Ancient DNA reveals lack of postglacial habitat tracking in the arctic fox

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How species respond to an increased availability of habitat, for example at the end of the last glaciation, has been well established. In contrast, little is known about the opposite process, when the amount of habitat decreases. The hypothesis of habitat tracking predicts that species should be able to track both increases and decreases in habitat availability. The alternative hypothesis is that populations outside refugia become extinct during periods of unsuitable climate. To test these hypotheses, we used ancient DNA techniques to examine genetic variation in the arctic fox (*Alopex lagopus*) through an expansion/contraction cycle. The results show that the arctic fox in midlatitude Europe became extinct at the end of the Pleistocene and did not track the habitat when it shifted to the north. Instead, a high genetic similarity between the extant populations in Scandinavia and Siberia suggests an eastern origin for the Scandinavian population at the end of the last glaciation. These results provide new insights into how species respond to climate change, since they suggest that populations are unable to track decreases in habitat avaliability. This implies that arctic species may be particularly vulnerable to increases in global temperatures.

climate change | evolutionary stasis | extinction | phylogeography | postglacial recolonization

The glacial cycles have influenced the distribution of organisms worldwide (1). Whereas cold periods have generally forced temperate species in North America and Europe to endure repeated isolations in southern refugia, the warmer interglacials have allowed them to expand northwards to recolonize previously glaciated regions (2). Genetic analysis has in recent years been used to study the patterns of postglacial recolonization after the last glaciation, where the genetic composition of recently recolonized regions is compared with that of refugial populations (2, 3).

However, this approach is of limited value if one or several glacial populations have gone extinct during the Holocene. Moreover, although phylogeographic inference based on modern samples can be used to investigate the process of recolonization, it cannot be used to directly study the process by which species contract. Current knowledge about this process is therefore limited, despite its importance for evolutionary theory. Darwin suggested that when species "moved first southward and afterward backwards to the north, in unison with the changing climate, they will not have been exposed during their long migration to any great diversity of temperature [..]. Hence, in accordance with the principles inculcated in this volume, these forms will not have been liable to much modification" (4). The idea that species are able to increase their distribution during periods of suitable climate and, in a similar way, literally contract when climate conditions become unsuitable was later formulated as the hypothesis of habitat tracking (5), which was proposed as an explanation for the high degree of evolutionary stasis observed in the fossil record (6). The alternative to habitat tracking during the contraction phase would be that populations inhabiting areas outside refugia become extinct when climate deteriorates (3, 7).

One possible approach to test these two hypotheses is to use ancient DNA technology to investigate if populations that inhabited geographic regions outside refugia during the expansion phase have contributed to the genetic composition of the refugial populations. For temperate species, this would require DNA to be recovered from samples predating the last interglacial, which would be problematic since samples of that age are on the limits of ancient DNA recovery. Arctic species, on the other hand, expand during glacials and contract during interglacials (8, 9). Testing the hypothesis of habitat tracking on an arctic species would thus be possible by comparing the genetic variation of southern Late Pleistocene populations with that of contemporary northern populations.

We have analyzed genetic variation in the arctic fox (*Alopex lagopus*), which had a large distribution during the last glacial maximum (LGM) and inhabited large parts of central and northeastern Eurasia (10–15). Today, the arctic fox is restricted to the tundra regions in the northern hemisphere, including regions that were glaciated during the LGM, such as Scandinavia (16). There are three possible hypotheses for the origin of the Scandinavian arctic fox: (*i*) Scandinavia was colonized from the south by foxes tracking the retreating ice edge at the end of the LGM, (*ii*) Scandinavia was colonized by foxes expanding from the ice-free regions in the east (e.g., Beringia) after the end of the LGM, and (*iii*) the arctic fox survived the LGM in a local Scandinavian refugium (17). These three scenarios are all plausible because Scandinavia has been colonized from both the south and east by other species (3), and LGM survival in a local refugium has been suggested for the Norwegian lemming (*Lemmus lemmus*) (18).

