

Research



Cite this article: Larsson P *et al.* 2019
Consequences of past climate change
and recent human persecution on
mitogenomic diversity in the arctic fox. *Phil.
Trans. R. Soc. B* **374**: 20190212.
<http://dx.doi.org/10.1098/rstb.2019.0212>

Accepted: 27 August 2019

One contribution of 17 to a discussion meeting
issue ‘The past is a foreign country: how much
can the fossil record actually inform
conservation?’

Subject Areas:

evolution, genetics, genomics

Keywords:

mitochondrial DNA, mitogenome, arctic fox,
bottleneck, climate change

Author for correspondence:

Love Dalén
e-mail: love.dalen@nrm.se

Electronic supplementary material is available
online at <https://doi.org/10.6084/m9.figshare.c.4669517>.

Consequences of past climate change and recent human persecution on mitogenomic diversity in the arctic fox

Petter Larsson^{1,2}, Johanna von Seth^{1,2}, Ingerid J. Hagen⁴,
Anders Götherström³, Semyon Androsov⁵, Mietje Germonpré⁶,
Nora Bergfeldt^{1,2}, Sergey Fedorov⁷, Nina E. Eide⁴, Natalia Sokolova^{8,9},
Dominique Berteaux¹⁰, Anders Angerbjörn², Øystein Flagstad⁴,
Valeri Plotnikov¹¹, Karin Norén², David Díez-del-Molino^{1,2}, Nicolas Dussex¹,
David W. G. Stanton¹ and Love Dalén^{1,2}

¹Department of Bioinformatics and Genetics, Swedish Museum of Natural History, Stockholm, Sweden

²Department of Zoology, and ³Department of Archaeology and Classical Studies, Stockholm University, Stockholm, Sweden

⁴Norwegian Institute for Nature Research, Trondheim, Norway

⁵Museum ‘Severnyi Mir’, Yakutsk, Russia

⁶Operational Direction ‘Earth and History of Life’, Royal Belgian Institute of Natural Sciences, Brussels, Belgium

⁷Mammoth Museum of Institute of Applied Ecology of the North, North-Eastern Federal University, Yakutsk, Republic Sakha (Yakutia), Russia

⁸Arctic Research Station of Institute of Plant and Animal Ecology, Ural Branch of Russian Academy of Sciences, Yamal-Nenets Autonomous District, Russia

⁹Arctic Research Center of Yamal-Nenets Autonomous District, Salekhard, Russia

¹⁰Canada Research Chair on Northern Biodiversity and Centre for Northern Studies, Université du Québec à Rimouski, Rimouski, Canada

¹¹Academy of Sciences of Sakha Republic, Lenin Avenue 33, Republic of Sakha, Yakutia, Russia

© DDDM, 0000-0002-9701-5940; ND, 0000-0002-9179-8593; LD, 0000-0001-8270-7613

Ancient DNA provides a powerful means to investigate the timing, rate and extent of population declines caused by extrinsic factors, such as past climate change and human activities. One species probably affected by both these factors is the arctic fox, which had a large distribution during the last glaciation that subsequently contracted at the start of the Holocene. More recently, the arctic fox population in Scandinavia went through a demographic bottleneck owing to human persecution. To investigate the consequences of these processes, we generated mitogenome sequences from a temporal dataset comprising Pleistocene, historical and modern arctic fox samples. We found no evidence that Pleistocene populations in mid-latitude Europe or Russia contributed to the present-day gene pool of the Scandinavian population, suggesting that postglacial climate warming led to local population extinctions. Furthermore, during the twentieth-century bottleneck in Scandinavia, at least half of the mitogenome haplotypes were lost, consistent with a 20-fold reduction in female effective population size. In conclusion, these results suggest that the arctic fox in mainland Western Europe has lost genetic diversity as a result of both past climate change and human persecution. Consequently, it might be particularly vulnerable to the future challenges posed by climate change.

This article is part of a discussion meeting issue ‘The past is a foreign country: how much can the fossil record actually inform conservation?’

1. Introduction

The field of conservation biology can be divided into two major paradigms [1]. One of these, the small population paradigm, is concerned with the threats

posed by small population size itself, such as genetic and demographic stochasticity. The second, termed the declining population paradigm, describes the extrinsic processes that cause small populations to become small in the first place. Ancient DNA has emerged as an increasingly popular tool to study both these paradigms. For example, the use of a temporal approach can enable quantification of changes in adaptive potential, as well as inbreeding levels and genetic load, in small populations [2]. In addition, the recovery of DNA from ancient remains makes it possible to sample across known past environmental changes, and consequently to identify factors that have led to population declines in wild populations [3,4].

The main external processes that are thought to cause population declines include overharvesting, habitat destruction, competition from invasive species, and climate change. In many species, it is likely that several of these processes have contributed to population declines, either simultaneously or one after the other. For example, the present-day population sizes in most Arctic species are likely the result of an interaction between prehistoric changes in climate and more recent anthropogenic impacts.

For Arctic species, the end of the Pleistocene was a period of dramatic shifts in habitat availability, which resulted in losses of genetic diversity and population replacements [5–7]. In general, the warming climate at the onset of the Holocene led to decreases in geographical distributions of Arctic taxa. However, it has been proposed that the underlying processes for these range contractions differed among species, either being characterized by population movements (habitat tracking; [8,9]) or local population extinctions [10]. In more recent times, human activities such as habitat destruction, pollution and hunting have contributed to local population decreases and extinctions [11], including in Arctic species. Especially during the last 100 years, these have led to demographic bottlenecks and ensuing losses in genetic variation [12].

One species that both contracted in range at the end of the last glaciation and has been affected by more recent human activities is the arctic fox (*Vulpes lagopus*). The arctic fox had a wide distribution during the last glaciation [13]. Today, however, the arctic fox is restricted to a circumpolar distribution along the northern fringe of continental Eurasia and North America, as well as on high-latitude islands such as Greenland, Iceland and Svalbard [14]. On a worldwide scale, the arctic fox is classified by the International Union for Conservation of Nature as Least Concern, but the population in Scandinavia is classified as Endangered in Regional Red List assessments. The population started declining owing to a severe demographic bottleneck at the beginning of the twentieth century [15]. Heavy hunting pressure fuelled by a profitable fur trade led to a decrease from several thousand to only a few hundred individuals [16,17]. Following this decline, the population has failed to recover, and the contemporary population consists of approximately 300 adult individuals [18]. The failure to recover could be related to the small population size, but also changes in the alpine ecosystems causing expansion of the red fox (*Vulpes vulpes*) as well as fading cycles of small rodents [19].

Previous studies on mitochondrial DNA variation in the arctic fox have indicated a loss of unique genetic variants as the species' range contracted at the end of the last glaciation, as well as a marked loss of genetic diversity in

connection with the human-induced bottleneck 100 years ago [10,20]. These studies have been based on short (less than 300 bp) DNA sequences from the mitochondrial control region, and the results may thus not be representative of the overall mitogenomic diversity. However, recent advances in DNA sequencing technology have now made it possible to recover mitogenome-scale datasets from ancient specimens at a reasonably low cost and effort [21,22].

