Biological Conservation 248 (2020) 108534

Contents lists available at ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/biocon

Genetic consequences of conservation action: Restoring the arctic fox (*Vulpes lagopus*) population in Scandinavia

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ARTICLE INFO

Keywords:
Genetic variation
Population structure
Connectivity
Dispersal
Metapopulation
Captive breeding and release

ABSTRACT

The Arctic fox (*Vulpes lagopus*) population in Fennoscandia experienced a drastic bottleneck in the late 19th century as a result of high hunting pressure. In the 1990s, despite nearly 70 years of protection, the population showed no signs of recovery. In order to mitigate the population decline and facilitate re-establishment, conservation actions including supplementary feeding and red fox culling were implemented in 1998, followed by the reintroduction of foxes from a captive breeding programme, starting in 2006. A positive demographic impact of these actions is evident from a doubling of the population size over the past decade. We used genetic data collected in eight subpopulations between 2008 and 2015 to address whether the recent demographic recovery has been complemented by changes in genetic variation and connectivity between subpopulations. Our results show that genetic variation within subpopulations has increased considerably during the last decade, while genetic differentiation among subpopulations has decreased. A marked shift in metapopulation dynamics is evident during the study period, suggesting substantially increased migration across the metapopulation. This shift followed the recolonization of an extinct subpopulation through the release of foxes from the captive breeding programme and was synchronized in time with the implementation of supplementary feeding and red fox culling in stepping stone patches between core subpopulations in mid-Scandinavia. Indeed, the increase in genetic variation and connectivity in the Scandinavian arctic fox population suggests that metapopulation dynamics have been restored, which may indicate an increase in the long-term viability of the population.

1. Introduction

Throughout the twentieth century, anthropogenic pressures have caused severe demographic declines and substantial fragmentation of natural populations (Brook et al., 2008; Murphy and Romanuk, 2014). For species occupying fragmented habitats, empirical studies as well as metapopulation- and population genetic theory (Hanski, 1998; Nei et al., 1975; Wright, 1931) emphasize the importance of connectivity for maintaining genetic variation within populations, and preserving the ability of species to rapidly adapt and persist (Stacey and Taper, 1992; Broquet et al., 2010).

Dispersal and genetic drift are the most prominent processes influencing genetic variation in animal populations (Slatkin, 1987; Clobert, 2012). When connectivity within a metapopulation becomes restricted, reduced gene flow and increased subpopulation isolation result in increased vulnerability to genetic drift and inbreeding (Frankham et al., 2002; Baguette et al., 2013). This can result in reduced genetic variation within subpopulations and increased genetic differentiation among subpopulations (Nei et al., 1975; Hanski, 1998). Loss of genetic variation and inbreeding may, in turn, reduce individual fitness, the ability to resist disease, and evolutionary potential, eventually driving species to extinction (Lacy, 1997; Frankham, 2005).

To avoid extinction of small and vulnerable populations, extensive conservation actions such as habitat restoration and translocation of individuals between subpopulations may be required (Che-Castaldo and Neel, 2016). The establishment or maintenance of habitat corridors and "stepping stone" habitat patches may contribute to restoration of landscape connectivity, affecting the target populations demographically as well as genetically (Riordan et al., 2015; Suarez-Rubio et al., 2015). Corridors and stepping stones have been shown to

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https://doi.org/10.1016/j.biocon.2020.108534
Received 27 September 2019; Received in revised form 3 March 2020; Accepted 20 March 2020
Available online 07 June 2020

increase movement rates of terrestrial mammals (Gilbert-Norton et al., 2010; Mech and Hallett, 2001), and to increase gene flow, thereby alleviating genetic threats (Aars and Ims, 1999; Hale et al., 2001). Likewise, reintroduction and translocation of individuals between subpopulations may increase population size and augment gene flow, thus maintaining genetic variation and mitigating the negative consequences of inbreeding (Storfer, 1999; Watson and Watson, 2015). Such strategies were for instance successfully used to re-establish a viable grey wolf population in Yellowstone national park (vonHoldt et al., 2008), and to facilitate the genetic rescue of the Florida panther (Johnson et al., 2010).

The remnant arctic fox (*Vulpes lagopus*) population in Scandinavia exhibits a typical metapopulation structure, consisting of several small and isolated subpopulations (Herfindal et al., 2010). Like many large carnivores in Scandinavia, the population experienced a major demographic and genetic bottleneck in the late 19th century (Nyström et al., 2006) as a result of high hunting pressure associated with a lucrative fur trade (Lönnberg, 1927). Despite protection across Fennoscandia in the early 1900s, the population showed little or no indication of recovery in the 60 years that followed (Hersteinsson et al., 1989).

While former persecution is accepted as the primary cause of the original population decline, other factors may have influenced the population's failure to recover. Invasion of red foxes (*Vulpes vulpes*) into habitat historically dominated by the arctic fox has increased interspecific competition and likely also intraguild predation from red foxes (Tannerfeldt et al., 2002). Dampened rodent cycles recorded in the northern hemisphere (Ims et al., 2008; Cornulier et al., 2013) have also had detrimental effects on the arctic fox, whose reproductive success is tightly linked to rodent abundance (Elmhagen et al., 2000). The vulnerability of the species is further increased by the small and fragmented nature of the remnant subpopulations, which increase their susceptibility to the threats of inbreeding and Allee effects (Loison et al., 2001).

At the end of the 20th century, the entire Scandinavian arctic fox population likely consisted of as few as 40–60 individuals (Angerbjörn et al., 2013), divided into three relatively isolated subpopulations (Dalén et al., 2006). Beginning in 1998, a number of large-scale conservation actions were implemented as part of Sweden and Norway's national action plans for arctic fox recovery. The goal of this intervention was to mitigate threats associated with increased red fox competition and food resource decline. Initially, these actions included red fox culling and supplementary feeding in extant arctic fox populations (Angerbjörn et al., 2013). In 2006, the first pups were released from the Norwegian Arctic Fox Captive Breeding Programme (Landa et al., 2017). In 2011, conservation intervention was further extended into potential stepping stone areas, with the aim of increasing connectivity and stimulating recolonization of previously inhabited arctic fox territories (see supplementary material for more details on the conservation actions).

Several studies have confirmed the positive demographic effect of supplementary feeding and red fox culling in the Scandinavian arctic fox population (e.g. Angerbjörn et al., 2013), and the release of captive-bred foxes has resulted in the recolonization of three historically extinct subpopulations (Landa et al., 2017). In 2015, monitoring revealed a minimum of 127 arctic fox litters born in Sweden and Norway (Ulvund and Wallén, 2018), implying that the population had more than doubled over a 15-year period.

Despite demographic recovery, however, populations may still suffer from reduced genetic variation due to genetic drift and inbreeding that occur at reduced population size. Furthermore, invasive management strategies such as translocation and reintroduction are associated with a number of risks including “contamination” or genetic swamping of unique remnant subpopulations (Bertram and Moltu, 1986; Price, 1989), as well as loss of genetic variation due to small founder group size (Berry, 1986; Maudet et al., 2002). Understanding how conservation actions affect spatial genetic structuring and levels of genetic variation within fragmented populations is thus essential for understanding the effects of conservation on population viability (Allendorf and Luikart, 2009), and for evaluating future conservation priorities.

