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RESEARCH

Low genetic polymorphism and no population genetic structure detected during the natural recolonization of a large carnivore to its previous range

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Abstract

In the face of ongoing habitat loss and fragmentation, examining the genetic effects of range expansion can provide insight into the resilience and adaptability of large carnivore populations returning to parts of their former range. This study investigates the genetic structure of the Eurasian lynx (*Lynx lynx*) population during its natural range expansion into southern Sweden, an area from which it had been extirpated for over a century. We utilized genomic data from 600 individual lynx collected throughout the recolonization period to assess heterozygosity, inbreeding, and genetic differentiation. Our results indicate no significant genetic structure or barriers to gene flow during this recolonization event, despite potential physical barriers such as lakes, farmland, and human infrastructure. Observed and expected heterozygosity, as well as the inbreeding coefficient did not show significant variation over time or across latitude, suggesting that connectivity with the source population was maintained. Spatial principal component analysis, cluster analysis, and discriminant analysis of principal components further supported these findings, showing little spatial or temporal structure. This lack of genetic structure contrasts with the experience of smaller and more isolated lynx populations, which have become inbred. Our study, thus, provides valuable insights into the natural range expansion of a large carnivore in human-dominated landscapes and underscores the importance of ensuring genetic connectivity for successful recolonization and conservation efforts.

Keywords Eurasian lynx · *Lynx lynx* · Recolonization · Range expansion · Population genomics · Individual heterozygosity · Cluster analysis

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Introduction

Across Europe, wide-ranging carnivores are recolonizing areas of their historic range, often passing through heavily human-modified landscapes to do so (Chapron et al. 2014). The ability of these species to expand primarily depends on human population density, human land use patterns, availability of suitable habitat, and legal protection (Cimatti et al. 2021). As population ranges expand, changes to the genetic structure of a population can be negligible if the expansion takes place gradually and if connectivity to the source population is retained. However, if a few founders become cut off or only have limited connectivity to the source population, founder effects and genetic drift can occur, leading to inbreeding and resulting in rapid genetic differentiation due to a shift in allele frequencies (Waits and Storfer 2015; Welles and Dlugosch 2018). This has occurred among large mammals including the Scandinavian wolf (*Canis lupus*),

whose population was founded by two individuals, and after growing to 400–500 individuals, is severely inbred (Åkesson et al. 2022). These effects can even occur on a relatively small geographic scale when significant barriers are present, as in the case of the puma (*Puma concolor*) in southern California, in which the smaller, western population is isolated from the larger eastern population by a barrier of human development, most notably a major highway (Gustafson et al. 2017).

On the Scandinavian Peninsula, the Eurasian lynx (*Lynx lynx*) was hunted nearly to extinction in the early part of the twentieth century (Lönnberg 1930; Curry-Lindahl 1951; Spong and Hellborg 2002; Rueness et al. 2003a). In Sweden, bounties for hunting lynx were removed in 1928 when legal protection was first implemented (Lönnberg 1930; Andrén et al. 2022a). Since then, the lynx population has gone through periods of complete protection and periods in which hunting has been permitted through limited quotas, and has now recovered and recolonized most of Sweden (Andrén et al. 2022b). In winter 2021/2022, the lynx population in Sweden was estimated at between 1200 and 1600 individuals (Odden and Frank 2022). Despite this population recovery, Scandinavian lynx retain low genetic diversity compared to other Eurasian lynx populations due to the historic bottleneck and lack of connectivity with the rest of the continent (Rueness et al. 2003a; Lucena-Perez et al. 2021; Mueller et al. 2022; Bazzicalupo et al. 2022, 2023).

At the time of protection, less than 100 individuals remained in one or two isolated populations in central Sweden (Andrén et al. 2002). As the lynx population recovered, its range expanded southward, and the first documented reproduction in southern Sweden was in 2003 (Hemmingmoore et al. 2020; Andrén et al. 2022b). However, the recolonization of lynx into southern Sweden took place more slowly than that of central Sweden (Andrén et al. 2022b). Between central and southern Sweden, a belt of agricultural land stretches along the narrow land area between Sweden's two largest lakes (Fig. 1). This region is also dominated by human development, with two of Sweden's major highways bisecting the area of central, and all ten of Sweden's most populous cities occurring within the southern third of the country (Fig. 1). Lynx have been shown to avoid agricultural land (Niedziałkowska et al. 2006) and areas of high road density (Kramer-Schadt et al. 2004; Basille et al. 2013; Ripari et al. 2022), and highways can create barriers to lynx dispersal (Zimmermann et al. 2007). Consequently, these potential barriers, both natural and human-made, could potentially limit lynx dispersal between southern and central Sweden, leading to genetic differentiation.

