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Multiple recolonization routes towards the north: population history of the Fennoscandian red fox (*Vulpes vulpes*)

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Abstract

Understanding the response of boreal species to past climate warming can help predict future responses toclimate change. In the northern hemisphere, the distribution and abundance of northern populations are influenced by prior glaciations. In this paper, we investigate the population history of the Fennoscandian red fox (*Vulpes vulpes*) a generalist carnivore currently undergoing range expansion in the tundra ecosystem. By analysing a 696 base pair sequence of the mitochondrial DNA (n=259) and two Y chromosome-specific microsatellite loci (n=120), we specifically investigated where the red fox survived the last glacial maximum and how Fennoscandia was recolonized. There was high genetic continuity across most of Fennoscandia and we identified at least two recolonization pathways: one from continental Europe and one from the northeast (Siberia). Mitochondrial haplotype diversity displayed a significant decline with increasing latitude, consistent with expectations of unidirectional colonization. Each region displayed signatures of recent demographic and/or range expansions. For Finland, an additional recolonization route was suggested from the mismatch distribution analysis and identification of novel haplotypes. We conclude that, as for many boreal generalist species, the Fennoscandian red fox originates from multiple refugia, suggesting it has benefited from diverse evolutionary histories, potentially enhancing its tolerance for different habitat conditions.

Key words: climate change, boreal invasion, phylogeography

Introduction

The present distribution and abundance of populations in northern boreal and Arctic regions are strongly influenced by historical glaciations (Hewitt 2000). The massive ice sheets, permafrost and drastically lowered temperatures caused southward contraction of temperate habitats (Darwin 1859, Hewitt 1996). The commonly described response of northern hemisphere species is through the contraction-expansion model where temperate populations contracted their distribution to southern refugia. According to this model, as the ice sheets melted and northern habitats became tolerable, they were recolonized from the south through "leading–edge" colonization (Hewitt 2000), a process in which species changed their range in response to shifting habitat suitability (Darwin 1859). The efficiency of this process is connected to species-specific characteristics like dispersal capacity and environmental tolerance, but also biotic factors such as species interactions in the new habitat (e.g. Jackson & Sax 2009).

In addition to southern refugia, there is accumulating support for the occurrence of cryptic, northern refugia in which remnants of populations survived the last glacial maximum (LGM) in high-latitude refugia (Stewart & Lister 2001, Stewart et al. 2010). The environmental conditions in glacial refugia can be important for determining not only what type of contemporary environments a population can establish in, but also how they respond to climate change (Aubry et al. 2009, Scoble & Lowe 2011). When a small population becomes isolated in a high-latitude refugium with colder climate, an enhanced selection pressure and adaptability to northern habitat conditions can be expected (Nosil et al. 2009, Stewart et al. 2010). An extensive assemblage of population history for 90 European species revealed that diverse refugium locations were common among generalist mammals in northern habitats (Bhagwat & Willis 2008). Understanding the past can augment our understanding of present processes as well as our ability to predict how species are likely to respond to future climate change (Dalén et al. 2007).

One example of a generalist mammal species with broad geographic distribution is the red fox (*Vulpes vulpes*). The red fox is capable of inhabiting diverse habitat types, from deserts in the south to mountain tundra in the north (Lariviére & Pasitschniak-Arts 1996). According to fossil records, the European red fox survived the LGM in the typical southern European refugia (Iberia, Italy, Balkan) as

well as farther north (i.e. France and Romania) (Kurtén 1968, Sommer & Nadachowski 2006). Since the 19th century, the red fox has undergone increases in abundance in many parts of Europe and/or geographic range expansions (Chautan et al. 2000, Selås & Vik 2006, Elmhagen & Rushton 2007). In Fennoscandia, the red fox emerged northwards and established in tundra areas during warmer periods of the 19th century (Hersteinsson & Macdonald 1992). The present northern red fox population increase in northern areas is most likely a consequence of both range expansion from boreal zones (boreal invasion) as well as demographic increases of the local northern populations (Norén et al. 2015, Norén et al. 2017). Through a combination of ancient and modern genetic data collected throughout Europe, low levels of phylogeographic structuring was recorded which was suggested to be a consequence of high dispersal and capacity to inhabit a wide variety of habitat types (Teacher et al. 2011). As a part of broader-scale studies, Edwards et al. (2012) suggested that northern European red foxes possibly originated from two different refugia. However, due to a limited sample size and lack of representation from important areas like Finland and south-central Norway, more in-depth analyses were recommended (Edwards et al. 2012).