In order to address this, we analyzed patterns of genetic variation and population differentiation in the fragmented mid-Scandinavian arctic fox population. This population consists of several core populations connected by smaller stepping stone habitat patches. Changes in genetic variation and structure are tightly linked to dispersal and reproductive contribution from migrants. In line with this, we addressed three specific questions: (i) Did genetic variation in core populations increase with the implementation of conservation actions? (ii) To what extent did dispersers from the different core populations contribute to the re-establishment of stepping stone populations? (iii) How was connectivity, dispersal, and genetic structure in the metapopulation influenced by the implementation of conservation actions?

Our study system provides an excellent opportunity to assess the role of dispersal in an increasing population. Given that re-colonization and demographic recovery have likely been facilitated by directed conservation actions (Angerbjörn et al., 2013), we expect reduced environmental resistance to promote increased migration and gene flow, leading to increased genetic variation within subpopulations and reduced genetic differentiation across the landscape.

2. Materials and methods

2.1. Study area and sample collection

Our study system is comprised of eight arctic fox subpopulations in mid-Scandinavia, covering an area of 65.222 km^2 and spanning a distance of 470 km, north to south (Fig. 1a). The subpopulations at Snøhetta, Sylane/Helags, and Børgefjell/Borgafjäll (hereafter named Snøhetta, Helags, and Borga) are referred to as “core” populations as they maintained relatively large and stable populations throughout the study. The remaining five subpopulations represent two “stepping stone” areas (Kjølifjellet and Forollhogna, hereafter named Kjøli, and Hestkjølen, Blåfjellet, and Skjækerfjella, hereafter named Lierne). These stepping stone areas likely play a role in facilitating dispersal between core populations. Many of the stepping stone habitat patches were recolonized during the course of the study, and as such, subpopulation sizes in both stepping stone areas were low throughout the study.

Sampling and genetic analyses were carried out by the Norwegian National Arctic Fox Monitoring Programme and the Swedish Arctic Fox Project. In Norway, hair and faeces samples were collected during winter monitoring of known arctic fox den sites. Between 2008 and 2015, 2620 hair and faecal samples were collected in Norway from: Snøhetta (n = 949), Kjøli (n = 263), Helags (n = 341), Lierne (n = 409), and Borga (n = 658) (Ulvund et al., 2018). In Sweden, tissue samples were collected during ear tagging of arctic fox pups during summer den controls and faecal samples were collected in winter during systematic den surveys. All Swedish samples were processed at Stockholm University, and complete genotypes for n = 290 tissue samples and n = 47 faecal samples were provided to supplement the Norwegian data for subpopulations occurring along the Norwegian/Swedish border: Helags, Lierne, and Borga.

2.2. Molecular analyses

Genomic DNA from faeces, hair, and tissue samples was extracted using the FastDNA SPIN Kit for Soil (MP Biomedicals, Santa Ana, Ca), the Maxwell 16 Tissue DNA Purification kit using the automatic Maxwell 16 instrument (Promega, Fitchburg, Wisconsin), and the DNeasy Blood & Tissue Kit (Qiagen, GmbH, Hilden, Germany), respectively, following the manufacturers protocols. Species determination - arctic fox, red fox, or wolverine (*Gulo gulo*) - was performed for extracted DNA from all faecal and hair samples following the faeces

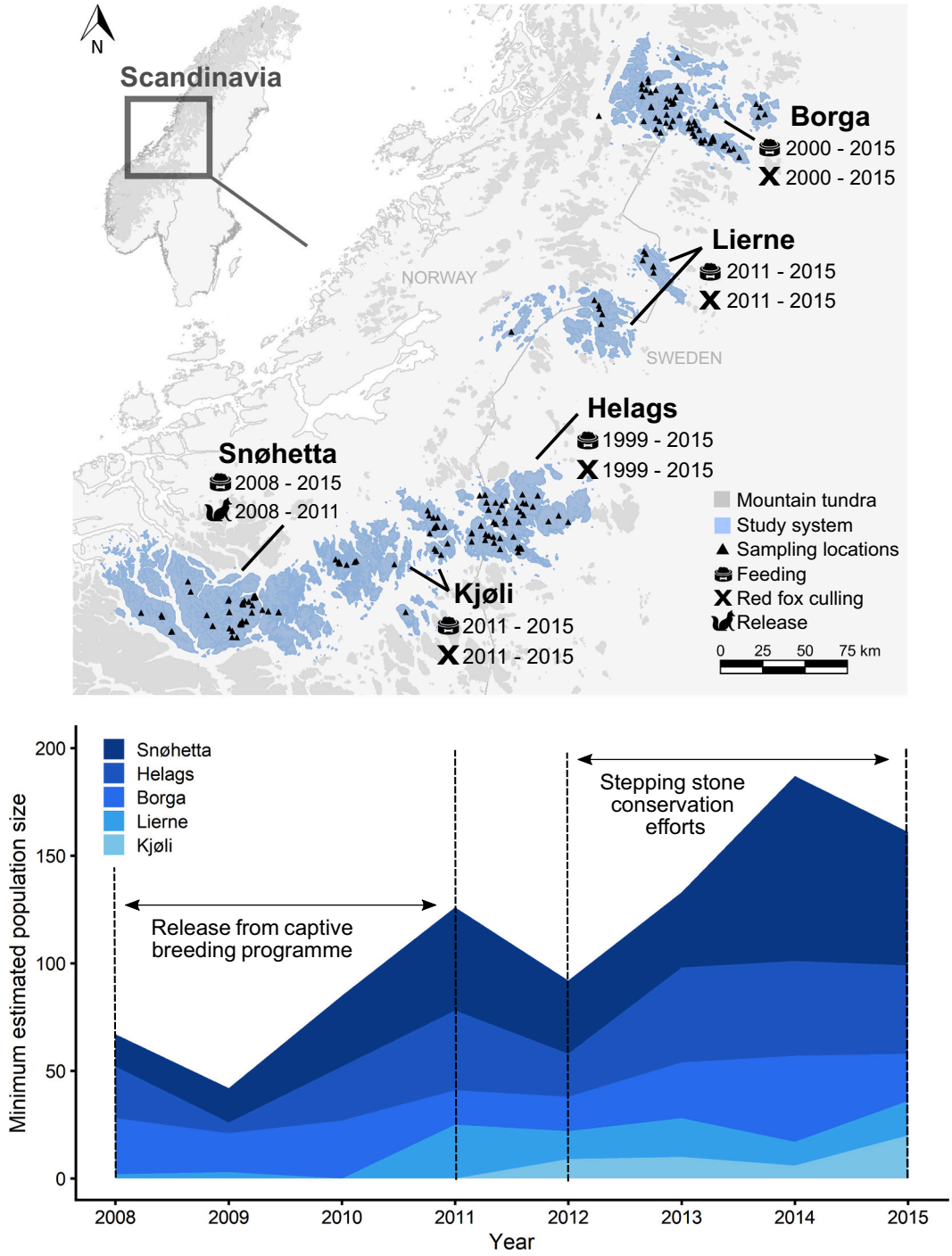

Fig. 1. (a) Map of the mountain tundra region in mid-Scandinavia. Light blue areas show the current distribution of the arctic fox in mid-Scandinavia, representing three core populations (Snøhetta, Helags, and Borga) and two stepping stone areas (Kjøli and Lierne). Black triangles indicate geographical sampling locations where arctic fox samples were collected between 2008 and 2015. The temporal span of conservation actions implemented in each region are summarized on the map. (b) Minimum arctic fox population size estimates in mid-Scandinavia between 2008 and 2015 based on DNA analysis of faeces, hair, and tissue samples, and supplemented with Trovan/Biomark chip recapture data.

identification method described by Dalén et al. (2004). Faecal DNA extraction and polymerase chain reaction (PCR) setup were performed in a work area dedicated for low-copy number DNA extractions in a room separated from the post-PCR laboratory. One negative control well was used in each 96 well PCR plate to monitor for contamination during extraction. See supplementary material for details on the molecular genetic analyses and quality control of consensus genotypes.