Population genetic structure can develop in different ways during colonization of a new area. At one extreme, the new population is established by two individuals at a single founding event and whose descendants reproduce among themselves, as in the case of the Scandinavian wolf population (Åkesson et al. 2016, 2022). In this case, there should be strong genetic differentiation between the source and the new population resulting from this founder effect, which can persist for generations (Ibrahim et al. 1996). Density blocking can also contribute to population structure in recolonized areas, in which high population densities in established areas can act as a barrier to movement, limiting the spread of alleles from the core population (Waters et al. 2013). At the other extreme, an expanding population can consist of many individuals dispersing long distances in all directions, while maintaining strong connectivity with the source population, which would result in no differentiation (Ibrahim et al. 1996). A population could also expand gradually, in which case we would expect "allele surfing," or random fixation of alleles that are present at the frontier of recolonization, which results in persistent population structure (Excoffier et al. 2009).

Given the various ways in which population structure can develop in an expanding population, it remains unclear whether dispersal barriers between central and southern Sweden influenced the genetic patterns of lynx recolonization. It is unlikely that population growth was limited due to small population size (Andrén et al. 2022b). However, we do not know whether the recolonization was shaped by a gradual southward expansion at the edge of the population in central Sweden, if a few individual lynx traversed the barriers and established separately from the source population, or if lynx continue to disperse long distances in both directions, maintaining panmixia with the source population. The degree to which the southern population may be divergent from the central population depends on the connectivity between the regions and to what extent the relatively small southern population has bred in isolation, especially early in its establishment. Lynx are capable of long-distance dispersal (Samelius et al. 2012), and we know they can traverse the sub-optimal habitat between central and southern Sweden (Hemmingmoore et al. 2020), although we do not know how many do so. By analyzing the genomic structure of the lynx population during the range expansion period, we are able to understand how the recolonization developed. In this study, we use population genomics to investigate how the lynx recolonization in southern Sweden likely occurred. Our three competing hypotheses are as follows:

1. If the lynx population in southern Sweden have been established by a few individuals whose descendants have created a largely separate, southern population, we expect genetic differentiation in terms of allele frequencies between central and southern lynx resulting from a founder effect.
2. If the lynx at the southern periphery of the population have maintained strong connectivity to the source popu-

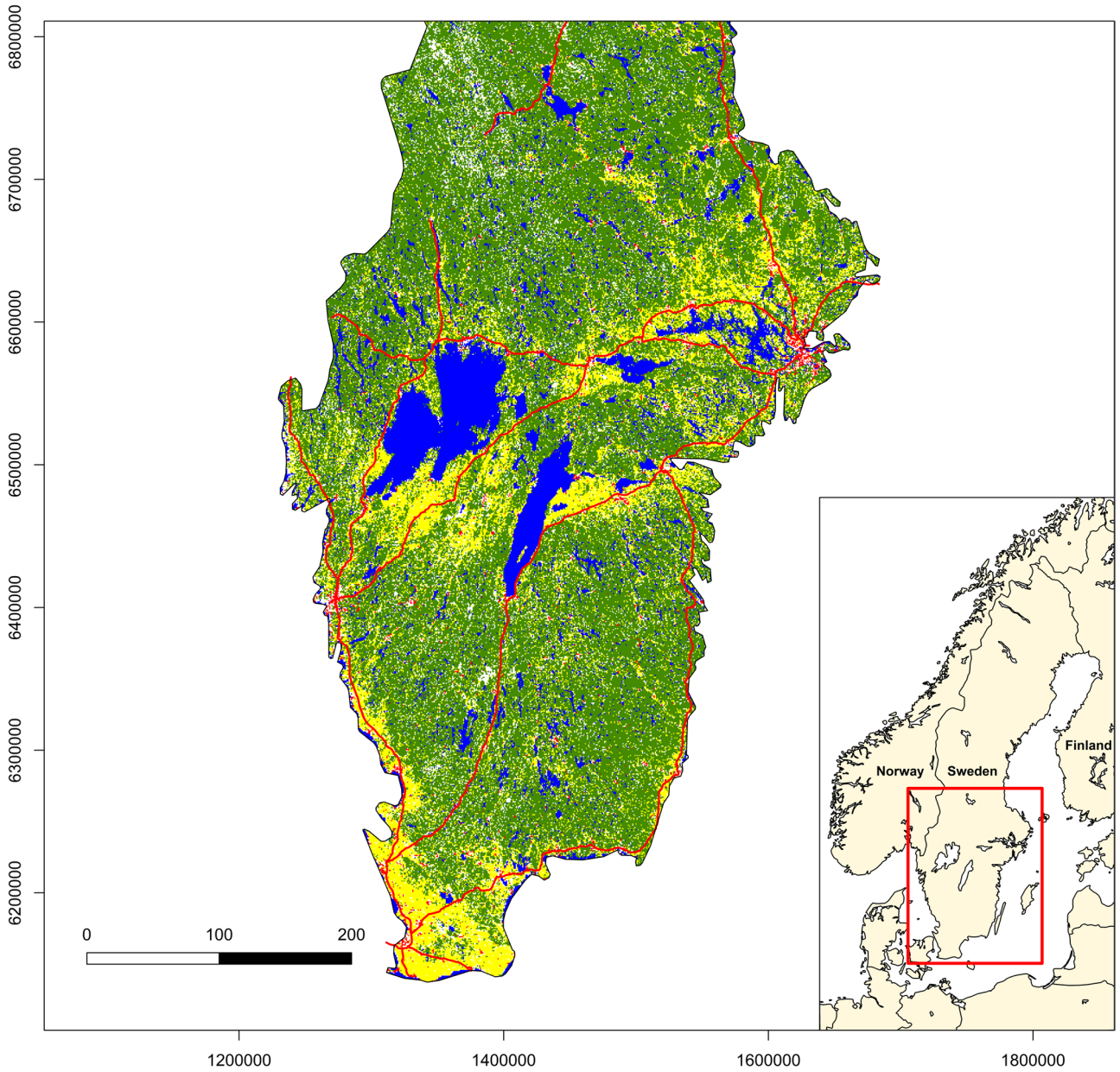

Fig. 1 Entire study area with forest (green), agricultural or grassland (yellow), urban areas and highways (red), and water (blue). Insert shows the location of the study area inside the red box

