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Genetic consequences of a demographic bottleneck in the Scandinavian arctic fox

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Demographic bottlenecks can result in a loss of genetic variation due to the bottleneck effect and subsequent genetic drift. The arctic fox population in Scandinavia went through a severe demographic bottleneck in the early 20th century, and is today classified as critically endangered. In this study, we investigated the pre-bottleneck genetic variation in Scandinavia and compared it to modern samples from Scandinavia and North Russia. Variation in the mtDNA control region and five microsatellite loci was examined through ancient DNA analysis on museum specimens. The microsatellite data from the museum specimens was further used to simulate the expected effect of the bottleneck. The arctic foxes in Scandinavia have lost approximately 25% of the microsatellite alleles and four out of seven mtDNA haplotypes. The results also suggest that the genetic differentiation between North Russia and Scandinavia has doubled over the last 100 years. However, the level of heterozygosity was significantly higher than expected from the simulations. This highlights both the advantage of using museum specimens and the importance of generating specific predictions in conservation genetics.

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Genetic variation is an important component for both short and long-term persistence of populations (England et al. 2003). The number of individuals within a population has a large influence on the amount of genetic variation that is maintained over time (Lande 1988). Rapid reductions in population size (i.e. demographic bottlenecks) are therefore often assumed to result in a loss of genetic variation (Wright 1969, Nei et al. 1975, Chakraborty and Nei 1977, Lacy 1987, Bouzat et al. 1998). Bottlenecked populations that are small for a long time could lose additional genetic variation as a consequence of intensified genetic drift (Garza and Williamson 2001) and could be subjected to inbreeding (Keller and Waller 2002). Loss of genetic variation can result in a lowered adaptability to changing environments (Lande 1988), and inbreeding may eventually result in inbreeding depression and a reduction in fitness (Lacy 1997, Amos and Balmford 2001, Larson et al. 2002). On the other hand, even a small number of immigrants from neighbouring populations can offset or even reverse the loss of genetic variation in a bottlenecked population (Crow and Kimura 1970, Vilá et al. 2003).

The arctic fox (*Alopex lagopus*) used to be a common sight in the 19th century in Sweden, Norway and norternmost Finland (from here on referred to as Scandinavia), with numbers probably exceeding 10 000 individuals during peak years (Tannerfeldt 1997). However, in the beginning of the 20th century the population suffered a severe demographic bottleneck. Heavy hunting pressure associated with a profitable fur trade rapidly reduced the population size down to a couple of hundred individuals (Lönnberg 1927). Despite being protected by law in Sweden, Norway and Finland the population has

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not recovered (Hersteinsson et al. 1989). Today, the arctic foxes in these countries number about 150 individuals and are considered to be critically endangered (Angerbjörn et al. 2004, Gärdenfors 2005). The main threats to the population's existence are shortage of food and interspecific competition with red foxes (*Vulpes vulpes*), but also the low population size itself (Hersteinsson et al. 1989, Linnell et al. 1999, Elmhagen 2003). Concerns have also been raised that escaped farm foxes may hybridise with wild arctic foxes. Norén et al. (2006) recently showed that escaped domestic farm foxes are present on the mountain tundra in Scandinavia, but whether hybridisation has taken place is unclear.

A general problem in bottleneck-studies has been that genetic analyses of extant populations only can allow researchers to infer population history rather than study it directly. However, the use of museum specimens has provided an opportunity to reconstruct past levels of genetic variation in populations and estimates of changes in populations' genetic composition over time (Taylor et al. 1994). Direct connections between a reduction in population size and a considerable loss of genetic variation have for example been demonstrated in greater prairie chickens (*Tympanuchus cupido*; Bouzat et al. 1998), whooping cranes (*Grus americana*; Glenn et al. 1999), northern elephant seals (*Mirounga angustirostris*; Weber et al. 2000), sea otters (*Enhydra lutris*, Larson et al. 2002) and North American grey wolves (*Canis lupus*, Leonard et al. 2005).

In this study, we used museum specimens to examine the genetic variation in the mtDNA and five microsatellite loci in the Scandinavian arctic fox population before the demographic bottleneck. Although bottlenecks generally lead to a loss of haplotypes, alleles and heterozygosity, it can be problematic to accurately quantify the expected decrease in variation without knowledge of the pre-bottleneck variability. To circumvent this problem, we used the historical data to simulate an expected loss in heterozygosity and compared these results to the observed genetic variation in contemporary Scandinavian arctic foxes. Furthermore, we compared the genetic variation in Scandinavia and North Russia to assess if the bottleneck has led to any changes in genetic differentiation between the two populations.

Material and methods

Samples and DNA extraction

Fifty-one samples from historical Scandinavian arctic foxes, collected between 1831 and 1924, were obtained from museums in Sweden and Finland (Appendix 1). Some of the historical samples were collected far outside the normal distribution of arctic foxes. These individuals are highly likely to be long distance migrants (Pulliainen 1965). The samples consisted of teeth, bone and skin samples originally obtained from Sweden and Finland (Fig. 1). Ear-biopsy samples from 31 modern Swedish foxes were collected between 1995 and 2004 (Fig. 1). We also used muscle samples from ten Russian arctic foxes (Fig. 1). Due to the large population size in North Russia, we assumed that the allele frequencies have not changed significantly over the last 100 years. It should therefore be possible to compare historical Scandinavia with North Russia.