2.3. Assembly of the final data set

Of the 2620 samples collected in Norway, 2072 samples were analyzed, 1557 samples were confirmed to be of arctic fox origin, and 945 of these were successfully amplified and genotyped for at least eight of the eleven applied arctic fox microsatellite loci. In order to combine the Swedish and Norwegian data, 30 samples were analyzed at both genetics labs, their genetic profiles compared, and the necessary calibration performed. This calibration was then applied to all Swedish

genotypes used in the study. Following combination of the Norwegian and Swedish data sets, all sample genotypes were matched using the EXCEL MS TOOLKIT 3.1 (Park, 2001) to detect and exclude all duplicate samples within years. After further supplementation with recapture data and data for intermediate years (see supplementary material for details), the final dataset included n = 868 observations of n = 606 unique individuals from eight subpopulations (with a maximum of 5% missing data per locus). Annual minimum subpopulation size estimates for all sampled subpopulations are shown in Fig. 1b.

2.4. Genetic diversity and subpopulation differentiation

Potential deviations from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) between pairs of loci were tested using Markov chain exact tests in GENEPOP 4.2 (Raymond and Rousset, 1995; Rousset, 2008). Probability of Identity (PI) was calculated using GENALEX 6.5 (Peakall and Smouse, 2006, 2012). See supplementary material for details and results of the HWE, LD, and PI tests.

Using GENALEX 6.5, yearly estimates of genetic diversity in each subpopulation were calculated as the average number of alleles per locus (n_A) and the average effective number of alleles per locus (A_E), which is the true diversity of the expected heterozygosity expressed as $1/(1\text{-}H_E)$. Genetic differentiation among subpopulations (F_{ST}; Weir and Cockerham, 1984) was estimated annually at the metapopulation level and for subpopulation pairs (pairwise F_{ST}) using GENALEX 6.5. As F_{ST}, strictly speaking, is a fixation index (Jost and Jost, 2008), Jost's *D* (Jost et al., 2018) was also calculated to estimate genetic differentiation annually at the metapopulation level and for subpopulation pairs (pairwise *D*).

Temporal variation in genetic diversity and differentiation estimates was assessed by comparing two subsets of data including all unique individuals present in two sampling periods: 2008–2009 (n = 90) and 2014–2015 (n = 288). Significant change in genetic diversity and metapopulation level F_{ST} between these periods was assessed using a linear mixed model (LMM) approach with the *lme4* package in R (Bates et al., 2014; R Core Team, 2016). LMMs were constructed separately for each diversity and differentiation parameter. For n_A and A_E, *sampling period* (2008/2009 vs. 2014/2015) and *subpopulation* were included as fixed factors, and *locus* as random factor (to account for interlocus variability as is performed in Soro et al. (2017)). Significant interactions between *sampling period* and *subpopulation* were also tested for all diversity parameters using two-way ANOVAs. For metapopulation level F_{ST} and Jost's *D, sampling period* was included as a fixed factor and *locus* as a random factor. Temporal variation in pairwise F_{ST} and *D* estimates was assessed using a linear model approach and pairwise *t*-tests in R (R Core Team, 2016). As a goodness of fit measure for our models we computed both the conditional and marginal coefficient of determination. This allowed us to quantify the variance accounted for by *sampling period* and/or *subpopulation* alone (marginal R^2, Nakagawa and Schielzeth, 2013) vs. the variance accounted for by marker variability (conditional R^2, Nakagawa and Schielzeth, 2013). See supplementary material for more details on the statistical analyses of genetic diversity and differentiation.

2.5. Population structure and dispersal

Genetic structure was estimated using two approaches. First, to identify genetic clusters in the metapopulation, a Bayesian Markov Chain Monte Carlo (MCMC) approach was employed using the software STRUCTURE 2.3.4 (Pritchard et al., 2000). All unique individuals were run in a single admixture model with correlated allele frequencies. Multi-year survivors were included once, in the first year they were sampled. The captive bred individuals released at Snøhetta were also included in this pooled dataset in order to visualize their contribution to the Snøhetta subpopulation. The predefined number of clusters (*K*) ranged from one to eight to include all potential geographical clusters, and ten independent runs were performed for each predefined number of clusters tested. In order to visualize temporal variation in assignment within subpopulations, samples were ordered chronologically within each subpopulation. As the Evanno method (Evanno et al., 2005) frequently identifies only the uppermost hierarchal structure in a dataset, we opted for the common sense approach, advocated for in the STRUCTURE manual (Pritchard et al., 2003), to determine the most probable and biologically reasonable number of clusters. See supplementary material for more details on the STRUCTURE analyses.

To further investigate and visualize temporal variation in population structure, a principle component analysis (PCA) was performed on the pooled dataset (including the captive bred individuals released at Snøhetta) using the R package *adegenet* (Jombart, 2008). The results were then visualized using a series of biplots highlighting the relevant subpopulations for each year.

In order to estimate the rate of contemporary migration between subpopulations, a Bayesian method based on multilocus genotypes was implemented using the software BAYESASS (Wilson and Rannala, 2003). This approach uses MCMC iterations to estimate the posterior probabilities of the migration matrix among sub-populations (Wilson and Rannala, 2003). Analyses were run on two subsets of the full data set comprised of all unique individuals from 2008/2009, and all unique individuals from 2014/2015, respectively.

3. Results

3.1. Temporal changes in genetic diversity and population differentiation

In the core populations (Snøhetta, Helags, and Borga), the average number of alleles per locus (n_A) showed a significant increase from 4.208 (± 0.248) in 2008/2009 to 5.208 (± 0.255) in 2014/2015 ($t = 4.176$, $p < .01$; Fig. 2a, Table 1). This increase was particularly prominent at Helags, where n_A increased by almost 50% between sampling periods ($t = 4.333$, $p < .01$). While n_A at Helags in 2008/2009 was significantly lower than at both Snøhetta and Borga (vs. Snøhetta: $t = -2.492$, $p < .05$; vs. Borga: $t = -3.045$, $p < .01$), there was no significant difference in mean n_A between these core populations by 2014/2015 ($F_2 = 1.000$, $p = .393$). Although our results show a tendency towards differing rates of change in n_A between core populations, the results of the ANOVA showed no significant interaction between *sampling period* and *subpopulation* ($F_{1,2} = 2.449$, $p = .101$). In our final model, *sampling period* and *subpopulation* accounted for 21.1% of the variance in n_A, whereas marker variability accounted for a total of 63.2%, showing the importance of taking interlocus variability into account when performing LMM (Soro et al., 2017).

In contrast to the results for n_A, the average effective number of alleles per locus (A_E) in the core populations showed no significant change between 2008/2009 and 2014/2015 ($t = 1.310$, $p = .198$; Fig. 2b, Table 1). There was, however, significant population level differences in A_E, with Snøhetta showing consistently higher A_E compared to both Borga and Helags throughout the study (vs. Borga: $t = 2.641$, $p < .05$; vs. Helags: $t = 3.012$, $p < .01$). In our final model, *subpopulation* accounted for 11.6% of the variance in A_E, while marker variability accounted for 49.6%.

