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Population structure in a critically endangered arctic fox population: does genetics matter?

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Abstract

The arctic fox (*Alopex lagopus*) in Scandinavia is classified as critically endangered after having gone through a severe decline in population size in the beginning of the 20th century, from which it has failed to recover despite more than 65 years of protection. Arctic foxes have a high dispersal rate and often disperse over long distances, suggesting that there was probably little population differentiation within Scandinavia prior to the bottleneck. It is, however, possible that the recent decline in population size has led to a decrease in dispersal and an increase in population fragmentation. To examine this, we used 10 microsatellite loci to analyse genetic variation in 150 arctic foxes from Scandinavia and Russia. The results showed that the arctic fox in Scandinavia presently is subdivided into four populations, and that the Kola Peninsula and northwest Russia together form a large fifth population. Current dispersal between the populations seemed to be very low, but genetic variation within them was relatively high. This and the relative F_{ST} values among the populations are consistent with a model of recent fragmentation within Scandinavia. Since the amount of genetic variation is high within the populations, but the populations are small and isolated, demographic stochasticity seems to pose a higher threat to the populations' persistence than inbreeding depression and low genetic variation.

Keywords: *Alopex lagopus*, assignment, gene flow, microsatellites, migration, relatedness

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Introduction

Arctic foxes (*Alopex lagopus*) are small carnivores that inhabit the tundra region throughout the Arctic (Audet *et al*. 2002). The arctic foxes that inhabit continental North America, Eurasia and east Greenland mainly prey on lemmings (*Dicrostonyx* spp. and *Lemmus* spp.), whereas foxes inhabiting other parts of Greenland, Iceland and Svalbard mainly utilize resources from the marine environment (e.g. Tannerfeldt & Angerbjörn 1998). Due to the differences in resource predictability between these two types of environments, arctic foxes have been classified into two ecotypes, 'lemming foxes' and 'coastal foxes' (Braestrup 1941; Tannerfeldt & Angerbjörn 1998).

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The arctic fox is an unusually mobile species that has a high dispersal rate (Shilyaeva 1968) and is capable of long-distance movements of over more than 1000 km (e.g. Eberhardt & Hansson 1978; Garrott & Eberhardt 1987; Strand *et al*. 2000). Indeed, arctic foxes were periodically observed outside the Swedish cities of Stockholm and Gothenburg and along the Finnish south coast in the 19th century (Nyström *et al*. 2006; see also Pulliainen 1965), having dispersed more than 500 km through boreal forest. Such long-distance dispersal movements seem to be particularly common in arctic foxes from the 'lemming' ecotype (Angerbjörn *et al*. 2004; Dalén *et al*. 2005). The long and frequent dispersal movements in the arctic fox have been suggested to be an adaptation to spatial synchrony in lemming fluctuations (Tannerfeldt & Angerbjörn 1998; Angerbjörn *et al*. 2001). On the other hand, dispersal could be positively density-dependent (Murray 1967), which would also generate a higher dispersal in 'lemming'

foxes due to overcrowding after lemming peaks (Bræstrup 1941).

The 'lemming' arctic foxes in Scandinavia (Sweden, Norway and Finnish Lapland) and the Kola Peninsula have previously been considered to belong to a discrete Fennoscandian population. So far, however, there has been no empirical data to support such a classification other than the observation that Fennoscandia constitutes a biogeographical unit for other species (Hallanaro & Pylvänäinen 2001) including the lemming (*Lemmus lemmus*) and that the arctic foxes in this region share a common history over the last 100 years. Until the 20th century, the arctic fox was common in Fennoscandia, but due to intensive hunting in the beginning of the 20th century, the population declined to a few hundred individuals (Lönnberg 1927). Despite being protected by law in Sweden, Norway and Finland in 1928, 1930 and 1940, respectively, the population has failed to recover. The combined population size in these countries has recently been estimated at a maximum of 120 individuals (Elmhagen *et al*. 2004), and the arctic fox is therefore classified as critically endangered (Rassi *et al*. 2001; Gärdenfors 2005). The situation on the Kola Peninsula is uncertain and the population size is unknown.

A range of explanations for the arctic fox's nonrecovery have been proposed, including intraguild interactions with red foxes (*Vulpes vulpes*), changes in habitat, including human disturbance, the loss of larger carnivores and irregularities in lemming fluctuations (Hersteinsson *et al*. 1989; Tannerfeldt *et al*. 1994, 2002). Furthermore, there are concerns that the small population size may have led to genetic drift, inbreeding depression and a decrease in population growth due to the allee effect (Linnell *et al*. 1999; Loison *et al*. 2001). Inbreeding depression has been shown to cause a decrease in litter size and juvenile survival in farmed arctic foxes (Nordrum 1994). Conservation efforts are currently being undertaken in Sweden, Norway and Finland. Within the joint Swedish-Finnish-Norwegian SEFALO+ project, the conservation actions are focused on red fox culling and supplementary feeding, and in Norway, a captive breeding project was initiated in 2000.

To effectively organize conservation actions for endangered populations, it is important to identify population substructure, rates of gene flow among subpopulations and the degree of isolation from other populations (Caughley 1994). However, it can be difficult, especially for endangered carnivores, to obtain sample sizes large enough to accurately describe population structure and to quantify dispersal. In this study, we investigate genetic variation in 10 microsatellite loci using faecal and tissue samples from 150 arctic foxes collected in Fennoscandia and northwest Russia over the last 16 years.