Each tooth and bone sample from the historical material was drilled with a Multitool (Robust) and 30–170 mg tooth or bone powder was collected from each sample. DNA was subsequently extracted from the tooth and bone powder using a silica-based method modified from Yang et al. (1998) and described by Bouwman et al. (2002). Museum skin samples and modern tissue samples were extracted using Qiagen's Dneasy tissue kit (Qiagen), following the manufacturer's instructions. Extracts from museum samples were eluted to a final volume of 100 μl.

Fig. 1. Map showing the current (dark grey areas) and historical (light grey areas) distribution of arctic foxes in mainland Europe. Sampling locations for the historical samples are shown with grey dots. The geographical origin of the modern samples is indicated by areas enclosed by dashed lines. Sample sizes for the modern samples are 31 for Scandinavia and five for each of the Russian locations.

mtDNA amplification and sequencing

All museum sample extracts were amplified for an approximately 330 base pair (bp) long fragment of the mitochondrial control region, using primers Pex1F and H3R (Dalén et al. 2002; Table 1). Samples that could not be amplified for 330 bp, were amplified for the same region but with three separate primer pairs (Pex1F/Pex1R, Pex2F/Pex2R or Pex2bF/Pex2bR and Pex3F/H3R), giving three overlapping fragments of approximately 150 bp each (Table 1).

Polymerase chain reactions (PCRs) were set up in 25 μl volumes, each containing 2.5 mM $MgCl_2$ (Qiagen), 0.4 mM dNTPs, 0.2 μM of each primer, 1 × PCR-buffer (Qiagen), 3 units (U) Hotstar Taq (Qiagen) and 5 μl of DNA extract. Amplifications were performed with a 10 min denaturation step at 94°C followed by 50 cycles of 20 s denaturation at 94°C, 30 s annealing at 51 or 53°C, 15 s of extension at 72°C, and a final 7 min extension step at 72°C. The PCR products were purified using the QIAquick Spin PCR purification kit (Qiagen), and sequenced following Dalén et al. (2005). The resulting consensus sequences were assigned to haploptypes and compared to previously published sequences from contemporary Scandinavian and Siberian foxes (Dalén et al. 2005).

Microsatellite genotyping

Variation in five microsatellite loci (771: Mellersh et al. 1997, 377: Ostrander et al. 1995, CXX173, CXX250 and CXX140: Ostrander et al. 1993) were investigated using fluorescently labeled canine-specific primers. For the museum samples, two multiplex PCRs were performed with the following combination of loci: (1) 140, 250 and (2) 173, 377. Locus 771 was amplified separately. PCRs were carried out in 15 μl volumes, each containing 2 mM (2.5 mM for 771) $MgCl_2$ (Qiagen), 0.64 mM dNTPs, 0.11–0.24 μM of each primer, 1 × PCR-buffer (Qiagen), 2.25 U Hotstar Taq polymerase (Qiagen) and 3 μl DNA extract. Amplifications were performed in a PTC-200 Programmable Thermal Controller with the following cycles: 95°C for 10 min; 3 cycles at 94°C for 30 s, 54°C for 20 s and 72°C for 5 s; 47 cycles at 94°C for 15 s, 54°C for 20 s and 72°C for 1 s; 72°C for 30 min. Modern samples were analysed following Norén et al. (2006). PCR products were separated electrophoretically using a CEQ 8000 DNA sequencer (Beckman Coulter) and allele sizes were scored against a size standard.

Table 1. Mitochondrial DNA primer sequences. Pex1F and H3R obtained from Dalén et al. (2002).

Primer	Sequence (5'–3')
Pex1F	TAAACTATTCCCTGATACTC
Pex1R	TTAAGCATAGTATGTCTTATG
Pex2F	CCATGCATATAAGCATGTAC
Pex2R	GATGGTTTCTCGAGGCATG
Pex2bF	ATGCCCCATGCATATAAGC
Pex2bR	TTGATGGTTTCTCGAGGC
Pex3F	TTAGTCCAATAAGGGATTTATC
H3R	CCTGAAGTAGGAACCAGATG

Precautions and authenticity

Due to the low copy number and degraded nature of ancient DNA (Cooper and Wayne 1998), precautions to avoid contamination were taken during the whole procedure. DNA extraction and PCR for the historical material took place in separate rooms in a laboratory dedicated to ancient DNA research. All working surfaces and equipment were washed with HCl or sodium hypochlorite and/or UV irradiated. Teeth and bone surfaces were removed with sandpaper before drilling. No more than eight samples were handled simultaneously in order to avoid cross-contamination between samples.

