

## Organelle Genetic Diversity and Phylogeography of Scots Pine (*Pinus sylvestris* L.)

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### Abstract

The paper reviews the present knowledge of Scots pine (*Pinus sylvestris* L.) diversity, historical and geographical distribution, based on mitochondrial and chloroplast DNA data. The observed differences in the estimates of genetic differentiation between different types of genomes suggest that both pollen and seed contribute significantly to gene flow within species. Organelles' diversity represents an important criterion which could be later applied in planning for future forest management and breeding through a better understanding of adaptation strategies of different Scots pine haplotypes. This analysis would provide valuable references when facing current day problems with climate change, species adaptation, and loss of forest with negative effects on biodiversity. Research on organelles' diversity could lead to important practical applications in areas such as traceability and eco-certification of forest products, and the identification of plant populations for conservation. Based on the results from earlier investigations, Scots pine in Europe can be divided into at least three evolutionary units (Spain, northern/central Europe and northern Fennoscandia), each with a different origin after glaciations. However, it must be emphasized that these interpretations are preliminary and further mitochondrial and chloroplast DNA data need to be analyzed in conjunction with evidence from pollen and fossil analysis.

**Keywords:** chloroplast and mitochondrial DNA, glacial refugia, Scots pine, spatial distribution

### Introduction

In plant ecology and evolution, the central focus is on understanding the processes that regulate similarities and differences between spatial genetic patterns in populations (Loveless and Hamrick, 1984). Plants and animals are directly influenced by the specific characteristics of their surrounding environment; therefore, spatial information is an important element to be considered when trying to understand genetic resources (Heywood, 1991), habitat connectivity and distribution of the organisms (Murphy *et al.*, 2008). If a geographical region is subdivided into smaller areas characterized by environmental heterogeneity, or genetic drift is acting (isolation by distance), a spatial genetic structure, variation in genotype frequencies among the subdivisions should be expected (Heywood, 1991). The distribution of genetic variation depends not only on the mating system, but also on pollen and seed dispersal, survival through life cycle stages and population density. Although gene movement in seed plants involves both pollen and seed, empirical data indicate that the development of spatial genetic structure within populations is more strongly influenced by seed than by pollen dispersal (Hamrick and Nason, 1996). Surveys of genetic variation of numerous taxa have revealed that patterns of subdivision and diversity are consistent with isolation in refugia during cold stages and geographic expansion during the interglacial periods (Hewitt, 2000). In this study,

we give an overview on a long-lived coniferous species, *Pinus sylvestris*, its evolution after the glacial period, and the diversity within and among its populations in Europe.

Quaternary climatic fluctuations have left contrasting historical footprints on the neutral genetic diversity patterns of existing populations of different tree species (Comes and Kadereit, 1998; Hewitt, 2000), providing information about the interaction between climatic niche and climate change sensitivity of the species. The cold periods of the Pleistocene had a dramatic impact on most of the species living in temperate regions (Webb, 1992), responding through migrations to regions where climatic condition allowed them to survive (Ibrahim *et al.*, 1996; Taberlet *et al.*, 1998). It is well known that the European tree flora was strongly affected by the late Neogene-Quaternary climate changes, experiencing dramatic range transformations (Huntley and Birks, 1983; Bennett *et al.*, 1991; Cheddadi *et al.*, 2005). Recent palaeobotanical studies have reported much wider distributions of boreal and alpine trees in Central and Eastern Europe during the last glacial maximum (LGM), although some species persisted also in northern refugia (Willis *et al.*, 2000; Stewart and Lister, 2001; Willis and van Andel, 2004). The predominant view in recent decades has been that the forests and trees were restricted to localized refugia in southern Europe during the - LGM (Bennett *et al.*, 1991; Brewer *et al.*, 2002; Hewitt, 2000). During this period, a large proportion of the European tree flora was lost due the onset of

the summer-dry Mediterranean climate and the repeated glaciations. The scarcity of warm, moist glacial refugia caused extinctions, which particularly affected frost- and drought-sensitive taxa (Svenning, 2003). The extinctions included numerous tree genera that form an important and diverse component of modern warm-temperate vegetation in East Asia and North America. Many thermophilous taxa (*P. pinea* and *P. halepensis*) have been subject to stronger demographic fluctuations in the past, as a consequence of their mal-adaptation to recurrent glacial cold stages, but altitudinal migrations have allowed the maintenance of large effective population sizes and genetic variation in cold-tolerant species (*P. uncinata*, *P. sylvestris* and *P. nigra*), especially in more humid regions (Soto et al., 2010).

In the present paper, a literature review was conducted using several keywords for studies on organelle DNA (oDNA) genetic diversity in Scots pine. Only studies referring to mitochondria and chloroplast genome analysis on Scots pine were taken into account. Correlation with phylogeographic reports based on pollen, macro- and megafossils was carried out with oDNA data, which was available with reference to Scots pine.