We addressed these hypotheses by comparing mitochondrial DNA (mtDNA) sequences retrieved from Late Pleistocene arctic foxes in midlatitude Europe with those from extant Siberian and Scandinavian arctic foxes. Furthermore, to avoid confounding effects of a recent bottleneck in Scandinavia caused by human overexploitation 100 years ago, which may have altered the population's genetic composition, we also included 42 prebottleneck samples from Scandinavia (19). For hypotheses
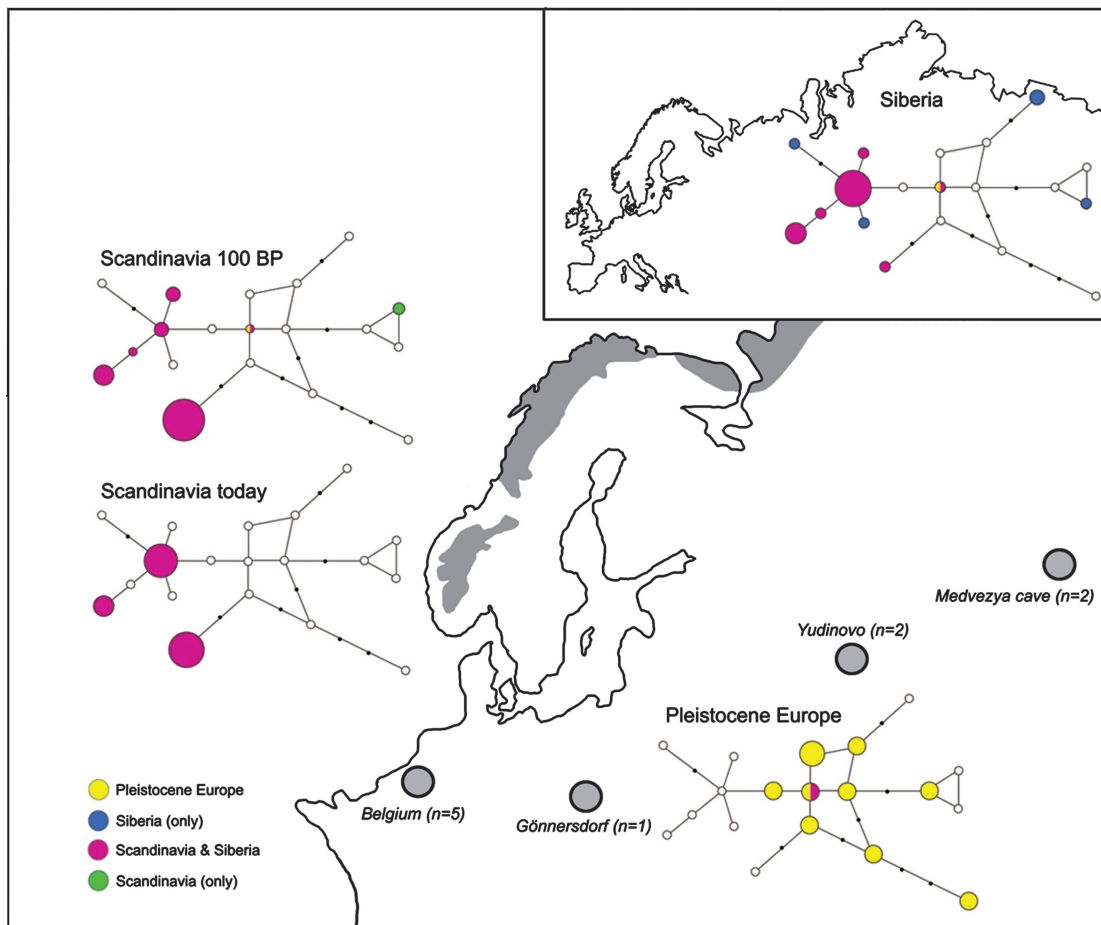
Fig. 1. Minimum spanning networks for the sampled populations. The size of each haplotype illustrates its relative frequency. Each branch represents one mutational step. Haplotypes missing in a particular population are shown with small unfilled circles, whereas overall missing haplotypes are shown with black dots. Gray areas represent the current distribution of arctic fox habitat, whereas gray circles indicate sample sites for Late Pleistocene samples.

(*i*) and (*ii*), we would expect to find Scandinavian mitochondrial DNA haplotypes that are identical to, or recently derived from, the haplotypes in the source population (2, 3), whereas hypothesis (*iii*) predicts that several unique haplotypes should have evolved in Scandinavia (18).

Results and Discussion

Late Pleistocene fossils were sampled from four locations in midlatitude Europe [Fig. 1; and see supporting information (SI) Table 1]. We used ancient DNA technology to retrieve 292 bp of the mitochondrial control region from seven specimens, and 103–209 bp from an additional three specimens. Although shorter, the latter sequences were unique compared with all other sequences, and their position could therefore be inferred in a minimum spanning network.