The purpose of the study was to investigate the current distribution and population structure of arctic foxes in Fennoscandia and northwest Russia. More specifically, we attempt to resolve whether the arctic foxes on the Kola Peninsula belong to the same population as the arctic foxes in Scandinavia, since this has consequences for estimating the total population size and thus the regional extinction risk. We also examine whether there is any further substructure within Scandinavia. The high dispersal rates observed for arctic foxes and their capability of long-distance dispersal even through forested areas predict that there should be little genetic differentiation and high levels of gene flow between geographical areas.

Previous genetic studies on arctic foxes have indicated that there is current gene flow from Russia into Scandinavia (Dalén *et al*. 2002, 2005; Nyström *et al*. 2006). Since the mountain tundra in Scandinavia is long and narrow in a north–southerly direction, gene flow from Russia could be expected to generate a gradual decline in genetic variation from north to south. Furthermore, if there is ongoing gene flow into Scandinavia, the use of high-resolution microsatellite markers should allow us to identify immigrants directly.

Materials and methods

Sampling procedure and DNA analysis

Tissue and blood samples ($n = 98$) as well as hair samples ($n = 3$) were collected between 1989 and 2004 from arctic foxes that were either live-caught within the Swedish and Norwegian arctic fox conservation projects, or obtained from animals found dead. Fox faecal samples ($n = 568$) were collected during systematic den surveys covering most of the tundra in Scandinavia and parts of the Kola Peninsula. The faecal samples were used in two ways. First, we used the faeces identification method by Dalén *et al*. (2004) to separate faeces from arctic foxes from those of red foxes and wolverines. The results obtained from this analysis were subsequently used in combination with data on where arctic foxes have been observed to reproduce to identify the current distribution of arctic foxes in Scandinavia. Second, a subset of the arctic fox faecal samples that were well preserved were genotyped for microsatellite variation ($n = 49$). In order to avoid any bias in the sampling procedure, only one individual or faecal sample per den and season was used for the microsatellite genotyping.

Whole genomic DNA was extracted from faecal samples using QIAGEN's stool kit, from muscle using the DNeasy tissue kit (QIAGEN) following the manufacturer's instructions or as described by Taggart *et al*. (1992), and hair samples as described by Gagneux *et al*. (1997). Faecal extraction and polymerase chain reaction (PCR) setup was performed in a work area dedicated for low-copy number DNA extractions, in a room separated from the post-PCR laboratory. Negative controls were used in all extractions to monitor contamination. Microsatellite variation was

analysed for 10 loci: CXX20, CXX140, CXX250, CXX173 (Ostrander *et al*. 1993), CPH3, CPH15, CPH9 (Fredholm & Winterø 1995), 758, 771 and 377 (Ostrander *et al*. 1995). PCR amplification of DNA extracts from tissue and blood samples was carried out following Norén *et al*. (2005). Amplification of DNA extracted from faeces and hair was carried out in 15 μL reactions containing 1.5 μL of DNA extract, 2 or 2.5 mM $MgCl_2$, 0.64 mM dNTPs, 0.16 or 0.2 μM of each primer, 1× PCR buffer and 0.75 U Hotstar *Taq* Polymerase (QIAGEN). The lower concentrations of $MgCl_2$ and primer was used for loci CXX173, 377, CXX140 and CXX250, whereas the higher concentrations were used for loci CXX20, CPH3, CPH15, CPH9, 758 and 771. PCR amplifications for tissue and blood samples were performed using a PTC-100 Programmable Thermal Controller (MJ Research Inc.), a GeneAmp PCR System 9700/9600 (Applied Biosystems) or MJ Research PTC-200 (VWR International) following Norén *et al*. (2005). For the faecal samples, we used the following cycle parameters: 95 °C for 15 min, followed by 40 cycles of 94 °C for 30 s, 54 °C for 30 s and 72 °C for 30 s, followed by 72 °C for 30 min. For locus 771, the annealing temperature was set to 56 °C. The resulting PCR products were separated electrophoretically on a CEQ 8000 automated sequencer (Beckman Coulter) and an ABI PRISM 310 Genetic Analyser (Applied Biosystems). Ten samples were analysed on both sequencers in order to calibrate for differences in PCR product size estimation between the sequencers.

The low quality and small number of DNA copies in faecal and hair samples can cause allelic dropout during PCR. To control for this, each amplification was replicated at least twice. Genotypes that still appeared homozygous after two replicates were replicated a third time. We subsequently used the formulae of Gagneux *et al*. (1997) to estimate the probability of receiving a false homozygote after *n* replicates.

Statistical analysis

We used a Bayesian Markov chain Monte Carlo (MCMC) approach to estimate the number of populations in our study area. The analyses were run using the software STRUCTURE (Pritchard *et al*. 2000) which calculates the likelihood [ln Pr($X\backslash K$)] for a pre-assigned number of genetic clusters (K) in the data set. The K value where the likelihood plateaued was chosen as the number of populations (Pritchard *et al*. 2000). In order to statistically evaluate where the likelihood values plateaued, we followed the approach by Rosenberg *et al*. (2001), running 20 replicates for values of K ranging from 1 to 10 with a burn-in length of 5000 followed by 50 000 iterations using the model of correlated allele frequencies (Falush *et al*. 2003). A Mann–Whitney U test (StatSoft Inc. 1999), corrected for multiple testing using sequential Bonferroni correction (Rice 1989), was used to test where the likelihood values plateaued.

