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ORIGINAL PAPER

Colonization routes of *Pinus sylvestris* inferred from distribution of mitochondrial DNA variation

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Abstract Understanding the present-day distribution of molecular variation requires knowledge about the history of the species. Past colonization routes and locations of refugia of Scots pine (*Pinus sylvestris*) were inferred from variation in mitochondrial DNA in material collected from 37 populations located in countries within, and immediately adjacent to the continent of Europe. Two mitochondrial regions, *nad*1 intron (exon B/C) and *nad*7 intron 1, were included in the study. Differentiation in maternally inherited mitochondria was high ($G_{ST}'=0.824$). Two new haplotypes were found at the *nad*7 intron 1. The occurrence of a 5-bp indel variant was restricted to the Turkish Kalabak population and a 32 bp only found in Central, Eastern, and Northern Europe. The complete absence of the 32-bp indel from the Mediterranean peninsulas supports the view that coniferous forests existed outside these areas during the last glacial maximum, and these populations contributed to the subsequent colonization of the northern parts of Europe. *P. sylvestris* shares features of its glacial and postglacial history with two other northern, cold-tolerant tree species, *Picea abies* and *Betula* sp. These three species differ from many other European trees for which pollen core and molecular evidence indicate colonization from southern refugia after the last glacial period.

Keywords *Pinus sylvestris* · Mitochondrial DNA · Postglacial colonization

Introduction

Pinus sylvestris (Scots pine) is the most widely distributed Eurasian conifer. It is found in a range of environments, soils, and climates from arid, mountainous areas of Spain and Turkey to subarctic forests of northern Scandinavia and Siberia. A large part of its current distribution was covered by the continental ice or was otherwise uninhabitable during the last glacial maximum (LGM) which occurred about 20,000 years ago (Willis et al. 1998; Svendsen et al. 1999). During the LGM, Scots pine was present in the ice-free regions of Southern and Central Europe, but it is also possible that it survived in Siberia and in more eastern parts of Europe (Willis and van Andel 2004). After the LGM, Scots pine expanded to new areas as the habitats became more suitable for it. Based on the European pollen records of the genus *Pinus*, its distribution started to expand about 16,000 years ago in Southern European regions, and it reached northernmost Scandinavia 7,800 years ago (Willis et al. 1998). However, little is known about the origin of the populations that colonized the northern parts of Europe.

The European pine pollen fossil records are exhaustive (Huntley and Birks 1983), but for the following reasons, they only provide an approximation of the species distribution. First, pollen disperses long distances, and second, pollen production tends to be low in severe environments (Sarvas 1962). Furthermore, *P. sylvestris* pollen cannot be distinguished morphologically from other Diploxylon pollen. In areas where several species of this same group exist or have existed, conclusions based on pollen occurrence are thus uncertain. Macro- and mega-fossil data, which are considered to be more reliable than pollen data, suggest that *P. sylvestris* survived in Hungary and the Czech Republic more than 30,000 years ago

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(reviewed by Willis and van Andel 2004). Based on genetic data, it is highly probable that there were some isolated populations in the Iberian and Italian Peninsulas during the LGM (Sinclair et al. 1999; Soranzo et al. 2000; Cheddadi et al. 2006). Little is known about the history of Scots pine east of Central Europe. The possibility that more eastern populations have also contributed to postglacial colonization of Europe cannot be ignored.

Scots pine occurs at present in very diverse habitats and exhibits genetic adaptation to local conditions in quantitative traits such as timing of growth (Hurme et al. 1997 and citations therein). However, nuclear genetic markers have not shown clear signs of genetic differentiation (Wang et al. 1991; Szmidt and Wang 1993; Karhu et al. 1996; Dvornyk 2001), mainly due to effective pollen-mediated gene flow (Muona and Harju 1989; Dvornyk et al. 2002). Some of the modern adaptive variations could have been present already in ancestral populations. Present differences in adaptive traits may just be due to these precolonization differences. The other option is that response to local conditions, for example climate–day length combination, has emerged during and after the colonization. To resolve the relative importance of these two processes resulting in adaptation, it is important to know the colonization history.