In this study, we use mitochondrial DNA and Y chromosome microsatellite markers to investigate the detailed population history of the Fennoscandian red fox. We specifically investigated (*i*) where the red fox survived the last glacial maximum, (*ii*) how Fennoscandia was recolonized, and (*iii*) if signatures of demographic and/or range expansion were detectable. Based on previous studies of species in northern Europe, we expected that the red fox originated from eastern or southern Europe (e.g. Hewitt 1999, 2000; Dalén et al. 2007, Teacher et al. 2011, Edwards et al. 2012). Considering the rapid northward expansion, an alternative scenario was that the Fennoscandian red fox population originated from a cryptic, northern refugium (Stewart et al. 2010), or that the region was recolonized from different directions and comprised of multiple genetic lineages.

Material and methods

Samples and DNA extraction

We assembled 259 red foxes, of which 79 originated from Sweden, 66 from Finland, 94 from Norway, 16 from Denmark, and 4 from the Kola Peninsula. These samples have previously been included in

papers addressing contemporary expansion patterns in Fennoscandia using autosomal microsatellite genotyping (Norén et al. 2015, Norén et al. 2017) or range-wide phylogeography (Statham et al. 2014). Among these 259 samples, 72 sequences were collected by Statham et al. (2014) and references therein. These sequences originated from Sweden (n=12), Norway (n=46), Denmark (n=10) and the Kola Peninsula (n=4) (Statham et al. 2014).

Red fox samples were initially collected from several different sources. Swedish samples were assembled from a parasite screening program (Swedish Veterinary Institute, SVA, 1997-2011) (n=63), or from Swedish local residents or hunters (n=4). All Finnish samples originated from a parasite screening program (Finnish Food Safety Authority, 2010-2012) (Evira) and six of the Danish samples (from the island Fyn) were tissue samples collected from the Naturama museum. From Norway, we assembled 25 faecal samples from south-central Norway collected by the Norwegian Institute for Nature Research, 23 tissue samples from northern Norway ('Fjellrev in Finnmark' project, Tromsø University). We extracted DNA from tissue and faecal samples using commercial kits from Qiagen Inc. following the procedures described in Norén et al. (2015).

Mitochondrial analysis

We amplified cytochrome-b (354 bp) and D-loop (342 bp) fragments in the mitochondrial genome following published protocols (Perrine et al. 2007, Aubry et al. 2009) using a PTC100 Programmable Thermal Controller (MJ Reseach Inc.). Each PCR setup was accompanied by negative controls from the extraction as well as PCR blanks. PCR products were checked on a 1.5% agarose gel electrophoresis and cleaned using the PCR Purification Kit (Qiagen) or Exo-Sap-It (Affymetrix). We sequenced the 5' portion of the cytochrome-b and D-loop fragments at the commercial lab Macrogen Inc. (*www.macrogen.com*). To verify the accuracy of our results, we sequenced the 3' portion of each unique novel (previously unpublished) haplotype in a separate PCR reaction. Mitochondrial sequences were manually aligned in BioEdit using previously published red fox data for haplotype determination (Hall 1999). To identify the refugial origin of Fennoscandian red foxes, we used 103 reference sequences downloaded from GenBank representing Eurasia (n=53) and the Middle East (n=50) (Statham et al. 2014). The relationship between consensus haplotypes were visualized in a minimum

spanning network in Arlequin 3.5.1.2 (Excoffier et al., 2005) and Hapstar 0.7 (Teacher & Griffiths 2011). Clade and sub-clade classification originate from Statham et al. (2014) and references therein.

Mitochondrial data analysis

Previous studies (Norén et al. 2015, Norén et al. 2017) revealed low levels of red fox population structure. We therefore estimated basic population genetic parameters within each country. Based on a total of 255 Fennoscandian sequences (after excluding the Kola Peninsula due to low sample size), we used Arlequin version 3.5.1.2 (Excoffier et al. 2005) to calculate haplotype and nucleotide diversity within each country as well as F_{ST} between countries. For significance testing, we used 10 000 permutations.