At the metapopulation level, genetic differentiation (F_{ST}) decreased by 35% over the study period, from 0.137 (± 0.019) in 2008/2009 to 0.089 (± 0.014) in 2014/2015 ($t = -4.176$, $p < .01$; Fig. 3a). In accordance with this, average pairwise F_{ST} between the core populations showed a gradual but non-significant decrease over time from 0.076 (± 0.021) in 2008/2009 to 0.050 (± 0.013) in 2014/2015 ($t = -1.039$, $p = .357$; Fig. 3b). This decrease was most apparent for pairwise F_{ST} between Helags-Snøhetta ($t = -1.952$, $p = .146$) and Borga-Helags ($t = -2.533$, $p = .085$). Pairwise F_{ST} between Snøhetta and Borga remained relatively stable and consistently lower throughout the study period, as is expected given the genetic background of the Snøhetta population (Fig. S3). The Jost's *D* estimates of population

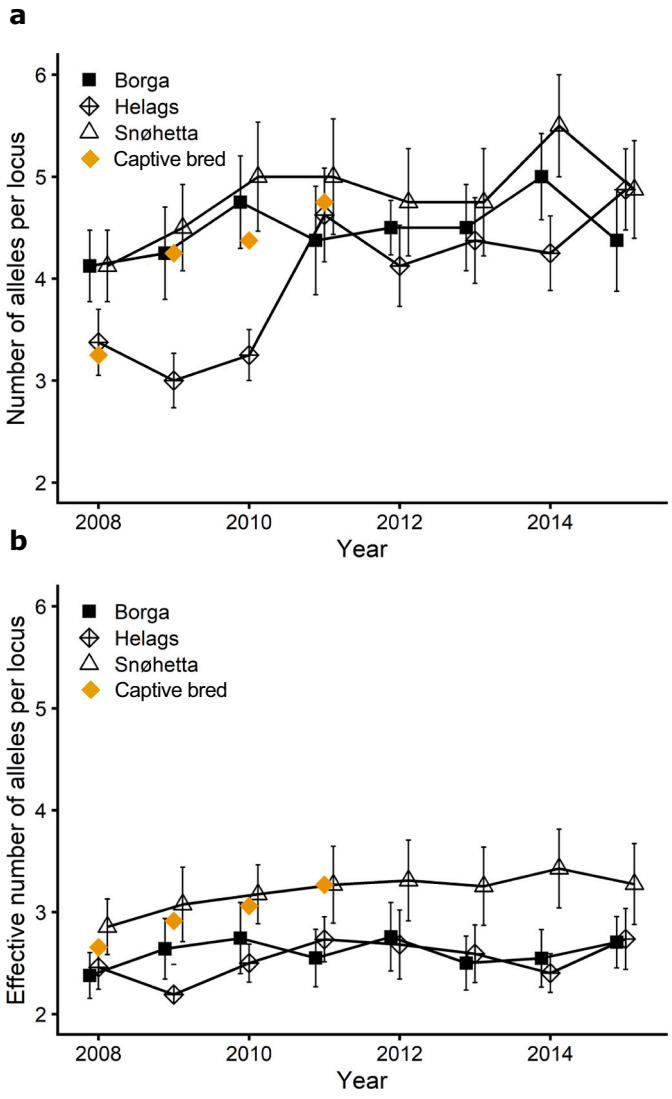

Fig. 2. Annual changes in (a) average number of alleles per locus (n_A) and (b) average effective number of alleles per locus (A_E) in core arctic fox subpopulations in mid-Scandinavia between 2008 and 2015. Error bars represent ±1 standard error of the estimates. Orange diamonds indicate average genetic diversity of captive bred individuals released at Snøhetta between 2008 and 2011.

differentiation (Fig. S2) showed the same pattern as the F_{ST} estimates (Fig. 3), both at the metapopulation level and for the pairwise comparisons between core populations.

In the stepping stone populations, the number of alleles per locus and the effective number of alleles per locus varied greatly over the course of the study, with the only visible increase occurring at Lierne (Fig. S1). Average pairwise F_{ST} between stepping stone populations and their adjacent core populations decreased over time at Lierne but not at Kjøli (Fig. 3c, d). Although these trends were difficult to interpret as the stepping stone populations were virtually extinct at the start of the study period, the considerable variation after re-establishment seems to reflect the significant impact of genetic drift, emigration, and immigration on diversity and differentiation in small stepping stone populations.

3.2. Population structure and migration

Based on the L(K) graphs visualizing the log likelihood of our data under different predefined values of K (Fig. S4), we concluded that the most appropriate number of genetic clusters describing the mid-Scandinavian arctic fox population between 2008 and 2015 was $K = 3$. The populations at Helags and Borga formed two distinct clusters, while the population at Snøhetta and the captive bred individual showed partial assignment to both the Borga lineage and a third unique lineage (Fig. 4). This third lineage likely represents genetic variation that is more common in arctic fox subpopulations further to the north, outside our study area, where some of the founders of the breeding programme originated (Fig. S3).

At the start of the study period, all foxes at Helags belong to one lineage (dark color), whereas foxes at Borga show a distinctly different genetic signature (intermediate color) (Fig. 5). Near the end of the release phase (2008 - 2011), we see the addition of a third lineage (light color; putative northern lineage) which is prevalent among the captive bred foxes. As expected, the re-established subpopulation at Snøhetta shows an almost identical genetic signature to that of the captive bred individuals and their released offspring, exhibiting a mix of the light-colored lineage, and the typical Borga lineage (intermediate color). After the release phase, there is an increasing mixture of lineages in all three core populations. This is particularly apparent in the south where we see an increase in the prevalence of the typical Helags lineage at Snøhetta and an increase in the prevalence of the other two lineages at Helags.

Patterns of assignment in the stepping stone populations were also of interest, as they provided insight into the origin of founders and migrants to these subpopulations. Throughout the study, the populations at Lierne showed an almost identical genetic signature to that of the Borga population. In contrast, the populations at Kjøli showed relatively high temporal variation in assignment in the initial years after recolonization, but seemed to assign more consistently to the Helags cluster by 2014. The STRUCTURE analyses show a gradual increase in assignment heterogeneity over time, both at the individual level (Fig. 4; Table 2) as well as at the population level (Fig. 5). The PCA results corroborate the main findings from the STRUCTURE analysis (Fig. 6). In 2008, prior to the release of captive bred foxes, the PCA shows two distinct genetic clusters, representing two apparently isolated

Table 1
Mean (± 1 standard error) number of alleles per locus (n_A), and effective number of alleles per locus (A_E) estimated in three core arctic fox subpopulations in mid-Scandinavia in 2008/2009 and 2014/2015. The mean difference (± 1 standard error) between the two sampling periods is also presented for each measure of genetic diversity and subpopulation. Statistically significant differences between time periods are highlighted in bold.