The history of colonization of forest trees also helps to deduce the climate and habitat conditions in different parts of Eurasia during the Ice Age. The extensive study of Petit et al. (2003) on the postglacial history of 25 European trees and shrubs clarified the history of European forests. It has been concluded that even if the refugia for most species were located in the same regions, the routes and dynamics of recolonization differ from species to species (Ferris et al. 1998; Petit et al. 2002). Further, more cold-tolerant species such as *Picea abies* and *Betula* sp. are likely to have more northern and eastern origins (Lagercrantz and Ryman 1990; Palme et al. 2003). Therefore, the history of Scots pine, the most widespread *Pinus* species, must be resolved from species-specific data.

In Pinaceae, mitochondrial DNA (mtDNA) is maternally inherited (Neale and Sederoff 1989 and citations therein; see Wagner et al. 1991 for exception; Wang et al. 1996). A lower effective population size, a clonal mode of evolution, and different means of gene flow of the organellar genomes have contrasting effects on the level of differentiation compared to the nuclear genome (Petit et al. 1993; Ennos 1994), especially in wind pollinated species such as *P. sylvestris*. In this study, we use data on mitochondrial markers to distinguish between the alternative locations of glacial refugia and postglacial colonization routes of Scots pine by examining the distribution of mitochondrial haplotypes with special emphasis on resolving the descent of Northern European populations.

Materials and methods

Sampling

We sampled 37 populations (containing 3–35 trees) covering most of the present Scots pine distribution in Europe and its neighboring countries (Table 1, Fig. 1). The total sample size was 714 trees. Material was provided by the Finnish Forest Research Institute, except for the Spanish samples, which were provided by SIA (Servicio de Investigación Agroalimentaria de la Diputación de Aragón) in Spain, and Northern Swedish and Russian samples, which were kindly provided by The Forestry Research Institute of Sweden and the Russian Academy of Sciences, Komi Science Center.

DNA extraction, polymerase chain reaction, sequencing, and polymerase chain reaction-restriction fragment length polymorphism

DNA was extracted from needles (Lapinjärvi, Lithuania, some of Kolari, Puebla de Lillo) with the DNeasy Plant Mini Kit (Qiagen) or from germinating seeds by FastDNA® Kit (Q-BIO Gene). Several mitochondrial regions were sequenced from a screening set consisting of at least 12 individuals from eight geographical regions to find polymorphisms. Both strands were sequenced using the Big Dye Terminator Kit v3.1 (Applied Biosystems). Multiscreen plates (Millipore) with Sephadex matrix (Sigma-Aldrich) were used for purification of sequencing products. Sequencing was conducted with ABI PRISM 377 and 3730 DNA Analyzer (Applied Biosystems).

The mitochondrial regions *nad*5a (intron 1), *nad*3-*rps*12, and *cox*2 (exon 1; Wu et al. 1998) were monomorphic in our primary screening set. Polymorphisms were found in regions *nad*7 intron 1. *nad*1 intron (exon B/C) was already known to be polymorphic in Europe (Soranzo et al. 2000). Primers for the polymorphic *nad*7 intron 1 region were originally developed for *Picea mariana* (Jaramillo-Correa et al. 2004), and the region includes a variable minisatellite motif in *Pinus banksiana* (Godbout et al. 2005). For amplifying a known polymorphic indel in *nad*1, diagnostic primers *nad*1 H and *nad*1 I (Soranzo et al. 2000) were used. Sequences have been deposited to GenBank (Accession numbers EU072470–EU072475).

The polymerase chain reaction (PCR) for *nad*1 had concentration 2.5 mM of $MgCl_2$, 0.2 μM of each primer, 0.2 mM of each deoxyribonucleotide triphosphate (dNTP), included 1 U of *Taq* polymerase (Invitrogen), and 20 ng of template DNA in 15 μl volume of 1X *Taq* buffer. The PCR reaction for *nad*7 had concentration 2.5 mM of $MgCl_2$, 0.5 μM of each primer, 0.3 mM of each dNTP, included 0.5 U of EXT polymerase (Finnzymes), and 20 ng of template DNA in 25 μl volume of 1× EXT buffer. Amplification protocol for *nad*1 was 4 min at 94°; followed

Table 1 Sampled *P. sylvestris* populations, their location, coordinates as close as available, type, sample size (*N*), mitochondrial haplotype frequencies, mitochondrial gene diversity $H_{(m)}$, and its standard deviation (SD)