lation during the southward expansion, we expect that the population to be genetically continuous.

3. Several founding events could have occurred, in which case we expect to see an initial difference in allele frequencies in the first generation, with the newly established population becoming more genetically similar to the source population with time as more founders enter the gene pool.

Materials and methods

Study area

Our study area consists of all of Sweden below the latitude of 6,738,000 in the Swedish National grid RT90 projection, which begins just over 200 km north of Stockholm

and encompasses the southernmost third of the country (Fig. 1). The study area is covered primarily in coniferous forest, the habitat most selected by lynx (Hemmingmoore et al. 2020), and is bisected by a belt of agricultural land. The region above this agricultural belt is referred to as "central Sweden" throughout this paper. The region below this agricultural belt is the southernmost part of the Swedish peninsula, surrounded by water on three sides. This area is referred to as "southern Sweden" throughout this paper.

DNA extraction and sequencing

We obtained tissue samples from the Swedish Veterinary Institute (SVA), which keeps samples and metadata from all lynx that are killed in traffic, legally shot, or found dead of other causes. We used restriction site associated DNA sequencing (RADseq) to sequence thousands of short DNA fragments for the purpose of identifying single nucleotide polymorphisms (SNPs) from 672 individual lynx samples, of which 600 were retained after filtering as described below (Catchen et al. 2013; Andrews et al. 2016; Rochette and Catchen 2017). Of these, samples from all lynx killed in southern Sweden prior to April 2017 (n = 101) were sequenced, with samples from central Sweden selected by the team for sequencing across a spatial and temporal gradient. Of the samples, 330 were male, 261 were female, and nine were of unknown sex. Three hundred five were legally shot, two were illegally shot, 148 were killed by a vehicle, 44 were found dead of disease or predation by another animal, and 101 died of unknown causes.

All lynx DNA was extracted using the phenol chloroform extraction method. Restriction-site associated DNA sequencing (RADseq) is a next generation sequencing (NGS) method that sequences short fragments of DNA around sites where the DNA has been cut with a restriction enzyme (Catchen et al. 2013). Each restriction enzyme cuts the DNA at specific places in the genome, so as long as the same enzyme is used, samples are cut in the same place. The DNA was digested in preparation for RAD sequencing using the Eco-RI restriction enzyme, and results were visualized using electrophoresis to confirm successful digestion. All samples were sequenced at SciLife Lab, National Genomics Infrastructure, Sweden, on Illumina HiSeq 2500 sequencers.

Data preparation and sequencing

Short read sequence data was assembled using the Stacks pipeline software (Catchen Lab, University of Illinois, USA) (Catchen et al. 2013; Rochette and Catchen 2017). Stacks allows for de-novo sequence alignment without the use of a reference genome, in which the sequences are aligned against one another. Stacks was run using the following parameters, m = 3, M = 5, and n = 4, where m is the number of identical reads required to recognize a site as a new putative allele, M is the number of mismatches allowed between alleles of a heterozygous sample, and n is the number of mismatches allowed between alleles within the population.

We filtered our sequence data using the VCFtools software v0.1.16 (Danecek et al. 2011). The unfiltered data set contained 72,092 SNPs. Markers with coverage lower than 15×, a minor allele frequency lower than 5%, and more than 25% missing data were excluded. Individuals with more than 25% missing data were excluded. The data was thinned to retain only sites more than 126 bases away from each other to remove linkage, as the RAD fragments in this analysis are all 126 base pairs long. Markers that were out of Hardy–Weinberg equilibrium with a $p <$ value of 0.005 or below were excluded. The final data set contained 1594 SNPs. We also excluded one sample from each pair that were killed on the same date within two km of each other (n = 7) to prevent a bias toward likely relatives.