The aim of this study was to examine the consequences of both the end-Pleistocene range contraction and more recent human persecution on the mitogenome diversity in the arctic fox. A better understanding of these past processes could help refine our ability to predict the impact of ongoing changes in climate and demographic bottlenecks. To investigate these questions, we generated a temporally sampled mitogenomic dataset that spanned from the Late Pleistocene to the present day, and included samples from mid-latitude Europe, northeastern Russia and Scandinavia. Because previous results have suggested that the Late Pleistocene mid-latitude European populations did not contribute to the present-day mitochondrial DNA gene pool [10], we expected to find relatively high sequence divergence between the ancient samples from mid-latitude Europe and present-day Scandinavia. By contrast, because the Scandinavian population has been proposed to originate from a postglacial expansion from Beringia [10], we expected low sequence divergence (i.e. direct ancestry) between Pleistocene samples from northeastern Russia and present-day Scandinavia. Additionally, we expected that a mitogenomic dataset would permit a more accurate assessment of the loss of genetic diversity and changes in effective population size during the last 100 years, compared to previous studies on shorter mitochondrial DNA sequences.

2. Material and methods

(a) Samples and data generation

We sampled 30 historical Scandinavian specimens, collected between 1845 and 1927, and 26 Late Pleistocene Eurasian remains that ranged in age from 30 000 to 12 000 years before present (BP) (electronic supplementary material, tables S1 and S2). The samples comprised mainly arctic fox teeth and bones, and also one complete frozen carcass (sample IN18-002; electronic supplementary material, figure S4). The frozen carcass was recovered from permafrost deposits in Belaya Gora (Yakutia, Russia), and was sent for dating at the Oxford Radiocarbon Accelerator Unit.

DNA extractions from the historical as well as the comparatively well-preserved permafrost Pleistocene bone and tooth samples were conducted following the protocol described in Ersmark *et al.* [23]. The non-permafrost Pleistocene samples were extracted using a method optimized for highly degraded samples [24], and the skin and claw samples were extracted using a method optimized to digest keratin-rich tissues [25].

For the ancient and historical samples, DNA libraries were constructed according to the protocol by Meyer and Kircher [26], but with an additional step before the blunt-end repair. Here, the samples were incubated with the uracil-specific excision reagent (USER) enzyme to remove DNA damage that is characteristic for historical and ancient samples. The DNA libraries were subsequently amplified through polymerase chain reaction (PCR) (using 12 to 16 cycles) together with sample-specific indexing primers. To purify and simultaneously conduct a size selection of the post-amplified libraries, Agencourt AMPure XP beads were used.

The concentration of each library was measured on a Bioanalyzer2100 (Agilent) and the libraries were subsequently pooled in equimolar ratios before being sent to the Science for Life Laboratories (SciLifeLab) in Stockholm for paired-end shotgun sequencing on the Illumina HiSeq 2500 High Output V4 platform with a 2×125 bp setup.

To minimize the risk of contamination, all pre-PCR work on the ancient samples was done in a dedicated ancient DNA laboratory at the Swedish Museum of Natural History (NRM) following standard practices [27]. Similarly, the work on historical samples was done in a second laboratory dedicated to work on degraded historical DNA. Both of these laboratories are physically separated from the modern DNA and post-PCR laboratories.

In addition to the Pleistocene and historical samples, raw genomic data were generated from 21 modern Scandinavian samples, seven farm fox samples (from fur farms), two modern Russian samples and one modern Canadian sample (electronic supplementary material, table S3). The rationale for including farm foxes was partly that these originate from, and thus represent, wild populations in Svalbard and North America. In addition, an earlier study had found a control region haplotype shared between farm and historical Scandinavian foxes [20], and we were interested in resolving whether this result held up when extending the data to a mitogenomic dataset. DNA from 20 samples was extracted at NRM using the Thermo Scientific KingFisher Cell and Tissue DNA kit, while DNA from 11 samples was extracted at the Norwegian Institute for Nature Research (NINA), Trondheim, using the Qiagen DNeasy Blood & Tissue Kit. The samples were subsequently sent to SciLifeLab, Stockholm, and the Genomics Core Facility at the NTNU, Trondheim, for Illumina TruSeq PCR-free library construction and deep-sequencing on the Illumina HiSeq X and Illumina HiSeq 4000 platforms, respectively.

(b) Data processing

The resulting sequence data were processed following the approach in Pecnerova *et al.* [21]. In brief, the sequencing reads were trimmed of adapters and paired reads were merged using the software SEQPREP 2.2 (<http://github.com/jstjohn/SeqPrep>). The merged reads were then mapped to a reference mitochondrial genome of the arctic fox (GenBank accession no. NC_026529.1), using the BWA aln algorithm [28]. Next, the alignments were converted from SAM to BAM format, coordinate sorted, indexed, and PCR duplicates were removed using SAMtools [29] and a custom script [21]. Mapped reads were subsequently extracted and imported into GENEIOUS 8.1.6 [30]. Consensus sequences were called for all samples separately, using the majority call rule with a minimum coverage of at least three independent reads. To estimate endogenous DNA content in the Pleistocene and historical samples, the raw reads from each sample were mapped to an internally generated de novo assembly of the arctic fox genome (J. von Seth, K. Norén, A. Angerbjörn, L. Dalén 2016, unpublished data), and the proportion of reads that mapped to the reference genome prior to duplicate removal was estimated with QUALIMAP 2 [31].

The mapped mitochondrial reads were inspected manually in GENEIOUS and a BLAST-search was conducted of all reads that contributed to a mutation, in order to identify potentially falsely mapped nuclear mitochondrial segments (numts). Out of a total of 119 segregating sites, only one modern sample contained a mutation that appeared to be derived from a numt (at site 55; sample 5963; electronic supplementary material, table S3). This site was therefore deleted in all samples. Ten samples showed a heterozygote pattern within a tandem repeat region in the D-loop. All samples had equal ratios of G and C in the 13th position of the 14 bp tandem repeat. Owing to this, the

tandem repeat region (252 bp) was excluded from the subsequent data analyses.