To test for signatures of recent expansions, we calculated Fu's *Fs* (Fu 1997) and Tajima's *D* (Tajima 1983) and tested for significance using 1000 replicates. A significant, negative value of these parameters indicate demographic expansion in population size. Furthermore, we applied a mismatch distribution approach for Sweden, Norway and Finland (n=239) to investigate the occurrence of a sudden demographic expansion versus a spatial expansion under a constant deme size within Fennoscandia. A population in demographic equilibrium usually display a multi-modal distribution of mismatches, whereas a population that has undergone a recent expansion displays a uni-modal mismatch distribution (Harpending 1994). We compared the observed distribution of mismatches with simulated models of a sudden, demographic expansion and range expansion using 10 000 bootstrap replicates. Based on the mismatch distribution, we calculated the sum of squared differences (SSD) and raggedness index (*r*) from the shape of the curve. Non-significant values of these parameters indicate population expansion. All of the analyses above were accomplished in Arlequin 3.5.1.2 (Excoffier et al. 2005). Also, as measures of admixture for each area, we used the software DnaSP6 (Rozas et al. 2017) to calculate Strobeck's *S* (1987).

We used the standard AMOVA approach (Excoffier et al. 1992) implemented in Arlequin 3.5.1.2 to investigate hierarchical patterns of genetic divergence within Fennoscandia (n=239) using three alternative groupings. First, based on the level of habitat connectivity we compared the relationship between Sweden and Norway to Finland (nb of groups=2). Second, if assuming that the tundra

mediates connectivity across borders, we compared Sweden, Norway and tundra regions of Finland to south-central Finland (nb of groups=2). Third, if assuming that the northernmost tundra red foxes are divergent from the boreal red foxes further south, we compared Sweden and south-central Norway to south-central Finland and tundra regions in northernmost Norway and Finland (nb of groups=3) (Table 3). Significance testing was accomplished using 10 000 permutations.

To test for latitudinal patterns in haplotype diversity that can arise from leading edge colonization, we constructed a grid covering Sweden, Norway and Finland where each cell covered an area of 20*20 km. We calculated an average coordinate for each cell which was correlated to the within cell haplotype diversity through a linear regression in R.

Y chromosome analysis

We genotyped 120 red fox males (Sweden: n=61, Finland: n=36 and Norway: n=23) from Norén et al. (2015) in two Y-chromosome specific microsatellite loci (Y29 and Y30; Statham et al. 2014) (Fig. 2). Amplification and fragment analysis followed the procedure described in Statham et al. (2014) using primers developed by Statham et al. (2014) and Natanaelsson et al. (2006). We grouped the alleles from the two Y chromosome loci into consensus haplotypes that were compared to previously published data on red fox males from Eurasia (n=47;) and North America (n=5) (Statham et al. 2014). To illustrate the relationship between the haplotypes, we used Network 5.0 (www.fluxus-engineering.com) to construct a median joining network (Bandelt et al. 1999). Loci were weighted inversely to the observed level of polymorphism (Statham et al. 2014).

Results

Distribution and structure of mitochondrial DNA variation

For the 259 analyzed individuals, we combined the cytochrome-b and D-loop sequences into 32 different 696 base pair consensus haplotypes. We recorded seven haplotypes in Denmark, seven in Norway, 16 in Sweden, 20 in Finland and two from the Kola Peninsula (Table 1). Among these, six had already been published (Statham et al. 2014), whereas 21 were novel to this study. All haplotypes we identified in this data set originated from the Holarctic clade, in one out of four different sub-clades

(VII, II, IX and I) (Statham et al. 2014) (Fig 1). Sub-clade VII was the most common and contained haplotypes sampled across Fennoscandia, including the most frequently sampled haplotype (present in 124 individuals). Previous studies have documented widespread occurrence of this sub-clade throughout Eurasia. Sub-clade I only included low frequency (n=3), divergent haplotypes that occurred exclusively in south-central parts of Finland (Fig. 1a).

The level of genetic divergence between countries (population pairwise F_{ST}) was highest between Denmark and the Fennoscandian Peninsula (Table 2). Within Fennoscandia, however, the level of divergence was considerably lower between Sweden-Norway than that between Norway-Finland and Sweden-Finland (Table 2). There was no significance for the grouping of Sweden-Norway versus Finland (F_{CT}=0.029, *P*=0.330), or Sweden-Norway-northern Finland versus south-central Finland (F_{CT}=0.162, *P*=0.251) (Table 3). The third setting where we used three groups consisting of (i) Sweden-southcentral Norway, (ii) tundra regions in northernmost Norway and Finland, and (iii) south-central Finland however showed a close to significant pattern of divergence (F_{CT}=0.110, *P*=0.067) (Table 3).

