considered. The subset of Harz individuals comprised only adult animals sampled in 2015 and 2016 to ensure a comparable sample size (N = 34). For the calculation of allelic richness ten random individuals were selected with the exception of Croatia and Slovakia, which had sample sizes smaller than that. Of the reintroduced populations (highlighted in grey) lynx from the Harz had highest levels of genetic diversity followed by the Vosges-Palatinate and Bohemian-Bavarian population. The Dinaric lynx population in Slovenia and Croatia showed the lowest diversity, while the autochthonous populations in Estonia, Latvia, Poland and Russia had highest levels with the Slovakian, which had the smallest sample size being below these. Compared to the autochthonous populations the diversity of lynx from the Harz was rather low. When plotting the total number of alleles against the number of samples, the same pattern as described above arises with autochthonous populations having higher genetic diversity than the reintroduced (Fig. S3).

Table S5. Genetic diversity of lynx populations in Europe. Reintroduced populations are highlighted in grey, others are autochthonous. N = number of individuals, Ar = allelic richness with N = 10, Ho = observed heterozygosity, uHe = unbiased expected heterozygosity. Based on six loci, microsatellite data published by Bull et al. (2016) and own data for the Harz lynx population.

Country/Population	N	Ar	Ho	uHe
Harz	34	3.33	0.50	0.53
Estonia	34	4.50	0.54	0.63
Latvia	29	4.83	0.56	0.71
Poland	18	4.17	0.55	0.56
Russia	10	4.33	0.57	0.71
Slovenia	12	2.83	0.28	0.44
Croatia	8	2.50	0.38	0.46
Bohemian-Bavarian	12	3.00	0.36	0.43
Vosges-Palatinate	23	3.17	0.36	0.50
Slovakia	6	2.83	0.50	0.52

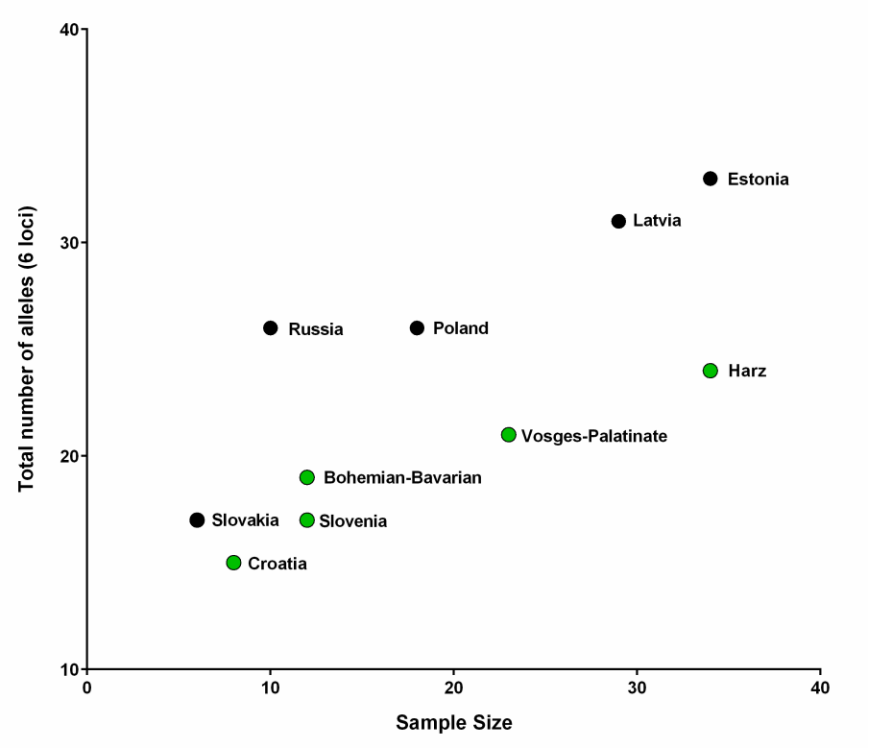

Figure S2: Total number of alleles across six loci plotted against sample size. Reintroduced populations are highlighted in green. Based on microsatellite data published by Bull et al. (2016) and own data for the Harz lynx population.

Publication III

Genome-wide diversity loss in reintroduced Eurasian lynx populations urges immediate conservation management

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Erklärung zu den Autorenanteilen / Declaration on the author contributions

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Was hat der Promovierende bzw. was haben die Koautoren beigetragen?*3

(1) Entwicklung und Planung

SAM: 60%, CN: 20%, SP: 10%, TER: 5%, CB: 5%

(2) zur Durchführung der einzelnen Untersuchungen und Experimente

Sample Collection: OA, CB, OK, PK, MK, AK, JK, LM, GO, KS, MS, TS, BT, AS, NG: all contributed to sample collection with number of samples in paraentheses

DNA Extraction: SAM: 85%, PK: 5%, JK: 5%, MS: 5%

Sample selection, quality control: SAM: 100%

(3) zur Erstellung der Datensammlung und Abbildungen

SAM: 100%

(4) zur Analyse und Interpretation der Daten

SAM: 70%, SP: 10%, CN: 10%, TER: 10%

(5) zum Verfassen des Manuskripts

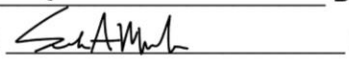
SAM: 80%, CN: 10%, all other co-authors contributed on the manuscript: 10%

Datum/Ort: 22.06.2021, Frankfurt a.M.

Unterschrift Promovend: 

Zustimmende Bestätigungen der oben genannten Angaben

Unterschrift Betreuer:  Datum/Ort: _____

Ggfs. Unterschrift *corresponding author* SAM:  Datum/Ort: 22.06.2021, Frankfurt a.M.

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Abstract (242/250)

Reintroductions may elevate negative effects by decreasing genetic variation caused by isolation, genetic drift and inbreeding if not assisted by careful population management. To assess the genetic consequences of reintroductions in large carnivores, we used the Eurasian lynx (Lynx lynx), which was the subject of several reintroduction attempts in the last 50 years. Although some restocking actions initially appeared successful, lynx recovery stagnated in recent years. To reveal potential genetic causes of slow lynx recovery in Europe, we examined genome-wide patterns of genetic diversity and inbreeding in 13,525 single nucleotide polymorphisms (SNPs) of all six successfully reintroduced populations, as well as twelve natural populations across Eurasia. All reintroduced populations showed lower genetic diversity and elevated levels of inbreeding compared to source and other natural populations. Recent inbreeding is prevalent in all reintroduced populations with varying degrees of severity; the most severe cases are those with the fewest number of founding individuals. Interestingly, we found evidence of lower genetic diversity and recent inbreeding in the source population for five reintroductions, begging the question if this source population can provide sufficient genetic diversity for future reintroduction projects. Given the observed genetic consequences, we advocate for standardized genomic assessment of populations, genetically assessing individuals prior to release, and careful consideration of the source population when considering future projects. Our study provides the most comprehensive look at reintroduced lynx populations to date and has broad implications for understanding the impact of reintroductions on large carnivore metapopulations.

Keywords: Lynx, reintroduction, high throughput sequencing, GBS

1 Introduction

Large carnivores exert a wide range of cascading ecological effects that regulate and maintain ecosystems and can enhance overall biodiversity (Ripple et al. 2014). Despite their important ecological role, these species struggle to persist in areas of high human population density (Packer et al. 2013). Many apex predators have already become locally extinct and the return of large carnivores into their historical range can play a key role in ecosystem restoration (Lipsey und Child 2007). One potential way to foster this return of large carnivores and reestablish their important ecosystem functions is active reintroduction. Numerous reintroduction projects across a wide array of taxa have been conducted in order to reestablish a species to their native range (La Haye et al. 2017; Frosch et al. 2014; Cochran-Biederman et al. 2015; Godefroid et al. 2011). The outcomes of such projects vary, likely because the conditions prior to release are difficult to assess and the results are challenging to predict before reintroduction takes place, despite careful planning (Armstrong and Seddon 2008). Some actions have led to positive results (vonHoldt et al. 2008; Moseby et al. 2018; Frosch et al. 2014), while others failed post-release or required constant restocking (Griffith et al. 1989; Fischer und Lindenmayer 2000). The reasons behind the overall low success rates of reintroduction attempts are not fully understood.

Genetic factors can have a major influence on the overall outcome of the reintroduction effort (Frankham 2009). In the short-term, the genetic composition of released individuals, the size of the founding population and outbreeding depression are major concerns (Keller and Waller 2002; Hayward and Somers 2009). Ensuring a large enough pool of unrelated released individuals to reach a sufficient number of genetic founders was found to be of particular importance for species with low reproduction rates (Noss et al. 1996) and to avoid inbreeding (Armstrong and Seddon 2008). Given the high spatial and food requirements as well as potential for human-wildlife conflict, carnivores are considered harder to translocate than many other species (Noss et al. 1996).

Once a population has been established, inbreeding, isolation, and small population size all contribute to further reduction of genetic diversity by genetic drift (Frankham 2005) that may result in the accumulation of deleterious mutations (Whitlock 2000) and inbreeding depression. Inbreeding is commonly observed in reintroductions with small founding populations (Hayward and Somers 2009). In extreme cases, inbreeding depression can lower individual fitness (Keller and Waller 2002), e.g. by an accumulation of deleterious mutations resulting in lowered fertility or decreased disease resistance (Xue et al. 2015). As a long-term consequence of accumulated inbreeding and reduced genetic variability, reintroduced populations may become more vulnerable to environmental change and possible extinction.

Modern advances in genetic and genomic methods enable the investigation of these potentially detrimental genetic consequences of founder effects and small effective population sizes on reintroduced populations (Xue et al. 2015). Genomic techniques allow deeper insight into population demography and genetic diversity, even if few individuals are sampled (Robinson et al. 2019). Traditionally, population monitoring is carried out through surveys or DNA-based monitoring using mitochondrial (mtDNA) sequence data and microsatellite analysis (Bull et al.

2016, Sindičić et al. 2013, Krojerová-Prokešová et al. 2019, Mueller et al. 2020, Breitenmoser-Würsten et al. 2003). These methods are prone to ascertainment bias and lack of comparability across laboratories, which present serious obstacles to match genetic diversity values across larger geographical scales and national borders as well as multiple populations and generations. Measures of genetic diversity and inbreeding derived from microsatellite markers have been shown to correlate only loosely with genome-wide heterozygosity estimates (Väli et al. 2008). In contrast, genomic methods such as restriction site associated DNA sequencing (RADseq) allow for high resolution genome-wide analysis of genetic diversity and inbreeding (Grossen et al. 2018) and can thus provide more detailed insights into the potential long-term viability and extent of inbreeding in reintroduced populations. Runs of homozygosity (ROH) analysis, , for instance, may be used to uncover recent inbreeding more accurately than traditional heterozygosity estimates that do not take the location of SNPs into account (Kardos et al. 2015, Forutan et al. 2018) and may thus inform managers of acute genetic threats to population health and viability (Kardos et al. 2018; Grossen et al. 2018).

The Eurasian lynx (*Lynx lynx,* Linnaeus 1758) is a suitable example to study genetic consequences of reintroduction using genome-wide markers due to diverse population histories and demography across the range, including a number of reintroduction attempts. It is a large solitary carnivore; its historical range stretched across the Palearctic from Western Europe to East Asia. During the 19th and 20th centuries, populations in Europe faced extensive persecution and became locally extinct in several regions (Chapron et al. 2014). During the 19th and 20th centuries, populations in Europe faced extensive persecution and became locally extinct in several regions (Chapron et al. 2014). Today, the Eurasian lynx is considered to have a large, stable population in the central and eastern parts of its vast range. A stable population is present in Finland, which underwent a significant bottleneck in the mid-20th century (Hellborg et al. 2002, Pulliainen 1968). The Scandinavian population (Sweden and Norway) experienced a severe bottleneck during the first half of the 20th century and is slowly recovering (Supplementary Table S2; Hellborg et al. 2002, Chapron et al. 2014). The Baltic population is exposed to considerable habitat fragmentation in its western-most part and has decreased in recent years (Supplementary Table S2; Schmidt et al. 2009).

Since 1971, 17 different reintroduction and translocation projects were implemented to restore populations of this elusive carnivore in Western and Central Europe (Linnell et al. 2009; Idelberger et al. 2021, in press; Molinari et al. 2021, in press). These projects faced a number of challenges and setbacks. Additionally, many projects released only a few individuals and could not adequately monitor the population post-release (Linnell et al. 2009). Further, human induced mortality, especially legal and illegal hunting and persecution, has impacted several populations negatively (Heurich et al. 2018; Breitenmoser-Würsten and Obexer-Ruff 2003; Sindičić et al. 2016).

Despite the hardships, some projects founded populations which experienced demographic growth in the years post-release. In the 1970s, lynx were translocated from the Slovakian part of the

Western Carpathians to four different reintroduction sites: two in Switzerland (Breitenmoser-Würsten et al. 2007; Breitenmoser and Baettig 1992), one in the Bavarian National Park, Germany (Červený and Bufka 1996) and one in Slovenia (Čop 1987; Figure 1). The number of released individuals varied in these first reintroductions from six in Slovenia, to 10-12 in the three other populations (Breitenmoser & Breitenmoser-Würsten 2008; Figure 1). No genetic information is available on founding individuals, however, two known sibling pairs were released in Slovenia. Additionally, the reintroduction in the Bavarian Forest National Park was later supplemented by 17 individuals reintroduced in the neighbouring Šumava National Park in the Czech Republic (Červený and Bufka 1996). Decades later, in 2001, individuals from both reintroduced populations in Switzerland were translocated to create a secondary population in the northeast (Robin and Nigg 2005). Around the same time, between 2000 and 2006, a reintroduction of 24 captive-bred individuals was conducted in the Harz Mountains in Germany (Anders and Sacher 2005).

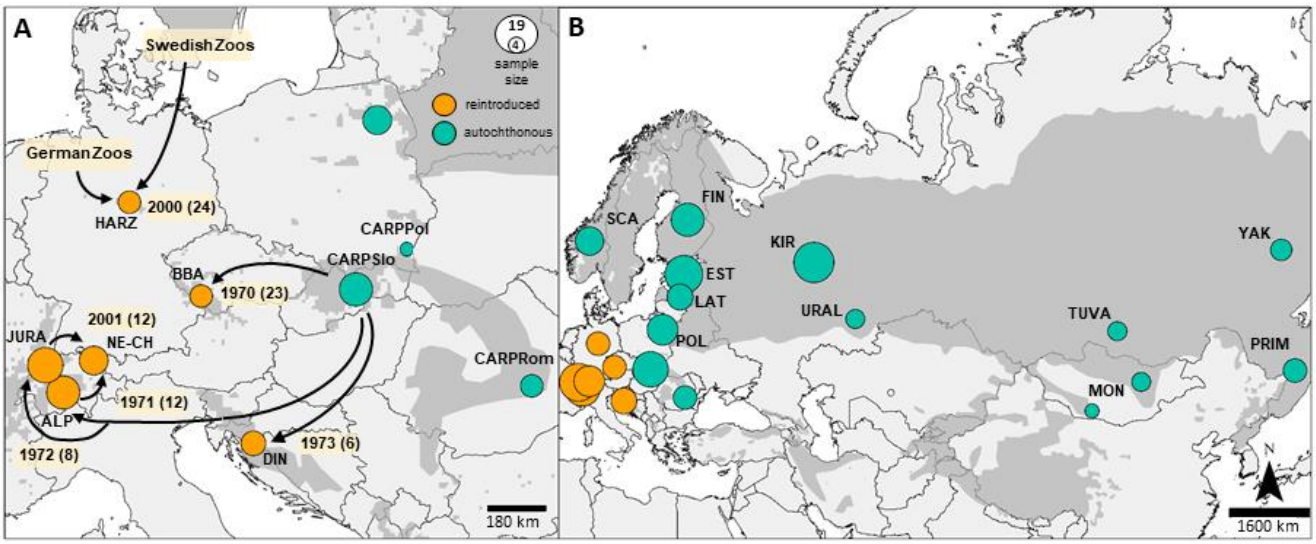

Figure 1. Map of sampled populations and reintroduction history of the Eurasian lynx. A) Sample size and source populations of six reintroduced populations (ALP, JURA, LUNO, BBA, DIN, and HARZ). The year denotes the time when reintroduction first began at each respective site (additional translocation years not shown) and the minimum number of individuals released in brackets. The Carpathian source population is also shown. Noticeably, we sampled from the entire Carpathian range, however, reintroductions were only sourced from CARPSlo. B) Sample locations (14) representing 11 natural populations used in this study.

However, nearly two decades after the last reintroductions, several populations are seeing noticeable changes in demography. The reintroduction in the Bohemian-Bavarian Forest has been subject to a high level of human induced mortality (Heurich et al. 2018), and the Dinaric population experienced a considerable decrease in population size since the early 2000s (Sindičić et al. 2013).

The Swiss reintroductions have experienced recent demographic growth after a period of presumed stagnation (Molinari-Jobin et al. 2017, Drouet-Hoguet et al. in press, Breitenmoser et al. 1998). All populations are currently monitored both through field and genetic methods (Appendix 1). Microsatellite analysis discovered that reintroduced populations display low genetic diversity (Bull et al. 2016; Breitenmoser-Würsten and Obexer-Ruff 2003; Mueller et al. 2020), some to the point of being in critical status (Sindičić et al. 2013). These findings have fuelled ongoing plans to connect these currently isolated populations to allow for sufficient gene-flow within a large European lynx metapopulation (Molinari-Jobin et al. 2010).

Given that lynx populations are faced with low genetic diversity, which affects the species' ability to survive in the long term, we aimed to provide the first genome-wide assessment of genetic diversity and levels of inbreeding in reintroduced and natural lynx populations. In particular, we aimed to answer the following questions: i) what is the extent of inbreeding and genome-wide genetic diversity loss in lynx reintroductions compared to natural populations? ii) is genetic erosion severe enough to warrant management through translocation and supplementary measures?

Our data constitute an important baseline for the currently envisioned Eurasian lynx conservation strategy to form a large, connected Central European lynx metapopulation which will be capable of maintaining a high level of genetic variability through gene flow among reintroduced and adjacent natural populations (Bonn Lynx Expert Group, in prep., Molinari –Jobin et al. 2010). We use the term natural populations to refer to non-reintroduced populations. Based on detected results we formulated recommendations for further conservation management of reintroduced lynx populations in Central Europe. Further, we discuss factors contributing to reintroduction outcomes and if exchange of animals among reintroduced Central European populations could enhance levels of genetic diversity.

2. Methods

2.1 Sample Collection and DNA Extraction

We obtained 308 samples from 14 different countries collected from 2000-2019 (Figure 1, Supplementary Table S1). We sampled six reintroduced populations; five sourced from the Slovak Carpathians (Swiss-Alpine (ALP), Swiss-Jura (JURA), North-Eastern Swiss (NE-CH), Bohemian-Bavarian-Austrian (BBA), and Dinaric (DIN)) and one from captive-bred individuals from German and Swedish zoos (HARZ) (Figure 1a, Appendix 1).

We also sampled the Slovak Carpathians (CARP) to investigate individuals from the source population. We included seven additional individuals that originate from the Polish and Romanian Carpathians, four of which were sequenced by Lucena-Perez et al. (2020) (Figure 1a, Supplementary Table S1). In addition, we sequenced samples from seven populations identified solely by geographical location, namely North-Eastern Poland (POL), Latvia (LAT), Estonia (EST), Finland (FIN), Scandinavia (SCA), Kirov (KIR), and Mongolia (MON). We included 35

samples from Lucena-Perez et al. (2020) from the Ural, Tuva, Yakutia (YAK), Primorsky Krai (PRIM) and MON populations to obtain the full distribution of Eurasian lynx populations for comparison.

Samples included mainly tissue [251], but also blood [17], dried skin [27], bone [7], hair [2], and feces [2]. For invasive samples, DNA was isolated using the Qiagen Blood and Tissue Kit following the manufacturer's protocols. We added an additional step to treat the samples with RNase A after lysis. The Genomic DNA Mini kit Tissue was used to extract DNA from bone and hair samples and the QIAamp DNA Stool Mini Kit was used to extract DNA from fecal samples, both following the manufacturer's protocols. We chose 190 samples for GBS, which met quality specifications and maintained equal sampling distribution. The extracts were diluted to 10-15 ng/µl to fit sequencing recommendations.

2.2 GBS Sequencing

Genomic DNA from the selected 190 samples was converted into nextRAD genotyping-by-sequencing libraries (SNPsaurus, LLC) as in Russello et al. (2015). Genomic DNA was first fragmented with Nextera DNA Flex reagent (Illumina, Inc), which also ligates short adapter sequences to the ends of the fragments. The Nextera reaction was scaled for fragmenting up to 25 ng of genomic DNA. Fragmented DNA was then PCR amplified with one of the primers matching the adapter and extending 10 nucleotides into the genomic DNA with the selective sequence GTGTAGAGCC. Thus, only fragments starting with a sequence that can be hybridized by the selective sequence of the primer will be efficiently amplified. The GBS libraries were sequenced on a HiSeq 4000 with one lane of 150 bp reads (University of Oregon).

Upon receiving the sequencing data, the raw reads were first trimmed to remove adapter sequences as well as stretches of low quality and ambiguous bases using Adapter Removal v2.3.0 (Lindgreen 2012). At this step, we added bam files of 39 individuals with whole genome sequences from a previous study (Lucena-Perez et al. 2020).