One negative extraction control was included for every fourth sample and separate PCR negative controls were used in all amplifications. The negative controls were continually screened and no evidence of contamination was observed. Double samples were also taken from 13 individuals. The samples from the same specimen produced identical results. Historical teeth and bone samples from two red foxes (*Vulpes vulpes*) were also analysed. The samples from the red foxes did not give any amplification products when amplified with the primers used for the arctic foxes (Pex1F and H3R). However, when amplified with primers specific for red foxes the samples produced the expected sequences.

As ancient DNA usually is degraded, the amplification success rate should decrease with increased amplicon length (Hummel et al. 1999, Wandeler et al. 2003). The fact that not all samples could successfully be amplified for the longer mtDNA fragment agrees with appropriate molecular behaviour. Samples with unique sequences (i.e. sequences not observed in any other individuals) were amplified and sequenced two additional times in order to identify possible misincorporated nucleotides.

A common genotyping error that can occur during work with ancient DNA is allelic dropout, which is a phenomenon when only one of the two alleles in a heterozygous locus is amplified. All successful amplifications were therefore repeated at least twice. Samples that still appeared homozygous after the two replicates were amplified two additional times. An estimate of the probability of receiving a false homozygote after a number of replicates (n) was calculated according to Gagneux et al. (1997): $P_{(\text{false homozygotes})} = K \times (K/2)^{n-1}$, where K is the observed number of allelic dropouts divided by all heterozygous individuals. A χ^2-test

(STATISTICA 5.5, StatSoft, inc.) was used to test for non-random distribution across loci.

Data analysis

The software Arlequin version 2.0 (Schneider et al. 2000) was used to calculate the genetic variability and to make population pairwise comparisons between historical Scandinavia, contemporary Scandinavia and North Russia. The nucleotide substitution model by Tamura and Nei (1993), with a gamma value of 0.7, was used in all mtDNA analyses (Dalén et al. 2005). Sequence variability was measured as the number of haplotypes (n_h) and haplotype diversity ($h \pm SE$) according to Nei (1987).

A linkage disequilibrium test (16 000 permutations and 100 random initial conditions) was used to test for the presence of a significant association between all pairs of microsatellite loci (Slatkin 1994), corrected for multiple testing using the Bonferroni correction (Rice 1989). Microsatellite genetic variation was measured as the total number of observed alleles (n_a), allelic richness (R_S) and average heterozygosity ($H_a \pm SE$, Tajima 1983). The observed heterozygosity (H_O) and heterozygosity expected from Hardy–Weinberg assumptions (H_E) were also calculated and deviations from Hardy–Weinberg equilibrium were tested using an exact test based on a Markov chain algorithm with a forecasted chain length of 100 000 steps and 3000 dememorisation steps (Levene 1949). A population pairwise F_{ST} analysis with 10 000 permutations was used to investigate the differentiation between all pairs of populations. Here, we also included previously published microsatellite data from Scandinavian farm foxes (Norén et al. 2006).

To estimate the number of alleles in each of the three populations, we plotted the cumulative number of alleles with increasing sample size. This was done using the "Doh assignment test calculator" available at http://www2.biology.ualberta.ca/jbrzusto/Doh.php. Each data set was randomly resampled 5000 times without replacement. The average values from these iterations were used to estimate the asymptote (a) of the curve using the function $y=(ax)/(b+x)$, where y is the cumulative number of alleles and b is the rate of decline of the slope. The values of a and b, and the 95% confidence intervals for the asymptote (CI_a), were calculated using the software Graphpad Prism (GraphPad Software v. 4.02). We also used the software FSTAT ver. 2.9.3 (Goudet 1995) to calculate allelic richness for each locus, taking into account the difference in sample size among populations. Differences in allelic richness (R_S) among populations were tested using Wilcoxon sign rank tests (STATISTICA 5.5, StatSoft, Inc.).

We simulated the effect of the bottleneck using the historical microsatellite data in the software BottleSim (Kuo and Janzen 2003). BottleSim simulates the actual population size rather than the effective population size and allows for both generation overlap and fluctuations in population size. We ran five simulations with different post-bottleneck population sizes to investigate the number of individuals needed to retain the observed amount of genetic variation in the current population. The time period in the simulations was set from 1900 until 1995, starting with a peak phase population size of 10 000 which gradually decreased to 100, 200, 400, 800 and 1200 individuals at the year 1927 and then was kept constant through the remainder of the simulation. Since the arctic fox population in Scandinavia closely follows the lemming cycle (Kaikusalo and Angerbjörn 1995), we incorporated a cycle into the simulations, based on empirical data (Angerbjörn et al. 2004). The population size was set to vary with a four-year cycle so that 100% of the individuals were simulated to breed in year one (peak phase), 5% of the individuals in years two and three (crash phase) and 25% in year four (increase phase). The estimate for the crash phase is overly conservative and may thus overestimate the level of genetic variation retained. Additional settings used in the simulations were a) maximum generation overlap, b) random mating, c) longevity = four years, d) age at first reproduction = one year, e) sex ratio = 1:1 and f) number of iterations = 1000. The setting for longevity is probably an overestimate since out of ca 350 foxes tagged within the Swedish Arctic Fox Conservation Project, less than five lived to reproduce beyond four years of age. Increasing the longevity, however, did not have any significant effect on the outcome of the simulations (data not shown).