Distribution and structure of Y chromosome variation

We identified three alleles in locus Y29 and five in locus Y30, which generated seven different allele combinations (haplotypes) (Fig. 2). The Y chromosome median joining network showed representation from previously described clades (Statham et al. 2014; Fig. 2a). In detail, clade 1 was previously described to contain haplotypes from Europe and the Middle East (Statham et al. 2014) and within this clade, we identified two novel Fennoscandian haplotypes as well as one previously recorded European haplotype (sampled in Serbia and Spain; Statham et al. 2014). Clade 2 was comprised by three haplotypes, one high frequency haplotype occurring throughout Finland, in northernmost Norway, north and central parts of Sweden as well as in Siberia. In addition to this, clade 2 contained one low-frequency haplotype from northern Finland and one from northern Sweden. Surprisingly, we identified one haplotype sampled exclusively within northern red fox habitats in all countries (n=11; Fig. 2) that occurred among previously published North American haplotypes (clade 3).

Signatures of expansion

Based on Fu's *Fs* and Tajima's *D* (Table 1), no significant expansion signatures were detected, except for Norway that displayed a weak, but significant negative value for Tajima's *D* (*P*=0.04). Within the total sample, however both measures (Tajima's *D*=-1.57, *P*=0.04; Fu's *Fs*=-8.07, *P*=0.024) generated significant negative values.

The average number of pairwise differences was 2.48 for Sweden, 1.92 for Norway and 5.19 for Finland. No areas showed significant signatures of admixture when measured as Strobeck's *S* (Table 1). The shape of the observed and simulated mismatch distribution curves indicated unimodal patterns suggesting recent expansion as the most likely process across the data set (Fig. 4a-c), except for Finland that displayed a tendency for bimodal mismatch distribution (Fig. 4d). Based on both SSD and raggedness index (*r*), we identified signatures following simulated scenarios of both range and demographic expansions across the data set (Table 4). The distribution of mitochondrial haplotype diversity was significantly correlated to latitude (r^2=0.434, *P*<0.001) with higher diversity in the south than in the north (Fig. 3), consistent with northwards range expansion.

Discussion

Earlier efforts to disseminate the population history of European red foxes have demonstrated an overall low resolution across most parts of Europe (Teacher et al. 2011, Edwards et al. 2012, Statham et al. 2014). These studies suggest that Fennoscandia may have been recolonized from multiple sources (Edwards et al. 2012, Statham et al. 2014). The four mitochondrial sub-clades we identified primarily occur in Eurasia. Sub-clades II and VII occur across Eurasia whereas sub-clade IX originate from continental Europe (Statham et al. 2014). This suggests that colonization from at least one southern refugia have had a strong impact on the present genetic composition in Fennoscandia. Colonization from a southern source was also supported by a cline in mitochondrial haplotype diversity that decreases towards the north (Fig. 3) which reflects the traditional expectation of leading edge colonization (Hewitt 1996). We also identified low-frequency representation from sub-clade I, which is basal in the Holarctic clade and has a widespread distribution in Africa, the Middle East and

Europe (Statham et al. 2014). Within this sub-clade, we identified the two most divergent haplotypes sampled exclusively in south-central parts of Finland and these lineages displayed the highest similarity to haplotypes previously sampled in the Middle East (e.g. Saudi Arabia, Iran, Iraq and Palestine; Statham et al. 2014; Fig. 1b). There has previously been a low number of representatives of sub-clade I in Ireland, Britain and Denmark (Statham et al. 2014). In comparison to these haplotypes, the Finnish lineage still has a closer relationship to the Middle East.

Based on Y chromosome data, we concluded that Fennoscandia was comprised of at least two male lineages of different origin. We identified a clear division between northern and southern habitats and support colonization from both southern (continental Europe) and eastern (Siberia) refugia. The identification of a haplotype previously identified in western parts of North America (Statham et al. 2014) was an unexpected finding. Whether this is a haplotype more widespread than previosuly thought, or have appeared through, for instance, introduction of genetic lineages from North American fam-bred foxes need to be addressed more thorough through extended sampling and increased resolution of Y chromosome markers.