Individual heterozygosity

We assessed the spatial and temporal development of genetic diversity within the population by looking at heterozygosity and its implications for inbreeding. Expected heterozygosity (H_E) at the individual level is the probability that two randomly selected alleles from a given locus would be different if the population is in Hardy–Weinberg equilibrium (HWE) (Waits and Storfer 2015). Observed heterozygosity (H_O) is the actual proportion of heterozygous genotypes in the sample. The inbreeding coefficient F_{IS}, calculated as $F_{IS} = (H_E - H_O)/H_E$), is a measure of the deviation of an individual from what would be expected under HWE (Waits and Storfer 2015). An inbreeding coefficient close to zero indicates low levels of inbreeding, and values close to one indicating high levels of inbreeding. Observed heterozygosity, expected heterozygosity, and the inbreeding coefficient were calculated using Plink version 1.9 (Chang et al. 2015). Beta regression models, which are generalized linear models (GLMs) with a beta distribution and logit-link function, were modeled in R (R Core Team 2021) using the package "betareg" (Cribari-Neto and Zeileis 2010) to test spatial and temporal effects on heterozygosity and inbreeding coefficients respectively. A beta distribution is appropriate for modeling response variables whose values are bounded by zero and 1. The range of possible values for heterozygosity are within this range, while those of the inbreeding coefficient are between − 1 and 1. We therefore transformed the value of the inbreeding coefficient using the formula $(F_{IS} + 1)/2$). Explanatory variables were date, latitude, and the interaction between the two. Response variables were observed heterozygosity (H_O) and transformed F_{IS}. Date is the date (in consecutive days) the animal was killed or the

remains were found, from December 28, 1993 to March 3, 2017. Y coordinate in the Swedish National grid RT90 projection and varies between 6,131,905 and 6,810,900, which were mean-centered. The beta regression model consists of two components: the mean (μ) and the precision (φ) of the beta distribution. The mean component is modeled as $\text{logit}(\mu) = \beta_0 + \beta_1 \times \text{date} + \beta_2 \times \text{latitude} + \beta_3 \times (\text{date} \times \text{latitude})$, and the precision component is modeled using the identity link. The precision component (φ) is modeled using the identity link function $\phi = \theta$. Here, θ is the estimated coefficient representing the precision of the beta distribution, indicating the level of dispersion around the mean μ.

Population structure

Population cluster analysis, spatial principal component analysis (sPCA), and discriminant analysis of principal components (DAPC) were conducted using the package "adegenet" (Jombart and Ahmed 2011) in R (R Core Team 2021) in order to assess structure within the population. Cluster analysis uses DAPC to group the samples into the most likely genetic clusters. We chose the number of genetic clusters based on that with the lowest BIC value according to the model. DAPC does not rely on an assumption of Hardy–Weinberg equilibrium or linkage disequilibrium, which makes it especially applicable to an expanding population (Jombart et al. 2010). In a DAPC, a PCA first transforms the data into a set of uncorrelated principal components, then uses these principal components to find linear combinations that maximize between-group variance and minimize within-group variance with the goal of separating genetic clusters. Spatial PCAs are principal component analyses that use spatial information between samples to account for autocorrelation and identify spatially structured genetic variation. It first calculates a covariance matrix based on the genomic and spatial information of each individual, then identifies the principal components of this variation. We used the K nearest neighbor connection network with 4 neighbors and 10 axes each of positive and negative spatial autocorrelation.

We divided the results of all three analyses into the three time periods representing pre-colonization (1993–2003), the colonization period (2004–2010), and the establishment period (2011–2017). This was done so the structure would be comparable over time, to be able to identify persistent or changing population structure. We tested the fixation index (F_{ST}) value, which measures differences in allele frequencies between populations (Holsinger and Weir 2009; Waits and Storfer 2015). For this analysis, we tested the differences when the population was divided by study area (n = 2), by time period (n = 3), and by both together (n = 6) using the $-F_{ST}$ function in Plink.

Results

Individual heterozygosity

Observed heterozygosity (H_O) was slightly lower than expected heterozygosity (H_E) through the whole study period and across the whole study area ($p = 0.002$; Welch's two-sample t-test; Fig. 2a and b). Mean H_E was 0.347 ± 0.001 SD and mean H_O was 0.343 ± 0.034 SD. The mean inbreeding coefficient value 0.014 ± 0.097 SD through the whole study period and study area, indicating inbreeding ($p = 0.002$, one-sample t-test; Fig. 2c and d). Although our analysis showed significant p values, the standard deviations in both cases far exceed the difference in means, which means that for any individual sample, the mean of the other group is within its standard deviation. This effect suggests that H_E and H_O effectively overlap, and F_{IS} overlaps 0. Neither heterozygosity nor inbreeding showed any development across the latitudinal gradient or through time. The GLMs confirmed these results, showing no significant effects of date and latitude or interaction date × latitude (all coefficients; $p > 0.5$; Table 1).

Population

Neither the cluster analysis nor spatial PCA revealed distinct genetic groups developing throughout the recolonization period (Fig. 3). The cluster analysis suggested two genetic groups that completely overlapped spatially. The spatial PCA was on a continuous scale and therefore did not divide the samples into distinct groups. As the population spreads southward, both the cluster analysis and sPCA showed a mixed population. Fixation index (F_{ST}) estimates showed low differentiation between spatial and temporal groups. For the area grouping (Central and South), the mean F_{ST} was 0.011. When the populations were divided by time period, the mean Fst estimate was even lower, at 0.003. When populations are divided by both time period and area, the mean F_{ST} estimate is 0.005. The weighted F_{ST} estimates are the same as the mean F_{ST} estimates when rounded to three decimal places (Table 2).