In total, 9 haplotypes were observed in the Pleistocene European population, compared with 10 haplotypes in the contemporary Siberian population. In Scandinavia, 7 haplotypes were observed before the bottleneck and 3 haplotypes after the bottleneck (Fig. 1). Eight of the Pleistocene European haplotypes were unique and had not been observed elsewhere in the world (9), despite extensive sampling of modern populations (20). In contrast, 6 of 7 haplotypes in Scandinavia were shared with Siberia, whereas only 1 was shared with the Pleistocene European population, and that haplotype was found also in Siberia (Fig. 1). The 7th haplotype in Scandinavia, although not shared with Siberia or Pleistocene Europe, is not unique and exists also in Greenland, Alaska, and Svalbard (9). In an analysis of molecular variance (AMOVA), the most probable geographic structure was with Pleistocene Europe in one group versus Siberia and both Scandinavian samples in the other group ($\phi_{CT} = 0.18$, $P < 0.001$, all other ϕ_{CT} values were <0.06 and nonsignificant).

Both founder effects during recolonization and demographic bottlenecks have been theorized to cause a loss of genetic variation. In the case of the arctic fox, comparatively few mtDNA haplotypes seem to have been lost as a consequence of the recolonization process, whereas human overexploitation 100 years ago caused a loss of ≈60% of the haplotypes. The small loss of mtDNA haplotypes during the postglacial recolonization is intriguing because the arctic fox is known for its capacity for long-distance dispersal. Such species are expected to display leptokurtic expansions (i.e., long-distance dispersers setting up colonies ahead of the main advance), which models have shown should lead to particularly high losses of genetic variation (2, 21). We suggest that the reason for the disparity between the models and our data are that, although leptokurtic dispersal may initially lead to lower genetic variation, the high gene flow inherent in this type of species will, over time, reverse this effect and allow a higher level of variation to be maintained.

The haplotype diversity was higher in Pleistocene Europe than in Siberia ($t_{33} = 7.74$, $P < 0.001$), which, in turn, had higher haplotype diversity than both pre- and postbottleneck Scandinavia ($t_{50} = 7.99$, $P < 0.001$ and $t_{28} = 5.94$, $P < 0.01$; see SI Table 2). The high haplotype diversity in the Pleistocene European population indicates a large effective population size. This is in agreement with the high frequency of arctic fox remains found

in palaeontological sites across Europe (10–15). As observed in contemporary populations around the arctic (9), there was no mtDNA phylogeographical structure within Pleistocene Europe, suggesting gene flow or a recent shared history within Europe (SI Fig. 2 and SI Table 1). This is congruent with the lack of osteometrical differences between the fossil Russian and West European foxes (11). However, the Pleistocene European foxes had smaller metatarsal and metacarpal bones, implying a smaller paw size, compared with contemporary Siberian foxes (11, 12), which is consistent with the genetic differentiation between Siberia and Pleistocene Europe found in this study.

The genetic similarity between Scandinavia and Siberia, and the absence of unique haplotypes in Scandinavia, suggest that Scandinavia was recolonized from Beringia in northeastern Siberia after the retreat of the Scandinavian ice sheet (Fig. 1). Consequently, the arctic foxes in midlatitude Europe became extinct during the contraction phase and their genes did not contribute to the makeup of present-day populations. This suggests that the arctic fox was unable to track the changing environment as the climate shifted, and there was thus no support for the hypothesis of habitat tracking (4, 5). Such inability to track decreases in habitat availability may be due to, for example, behavioral constraints or that the habitat shifts faster than the species is capable of dispersing. However, the arctic fox is a highly mobile species (9), suggesting that lack of habitat tracking may be a general pattern among species. This implies that the explanation for evolutionary stasis (22) during the Quaternary may be found inside, rather than outside, refugia.

The results in this study have far-reaching implications for our understanding of how species respond to climate change, because they provide empirical evidence for how populations behave when the distribution of their habitats shift. The habitat-tracking hypothesis (4, 5) assumes similar processes during both the expansion and contraction phase. However, as opposed to when species expand in range to occupy newly available habitats, a decrease in habitat availability may cause local extinction of populations. This confirms the significance of climate change as an agent for extinction (23), and demonstrates the importance of refugia for the long-term persistence of species. Our results also highlight an important difference between arctic and temperate species in the light of current climate change. If lack of habitat tracking is a general phenomenon during range contraction, arctic species may be unable to track the shifting habitat as the temperature increases. This may result in losses of genetic variation as local populations become extinct.