Parameter	Subpopulation	2008/2009		2014/2015		Difference			
		n	Mean ± SE	n	Mean ± SE	Mean ± SE	df	*t*-Value	*p*-Value
n_A	Borga	36	4.750 ± 0.412	56	5.125 ± 0.479	0.375 ± 0.183	7	2.049	0.080
	Snøhetta	20	4.500 ± 0.423	105	5.500 ± 0.500	**1.000 ± 0.267**	7	3.742	**0.007**
	Helags	29	3.375 ± 0.324	83	5.000 ± 0.378	**1.625 ± 0.375**	7	4.333	**0.003**
A_E	Borga	36	2.517 ± 0.241	56	2.688 ± 0.276	0.172 ± 0.239	7	0.718	0.496
	Snøhetta	20	2.974 ± 0.342	105	3.390 ± 0.393	0.415 ± 0.242	7	1.717	0.130
	Helags	29	2.466 ± 0.234	83	2.576 ± 0.225	0.111 ± 0.131	7	0.846	0.425

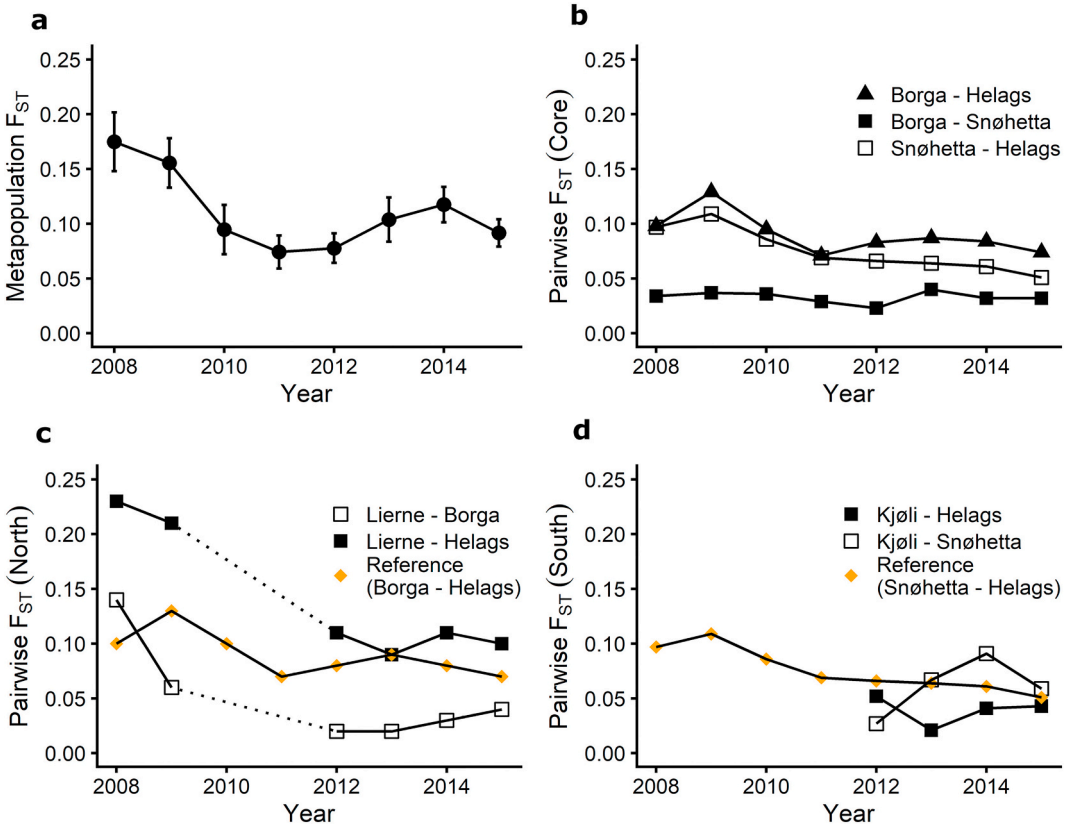

Fig. 3. (a) Annual levels of genetic differentiation (F_{ST}) in the mid-Scandinavian arctic fox metapopulation between 2008 and 2015. Error bars represent ±1 standard error of the estimates. (b) Average pairwise differentiation (pairwise F_{ST}) between core arctic fox subpopulations in mid-Scandinavia between 2008 and 2015. (c) Yearly pairwise F_{ST} estimates between the northern stepping stone populations at Lierne and their surrounding core populations (Borga and Helags), and (d) Yearly pairwise F_{ST} estimates between the southern stepping stone populations at Kjøli and their surrounding core populations (Helags and Snøhetta). Orange diamonds in figures (c) and (d) specify differentiation between the core populations surrounding the northern and southern stepping stone patches, respectively. Dotted lines indicate interpolated values for intermediate years with missing data.

populations at Helags and Borga. During the release phase, most foxes at Helags still show a distinct genetic signature, while foxes from Borga and Snøhetta strongly overlap with the captive bred foxes. This distinct clustering dwindles over time, and by the end of the study period most populations overlap to some degree in genetic space.

Lower genetic differentiation, larger overlap in genetic space, and a higher proportion of admixed individuals strongly suggest increased gene flow across the study area, which is also supported by the BAYESASS analyses (Table 2, S5). At Helags, the immigration rate showed a statistically significant two- to threefold increase during the course of our study. Similarly, at Borga, the Bayesian estimate indicates increased immigration, although this was not statistically significant. Throughout the study period, the immigration rate at Snøhetta was substantially higher than at both Helags and Borga, reflecting the populations origin from multiple sources through the captive breeding programme. High immigration rates were also estimated for the recently re-established stepping stone populations at Lierne and Kjøli. Despite this, Lierne showed a significantly decreased immigration rate over time, which implies that the degree of self-recruitment increased during the short time since re-colonization.

Increased connectivity during the course of our study is also supported by the direct observation of migrants between subpopulations. Eight migrants were identified in our dataset, and all migration events occurred between 2012 and 2015. Three foxes migrated from core populations to adjacent stepping stone patches, another three foxes dispersed from stepping stone patches to adjacent core populations, one fox migrated between stepping stone areas, and a final fox migrated between the two most distantly located core populations, Snøhetta and Borga. These results demonstrate the dispersal capacity of the arctic fox, and emphasize the importance of the stepping stone areas for metapopulation dynamics in our study system.

4. Discussion

Over the past decade, the arctic fox population in mid-Scandinavia has more than doubled in size and several historically occupied habitat patches have been recolonized. In parallel with these demographic changes, and in accordance with our predictions, our results confirm that genetic diversity within subpopulations has increased, while genetic structuring and differentiation between subpopulations have decreased. These genetic responses strongly suggest increased connectivity across the metapopulation, and this was directly supported by the Bayesian assessment of migration rate and admixture. Conservation efforts implemented to support the endangered arctic fox population have likely influenced these changes both directly and indirectly, by reducing environmental resistance, facilitating increased reproduction, and promoting dispersal.

Our study system comprises three core populations with different population histories. Borga, to the north, has had a relatively stable population size over time. In contrast, Helags experienced a dramatic bottleneck in the late 1900s and the current population can be traced back to a handful of founders (Norén et al., 2016). The subpopulation at Snøhetta went extinct in the 1990s and was re-established by released arctic foxes from the Norwegian Captive Breeding Programme (Landa et al., 2017). Furthermore, at the start of the study, there were very few arctic fox subpopulations occupying the stepping stone patches between