Country	Location	Latitude (North)	Longitude	Type	*N*	Haplotype				$H_{(m)}$ (SD)
						a	*b*	*c*	*d*	
Finland	Utsjoki	69°38′	27°07′	Natural population	23	0	0	23	0	0.00 (0.00)
Finland	Inari	69°08′	27°13′	Natural population	19	3	0	16	0	0.28 (0.12)
Finland	Kolari	67°11′	24°03′	Natural population	34	16	0	18	0	0.51 (0.02)
Finland	Salla	67°13′	29°12′	Natural population	15	2	0	13	0	0.25 (0.13)
Sweden	Leiporova	67°02′	21°17′	Natural population	10	9	0	1	0	0.20 (0.15)
Finland	Rovaniemi	66°22′	26°45′	Natural population	12	3	0	9	0	0.41 (0.13)
Russia	Usinsk	66°05′	57°30′	Natural population	15	15	0	0	0	0.00 (0.00)
Russia	Ust-Chilma	65°23′	52°21′	Natural population	15	15	0	0	0	0.00 (0.00)
Russia	Archangel region	64°40′–61°11′	38°05′–46°30′	Natural population	9	7	0	2	0	0.39 (0.16)
Finland	Kälviä	63°45′	24°05′	Natural population	29	0	0	29	0	0.00 (0.00)
Sweden	Hede flygplats	62°42′	13°78′	Natural population	7	6	0	1	0	0.29 (0.20)
Russia	Karelia region	61°41′–62°55′	30°40′–36°40′	Natural population	5	3	0	2	0	0.60 (0.18)
Russia	Surgut	61°25′	73°20′	Natural population	16	16	0	0	0	0.00 (0.00)
Russia	St. Petersburg region	61°00′–58°45′	28°36′–33°30′	Natural population	19	2	0	17	0	0.20 (0.11)
Finland	Uusikaupunki	60°52′	21°20′	Natural population	35	26	0	9	0	0.39 (0.07)
Finland	Lapinjärvi	60°37′	26°08′	Natural population	17	6	0	11	0	0.49 (0.08)
UK	Eastern Scotland	57°03′	03°16′	Natural population	10	10	0	0	0	0.00 (0.00)
UK	Scotland, Achnashellach	57°31′	–05°37′	Natural population	3	3	0	0	0	0.00 (0.00)
Sweden	Norra-Gullabo	56°28′	15°55′	Natural population	30	21	0	9	0	0.43 (0.07)
Russia	Moscow region	56°20′–54°54′	35°21′–39°01′	Natural population	20	8	0	12	0	0.51 (0.06)
Lithuania	Nemencine	54°50′	25°30′	Natural population	14	12	0	2	0	0.26 (0.14)
Holland	Gruppenvorst	52°06′–52°30′	05°39′–06°27′	Seed orchard	30	30	0	0	0	0.00 (0.00)
Belgium	Sart-Custine, Harre	49°58′–50°20′	4°55′–5°38′	Seed orchard	20	20	0	0	0	0.00 (0.00)
Germany	Teisendorf, Bavaria	50°15′	10°30′	Seed orchard	18	18	0	0	0	0.00 (0.00)
Poland	Radom	50°41′	20°05′	Natural population	30	21	0	9	0	0.43 (0.07)
Slovakia		47°44′–49°37′	16°50′–22°34′	Seed orchard	30	25	0	5	0	0.29 (0.09)
France	Haguenau	48°51′	07°52′	Natural population	30	30	0	0	0	0.00 (0.00)
Austria	Oberloisdorf	47°26′	16°29′	Natural population	18	8	0	10	0	0.52 (0.05)
Spain	Baza	37°22′	−02°50′	Natural population	26	24	2	0	0	0.15 (0.09)
Spain	Borau	42°40′	−00°34′	Natural population	21	0	21	0	0	0.00 (0.00)
Spain	Bronchales	40°30′	−01°39′	Natural population	7	0	7	0	0	0.00 (0.00)
Spain	Gudar	40°25′	−0°41′	Natural population	22	0	22	0	0	0.00 (0.00)
Spain	Javierregay	42°35′	−0°25′	Seed orchard	29	0	29	0	0	0.00 (0.00)
Spain	Morrano	42°13′	−0°6′	Natural population	22	0	22	0	0	0.00 (0.00)
Spain	Publa de Lillo	43°04′	−5°15′	Natural population	8	8	0	0	0	0.00 (0.00)
Spain	Valsain	40°49′	−04°02′	Natural population	16	13	3	0	0	0.33 (0.13)
Turkey	Kalabak	39°27′	30°18′	Natural population	30	15	0	0	15	0.52 (0.02)
				Total	714	395	106	198	15	

by 35 cycles of 45 s at 94°, 1 min at 53°, 1 min 30 s at 72°; and finally one cycle for 10 min at 72°, and for *nad*7, 3 min at 94°; followed by 38 cycles of 1 min at 94°, 1 min 30 s at 59°, 2 min 30 s at 72°; and finally one cycle 20 min at 72°.