Discussion

This study provided the unique opportunity to track the genomic development of the lynx population as it recolonized an area of its historic range from which it had been extirpated for at least a century. The study period began before the first documented lynx reproduction in southern Sweden, which allowed us to assess the structure of the source population prior to recolonization and how that

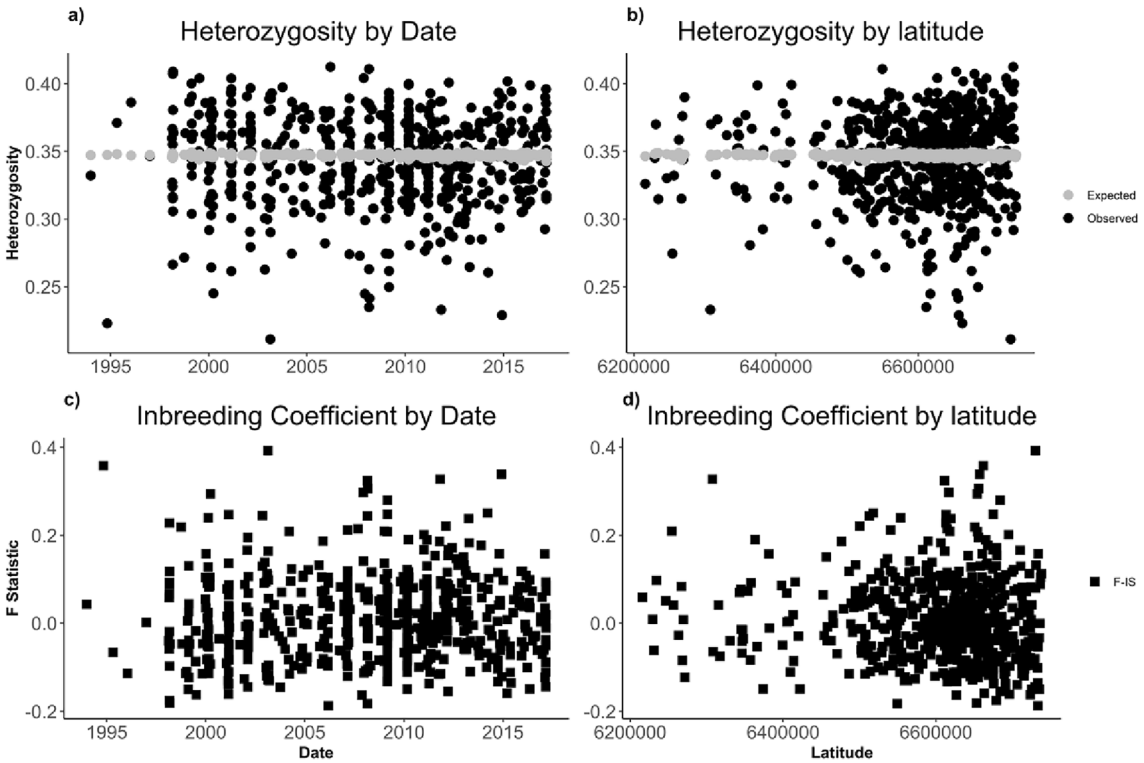

Fig. 2 **A** Observed and expected heterozygosity by individual, plotted by date. **B** Observed and expected heterozygosity plotted by latitude. **C** F_{IS} inbreeding coefficient, plotted by date. **D** F_{IS} inbreeding coefficient, plotted by latitude

Table 1 Parameter estimates for the effects of date (December 28, 1993 to March 3, 2017, day 1 = December 28, 1993), latitude (Y coordinate in the Swedish National grid RT90 projection, 6,131,905 to 6,810,900) and the interaction between them on observed heterozygosity (H_O) and transformed inbreeding coefficient (F_{IS})

Variable	Estimate	Std Error	Z statistic	p-value
Observed heterozygosity, H_O				
Intercept	−6.39e−01	4.68e−02	−13.637	<2e−16
Date	−9.90e−07	3.29e−06	−0.301	0.763
Latitude	2.53e−07	5.21e−07	0.486	0.627
Date × latitude	−1.27e−11	3.44e−11	−0.369	0.712
φ (phi)	208.4	12	17.36	<2e−16
Inbreeding coefficient (F_{IS})				
Intercept	1.188e−02	6.002e−02	0.198	0.843
Date	1.152e−06	4.215e−06	0.273	0.785
Latitude	−4.072e−07	6.668e−07	−0.611	0.541
Date × latitude	2.108e−11	4.403e−11	0.479	0.632
φ (phi)	113.896	6.547	17.4	<2e−16

structure shifted and developed as the population expanded. Although this timeframe is too short to see signatures of evolutionary change, allele frequencies can shift within a single generation if a group is geographically or genetically separated from the source population through founder effects or genetic drift (Waits and Storfer 2015). We found no evidence of structure within the lynx population during its natural range expansion into southern Sweden despite the expectation that decreased land area between the lakes, open expanses of farmland, and highways could have posed barriers to their recolonization. We would expect to see differentiation in a single generation if the recolonizing lynx originated from a small group of dispersers and faced barriers to gene flow between itself and the source population, with no such structure if the recolonization was characterized by a large number of lynx dispersing southward while maintaining connectivity with the rest of the population. Our results clearly show the latter situation, in which the lynx recolonizing southern Sweden retained connectivity to the population in central Sweden.