After filtering, the cleaned reads were mapped to the Eurasian lynx reference genome (Abascal et al. 2016) using BWA-MEM (v 0.7.12-r1039) (Li and Durbin 2009). We performed SNP calling using samtools (v1.9) (Li et al. 2009) mpileup function. The called raw SNPs were filtered using the following criteria: 1) individual samples with missing data above 65% or with less than 5.0X average coverage across SNPs were excluded; 2) loci with missing data in 30% of the samples, minor allele frequency (MAF) lower than 0.05, or depth lower than 3.0X and higher than 70X were removed; 3) loci with genotype quality less than 20 were excluded. In addition to these criteria, the SNPs were also pruned to account for linkage disequilibrium using $r^2 > 0.8$ in 100kb windows using bcftools v1.9 (Li et al. 2009). For detailed information on programs and parameters see Appendix 2.

2.3 Analysis of Population Structure

We first performed a principal component analysis (PCA) using PLINK (v1.9) (Chang et al. 2015), which requires no a priori information on populations allowing an unbiased estimation of the general trends in allele frequencies. PLINK uses a variance-standardized relationship matrix, taking each observed SNP covariance over the SNP's variance. Next, we calculated genotype likelihoods with the samtools model in ANGSD (v0.930) (Korneliussen et al. 2014) to estimate likelihoods with a SNP p-value of 1e−6. We then used ADMIXTURE (v 1.3.0) (Alexander and Lange 2011) to infer individual ancestries from the SNP dataset from a K=1 to K=18 with 50 repetitions of each K to investigate convergence patterns. We consolidated the runs with CLUMPAK (Kopelman et al. 2015) and estimated the optimal K value based on the Evanno method (Evanno et al. 2005). We looked at population histories by utilizing Treemix (v1.13) (Pickrell and Pritchard 2012) to determine a maximum likelihood tree for the sampled populations using the available *Lynx rufus* genome as a root. Treemix can also infer the number of admixture events, so we examined trees that had 0-5 migration events. Lastly, we calculated population F_{ST} values with ANGSD. A two-dimensional site frequency spectrum (2dSFS) is generated for each pair of populations by estimating the folded SFS. Here, we used the reference genome as the ancestral, as no ancestral genotypes are available. We generated the weighted F_{ST} estimates from the 2dSFS.

2.4 Genetic Diversity Measures

Tajima's D was calculated with ANGSD, also using the 2dSFS, but then calculating theta for each loci to generate an overall Tajima's D statistic. We utilized STACKS populations (v.2.41) (Catchen et al. 2013) to estimate population wide genomic diversity statistics including heterozygosity values, Pi, and private alleles within each population. We used PLINK to estimate heterozygosity values based on allele frequency information.

2.5 Inbreeding

We investigated the extent of inbreeding across all populations. We used the filtered SNPs and further filtered for sex-linked markers as these can influence inbreeding estimates (Supplementary Figure S2) (Humble et al. 2020). We first mapped the SNP flanking regions using BWA MEM default parameters to the domestic cat genome as it is assembled to the chromosome level. The 349 loci that were located on the X chromosome were removed from inbreeding analysis (Supplementary Figure S1). We used ANGSD to create genotype likelihoods and subsequently analyzed individual inbreeding levels. As we suspected inbreeding in reintroduced populations based on available monitoring data, we aimed to use a method that is capable of handling populations that may not fit the assumptions of Hardy-Weinberg equilibrium (HWE). Therefore, we used ngsF (Vieira et al. 2013) to calculate the inbreeding coefficient (*F*) as it is not reliant on allele frequencies, but utilizes an expectation-maximization (EM) algorithm that is robust to the uncertainty of assigned genotypes.

We also estimated the number of runs of homozygosity (ROH) present in the sampled individuals after sex-linked markers were removed. ROH were estimated using PLINK –homozyg function following parameters used by Humble et al. (2020) and Grossen et al. (2018), namely—homozyg-window-het 1, --homozyg-window-missing 5, --homozyg-window-threshold 0.05, --homozyg-het 1, --homozyg-kb 1000, and—homozyg-gap 1000. In order to identify and call a region as ROH we used—homozyg-window-snp 15 and—homozyg-snp 15. We determined the length needed to consider a region as a ROH using -homozyg-density 150. These three parameters were in accordance to recommendations by Kardos et al. (2015), which gives density and SNP thresholds based on the number of SNP loci available for analysis. Individual inbreeding estimates were then calculated as the proportion of the genome in ROH (F_{ROH}) by taking the total ROH length (Kb) over the total length of the genome (2.4 Gb).

2.6 Relatedness

Subsequently, we estimated relatedness between individuals of each population. First, we filtered the dataset to obtain highly polymorphic SNPs with little missing data using vcftools (--min-maf 0.3 and –max-missing 0.9), which resulted in 4124 SNPs to be used for relatedness calculations. We once again used the genotype likelihoods created from ANGSD, this time following the GATK method (-GL 2) as it may provide better estimates for relatedness measures (Waples et al. 2018). The genotype likelihoods were then analyzed with ngsRelate (Korneliussen and Moltke 2015; Hanghøj et al. 2019) to estimate the pairwise relatedness values for individuals within each population using the R0, R1 and KING-robust kinship (KING) coefficients. We chose this method because it is not reliant on allele frequencies and is robust to SNP ascertainment bias, and is therefore a good candidate when studying small populations of non-model organisms where a chromosome level reference genome is not available (Waples et al. 2018; Brüniche-Olsen et al. 2019). We also constricted the analysis to intrapopulation assessment of relatedness as population structuring can potentially lead to bias in the results (Conomos et al. 2016; Thornton et al. 2012). We then identified the pairwise relationships using the inference criteria outlined in Manichaikul et al. (2010). These inference criteria can distinguish between unrelated, third-degree, second-degree (half-siblings, grandparents, etc.), and full sibling/parent-offspring. To further distinguish between parent-offspring and full-sibling, we used R1 and R0 values to determine these pairs as outlined in Brüniche-Olsen et al. (2019) and Waples et al. (2018). We complimented this analysis with relatedness estimation using PLINK–genome function as this uses allele frequency data. We excluded samples with an average coverage of < 10x as this can lead to an overestimation of relatedness due to uncaptured alternative alleles and estimates PI_HAT, the overall proportion of the genome that is identical by descent (IBD).

3 Results

3.1 GBS Sequencing

Library preparation and sequencing was carried out successfully in 190 samples chosen for sequencing with an average of 656,578 unique reads per sample. Mapping to the Eurasian lynx reference genome (PRJEB12609, Abascal et al. 2016) resulted in an average alignment of 95.77%. Three samples mapped below 65%, which were subsequently removed from analysis. Another seven samples exhibited low coverage and seven samples had >65% missing data across SNPs and were removed from analysis. The 39 samples from Lucena-Perez et al. (2020) were already mapped to the reference genome before SNP calling for downstream analysis. These remaining 212 individuals formed the basis for our analysis. The samples had an average coverage of 18.6X and 14.6% missing data in called loci (Supplementary Figure S2). After SNP and linkage disequilibrium filtering, 13,525 SNPs were utilized for analysis. SNPs were in general evenly distributed across chromosomes when mapped to the domestic cat genome (Supplementary Figure S1).

3.2 Population Structure

We first examined population structuring through PCA analysis, which provided support for 3 subdivisions within the sampled individuals (Figure 2). Bayesian population structure analysis confirmed this structuring with K=2 as the optimal value using the Evanno method (Supplementary Table S3). Besides K=2 the Evanno method found subsequent peaks at K=4 and K=7 (Supplementary Table S3), suggesting an additional sub-structuring within the defined clusters (Figure 2). We found significant variation within the Carpathian population based on geographic location (Supplementary Figure S3). Pairwise F_{ST} values revealed similar genetic structuring to PCA or Admixture results (Supplementary Figure S4). Results from maximum-likelihood phylogenetic analysis supported the separation between the Carpathian lineage and the Asian and European subspecies. It placed the HARZ as an intermediate between the Asian and European populations and when Treemix considered possible migration events, indications of gene-flow between DIN and BBA were suggested (Supplementary Figure S5 and S6).

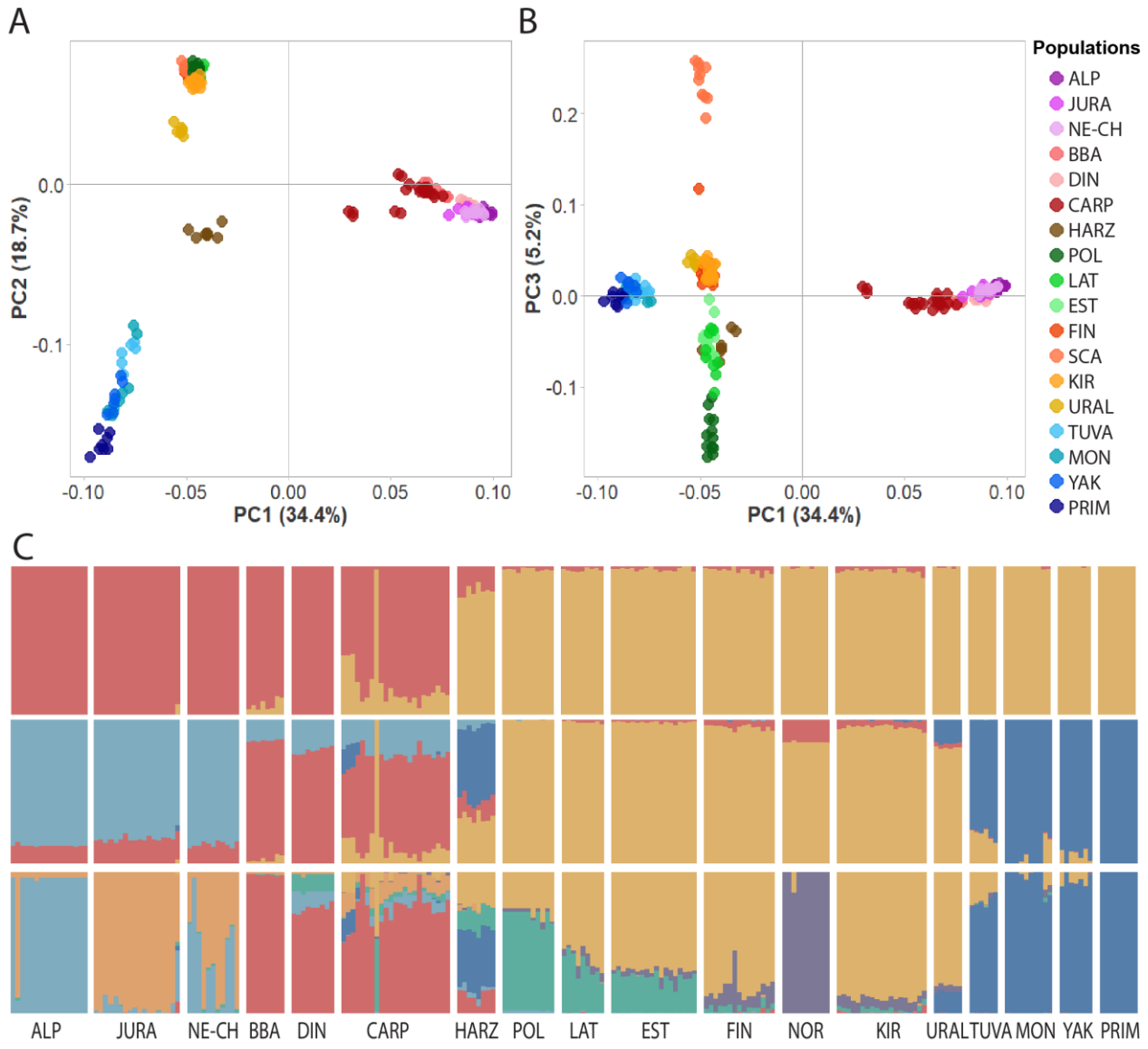

Figure 2. Relationship between individuals based on 13,525 SNP sites identified from nextRAD sequencing. A) The first PC axis separates the Carpathian origin samples from central Europe and Asian samples and the second axis separates European from Asian samples (upper left). B) The third PC shows separation among Baltic and Scandinavian populations (upper right). C) Admixture results showing K=2, K=4, and K=7, showing population separation. Divisions among reintroduced populations can already be seen in K=4.

3.3 Genomic Diversity

Calculations of individual heterozygosity demonstrated lower observed heterozygosity values in reintroduced populations compared to natural populations (reintroduced 0.17, natural 0.25) (Figure 3). In particular, Carpathian sourced reintroductions had the lowest observed heterozygosity out of all populations (Figure 3). Among natural populations, the SCA, FIN and CARP populations had slightly lowered values. No private alleles were found between populations and no significant Tajima's D values were identified (Supplementary Table S4).

3.4 Inbreeding

Overall, reintroduced populations showed inflated levels of inbreeding ($F_{reintroduced} = 0.40$, $F_{natural} = 0.26$; Supplementary Figure S7). The JURA and HARZ populations were the only exception (0.35 and 0.27 respectively). On the other hand, SCA (0.44) and FIN (0.34) had elevated levels of inbreeding compared to other natural populations.

To investigate the amount of recent inbreeding we calculated the number and length of ROH present in the genome. We found a total of 6,348 ROH throughout the genome. F_{ROH} as the proportion of the genome in ROH ranged from 0-0.19 across all individuals (Figure S7, reintroduced 0.020, natural 0.007). ROH length was correlated with SNP density, specifically in short ROH (< 5 Mb). In long ROH (> 5 Mb), however, this correlation was no longer present (Supplementary Figure S8). Both the total number of ROH and the total number of long ROH (>5 Mb) varied among populations; reintroduced populations on average had longer ROH than autochthonous populations (Figure 4). Among reintroduced populations, 98% of individuals had ROH longer than 5Mb, as compared to 71% of individuals from natural populations. In stable populations with large population sizes (Supplementary Table S2), the median total length of ROH >5 Mb was 4.84 Mb compared to 27.42 Mb in reintroduced populations. When the CARP population was split into groups based on region, the western edge showed elevated levels of recent inbreeding (Supplementary Figure S9). We identified longer ROH and higher F_{ROH} in individuals sourced from the Slovak Carpathians when compared to the captive-sourced reintroduced HARZ population (Supplementary Figure S10).

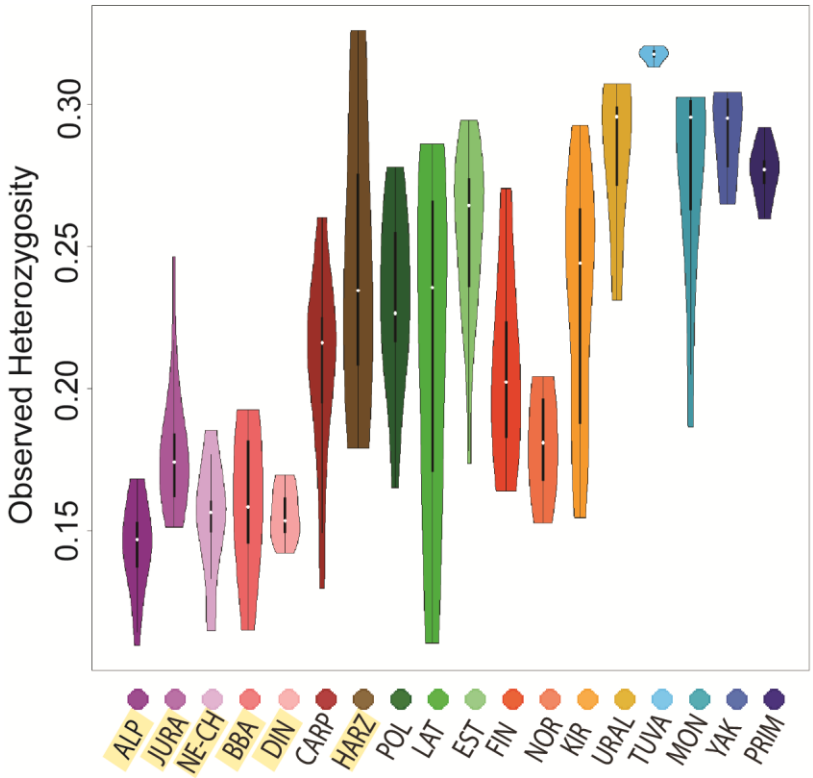

Figure 3. Observed heterozygosity calculated for each population. White dot shows the mean value and black line shows the range of values present in the population. Reintroduced populations are highlighted in yellow.

3.5 Relatedness values

We calculated pairwise relatedness estimates for 378 pairs of reintroduced individuals and 943 pairs of wild individuals (Figure 5). Among reintroduced individuals, three pairs were identified as full siblings, 16% were second-degree relatives, 20% were third-degree relatives and 60% were unrelated. In natural populations, we discovered two first-degree relatives, 1% second-degree, 8% third-degree, while the vast majority were unrelated (91%).

The complimentary analysis in PLINK resulted in an elevated PI_HAT across reintroduced individuals (Supplementary Figure S11). Pairwise relatedness assignments resulted in a much higher proportion of unassignable relationships; 13% of reintroduced and 2% of natural populations were not assigned.

4. Discussion

Our study aimed to quantify the extent of genetic diversity loss and inbreeding in reintroduced populations of Eurasian lynx across Western and Central Europe. By utilizing genome-wide SNP markers and population genomic techniques, we have identified patterns of population structuring among natural and reintroduced populations across the lynx distribution (Figure 2). Within reintroduced populations, we found signs of genetic drift and lower observed heterozygosity in comparison to their natural counterparts (Figure 2). Further, using three different inbreeding and relatedness methods, we showed that reintroduced populations exhibit elevated inbreeding values and evidence of recent inbreeding is prevalent in almost all reintroduced individuals. Our analyses allow a nuanced understanding of the genetic consequences of reintroduction in large carnivores. The second aim of this study was to evaluate if subsequent action should be taken in reintroduced populations to maintain the populations through genetic rescue. Genetic rescue aims to decrease the probability of extinction by increasing gene-flow through translocation (Hohenlohe et al. 2021; Whiteley et al. 2015). Identifying populations under stress and providing early conservation action can increase the success of translocation projects (Griffith et al. 1989; Wolf et al. 1996). We found varying degrees in the severity of genetic diversity loss, which strongly suggests that conservation action is vital to the long-term sustainability of these populations and a focus on genomic assessment of populations is needed.

4.1 Genomic consequences in reintroduced populations

Our results show that signatures of genetic drift and lower genetic diversity are prevalent across all reintroduced populations to varying degrees of severity. The assessment of the degree of relatedness between individuals of each population revealed a higher number of first and second degree relationships in reintroduced lynx populations, providing additional evidence for genetic similarity among individuals within the reintroduced populations. We identified high rates of recent inbreeding in reintroduced populations, the worst of which was found in populations with the lowest number of released individuals (Figure 4). Traditional methods of calculating inbreeding are limited as they make no distinction between distant and recent inbreeding. This distinction is of considerable importance as the latter has a more significant influence on population health and viability as purging the deleterious alleles from the genome has not yet occurred (Kardos et al. 2018; Robinson et al. 2019). It is particularly important to quantify ROH burden in reintroduced populations as inbreeding depression is suspected to be more severe in the wild, making early intervention vital (Crnokrak und Roff 1999; Ralls et al. 1988). Here, we found that natural populations exhibited values seen in other stable populations (Humble et al. 2020; van der Valk et al. 2020), while reintroduced populations are consistent with available information regarding ROH in small, isolated mammalian species using GBS data (Schurink et al. 2019; Grossen et al. 2018). Given that ROH have been linked to fitness related changes (Xue et al. 2015; Robinson et al. 2019), we can assume that populations with a larger ROH burden are at a higher risk of extinction. There remains limited data on life history-related traits that can be impacted by inbreeding for reintroduced lynx in Western and Central Europe, but we can assume that

inbreeding depression may already impact reintroduced populations or will do so in future if these remain isolated. This is particularly true as ROH values derived from GBS are likely an underestimate of true ROH presence in the genome as studies using whole genomes found considerably higher levels of ROH (Kardos et al. 2018).

Besides elevated inbreeding we found that the number of released animals has a long-term effect on the genetic characteristics of the established lynx populations. The limited signatures of genetic drift and comparable inbreeding levels in the BBA population in comparison to the source population can, in part, be attributed to the relatively large total number of released individuals. There was a total of 23-28 individuals released at two sites, with likely 18 that could have contributed to the founding population (Appendix 1). It was the largest reintroduction of Slovak Carpathian lynx; other reintroduction projects that began around the same time (DIN, ALP, JURA) released between 6-12 animals (Breitenmoser et al. 1998; Čop 1987). It appears plausible that those numbers of released individuals were too low to prevent significant drift and loss of genome-wide heterozygosity.

The Dinaric population has one of the highest rates of inbreeding, in accordance with the fact that closely related individuals were present among released founders (Koubek and Červený 1996). Ensuring no related or inbred individuals are released will help buoy the genetic variation within already established populations. Similarly high rates of inbreeding equivalent to the Dinaric population were found in the Swiss Alpine population, which was established from twice the number of released individuals (Figure 4). However, releases took place across 5 different sites and it was suspected that the area where 8 individuals were released gave rise to the current population (Breitenmoser et al. 1998). Given that the number of genetic founders is likely considerably lower than the released individuals for all populations (Mueller et al. 2020), the Swiss Alpine populations may derive from few genetic founders despite currently observed population growth. We cannot deny that unpredictable stochastic effects may also contribute, in part, to high inbreeding and genetic diversity loss in certain populations. However, reintroductions that released at least 20 animals showed lower recent inbreeding here, which is comparable to the natural Carpathian population. Therefore, present and future reintroductions should focus on maximizing genetic founders, which likely means releasing higher number of individuals.