(c) Computational analyses

A Bayesian phylogeny was reconstructed in BEAST 2.0 [32], using date calibrated tips, the HKY + I substitution model and a uniform prior of the mutation rate of the mitochondrial genome set to 6.1×10^{-8} to 1.0×10^{-7} site⁻¹ yr⁻¹ (calculated for grey wolf, *Canis lupus* [33]). The substitution model was selected according to the Bayesian information criterion in JMODELTEST2 [34]. We investigated genetic variability in arctic foxes between time periods, using a median-joining network created in POPART 1.7 [35]. We also estimated population genetic diversity parameters, such as haplotype and nucleotide diversity, with DNASP v5 [36]. Haplotype accumulation curves were constructed through random permutation subsampling without replacement using the functions haploAccum() and plot.haploAccum() supplied in the SPIDER package in R [37]. We also used an approximate Bayesian computation (ABC) approach [38,39] to estimate changes in female effective population size (N_{ef}) over the last 150 years. Our model included a single human-induced decline occurring some 90–120 years ago, a generation time of 3 years and mean rate ranging from 1.85×10^{-7} to 3.00×10^{-7} substitutions generation⁻¹ (electronic supplementary material, table S4; [33]). In the model, we set the sampling times for modern and historical samples at $t = 0$ generations and $t = 40$ generations in the past, respectively. We used seven summary statistics and estimated posterior parameter distributions by retaining the 10 000 datasets (1%) with the smallest Euclidean distances to the observed dataset.

3. Results

(a) Data processing and final datasets

Eleven out of the 26 Pleistocene samples and 20 out of the 30 historical samples had an endogenous DNA content above 10% (electronic supplementary material, tables S1 and S2). Among the non-permafrost Pleistocene samples, originating from mid-latitude Europe and Russia, one-third of the samples had endogenous DNA content above 10%. The average endogenous DNA content for the Pleistocene permafrost samples was 72% (electronic supplementary material, table S2). Samples with endogenous DNA content exceeding 10% are generally considered highly suitable for whole-genome sequencing, and these results, therefore, suggest that a large proportion of our samples could be used in future studies of autosomal genetic diversity.

The complete arctic fox carcass from Belaya Gora was dated to $18\,350 \pm 130$ radiocarbon years BP (radiocarbon laboratory reference OxA-38194), and was calibrated using the international calibration curve 'IntCal13' [40,41], to 22 468–21 887 (95.4%) cal BP.

The low-coverage sequencing, variable endogenous DNA content and high clonality in the Pleistocene samples did not permit reconstruction of entire mitogenomes from all the samples. Thus, in order to maximize the number of Pleistocene samples included in the comparisons, we compiled a reduced alignment comprising 3954 nucleotide sites (at greater than 3x coverage). However, to obtain an even more accurate estimate of the loss of mitogenomic diversity during the last 100 years, we also constructed a second alignment that comprised 13 224 sites (at greater than 3x coverage), which excluded all Pleistocene samples. Taken

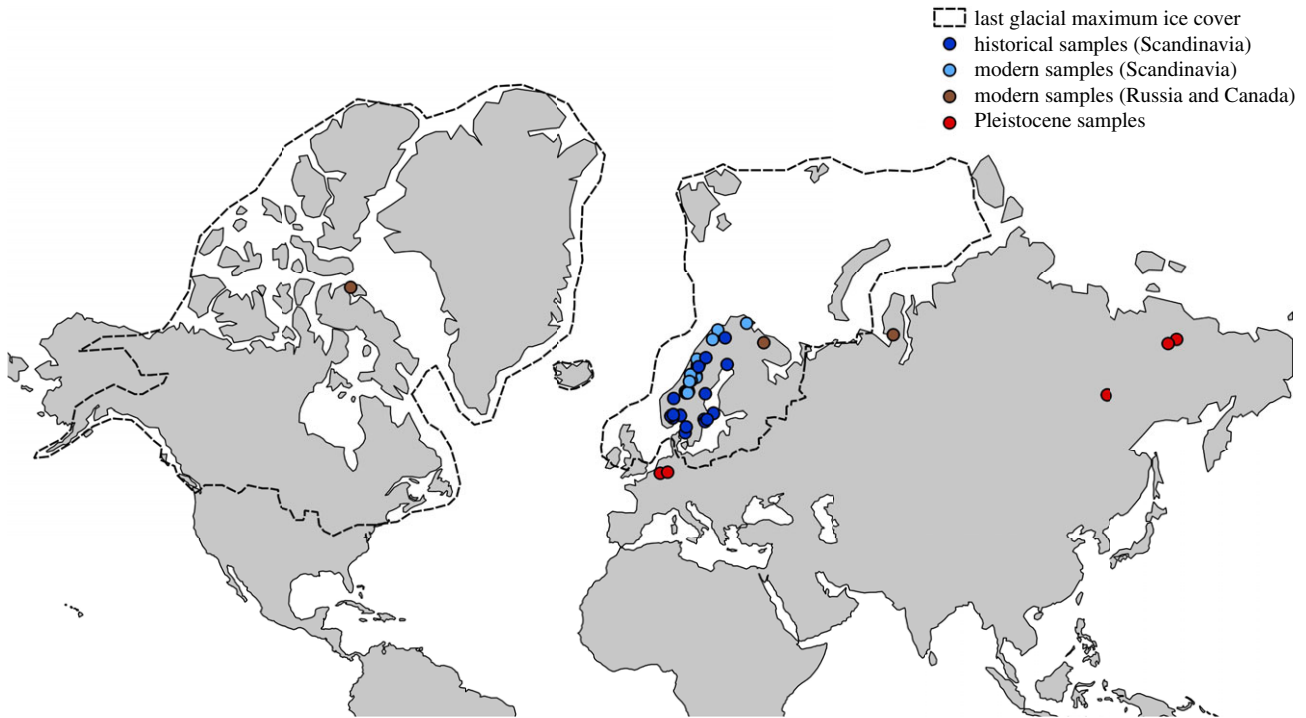


Figure 1. Geographical distribution of the samples used in this study. Light and dark blue dots represent modern and historical samples, respectively. Red dots represent Pleistocene samples and brown dots represent modern samples from Canada and Russia. Striped black lines represent the ice cover during the last glacial maximum [42]. (Online version in colour.)

together, both datasets comprised five Pleistocene samples (two from Western Europe and three from Beringia), 16 historical samples, and all 24 modern samples (including the modern samples from Russia and Canada), plus seven modern farm fox samples (figure 1).

(b) Phylogeny and median-joining network

The Bayesian phylogeny (figure 2) suggested that the most recent common female ancestor lived *ca* 87 000 years BP (mean: 87 038 BP; 95% highest posterior density (HPD) = 44 571–153 339 BP). The phylogeny also revealed that all present-day, and most Late Pleistocene, mitogenome haplotypes can be divided into two separate clades with a posterior probability node support of 0.95. The split into these two clades was estimated to have occurred *ca* 66 000 years BP (mean: 65 756 BP; 95% HPD = 33 196–122 310 BP). There was no apparent phylogeographic structure within or between the clades. Clade A included historical and modern Scandinavian, Canadian and farm arctic foxes, and Pleistocene Russian and Pleistocene western European arctic foxes. Interestingly, the mitogenomic dataset allowed us to demonstrate that historical Scandinavian and farm foxes do not share the same haplotype, meaning that free-ranging present-day foxes with the farm fox haplotype now can be conclusively assigned as having farm fox ancestry (see [43]). Clade B included modern and historical Scandinavian, modern Russian, Pleistocene Russian and Pleistocene western European arctic foxes (figure 2). The median-joining haplotype network illustrates the separate clades, with the Pleistocene Russian haplotype C1 placed in an intermediary position between the two clades (figure 3).