Taken together, this study adds to a growing body of evidence pointing towards multi-refugial origin of the Fennoscandian red fox. The genetic lineages in present Fennoscandia most likely represent origin from both traditional southern refugia as well as potentially cryptic refugia in unexpected locations. Most likely, Fennoscandia was recolonized from the south through a land bridge that provided connection to continental Europe until 9.3 thousand years before present (Herman et al. 2014), and from the east via Karelia, Russia. In addition to the main colonization routes described above, a unique mitochondrial lineage recorded exclusively in south-central Finland is a novel finding. This lineage can reflect an additional colonization route from a more divergent source that spread through eastern parts of continental Europe. This lineage has however not spread further than central parts of Finland. The geographic restriction of this lineage may reflect an early colonization event into Finland facilitated by an ice-free corridor while an ice sheet still covering the rest of Finland could have prevented further northwards expansion. Deglaciation of Finland occurred from the south-east to the north-west (Kurimo 1982) which corresponds well to the hypothetical red fox immigration route (Fig. 1b). Alternatively, a late colonization event occurring after most of Finland already was

colonized could have caused the same pattern. If the red fox already had established in most of Finland, further northwards expansion could have been limited by intra-specific competition, intra-guild predation or lack of appropriate habitats (e.g. Jackson & Sax 2009). The observed genetic division between western and eastern regions (measured as population pairwise F_{ST}) is most likely due to the ice sheet that covered northern Sweden (Lundqvist & Mejdahl 1995) and prevented connectivity on a longitudinal scale.

We also addressed whether past expansion patterns were detectable in the data set. The mitochondrial data showed signatures of both demographic and geographic expansions (Table 1, 3, Fig. 4). All regions displayed signatures of both demographic and geographic expansions in the recent past (Fig. 4b-d). The tendency for a bi-modal distribution of mismatches in Finland (Fig. 4d) however suggest a more complex pattern of population history (Harpending 1994). A possible scenario is that the bi-modal distribution of mismatches within Finland reflects post-glacial expansion and colonization processes from at least two different refugia at two different points in time.

Since most of the biota recolonized Fennoscandia from glacial refugia as the ice sheet melted, it is possible that similar processes occurred also in other generalist species. Recolonization of Scandinavia from multiple directions have been recorded in brown bears (*Ursus arctos*; Taberlet & Bouvet 1994; Bray et al. 2013), red deer (*Cervus elaphus*; Skog et al. 2009), martens (*Martes martes*; Davison et al. 2001), moose (*Alces alces*; Niedzialkowska et al. 2014) and bark beetles (*Ips typographus*; Mayer et al. 2014). More specifically, colonization from eastern refugia via the Kola Peninsula and Finland has, for instance, been found in the brown bear (Taberlet & Bouvet 1994), the wood lemming (*Myopus schisticolor*; Fedorov et al. 1995) and the common shrew (*Sorex araneus*; Lundqvist et al. 2011). The diverse refugial origin in boreal species is in sharp contrast to tundra species that usually show simple pattern of colonizing Scandinavia (Flagstad & Roed 2003, Dalén et al. 2007, Lagerholm et al. 2014, Lagerholm et al. 2017, Smith et al. 2017).

Survival in refugia further north may bring evolutionary consequences related to local adaptations to northern habitats (Bhagwat & Willis 2008, Lagerholm et al. 2014). Furthermore, survival in multiple glacial refugia located in contrasting environments are suggested as a common feature for generalist northern mammals (Bhagwat & Willis 2008). Concerning the ongoing climate change and

invasion and establishment of red foxes on the Fennoscandian tundra (Elmhagen et al. 2015, Elmhagen et al. 2017), we suggest that the diverse evolutionary background of the red fox have contributed to the ongoing boreal invasion. Accordingly, populations surviving in northern refugia could thus expand and establish in northern habitats more efficiently than conspecifics originating from southern refugia (Hewitt 1996, Stewart & Lister 2001, Hewitt 2004, Stewart et al. 2010). Thus, the survival of a population in a northern refugium such as Siberia may have contributed to the efficient establishment of red foxes in the tundra ecosystem.