We used the software EASYPOP (Balloux 2001) to investigate how much effect novel mutations can have on the heterozygosity of a small population over a time span of 100 years (25 generations). We used the harmonic mean as an approximation to a fluctuating post-bottleneck population size of 200 and the settings random mating, free recombination among loci, equal sex ratio, the KAM model of mutation, a maximum of seven allelic states with maximal initial variation and 20 replicates for each run. Using these settings, we were able to obtain a similar outcome as the simulation for 200 individuals in BottleSim. We then repeated the simulation described above using a very high mutation rate ($\mu = 0.01$; Epperson 2005).

Results

mtDNA analysis

Consensus sequences of 292 bp were obtained from 42 of the 51 historical samples. Although misincorporated nucleotides were detected in seven samples, majority rule consensus sequences were obtained after three

independent amplifications. Seven mitochondrial haplotypes, all previously reported for arctic foxes (Dalén et al. 2005), were detected in the historical Scandinavian population (Appendix 1). The three haplotypes observed in contemporary Scandinavian arctic foxes, H1, H3 and H7, (Strand et al. 1998, Dalén et al. 2002) were also found in the historical material. H3 was the most common haplotype in the historical material and was found in 62% of the samples, compared to 47% in the contemporary population. Haplotypes H1 and H7 had increased in frequency from 7 to 35% and 14 to 18% respectively (Dalén et al. 2002). Four of the seven haplotypes in the historical Scandinavian population were shared with North Russia (6 out of 7 compared to all of Siberia, Dalén et al. 2005).

Comparison of historical and contemporary Scandinavia surprisingly revealed a higher haplotype diversity ($h \pm SE$) after the demographic bottleneck (0.60 ± 0.01 vs 0.65 ± 0.01; $t_{60} = 3.11$, $p < 0.01$). However, the haplotype diversity in North Russia (0.80 ± 0.03) was higher than in both historical and contemporary Scandinavia ($t_{50} = 7.99$, $p < 0.001$ and $t_{28} = 5.94$, $p < 0.01$ respectively).

Microsatellite analysis

Twenty-one of the individuals from historical Scandinavia (Appendix 1) along with 31 individuals from contemporary Scandinavia and ten individuals from North Russia were successfully genotyped for all five microsatellite loci. Allele sizes and most genotypes appeared consistent and no sample gave more than two alleles per locus when replicates were compared. Allelic dropout was however detected in 16% of all heterozygous loci and the probability of receiving a false homozygote after two replicates was estimated to 0.01. After two more replicates were performed for the homozygotes, the probability of receiving a false homozygote was less than one in ten thousand ($p = 0.00009$). No differences were found between the loci in regard to the number of allelic dropouts ($\chi^2 = 6.5$, $df = 4$, $p = 0.17$).

In the historical material a total of 34 alleles were observed, compared to 27 alleles in contemporary Scandinavia. Eleven of the alleles observed in the historical population were not observed in the contemporary population. Four alleles in the contemporary population were not observed in the historical samples, but three of these were observed in North Russia. Although the sample size was very small in North Russia, the total number of observed alleles was 35.

To calculate the total number of alleles in each population, we plotted the cumulative number of alleles with increasing sample size (Fig. 2). The estimated number of alleles in historical Scandinavia was 40.3 (95% $CI_a = 38.8 - 41.9$), which was higher than the 30 alleles estimated for contemporary Scandinavia (95% $CI_a = 29.7 - 30.3$). The number of alleles estimated for North Russia was 49.7 (95% $CI_a = 48.2 - 51.1$), which was higher than in both the Scandinavian samples. There was also a significant difference in allelic richness between the populations (Wilcoxon sign rank test; $Z = 2.02$, $p < 0.05$ for all comparisons), where North Russia had the highest allelic richness and contemporary Scandinavia the lowest (Table 2).

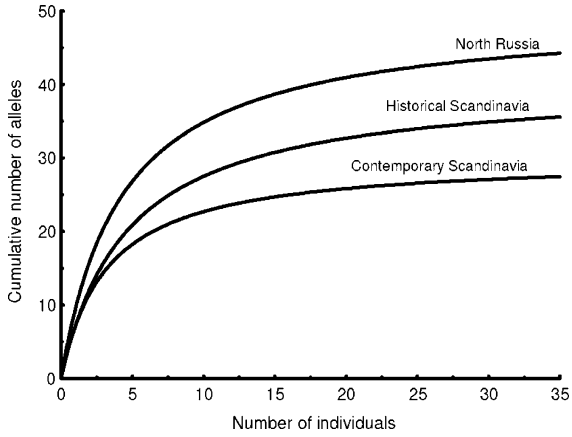

Fig. 2. Cumulative number of microsatellite alleles with increasing sample size for North Russia, historical and contemporary Scandinavia. The estimates are based on averages from 5000 randomised iterations.