(c) Changes in mitogenomic variation and N_{ef} in Scandinavia

In Scandinavia, we identified 10 and five haplotypes in the historical and modern samples, respectively, in the alignment

comprising 13 224 bp of the mitochondrial genome (electronic supplementary material, figures S1 and S5). Using the shorter alignment including the Pleistocene samples, we found eight and three haplotypes in the historical and modern populations, respectively (figures 3 and 4). The loss of haplotypes in the Scandinavian arctic fox population also resulted in a significant decline in nucleotide diversity ($\pi \pm$ s.e.) (0.00245 ± 0.00008 versus 0.00211 ± 0.00006 ; $t_{37} = 3.39$, $p < 0.01$) and haplotype diversity ($Hd \pm$ s.e.) (0.917 ± 0.012 versus 0.762 ± 0.014 ; $t_{37} = 7.85$, $p < 0.001$) (figure 5). In the modern population, three haplotypes were shared with the historical population, while two haplotypes were private: A10 was found in two samples from Lierne and two samples from Borga, and B9 was found in two samples from Vindelfjällen and two samples from Borga (electronic supplementary material, figure S1 and table S3). The ABC analysis supported an almost 20-fold reduction in female effective population size with N_{ef} declining from approximately 3650 to approximately 220 over the last 150 years (electronic supplementary material, table S4).

4. Discussion

(a) Consequences of past climate change

In contrast to earlier studies, the recovery of a mitogenomic dataset allowed us to generate a robust phylogeny and an assessment of divergence times among lineages. This showed that the most recent female common ancestor of the arctic fox probably lived less than 100 000 BP (figure 2). This is consistent with previous suggestions that the arctic fox went through a bottleneck during the warm Eemian interglacial [44], which had its peak *ca* 125 000 BP with average temperatures in the Arctic about 2–5 degrees warmer than today [45]. Our results also showed that the

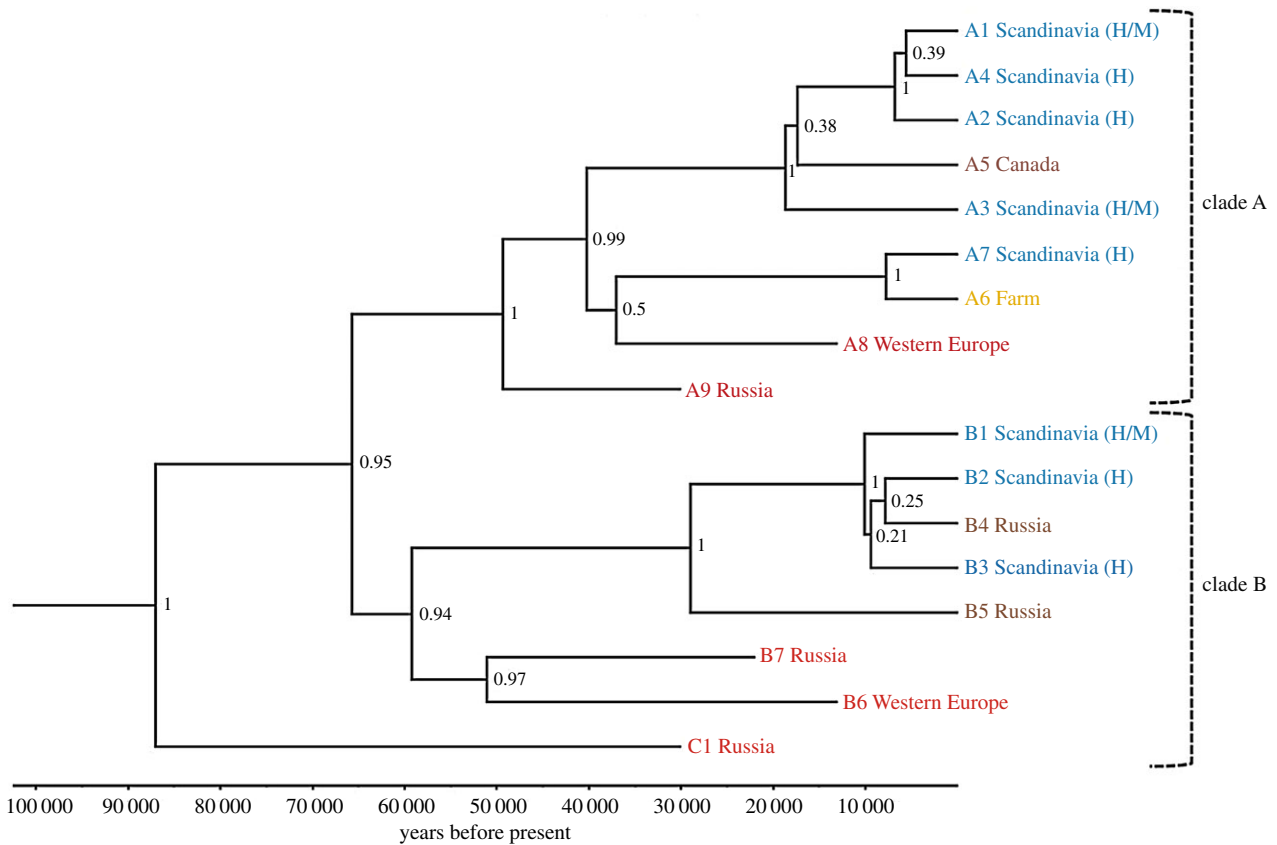


Figure 2. Phylogeny of arctic fox haplotypes based on 3954 bp of the mitogenome. Haplotypes are labelled after clade and location, where Scandinavian haplotypes found in historical samples are indicated with an H and modern samples with an M. Posterior values for node support are shown for each node. (Online version in colour.)

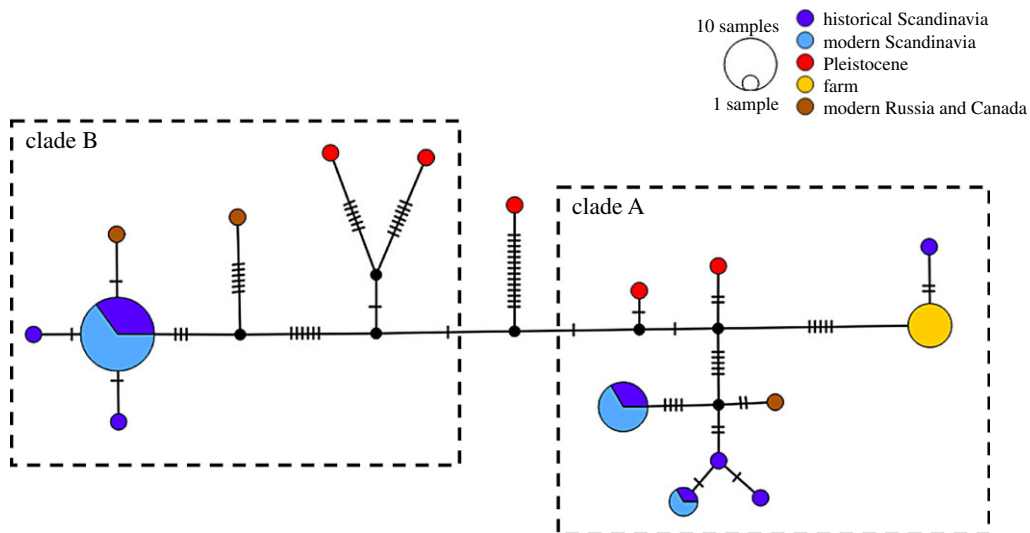


Figure 3. Median-joining haplotype network based on 3954 bp of the mitogenome. Small crossing bars represent mutations between haplotypes. Colours correspond to sample population. Dotted lines indicate clade A and clade B as identified in the BEAST analysis (figure 2). (Online version in colour.)