Deviation from Hardy–Weinberg equilibrium and linkage disequilibrium was investigated using the software ARLEQUIN (Schneider *et al*. 2000). Analyses of Hardy–Weinberg equilibrium were computed using a Markov chain with a chain length of 100 000 and 3000 dememorization steps. The test for linkage disequilibrium was performed using 100 initial conditions followed by 1600 permutations. The observed heterozygosity (H_O) and heterozygosity expected under Hardy–Weinberg equilibrium (H_E) were calculated and deviations from this equilibrium were tested using a Markov chain algorithm with 100 000 iterations and 3000 dememorization steps (Schneider *et al*. 2000).

Genetic differentiation among populations, measured as pairwise F_{ST} values, were calculated in ARLEQUIN and were tested for significance using 10 000 permutations (Schneider *et al*. 2000). The number of migrants per generation (Nm) under migration/drift equilibrium was estimated using the equation $Nm \approx (1 - F_{ST})/4\,F_{ST}$ (Wright 1951). The probability of finding two siblings with identical genotypes (PI_{SIBS}) was calculated following Taberlet & Luikart (1999), using the frequencies of observed alleles in each population estimated in STRUCTURE (Pritchard *et al*. 2000).

Simulations using the software EASYPOP (Balloux 2001) were carried out to investigate historical causes of differentiation among populations. We simulated two different scenarios where populations were arranged in a linear stepping-stone model. The first scenario was a transition from a single panmictic population into four completely isolated populations, using five different settings for the number of generations in isolation (Table 5). The second scenario involved four separate populations with constant gene flow among them, again using five different settings for the proportion of migration (Table 5). All simulations were replicated 10 times. The population size for the four populations were set at 50, 40, 20 and 10 individuals assuming 10 loci with an initial maximum variation of 10 alleles each, equal sex ratio, random mating and no mutations. Under the assumption of a linear stepping-stone model, these scenarios generate different predictions for the genetic distances among populations. In a scenario of continuous gene flow, neighbouring populations would be less differentiated than non-neighbouring populations, whereas a complete isolation would cause populations to drift apart randomly. F_{ST} values were computed from the simulated data using the software ARLEQUIN as described above. Correlation analyses, corrected for multiple testing using a Bonferroni correction (Rice 1989), was used to test for significant association between simulated and observed F_{ST} values.

In addition to low gene flow and fragmentation, genetic differentiation among populations can also be caused by local bottlenecks and founder events. Such reductions in

effective population size cause a temporary excess of heterozygote genotypes relative to the number of alleles in the population (Cornuet & Luikart 1996). The software BOTTLENECK (Cornuet & Luikart 1996) was used to detect if there were any signatures of such bottlenecks/founder events. The microsatellite loci were assumed to evolve following a two-phased model of mutation with 10% multistep changes and a Wilcoxon sign rank test was used to determine whether the populations exhibited a significant number of loci with heterozygote excess.

In order to identify dispersers between populations, we used three different approaches. First, we used STRUCTURE to calculate each individual's membership coefficient (Q) to the clusters identified as described above. Second, we used the assignment test incorporated in STRUCTURE, applying the settings prior population information and identification of individuals with mixed origin. This allowed us to calculate the posterior probability that an individual is from each of the K populations (Pritchard *et al*. 2000). Third, we used the method by Rannala & Mountain (1997) implemented in GENECLASS2 (Piry *et al*. 2004). Instead of assigning each individual to the population it was most likely to originate from, we used an exclusion approach where individuals were excluded from populations at the 0.05 level. Genotypes that were excluded from all except one population were either assigned as resident or immigrant, depending on whether they genetically belonged to the population they were sampled in or not.

In addition to investigate dispersal among populations, we also investigated genetic patterns within populations by analysing the association between pairwise relatedness and geographical distance between individuals. This was carried out for a subset of the samples where exact coordinates were available for the samples (n = 103). The analysis was performed using the software SPAGEDI (Hardy & Vekemans 2002) with Queller & Goodnight's (1989) estimator for pairwise relatedness (r). The statistic was calculated using the allele frequencies within each population and was restricted to within-population pairs (Hardy & Vekemans 2002). Significance tests were carried out with a permutation approach (10 000 permutations) incorporated in SPAGEDI.

We used a Spearman rank correlation (StatSoft Inc. 1999) to investigate if there was a decline in the average number of alleles (n_a) and average expected heterozygosity (H_a) from north to south. We also investigated if there was decrease in individual heterozygosity from north to south. To do this, we calculated the geographical distance (ARCGIS DESKTOP version 8.3) between each individual (n = 103) and the nearest tundra on the Kola Peninsula. The geographical distances were subsequently analysed in a linear regression (StatSoft Inc. 1999) against individual heterozygosity and d^2, which is an alternative measure of individual heterozygosity taking into account the difference in repeat numbers among alleles (as in Coulson *et al*. 1998).

Results

Two hundred seventeen of the 568 faecal samples were positively identified as being from arctic foxes (Table 1). These samples originated from seven mountain areas, reflecting the current distribution of arctic foxes in Scandinavia (Fig. 1). This distribution is also supported by the observation that 98% of the litters born during the last five years were found within these mountain areas (Table 1). Forty-nine of the faecal samples, along with 101 tissue, blood and hair samples, were successfully genotyped for all 10 microsatellite loci. The probability of receiving a false homozygote after three replicates in the faecal and hair samples was estimated as 0.007.