Conclusions

In pace with increasing temperatures, northwards expansion of generalist species from boreal habitats is one of the most commonly described consequences. This study has investigated the population history in a boreal generalist species already expanding into the tundra ecosystem. This study clearly demonstrated that the red fox recolonized the Fennoscandian Peninsula from multiple refugia located at different latitudes and longitudes (*i* and *ii*) and that signatures indicating both demographic and geographic expansions can be identified across the study area (*iii*). Fennoscandia is thus comprised of a mosaic of red fox genetic lineages originating from traditional southern as well as more cryptic refugia and we suggest that the distinct evolutionary histories may accelerate the boreal expansion process and capacity to establish in northern ecosystems. Separate evolutionary histories may be a common trait for present generalist populations currently expanding into novel ecosystem and the question should be addressed in the light of a functional genetic perspective.

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Table 1. Sample size and genetic variation for mitochondrial and Y chromosome DNA in the Fennoscandian red fox population. For mitochondrial DNA, population admixture and expansion parameters (Strobecks's *S*, Tajima's *D* and Fu's *Fs* values). Significant values are shown with an asterix (*).

		Mitochondrial DNA							Y chromosome	
Area	*n*	*No. haplotypes*	*Novel haplotypes*	*Gene diversity*	*Nucleotide diversity*	*Tajimas D*	*Fu's Fs*	*Strobeck's S*	*n*	*No. haplotypes*
Norway	94	7	1	0.538 +/- 0.073	0.003 +/- 0.002	-1.55*	0.169	0.643	15	5
Sweden	79	16	9	0.557 +/- 0.069	0.003 +/- 0.002	-1.24	-1.88	0.932	57	6
Finland	66	20	10	0.717 +/- 0.057	0.008 +/- 0.004	-1.08	-1.37	0.876	36	6
Denmark	16	7	5	0.750 +/- 0.127	0.014 +/- 0.008	0.535	3.2	0.114	NA	NA
Kola	4	2	0							

Table 2. Pairwise F_{ST} between countries for mitochondrial DNA (below diagonal) with corresponding *P* values (above diagonal).

Area	**Norway**	**Sweden**	**Finland**	**Denmark**
Norway	*	0.002	<0.001	0.002
Sweden	0.038	*	<0.001	0.002
Finland	0.086	0.076	*	0.003
Denmark	0.086	0.414	0.285	*

Table 3. Standard AMOVA comparing the distribution of genetic variation within and between hierarchical groupings in Fennoscandia (n=239) using three alternative settings. Significant or close to significant P values are shown in bold text.

	Groups=2 • *Sweden-Norway* • *Finland*			**Groups=2** • *Sweden-Norway-N. Finland* • *SC. Finland*			**Groups=3** • *Sweden-SC. Norway* • *N.Norway-N.Finland* • *SC. Finland*		
Level	*% of variation*	*F*	*P*	*% of variation*	*F*	*P*	*% of variation*	*F*	*P*
Among groups (Va)	2.99	F_{CT}=0.029	0.330	16.18	F_{CT}=0.162	0.251	10.99	F_{CT}=110	**0.067**
Among populations between groups (Vb)	7.12	F_{SC}=0.073	**<0.001**	4.53	F_{SC}=0.054	**<0.001**	0.46	F_{SC}=0.005	0.152
Within populations (Vc)	89.89	F_{ST}=0.101	**<0.001**	79.28	F_{ST}=0.207	**<0.001**	88.55	F_{ST}=0.115	**<0.001**

Table 4. Mismatch distribution parameters (SSD and raggedness index) with corresponding *P* values (from 10 000 bootstrap replicates) assuming a model of sudden demographic expansion versus a spatial expansion under constant deme size for each country separately as well as the full data set (n=239). Bold values indicate significant signatures of expansion.

	Sudden demographic expansion				**Range expansion**			
Region	*Sum of squared deviation (SSD)*	*P(SSD)*	*Raggedness index (r)*	*P (r)*	*Sum of squared deviation (SSD)*	*P(SSD)*	*Raggedness index (r)*	*P (r)*
Norway	0.229	<0.001	**0.102**	**1.0**	**0.014**	**0.660**	**0.102**	**0.850**
Sweden	0.378	<0.001	**0.125**	**1.0**	**0.019**	**0.775**	**0.125**	**0.804**
Finland	**0.068**	**0.078**	0.118	0.044	**0.030**	**0.673**	**0.118**	**0.650**
Fennoscandia	**0.047**	**0.430**	**0.430**	**0.620**	**0.014**	**0.890**	**0.059**	**0.870**