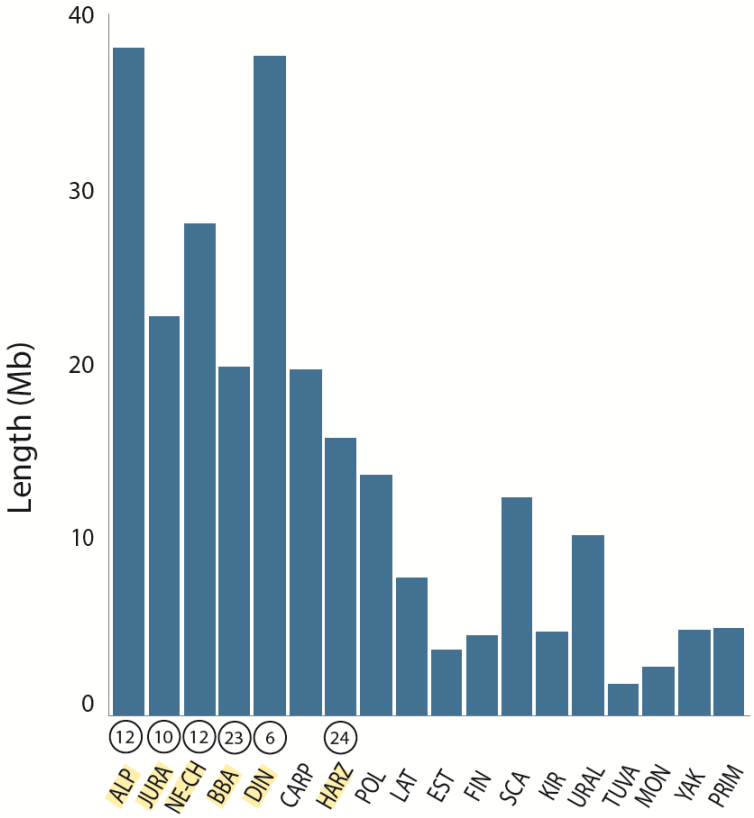

Figure 4. Average total length of ROH >5 Mb long in each population estimated from 13,525 SNPs. ROH segments longer than 5 Mb are likely due to recent inbreeding events. Reintroduced populations are highlighted in yellow with corresponding number of released individuals.

In addition to founder size, reintroduction source and time since reintroduction also impact trends in genetic diversity loss and inbreeding. The captive-sourced HARZ population exhibited higher observed heterozygosity and lower inbreeding than wild sourced reintroductions (Figure 3 & 4). This can likely be explained by the mixture of different lineages, which enabled the inclusion of varied alleles within the population. We must also consider the time since release, as the HARZ population is a comparatively recent reintroduction and there is evidence for ongoing genetic depletion in this population as well (Mueller et al. 2020). Despite these findings we also found examples of successful preservation of substantial variation within reintroductions of wild sourced individuals. The JURA population, despite having a relatively low number of founders (8-10) and experiencing strong signatures of genetic drift, has maintained a higher level of genetic variation than other reintroductions from the same source. We suspect that the JURA population achieved a more diverse set of genetic founders. Genetic testing prior to release could offer a method for evaluating genetic variation in individuals and better forecast if sufficient diversity is passed to the subsequent population.

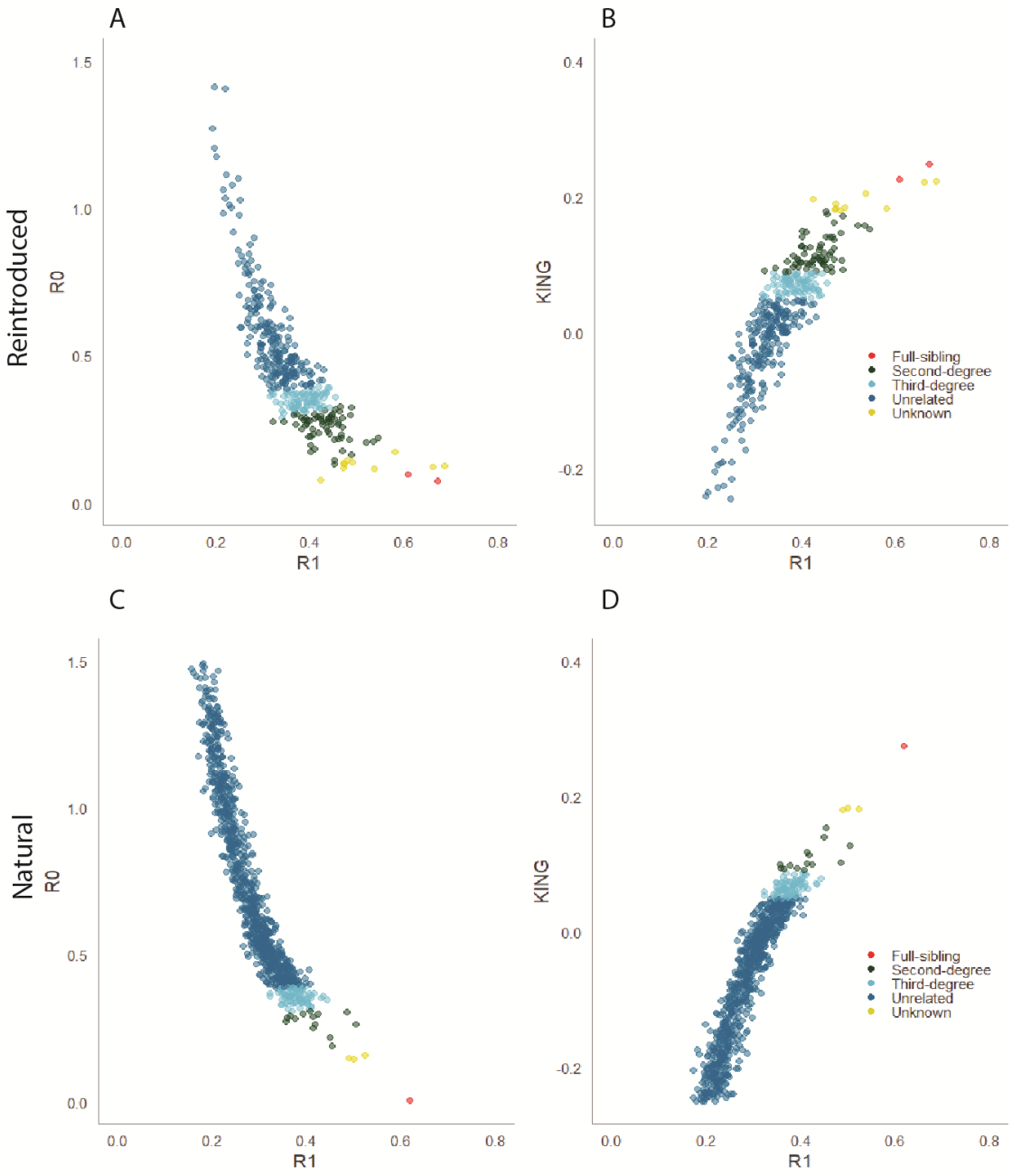

Figure 5. Relatedness estimates among 378 reintroduced pairs (a, b) and 943 pairs of autochthonous pairs (c, d). R1 and R0 estimates (a, c) are used to distinguish between parent-offspring and full-sibling relationships, and KING and R1 ratios (b, d) are used to define relationship classes (1st, 2nd, 3rd degree relationships).

4.2 Genetic Structure of natural Eurasian lynx populations

In general, our results support the known demographic histories of natural Eurasian lynx populations (Lucena-Perez et al. 2020) and provide evidence that the genetic consequences of past bottlenecks are still visible despite recovery of European populations in the last half of the 20th century. The lower genetic diversity observed in Finland and Scandinavia can be explained by the bottleneck during the 20th century that affected both populations (Hellborg et al. 2002). The comparatively low ROH values in the Finnish populations despite elevated inbreeding values calculated as a function of heterozygosity indicate that while evidence of a past, less severe, bottleneck remains visible, the current inbreeding levels within this population is low. The Finnish connection to the larger KIR (Ratkiewicz et al 2014) population has likely facilitated gene-flow and the partial return to pre-bottleneck composition. In contrast, the Scandinavian population remains genetically distinct despite evidence of demographic growth (Chapron et al. 2014). Its elevated ROH values and increased inbreeding values (F) suggest that the genetic signatures of severe bottlenecks are visible beyond the point of demographic recovery.

In the Baltic region, our results support previous studies suggesting that the northeastern Polish population is partially isolated and has experienced bottlenecks over the last century (Schmidt et al. 2009, Ratkiewicz et al. 2014). This isolation indicates the need to maintain avenues for gene-flow within the Baltic region. The more stable Asian populations show low values for long ROH, along with populations in European Russia, Finland and Estonia. One exception is the Ural population where we found elevated levels of long ROH. The Ural population appeared to be a somewhat permeable barrier between the eastern most populations and the Kirov region in Russia, which is supported by previous studies using mtDNA (Figure 2, Rueness et al. 2014). It remains unclear what biological or sampling factors could be contributing to the presence of longer ROH within this population.

4.3 Carpathian Population Structure

It is worthwhile to take a closer look at the lynx population from the Carpathian Mountains, as this region served as founding stock for most reintroductions within the study area and is still the main reintroduction source for ongoing reintroductions (i.e. the Dinaric region and Southwestern Germany). The Carpathian lineage has a shared phylogenetic past with the Baltic states until it served as an isolated forest refugium during the last ice age, resulting in the presence of a single haplotype (H4) in this region (Lucena-Perez et al. 2020; Horáček 1993; Ďurišová 2005). Despite being considered a large, continuous habitat, lynx within the Western Carpathians experienced significant fluctuations in population size over the last century (Hell 1968; Jamnicky 1997). The first significant reduction happened between 1930 and 1934, mainly as a result of strong hunting pressure (Jamnicky 1997). The population recovered quickly after protection, and by 1964 it was again present across the Western Carpathians (Hell 1968). Recent studies along the western edge of the Carpathians revealed elevated levels of inbreeding and population structuring (Krojerová-

Prokešová et al. 2019; Kubala et al. 2020). Therefore, the reduced heterozygosity within the Carpathians appears to be the result of a complex historic and recent demographic history. Importantly, our sample size from the Romanian Carpathians (n=3) was too low to encompass the full distribution and future studies can look at these trends in further detail with more comprehensive sampling.

The Carpathian population exhibited longer ROH and larger F_{ROH} than other natural populations, even when sub-divided by region to account for increased inbreeding at the western-most edge of the distribution (Krojerová-Prokešová et al. 2019). Similar to trends seen in the Scandinavian population, this can be partially explained as a genetic signature of the known bottleneck in the 20^{th} century despite subsequent demographic recovery. However, given that ROH levels are comparable to reintroduced populations, it may be an early indication of ongoing sub-structuring and isolation within the Carpathian range. Given the low sample sizes of the Polish and Romanian Carpathians, future studies should consider investigating the historic and current impacts of population size across the Carpathian range. In either case, geographically isolated populations, located at the margins of the species distribution may harbor rare genetic variants that are important for species survival under changing environmental conditions. Therefore, it is critical that continued monitoring and mitigation of threats that the Carpathian population currently faces are addressed. Given the result of lowered genome-wide diversity and increased inbreeding in Carpathian lynx we stress that individuals captured for future translocation should be given particular care to ensure that genetically diverse individuals are being chosen for reintroduction, which needs the involvement of genetic testing at an early stage in the course of reintroduction efforts involving wild-captured lynx.

4.4 Conclusions

Examining the genomic consequences of reintroduction across multiple lynx populations has been identified as a key action needed for lynx management in Europe for the last decade (Boitani et al. 2015; Molinari-Jobin et al. 2010). Here, we provide the first comprehensive look at genetic diversity loss across reintroduced and natural populations of Eurasian lynx. We found genetic impoverishment and evidence of inbreeding in all reintroduced populations at differing degrees of severity. Swiss-Alpine and Dinaric populations appear to be at highest risk of experiencing negative consequences of inbreeding. Other populations, in a less critical state, still show evidence of drift, inbreeding and diversity loss at higher rates than natural populations. This raises concerns for the future of reintroduced lynx populations in West and Central Europe. We strongly suggest for the supplementation of lynx populations through translocation from natural populations to supplement inbred and vulnerable populations. Plans for deciding on a source population need to be discussed noting that the Carpathian population shows signs of recent inbreeding across the population range, which urgently requires closer inspection throughout the Carpathian range.

Translocations from other reintroductions could potentially boost genetic diversity, especially when individuals are taken from populations with low observed inbreeding (JURA, BBA). While this would also be applicable to the relatively diverse HARZ population, the finding of substantial Siberian lynx ancestry in this reintroduction at least questions its use as potential source of supplementation of other populations.

In sum, our study provides a scientific basis which can inform ongoing and future conservation action aiming to restore genetic diversity and build a large, interconnected lynx metapopulation throughout Western and Central Europe.

Acknowledgements
Funding for this study was generated through the wildlife genetics analysis service at the Senckenberg Conservation Genetics Section, Gelnhausen, Germany. Additional funds came from the Landes-Offensive zur Entwicklung Wissenschaftlich-ökonomischer Exzellenz Center for Translational Biodiversity Genomics (LOEWE-TBG) of the German Federal State of Hesse. S.A.M is funded through the DAAD. We thank everyone involved in collecting samples as this constitutes a tremendous amount of work and our study would not be possible without it.

Author Contributions
S.A.M, T.E.R, S.P, C.B-W and C.N designed the study. All co-authors contributed samples, S.A.M, J.K, P.K and M.S carried out DNA extraction and preparation for HTS. S.A.M analyzed the data. S.A.M and C.N wrote the manuscript and all authors were involved in revision and editing the final manuscript.

Conflict of interests
None declared.

Supplementary Information

Supplementary PDF and Excel file can be found attached.

Data availability statement

Sequencing data associated with this study will be found on Dryad and all codes used in analysis can be found at https://github.com/sa-mueller/lynx_2021

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Supplementary Material

Genome-wide diversity loss in reintroduced Eurasian lynx populations urges immediate conservation management

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Appendices

Appendix 1: History of Lynx Reintroductions in Europe 121

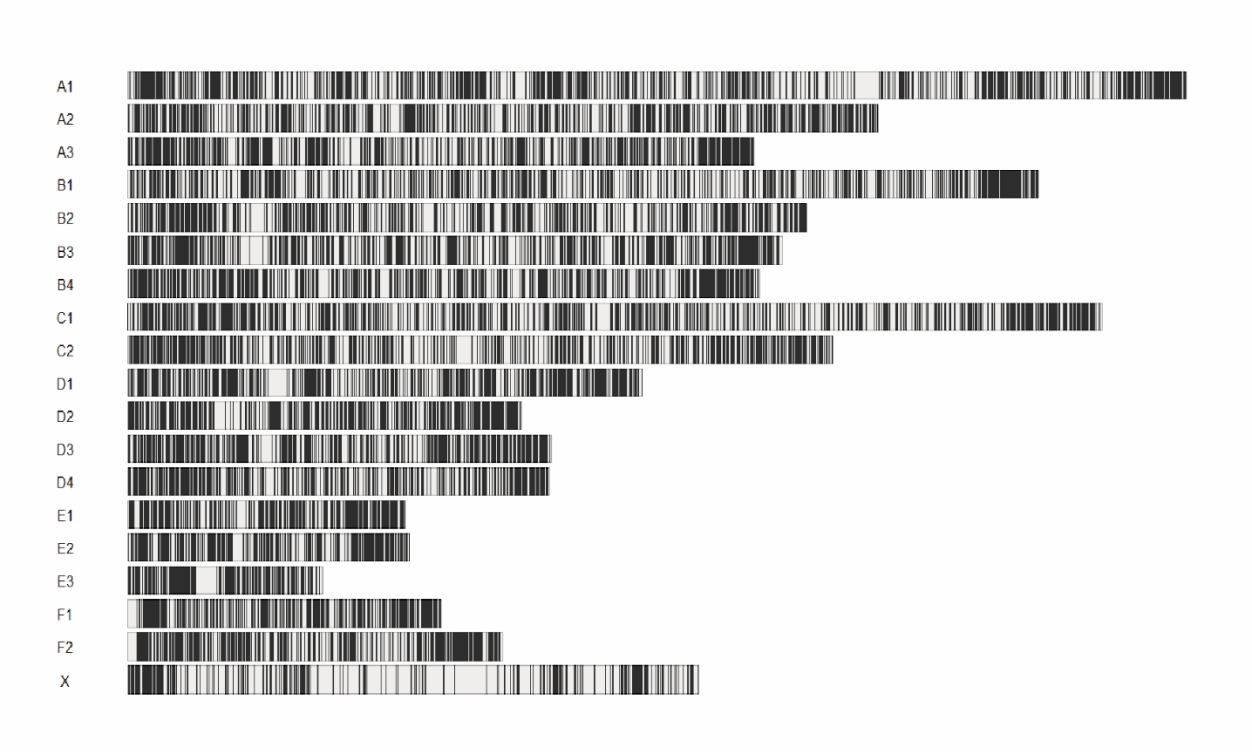


Figure S1. 13525 SNP markers that were used for analysis mapped to the domestic cat genome. 349 SNP loci mapped onto the X-chromosome. The rest of the SNP loci were evenly distributed across chromosomes.

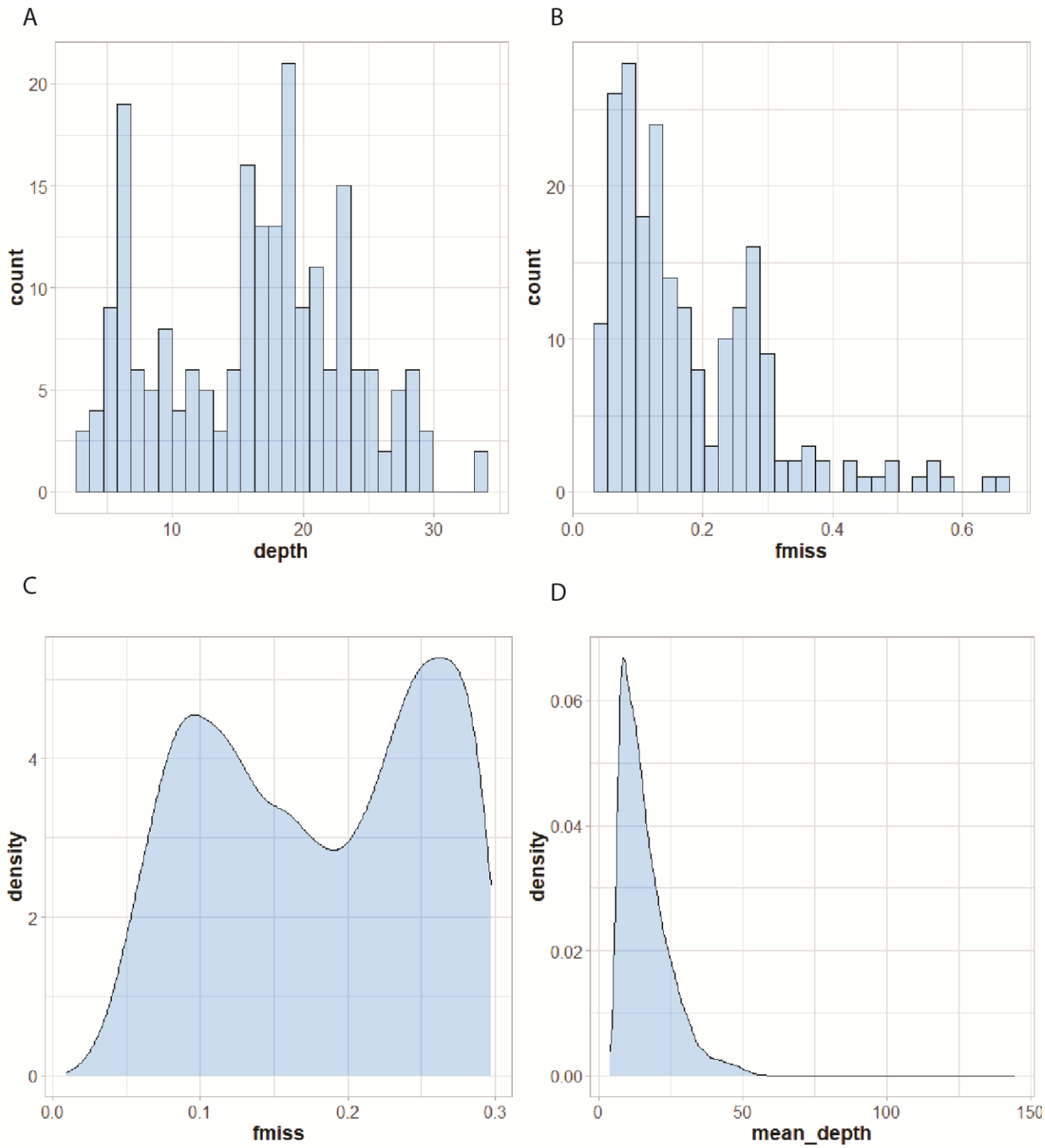


Figure S2. Quality of 212 Eurasian lynx samples across 13525 loci used for analysis. (A) average depth per individual (B) frequency of missing data per individual (C) frequency of missing data across loci and (D) average depth across all loci.