The 31-bp indel in *nad*1 intron (exon B/C) was scored by electrophoresis of the PCR product through 2% agarose gel. The polymorphisms in *nad*7 were initially scored by sequencing 90 individuals from various populations, but as no additional polymorphisms were found, the rest of the samples were genotyped by digesting 3 µl of PCR products with 0.5 U of DraII and electrophoresing through 2% agarose gel.

In this study, mitochondrial haplotypes *a* and *b* are combinations of *nad*1 types *a* and *b* in Soranzo et al. (2000) and the most common type in *nad*7. Haplotypes *c* and *d* are combinations of rare types in *nad*7 with *a* of Soranzo et al. (2000) at *nad*1.

Data analysis

Sequence contigs were assembled with SequencherTM 4.0.5 (Gene Codes Corporation) and aligned with Clustal X (1.83; Thompson et al. 1997). GeneDoc (2.6.002;

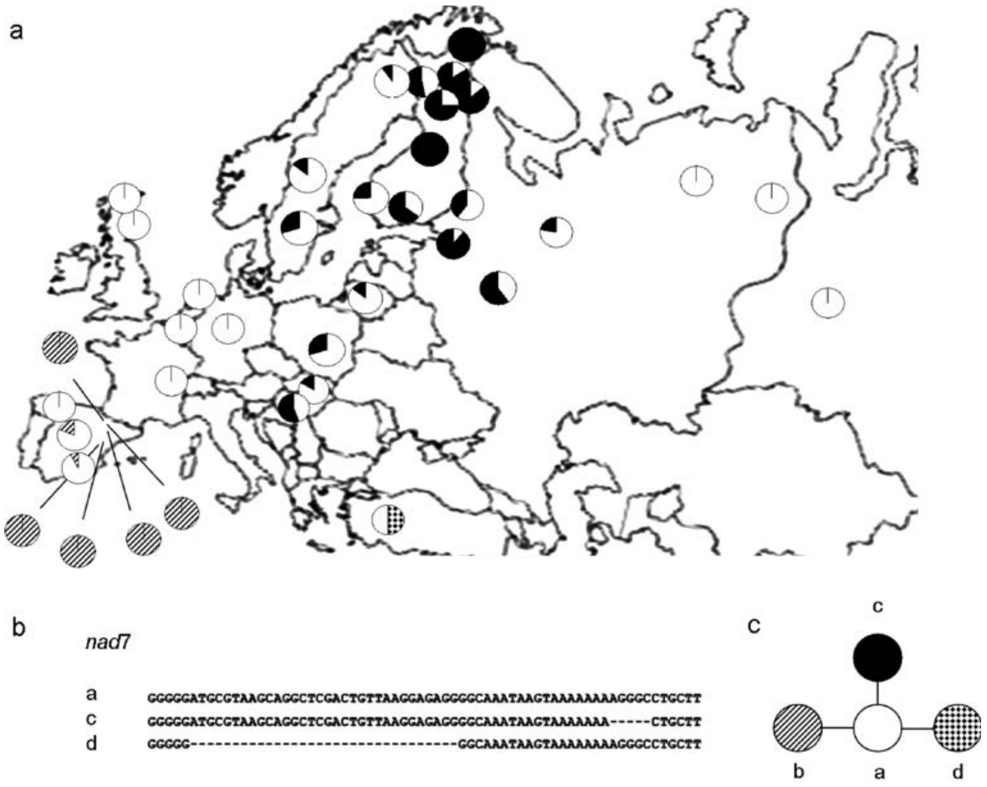

Fig. 1 **a** Pie diagrams describe the distribution of mitochondrial haplotypes in *P. sylvestris* populations. *Patterns* in diagrams are equivalent to the patterns in haplotype network. For sample sizes, see Table 1. **b** Sequences of all three variants in the polymorphic region of mitochondrial *nad*7 intron 1. **c** Mitochondrial haplotype network with one indel mutation between the neighboring haplotypes

Nicholas et al. 1997) was used for editing the alignment manually. A standardized measure of G_{ST}, G_{ST}' was calculated according to Hedrick (2005) to remove the effect of level of polymorphism on the estimate.