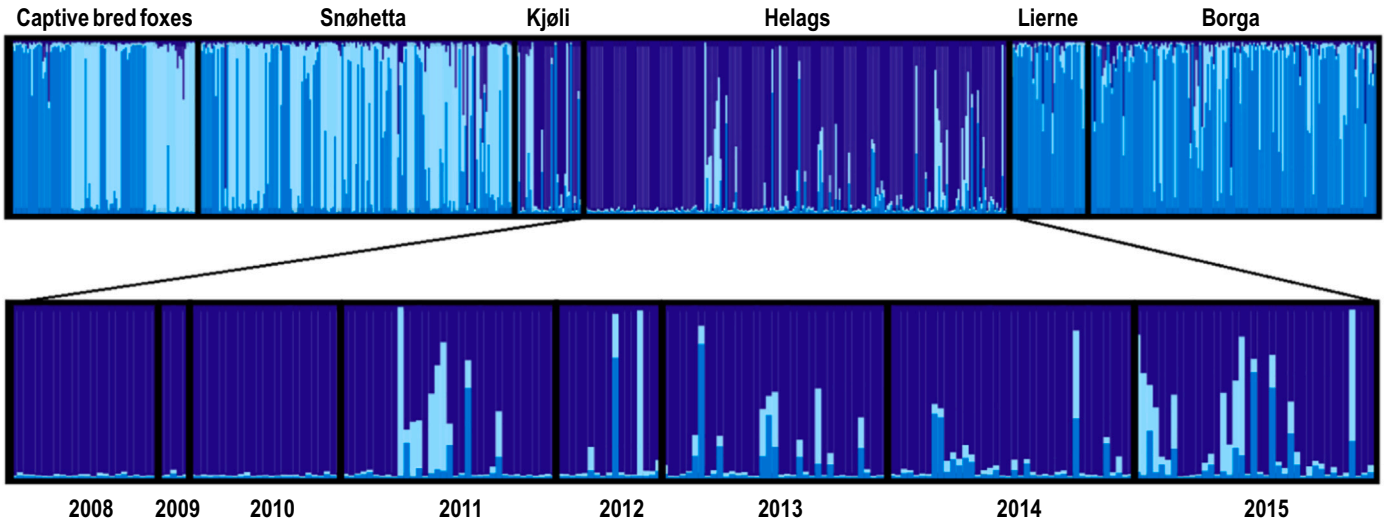

Fig. 4. Assignment of all unique arctic foxes in mid-Scandinavia (including captive bred and released foxes) to $K = 3$ genetic clusters, as inferred by the STRUCTURE analysis. Individuals are ordered temporally within each subpopulation. Cases where individuals and subpopulations show partial assignment to multiple clusters indicate admixture and immigration. The highlighted segment illustrates the increased levels of admixture, here exemplified for the core population at Helags.

Borga, Helags, and Snøhetta. These distinctions are important to bear in mind when interpreting the patterns of genetic diversity and structure.

In fragmented landscapes, species are highly dependent on sufficient connectivity to avoid loss of genetic variability (Spielman and Frankham, 1992; Hanski, 1998; Broquet et al., 2010). In the case of the arctic fox in Scandinavia, increasing fragmentation of remnant subpopulations was accompanied by a 25% loss of genetic diversity during the course of the past 100 years (Nyström et al., 2006). Indeed, before the release of captive bred foxes at Snøhetta and the more recent implementation of conservation efforts in the stepping stone populations, most arctic fox subpopulations in Scandinavia were highly isolated (Dalén et al., 2006). Our estimates of genetic diversity from 2008/2009 at Borga and Helags are comparable with those reported by Dalén et al. (2006) for the same subpopulations and largely the same loci 10 years earlier. This suggests that the increase in genetic diversity observed in our study did not occur immediately following the initial implementation of red fox culling and supplementary feeding between 1998 and 2008 (Fig. 1a). Rather, the increase seems to have occurred as a delayed response to these actions, and/or as a result of restoring the formerly extinct population at Snøhetta through the release of captive bred foxes (2008–2011) and the additional implementation of conservation actions in the stepping stone patches (2011 – 2015).

While genetic diversity showed a general increase in all subpopulations, the observed changes were most pronounced in the severely inbred subpopulation at Helags where average allelic diversity increased by 50% between 2008/2009 and 2014/2015. Multiple studies indicate that outcrossing by just a few individuals into a small and inbred population may replenish genetic variation, thereby preventing inbreeding depression and increasing fitness through genetic rescue (Spielman and Frankham, 1992; Ingvarsson, 2001; Johnson et al., 2010; Åkesson et al., 2016). Norén et al. (2016) showed that the arctic fox subpopulation at Helags originated from only five founders and reported significant inbreeding depression in both reproduction and survival at Helags between 2000 and 2009. The increase in genetic diversity at Helags from 2011 onwards suggests that the arrival and successful reproduction of one or more immigrants in 2010 may have prompted a genetic rescue effect. Indeed, Hasselgren et al. (2018) reported the arrival and successful reproduction of three male arctic foxes at Helags in 2009/2010, all originating from the captive breeding programme; released at Snøhetta in 2009. It appears that these three males and their offspring had a strong genetic impact on this subpopulation, contributing to the substantial increase in average allelic diversity.

Despite a significant increase in the average number of alleles per locus during the study period, no significant change was detected in the average effective number of alleles per locus (Table 1). One explanation for this discrepancy could be that the temporal scale of our study was not long enough to capture changes in this diversity measure, which is an equivalent to expected heterozygosity ($1/1\text{-}H_E$). In comparison, the release of eight captive-bred Florida panther (*Puma concolor couguar*) individuals into a highly inbred population resulted in a substantial increase in heterozygosity over a 10-year period (Johnson et al., 2010). In this case, however, it took more than four years before a detectable response in heterozygosity was observed. It is also worth pointing out that despite small subpopulation sizes, at which high rates of genetic drift and inbreeding are expected (Wright, 1931; Hanski, 1998), no decrease in the effective number of alleles was detected in our target subpopulations. This suggests that although there was no significant increase in the average effective number of alleles per locus, the management actions (and dispersal) at the very least, constributed to maintaining genetic variation within subpopulations.

Throughout the course of the study, the subpopulation at Snøhetta showed a consistently and significantly higher number of alleles compared to the subpopulations at both Helags and Borga. The high levels of genetic variation in the Snøhetta subpopulation may be explained by the diverse background of the captive-bred individuals released at Snøhetta between 2008 and 2011, founded by breeders from the remnant arctic fox populations throughout Scandinavia (Fig. S3; Landa