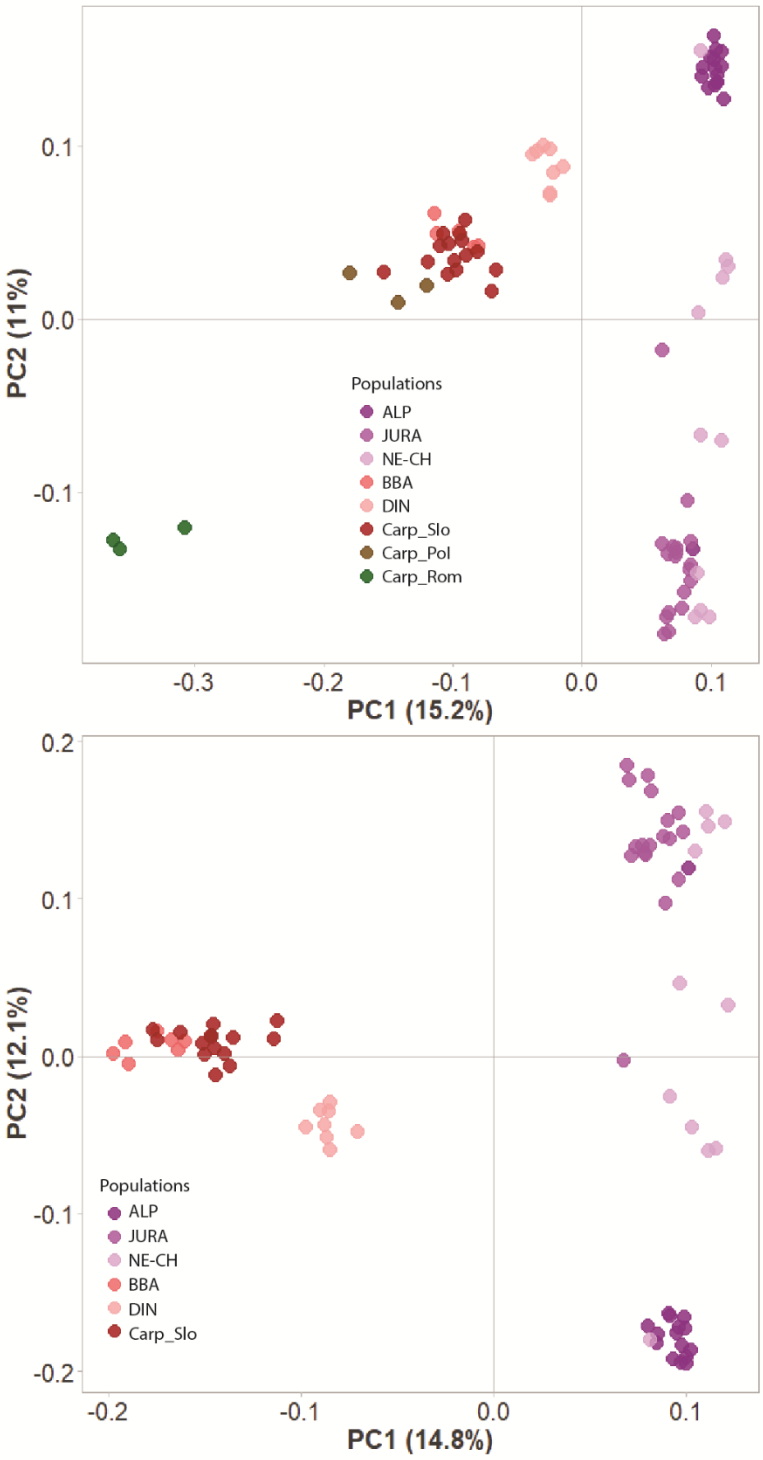


Figure S3. PCA analysis including entire sampled Carpathian population (above) including coloring for different regions within the Carpathian Mountains and PCA analysis including only the Slovakian Carpathian Population which was the source location for translocation to reintroduced populations in Central Europe (below).

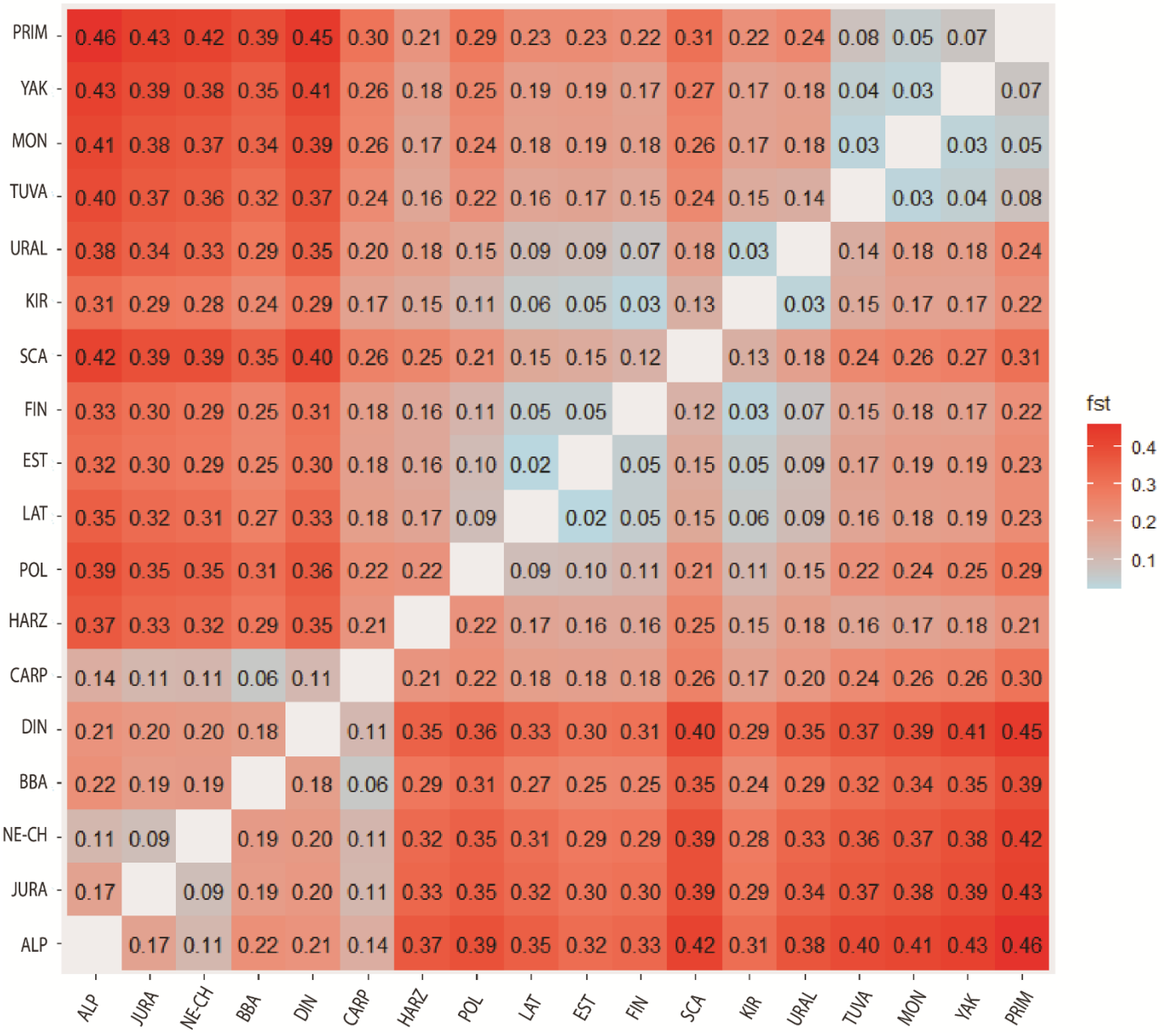


Figure S4. Weighted population wide pairwise F_{ST} estimates showing the relative population differentiation on a sliding color scale.

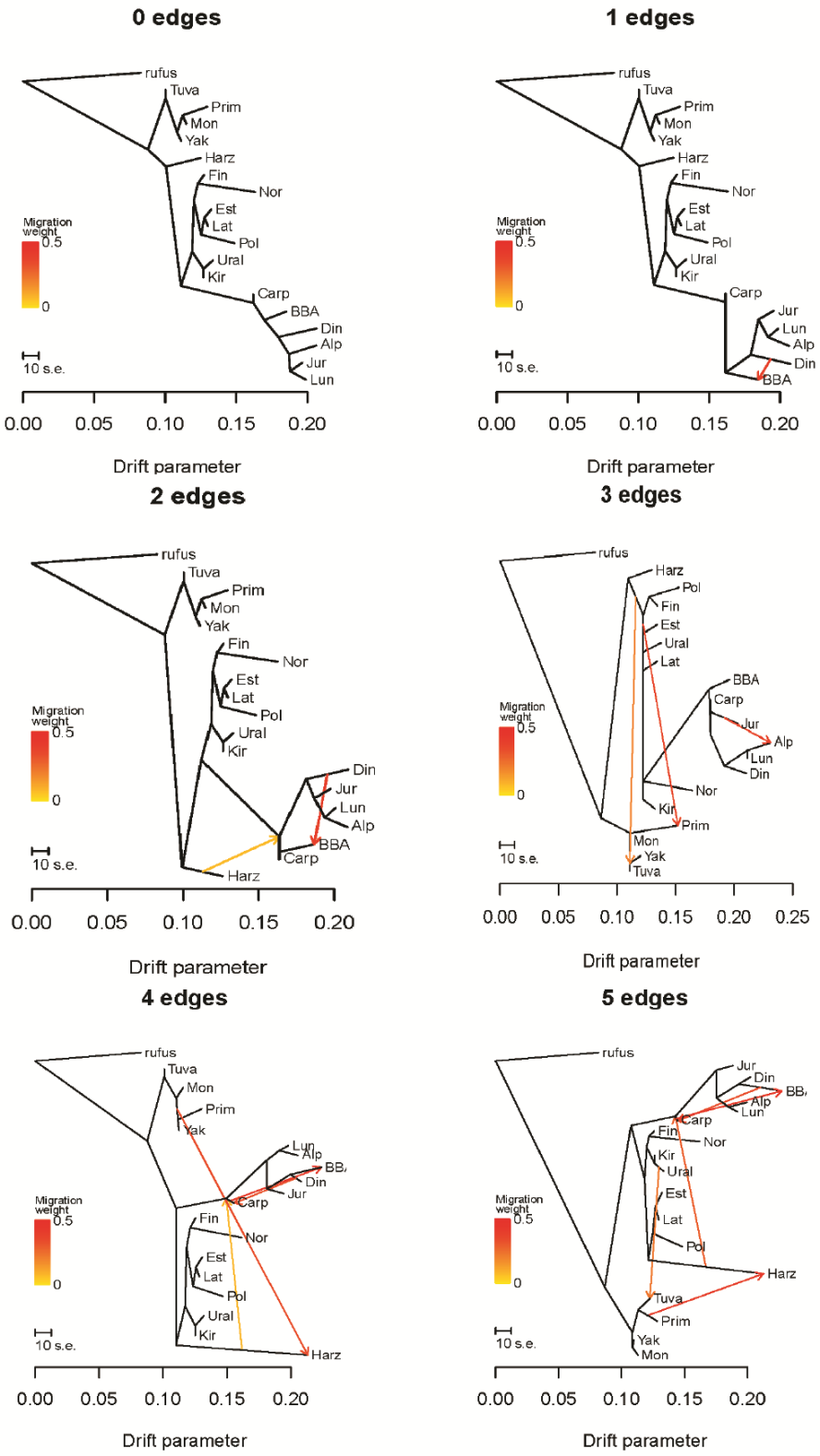


Figure S5. Treemix output showing the maximum likelihood tree with 0 to 5 migration events and the relative weight of these migration events by color.

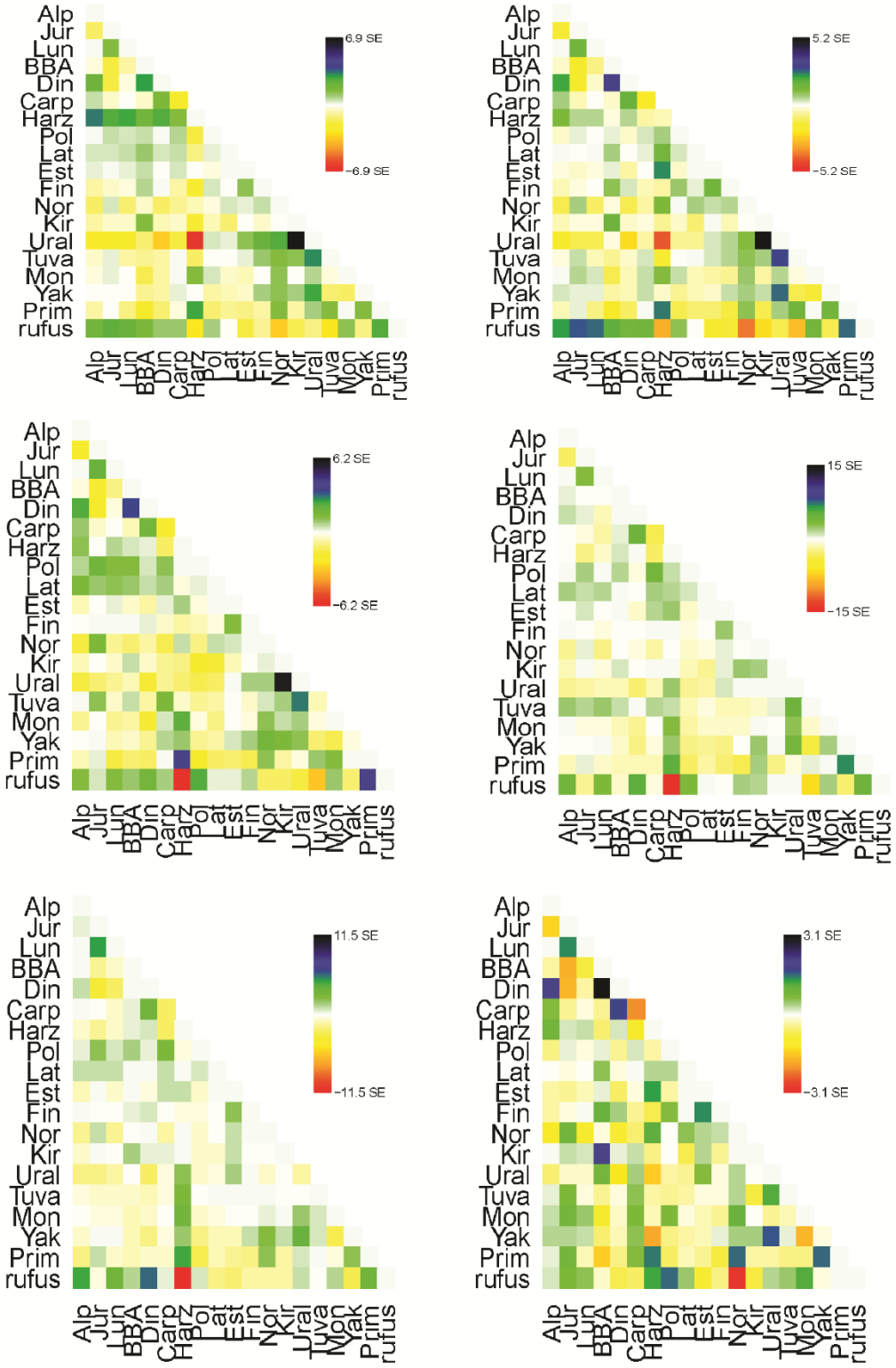


Figure S6. Treemix output showing the residual fit from the maximum likelihood tree.

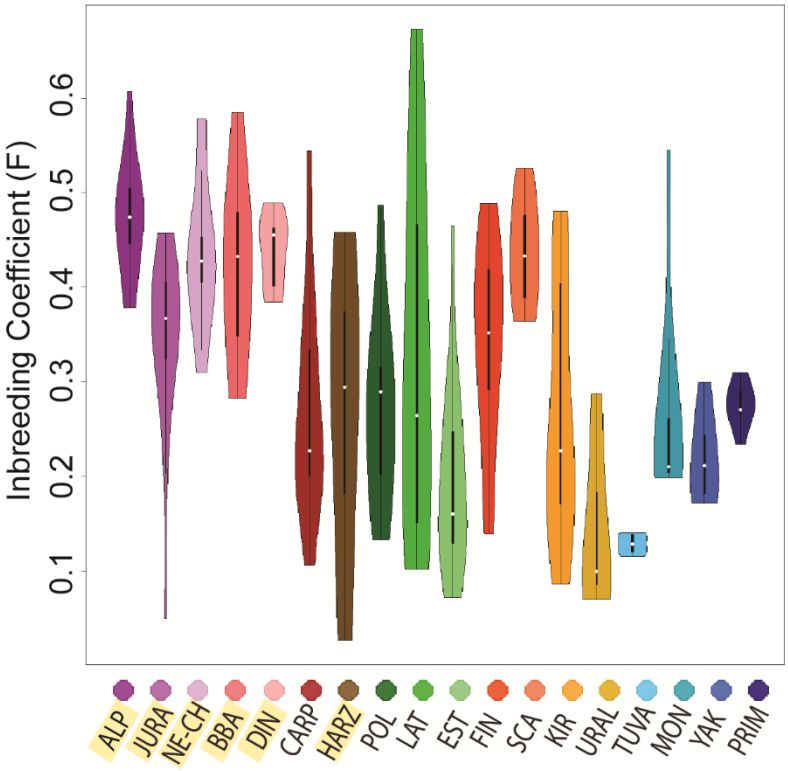


Figure S7. Inbreeding coefficient (F) calculated for each population. White dot shows the mean value and black line shows the range of values present in the population. Reintroduced populations are highlighted in yellow.

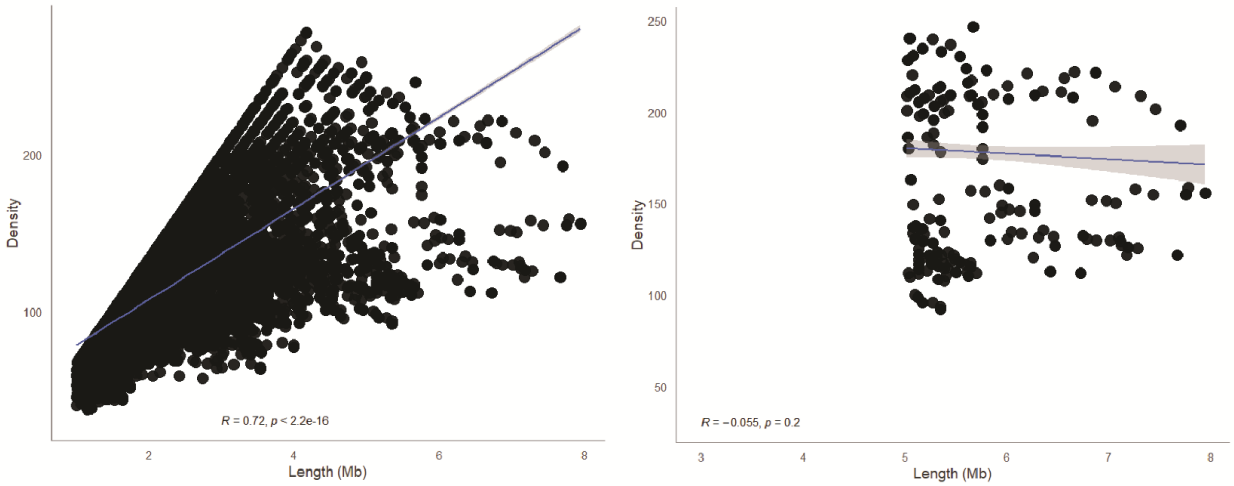


Figure S8. Correlation between SNP densities and length of ROH across all inferred ROH (left) ROH >5 Mb (right).