In order to investigate the number of populations in our study area, we performed a genetic clustering analysis computed in STRUCTURE. In this analysis, $\ln \Pr(X \backslash K)$ plateaued at five genetic clusters (K = 5). K = 4 was significantly lower than K = 5 (Mann–Whitney U test, $P < 0.0001$), whereas K = 5 was not significantly different from K = 6 or K = 7. Also, the highest mean likelihood value [$\ln \Pr(X \backslash K) = -4247$] was observed when K = 5 (Table 2). These five genetic clusters corresponded to five geographical areas (Fig. 1): Russia, North Scandinavia,

Table 1 The number of faecal samples analysed with the species identification method, how many of these that had arctic fox origin, and the number of litters born are shown for each geographical region for the period 2000–2004. The table also shows the total number of samples genotyped for each region. The first seven regions, comprising the current distribution of arctic foxes in Scandinavia, are shown in Fig. 1 as dark grey areas from north to south

	Hardanger-vidda	Helags	N. Tröndelag Lierne Stekenjokk	Vindelfj. Saltfjellet	Padjelanta Sitas	Rosto Troms	Finnmark	Finland	Outside main areas
Analysed for species ID	22	69	127	137	38	25	68	71	11
From arctic fox	12	33	69	52	13	13	25	0	0
Number of litters	4	12	23	16	2	2	6	0	1
Samples genotyped	11	17	32	43	6	7	8	0	5

Fig. 1 Map of the mountain tundra regions in mainland Europe. Dark grey areas show the current distribution of arctic foxes. Light grey areas illustrate mountain tundra no longer inhabited by arctic foxes. Land areas in white illustrate the distribution of forest. The five arctic fox populations are as follows: Russia ($n = 21$), North Scandinavia ($n = 64$), Central Scandinavia ($n = 32$), South Scandinavia ($n = 17$) and Southwest Scandinavia ($n = 11$) and are encircled by dashed line. Sampling locations in Russia are indicated by stars (sample sizes were 11 for the Kola Peninsula and five for each of the other two locations). The Snøhetta/Dovrefjell region, a possibly extinct population, and the Kola Peninsula are also shown.

Central Scandinavia, South Scandinavia and Southwest Scandinavia. The Snøhetta/Dovrefjell region could not be reliably placed in any of the clusters. One of the individuals had a high proportion of ancestry in the Southwest Scandinavian cluster, whereas the other four had shared ancestry between the Southwest and South Scandinavian clusters.

Average expected heterozygosity (H_a) and average number of alleles (n_a) varied between the populations, but were in both cases highest in Russia and lowest in South Scandinavia (Table 3). The estimated PI_{SIBS} values were low in all populations and ranged from *c.* 1×10^{-4} in Russia to *c.* 1×10^{-3} in South Scandinavia (Table 3). Linkage disequilibrium was observed in 12 of the 200 comparisons made. There was however, no consistency among the populations. All associations were observed only in one of the five subpopulations, except the association between loci CXX173 and 377, which was observed in both South Scandinavia and Southwest Scandinavia. This indicates that the disequilibrium observed is not caused by physical linkage, but rather that it could be the result of close relatives in the sample. All loci were in Hardy–Weinberg equilibrium in all populations, with four exceptions (Table 3). Although we cannot rule out the possibility of null alleles in some of the populations, it should be pointed out that the deviations were only significant at the $0.01 < P < 0.05$ level and could be a consequence of the large number of tests made ($n = 50$).

Table 2 Estimated mean likelihoods of data [ln Pr($X\backslash K$)] and posterior probabilities [$P(K\backslash X)$] for different numbers of genetic clusters (K). The number of genetic clusters corresponds to the number of hypothesized populations in the study area

K	Mean ln Pr($X\backslash K$)	$P(K\backslash X)$
1	−4741	0.000
2	−4490	0.000
3	−4391	0.000
4	−4308	0.000
5	**−4247**	**0.999**
6	−4300	0.000
7	−4259	0.000
8	−4514	0.000
9	−4371	0.000
10	−4622	0.000

Genetic differentiation was moderate among most populations ($F_{ST} = 0.06–0.12$), except for between Southwest Scandinavia and the other Scandinavian populations where the F_{ST} values were between 0.19 and 0.20 (Table 4). Consequently, the estimated number of migrants per generation under migration/drift equilibrium ranged from 0.97 to 4.12 (Table 4). In the assignment tests, the percentage of individuals that could be excluded from all except one population was 97% (STRUCTURE with prior population information), 72% (GENECLASS2) and 44% (STRUCTURE without prior population information). One immigrant genotype from Russia to North Scandinavia was detected in all three analyses. Two additional immigrant genotypes were detected from North Scandinavia to Central Scandinavia (one in both STRUCTURE without prior population information and GENECLASS2, and one only in GENECLASS2). It should be pointed out though, that 45 of the samples came from cubs trapped during the breeding season, who cannot by definition be immigrants. However, the analysis in STRUCTURE, using prior population information, also computes posterior probabilities for individuals having mixed origin, but no such individuals could be detected in our data set (the four individuals that could not be excluded from all except one population had posterior probabilities ranging from 0 to 0.35 of having mixed