No change over time or across space

None of our results show any indication of structure in time or space. Although the p-values were significant for individual heterozygosity and inbreeding, the standard deviation in observed heterozygosity overlapped the mean in expected heterozygosity, and the standard deviation in F_{IS}, the inbreeding coefficient, overlapped zero, suggesting little effect. We found no discernible difference between

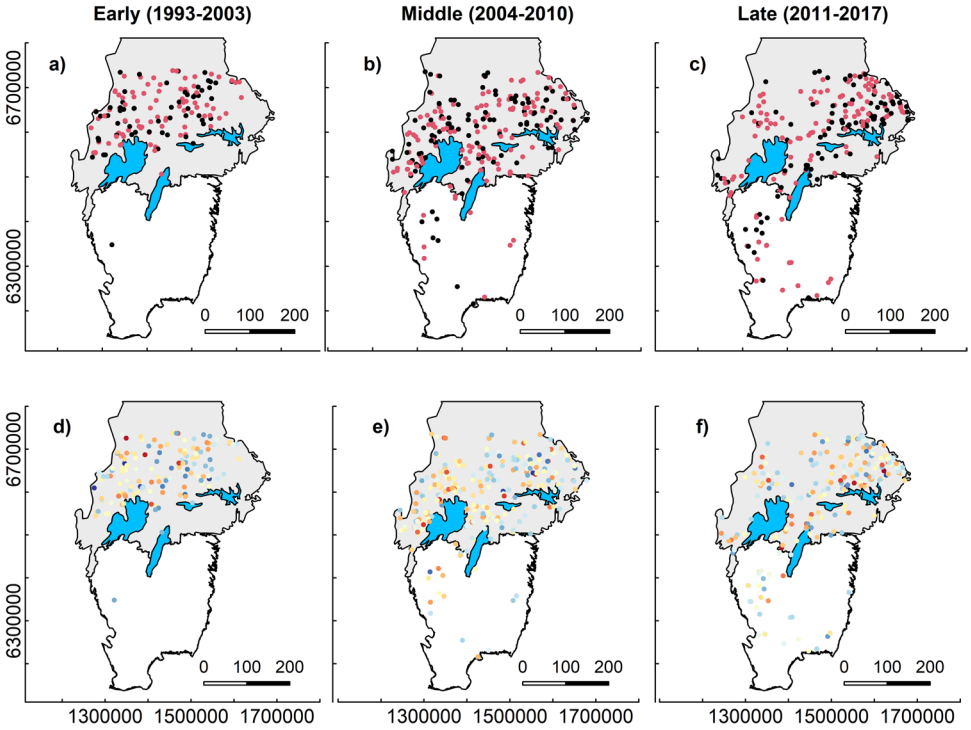

Fig. 3 Spatial distribution of cluster analysis and spatial PCA analysis. **a–c** Cluster analysis results for each of the three time periods. **d–f** Spatial PCA results for each of the three time periods. Central Sweden is grey and southern Sweden is white

Table 2 Mean F_{ST} values between populations

	Population division		
	Area (central/south)	Time period	Time period and area
Number of populations	n = 2	n = 3	n = 6
Mean F_{ST}	0.011	0.003	0.005
Weighted F_{ST}	0.011	0.003	0.005

lynx in central and southern Sweden, as neither date nor latitude affected individual heterozygosity or the inbreeding coefficient. The fact that lynx in southern Sweden did not become more divergent in terms of heterozygosity or level of inbreeding over time suggests that the southern population is not isolated from the source population. In contrast, the lynx of the French Jura mountains, which is isolated from other lynx populations, has experienced a decline in observed heterozygosity and increase in inbreeding over time (Huvier et al. 2023). The Bohemian-Bavarian-Austrian (BBA) lynx population experienced an initial decline in genetic diversity following reintroduction, but has since improved despite isolation (Gajdárová et al. 2023). This contrasts with our results, which show no change as they expanded southward.

At the population level, we also did not identify population structure within our study area and across the recolonization period. The cluster analysis suggested that two distinct clusters best fit the data, with no clear spatial or temporal division between them (Fig. 3a–c). Similarly, the results of our spatial PCA (sPCA) do not demonstrate a clear spatial or temporal pattern (Fig. 3d–f), nor did the DAPC. Previous work has identified cryptic structure within the Scandinavian lynx population. Hellborg et al. (2002) and found results that were consistent with isolation by distance. Rueness et al. (2003a) found an east–west divide that could not be explained by isolation by distance alone. However, our study area falls within a single cluster as identified by each of these two previous studies, so our results are not unexpected in the context of these earlier findings. The fixation index (F_{ST}), which compared the different spatial and temporal groups as if they were separate populations, suggested little differentiation between central and southern Sweden, and across the recolonization time periods. This lack of temporal development, especially between regions, suggests little barrier effect and no isolation.