No significant differences in average heterozygosity were found between historical and contemporary Scandinavia or historical Scandinavia and North Russia ($t_{102} = 0.89$, $p = 0.37$ and $t_{60} = 1.30$, $p = 0.20$ respectively; Table 2). The average heterozygosity was however significantly lower in contemporary Scandinavia than in North Russia ($t_{80} = 2.04$, $p < 0.05$; Table 2).

Departures from Hardy–Weinberg equilibrium were not significant for any of the loci in contemporary Scandinavia or North Russia. One locus (CXX250) in historical Scandinavia showed a significant deviation from Hardy–Weinberg equilibrium that was caused by a deficit of heterozygosity (Table 2). No significant deviations from linkage equilibrium were observed after correcting for multiple testing.

The level of differentiation between contemporary Scandinavia and North Russia was higher ($F_{ST} = 0.082$, $p < 0.00001$) compared to the differentiation between the historical population and North Russia ($F_{ST} = 0.040$, $p < 0.01$). Furthermore, the arctic foxes in contemporary Scandinavia were significantly differentiated from the historical population ($F_{ST} = 0.035$, $p < 0.002$, Fig. 3). When farm foxes were compared to North Russia, historical Scandinavia and contemporary Scandinavia, the F_{ST} values were 0.302, 0.318 and 0.348 respectively ($p < 0.00001$ in all cases).

The simulations in BottleSim indicated that the average heterozygosity should have decreased to

Table 2. Microsatellite genetic variation within the five loci for each population. R_S is the allelic richness, H_O is the observed heterozygosity and H_E is the expected heterozygosity from Hardy–Weinberg assumptions. H_O values marked with (*) indicate deviations from Hardy Weinberg equilibrium ($p < 0.05$). The average heterozygosities (H_a) are presented with standard error (SE). The total number of heterozygotes (N_{Het}) and the number of allelic dropouts (N_{AD}) are presented for historical Scandinavian samples. Sample size for each locus and population in parentheses.

Locus	Historical Scandinavia (n = 21)					Contemporary Scandinavia (n = 31)			Contemporary North Russia (n = 10)		
	N_{Het}	N_{AD}	R_S	H_O	H_E	R_S	H_O	H_E	R_S	H_O	H_E
CXX140	14	4	4.4	0.67	0.73	3.9	0.61	0.58	7.0	1.00	0.83
CXX250	13	0	5.8	0.62*	0.74	5.3	0.71	0.76	6.0	0.80	0.84
771	16	1	7.9	0.76	0.85	7.0	0.85	0.88	9.0	0.90	0.89
CXX173	12	2	3.9	0.57	0.58	3.7	0.55	0.51	4.0	0.80	0.72
377	12	4	4.9	0.57	0.51	3.3	0.32	0.37	9.0	0.90	0.90
$H_a \pm SE$				0.68 ± 0.06			0.61 ± 0.05			0.83 ± 0.11	

0.11–0.27 when using the most realistic assumptions on the post-bottleneck population size (100–200 individuals). Only the simulation where the number individuals was set to 1200 during peak years retained the amount of variation observed in the contemporary population (Fig. 4). The analyses in EASYPOP showed that a mutation rate of $\mu = 0.01$ resulted in an increase in heterozygosity by 0.11 compared to when the mutation rate was set to zero.

Discussion

DNA from museum specimens and methodology

More than 80% of the historical samples were successfully amplified for the entire 330 bp mtDNA fragment or the three 150 bp fragments. Teeth gave the highest success rate (94%), followed by bone (80%) and skin samples (50%). The success rate for the nuclear microsatellites was lower than for the mtDNA. However, 50% of the samples used in the mtDNA analysis were also successfully genotyped for all loci. Again, teeth had the highest success rate, followed by bone and skin (50, 45 and 20% respectively). This confirms the results from previous studies where bone and teeth are the tissues that have been reported to preserve DNA best over time (Greenwood et al. 1999).

Misincorporated nucleotides were found in 17% of the samples. This may be a consequence of the high number of cycles used in the PCR, which resulted in successful amplification of extracts with very few starting templates. Although misincorporated nucleotides previously have been identified with multiple amplifications in for example DNA molecules from 25 000–50 000 years old bones and teeth (Hofreiter et al. 2001), there are no such reports in studies on ca 100 years old material (Glenn et al. 1999, Larson et al. 2002, Flagstad et al. 2003, Leonard et al. 2005). This demonstrates that precautions to identify erroneous bases also should be taken when analysing relatively young museum specimens.