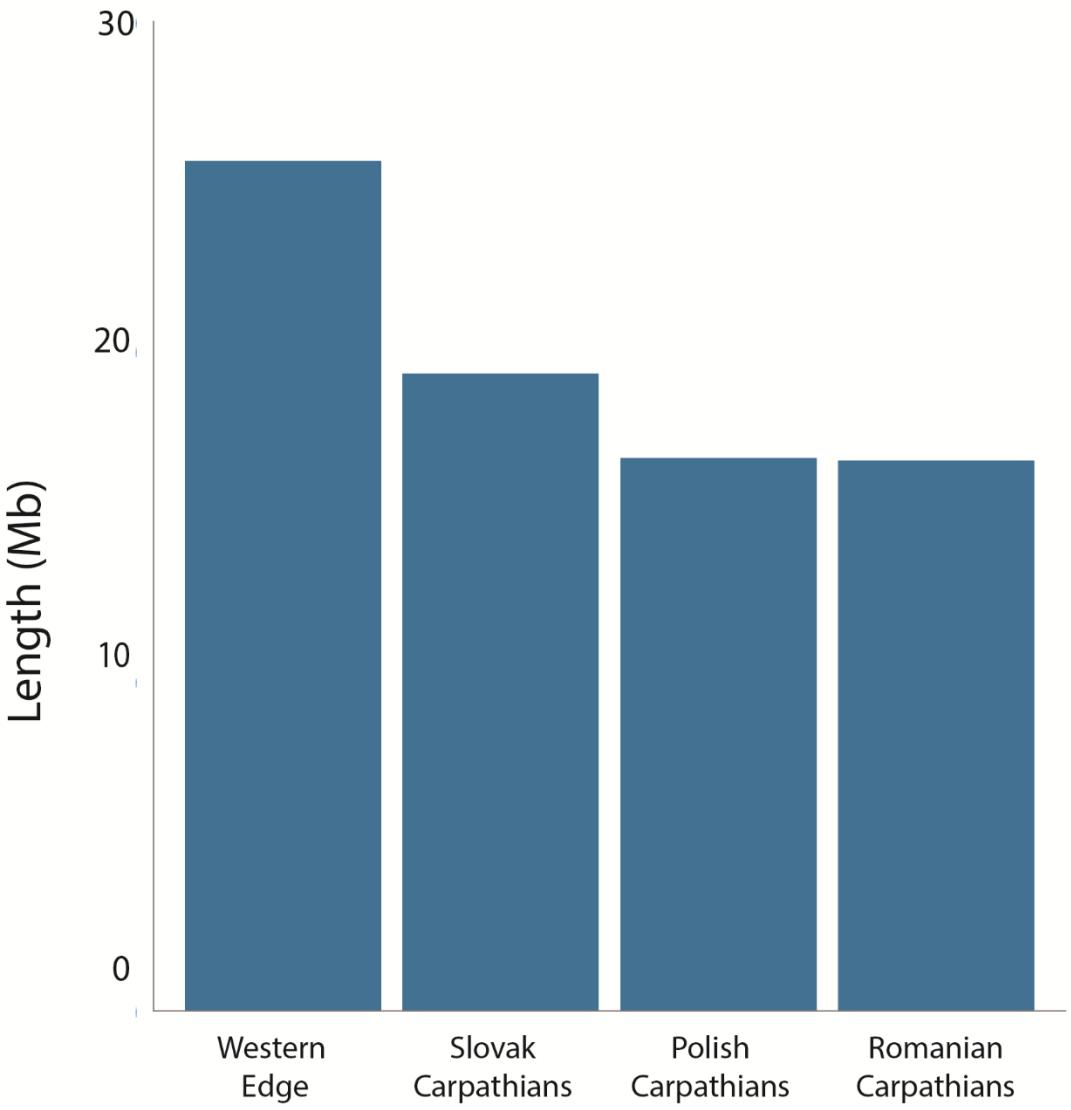
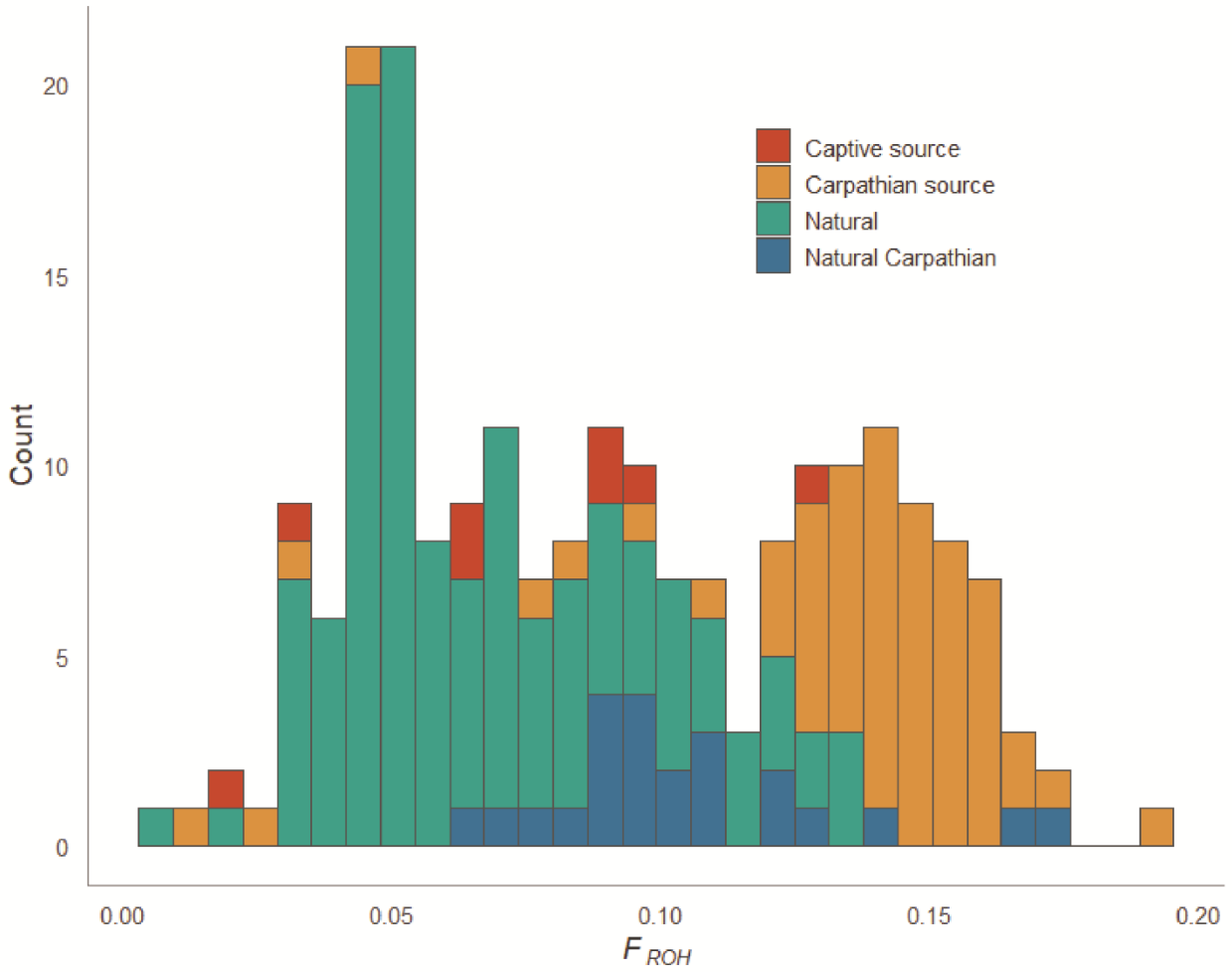
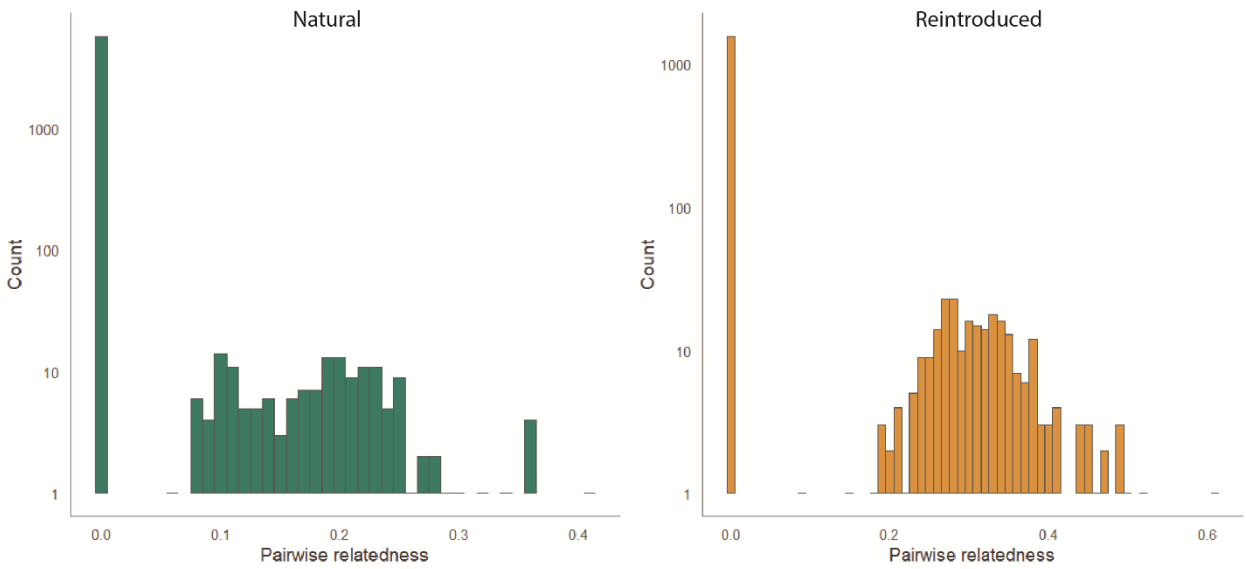


Figure S9. Average total length of ROH >5 Mb long estimated from 13,525 SNPs within the different regions of the Carpathian Mountains; showing additionally samples from the western edge in the Czech Republic, where higher inbreeding has been observed. ROH segments longer than 5 Mb are likely due to recent inbreeding events.



S10. Genomic patterns of homozygosity, F_{ROH} values, per individual further categorized by reintroductions from zoos and wildlife parks, reintroductions sourced from the Slovak Carpathians, wild born individuals from the Carpathian Mountains and all other natural populations.



S11. Genomic relatedness values from all possible pairwise comparisons across all natural (left) and reintroduced (right) populations of Eurasian lynx in the study area. Genomic relatedness was calculated as the proportion of the genome which is identical by descent (IBD) as evaluated from allele frequencies across the entire range as estimated in PLINK.

Appendix 1: History of Sampled Eurasian Lynx Reintroductions in Europe

Over the last 50 years, 17 reintroduction attempts of Eurasian lynx have been carried out in Central Europe (Linnell et al. 2009, Idelberger et al. 2021, in press; Molinari et al. 2021 in press). Many of these reintroduction attempts failed post-release. For this study, we sampled six surviving reintroduced populations. Each population history including number of founder, current status, and challenges to population growth all have an impact on the genetic make-up and status which will continue to shape the population demography for decades to come. A brief summary of each reintroduction project can be found below.

Harz (Harz National Park, Germany)

The Harz population is located primarily within the Harz National Park in central Germany. This region was previously lynx habitat until the species was expatriated over 200 years ago. This reintroduction was founded by 24 individuals (9 males, 15 females) that were raised in captivity in German and Swedish wildlife parks and zoos (Mueller et al. 2020). These individuals were released between 2000 and 2006. Ten additional individuals were released illegally or escaped from nearby wildlife parks. Four of these escaped individuals were recaptured and removed from the wild due to their habilitation to humans. Additionally, 7 individuals died shortly after release without reproducing. The first evidence of reproduction in the wild was reported in 2002, with multiple reproductions documented in the following years (Anders and Sacher 2005). According to the monitoring data of the German federal states, the current Harz lynx population has at least 100 individuals (including 17 reproducing females with 34 juveniles) documented through genetic analysis and other monitoring methods (BfN 2021). The population shows a demographic and range expansion. However, the overall genetic diversity is dependent on relatively few reproducing individuals (Mueller et al. 2020).

NE-CH (North-eastern Switzerland)

The NE-CH population is situated in the cantons of St. Gallen, Zurich, Thurgau, and both Appenzells in north-eastern, Switzerland. This population was founded between 2001 and 2008 when a total of 12 lynx were translocated from the populations in the north-western Alps and the Jura Mts into north-eastern Switzerland (Robin and Nigg 2005). The first reproduction in this area was observed in 2002, and subsequent litters were also closely monitored. Between 2002 and the beginning of 2012, 16 litters with at least 31 young had been documented (pers.comm. KORA). There has been evidence of long distance male dispersal from this region as well as dispersal from the Jura population into this region. This dispersal could predict that migration between these populations is possible. Therefore, the outlook of this population relies on the demography of surrounding populations as well as the possible connection to other European populations.

Jura (Switzerland)

In the Jura Mountains, Switzerland, lynx persisted despite hunting pressure until the end of the 19th century (Breitenmoser & Baettig 1992). The last reported evidence of lynx in the area was in

1830 (Schauenberg 1969). Lynx were then absent in this region until the early 1970s, when 8 to 10 lynx from the Czech Carpathian Mountains were released. Monitoring of the reintroduced population began retrospectively a decade later (Breitenmoser et al. 2007; Capt 2007). In 2007, the resident population was estimated around 56-101 individuals (Zimmermann and Breitenmoser 2007; Capt 2007). The Jura population has shown demographic growth and expansion, however, like other reintroduced populations remains isolated and susceptible to the negative effects of genetic drift and inbreeding (Zimmermann and Breitenmoser 2007). The population in the Jura Mts is estimated to be 150 independent individuals (Drouet-Hoguet et al. 2021).

North-western Alps (Switzerland)

The lynx population in the north-western Alps has evolved after the lynx reintroductions from the Slovak Carpathians in the cantons of Obwalden and Vaud in the early 1970s (Breitenmoser and Breitenmoser-Würsten 2008). Here, at least 12 lynx were released into the area and showed expansion into surrounding regions as well as population increase (Breitenmoser and Haller 1993, Breitenmoser & Breitenmoser-Würsten 2008). It is unknown how many of these originally released animals contributed to the current population, however, preliminary analysis suggests that the Alpine lynx population has an higher level of inbreeding than other reintroduced populations.

Dinaric (Croatia/Slovenia)

On March 2nd 1973 three females and three males, live captured in1971 and 1972 in Slovakian Carpathians, were released to Kočevje forests of Slovenia (Čop 1987; Koubek and Červený 1996). These lynx already included two pairs of related animals (mother and son; brother and sister) (Koubek and Červený 1996). The newly established population encountered favorable habitat with abundant prey base, and all three females produced offspring in the 1st year. The population rapidly expanded, with lynx appearing in Croatia almost immediately in 1974, and in Bosnia and Herzegovina in 1980 (Čop 1987). Out of all Eurasian lynx populations studied until now (Hellborg et al. 2002; Spong and Hellborg 2002; Rueness et al. 2014; Breitenmoser-Würsten and Obexer-Ruff 2003; Schmidt et al. 2009; Davoli et al. 2013; Bull et al. 2016; Krojerová-Prokešová et al. 2019) the Dinaric population has the lowest microsatellite diversity (Sindičić et al. 2013).

Bohemian-Bavarian-Austrian (Czech Republic, Germany and Austria)

The Eurasian lynx was extirpated from the Šumava Mountains and surrounding region, which covers parts of the Czech Republic, Austria and Germany in the late 1800s (Bufka and Červený 1996). Sporadic presence of lynx was observed throughout the early-mid 1900s, which were thought to be dispersing individuals from the Slovak Carpathians (Wölfl et al. 2001). Reintroduction of lynx first began in the Bavarian National Forest and it is estimated 5 to 10 lynx were released in the early 1970s. Later, 17 lynx were released in the Czech Šumava Mountains in the 1980s (Červený and Bufka 1996). The population has been monitored both in all three countries since the 1990s. Despite population growth, poaching has been a major driver of low population size (Heurich et al. 2018; Müller et al. 2014). This genetic status of this population was also

explored and found to be extremely low levels of genetic diversity, similar to the Dinaric population, despite having some gene flow into the population (Bull et al. 2016). The authors therefore classify this population as stagnate (Port et al. 2020; Bull et al. 2016).

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Table S1. List of Samples including, species, their status, which geographically defined population, country, if we used the sample for RADseq, the average sequencing depth across all loci, sample type, sampling date, the geographic sampling coordinates when known, and the sex of the animal if provided.

Sample Name	Subspecies	Status	Population	Country of Origin	Sequenced	Ave. Depth	Sample Type	Sampling Date	Latitude	Longitude	Sex
A_CH_W14-2427	*Lynx lynx carpathicus*	Reintroduced	Alpine	Switzerland	No		Tissue	2014.08.03	46.67148	7.56148	UK
A_CH_W16-1337	*Lynx lynx carpathicus*	Reintroduced	Alpine	Switzerland	Yes	15.8972	Tissue	2016.08.08	46.37888	6.96042	F
A_CH_W16-1981	*Lynx lynx carpathicus*	Reintroduced	Alpine	Switzerland	Yes	18.5107	Tissue	2016.09.24	46.78456	8.14445	UK
A_CH_W16-2111	*Lynx lynx carpathicus*	Reintroduced	Alpine	Switzerland	Yes	22.0358	Tissue	2016.10.10	46.25943	6.87813	M
A_CH_W16-2138	*Lynx lynx carpathicus*	Reintroduced	Alpine	Switzerland	Yes	0.316357	Tissue	2016.10.11	46.75891	8.03062	UK
A_CH_W16-2542	*Lynx lynx carpathicus*	Reintroduced	Alpine	Switzerland	Yes	16.8195	Tissue	2016.11.13	46.94366	8.26185	F
A_CH_W16-3134	*Lynx lynx carpathicus*	Reintroduced	Alpine	Switzerland	Yes	10.9468	Tissue	2016.12.28	46.68482	7.78895	F
A_CH_W16-3167	*Lynx lynx carpathicus*	Reintroduced	Alpine	Switzerland	Yes	10.9079	Tissue	2017.01.01	46.7538	8.15735	F
A_CH_W16-8805	*Lynx lynx carpathicus*	Reintroduced	Alpine	Switzerland	Yes	19.3025	Tissue	2015.10.21	46.21891	7.24916	F
A_CH_W17-5711	*Lynx lynx carpathicus*	Reintroduced	Alpine	Switzerland	Yes	19.4567	Tissue	2017.07.18	46.60583	7.47434	M
A_CH_W17-6391	*Lynx lynx carpathicus*	Reintroduced	Alpine	Switzerland	Yes	16.7508	Tissue	2017.09.07	46.47428	6.84056	F
A_CH_W17-6558	*Lynx lynx carpathicus*	Reintroduced	Alpine	Switzerland	Yes	18.8403	Tissue	2017.09.18	46.78165	7.31751	F
A_CH_W17-6812	*Lynx lynx carpathicus*	Reintroduced	Alpine	Switzerland	Yes	19.4161	Tissue	2017.10.09	46.61947	7.68364	M
A_CH_W17-6860	*Lynx lynx carpathicus*	Reintroduced	Alpine	Switzerland	Yes	19.7734	Tissue	2017.10.11	46.24734	7.37251	M
A_CH_W17-7074	*Lynx lynx carpathicus*	Reintroduced	Alpine	Switzerland	Yes	19.3914	Tissue	2017.10.28	46.80177	7.57371	M
A_CH_W17-7086	*Lynx lynx carpathicus*	Reintroduced	Alpine	Switzerland	Yes	18.6042	Tissue	2017.10.04	46.69327	7.58171	UK
A_CH_W18-2217	*Lynx lynx carpathicus*	Reintroduced	Alpine	Switzerland	Yes	11.5581	Tissue	2018.03.22	46.4553	6.91181	F
A_CH_W18-8536	*Lynx lynx carpathicus*	Reintroduced	Alpine	Switzerland	Yes	18.1028	Tissue	2018.02.19	46.63777	8.00813	F
BBA_CZ_ch600	*Lynx lynx carpathicus*	Reintroduced	Bohemian-Bavarian-Austrian	Czech Republic	Yes	0.606725	Tissue	2014			M
BBA_CZ_ch601	*Lynx lynx carpathicus*	Reintroduced	Bohemian-Bavarian-Austrian	Czech Republic	Yes	16.5124	Tissue	2014			M
BBA_CZ_ch937	*Lynx lynx carpathicus*	Reintroduced	Bohemian-Bavarian-Austrian	Czech Republic	Yes	3.17421	Tissue	2016.12.06			M
Carp_CZ_ch954	*Lynx lynx carpathicus*	Reintroduced	Carpathian	Czech Republic	Yes	19.1345	Tissue	2017.10.24			F
BBA_CZ_LL1a	*Lynx lynx carpathicus*	Reintroduced	Bohemian-Bavarian-Austrian	Czech Republic	Yes	1.63115	Tissue	2003.03.27			M
BBA_CZ_LL3a	*Lynx lynx carpathicus*	Reintroduced	Bohemian-Bavarian-Austrian	Czech Republic	Yes	7.73072	Tissue	2003.03.20			M
BBA_CZ_LL4a	*Lynx lynx carpathicus*	Reintroduced	Bohemian-Bavarian-Austrian	Czech Republic	Yes	3.19621	Tissue	2000.02.14			F
BBA_CZ_LL5a	*Lynx lynx carpathicus*	Reintroduced	Bohemian-Bavarian-Austrian	Czech Republic	Yes	18.3346	Tissue	2008.02.26			F
BBA_CZ_LL6a	*Lynx lynx carpathicus*	Reintroduced	Bohemian-Bavarian-Austrian	Czech Republic	Yes	19.6225	Tissue	2004.06.09			F
BBA_CZ_Y170422	*Lynx lynx carpathicus*	Reintroduced	Bohemian-Bavarian-Austrian	Czech Republic	Yes	34.3785	Tissue	2015.03.03	49.1557	13.4409	F
BBA_CZ_Y170424	*Lynx lynx carpathicus*	Reintroduced	Bohemian-Bavarian-Austrian	Czech Republic	Yes	23.6089	Tissue	2015.05.10	49.0872	13.4804	F
BBA_CZ_ch224	*Lynx lynx carpathicus*	Natural	Carpathian	Czech Republic	Yes	17.0507	Tissue	2002			F
Carp_CZ_ch262	*Lynx lynx carpathicus*	Natural	Carpathian	Czech Republic	No		scat	2013.02.05			M
Carp_CZ_ch939a	*Lynx lynx carpathicus*	Natural	Carpathian	Czech Republic	No		hairs	2017.06.15			M
Carp_CZ_Y170423	*Lynx lynx carpathicus*	Natural	Carpathian	Czech Republic	Yes	23.3628	Tissue	2017.04.30	49.2260	18.0470	F
Carp_SK_1	*Lynx lynx carpathicus*	Natural	Carpathian	Slovakia	Yes	13.0286	Tissue	2014	49.035	19.573	UK
Carp_SK_2	*Lynx lynx carpathicus*	Natural	Carpathian	Slovakia	Yes	12.2646	Tissue	2007	48.7935	20.655	UK
Carp_SK_3	*Lynx lynx carpathicus*	Natural	Carpathian	Slovakia	Yes	12.2669	Tissue	2013	48.7935	20.655	UK
Carp_SK_4	*Lynx lynx carpathicus*	Natural	Carpathian	Slovakia	Yes	12.906	Tissue	2016			UK
Carp_SK_5	*Lynx lynx carpathicus*	Natural	Carpathian	Slovakia	Yes	12.2261	Tissue	2017	49.3555	18.694	UK
Carp_SK_ch167	*Lynx lynx carpathicus*	Natural	Carpathian	Slovakia	Yes	12.3473	Blood	2012.08.20			F
Carp_SK_ch236a	*Lynx lynx carpathicus*	Natural	Carpathian	Slovakia	No		Tissue	2012			F
Carp_SK_ch278	*Lynx lynx carpathicus*	Natural	Carpathian	Slovakia	Yes	16.5214	Tissue	2011			F
Carp_SK_ch338	*Lynx lynx carpathicus*	Natural	Carpathian	Slovakia	Yes	14.8923	Tissue	2013			M
Carp_SK_ch568	*Lynx lynx carpathicus*	Natural	Carpathian	Slovakia	No		hairs	2014.04.13			M
Carp_SK_ch727	*Lynx lynx carpathicus*	Natural	Carpathian	Slovakia	No		scat	2016.01.26			F
Carp_SK_Leb10a	*Lynx lynx carpathicus*	Natural	Carpathian	Slovakia	No		museum skull	2004			M
Carp_SK_Leb215	*Lynx lynx carpathicus*	Natural	Carpathian	Slovakia	Yes	0.686712	museum skull	1993.03.21			F
Carp_SK_Leb216	*Lynx lynx carpathicus*	Natural	Carpathian	Slovakia	No		museum skull	1994.02.16			F
Carp_SK_Leb3a	*Lynx lynx carpathicus*	Natural	Carpathian	Slovakia	No		museum skull	2013			F
Carp_SK_Leb44	*Lynx lynx carpathicus*	Natural	Carpathian	Slovakia	No		museum skull	1977.03.11			M
Carp_SK_Leb4a	*Lynx lynx carpathicus*	Natural	Carpathian	Slovakia	No		museum skull	2003			M
Carp_SK_Leb9a	*Lynx lynx carpathicus*	Natural	Carpathian	Slovakia	No		museum skull	2002			M
Carp_SK_PK10	*Lynx lynx carpathicus*	Natural	Carpathian	Slovakia	Yes	17.5154	Tissue	2013	49.31	19.521	UK
Carp_SK_PK6	*Lynx lynx carpathicus*	Natural	Carpathian	Slovakia	Yes	16.7728	Tissue	2014	48.7935	20.655	UK
Carp_SK_PK7	*Lynx lynx carpathicus*	Natural	Carpathian	Slovakia	Yes	21.5333	Tissue	2017	48.7935	20.655	UK
Carp_SK_PK8	*Lynx lynx carpathicus*	Natural	Carpathian	Slovakia	Yes	20.1596	Tissue	2016			UK
Carp_SK_PK9	*Lynx lynx carpathicus*	Natural	Carpathian	Slovakia	Yes	21.261	Tissue	2018	49.174	20.296	UK
D_Y120029	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	No		Tissue	2007.12.19	51.895321	10.38574	F
D_Y120030	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	No		Tissue	2008.11.03	51.836838	10.182847	F
D_Y120032	*Lynx lynx lynx*	Reintroduced	Harz	Sweden	No		Tissue	2009.02.13	56.466671	12.93333	F
D_Y120035	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	Yes	5.35116	Blood	2009.02.21	51.792953	10.800783	M
D_Y120036	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	No		Blood	2008.12.18	51.836838	10.182847	F
D_Y120040	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	Yes	21.3301	Blood	2007.11.29	51.895321	10.385737	F
D_Y120043	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	Yes	15.2584	Blood	2008.03.17	51.87	10.61	M
D_Y120048	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	Yes	15.5849	Blood	2010.11.22	51.87	10.61	F
D_Y120050	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	Yes	22.0571	Blood	2011.06.22	51.91	10.40	F
D_Y120062	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	Yes	19.5098	Blood	2003.01.01	52.25	8.07	F
D_Y120074	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	No		Blood	2003.06.23	51.18	9.05	M
D_Y120075	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	No		Blood	2003.06.23	51.180840	9.054200	M
D_Y120077	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	No		Blood	2003.01.01	51.49	9.66	F
D_Y120083	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	No		Blood	2003.01.01	51.49	9.66	F