Table 3 Microsatellite variation in arctic foxes measured as observed (H_O) and expected (H_E) heterozygosities for each locus and population. H_O values marked with an asterisk (*) indicate deviations from Hardy–Weinberg equilibrium at the $0.01 < P < 0.05$ level. Average expected heterozygosities (H_a) and average number of alleles (N_a) are shown with standard errors (SE). The probabilities of identity among siblings (PI_{SIBS}) are also shown. Populations are abbreviated as follows: Russia (RU), North Scandinavia (NS), Central Scandinavia (CS), South Scandinavia (SS) and Southwest Scandinavia (SW)

	RU (n = 21)		NS (n = 64)		CS (n = 32)		SS (n = 17)		SW (n = 11)	
Locus	H_O	H_E	H_O	H_E	H_O	H_E	H_O	H_E	H_O	H_E
CPH3	0.90	0.92	0.81	0.81	0.90	0.78	0.94	0.69	0.80	0.87
CPH9	0.81	0.76	0.47	0.47	0.75	0.65	0.82	0.68	0.73	0.68
CPH15	0.57	0.56	0.33	0.35	0.81	0.69	0.35	0.36	0.64	0.69
CXX20	0.81	0.86	0.78	0.80	0.84	0.81	0.76	0.75	0.67	0.76
CXX140	0.86	0.76	0.76	0.72	0.68	0.69	0.59	0.47	0.09	0.18
CXX173	0.80	0.73	0.38*	0.51	0.48	0.52	0.76	0.59	0.45	0.63
CXX250	0.86	0.88	0.77	0.70	0.56*	0.65	0.65	0.68	0.82	0.79
377	0.80	0.84	0.48*	0.53	0.39	0.43	0.31	0.34	0.91*	0.77
758	0.90	0.78	0.76	0.77	0.58	0.64	0.82	0.76	0.91	0.77
771	0.95	0.89	0.88	0.84	0.84	0.76	0.71	0.68	0.73	0.69
$H_a \pm$ SE	0.77 ± 0.06		0.62 ± 0.03		0.63 ± 0.04		0.58 ± 0.05		0.61 ± 0.07	
$N_a \pm$ SE	8.3 ± 0.75		6.7 ± 0.47		5.1 ± 0.43		3.8 ± 0.25		4.8 ± 0.36	
PI_{SIBS}	0.00005		0.00041		0.00041		0.00092		0.00051	

Table 4 Genetic differentiation between populations (measured as F_{ST}) above diagonal and estimated number of migrants per generation (N_m) below diagonal. All populations were significantly differentiated ($P < 0.00001$) from each other. Population abbreviations are as in Table 3

	RU	NS	CS	SS	SW
RU	*	0.06	0.08	0.09	0.08
NS	4.12	*	0.06	0.08	0.19
CS	3.07	3.87	*	0.12	0.20
SS	2.52	2.77	1.91	*	0.19
SW	2.83	1.06	0.97	1.04	*

origin). Three of these individuals had Q values split between the population they were sampled in and a neighbouring population. The fourth individual was sampled in Southwest Scandinavia, but had a Q value that was divided between South Scandinavia and Central Scandinavia. Within the populations, there was no support for a decrease in pairwise relatedness with increasing geographical distance among individuals (P two-sided test = 0.86; Fig. 2).

The results from the simulations in EASYPOP, which were carried out to distinguish between possible causes of population differentiation, are summarized in Table 5. Overall, the empirically observed F_{ST} values seemed to be better correlated to the simulated F_{ST} values obtained under a scenario of recent isolation than the F_{ST} values obtained under a scenario of continuous gene flow (Table 5). In the BOTTLENECK analysis, none of the populations displayed any signs of recent reductions in effective population size (one-tailed P values for heterozygote excess ranged from 0.25 to 0.81).

Although average expected heterozygosity (H_a) was higher in Russia than in the Scandinavian populations, there was no significant decline in H_a from north to south (Spearman rank correlation, $P = 0.10$) or between geographical distance from Russia and individual heterozygosity ($P = 0.86$). There was, however, a significant decline from north to south in average number of alleles (Spearman rank correlation, $r^2 = 0.81$; $P = 0.04$; Fig. 3). There was also a significant decline in d^2, a measure that takes into account the size difference between alleles, with geographical distance from Russia (simple regression, $r^2 = 0.07$; $P = 0.004$; Fig. 3).

Discussion

Population structure and dispersal

The arctic fox is not continuously distributed across the Scandinavian mountain tundra. Instead, the data from the number of observed litters in each region and faecal analyses show that the arctic foxes mainly persist in seven mountain tundra areas (Fig. 1). The arctic foxes inhabiting these areas are divided into four genetically distinct populations, where the northernmost, North Scandinavia, is substantially larger than the others and includes four of the seven areas described above (Fig. 1). The fifth population, Russia, encompasses both the Kola Peninsula and northwest Russia. Common to all these populations is that they are situated in areas composed of more or less continuous mountain tundra (and in the case of Russia, the White Sea which is frozen in winter), which are separated from the other populations by forested areas (Fig. 1). A recent