Figure 1. (a) Minimum spanning network of the 696 base pair mitochondrial sequence in 259 red foxes sampled in Sweden, Norway, Finland and Denmark. Geographic distribution is shown in color and frequency of occurrence in haplotype size; (b) geographic distribution of the Holarctic subclades found in our data set with potential post-glacial colonization routes are shown with arrows. Reference data and Holarctic subclade designation is obtained from Statham et al. (2014) and references therein

Figure 2. Median joining network for Y- chromosome haplotypes based on two microsatellite loci in 120 red foxes sampled in Finland, northern Norway and Sweden. Clade designation and reference males from Europe, Siberia, Middle East, Asia and North America was obtained from Statham et al. (2014).

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The version of record Wallen, J. et al 2018 Multiple recolonization routes towards the north: Population history of the Fennoscandian red fox is available online at
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Figure 3. Relationship between mitochondrial haplotype diversity and latitude across Fennoscandia (n=239, r^2=0.434, *P*<0.001). Each circle represents the number of unique haplotypes relative to the sample size in a cell covering 20*20km. Shaded areas represent the location of *Limes norrlandicus* (the transition between nemoral and boreal zones) and the *Polar Circle* (the transition to the northern polar region).

Figure 4. Observed and simulated mismatch distribution (bars) under simulated scenarios of range (black line) and sudden, demographic (dashed) expansions for (a) Fennoscandia in total, (b) Norway, (c) Sweden, and (d) Finland. Note the x-axis scale differences.

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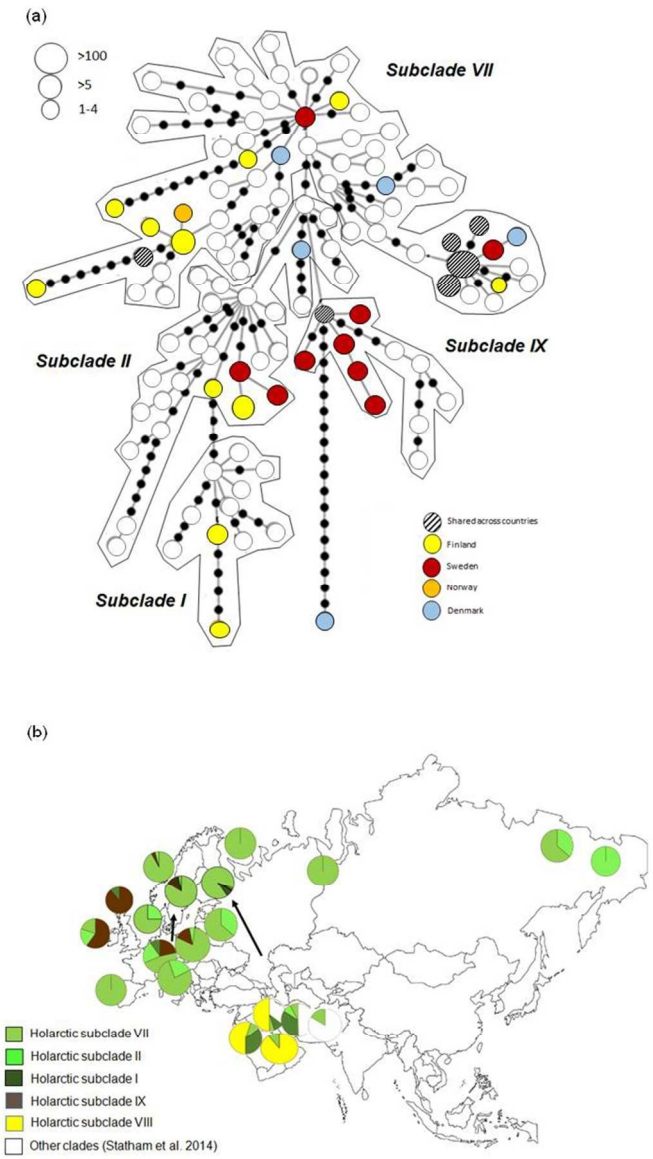