Materials and Methods

Sampling. Late Pleistocene samples for DNA analysis were collected from Belgium ($n = 17$), Germany ($n = 9$), and southwestern Russia ($n = 17$; SI Table 1). Approximately 50 mg of bone powder was sampled by using a Multitool drill. DNA sequences from the extant populations in Scandinavia ($n = 20$) and Siberia ($n = 25$) as well as the prebottleneck population in Scandinavia ($n = 42$) were gathered from published data sets (9, 19).

DNA Analysis. DNA was extracted with a collagenase and phosphate buffer-extraction protocol (24). After this, the mtDNA control region was lifted from the extract as described (24) by using biotinylated probes (primers Pex1R, Pex2F, Pex2R, and Pex3F) (19). Amplifications were done by using three overlapping primer pairs (19), each pair amplifying ≈150 bp. PCRs were set up in 25-μl volumes containing 0.2 μM solutions of each primer, 1× PCR-buffer (Qiagen, Valencia, CA), 2.5 mM MgCl$_2$ (Qiagen), 0.4 mM dNTPs, 3 units Hotstar Taq (Qiagen), and 9 μl of DNA extract. The PCR profile was 10-min denaturation at 95°C, followed by 55 cycles of 30-s denaturation at 94°C, 30-s annealing at 50°C, 30 s of extension

at 72°C, and a final 7-min extension step at 72°C. All PCR products were cloned by using JM109-competent cells and pGEM-T Vector System II cloning kit (both from Promega, Madison, WI), and resulting products were cleaned with ExoSAP-IT (USB, Cleveland, OH). Sequencing reactions were performed by using the DYEnamic cycle sequencing kit (Amersham Biosciences, Piscataway, NJ) and were analyzed on a MegaBACE 1000 (Amersham Biosciences).

Precautions and Authentication. Standard precautions for ancient DNA work were taken (25). Extractions were done in an ancient DNA laboratory (Madrid, Spain), physically isolated from the post-PCR laboratory, where no work with modern fox samples had taken place. All equipment and working surfaces were sterilized by using HCl, sodium hypochlorite, or UV-light. To monitor contamination, and to avoid a bias caused by the carrier effect (26), samples from Late Pleistocene horses (*Equus caballus*), to which the arctic fox primers should not anneal, were used as negative controls (one horse sample for every two arctic fox samples). Ancient DNA can contain damages that cause misincorporated bases during PCR (27). To identify such misincorporated bases, all amplicons were cloned, and an average of 10.5 (SE ± 0.9) clones per sample were sequenced. A subset of five samples were also sent to the Archaeological Research Laboratory in Stockholm, Sweden, for independent replication, by using the methods described above. None of the negative controls produced arctic fox DNA. However, one sample gave a DNA sequence identical to red fox (*Vulpes vulpes*). This sample was one of the Russian canine teeth, which are difficult to morphologically identify down to species level. Contamination from dog (*Canis familiaris*) was observed in two samples (once during initial work and once during replication). During replication, a PCR product contamination was observed for one of the fragments. Results for this fragment were therefore excluded from the replication. However, one of the other fragments was successfully replicated for one sample. This sequence, which has not been observed in any other arctic fox, was identical to the one previously retrieved.

Data Analysis. Clone sequences were aligned by using Sequencher 4.1.4 (Gene Codes, Ann Arbor, MI) and were collapsed to majority-rule consensus sequences. Haplotype and nucleotide diversities and a minimum spanning network were computed in Arlequin ver. 3.01 (28), by using previously described parameters (9). To investigate geographical structuring of genetic variation, we used an AMOVA with 10,000 permutations (29). We performed four AMOVAs with different hierarchical groupings: [Pleistocene Europe vs. Siberia and both Scandinavian samples], [Siberia vs. Pleistocene Europe and both Scandinavian samples], [Siberia and prebottleneck Scandinavia vs. Pleistocene Europe and postbottleneck Scandinavia], and [Siberia and postbottleneck Scandinavia vs. Pleistocene Europe and prebottleneck Scandinavia]. The most probable geographic structure was assumed to be represented by the groupings that maximized values of ϕ_{CT} (30). Although cloning of PCR products allowed us to identify several misincorporated bases, it is possible that some damage remained because of a few starting templates in the PCR. Because the extraction method we used lifts out all template copies from the extract, we could not perform multiple amplifications from the same extract. As an additional precaution (31), we therefore treated C/G to A/T nucleotide substitutions (27) that were observed only in one single sample (compared with both modern and ancient samples) as unknown characters in the phylogenetic analysis. It should be noted that such characters do not have any phylogenetic signal. This approach did therefore not change the tree topology but reduced branch length in some cases. Diversity analyses were done with and without these characters being treated as unknown.

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