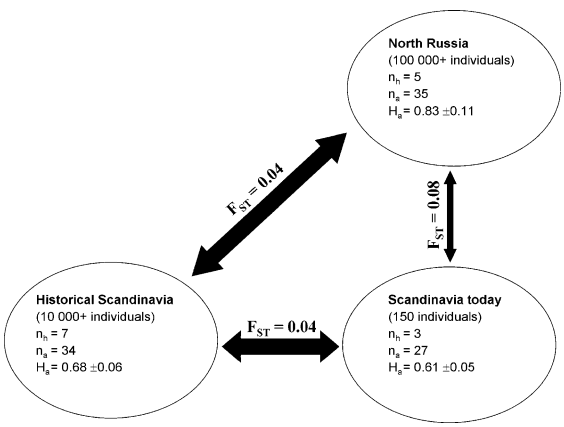

Fig. 3. Genetic variation within and genetic distances between Scandinavia and North Russia. Genetic variation is shown as number of mtDNA haplotypes (n_h), total number of observed alleles (n_a) and average heterozygosity ($H_a \pm SE$). Genetic distances are shown as F_{ST} where the arrows are inversely proportional to the F_{ST}-values.

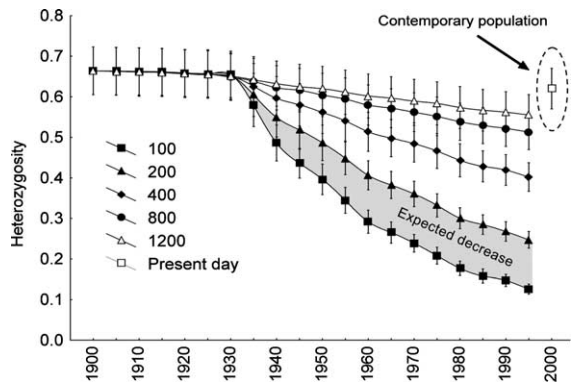

Fig. 4. Results from the simulations using the pre-bottleneck microsatellite data. Error bars represent standard error. Five different post-bottleneck population sizes were used ranging from 100 to 1200 individuals. The results show that approximately 1200 individuals were needed to retain a heterozygosity similar to the one observed in the present day population, which is almost tenfold the estimated actual post-bottleneck population size of 100–200 individuals.

In the microsatellite analysis, allelic dropout was detected in 16% of the heterozygous loci. Given this ratio, the estimated probability of undetected dropouts after four replicates was very low. However, we cannot rule out that the observed heterozygote deficiency in locus CXX250 is a result of allelic dropout despite the four replicates. Alternatively, it is due to the presence of null alleles or a subdivision of the historical population (Wahlund 1928).

Changes in genetic structure over time

Populations on peninsulas generally have lower genetic diversity than mainland populations (Crow and Kimura 1970, Vilá et al. 1999, Randi et al. 2000). For example, the Scandinavian wolf, wolverine, lynx and brown bear populations display very low mtDNA variability (1–2 haplotypes), which most likely is a result of their colonisation history rather than recent reductions in population size (Waits et al. 2000, Walker et al. 2001, Hellborg et al. 2002, Flagstad et al. 2003). In contrast, we found seven mtDNA haplotypes in Scandinavia before the bottleneck. This could be a result of the frequent long range migrations in the arctic fox (Pulliainen 1965, Eberhardt and Hanson 1978), which has been suggested to have erased the historical patterns of post-glacial colonisation (Dalén et al. 2005).

We had expected the demographic bottleneck in Scandinavia almost 100 years ago to result in a considerable loss of genetic variation. Indeed, the results showed that four out of seven mtDNA haplotypes (ca 60%) no longer exist in the contemporary population. Allelic richness was significantly lower after the bottleneck, and the results suggested that approximately 25% of the microsatellite alleles have been lost (Fig. 2). There was however no significant difference in average heterozygosity between the historical and contemporary population. It is therefore clear that a certain amount of genetic variation has been lost, but the question remains whether this loss is as high as expected given the severity and persistence of the bottleneck. The 25% loss of microsatellite alleles in Scandinavia is relatively small compared to other bottlenecked populations. In for example sea otters, a similar reduction in population size resulted in a loss of at least 62% of the alleles and 43% of the expected heterozygosity (Larson et al. 2002). Further, the Scandinavian wolf population lost approximately 40% of the allelic diversity and 30% of the heterozygosity as a consequence of a severe decline in population size (Flagstad et al. 2003).

We used the microsatellite variation observed in the historical population to simulate the expected change in heterozygosity, which resulted in an expected decreased to between 0.11–0.27. However, this is significantly lower than the actual average heterozygosity for the arctic foxes ($H_a = 0.61 \pm 0.05$) in contemporary Scandinavia (Fig. 4). The post-bottleneck population size needed to retain such a high level of heterozygosity was 1200 individuals (Fig. 4), which is almost ten times higher than the recorded post-bottleneck population size of 100–200 individuals (Angerbjörn et al. 2004).