Mitochondrial gene diversities (which in the case of haploid data is the probability of two randomly chosen alleles being different in the sample) and their standard deviations (Nei 1987) for each population were calculated with Arlequin version 2000 (Schneider et al. 2000). Average population diversity, total diversity, and estimates of differentiation parameter for unordered (H_S, H_T, G_{ST}) and ordered (V_S, V_T, N_{ST}) haplotypes were obtained with the software PERMUT (http://www.pierroton.inra.fr/genetics/labo/Software/Permut/). PERMUT was also used for testing whether N_{ST} is higher than G_{ST} for the mitochondrial data by permuting rows and columns of distance matrices between alleles (Pons and Petit 1996). G_{ST} is an F_{ST} estimate that takes into account only the allele frequencies, whereas N_{ST} makes use of the distance between different alleles. A higher N_{ST} than G_{ST} indicates phylogeographic structure within populations (Pons and Petit 1996).

The software package SPAGeDi 2.1 (Hardy and Vekemans 2002) was used to analyze the relationship between geographic and genetic distance. In a regression analysis, genetic distance was defined as $F_{ST}/(1-F_{ST})$ and geographic distance as the natural logarithm of distance as kilometers (Rousset 1997). A pattern of isolation by distance is detected as significant positive regression coefficient. Significance was tested by permuting population locations among populations (Mantel's test). To examine whether the pattern of isolation by distance was especially strong in some specific distance class, estimates of genetic differentiation were binned by geographical distance. The level of differentiation was scored in six distinct classes for every 1,000 km from 1,000 to 6,000 km.

Results

Patterns and amount of genetic diversity

We found altogether four mitochondrial haplotypes (Fig. 1). None of the 90 samples sequenced or 624 samples genotyped by PCR-restriction fragment length polymorphism (RFLP) in this study showed signs of heteroplasmy as expected, given the maternal inheritance of mtDNA. New polymorphisms were found in the *nad*7 region, where two indels (32 bp and 5 bp) resulted in three different types in this region (Fig. 1b). There was one common mitochondrial haplotype (*a*), and the other three haplotypes (*b*, *c*, and *d*) were all within one mutational (insertion or deletion) step from it (Fig. 1c). The 32-bp indel is not the same as found by Godbout et al. (2005) in *P. banksiana*.

The mitochondrial gene diversities varied from 0 to 0.6 (Table 1). Total gene diversity (H_T) was 0.583, average gene diversity (H_S) 0.201, and between population component of diversity, G_{ST} 0.655. The standardized genetic differentiation, G_{ST}', was 0.824. The ordered and unordered differentiation statistics were quite similar: N_{ST} was 0.700 and slightly but not significantly higher than G_{ST} when tested with 1,000 permutations. There was a pattern of isolation by distance between pairwise mitochondrial genetic differentiation and geographic distance. The regression coefficient between $F_{ST}/(1-F_{ST})$ and natural logarithm of distance as kilometers between 37 populations was significantly greater than zero (P=0.0012, one-sided test, population locations permuted among all populations). However, the distance explained only 3% of the variation in genetic distance (r^2=0.029). In the analysis of genetic differentiation by distance classes, the isolation by distance was significant up to distance class of 4,000 km (Fig. 2).

Distribution of mitochondrial haplotypes

The most common haplotype, *a*, was present in all geographical areas and is probably the ancestral type (Fig. 1a). The indel in the *nad*1 (haplotype *b*) is restricted to the Iberian Peninsula. Two haplotypes which have not been previously found, *c* and *d*, had their own characteristic distributions. Haplotype *d* was restricted to the Kalabak population in Turkey only. Haplotype *c* was found in central, northern, and eastern parts of Europe but was absent from Western Europe, the Iberian Peninsula, Turkey, and Scotland. Three easternmost Russian populations also had only the mitochondrial type *a*. Two Finnish populations, Kälviä and Utsjoki, were monomorphic for type *c*. Type *c* is the most common haplotype in the northern Finnish populations and the Moscow and St. Petersburg region. The frequency of type *c* gradually diminishes towards west and east from these regions. There is a large difference in frequency of type *c* between northern Finland and northern Sweden (0.53 in Kolari vs 0.1 in Leiporova). The observation is supported by data set of 18 additional single trees sampled from different locations in the northern Sweden (data not presented in Table 1 and Fig. 1), where only one tree was of type *c*.