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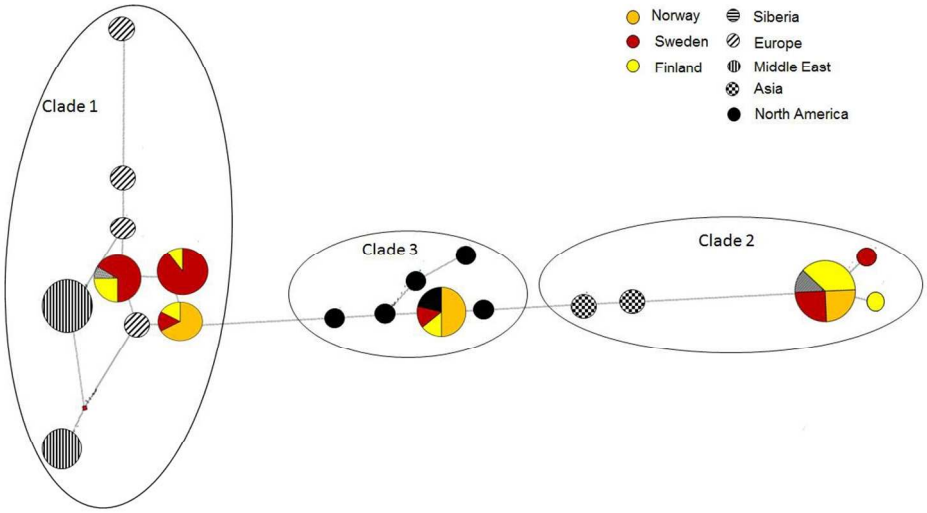

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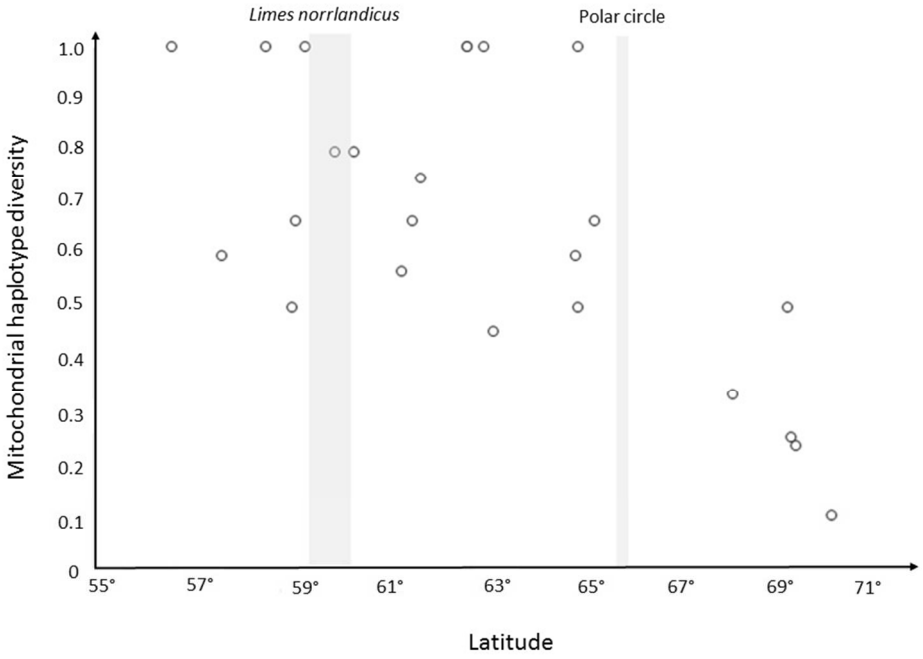

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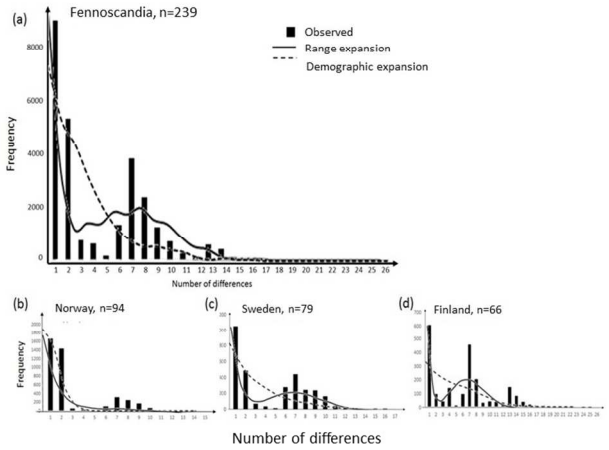

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