mitogenomes in present-day arctic foxes can be divided into two distinct clades, which diversified approximately 60 000 years ago. The reason for this diversification into two clades remains to be investigated, but could be related to the onset of rapid stadial-interstadial fluctuations in temperature during Marine Isotope Stage 3 [46]. Moreover, it is noteworthy that there seems to have been no clear phylogeographical structure during at least the last 30 000

years, which probably is a result of the high dispersal capability of the arctic fox (see also [44]). Even though the large ice sheets covering much of northern Europe and North America probably were a poor habitat for arctic foxes, these animals are capable of long movements over the polar pack ice [47]. They would therefore still have been able to disperse and admix throughout the circumpolar area during the Late Pleistocene [48].

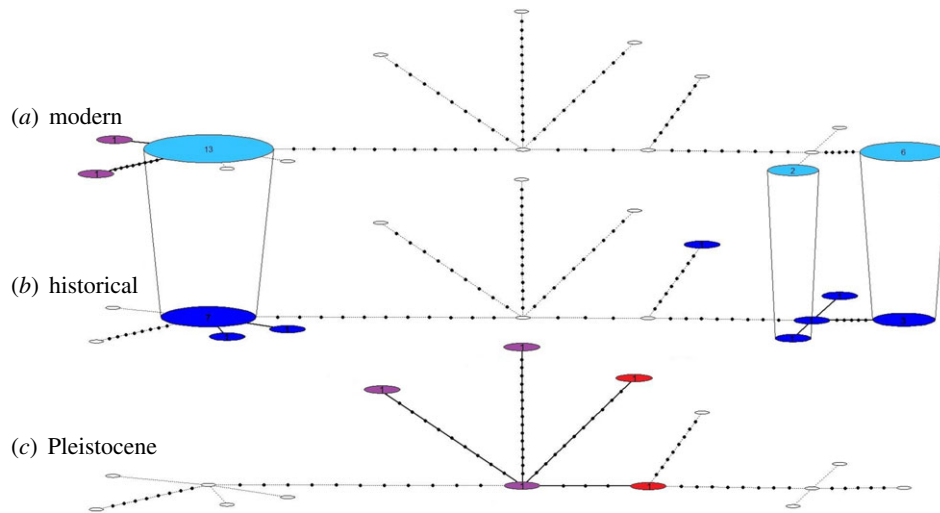


Figure 4. Temporal haplotype network of samples from Eurasia based on 3954 bp of the mitogenome. Circles represent haplotypes and numbers represent sample sizes. (a) Haplotypes found in modern-day Eurasia. (b) Haplotypes found in the historical Scandinavian population. (c) Pleistocene haplotypes. Mid-latitude European samples are shown in red, Russian samples are shown in purple, whereas Scandinavian historical and modern samples are shown in dark and light blue, respectively. Empty circles represent haplotypes absent in the given time period. Haplotypes found in multiple time periods are connected with vertical lines. (Online version in colour.)

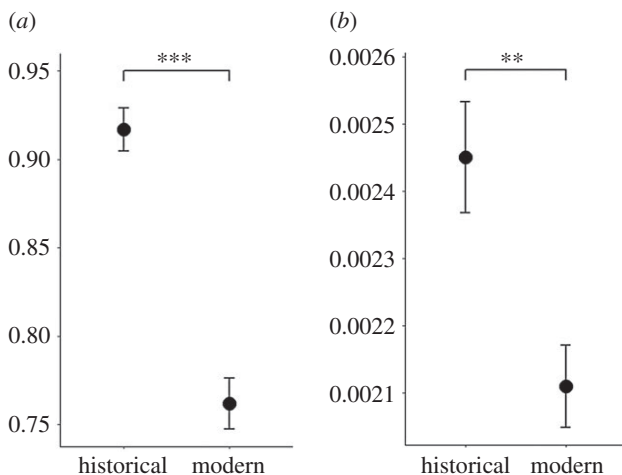


Figure 5. Haplotype diversity (a) and nucleotide diversity (b) in the historical and contemporary Scandinavian arctic fox populations. Asterisks indicate significant differences among time periods, where $**p < 0.01$ and $***p < 0.001$. Error bars depict standard error.

The two ancient western European samples, which are approximately 12 000 years old, were not closely related to any of the modern or historical samples (figures 2 and 4). While these two samples do not by themselves permit a comprehensive assessment of the mid-latitude European population's genetic diversity, when combined with the results from Dalén *et al.* [10] they reinforce the conclusion that the mid-latitude European population did not contribute to the present-day mitochondrial diversity in the arctic fox. Together, these studies therefore support the hypothesis that the mid-latitude European arctic fox population went extinct at the end of the last glaciation, and thus that the species' range contraction was driven by local extinction events rather than postglacial habitat tracking.

An alternative hypothesis is that the mid-European population did in fact recolonize the Scandinavian Peninsula during the early Holocene, when previously glaciated areas became suitable habitats, but that it subsequently went

extinct later in the Holocene. Subfossil records of arctic foxes in Norway suggest a gap in the fossil record between *ca* 9000 BP and *ca* 5000 BP, coinciding with a warm period in the mid-Holocene. It has been hypothesized that the arctic fox disappeared from the Scandinavian Peninsula during this period, and that another recolonization event occurred around 5000 BP [49]. Although this scenario would also imply that the arctic foxes failed to track the climate-induced changes in habitat, it is thus uncertain whether this occurred during the Pleistocene/Holocene transition or during the subsequent Holocene warm peak. To examine this further, genetic data from postglacial Scandinavian samples that are older than 9000 BP would be needed.

Intriguingly, and contrary to our expectations, the Pleistocene Russian samples from Beringia were also genetically distinct from the present-day population in Scandinavia (figures 2 and 4). It should be noted that the small sample size ($n = 3$) does not allow us to exclude that the Scandinavian population originates from the Beringian Late Pleistocene population. However, although speculative, these preliminary findings could suggest that the postglacial recolonization of Scandinavia originated from another, yet unsampled, Late Pleistocene population of arctic foxes, which in turn could also imply that a local extinction/recolonization event may have taken place in Beringia at the end of the last glaciation. This hypothesis is consistent with earlier findings of end-Pleistocene population turnover in several other Arctic taxa in Beringia [5,6].