Scandinavian lynx population

While the lack of change in structure suggests no isolation between the central source population and newly-established southern population, the Scandinavian lynx population as a whole is among the Eurasian lynx populations with the lowest diversity by a variety of measures. The Scandinavian lynx was long thought to contain only a single mitochondrial haplotype (Hellborg et al. 2002; Rueness et al. 2014; Ratkiewicz et al. 2014). Although a second mtDNA haplotype was discovered in this population, its haplotype diversity remained

low at 0.14 (Lucena-Perez et al. 2020). Studies using microsatellite markers identified lower expected heterozygosity in Scandinavian lynx than those in Finland and the Baltics (Hellborg et al. 2002), and lower expected heterozygosity in Norway than in eight other countries, above only lynx in the Białowieża Primeval Forest in Poland (Ratkiewicz et al. 2014). Data derived from whole genome sequencing (WGS) have also shown signs of isolation and inbreeding, with long runs of homozygosity (Mueller et al. 2022) and a low effective population size, Lucena-Perez et al. 2020, 2021; Bazzicalupo et al. 2022). Mueller et al. (2022), using RADseq data, found lower expected and observed heterozygosity than we did in this study, at 0.236 and 0.180 respectively for lynx samples from Norway. Our results show higher heterozygosity and lower inbreeding than those reported by Mueller et al. (2022), as well as lower inbreeding than Bazzicalupo et al. (2022). The long runs of homozygosity observed in Mueller et al. (2022) and the relative lack of inbreeding in our results could partly be explained by methodological differences. Their ROH analysis was based on both WGS and RADseq data, whereas we did not conduct ROH analysis since our study relied solely on RADseq data, which only provides short reads. However, given that Mueller et al. (2022) found lower heterozygosity than we did when only analyzing their RADseq data, demographic factors may also play a role. Mueller et al. (2022) used samples from Norway, whereas our data is from Sweden. Although Norwegian and Swedish lynx belong to the same Scandinavian population, management practices differ between the two countries, with Norway maintaining lynx populations at lower levels than in Sweden through licensed hunting (Andrén et al. 2002). Further analysis of samples from a wider geographic area within Scandinavia would be required to assess the extent to which this variation in management policy contributes to differences within the Scandinavian lynx population, which could inform an interesting follow-up study.

The relatively low diversity of Scandinavian lynx compares to other populations with similar historical bottlenecks (Bazzicalupo et al. 2022), although Scandinavia's geographic isolation from the rest of Europe also contributes to the isolation of the Scandinavian lynx from the rest of the continent (Hellborg et al. 2002; Ratkiewicz et al. 2012, 2014; Rueness et al. 2014). The lynx populations that are less diverse or more inbred than Scandinavian lynx are those that are small, reintroduced, or isolated. Mueller et al. (2022) found Norway to the lowest observed heterozygosity of any natural population, but ahead of five of six reintroduced populations. Bazzicalupo et al. (2022) found Norwegian lynx to be above only the endangered Balkan population according to one measure of autosomal diversity, Watterson's theta (θW). Although Huvier et al. (2023) found higher observed and expected heterozygosity of lynx in the French Jura mountains than we did in central and southern Sweden, their population numbers, heterozygosity, and inbreeding coefficient is in decline due to isolation, whereas our values did not change throughout the course of the recolonization. In addition, microsatellite markers can lead to higher estimates of heterozygosity than SNPs, and the values for the French Jura population, at 0.39 H_E are lower than every population assessed with microsatellite markers by Ratkiewicz et al. (2014), who found lynx in Norway to have a H_E value of 0.50. The Bohemian-Bavarian Austrian population also faced an increase in inbreeding and a decline in observed and expected heterozygosity following reintroduction, which has since partially recovered (Gajdárová et al. 2023). The heterozygosity values of this population remained above 0.4 for the entirety of the 35 year study period, below the values found for the Scandinavian lynx in the same study, which were 0.50 for both H_E and H_O, but higher than our heterozygosity results. Relatively high heterozygosity and low inbreeding have persisted in the BBA population despite isolation, due in large part to its population size. These examples of lynx populations through time suggest that the genetic diversity of the Scandinavian lynx has been maintained at a higher level than many smaller or reintroduced populations, potentially due to its relatively large population size and connectivity within Scandinavia.

Maintaining genetic connectivity during range expansion

When large mammals face geographic barriers in their attempts to recolonize areas of former range, their populations become fragmented and inbred (Waits and Storfer 2015), as in the case of the Scandinavian wolf (Liberg et al. 2005; Åkesson et al. 2016), puma in southern California (Gustafson et al. 2017), and the Florida Panther, another puma population in the United States (Johnson et al. 2010). These effects occur even when the species has a high dispersal capability and does not show signatures of isolation in areas without significant barriers, e.g. pumas in Wyoming (Anderson et al. 2004). Even when a population grows quickly, the genetic signatures of founder effects persist if the population was founded by relatively few individuals (Grossen et al. 2018). However, barriers to population expansion can exist even where there is no obvious geographic obstruction, as in the case of Canadian lynx in northeastern North America (Rueness et al. 2003b). We found no evidence of isolation or founder effects, which suggests that the expanding lynx population in our study area did not face significant barriers to reaching southern Sweden, and continued to mix with the source population. Range expansions can maintain genetic diversity when there is sufficient gene flow between newly established populations and the source population, as high migration rates prevent the strong genetic drift and founder effects that characterize isolated populations

(Waits and Storfer 2015). The lack of genetic structuring as the recolonization progressed southward implies that the population did not experience isolation or founder effects, and was driven by continuous gene flow, which prevented the allele fixation that can result from genetic surfing at the frontier of recolonization (Excoffier et al. 2009; Excoffier and Ray 2008). Additionally, there is no indication of density blocking, or density-dependent dispersal restrictions that could have contributed to genetic structuring, further supporting the idea that movement and mixing were largely unrestricted during the expansion (Excoffier and Ray 2008; Waters et al. 2013).