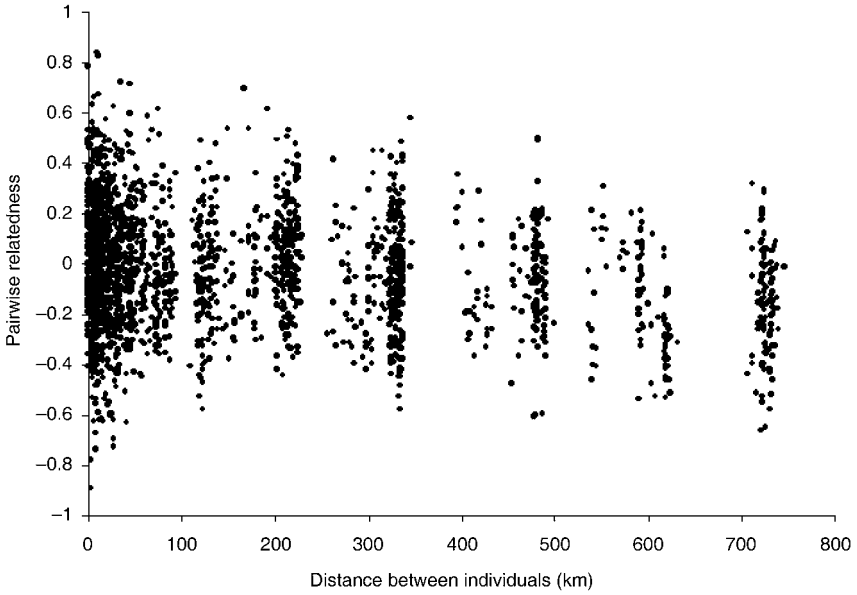

Fig. 2 Pairwise relatedness (r in Queller & Goodnight 1989) and geographical distance between individuals. There was no significant relationship between the two parameters ($P = 0.86$).

Table 5 Results from simulations in EASYPOP, assuming either continuous gene flow or a recent isolation. The expected pairwise F_{ST} values (populations abbreviated as in Table 3) are averages from 10 replicates. Significant correlations (r^2) between simulated and observed F_{ST} values are indicated with an asterisk (*)

		Expected F_{ST} values among populations						
		NS vs. CS	NS vs. SS	NS vs. SW	CS vs. SS	CS vs. SW	SS vs. SW	r^2
Proportion migrants	0.01	0.41	0.53	0.60	0.38	0.54	0.56	0.44
(gene flow model)	0.05	0.10	0.18	0.33	0.12	0.27	0.21	0.65
	0.10	0.04	0.10	0.18	0.06	0.15	0.11	0.67
	0.15	0.02	0.06	0.10	0.05	0.08	0.05	0.54
	0.20	0.02	0.04	0.09	0.02	0.08	0.05	0.60
Generations of isolation	40	0.35	0.49	0.51	0.46	0.51	0.71	0.46
(recent isolation model)	20	0.22	0.30	0.36	0.28	0.36	0.44	0.76
	15	0.15	0.21	0.31	0.26	0.33	0.44	0.77
	10	0.11	0.15	0.21	0.19	0.23	0.31	0.71
	5	0.04	0.09	0.16	0.10	0.17	0.19	0.94*

survey (Elmhagen *et al*. 2004) reported a 'best case' estimate of 120 adults in Scandinavia. Assuming that the number of litters born during the period 2000–2004 (Table 1) reflects differences in population size among the populations, this would mean that there are approximately 10 adult arctic foxes in Southwest Scandinavia, 20 in South Scandinavia, 40 in Central Scandinavia and 50 in North Scandinavia.

Historical records of Scandinavian arctic foxes dispersing more than 500 km through forested areas suggest that the observed subdivision into four Scandinavian populations may be a recent phenomenon. The genetic differentiation among the populations can have several causes, for example local bottlenecks, extinction followed by founder events, relatively low but continuous gene flow or a recent fragmentation. We did not find any evidence for recent bottlenecks or founder events in any of the populations. Differentiating between continuous gene flow and recent fragmentation can be difficult. However, assuming a linear stepping-stone model, the observed F_{ST} values seemed to fit better with a model of isolation than with a model of continuous gene flow. The results also suggest that the observed F_{ST} values among the populations are not consistent with an isolation since the bottleneck in the 1920s. Instead, the isolation seems to have occurred more recently. The hypothesis of a recent fragmentation is also supported by the results from the population assignment tests, which suggested that current dispersal among populations is very low. One possible cause of such a recent fragmentation may be a recent altitudinal expansion of red foxes, reducing the amount of available high-quality arctic fox habitat (Elmhagen *et al*. 2002). Although human activity may have contributed indirectly to the expansion of red foxes, it is unlikely to have had