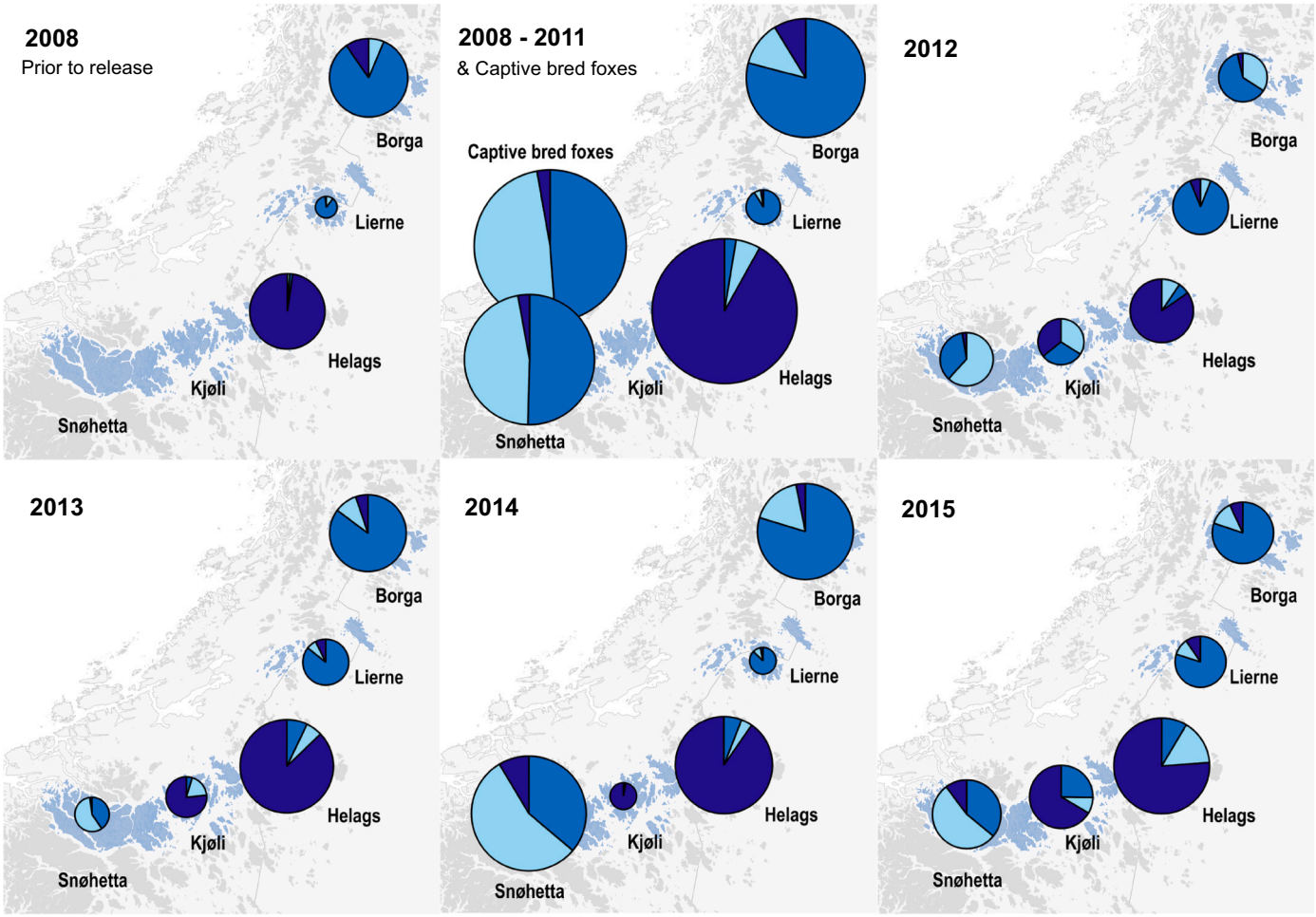

Fig. 5. Maps showing subpopulation level assignment to $K = 3$ genetic clusters, as inferred by STRUCTURE analysis of all unique arctic foxes in mid-Scandinavia (including captive bred and released arctic foxes) between 2008 and 2015. Cases where subpopulations show partial assignment to multiple clusters indicate admixture and immigration. Assignment pies are scaled based on subpopulation size, the largest pies represent n ≥ 50.

et al., 2017). Genetic analysis of two reintroduced populations of rocky mountain wolves with founders originating from multiple source populations showed similar results, with higher expected heterozygosity compared to either of their source populations (Forbes and Boyd, 1997).

Despite the growing popularity of reintroduction and supplementation programs, very few other studies have demonstrated comparable results in terms of both population growth and maintenance of genetic diversity after reintroduction (Wolf et al., 1996; Fischer and Lindenmayer, 2000). On the contrary, several studies examining isolated reintroduced populations have reported significant reductions in genetic variability after reintroduction (Broders et al., 1999; Williams et al., 2000; Hedrick et al., 2001), often as a result of insufficient founder population size (Nei et al., 1975; Berry, 1986;

Table 2
Immigration rates at the start and end of our study period (2008–2015) in three core arctic fox populations and two stepping stone areas as estimated from the Bayesian approach implemented in BAYESASS. For each population/stepping stone area we also estimated the proportion of admixed individuals, i.e. foxes that showed a maximum of 75% ancestry from any single genetic lineage based on the results of the STRUCTURE analyses.

Population	2008/2009			2014/2015		
	Immigration rate	95% CI	Proportion admixed	Immigration rate	95% CI	Proportion admixed
Borga	0.046	0.036–0.055	0.139	0.057	0.044–0.070	0.218
Lierne	0.290	0.271–0.309	0.000	0.149	0.122–0.176	0.143
Helags	0.032	0.023–0.040	0.000	0.067	0.055–0.080	0.167
Kjøli				0.219	0.196–0.241	0.050
Snøhetta	0.285	0.271–0.299	0.050	0.229	0.220–0.238	0.312

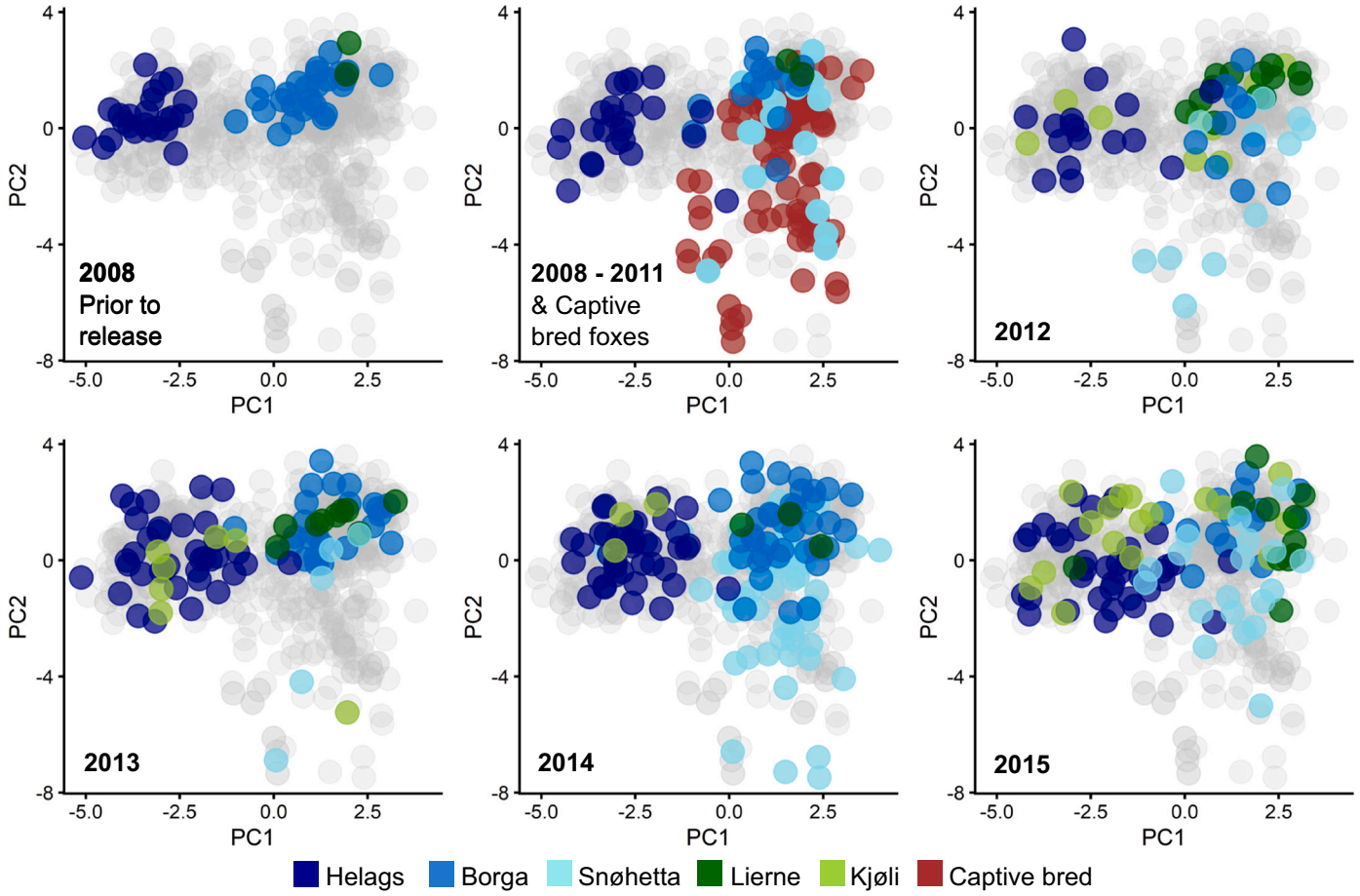

Fig. 6. Principal component analysis showing the genetic clustering of arctic foxes in three core populations (Snøhetta, Helags, and Borga) and two stepping stone areas (Kjøli and Lierne) in mid-Scandinavia from 2008 to 2015. All unique individuals from the entire study period were analyzed together (grey symbols in the background). For each year or period displayed, all individuals detected for the first time in that year are highlighted, and different colours represent the population/area where they were sampled. (For interpretation of the references to color in this figure legend, the reader is referred to the online version of this chapter.)