Lynx avoid sub-optimal habitat such as human settlements and roads (Ripari et al. 2022), but that they are able to cross them in pursuit of more suitable habitat, which is abundant in southern Sweden (Hemmingmoore et al. 2020). Although lynx can pass through human modified landscapes, they can only effectively use areas of human infrastructure when forest is also available (Niedziałkowska et al. 2006; Cimatti et al. 2021; Oeser et al. 2023). Our results suggest that the agricultural belt, large lakes, and human infrastructure that create a natural border between central and southern Sweden do not present barriers to lynx movement, as a large number of lynx must have dispersed through this area, and must continue to do so in order to maintain genetic connectivity between central and southern Sweden. The functional connectivity between central and southern Sweden contrasts with that of many other lynx habitats in Europe, which occur in more fragmented and human dominated landscapes (Cimatti et al. 2021; Iannella et al. 2024). These small and fragmented populations have faced loss of genetic diversity due to small population sizes, poor connectivity, and human persecution (Sindičić et al. 2013; Chapron et al. 2014; Bull et al. 2016; Gajdárová et al. 2022; Port et al. 2021; Bazzicalupo et al. 2022; Huvier et al. 2023). An expanded protected area network is one solution for lynx connectivity in Europe more broadly (Iannella et al. 2024), but it is not necessary in our study area specifically, as the largest protected area in southern Sweden is smaller than a single lynx home range, and lynx successfully pass use human-modified landscapes (Hemmingmoore et al. 2020). Therefore, while lynx in many parts of Europe face genetic isolation due to habitat fragmentation and anthropogenic barriers (Iannella et al. 2024) the Swedish population has expanded without evidence of dispersal barriers. This suggests that maintaining natural connectivity and sufficient habitat outside the protected area network has contributed to the success of the recolonization in Sweden.

Limitation in sampling design

This study follows a naturally occurring range expansion of a wild animal, and therefore our sampling scheme is based on a natural experiment rather than a strict experimental design. We used all the samples collected by SVA from southern Sweden during this period, and 69% of samples from central Sweden for the same period. The sample locations relied upon where lynx died, and were therefore not random. Firstly, 52% of our samples came from lynx that were shot, either as part of a licensed hunt or with special lethal control permits due to conflict with livestock. Licensed hunting of lynx only occurs in areas that have met their population targets as set by management the previous year. During the recolonization period, there were periods in which lynx hunting quotas were granted in parts of central Sweden and years in which they were not. In southern Sweden, lynx hunting permits were not issued until 2015, although lethal control permits could be issued, and therefore fewer lynx were sampled before 2015. This biases selection toward areas in which lynx have already sufficiently recolonized for license hunting to occur, although lethal control can be permitted everywhere. Of the 24% that were killed in traffic, these accidents necessarily occurred in areas with human infrastructure. Even for those lynx that were found dead of natural causes, the fact that they were found at all shows that humans were present in the area in which they were found. Therefore, there is likely an over-representation of samples from areas of human infrastructure and activity. Despite this limitation in sampling pattern, we expect our results to be robust and indicative of the true population structure because as few as 25–50 representative samples per population provides enough information to accurately assess structure (Waples and Gaggiotti 2006), and we used over 600 samples. However, caution should be employed when using opportunistic sampling that does not cover a broad representation of the population, or when extrapolating or comparing results with areas that are less represented.

Conclusion

This study was the first to evaluate Swedish lynx from a genetic or genomic perspective since their range expansion into southern Sweden. Although the recovery of the Scandinavian lynx population began nearly a century ago, the last phase occurred over the course of just a few lynx generations, since the turn of the twenty-first century. Previous studies on this recolonization event showed that lynx are able to disperse between central and southern Sweden (Samelius et al. 2012; Hemmingmoore et al. 2020) and appear not to have faced limitations in mate choice as they progressed into the south (Andrén et al. 2022b). However, we had no view of whether this expansion was driven by relatively few founders that managed to make it across difficult barriers, or if these obstructions posed no barriers at all. This study has demonstrated that lynx are able to maintain

connectivity even across areas of relatively high anthropogenic disturbance and away from protected areas. These results highlight the importance of maintaining functional connectivity in lynx conservation and range expansion, as lynx populations across Europe continue to expand through human dominated landscapes.

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Data availability The data sets generated and analyzed in this paper are available upon reasonable request from the corresponding author. Sequence data that support the findings of this study have been deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) with the Project ID PRJNA1225917.

Declarations

Conflict of interest The authors declare no competing interests.

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