D_Y120090	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	No		Blood	2005.05.10	51.74	9.52	F
D_Y120092	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	Yes	21.0732	Blood	2005.05.10	51.744202	9.518050	F
D_Y130056	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	No		Blood	2012.04.02	51.87	10.20	M
D_Y130064	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	Yes	7.97943	Blood	2013.04.30	51.485229	9.6611	M
D_Y150091	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	Yes	10.3049	Tissue	2015.03.08	51.873402	10.19147	F
D_Y150092	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	Yes	17.7438	Tissue	2015.03.09	51.7071	10.87332	F
D_Y150100	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	Yes	20.4242	Tissue	2014.12.12	51.87302	10.253892	M
D_Y160048	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	Yes	0.511916	Tissue	2015.12.05	51.55938	10.43277	F
D_Y160072	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	No		Tissue	2015.11.06	51.340538	9.663031	F
D_Y160117	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	No		Tissue	2015.06.25	51.784908	10.12184	F
D_Y160125	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	Yes	16.6145	Tissue	2015.11.30	51.355438	9.683354	F
D_Y160270	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	No		Blood	2016.04.15	51.77158	10.57911	F
D_Y170346	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	Yes	18.198	Tissue	2017.06.13	51.729115	10.538735	M
D_Y180226	*Lynx lynx lynx*	Reintroduced	Harz	Deutschland	Yes	17.4343	Tissue	2017.11.27	51.756802	10.99615	M
Din_BIH_M1YE6	*Lynx lynx carpathicus*	Reintroduced	Dinaric	Slovenia	Yes	19.2773	Tissue	Unknown			UK
Din_BIH_M1YEE	*Lynx lynx carpathicus*	Reintroduced	Dinaric	Slovenia	Yes	17.6959	Tissue	Unknown			UK
Din_HR_M1YE7	*Lynx lynx carpathicus*	Reintroduced	Dinaric	Slovenia	Yes	17.0112	Tissue	Unknown			UK
Din_HR_M1YE8	*Lynx lynx carpathicus*	Reintroduced	Dinaric	Slovenia	Yes	3.33843	Tissue	Unknown			UK
Din_HR_M1YEA	*Lynx lynx carpathicus*	Reintroduced	Dinaric	Slovenia	Yes	17.6885	Tissue	Unknown			UK
Din_HR_M1YEC	*Lynx lynx carpathicus*	Reintroduced	Dinaric	Slovenia	Yes	20.433	Tissue	Unknown			UK
Din_HR_M2AYA	*Lynx lynx carpathicus*	Reintroduced	Dinaric	Slovenia	Yes	20.1974	Tissue	Unknown			UK
Din_SI_CP.0XM0	*Lynx lynx carpathicus*	Reintroduced	Dinaric	Slovenia	Yes	20.1864	Tissue	Unknown			UK
Din_SI_M1YE5	*Lynx lynx carpathicus*	Reintroduced	Dinaric	Slovenia	Yes	15.1593	Tissue	Unknown			UK
E_1	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2010.12.27	59.0279697	23.51713034	F
E_10	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2012.01.03	59.0512472	24.56572446	F
E_1071	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2010.12.16	59.3119726	26.18331622	M
E_11	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2012.02.18	58.5815356	26.9765169	F
E_1111	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2010.12.29	58.5856089	24.82654933	M
E_1132	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2010.12.21	57.6041713	26.38611431	F
E_1139	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2011.01.02	57.9820167	26.4470414	F
E_1154	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2011.01.11	57.6423157	26.69387381	F
E_12	*Lynx lynx lynx*	Natural	Baltic	Estonia	Yes	20.6964	Tissue	2012.01.14	57.9876656	24.49005275	F
E_13	*Lynx lynx lynx*	Natural	Baltic	Estonia	Yes	22.0121	Tissue	2012.01.15	58.3439358	24.20537318	M
E_14	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2012.01.14	57.9943835	26.44153772	M
E_15	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2012.01.21	57.6886765	26.2640753	M
E_16	*Lynx lynx lynx*	Natural	Baltic	Estonia	Yes	27.5282	Tissue	2012.02.03	58.0867246	27.31633265	M
E_17	*Lynx lynx lynx*	Natural	Baltic	Estonia	Yes	26.4592	Tissue	2012.01.09	59.1382637	26.38896383	F
E_18	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2012.01.22	59.4829419	26.18454465	F
E_19	*Lynx lynx lynx*	Natural	Baltic	Estonia	Yes	30.8645	Tissue	2012.11.06	57.9810131	26.83452446	F
E_2	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2011.01.25	59.0490458	23.72596639	M
E_20	*Lynx lynx lynx*	Natural	Baltic	Estonia	Yes	6.9808	Tissue	2012.12.02	58.6526901	23.78221847	F
E_21	*Lynx lynx lynx*	Natural	Baltic	Estonia	Yes	20.5625	Tissue	2013.02.10	57.6737029	26.89005987	F
E_22	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2012.12.08	57.734232	27.44628144	M
E_23	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2013.01.12	58.8508762	26.7729653	F
E_24	*Lynx lynx lynx*	Natural	Baltic	Estonia	Yes	17.428	Tissue	2012.12.08	58.433146	24.7966476	F
E_25	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2012.12.30	58.6627613	24.5498199	F
E_26	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2013.01.10	58.3474538	26.18704875	M
E_27	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2012.12.29	58.6050849	26.29947412	M
E_28	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2013.02.01	59.0111048	25.1326307	M
E_29	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2013.02.23	58.8164445	25.15753855	M
E_3	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2011.01.08	58.6216108	25.18405735	M
E_30	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2013.01.16	58.1123983	26.82072313	M
E_31	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2013.02.03	58.2088628	25.8914133	M
E_32	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2013.02.23	58.4778485	25.50154579	M
E_33	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2012.12.08	58.9257097	26.01639015	M
E_34	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2014.01.10	59.2035682	24.61743097	F
E_35	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2014.02.09	59.3500973	26.00708542	M
E_36	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2014.02.25	57.7442456	26.88077322	M
E_37	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2011.02.08	59.4321326	25.03502098	F
E_38	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2011.02.05	59.305304	24.27537864	M
E_39	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2012.01.03	58.7213853	26.90868572	M
E_4	*Lynx lynx lynx*	Natural	Baltic	Estonia	Yes	30.2543	Tissue	2010.11.02	58.9508598	22.90753376	M
E_40	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2012.12.04	59.3291078	27.53838324	M
E_41	*Lynx lynx lynx*	Natural	Baltic	Estonia	Yes	25.8605	Tissue	2013.01.19	58.7237595	26.34958169	F
E_42	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2009.01.18	57.5658563	26.55883982	F
E_43	*Lynx lynx lynx*	Natural	Baltic	Estonia	Yes	24.8257	Tissue	2010.01.09	59.3046898	25.50332819	M
E_44	*Lynx lynx lynx*	Natural	Baltic	Estonia	Yes	22.9849	Tissue	2010.02.13	59.290458	26.69240478	F
E_45	*Lynx lynx lynx*	Natural	Baltic	Estonia	Yes	26.8289	Tissue	2010.01.13	59.0526215	27.06618964	M
E_46	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2010.01.02	59.1566638	27.27646007	F
E_47	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2010.02.14	57.8878733	27.42855412	F
E_48	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2009.12.30	58.2553151	24.72777412	F
E_49	*Lynx lynx lynx*	Natural	Baltic	Estonia	Yes	25.2856	Tissue	2008.01.11	58.2735081	25.3358366	F
E_5	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2011.12.04	58.9443042	25.77394495	F
E_50	*Lynx lynx lynx*	Natural	Baltic	Estonia	Yes	23.5937	Tissue	2011.01.22	59.4426989	26.70015855	M

E_6	*Lynx lynx lynx*	Natural	Baltic	Estonia	Yes	21.4802	Tissue	2011.12.12	58.1728856	26.29522519	F
E_7	*Lynx lynx lynx*	Natural	Baltic	Estonia	Yes	22.2992	Tissue	2012.01.03	59.4692681	25.54529967	F
E_8	*Lynx lynx lynx*	Natural	Baltic	Estonia	No		Tissue	2012.01.14	57.6019375	27.27724364	F
E_9	*Lynx lynx lynx*	Natural	Baltic	Estonia	Yes	23.1149	Tissue	2012.01.14	58.747917	25.75095525	M
F_F6407	*Lynx lynx lynx*	Natural	Karelian	Finland	No		Tissue	2007.09.21	60.798999	25.732742	M
F_F6435	*Lynx lynx lynx*	Natural	Karelian	Finland	No		Tissue	2007.10.16	61.053679	23.954309	M
F_F6457	*Lynx lynx lynx*	Natural	Karelian	Finland	Yes	29.6017	Tissue	2007.11.12	61.451804	24.120179	M
F_F6476	*Lynx lynx lynx*	Natural	Karelian	Finland	No		Tissue	2007.12.01	64.018119	28.513357	F
F_F6477	*Lynx lynx lynx*	Natural	Karelian	Finland	No		Tissue	2007.12.04	63.049765	28.447075	M
F_F6489	*Lynx lynx lynx*	Natural	Karelian	Finland	No		Tissue	2007.12.05	60.740134	28.011934	M
F_F6491	*Lynx lynx lynx*	Natural	Karelian	Finland	No		Tissue	2007.12.02	62.971202	26.546564	F
F_F6492	*Lynx lynx lynx*	Natural	Karelian	Finland	No		Tissue	2007.12.05	62.534971	29.242036	F
F_F6506	*Lynx lynx lynx*	Natural	Karelian	Finland	No		Tissue	2008.01.08	64.374311	27.316774	M
F_F6515	*Lynx lynx lynx*	Natural	Karelian	Finland	No		Tissue	2008.01.09	60.523392	27.593729	M
F_F6530	*Lynx lynx lynx*	Natural	Karelian	Finland	No		Tissue	2008.01.12	62.667448	25.636994	M
F_F6537	*Lynx lynx lynx*	Natural	Karelian	Finland	No		Tissue	2008.01.26	60.745411	25.468228	M
F_F6547	*Lynx lynx lynx*	Natural	Karelian	Finland	No		Tissue	2008.01.27	62.674408	26.553042	F
F_F6563	*Lynx lynx lynx*	Natural	Karelian	Finland	Yes	30.4883	Tissue	2008.02.09	63.12752	27.010029	M
F_F6568	*Lynx lynx lynx*	Natural	Karelian	Finland	No		Tissue	2008.01.27	61.439162	26.530848	F
F_F6569	*Lynx lynx lynx*	Natural	Karelian	Finland	Yes	28.4955	Tissue	2008.02.02	61.681511	22.401894	M
F_F6570	*Lynx lynx lynx*	Natural	Karelian	Finland	Yes	34.4752	Tissue	2008.02.02	61.344925	22.310138	M
F_F6578	*Lynx lynx lynx*	Natural	Karelian	Finland	Yes	26.0573	Tissue	2008.02.10	63.15778	28.588337	M
F_F6582	*Lynx lynx lynx*	Natural	Karelian	Finland	Yes	25.0964	Tissue	2008.02.18	65.30227	29.45172	M
F_F6617	*Lynx lynx lynx*	Natural	Karelian	Finland	Yes	28.0052	Tissue	2008.05.02	65.166144	29.370136	M
F_F6630	*Lynx lynx lynx*	Natural	Karelian	Finland	Yes	29.5077	Tissue	2008.04.17	62.676535	30.935616	F
F_F6631	*Lynx lynx lynx*	Natural	Karelian	Finland	Yes	28.3645	Tissue	2008.01.09	62.549976	21.109008	F
F_F6633	*Lynx lynx lynx*	Natural	Karelian	Finland	Yes	17.7823	Tissue	2008.08.10	61.543126	24.067464	F
F_F6634	*Lynx lynx lynx*	Natural	Karelian	Finland	No		Tissue	2007.12.21	62.10479	28.961163	F
F_F6635	*Lynx lynx lynx*	Natural	Karelian	Finland	Yes	24.6004	Tissue	2008.17.08	61.454472	24.141116	M
F_F6662	*Lynx lynx lynx*	Natural	Karelian	Finland	Yes	29.6765	Tissue	2008.28.08	62.76064	30.034764	M
F_F6667	*Lynx lynx lynx*	Natural	Karelian	Finland	Yes	7.59236	Tissue	2008.21.08	60.424306	22.513709	M
F_F6668	*Lynx lynx lynx*	Natural	Karelian	Finland	No		Tissue	2008.07.04	63.079473	25.858205	F
F_F6669	*Lynx lynx lynx*	Natural	Karelian	Finland	Yes	22.2504	Tissue	2008.10.08	62.53	25.77537	F
F_F6670	*Lynx lynx lynx*	Natural	Karelian	Finland	Yes	13.0287	Tissue	2008.06.15	63.737793	26.726671	M
J_CH_W16-0081	*Lynx lynx carpathicus*	Reintroduced	Jura	Switzerland	Yes	15.1093	Tissue	2016.05.03	47.00796	6.83234	F
J_CH_W16-1425	*Lynx lynx carpathicus*	Reintroduced	Jura	Switzerland	No		Tissue	2016.08.11	47.00796	6.83234	M
J_CH_W16-2393	*Lynx lynx carpathicus*	Reintroduced	Jura	Switzerland	Yes	17.0898	Tissue	2016.11.01	47.33318	7.44348	F
J_CH_W16-2394	*Lynx lynx carpathicus*	Reintroduced	Jura	Switzerland	Yes	16.5118	Tissue	2016.11.01	47.33318	7.44348	M
J_CH_W16-2427	*Lynx lynx carpathicus*	Reintroduced	Jura	Switzerland	Yes	12.6484	Tissue	2016.11.04	46.96215	6.80928	F
J_CH_W16-3160	*Lynx lynx carpathicus*	Reintroduced	Jura	Switzerland	Yes	10.0496	Tissue	2016.12.29	47.23608	7.48459	M
J_CH_W16-8810	*Lynx lynx carpathicus*	Reintroduced	Jura	Switzerland	Yes	15.4411	Tissue	2016.02.04	47.40635	7.0854	F
J_CH_W16-9054	*Lynx lynx carpathicus*	Reintroduced	Jura	Switzerland	Yes	8.92739	Tissue	2016.02.21	47.01301	6.90436	M
J_CH_W16-9429	*Lynx lynx carpathicus*	Reintroduced	Jura	Switzerland	Yes	18.4375	Tissue	2016.03.20	47.30171	7.37772	F
J_CH_W17-3242	*Lynx lynx carpathicus*	Reintroduced	Jura	Switzerland	Yes	17.8372	Tissue	2017.01.07	47.31789	7.54478	M
J_CH_W17-3728	*Lynx lynx carpathicus*	Reintroduced	Jura	Switzerland	Yes	18.4327	Tissue	2017.02.12	47.36797	7.1627	M
J_CH_W17-6418	*Lynx lynx carpathicus*	Reintroduced	Jura	Switzerland	Yes	17.5761	Tissue	2017.09.11	47.16719	7.0415	F
J_CH_W17-6452	*Lynx lynx carpathicus*	Reintroduced	Jura	Switzerland	Yes	16.1572	Tissue	2017.09.18	47.37356	7.88271	F
J_CH_W17-6732	*Lynx lynx carpathicus*	Reintroduced	Jura	Switzerland	Yes	9.10833	Tissue	2017.09.30	46.87144	6.50963	F
J_CH_W17-6794	*Lynx lynx carpathicus*	Reintroduced	Jura	Switzerland	Yes	2.03774	Tissue	2017.10.07	47.36029	7.5574	M
J_CH_W17-6922	*Lynx lynx carpathicus*	Reintroduced	Jura	Switzerland	Yes	18.6722	Tissue	2017.10.17	47.05444	7.23832	F
J_CH_W17-7072	*Lynx lynx carpathicus*	Reintroduced	Jura	Switzerland	Yes	19.1495	Tissue	2017.10.27	47.15337	6.90191	F
J_CH_W17-7186	*Lynx lynx carpathicus*	Reintroduced	Jura	Switzerland	Yes	17.0492	Tissue	2017.11.06	47.09565	7.16383	M
J_CH_W17-7266	*Lynx lynx carpathicus*	Reintroduced	Jura	Switzerland	No		Tissue	2017.11.08	47.23018	6.88104	F
J_CH_W18-3157	*Lynx lynx carpathicus*	Reintroduced	Jura	Switzerland	Yes	19.1571	Tissue	2018.06.07	47.13164	7.17346	F
J_CH_W18-7898	*Lynx lynx carpathicus*	Reintroduced	Jura	Switzerland	Yes	4.23448	Tissue	2018.01.03	46.6973	6.45061	M
J_CH_W18-8506	*Lynx lynx carpathicus*	Reintroduced	Jura	Switzerland	No		Tissue	2018.02.17	47.00909	6.90423	F
L_1	*Lynx lynx lynx*	Natural	Baltic	Latvia	No		Tissue	2010.01.02	57.091	24.630	F
L_11	*Lynx lynx lynx*	Natural	Baltic	Latvia	Yes	26.5922	Tissue	2009.12.05	56.676	22.608	F
L_14	*Lynx lynx lynx*	Natural	Baltic	Latvia	No		Tissue	2010.02.14	57.738	24.425	M
L_16	*Lynx lynx lynx*	Natural	Baltic	Latvia	No		Tissue	2009.12.01	56.314	27.577	F
L_20	*Lynx lynx lynx*	Natural	Baltic	Latvia	Yes	18.9106	Tissue	2009.12.12	56.377	28.145	F
L_24	*Lynx lynx lynx*	Natural	Baltic	Latvia	Yes	24.7586	Tissue	2010.01.09	56.535	22.614	F
L_3	*Lynx lynx lynx*	Natural	Baltic	Latvia	No		Tissue	2010.01.02	56.963	22.873	F
L_34	*Lynx lynx lynx*	Natural	Baltic	Latvia	Yes	24.4076	Tissue	2010.11.15	57.287	22.910	F
L_4	*Lynx lynx lynx*	Natural	Baltic	Latvia	No		Tissue	2009.12.27	57.321	22.821	F
L_42	*Lynx lynx lynx*	Natural	Baltic	Latvia	No		Tissue	2010.12.18	56.728	21.539	M
L_45	*Lynx lynx lynx*	Natural	Baltic	Latvia	Yes	15.6599	Tissue	2011.02.07	57.671	24.851	M
L_48	*Lynx lynx lynx*	Natural	Baltic	Latvia	Yes	7.21548	Tissue	2010.03.18	57.242	22.018	M
L_49	*Lynx lynx lynx*	Natural	Baltic	Latvia	No		Tissue	2009.12.07	57.504	25.400	F
L_50	*Lynx lynx lynx*	Natural	Baltic	Latvia	No		Tissue	2010.05.31	56.519	22.442	M
L_51	*Lynx lynx lynx*	Natural	Baltic	Latvia	No		Tissue	2009.12.19	57.441	26.260	F
L_53	*Lynx lynx lynx*	Natural	Baltic	Latvia	Yes	11.9349	Tissue	2010.12.21	56.783	24.564	M
L_54	*Lynx lynx lynx*	Natural	Baltic	Latvia	No		Tissue	2011.02.04	57.325	24.659	F
L_55	*Lynx lynx lynx*	Natural	Baltic	Latvia	Yes	14.122	Tissue	2011.12.07	57.211	25.330	F