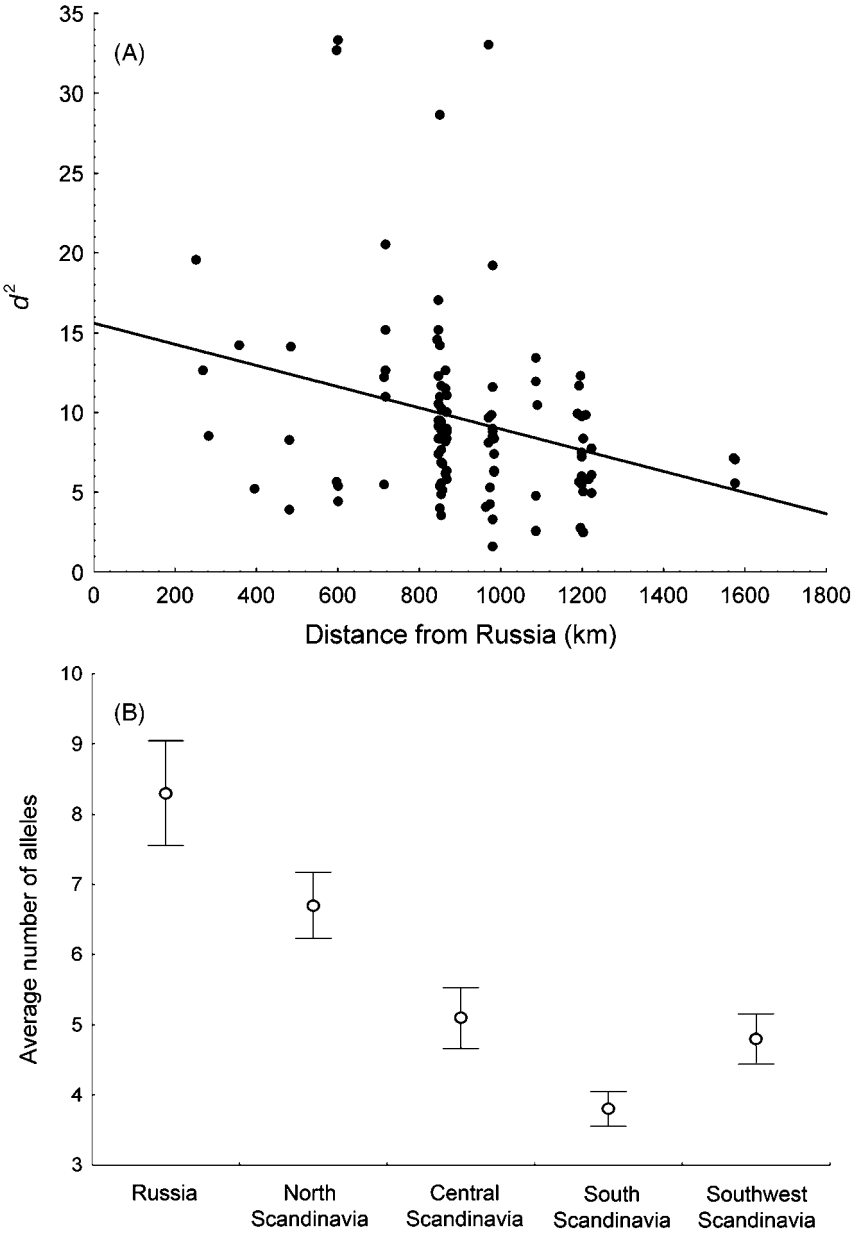

Fig. 3 Patterns of genetic variation from north to south. (A) There was a significant decrease in heterozygosity at the individual level (d^2) with increasing distance from Russia ($P = 0.004$). (B) The average number of alleles decreased significantly ($P = 0.04$) from north to south (bars represent standard error).

any direct effect on the arctic foxes, since the mountain tundra in Scandinavia is more or less uninhabited.

Within the Scandinavian populations, we did not find any relationship between pairwise relatedness and geographical distances among individuals (Fig. 2). Although this analysis may suffer from low statistical power, it does suggest that some individuals disperse for relatively long distances within the populations, despite the fact that the low population density implies a high availability of empty territories within a few kilometres to natal dens.

The observation that dispersal seems to be high within continuous mountain habitats, but low between populations is also supported by the data available from ear tagged foxes in populations North Scandinavia, Central Scandinavia and South Scandinavia. Of a total of 28 tagged cubs resighted as adults, none has dispersed from one population to another. Within the North Scandinavian population, however, movements of more than 400 km have been recorded.

Patterns of genetic variation

Average expected heterozygosity was similar in all Scandinavian populations. Although the heterozygosity in Scandinavia was lower than in Russia, it is still relatively high compared to other canids (e.g. Roy *et al*. 1994; Wandeler *et al*. 2003; Schwartz *et al*. 2005), especially considering the

recent demographic bottleneck and the finding that Scandinavia is subdivided into four populations (Wahlund 1928). In a recent study comparing extant animals with old museum samples, Nyström *et al*. (2006) found that the average expected heterozygosity had not decreased after the bottleneck 100 years ago, and suggested that this was a result of postbottleneck gene flow from Russia.

There was a significant decline in d^2 and average number of alleles from north to south (Fig. 3). A similar gradual decline in mitochondrial DNA variation has also previously been described for the mitochondrial DNA in arctic foxes (Strand *et al*. 1998). The hypothesis of gene flow from Russia into Scandinavia predicted that we would find such a gradual decline in genetic variation from north to south in Scandinavia. However, an equally parsimonious explanation to this pattern is that the population size for each of the populations decreases from north to south. Genetic drift, resulting in a loss of genetic variation, may therefore have had a larger impact in more southerly populations.

An immigrant genotype from Russia to Varangerhalvøya in northernmost Scandinavia was identified in all three assignment tests. Somewhat peculiar however, this sample was taken from a cub trapped at a den in Varangerhalvøya. Therefore, although carrying an immigrant genotype, this individual cannot be an immigrant. More likely is that both parents were immigrants from Russia. Unfortunately, we did not have DNA samples from either of the parents.