Discussion

Geographical patterns of genetic diversity

The level of mitochondrial gene diversity outside the Iberian Peninsula was considerably higher than was previously detected due to the discovery of the previously undescribed variation in *nad*7. The indel variant of *nad*1 was restricted to Spain and has been detected in earlier studies (Sinclair et al. 1999; Soranzo et al. 2000; Cheddadi et al. 2006). In earlier studies, all continental populations outside the Iberian Peninsula were fixed for a single mitochondrial haplotype (Sinclair et al. 1999), with few exceptions (Soranzo et al. 2000; Cheddadi et al. 2006). The higher level of variation results both from geographically more extensive sampling and the better resolution in mitochondrial markers used in this paper. Nonetheless, some of the individual populations exhibit no mitochondrial variation at all. Many natural Western European populations are fixed for type *a* which is predominant also in the easternmost part of the distribution. This is probably due to lack of resolution in our markers rather than a common origin of Eastern Russian and Western European populations. It is likely that the addition of more markers would

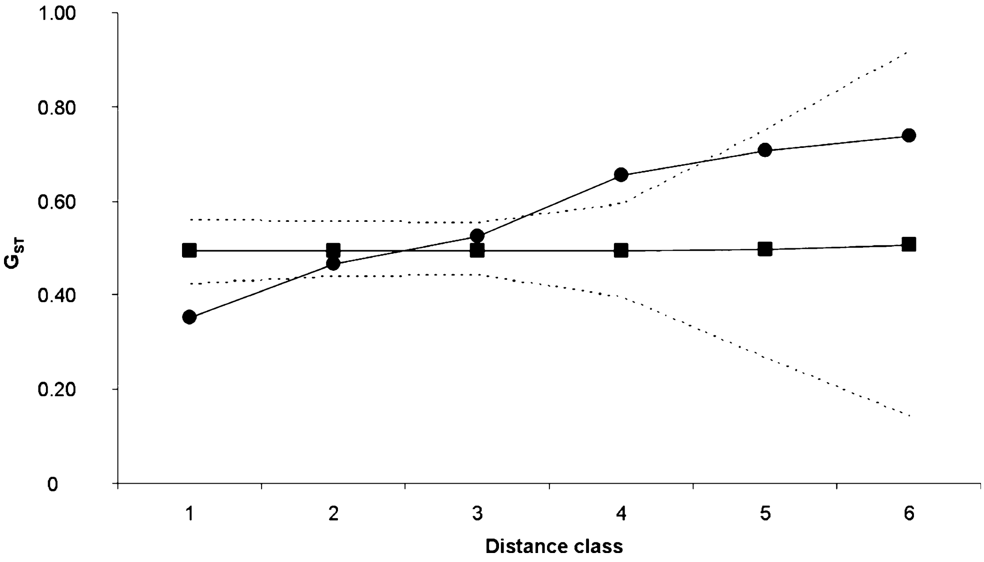

Fig. 2 Observed mean G_{ST} (*dots*) for six distance classes (×1,000 km). Means (*squares*) and 95% confidence intervals (*dashed lines*) of simulated G_{ST} values from permutation of population locations among all populations

result in higher levels of mitochondrial haplotype diversity. However, mtDNA has very low variability, as screening of many regions did not find much variation.

In *P. sylvestris*, the amount of differentiation in mtDNA was very much higher than what was previously documented in nuclear markers, as expected. The lower effective population size and more restricted dispersal in seeds vs pollen result in definite genetic structure in maternally inherited DNA (Ennos 1994). Similar relationships have been observed in other pine species, e.g., in *Pinus contorta* (Dong and Wagner 1993, 1994) and *Pinus flexilis* (Latta and Mitton 1997; Mitton et al. 2000).