L_58	*Lynx lynx lynx*	Natural	Baltic	Latvia	Yes	19.6118	Tissue	2008.12.22	57.264	27.128	F
L_9	*Lynx lynx lynx*	Natural	Baltic	Latvia	Yes	24.469	Tissue	2009.12.20	55.872	27.209	M
L_CH_W16-1191	*Lynx lynx carpathicus*	Reintroduced	Luno	Switzerland	Yes	16.6675	Tissue	2016.07.27	47.13318	9.4244	M
L_CH_W16-1948	*Lynx lynx carpathicus*	Reintroduced	Luno	Switzerland	Yes	16.788	Tissue	2016.09.25	47.33622	9.26455	M
L_CH_W16-2142	*Lynx lynx carpathicus*	Reintroduced	Luno	Switzerland	Yes	5.84035	Tissue	2016.10.12	47.39422	9.15513	F
L_CH_W16-2848	*Lynx lynx carpathicus*	Reintroduced	Luno	Switzerland	Yes	17.3343	Tissue	2016.12.06	47.28837	9.08842	F
L_CH_W16-2892	*Lynx lynx carpathicus*	Reintroduced	Luno	Switzerland	Yes	16.5594	Tissue	2016.09.22	47.13689	9.27145	F
L_CH_W17-6294	*Lynx lynx carpathicus*	Reintroduced	Luno	Switzerland	Yes	18.8556	Tissue	2017.08.31	47.15328	9.40345	M
L_CH_W17-6524	*Lynx lynx carpathicus*	Reintroduced	Luno	Switzerland	Yes	16.4144	Tissue	2017.09.16	47.22557	9.20187	F
L_CH_W17-6631	*Lynx lynx carpathicus*	Reintroduced	Luno	Switzerland	Yes	19.3014	Tissue	2017.09.23	47.26952	9.2614	F
L_CH_W17-7555	*Lynx lynx carpathicus*	Reintroduced	Luno	Switzerland	Yes	20.2548	Tissue	2017.11.24	47.07768	9.39152	F
L_CH_W18-1826	*Lynx lynx carpathicus*	Reintroduced	Luno	Switzerland	Yes	20.8066	Tissue	2018.02.21	47.18555	9.41349	F
L_CH_W18-2407	*Lynx lynx carpathicus*	Reintroduced	Luno	Switzerland	Yes	8.69154	Tissue	2018.04.08	47.36179	9.46626	F
M_579	*Lynx lynx isabellinus*	Natural	Omnogovi	Mongolia	Yes	19.0681	Dried Skin	2017.09.01	43.134	101.024	UK
M_580	*Lynx lynx isabellinus*	Natural	Omnogovi	Mongolia	Yes	13.6722	Dried Skin	2017.09.01	43.276	104.165	UK
N_M407590	*Lynx lynx lynx*	Natural	Scandinavian	Norway	Yes	21.1408	Tissue	2015.02.02	59.051448	9.832993	M
N_M407595	*Lynx lynx lynx*	Natural	Scandinavian	Norway	Yes	20.5157	Tissue	2015.02.01	65.330606	14.366471	F
N_M407707	*Lynx lynx lynx*	Natural	Scandinavian	Norway	No		Tissue	2015.03.27	59.440519	7.843569	F
N_M491940	*Lynx lynx lynx*	Natural	Scandinavian	Norway	Yes	24.9153	Tissue	2016.01.22	69.611157	20.340033	F
N_M492003	*Lynx lynx lynx*	Natural	Scandinavian	Norway	No		Tissue	2016.02.07	63.789354	10.588822	F
N_M492013	*Lynx lynx lynx*	Natural	Scandinavian	Norway	Yes	21.9544	Tissue	2016.02.08	65.776475	13.289279	M
N_M492056	*Lynx lynx lynx*	Natural	Scandinavian	Norway	Yes	24.6202	Tissue	2016.02.19	58.51787	6.765634	M
N_M493462	*Lynx lynx lynx*	Natural	Scandinavian	Norway	No		Tissue	2017.02.24	69.879547	25.180133	M
N_M494891	*Lynx lynx lynx*	Natural	Scandinavian	Norway	No		Tissue	2018.02.01	62.945929	11.109425	F
N_M494895	*Lynx lynx lynx*	Natural	Scandinavian	Norway	No		Tissue	2018.02.01	64.509992	11.600299	M
N_M494931	*Lynx lynx lynx*	Natural	Scandinavian	Norway	No		Tissue	2018.02.08	62.898166	7.079221	M
N_M494991	*Lynx lynx lynx*	Natural	Scandinavian	Norway	No		Tissue	2018.02.23	69.86626	21.985827	M
N_M494992	*Lynx lynx lynx*	Natural	Scandinavian	Norway	Yes	26.4119	Tissue	2018.02.21	61.891228	8.405619	M
N_M495135	*Lynx lynx lynx*	Natural	Scandinavian	Norway	No		Tissue	2018.03.14	60.617881	6.555738	M
N_M496536	*Lynx lynx lynx*	Natural	Scandinavian	Norway	No		Tissue	2019.02.01	61.44482	10.248134	F
N_M496611	*Lynx lynx lynx*	Natural	Scandinavian	Norway	Yes	29.7183	Tissue	2019.02.12	67.17838	14.936349	F
N_M496636	*Lynx lynx lynx*	Natural	Scandinavian	Norway	Yes	20.1143	Tissue	2019.02.24	58.415055	8.29809	M
N_M496790	*Lynx lynx lynx*	Natural	Scandinavian	Norway	No		Tissue	2019.03.09	68.234715	16.75649	F
N_M496868	*Lynx lynx lynx*	Natural	Scandinavian	Norway	Yes	19.3074	Tissue	2019.03.15	64.121386	13.925708	M
N_M496989	*Lynx lynx lynx*	Natural	Scandinavian	Norway	Yes	27.5542	Tissue	2019.03.14	60.164219	9.023418	F
P_1	*Lynx lynx lynx*	Natural	Baltic	Poland	Yes	24.001	Tissue	2001.11.20	52.954366	23.595467	F
P_124	*Lynx lynx lynx*	Natural	Baltic	Poland	No		Tissue	2007.07.01	49.654874	19.74398	UK
P_125	*Lynx lynx lynx*	Natural	Baltic	Poland	No		Tissue	2007.07.01	49.665477	19.711629	UK
P_129	*Lynx lynx lynx*	Natural	Baltic	Poland	No		Tissue	2008.02.13	53.09138	23.57397	F
P_13	*Lynx lynx lynx*	Natural	Baltic	Poland	Yes	23.8114	Tissue	2002.12.07	53.213438	23.690419	F
P_143	*Lynx lynx lynx*	Natural	Baltic	Poland	Yes	29.6978	Tissue	2008.04.24	52.768522	23.945538	F
P_145	*Lynx lynx lynx*	Natural	Baltic	Poland	No		Tissue	2008.12.15	53.069809	23.778962	M
P_147	*Lynx lynx lynx*	Natural	Baltic	Poland	No		Tissue	2008.12.21	50.218161	23.234611	M
P_161	*Lynx lynx lynx*	Natural	Baltic	Poland	Yes	18.183	Tissue	2009.03.31	53.591992	21.302052	M
P_163	*Lynx lynx lynx*	Natural	Baltic	Poland	Yes	18.9499	Tissue	2009.07.17	53.630747	21.725214	F
P_165	*Lynx lynx lynx*	Natural	Baltic	Poland	No		Tissue	2010.04.12	53.212887	23.616378	F
P_174	*Lynx lynx lynx*	Natural	Baltic	Poland	Yes	16.517	Tissue	2010.08.23	52.680726	23.743456	F
P_175	*Lynx lynx lynx*	Natural	Baltic	Poland	Yes	28.8791	Tissue	2010.09.02	49.746569	22.319933	F
P_21	*Lynx lynx lynx*	Natural	Baltic	Poland	No		Tissue	2001.02.19	52.84393	23.373986	F
P_325	*Lynx lynx lynx*	Natural	Baltic	Poland	Yes	19.4542	Tissue	2013.02.10	50.094879	23.120036	M
P_35	*Lynx lynx lynx*	Natural	Baltic	Poland	Yes	24.7239	Tissue	2004.12.27	53.073323	23.44457	M
P_4	*Lynx lynx lynx*	Natural	Baltic	Poland	Yes	20.6608	Tissue	2002.02.17	50.42291	20.907366	F
P_420	*Lynx lynx lynx*	Natural	Baltic	Poland	No		Tissue	2015.11.25	52.4548	23.3606	UK
P_561	*Lynx lynx lynx*	Natural	Baltic	Poland	No		Tissue	2016.02.28	52.4946	23.4716	F
P_562	*Lynx lynx lynx*	Natural	Baltic	Poland	Yes	25.2251	Tissue	2016.02.28	52.515	23.44	F
P_573	*Lynx lynx lynx*	Natural	Baltic	Poland	No		Tissue	2017.01.02	52.4852	23.3836	F
P_578	*Lynx lynx lynx*	Natural	Baltic	Poland	Yes	18.1335	Tissue	2017.04.21	49.3836	21.1441	F
P_583	*Lynx lynx lynx*	Natural	Baltic	Poland	No		Tissue	2018.11.13	52.67078	23.85154	F
P_584	*Lynx lynx lynx*	Natural	Baltic	Poland	No		Tissue	2019.01.25	53.30818	23.527135	M
P_586	*Lynx lynx lynx*	Natural	Baltic	Poland	Yes	23.4716	Tissue	2019.02.04	52.41168	23.2168	UK
P_589	*Lynx lynx lynx*	Natural	Baltic	Poland	No		Tissue	2019.02.15	53.8096	22.9664	UK
P_592	*Lynx lynx lynx*	Natural	Baltic	Poland	Yes	23.7698	Tissue	2019.04.06	53.331153	23.364324	UK
P_90	*Lynx lynx lynx*	Natural	Baltic	Poland	Yes	28.598	Tissue	2006.01.01	53.12199	23.568644	F
P_97	*Lynx lynx lynx*	Natural	Baltic	Poland	Yes	15.7702	Tissue	2000.01.01	53.569337	23.428149	UK
P_98	*Lynx lynx lynx*	Natural	Baltic	Poland	No		Tissue	2007.05.18	52.891802	23.51427	M
R_18	*Lynx lynx lynx*	Natural	Kirov region	Russia	Yes	9.31224	Dried Skin	2002.12.01	59.692931	48.363816	UK
R_19	*Lynx lynx lynx*	Natural	Kirov region	Russia	No		Dried Skin	2002.12.01	59.427547	48.904158	UK
R_20	*Lynx lynx lynx*	Natural	Kirov region	Russia	Yes	13.3869	Dried Skin	2002.12.01	59.642559	52.59096	UK
R_286	*Lynx lynx lynx*	Natural	Kirov region	Russia	Yes	22.3309	Dried Skin	2010.10.08	58.56044	50.845727	UK
R_287	*Lynx lynx lynx*	Natural	Kirov region	Russia	Yes	16.8256	Dried Skin	2010.10.08	60.0479	47.874313	UK
R_288	*Lynx lynx lynx*	Natural	Kirov region	Russia	Yes	18.7815	Dried Skin	2010.10.08	60.704656	47.805031	UK
R_289	*Lynx lynx lynx*	Natural	Kirov region	Russia	Yes	23.9277	Dried Skin	2010.10.08	59.988323	47.909056	UK
R_290	*Lynx lynx lynx*	Natural	Kirov region	Russia	Yes	16.4349	Dried Skin	2010.10.08	59.664581	50.582933	UK
R_291	*Lynx lynx lynx*	Natural	Kirov region	Russia	Yes	22.5331	Dried Skin	2010.10.08	59.815529	47.423128	UK

R_292	*Lynx lynx lynx*	Natural	border Kirov/Perm	Russia	Yes	23.8764	Dried Skin	2010.10.08	59.786398	53.598506	UK
R_293	*Lynx lynx lynx*	Natural	border Kirov/Perm	Russia	Yes	19.7347	Dried Skin	2010.10.08	59.678857	53.391106	UK
R_294	*Lynx lynx lynx*	Natural	border Kirov/Perm	Russia	Yes	26.2118	Dried Skin	2010.10.08	59.513706	53.352698	UK
R_295	*Lynx lynx lynx*	Natural	Kirov region	Russia	Yes	24.0608	Dried Skin	2010.10.08	59.513706	53.352698	UK
R_296	*Lynx lynx lynx*	Natural	Kirov region	Russia	Yes	21.2283	Dried Skin	2010.10.08	59.834552	47.740308	UK
R_297	*Lynx lynx lynx*	Natural	Kirov region	Russia	Yes	21.2643	Dried Skin	2010.10.08	59.2419	50.451323	UK
R_298	*Lynx lynx lynx*	Natural	Kirov region	Russia	Yes	9.25544	Dried Skin	2002.11.01	59.880919	50.340353	UK
R_299	*Lynx lynx lynx*	Natural	Kirov region	Russia	Yes	19.5595	Dried Skin	2009.01.01	60.219949	48.716519	M
R_300	*Lynx lynx lynx*	Natural	Kirov region	Russia	Yes	5.68253	Dried Skin	2002.11.01	60.932755	50.051502	F
R_310	*Lynx lynx lynx*	Natural	unknown	Russia	No		Dried Skin	1998.01.01			UK
R_311	*Lynx lynx lynx*	Natural	unknown	Russia	No		Dried Skin	2010.01.01			UK
R_574	*Lynx lynx lynx*	Natural	Novgorod	Russia	Yes	24.4962	Dried Skin	2017.04.10	57.2318	44.5815	UK
R_575	*Lynx lynx lynx*	Natural	Novgorod	Russia	Yes	19.9969	Dried Skin	2017.04.10	57.2318	44.5815	UK
R_576	*Lynx lynx lynx*	Natural	Novgorod	Russia	No		Dried Skin	2017.04.10	57.2318	44.5815	UK
R_577	*Lynx lynx lynx*	Natural	Kirov region	Russia	Yes	30.3756	Tissue	2016.12.01	57.2541	49.325	M
T_581	*Lynx lynx isabellinus*	Natural	Tajikistan	Tajikistan	Yes	26.6834	Dried Skin	2013.01.01	39.0048	73.3659	UK
c_ll_cr_0206	*Lynx lynx carpathicus*	Natural	Carpathian montains	Romania	Yes, by Lucena	7.54133	BAM file	NA	25.355794	45.382645	M
c_ll_cr_0207	*Lynx lynx carpathicus*	Natural	Carpathian montains	Romania	Yes, by Lucena	8.68415	BAM file	NA	22.1241	45.235094	F
c_ll_cr_0209	*Lynx lynx carpathicus*	Natural	Carpathian montains	Romania	Yes, by Lucena	8.62264	BAM file	NA	22.1241	45.235094	F
c_ll_cr_0212	*Lynx lynx carpathicus*	Natural	Carpathian montains	Poland	Yes, by Lucena	19.2437	BAM file	NA	22.2651	49.422808	M
c_ll_ka_0184	*Lynx lynx wrangeli*	Natural	Mongolia (Central/Khentii aym	Mongolia	Yes, by Lucena	6.96814	BAM file	NA	108.381100	48.224300	M
c_ll_ka_0186	*Lynx lynx wrangeli*	Natural	Mongolia (Central/Khentii aym	Mongolia	Yes, by Lucena	6.86957	BAM file	NA	108.45	48.243500	F
c_ll_ka_0188	*Lynx lynx wrangeli*	Natural	Mongolia (Central/Khentii aym	Mongolia	Yes, by Lucena	6.00429	BAM file	NA	110.2911	48.372100	M
c_ll_ka_0189	*Lynx lynx wrangeli*	Natural	Mongolia (Central/Khentii aym	Mongolia	Yes, by Lucena	5.92381	BAM file	NA	110.291100	48.372100	M
c_ll_og_0181	*Lynx lynx isabellinus*	Natural	Mongolia (Omnogovi)	Mongolia	Yes, by Lucena	6.79024	BAM file	NA	101.252	43.133000	M
c_ll_og_0187	*Lynx lynx isabellinus*	Natural	Mongolia (Omnogovi)	Mongolia	Yes, by Lucena	5.63946	BAM file	NA	101.252	43.133000	F
c_ll_to_0190	*Lynx lynx wrangeli*	Natural	Mongolia (Central/Khentii aym	Mongolia	Yes, by Lucena	7.33784	BAM file	NA	108.162300	47.235500	F
c_ll_to_0191	*Lynx lynx wrangeli*	Natural	Mongolia (Central/Khentii aym	Mongolia	Yes, by Lucena	7.71857	BAM file	NA	108.411	47.374800	M
c_ll_tu_0153	*Lynx lynx wrangeli*	Natural	Tuva Republic	Russia	Yes, by Lucena	8.10689	BAM file	NA	96.32	51.29	F
c_ll_tu_0157	*Lynx lynx wrangeli*	Natural	Tuva Republic	Russia	Yes, by Lucena	7.80348	BAM file	NA	96.32	51.29	M
c_ll_tu_0158	*Lynx lynx wrangeli*	Natural	Tuva Republic	Russia	Yes, by Lucena	8.0083	BAM file	NA	96.32	51.29	F
c_ll_tu_0159	*Lynx lynx wrangeli*	Natural	Tuva Republic	Russia	Yes, by Lucena	7.73842	BAM file	NA	96.32	51.29	F
c_ll_tu_0165	*Lynx lynx wrangeli*	Natural	Tuva Republic	Russia	Yes, by Lucena	7.76115	BAM file	NA	96.32	51.29	F
c_ll_tu_0166	*Lynx lynx wrangeli*	Natural	Tuva Republic	Russia	Yes, by Lucena	8.05401	BAM file	NA	96.32	51.29	M
c_ll_ur_0194	*Lynx lynx lynx*	Natural	Ural Mountains	Russia	Yes, by Lucena	11.0239	BAM file	NA	59.383300	56.253	F
c_ll_ur_0195	*Lynx lynx lynx*	Natural	Ural Mountains	Russia	Yes, by Lucena	11.7718	BAM file	NA	60.7494	55.858	M
c_ll_ur_0196	*Lynx lynx lynx*	Natural	Ural Mountains	Russia	Yes, by Lucena	12.3771	BAM file	NA	60.7494	55.858	M
c_ll_ur_0199	*Lynx lynx lynx*	Natural	Ural Mountains	Russia	Yes, by Lucena	13.1596	BAM file	NA	59.471900	55.105400	M
c_ll_ur_0200	*Lynx lynx lynx*	Natural	Ural Mountains	Russia	Yes, by Lucena	12.6612	BAM file	NA	59.0197	55.022	M
c_ll_ur_0203	*Lynx lynx lynx*	Natural	Ural Mountains	Russia	Yes, by Lucena	13.3973	BAM file	NA	57.214500	54.595800	F
c_ll_vl_0107	*Lynx lynx wrangeli*	Natural	Primorsky Krai	Russia	Yes, by Lucena	6.50257	BAM file	NA	136.282171	44.56845	F
c_ll_vl_0108	*Lynx lynx wrangeli*	Natural	Primorsky Krai	Russia	Yes, by Lucena	11.8787	BAM file	NA	135.344507	45.173784	F
c_ll_vl_0109	*Lynx lynx wrangeli*	Natural	Primorsky Krai	Russia	Yes, by Lucena	6.04097	BAM file	NA	136.435276	45.395998	M
c_ll_vl_0110	*Lynx lynx wrangeli*	Natural	Primorsky Krai	Russia	Yes, by Lucena	7.07492	BAM file	NA	132.545074	49.0533	M
c_ll_vl_0112	*Lynx lynx wrangeli*	Natural	Primorsky Krai	Russia	Yes, by Lucena	28.9235	BAM file	NA	137.03344	45.512599	F
c_ll_vl_0113	*Lynx lynx wrangeli*	Natural	Primorsky Krai	Russia	Yes, by Lucena	8.36169	BAM file	NA	137.03344	45.512599	M
c_ll_vl_0128	*Lynx lynx wrangeli*	Natural	Primorsky Krai	Russia	Yes, by Lucena	7.96396	BAM file	NA	137.255084	45.542711	M
c_ll_vl_0132	*Lynx lynx wrangeli*	Natural	Primorsky Krai	Russia	Yes, by Lucena	8.31332	BAM file	NA	137.255084	45.542711	M
c_ll_ya_0138	*Lynx lynx wrangeli*	Natural	Yakutia Republic	Russia	Yes, by Lucena	7.94849	BAM file	NA	132.4998	59.5437	M
c_ll_ya_0139	*Lynx lynx wrangeli*	Natural	Yakutia Republic	Russia	Yes, by Lucena	7.97872	BAM file	NA	129.121768	61.112123	M
c_ll_ya_0142	*Lynx lynx wrangeli*	Natural	Yakutia Republic	Russia	Yes, by Lucena	8.29523	BAM file	NA	136.103667	66.531709	M
c_ll_ya_0143	*Lynx lynx wrangeli*	Natural	Yakutia Republic	Russia	Yes, by Lucena	7.8525	BAM file	NA	136.103667	66.531709	M
c_ll_ya_0145	*Lynx lynx wrangeli*	Natural	Yakutia Republic	Russia	Yes, by Lucena	8.26097	BAM file	NA	129.264323	61.533584	M
c_ll_ya_0146	*Lynx lynx wrangeli*	Natural	Yakutia Republic	Russia	Yes, by Lucena	22.9182	BAM file	NA	130.404470	60.463441	M
c_ll_ya_0147	*Lynx lynx wrangeli*	Natural	Yakutia Republic	Russia	Yes, by Lucena	8.07335	BAM file	NA	127.181614	60.45554	F