Maudet et al., 2002). The use of captive breeding in reintroduction programs can also pose a risk, as the captive environment may erode the genetic basis for important morphological, physiological, and behavioral traits via artificial selection (Christie et al., 2012). If this occurs, individuals may exhibit reduced fitness and be unsuited for life in the wild (McPhee, 2004; Araki et al., 2007).

The Norwegian Arctic Fox Captive Breeding Programme took into account both disease risk and genetic background when capturing foxes from the wild remnant subpopulations to establish the breeding pool (Landa et al., 2017). To minimize adaptation to captivity, appropriate replacement protocols were established so that no single founder line would exceed three generations in captivity. Additionally, a substantial number of individuals were released in multiple release events to avoid loss of genetic variation due to founder effects.

In parallel with the documented changes in genetic diversity, a considerable decrease in genetic differentiation between subpopulations was observed, as is expected with increased connectivity and interpopulation dispersal (Slatkin, 1987; Wade and McCauley, 1988; Hale et al., 2001). This decrease was most pronounced (35%) at the metapopulation level. The unproportionally high level of differentiation observed during the first half of the study period is likely the result of founder effects during recolonization of the stepping stone patches (cf. Wade and McCauley, 1988). Over time, genetic differentiation became less pronounced, as new reproducing immigrants continued to supplement growth in the recolonized stepping stone populations, simultaneously contributing to a more similar genetic composition to their neighboring source populations. These results show that the stepping stone patches have the ability to support dispersers, leading to reduced environmental resistance across the metapopulation (cf. Baguette et al., 2013). The BAYESASS analyses also demonstrate an increased degree of self-recruitment in the stepping stone patches at Lierne, suggesting that not only migrants, but also "native stepping stone foxes" contribute to sustaining these highly important intermediate populations.

The profound influence of immigrants on the genetic composition in the stepping stone populations is further highlighted by large interannual variation in assignment. This was particularly the case for the stepping stone populations between Snøhetta and Helags (Kjøli), where the proportion of assignment to potential source populations varied

considerably throughout the study period, but seemed to approach an assignment equilibrium reflecting their geographical proximity to the core populations surrounding them. Contrastingly, assignment patterns for the stepping stone populations at Lierne were quite stable, with a consistent genetic signature similar to that at Borga. This may suggest that there is less environmental resistance, and hence higher connectivity, between Lierne and Borga compared to Lierne and Helags. It may also suggest an established migration route, since the Borga subpopulation has been historically stable, while the Helags subpopulation has been substantially reduced. Interestingly, recolonization of the stepping stone patches seems to correspond in time with the release and subsequent reproduction of captive-bred foxes and the implementation of conservation efforts in the stepping stone areas (Fig. 1ab). The implementation of red fox culling and supplementary feeding in the stepping stone patches may have reduced the risk of dispersal mortality, contributing to increased connectivity and restoration of natural metapopulation dynamics.

Considering the four-year generation time of the arctic fox and the relatively short temporal scale of this study, the documented genetic and demographic responses occurred surprisingly rapidly, confirming increased connectivity within the system. As connectivity is essential for both local and global population persistence in metapopulations (Hanski, 1998; Hanski and Gaggiotti, 2004), these results also suggest an increase in the long-term viability of the Scandinavian arctic fox population. Indeed, for species like the Scandinavian arctic fox, living in fragmented habitats and relying on fluctuating prey resources, dispersal and settlement of immigrants may be the key to population persistence (Loison et al., 2001).

Overall, our results emphasize the role of dispersal as the underlying mechanism influencing changes in genetic variation and differentiation and suggest a connection between the observed genetic changes and the implementation of conservation actions. As dispersal appears to be a determining factor in the maintenance of healthy metapopulation dynamics, it is essential to fully understand the mechanisms influencing dispersal events. Future studies should aim at understanding factors that trigger dispersal and recolonization, as well as factors that hinder movement, increase environmental resistance, and reduce connectivity.

4.1. Implications for conservation management

Our study confirms the genetic success of an intensive conservation programme, implementing a variety of population reinforcing actions, and covering both large core populations and smaller fragments of suitable habitat (stepping stone patches). Indeed, restoration of a formerly abundant core population, intensive conservation actions in two existing core populations, and targeted actions in stepping stone patches led to the restoration of natural metapopulation dynamics in the mid-Scandinavian arctic fox population. These results suggest that conservation efforts may be more successful when focused in neighboring subpopulations at a regional scale, rather than scattered across selected subpopulations at a wider geographic scale.

CRediT authorship contribution statement

Elisa Keeling Hemphill: Conceptualization, Investigation, Writing - original draft. **Øystein Flagstad:** Conceptualization, Investigation, Writing - review & editing, Project administration, Funding acquisition. **Henrik Jensen:** Conceptualization, Writing - review & editing. **Karin Nóren:** Investigation, Writing - review & editing, Project administration, Funding acquisition. **Johan Fredrik Wallén:** Investigation, Writing - review & editing. **Arild Landa:** Investigation, Writing - review & editing, Project administration. **Anders Angerbjörn:** Investigation, Writing - review & editing, Project administration. **Nina E. Eide:** Conceptualization, Investigation, Writing - review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors have no competing interests to declare.

Acknowledgements

We are grateful to all the people who collected samples in the field, and the laboratory technicians who have assisted in genotyping these samples. All fieldwork and handling of animals in Sweden followed Swedish law and was carried out under the auspices of ethics permits, approval from the Swedish board of Agriculture, and the Swedish Environmental Protection Agency (A130-07, A131-07, A36-11, A18-14,A19-14, 412-7884-07 Nv, NV-01959-14). The Arctic Fox Monitoring Programme in Norway (contract 18087019) and the Norwegian Captive Breeding Programme (contract 19087015) contributed a significant amount of data to this study and are funded by the Norwegian Environmental Agency. This study was part of ECOFUNC funded by the Research Council of Norway (grant 244557/E50). Additional funding was provided by EU-LIFE through SEFALO+ (LIFE03 NAT/S/000073), EU/Interreg Sweden-Norway though Felles Fjellrev I and II (N30441-5-10 / 09/10, 304-4159-13, 20200939 IR 2016-01), and WWF Sweden. Stockholm University had funding from the Swedish Research Council FORMAS (no. 2015–1526). The work was also partly supported by the Research Council of Norway through its Centres of Excellence funding scheme (grant 223257).

Role of the funding sources

The sponsors of this study have had no influence on the interpretation of data; in the writing of the manuscript; or in the decision to submit the paper for publication.

Data accessibility

All genetic data applied in this study are available upon request from the authors.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocon.2020.108534.

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