P. sylvestris seeds are wind dispersed, and the species has mostly continuous distribution, although there are some isolated populations at the margins of distribution. We thus expect a pattern of isolation by distance in genetic differentiation (Wright 1943). Although the number of haplotypes was low, isolation by distance was observed. It extended at least up to distance class of 4,000 km. Mitochondrial types separated by two indels were never found in the same population, which indicates some degree of phylogeographical pattern. However, N_{ST} and G_{ST} did not differ significantly. This is probably due to low power of the test, as there were only four haplotypes.

Recolonization of Scots pine from different refugia since the last glacial maximum

The distribution of the haplotypes supports the view that populations in the Iberian Peninsula are relicts that have not contributed to the recolonization of the rest of Europe as already concluded by Soranzo et al. (2000) and Sinclair et al. (1999). The distribution of haplotypes *a* and *b* in our study is consistent with the results of Soranzo et al. (2000) and Cheddadi et al. (2006), although in the current study, haplotype *b* was not detected outside the Iberian Peninsula. The Scots pine population in Turkey probably did not have a role in colonization because it is isolated from the main distribution. The private Turkish haplotype has not been found from any population earlier. It is probable that there was a glacial refugium in Asia Minor, and populations in this region may be more connected to Asian than European Scots pine populations. To verify the role of Asia Minor in colonization history of *P. sylvestris*, more populations from Asia Minor and adjacent regions need to be studied.

The previously undetected mitotype *c* was present in Central Europe, Scandinavia, and Eastern Europe. The origin of this type is not clear, but most probably it is neither from the Iberian Peninsula, Italian Peninsula, nor Turkey. Turkey and the Iberian Peninsula can be ruled out based on our results and those of Soranzo et al. (2000) and Cheddadi et al. (2006). The Italian Peninsula is one of the main hypothetical locations of glacial refugia for many species (Taberlet et al. 1998). Our study did not address the question, but the recent results of Cheddadi et al. (2006) show that Scots pine populations from Italian Peninsula have not contributed to postglacial colonization of Central and Northern Europe. For *P. abies*, another cold-tolerant European conifer, the Carpathians have been suggested as refugial area (Gugerli et al. 2001). However, the role of the Carpathians and Balkan Peninsula in colonization history of *P. sylvestris* remains unresolved, as our sampling does not cover these areas adequately.

According to comparative studies based on phylogeography of ten species, two main postglacial colonization routes meet in Central Sweden (Taberlet et al. 1998). One is from the northeast, and the other one from the southwest. Haplotype *c* has a low frequency in northern Sweden in contrast with higher frequencies in northern Finnish populations. Sinclair et al. (1999) suggested dual migration of *P. sylvestris* to northern Scandinavia and Finland from the northeast and the south. The difference in the frequency of haplotype *c* between northern Scandinavia and Finland are concordant with that hypothesis. However, type *c* is quite common in southern Sweden, and it is possible that the observed differences in haplotype frequencies between Scandinavia and Finland result from drift. They might have the same origin, but drift could have resulted in a high frequency of haplotype *a* in Sweden and haplotype *c* in Finland.

The northern cold-tolerant species, *P. sylvestris*, *Betula*, and *P. abies* are widely distributed in Eurasia. The distribution of chloroplastic and mitochondrial haplotypes of *P. abies* and chloroplastic haplotypes of *Betula pendula* in eastern Russia and in Northern and Central Europe (Sperisen et al. 1998; Vendramin et al. 2000; Palme et al. 2003) resemble the distribution of haplotypes in Scots pine. Our results are in accordance with the hypothesis that there was a refugium near Hungary and the Czech Republic, as suggested by macrofossil data from Central and Eastern Europe (reviewed in Willis and van Andel 2004). However, the highest frequencies of type *c* are relatively far north: northern Finland, St. Petersburg, and Moscow, which would suggest more eastern and northern origin of type *c*. Genetic data imply that one of the origins of current European *P. abies* is in the region of Moscow (Lagercrantz and Ryman 1990), as suggested by pollen analyses (Huntley and Birks 1983). To resolve whether Northern European *P. sylvestris* has an eastern origin as *P. abies* or a past refugium in Central Europe, denser sampling in central, northern, and eastern parts of Europe and preferably also more mitochondrial markers are needed.

These results are consistent with the view that during the last glacial maximum, there have been populations of cold-tolerant tree species such as *P. sylvestris*, *P. abies*, and *Betula* outside the Mediterranean peninsulas. The two northern conifers and *Betula* thus have a very different glacial and postglacial history compared to many European angiosperm tree species.

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