The small number of arctic foxes in each of the populations and the lack of evidence for any significant amount of dispersal among them is difficult to reconcile with the high genetic variation in the populations (see also Nyström *et al*. 2006). However, one possible explanation of this observation may be the proposed recent fragmentation discussed above. A historically higher connection, both within Scandinavia and between Scandinavia and Russia may have maintained a relatively high amount of variation within Scandinavia, and the proposed fragmentation may be so recent that there has not been time for genetic drift to affect the populations. The observation of an immigrant genotype from Russia suggests that genetic variation may continue to be maintained in the North Scandinavian population, whereas a future loss of genetic variation through genetic drift in the more southerly populations seems likely.

Implications for conservation

An important finding in this study is that the arctic foxes on the Kola Peninsula genetically belong to Russia and that there are four genetically distinct populations within Scandinavia. It is therefore clear that Fennoscandia does not constitute a biogeographical unit for arctic foxes. Instead of there being one large Fennoscandian population with a population size of 120 individuals, there are four populations in Scandinavia alone. This suggests that the risk of extinction in Scandinavia through demographic stochasticity is higher than previously thought.

In the last 20 years, arctic foxes have disappeared from at least two mountain areas that historically have been of good quality. In Snøhetta/Dovrefjell in southern Norway (between Southwest Scandinavia and South Scandinavia), arctic foxes have not been known to reproduce since 1995 (Linnell *et al*. 1999). Four of the samples in this study were samples collected before 1995 in Snøhetta/Dovrefjell, but these could not be reliably assigned to any of the genetic clusters in the structure analysis. The fifth sample, which was closely associated with Southwest Scandinavian foxes, was collected in 1999 and is suspected of being an escaped farm fox (Norén *et al*., in preparation). It is possible that Snøhetta/Dovrefjell represents a now extinct population, but that our sample size was too small to identify it. Similarly, there have not been any confirmed litters born in Finland since 1996 (Kaikusalo *et al*. 2000). Despite analysing 71 faecal samples with alleged arctic fox origin over the last four years, we have not been able to confirm the presence of arctic foxes in Finland. It therefore seems likely that the arctic fox is extinct in Finland. However, the Finnish mountain tundra is close and connected to currently inhabited areas in Sweden and Norway so the chances of a reestablishment through natural dispersal are good.

The results suggested that the subdivision into four populations may be a recent phenomenon. The observed F_{ST} values may therefore not represent any meaningful biological differences among the populations. However, from a demographic point of view, the four populations in Scandinavia should be considered as separate management units (Moritz 1994). The current management of the arctic fox in Scandinavia is mainly focused on supplementary feeding, red fox control and captive breeding. Since dispersal between the populations seems to be very low, it is likely that conservation measures must be taken in all populations in order to maintain as much of the arctic fox's historical distribution and as much of the remaining genetic variation as possible. Although the genetic variation is relatively high at present, the low population size in each of the populations implies that there is a risk that genetic drift will lead to a decrease of variation in the future. Furthermore, the linear distribution of these populations suggest that extinction of one of the populations could further disrupt the connectivity within Scandinavia, which in turn could lead to a nonlinear increase in extinction risk in Scandinavia as a whole (Hanski 1998). However, it is possible that the current dispersal rate among the populations is too low to maintain the populations in a long perspective, even if all four populations persist. Given the risk of inbreeding and the fluctuations in population size caused by the lemming cycle, it is likely that several dispersers per generation are necessary to avoid disruptive effects from genetic drift within the populations (Mills &

Allendorf 1996). Additionally, the difficulty in finding unrelated partners within the populations may have resulted in an allee effect (Courchamp *et al*. 1999), which would further increase the risk of local extinctions (Loison *et al*. 2001).

One possible approach to prevent negative effects of genetic drift, inbreeding and the allee effect is to augment the populations with foxes from the captive breeding project. Alternatively, individuals could be translocated directly between the Scandinavian populations, which would in effect mimic an increased dispersal rate among the populations. The most effective approach to reverse a possible inbreeding depression in Scandinavia may, however, be to translocate arctic foxes from Russia to Scandinavia. Although gene flow from Russia to Scandinavia seems to occur naturally, it probably was higher 100 years ago (Nyström *et al*. 2006), and may today be too low to counteract inbreeding depression.

Further studies are needed to investigate if there is an ongoing inbreeding depression in the Scandinavian populations. The population size of each population also needs to be better investigated. This could be accomplished by genotyping systematically collected faecal samples (Kohn *et al*. 1999). The low probabilities of identity among siblings (PI_{SIBS} = 0.00041–0.00092) in the populations suggest that the microsatellite loci used in this study have a high enough resolution for this purpose. However, more than three replicates may be needed in the faecal analyses in order not to overestimate the population size.

This study highlights the utility of population genetics as a tool in conservation, not only to describe levels of genetic variation and risks of inbreeding, but also to identify management units from a demographic perspective. First, using a method of species identification, we were able to describe the arctic fox's current distribution in Scandinavia. Second, genotyping of samples collected from throughout this distribution allowed us to resolve the number of populations within this region. The relatively high heterozygosity in the Scandinavian arctic fox suggests that inbreeding and low genetic variation may not be an important threat at present. However, the subdivision into four small isolated populations suggests a high risk of local extinctions through demographic stochasticity. Since dispersal is low, natural recolonization of formerly occupied habitats seems unlikely. Although inbreeding and low genetic variation may not be a large threat today, the small number of individuals in each of the populations suggests that these factors may become a problem in the near future.

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