Table S3. Optimal K values as estimated through the Evanno method (Evanno et al. 2005) and mean estimated ln(Pr(X|K) probability.

Delta(K=2)	1941.06639	Ln'(2)	288792.114
Delta(K=3)	3.1171018	Ln'(3)	98482.9137
Delta(K=4)	4.87283377	Ln'(4)	41509.0429
Delta(K=5)	0.80511199	Ln'(5)	34279.9384
Delta(K=6)	0.98357925	Ln'(6)	32444.4486
Delta(K=7)	1.17864252	Ln'(7)	28352.4165
Delta(K=8)	0.40535514	Ln'(8)	23396.6261
Delta(K=9)	0.49181714	Ln'(9)	21505.1838
Delta(K=10)	0.5174569	Ln'(10)	19434.9606
Delta(K=11)	0.44469931	Ln'(11)	16856.5133
Delta(K=12)	0.49511495	Ln'(12)	14867.3031
Delta(K=13)	0.3958948	Ln'(13)	13321.5743
Delta(K=14)	0.12412822	Ln'(14)	11669.5276
Delta(K=15)	0.41353504	Ln'(15)	12155.1251
Delta(K=16)	0.44672529	Ln'(16)	10569.9817
Delta(K=17)	0.43817485	Ln'(17)	9134.41126
Delta(K=18)	0.40828073	Ln'(18)	10332.699
Delta(K=19)	0.36436756	Ln'(19)	9119.8105
		Ln'(20)	8202.54744

Table S4. Genomic Diversity statistics by population, as defined by geographic location.

Population	Individuals	Private Alleles	Obs.Het	Exp.Het	Pi	F
Alp	16	0	0.14	0.16	0.17	0.48
Jur	18	0	0.18	0.18	0.19	0.35
Lun	11	0	0.15	0.17	0.18	0.45
BBA	8	0	0.16	0.17	0.19	0.42
Din	9	0	0.16	0.16	0.17	0.45
Carp	23	0	0.21	0.25	0.25	0.26
Harz	8	0	0.25	0.25	0.27	0.33
Pol	11	0	0.23	0.24	0.26	0.29
Lat	9	0	0.22	0.26	0.28	0.24
Est	18	0	0.25	0.28	0.29	0.21
Fin	15	0	0.21	0.27	0.29	0.36
Nor	10	0	0.18	0.22	0.23	0.39
Kir	19	0	0.24	0.28	0.29	0.28
Ural	6	0	0.28	0.26	0.29	0.15
Tuva	6	0	0.32	0.28	0.31	0.15
Mon	8	0	0.27	0.26	0.29	0.27
Yak	7	0	0.29	0.27	0.29	0.23
Prim	10	0	0.27	0.25	0.27	0.28

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Zusammenfassung

Die Ära der Genomik eröffnet der Naturschutzforschung bisher unerreichte Möglichkeiten bei der Untersuchung von Fragestellungen zur Wiederansiedlung von Wildtieren in ihren historischen Verbreitungsgebieten. Die Etablierung von kosteneffektiven DNA-Sequenziermethoden erlaubt hierbei die umfassende Analyse genomweiter genetischer Marker von einer großen Anzahl von Individuen; auch wenn ausschließlich Proben von geringer DNA-Qualität oder -Quantität zur Verfügung stehen. Eine der Anwendungsmöglichkeiten dieser modernen genomischen Methoden besteht in der Untersuchung der Auswirkungen von Wiederansiedlung auf den genetischen Status verdrängter oder ausgestorbenen Arten. Dies spielt eine wichtige Rolle bei der Bewertung von entsprechenden Projekten, da bisher nicht detailliert bekannt war, inwiefern sich die genetische Vielfalt der eingeführten Gründertiere sich auf die Etablierung und Persistenz der Spezies auf Populations- und Metapopulationsebene auswirkt. Die Beantwortung dieser Fragen ist von grundlegender Bedeutung für die Entwicklung evidenzbasierter Management-Strategien der betroffenen Wildtierarten. Beispielhaft für die Implikationen von Wiederansiedlungsprojekten sind die Wiederkehr des Eurasischen Luchs (*Lynx lynx*) und der Europäischen Wildkatze (*Felis silvestris*). Bei beiden Arten handelt es sich um heimlich lebende Beutegreifer, die in Eurasien beheimatet sind und Gegenstand mehrerer Wiederansiedlungsversuche in ihrem ursprünglichen Verbreitungsgebiet waren. Im 19. und 20. Jahrhundert wurde der Eurasische Luchs in West- und Mitteleuropa aufgrund zunehmender Lebensraumzerschneidung und Verfolgung ausgerottet. Auch die Europäische Wildkatze war der Verfolgung durch den Menschen ausgesetzt und lebte nur noch in wenigen Refugien in West- und Mitteleuropa. Nach der Unterschutzstellung in den 1950er Jahren, begannen in den 1970er und 80er Jahren Wiederansiedlungsprojekte für beide Arten und noch immer sind derzeit mehrere Projekte im Gange oder in Planung. Trotz dieser Fokussierung auf den Schutz der Feliden wurde bisher wenig in Betracht gezogen welche Auswirkungen diese Wiederansiedlungen auf die genetische Zusammensetzung der angesiedelten Populationen haben und ob die Populationen aus genetischer Sicht langfristige Überlebensfähigkeit haben. Da sowohl für den Luchs als auch die Wildkatze bisher noch keine umfassenden genomischen Untersuchungen hierzu durchgeführt wurden, bestehen weiterhin Forschungsbedarf bezüglich der genetischen Konsequenzen und Signaturen der bereits durchgeführten Wiederansiedlungsmaßnahmen. In meiner

Dissertation untersuche ich anhand genomischer Daten von Luchs und Wildkatze die jeweilige genetische Demographie, Populationsstruktur, Diversität und Inzucht auf Populations- sowie Metapopulationsebene. In der Einleitung gebe ich Hintergrundinformationen zur Wiederansiedlung, ihrer Rolle im Naturschutz und den genetischen Folgen für Populationen, insbesondere für Populationen von Apex- und Mesokarnivoren, welches den Kontext für die nachfolgenden Kapitel dieser Dissertation bildet. Zusätzlich werden die wesentlichen Ziele der Arbeit wie folgt erläutert:

1. Wie können genetische Methoden am besten eingesetzt werden, um den Erfolg der Wiederansiedlung von Feliden zu überwachen?

2. Wie groß ist der Verlust an genetischer Vielfalt und Inzucht bei der Wiederansiedlung von Feliden im Vergleich zu natürlichen Populationen? Setzt sich dieser Verlust im Laufe der Zeit fort oder findet er hauptsächlich in der frühen Phase der Wiederansiedlung statt und ist auf einen Gründereffekt zurückzuführen?

3. Welche Faktoren tragen zu den beobachteten Mustern im Verlust der genetischen Vielfalt und Inzucht bei?

4. Wie können wir die Ergebnisse der genetischen Bewertung in Schutzmaßnahmen und Überwachung umsetzen, um die Lücke zwischen Forschung und Umsetzung zu schließen und den Erfolg zukünftiger Wiederansiedlungen von Feliden in Europa und anderswo zu verbessern?

In dieser Arbeit wollte ich herausfinden, ob genetische Methoden verwendet werden können, um das Monitoring von wiederangesiedelten Populationen nach der Auswilderung zu erweitern, insbesondere mehrere Jahre oder Jahrzehnte nach der Wiederansiedlung. Genetische Methoden wie mtDNA, Mikrosatelliten, SNP-Genotypisierung und GBS-Sequenzierung können verwendet werden, um die genetische Vielfalt, potenzielle Inzucht und die Strukturierung der Population zu untersuchen (Schwartz et al. 2007). Ich werde zunächst die in jeder Publikation verwendeten Techniken und ihren Beitrag zu unserem Verständnis des genetischen Monitorings von Wiederansiedlungspopulationen erläutern und Methoden für das Monitoring von Wiederansiedlungspopulationen von Feliden diskutieren, einschließlich der Frage, wie man diese Methoden am besten anwendet.

In Publikation I verwendeten wir eine Kombination aus Mikrosatelliten- und SNP-Genotypisierung zusammen mit mtDNA-Haplotypen auf einer kleinräumigen Skala, um die populationsgenetische Strukturierung innerhalb einer Wildkatzenpopulation in einer Mittelgebirgsregion in Deutschland zu beschreiben. Wir fanden Hinweise auf ein rezentes demographisches Wachstum, das eine kontinuierliche Population darstellt. Die Analyse der genetischen Diversität und der Strukturierung der Population zeigte keine signifikanten Unterschiede zwischen Individuen, die aus natürlichen und wiederangesiedelten Regionen stammen. Die mtDNA-Haplotypen zeigten jedoch, dass die genetischen Spuren der vergangenen Wiederansiedlung, bestehend aus ca. 600 Individuen über einen Zeitraum von 24 Jahren, in der Region noch vorhanden waren. Diese wiederangesiedelte Population zeigte Anzeichen für eine Expansion in die umliegenden Regionen und vermischte sich mit einer natürlichen Population am nördlichen Rand ihrer Verbreitung, wie die räumliche Strukturierung aus der sPCA-Analyse zeigte. Wir fanden heraus, dass die Expansion in neue Gebiete durch die Ausbreitung von Männchen vorangetrieben wird, da Weibchen, die einen bestimmten, mit der Wiederansiedlung assoziierten mtDNA-Haplotyp tragen, nur in dem bekannten Wiederansiedlungsgebiet nachgewiesen werden. Auf größerer Ebene unterstreicht dieser Fall den Nutzen des Einsatzes mehrerer genetischer Methoden zur Bestimmung der Persistenz von Wiederansiedlungen, selbst wenn keine Felddaten verfügbar sind. Es war notwendig, eine Kombination von genetischen Methoden zu verwenden. Dies ist wahrscheinlich bei allen Wiederansiedlungsprogrammen der Fall, da jede Methode einen Einblick in einen anderen Bereich gibt, der für das Gesamtbild wichtig ist. Selbst Jahre nach der Wiederansiedlung können genetische Methoden bisher unbekannte Einblicke in den Erfolg einer bestimmten Wiederansiedlung bieten, was auf eine Vielzahl von Wiederansiedlungen anwendbar ist, deren Erfolg unbekannt bleibt (Armstrong und Seddon 2008).

In Publikation II verfolgten wir die Populationsentwicklung und die genetische Diversität im Laufe der Zeit in einer wiederangesiedelten Luchspopulation in Mitteleuropa. Diese Population ist die einzige wiederangesiedelte Luchspopulation, bei der seit der Auswilderung ein Monitoring stattfand. Weshalb sie sich eignet die Auswirkungen der Wiederansiedlung auf die genetische Populationsstruktur der Raubkatzen zu bestimmen. Wir nutzten mtDNA-Haplotypen und Mikrosatelliten-Analysen in Verbindung mit demographischen Monitoring-Methoden, wie Kamerafallen und Telemetrie-Nachweise, um die demographische Geschichte seit der ersten Auswilderung in dieser Population zu rekonstruieren. Wir fanden heraus, dass die Population nach der Wiederansiedlung einen demographischen Flaschenhals durchlief, da wir Nachweise von 7 genetischen Gründerindividuen der 24 freigelassenen Individuen fanden. Darauf folgte eine demographische und räumliche Ausdehnung der Population im darauffolgenden Jahrzehnt.

Diese demografischen Trends wurden durch die genetische Untersuchung widerlegt, die ein erhöhtes Niveau an gemessener und vermuteter Heterozygotie in den Jahren direkt nach der Wiederansiedlung ergab, gefolgt von einem langsamen Rückgang der genetischen Vielfalt im Laufe der Zeit. Wir fanden auch heraus, dass das Wachstum der Population von relativ wenigen, gut etablierten und hoch reproduktiven Individuen abhängt Das deutet daraufhin, dass eine weitere genetische Erosion stattfinden wird, da nur wenige Individuen zum Genpool beitragen.

Mehrere Studien haben nahegelegt, dass Feliden aufgrund ihrer hohen räumlichen Anforderungen und geringen Populationswachstumsraten nur schwer wiederangesiedelt werden können (Noss et al. 1996; Buk et al. 2018; Abascal et al. 2016), aber meine Studie ist eine der wenigen, die eine Populationsbildung in der Praxis zeigt . Hier war das genetische Monitoring unerlässlich, um den Verlust der genetischen Vielfalt zu quantifizieren, Einblicke in die effektive Populationsgröße zu erhalten und die Verteilung des Reproduktionserfolgs zu ermitteln, womit die Populationsmonitoring und die Identifizierung potenziell zuwandernder Tiere unterstützt werden. All diese Faktoren beeinflussen den Erfolg einer Wiederansiedlung. Ohne die genetischen Informationen hätte die Population stabil und wachsend gewirkt, was darauf hindeutet, dass weitere Überwachung und Maßnahmen nicht notwendig gewesen wären. Nur wenn wir die Anzahl der Allele, wie sie auf einer zeitlichen Skala berechnet werden, zusammen mit den Stammbauminformationen einbeziehen, können wir eine Abnahme der genetischen Vielfalt trotz des demografischen Anstiegs tatsächlich erkennen. Darüber hinaus zeigt diese Studie, dass die Population in den Jahren nach der Auswilderung wahrscheinlich Schwankungen in der gesamten genetischen Diversität erfährt und eine konsequente zeitliche Überwachung der beste Weg ist, um Veränderungen zu beobachten und Ergebnisse besser vorhersagen zu können. Daher sind genetische Methoden am hilfreichsten, wenn sie so früh wie möglich in die Planungen der Wiederansiedlung zur Wiederansiedlung integriert werden: von der Gründungspopulation in ein routiniertes Monitoring, insbesondere in den Jahrzehnten nach der Freilassung.

In Publikation III haben wir alle überlebenden wiederangesiedelten Luchspopulationen und 11 natürliche Populationen aus ganz Eurasien beprobt, um den aktuellen genetischen Status von wiederangesiedelten Luchsen vergleichend zu bewerten. Der Vergleich von wiederangesiedelten Populationen mit natürlichen Populationen wurde als wichtiger Parameter für die Bewertung des Wiederansiedlungserfolgs anerkannt, jedoch sind vergleichende Studien nach wie vor schwierig zu realisieren, vor allem aufgrund der Finanzierung und der Ressourcen für Langzeitstudien (Monks et al. 2012). Während bei allen wiedereingeführten Luchspopulationen im Rahmen

nationaler Programme ein routinemäßiges Monitoring durchgeführt wird, verwendet jedes Labor unterschiedliche Methoden, was einen Vergleich erschwert. Daher haben wir alle Populationen beprobt und mit Hilfe der nextRAD-Sequenzierung 13.525 genomweite SNPs für die Untersuchung gewonnen, was einen Vergleich über alle Populationen hinweg ermöglicht. Diese Dichte an SNP-Markern erlaubt eine tiefergehende Analyse, die mit anderen genetischen Techniken nicht möglich ist und kann eine sicherere Betrachtung des aktuellen Populationsstatus liefern, da aktuelle genetische Trends, vor allem die aktuelle Inzucht, von vergangenen Ereignissen getrennt werden können. Wir fanden einen Verlust der genetischen Vielfalt in allen Populationen der wiederangesiedelten Luchse in unterschiedlichem Ausmaß. Wiederangesiedelte Populationen wiesen in einigen Fällen alarmierende Raten aktueller Inzucht auf, wobei die höchsten Werte in den Populationen mit der geringsten Anzahl freigesetzter Gründerindividuen auftraten. Wir fanden auch heraus, dass die Ausgangspopulation von fünf der wiederangesiedelten Populationen genetische Verarmung und hohe Inzuchtraten aufweist, was die Frage aufwirft, ob diese Population eine ausreichende genetische Vielfalt für eine Wiederansiedlung bieten kann. Diese vergleichende Analyse ermöglichte die Ermittlung klarer Referenzwerte hinsichtlich des Niveaus der genetischen Diversität und der Inzucht. Dies kann die Grundlage für eine genaue Zuweisung von Schutzmaßnahmen bei Populationen bilden, die am meisten von den negativen Konsequenzen geringer Populationsgröße und Inzucht gefährdet sind.

In der abschließenden Diskussion gehe ich darauf ein, wie genetische Methoden beim Monitoring von Felidenarten nach der Wiederansiedlung angewendet werden können, um Fragen zur genetischen Zusammensetzung nach der Auswilderung zu beleuchten. Die Methoden, die in diesen Studien und in zukünftigen Arbeiten eingesetzt werden, werden stark von den Forschungsfragen abhängen, die wir beantworten wollen. Zusätzlich untersuche ich, was uns Wiederansiedlungen von zwei schwer erfassbaren Feliden über die Treiber der beobachteten genetischen Muster sagen können, einschließlich Anzahl der Gründerindividuen, Ausgangspopulation, Umweltfaktoren und Populationswachstum. Ich habe herausgefunden, dass die Muster des Verlustes der genetischen Vielfalt nicht klar definiert sind, jedoch können Managementmaßnahmen ergriffen werden, um die negativen Auswirkungen von Wiederansiedlungen zu mildern. Zu diesen Managementmaßnahmen gehören weitere Umsiedlungen, die Einführung einer ausreichenden Anzahl von freigelassenen Individuen und die Lage der Wiederansiedlung in der Nähe natürlicher Populationen. All diese Maßnahmen können die genetische Drift und Inzucht minimieren, zwei Faktoren, die sich negativ auf kleine Populationen auswirken. Letztendlich bieten die in dieser Dissertation entwickelten Mittel eine

solide Basis und Grundlage für zukünftige Arbeiten bezüglich der Folgen von Wiederansiedlungen. Dies ist besonders wichtig, da die Anzahl der Arten schrumpft und in den kommenden Jahren Wiederansiedlungen sowohl der Europäischen Wildkatze als auch des Eurasischen Luchses sowie vieler anderer Arten geplant sind.