(b) Consequences of recent human persecution

Our results show that the Scandinavian arctic fox population lost at least 50% of its mitochondrial haplotypes as a result of the demographic bottleneck that started at the turn of the twentieth century (figure 4; electronic supplementary material figure S2). Moreover, compared to previous studies [20], the higher resolution of the mitogenomic sequences allowed for better estimations of both haplotype and nucleotide diversity, revealing previously unknown significant

losses following the bottleneck (figure 5). The coalescent simulations coupled with ABC analyses suggested an approximately 20-fold decline in N_{ef} , which is roughly in line with expectations based on historical records [16,17].

We found five previously unknown haplotypes, three in the historical population and two in the contemporary population. The two new haplotypes found in the modern population were not shared with the historical population. A possible explanation for these private haplotypes in the modern population could be insufficient sampling in the historical population. Haplotype accumulation curves suggested that the asymptote for the modern population was reached at five haplotypes, while the haplotype accumulation curve for the historical population failed to reach the asymptote (electronic supplementary material, figure S2). Further sampling might thus identify these two modern haplotypes (A10 and B9; electronic supplementary material, figure S1 and table S3) in historical samples. It is also possible that the private haplotypes are the result of random mutations. However, taking the mutation rate of the mitochondrial genome into account, it seems highly unlikely that this would have happened in two individuals in less than 150 years. A third possible explanation would be recent immigration events from the population in Russia, which would be in line with results from previous studies [20,50].

Finding only 10 haplotypes in the pre-bottleneck population may seem relatively low, even when taking into account that further sampling probably would have revealed additional haplotypes (electronic supplementary material, figure S2). Such a low number of haplotypes could be a consequence of Scandinavia being an isolated peninsula with a recent glaciation history. Populations on peninsulas generally have less genetic diversity than mainland populations [51,52]. Moreover, populations in previously glaciated regions are expected to have a reduced genetic diversity because there has not been enough time since colonization to reach mutation-drift equilibrium (e.g. [53]). Indeed, results from studies on other historical Scandinavian carnivore populations (pre-dating recent bottlenecks) have shown that the Scandinavian grey wolf, brown bear (*Ursus arctos*), lynx (*Lynx lynx*) and wolverine (*Gulo gulo*) also have relatively low levels of mitochondrial diversity [54–57]. Thus, the relatively recent colonization of the region, which started with the contraction of the Scandinavian ice sheet at the end of the last glaciation, combined with a small number of founders and comparative isolation of Scandinavia as a peninsula, could explain the low diversity of the pre-bottleneck Scandinavian arctic fox population.

(c) Conservation implications

The distribution of the Arctic biota decreased considerably during the transition from the last glaciation to the present interglacial. Our results suggest that a contraction in available habitat (during the Pleistocene/Holocene transition or the mid-Holocene) caused local subpopulations to become extinct, which in turn led to losses of unique genetic diversity. This implies that the genetic diversity in many Arctic species probably was reduced even before the impact of human activities that accelerated during the industrial revolution. This might have affected the adaptive potential in the arctic fox as well as other cold-adapted species, thus increasing the impact of subsequent anthropogenic activities.

Furthermore, there are suggestions that climate change has again started to affect the Scandinavian arctic fox population in recent decades, through an expansion and ensuing inter-specific competition from red foxes that have moved into mountain areas previously inhabited by the arctic fox [58]. Assuming that these consecutive losses of mitogenomic diversity, owing to climate change and anthropogenic impact, also reflect changes in autosomal genetic diversity, this would imply that the arctic fox might be particularly vulnerable to the future challenges entailed by climate change.

Importantly, these findings indicate that climate-driven range contractions do not simply lead to a reduction in population size, but also that local populations become extinct without contributing to the gene pool of populations further north. This implies that future climate-induced range contractions may lead to larger losses of genetic diversity than predicted from simplistic models based solely on population size. Moreover, unique genetic variants (e.g. local adaptations) in southern populations will probably be lost as these populations become extinct. At a broader level, such a lack of habitat tracking needs to be accounted for in climate-based models that predict future extinction rates [59], and raises a concern about the efficiency of conservation corridors [60] designed to enable species to move into new habitats as the climate changes.

In the case of the Scandinavian arctic fox, this population is currently fragmented into several relatively isolated subpopulations along the Scandes mountain range [50]. Our results suggest that future changes in tree-line extent and red fox distribution will probably lead to a northwards contraction of the arctic fox's distribution, and thus a loss of any genes unique to the southernmost subpopulations.

Data accessibility. The mtDNA data are available in the form of BAM files, which can be downloaded at the European Nucleotide Archive (EA) under accession number PRJEB34472.

Authors' contributions. P.L., J.v.S., A.G. and L.D. conceived and designed the study. P.L., J.v.S., I.J.H., N.B. conducted laboratory analyses, where acquisition of data was contributed by S.A., M.G., S.F., N.E.E., N.S., D.B., A.A., Ø.F., V.P. and K.N. Computational analyses of the data were done by P.L. and N.D., with assistance from D.D.d.M. and D.W.G.S. P.L. wrote the manuscript, with input from all coauthors.

Competing interests. The authors have no competing interests.

Funding. D.W.G.S. acknowledges support from a Marie Skłodowska-Curie Intra-European Fellowship (grant no. 796877). D.D.d.M. was supported through a Carl Trygger's scholarship (grant no. CTS17:109). N.S. was supported by the Russian Foundation of Basic Research (RFBR) through grant no. Arctic-18-05-60261. The genetic analyses were funded by Stockholm University, a grant from the Norwegian Research Council (project no. 244557/E50) and two grants from Formas (project nos 2015-676 and 2018-01640).

Acknowledgements. The authors thank Verena Kutschera and Marcin Kierczak at the National Bioinformatics Infrastructure Sweden for bioinformatics support. We also acknowledge support from the Science for Life Laboratory, the National Genomics Infrastructure, and UPPMAX for providing assistance in massive parallel sequencing and computational infrastructure. We especially thank Øystein Wiig at the Natural History Museum in Oslo, Daniela Kalthoff at the Swedish Museum of Natural History and Friederike Johansson at the Natural History Museum of Gothenburg for helping out with sampling. We are also grateful to the Royal Belgian Institute of Natural Sciences in Brussels, the Natural History Museum in London, the Natural History Museum of Bern and the Russian Academy of Sciences for making their collections available for sampling.