There can be several explanations to why a demographic bottleneck does not cause a decrease in genetic variation. Fragmentation of the post-bottleneck population can preserve allelic diversity if different alleles become fixed in different subpopulations (Chesser et al. 1980). Although this to some extent could explain the relatively high allelic diversity in contemporary Scandinavia, it does not explain the high observed heterozygosity (Table 2) since fragmentation would increase loss of heterozygosity rather than preserve it (Wahlund 1928). An alternative explanation is that the high heterozygosity in the contemporary population is a result of a high mutation rate in the loci studied (Epperson 2005). However, the results from the simulations in EASYPOP suggested that not even a very high mutation rate ($\mu = 0.01$) is enough to maintain the amount of heterozygosity observed. In addition, such an explanation is not in concordance with comparable demographic bottlenecks in other species.

Introduction of new alleles through hybridisation between farm and wild foxes is another possible explanation to the current high variation in Scandinavia. However, the higher genetic differentiation between contemporary Scandinavia and farm foxes ($F_{ST} = 0.348$) than between historical Scandinavia and farm foxes ($F_{ST} = 0.318$) suggests that hybridisation has not introduced any significant amount of genetic variation. It therefore seems unlikely that a population subdivision, high mutation rate, or hybridisation can explain the high variation observed in contemporary Scandinavian arctic foxes.

A fourth explanation to the high level of variation in the contemporary population is a post-bottleneck gene flow from another wild population. This has previously been suggested as an explanation to the relatively high number of mtDNA haplotypes observed in contemporary Scandinavia (Dalén et al. 2002). Due to the ice-free coast of northern Scandinavia (Dalén et al. 2002) the only reasonable source is North Russia. The concept of gene flow from North Russia into Scandinavia is further supported by the observation that three alleles, not observed in the historical population, were shared between contemporary Scandinavia and North Russia.

The genetic differentiation between Scandinavia and North Russia, measured as F_{ST}, has doubled over the last 100 years. We can find two possible explanations to this. First, genetic drift in the small post-bottleneck population could have led to changes in allele frequencies within Scandinavia. This is also supported by the observation that two of the three mtDNA haplotypes

have changed considerably in frequency. Second, the amount of gene flow into Scandinavia may have decreased in connection with the bottleneck. This could in particular be the case if the population size on the Kola Peninsula has declined, since the Kola Peninsula may act as an intermediate area in a stepping stone model (Crow and Kimura 1970).

Immigration from North Russia may be an important factor for the maintenance of genetic variation in Scandinavia. Although it is possible that post-bottleneck gene flow from North Russia has allowed the population to maintain a relatively high level of heterozygosity, several alleles and mtDNA haplotypes have been lost and the genetic differentiation between North Russia and Scandinavia has doubled. If the observed loss of variation is a result of genetic drift over the last 100 years, this indicates that the genetic variation in Scandinavia cannot be sustained in a longer perspective unless the population size increases.

Our interpretation of the current genetic variation in Scandinavia might have been different in several ways, had we not had the information obtained from the museum specimens. The lower genetic variation in Scandinavia compared to North Russia could have been viewed as a direct consequence of the demographic bottleneck. This study shows that both allelic diversity and heterozygosity was lower in Scandinavia also before the bottleneck. Thus, although some microsatellite diversity has been lost, the loss is actually less than could be expected from a comparison with North Russia. In fact, the average heterozygosity has not decreased at all. Furthermore, the degree of differentiation may have been viewed as too high to permit translocation of arctic foxes from Russia (IUCN 1987). However, the results in this study suggest that there used to be a higher connection between North Russia and Scandinavia before the bottleneck, which should be taken into account if translocation is considered in the management of the Scandinavian arctic fox.

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Appendix 1. Samples from historical Scandinavian arctic foxes used in the study. Teeth (t), bone (b) and skin (s) samples successfully amplified and sequenced for the 332 bp mtDNA fragment or all three 150 bp fragments are indicated (+). The corresponding haplotype is named according to Dalén et al. (2005). Samples successfully amplified for all five microsatellite loci are indicated (+).