- Caughley G. 1994 Directions in conservation biology. *J. Anim. Ecol.* **63**, 215–244. (doi:10.2307/5542)
- Díez-del-Molino D, Sánchez-Barreiro F, Barnes I, Gilbert MTP, Dalén L. 2018 Quantifying temporal genomic erosion in endangered species. *Trends Ecol. Evol.* **33**, 176–185. (doi:10.1016/j.tree.2017.12.002)
- Shapiro B *et al.* 2004 Rise and fall of the Beringian steppe bison. *Science* **306**, 1561–1565. (doi:10.1126/science.1101074)
- Brace S *et al.* 2012 Serial population extinctions in a small mammal indicate Late Pleistocene ecosystem instability. *Proc. Natl Acad. Sci. USA* **109**, 20 532–20 536. (doi:10.1073/pnas.1213322109)
- Leonard JA, Vilà C, Fox-Dobbs K, Koch PL, Wayne RK, Van Valkenburgh B. 2007 Megafaunal extinctions and the disappearance of a specialized wolf ecomorph. *Curr. Biol.* **17**, 1146–1150. (doi:10.1016/j.cub.2007.05.072)
- Palkopoulou E *et al.* 2016 Synchronous genetic turnovers across western Eurasia in Late Pleistocene collared lemmings. *Glob. Chang. Biol.* **22**, 1710–1721. (doi:10.1111/gcb.13214)
- Lagerholm VK *et al.* 2014 On the origin of the Norwegian lemming. *Mol. Ecol.* **23**, 2060–2071. (doi:10.1111/mec.12698)
- Eldredge N. 1989 *Macroevolutionary dynamics*. New York, NY: McGraw-Hill.
- Lagerholm VK *et al.* 2017 Range shifts or extinction? Ancient DNA and distribution modelling reveal past and future responses to climate warming in cold-adapted birds. *Glob. Chang. Biol.* **23**, 1425–1435. (doi:10.1111/gcb.13522)
- Dalén L, Nyström V, Valdiosera C, Germonpré M, Sablin M, Turner E, Angerbjörn A, Arsuaga JL, Götherström A. 2007 Ancient DNA reveals lack of postglacial habitat tracking in the arctic fox. *Proc. Natl Acad. Sci. USA* **104**, 6726–6729. (doi:10.1073/pnas.0701341104)
- Ceballos G, Ehrlich PR, Barnosky AD, García A, Pringle RM, Palmer TM. 2015 Accelerated modern human-induced species losses: entering the sixth mass extinction. *Sci. Adv.* **1**, e1400253. (doi:10.1126/sciadv.1400253)
- Wandeler P, Hoecx PEA, Keller LF. 2007 Back to the future: museum specimens in population genetics. *Trends Ecol. Evol.* **22**, 634–642. (doi:10.1016/j.tree.2007.08.017)
- Sommer R, Benecke N. 2005 Late-Pleistocene and early Holocene history of the canid fauna of Europe (Canidae). *Mamm. Biol.* **70**, 227–241. (doi:10.1016/j.mambio.2004.12.001)
- Audet AM, Robbins CB, Larivière S. 2002 *Alopex lagopus*. *Mamm. Spec.* **31**, 1–10. (doi:10.1644/1545-1410(2002)713<0001:AL>2.0.CO;2)
- Westling A. 2015 *Rödlistade arter i sverige 2015*. Uppsala, Sweden: ArtDatabanken SLU.
- Tannerfeldt M. 1997 *Population fluctuations and life history consequences in the arctic fox*. Stockholm, Sweden. Department of Zoology, Stockholm University.
- Lönnberg E. 1927 Fjällrävstammen i Sverige 1926. *Kungliga Svenska Vetenskapsakademiens skrifter i naturskyddsärenden 7*. Stockholm, Sweden: The Royal Swedish Academy of Sciences.
- Ulvund K, Wallén J. 2018 Övervakning av fjellrev 2018. Norsk Institutt for Naturforskning (NINA) and Naturhistoriska riksmuseet (NRM).
- Angerbjörn A *et al.* 2013 Carnivore conservation in practice: replicated management actions on a large spatial scale. *J. Appl. Ecol.* **50**, 59–67. (doi:10.1111/1365-2664.12033)
- Nyström V, Angerbjörn A, Dalén L. 2006 Genetic consequences of a demographic bottleneck in the Scandinavian arctic fox. *Oikos* **114**, 84–94. (doi:10.1111/j.2006.0030-1299.14701.x)
- Pečnerová P *et al.* 2017 Mitogenome evolution in the last surviving woolly mammoth population reveals neutral and functional consequences of small population size. *Evol. Lett.* **1**, 292–303. (doi:10.1002/evl3.33)
- Posth C *et al.* 2016 Pleistocene mitochondrial genomes suggest a single major dispersal of non-Africans and a late glacial population turnover in Europe. *Curr. Biol.* **26**, 827–833. (doi:10.1016/j.cub.2016.01.037)
- Ersmark E, Orlando L, Sandoval-Castellanos E, Barnes I, Barnett R, Stuart A, Lister AM, Dalén L. 2015 Population demography and genetic diversity in the Pleistocene cave lion. *Open Quat.* **1**, 1–14. (doi:10.5334/oq.aa)
- Dabney J *et al.* 2013 Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proc. Natl Acad. Sci. USA* **110**, 15 758–15 763. (doi:10.1073/pnas.1314445110)
- Gilbert MTP *et al.* 2007 Whole-genome shotgun sequencing of mitochondria from ancient hair shafts. *Science* **317**, 1927–1930. (doi:10.1126/science.1146971)
- Meyer M, Kircher M. 2010 Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harb. Protoc.* **2010**, db.prot5448. (doi:10.1101/pdb.prot5448)
- Gilbert MTP, Bandelt H-J, Hofreiter M, Barnes I. 2005 Assessing ancient DNA studies. *Trends Ecol. Evol.* **20**, 541–544. (doi:10.1016/j.tree.2005.07.005)
- Li H, Durbin R. 2009 Fast and accurate short read alignment with Burrows–Wheeler transform. *Bioinformatics* **25**, 1754–1760. (doi:10.1093/bioinformatics/btp324)
- Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. 2009 The sequence alignment/map format and SAMtools. *Bioinformatics* **25**, 2078–2079. (doi:10.1093/bioinformatics/btp352)
- Kearse M *et al.* 2012 Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**, 1647–1649. (doi:10.1093/bioinformatics/bts199)
- Okonechnikov K, Conesa A, García-Alcalde F. 2016 Qualimap 2: advanced multi-sample quality control for high-throughput sequencing data. *Bioinformatics* **32**, 292–294. (doi:10.1093/bioinformatics/btv566)
- Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C-H, Xie D, Suchard MA, Rambaut A, Drummond AJ. 2014 BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Comput. Biol.* **10**, e1003537. (doi:10.1371/journal.pcbi.1003537)
- Koblmüller S, Vilà C, Lorente-Galdos B, Dabad M, Ramirez O, Marques-Bonet T, Wayne RK, Leonard JA. 2016 Whole mitochondrial genomes illuminate ancient intercontinental dispersals of grey wolves (*Canis lupus*). *J. Biogeogr.* **43**, 1728–1738. (doi:10.1111/jbi.12765)
- Darriba D, Taboada GL, Doallo R, Posada D. 2012 jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods* **9**, 772. (doi:10.1038/nmeth.2109)
- Bandelt HJ, Forster P, Rohl A. 1999 Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* **16**, 37–48. (doi:10.1093/oxfordjournals.molbev.a026036)
- Librado P, Rozas J. 2009 DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**, 1451–1452. (doi:10.1093/bioinformatics/btp187)
- Brown SDJ, Collins RA, Boyer S, Lefort M-C, Malumbres-Olarte J, Vink CJ, Cruickshank RH. 2012 Spider: an R package for the analysis of species identity and evolution, with particular reference to DNA barcoding. *Mol. Ecol. Resour.* **12**, 562–565. (doi:10.1111/j.1755-0998.2011.03108.x)
- Beaumont MA, Zhang W, Balding DJ. 2002 Approximate Bayesian computation in population genetics. *Genetics* **162**, 2025–2035.
- Cornuet J-M, Pudlo P, Veysier J, Dehne-Garcia A, Gautier M, Leblois R, Marin J-M, Estoup A. 2014 DIYABC v2.0: a software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphism, DNA sequence and microsatellite data. *Bioinformatics* **30**, 1187–1189. (doi:10.1093/bioinformatics/btt763)
- Reimer PJ *et al.* 2013 IntCal13 and Marine13 radiocarbon age calibration curves 0–50,000 years cal BP. *Radiocarbon* **55**, 1869–1887. (doi:10.2458/azu_js_rc.55.16947)
- Ramsey CB, Lee S. 2013 Recent and planned developments of the program OxCal. *Radiocarbon* **55**, 720–730. (doi:10.1017/S003822200057878)
- Ehlers J, Gibbard P. 2011 Quaternary glaciation. In *Encyclopedia of snow, ice and glaciers* (eds VP Singh, P Singh, UK Haritashya), pp. 873–882. Dordrecht, The Netherlands: Springer.
- Norén K, Dalén L, Kvåløy K, Angerbjörn A. 2005 Detection of farm fox and hybrid genotypes among wild arctic foxes in Scandinavia. *Conserv. Genet.* **6**, 885–894. (doi:10.1007/s10592-005-9075-8)
- Dalén L, Fuglei E, Hersteinsson P, Kapel CMO, Roth JD, Samelius G, Tannerfeldt M, Angerbjörn A. 2005