Accession number	Origin	Year of collection	Sample	mtDNA 332 bp/ 3 × 150 bp	Haplotype	Microsatellite amplification
A60 2466[A]	Norrbotten, Sweden	1831	s	−/+	H3	−
A60 6581[A]	Haparanda, Sweden	1832	t+b	+	H3	−
A59 5070[A]	Jämtland, Sweden	1841	s	−	−	−
A59 5169[A]	Jämtland, Sweden	1843	s	−	−	−
A58 3651[A]	Jämtland, Sweden	1845	t+b	+	H3	−
A58 5582[A]	Jämtland, Sweden	1845	t+b	+	H3	−
A60 1196[A]	Jämtland, Sweden	1845	t+b	−	−	−
A58 3644[A]	Södermanland, Sweden	1848	t+b	−	−	−
760[B]	Gothenburg, Sweden	1848	t	−/+	H7	−
A60 1198[A]	Kvikkjokk, Sweden	1852	t+b	+	H9	+
A59 1188[A]	Kvikkjokk, Sweden	1853	t+b	+	H7	+
A58 3646[A]	Kvikkjokk, Sweden	1853	b	+	H7	−
12[C]	Kvikkjokk, Sweden	1861	s	−/+	H7	+
761[B]	Trollhättan, Sweden	1861	t	−/+	H3	−
762[B]	Onsala, Sweden	1863	s	−	−	−
A58 9501[A]	Stockholm, Sweden	1867	b	+	H2	−
3329[C]	Lappland, Sweden	1867	t	+	H3	+
3338[C]	Lappland, Sweden	1867	b	+	H3	+
6672[B]	Gothenburg, Sweden	1873	t	−/+	H3	−
A58 1016[A]	Södertälje, Sweden	1877	b	−	−	−
763[B]	Sjabatjakkvaara, Sweden	1877	t	+	H3	+
10[C]	Uppland, Sweden	1878	s	−	−	−
A58 1017[A]	Härnösand, Sweden	1881	s	+	H3	+
A60 1192[A]	Munsö, Sweden	1892	t+b	+	H3	+
A58 0192[A]	Munsö, Sweden	1892	b	+	H3	−
A59 0205[A]	Runmarö, Sweden	1896	t+b	−/+	H1	−
765[B]	Onsala, Sweden	1896	t	+	H1	+
764[B]	Gothenburg, Sweden	1896	s	−/+	H1	−
7[C]	Lappland, Sweden	1896–97	s	−/+	H3	−
6[C]	Lappland	1896–97	s	−	−	−
A58 3188[A]	Västmanland, Sweden	1900	b	−	−	−
A60 2585[A]	Tärnaby, Sweden	1904	t+b	−/+	H3	+
A59 1586[A]	Tärnaby, Sweden	1907	t+b	+	H3	+
A60 1251[A]	Tillberga, Sweden	1924	t+b	+	H7	+
33.147[D]	Finland	1857	t	−/+	H3	−
A58 1525[A]	Kunmlinge, Åland, Finland	1900	b	+	H3	+
A60 4001[A]	Åland, Finland	1900	t+b	−/+	H3	+
1301[D]	Ivalo, Finland	1909	t	+	H3	−
1345[D]	Finland	1912	t	+	H7	−
2244[D]	Könkämälaakso, Finland	1912	t	+	H3	+
2233[D]	Könkämälaakso, Finland	1912	t	−/+	H3	+
2239[D]	Könkämälaakso, Finland	1912	t	−/+	S2	−
2241[D]	Könkämälaakso, Finland	1912	t	+	H3	−
2236[D]	Könkämälaakso, Finland	1912	t	+	H3	−
2243[D]	Könkämälaakso, Finland	1912	t	−/+	H3	−
2238[D]	Könkämälaakso, Finland	1912	t	−/+	S2	+
2237[D]	Könkämälaakso, Finland	1912	t	+	H9	+
2234[D]	Könkämälaakso, Finland	1912	t	+	H3	+

Appendix 1 (*Continued*)

Accession number	Origin	Year of collection	Sample	mtDNA 332 bp/ 3 × 150 bp	Haplotype	Microsatellite amplification
2240[D]	Könkämälaakso, Finland	1912	t	+	H3	+
2235[D]	Könkämälaakso, Finland	1912	t	−/+	S2	+
2242[D]	Könkämälaakso, Finland	1912	t	+	H5	−

*) Samples were obtained from four different museums indicated by superscript letters: Swedish Museum of Natural History, Stockholm (A), Museum of Natural History, Gothenburg (B), Museum of Evolution, Uppsala (C), Finnish Museum of Natural History, Helsinki (D)

Appendix 2. Observed allele frequency distribution for historical Scandinacia (HS), contemporary Scandinavia (CS) and North Russia (NR).

Locus	Allele	HS	CS	NR	Locus	Allele	HS	CS	NR
CXX140	136	0.02	–	0.20	377	175	–	–	0.20
	140	–	0.16	–		177	0.05	–	0.05
	142	–	–	0.10		179	0.69	0.81	0.25
	144	0.26	0.13	0.10		181	0.05	–	0.10
	146	0.02	–	0.15		183	0.12	0.03	0.15
	148	0.40	0.61	0.35		185	0.05	0.08	0.05
	150	0.02	0.10	–		187	0.05	–	0.10
	152	0.26	–	0.05		189	–	0.08	0.05
	154	–	–	0.05		193	–	–	0.05
CXX173	122	0.17	0.11	0.25	771	94	0.07	–	0.05
	124	0.10	0.06	0.20		96	0.02	–	0.05
	126	0.62	0.68	0.45		98	0.05	0.11	0.05
	128	0.12	0.15	0.10		100	0.10	0.19	0.10
CXX250	123	0.02	0.10	0.25		102	0.29	0.11	0.20
	125	0.43	0.37	0.35		104	0.21	0.02	0.15
	127	0.19	0.10	–		106	0.05	0.02	0.10
	129	–	–	0.05		108	0.14	0.27	0.25
	131	0.07	0.10	0.15		112	0.02	0.10	–
	133	0.21	0.31	0.15		114	0.05	0.15	–
	135	–	0.03	0.05		118	–	0.03	0.05
	137	0.02	–	–					
	139	0.02	–	–					
	147	0.02	–	–					