- Population history and genetic structure of a circumpolar species: the arctic fox. *Biol. J. Linn. Soc. Lond.* **84**, 79–89. (doi:10.1111/j.1095-8312.2005.00415.x)
45. Funder S, Hjort C, Landvik JY, Nam S-I, Reeh N, Stein R. 1998 History of a stable ice margin: east Greenland during the Middle and Upper Pleistocene. *Quat. Sci. Rev.* **17**, 77–123. (doi:10.1016/S0277-3791(97)00082-6)
46. Van Meerbeeck CJ, Renssen H, Roche DM. 2008 How did Marine Isotope Stage 3 and Last Glacial Maximum climates differ? Perspectives from equilibrium simulations. *Clim. Past Discuss.* **4**, 1115–1158. (doi:10.5194/cpd-4-1115-2008)
47. Tarrowx A, Berteaux D, Bêty J. 2010 Northern nomads: ability for extensive movements in adult arctic foxes. *Polar Biol.* **33**, 1021–1026. (doi:10.1007/s00300-010-0780-5)
48. Norén K, Carmichael L, Dalén L, Hersteinsson P. 2011 Arctic fox *Vulpes lagopus* population structure: circumpolar patterns and processes. *Oikos* **120**, 873–885. (doi:10.1111/j.1600-0706.2010.18766.x)
49. Frafjord K, Hufthammer AK. 1994 Subfossil records of the arctic fox (*Alopex lagopus*) compared to its present distribution in Norway. *Arctic* **47**, 65–68. (doi:10.14430/arctic1272)
50. Dalén L *et al.* 2006 Population structure in a critically endangered arctic fox population: does genetics matter? *Mol. Ecol.* **15**, 2809–2819. (doi:10.1111/j.1365-294X.2006.02983.x)
51. Randi E, Lucchini V, Christensen MF, Mucci N, Funk SM, Dolf G, Loeschcke V. 2000 Mitochondrial DNA variability in Italian and East European wolves: detecting the consequences of small population size and hybridization. *Conserv. Biol.* **14**, 464–473. (doi:10.1046/j.1523-1739.2000.98280.x)
52. Vilà C, Amorim IR, Leonard JA, Posada D. 1999 Mitochondrial DNA phylogeography and population history of the grey wolf *Canis lupus*. *Mol. Ecol.* **8**, 2089–2103. (doi:10.1046/j.1365-294x.1999.00825.x)
53. Sage RD, Wolff JO. 1986 Pleistocene glaciations, fluctuating ranges, and low genetic variability in a large mammal (*Ovis dalli*). *Evolution* **40**, 1092–1095. (doi:10.1111/j.1558-5646.1986.tb00576.x)
54. Flagstad Ø, Walker CW, Vilà C, Sundqvist A-K, Fernholm B, Hufthammer AK, Wiig Ø, Koyola I, Ellegren H. 2003 Two centuries of the Scandinavian wolf population: patterns of genetic variability and migration during an era of dramatic decline. *Mol. Ecol.* **12**, 869–880. (doi:10.1046/j.1365-294X.2003.01784.x)
55. Xenikoudakis G, Ersmark E, Tison J-L, Waits L, Kindberg J, Swenson JE, Dalén L. 2015 Consequences of a demographic bottleneck on genetic structure and variation in the Scandinavian brown bear. *Mol. Ecol.* **24**, 3441–3454. (doi:10.1111/mec.13239)
56. Hellborg L *et al.* 2002 Differentiation and levels of genetic variation in northern European lynx (*Lynx lynx*) populations revealed by microsatellites and mitochondrial DNA analysis. *Conserv. Genet.* **3**, 97–111. (doi:10.1023/A:1015217723287)
57. Walker CW, Vilà C, Landa A, Lindén M, Ellegren H. 2001 Genetic variation and population structure in Scandinavian wolverine (*Gulo gulo*) populations. *Mol. Ecol.* **10**, 53–63. (doi:10.1046/j.1365-294X.2001.01184.x)
58. Elmhagen B *et al.* 2017 Homage to Hersteinsson and Macdonald: climate warming and resource subsidies cause red fox range expansion and arctic fox decline. *Polar Res.* **36**, 3. (doi:10.1080/17518369.2017.1319109)
59. Thomas CD *et al.* 2004 Extinction risk from climate change. *Nature* **427**, 145–148. (doi:10.1038/nature02121)
60. Mawdsley JR, O'malley R, Ojima DS. 2009 A review of climate-change adaptation strategies for wildlife management and biodiversity conservation. *Conserv. Biol.* **23**, 1080–1089. (doi:10.1111/j.1523-1739.2009.01264.x)