#### *Scots pine (Pinus sylvestris L.) distribution*

The origin of the genus *Pinus* is thought to be in early to middle Mesozoic (Millar, 1998). Fossil records suggest that ancient species of *Pseudoaraucaria* and *Pityostrobus*, closely related to pines, may have provided the ancestral gene pool of pines (Millar, 1998). Pines cover 37% of the world's total land and 70% of the northern hemisphere forest (Mirov, 1967). Among all the pine species, Scots Pine (*P. sylvestris* L.) has the largest geographic distribution, which ranges from northern Scandinavia to southern Spain and from western Scotland to the Okhotsk Sea in eastern Siberia. Within its range, populations of Scots pine can be found at different altitudes; in the north, it is present from sea level to 1,000 m, while in the southern most limit it is found at 1,200-2,600 m altitude (i.e., in Spanish Sierra Nevada). As a consequence of its vast distribution, Scots pine has adapted to a large variety of soils and climates - from the arid mountains of Spain and Asia Minor to the subarctic forests of Northern Scandinavia and Siberia. It is therefore not surprising that adaptation has followed a cline with reference to quantitative traits, such as timing of budset, growth, frost hardiness and seed size. Based on these traits, temperature and light are considered to be the most important environmental cues (Vaartaja, 1959; Eiche, 1966; Eriksson, 1980; Mikola, 1982; Aho, 1994; Hurme et al., 1997; Notivol et al., 2007). For example, latitudinal transfers to the north or to higher altitudes usually result in highly increased mortality. Also, latitudinal transfers to the south seem to improve their survival and growth, although not at the same level as the local southern trees (Partanen and Beuker, 1999; Eriksson, 1980). Strong local adaptation contrasts with a lack of clear differentiation when the genetic structure is analyzed with neutral markers (Wang et al., 1991; Karhu et al., 1996; Dvornyk et al., 2002; Garcia-Gil et al., 2003).

Scots pine is considered as a relict from the Tertiary (Mirov, 1967), having a very complex history related to possible refugial areas scattered throughout Europe (Birks, 1989; Tantau et al., 2006; Willis and van Andel, 2004). Studies based on pollen, macro- and mega- fossil records in sediment profiles and historical data show that Scots pine distribution started to expand about 16,000 years ago (y.a) in southern Europe, reaching northern Scandinavia about 7,800 y.a (Tantau et al., 2006; Willis, 1998; Critchfield and Little, 1966; Godwin, 1956). Phylogeographic investigation based on the spatial-temporal population dynamics and structure with organelle DNA markers inferred at the sequence level has shown that the largest refugia of Scots pine were localized in the Balkans, Alps and Iberian Peninsula (Bennett et al., 1991; Huntley and Birks, 1983). Pine mitochondrial and chloroplast DNA variants provide further evidence for multiple origins after the last glaciation, mainly from the South (Sinclair et al., 1999; Soranzo et al., 2000).

#### *Mitochondrial and chloroplast genome*

Plants, in contrast to other eukaryotes, carry two organelle genomes (mitochondria and chloroplasts), which are uniparentally inherited (reviewed by Birky, 1995; Birky, 2001; Mogensen, 1996). Therefore, the comparison with nuclear markers, which are biparentally inherited, has been applied to infer the relative dispersal ability of males (i.e. pollen) and females (i.e. seeds). Organelle genomes are transmitted in multiple copies during mitosis and meiosis, and for this reason they are subject to random drifts within and between individuals. The uniparentally inherited, haploid and nonrecombinant nature of organelle genomes makes them very useful tools in evolutionary studies (reviewed by Petit et al., 2005), which is reflected in the high levels of intraspecific variability (Birky, 1988). There are two consequences of uniparental inheritance: (1) existence of half effective population size ( $N_e$ ) as compared to nuclear markers (biparentally inherited) in outcrossing hermaphrodites, and (2) presence of restricted recombination. A reduced effective population size ( $N_e$ ) results in higher rate of genetic drift for organelle DNA (Bucci et al., 2007; Nasri et al., 2008; Vendramin et al., 2008), which is twice than that of the rate for nuclear DNA (Palumbi et al., 2001), and also results in higher spatial structure (Latta, 2006).

Plant population studies based on organelle markers rely more on chloroplast DNA (cpDNA) markers (175 species), than on the mitochondrial DNA (mtDNA) markers (34 species) (Petit et al., 2005). Therefore, these haploid markers provide strong clue concerning seed migration (Petit et al., 2005) and phylogenetic complications (Samuel et al., 2005).

Studies in seed plants show greater genetic differentiation for both mtDNA and cpDNA compared to nuclear genomes (McCauley, 1994; reviewed by Korpelainen, 2004). Gene flow for maternally transmitted mtDNA is constrained to seed dispersal, which travels shorter distance than pollen, resulting in increased differentiation

between populations. A reduced effective population size determines genetic difference and variability in the case of the paternally inherited cpDNA (McCauley, 1995). High levels of G<sub>st</sub> for organelle markers were first calculated in the 1990s in *Quercus* (Kremer, 1991), giving a clear conclusion that subdivision at cpDNA markers level is considerably larger than at nuclear level.

In conifers (Petit *et al.*, 2005), earlier investigations report that G<sub>st</sub> is always larger for mtDNA markers than for the cpDNA. For example, in Scots pine F<sub>st</sub> values were estimated at 0.370 and 0.028 for mtDNA and nuclear DNA respectively (Sinclair *et al.*, 1998). In most conifers and pines, the mitochondrion genome is maternally inherited, while the chloroplast genome is paternally inherited (Neale and Sederoff, 1989; Korpelainen, 2004). Also, in most angiosperms, organelles are maternally transmitted and always show higher F<sub>st</sub> values than the nuclear markers (Latta, 2004).

**Discussion on literature data**

Only studies referring to mitochondrial and chloroplast genome analysis on Scots pine were taken into

account for the present bibliographical investigation. Whenever possible oDNA data were correlated with phylogeographic reports from pollen, macro- and megafossils. Several contributions regarding genetic variation among European populations of *P. sylvestris*, revealed by values of genetic parameters using mitochondrial and chloroplast data, are presented in Table1.

Powell *et al.* (1995) analyzed three species of pine: *P. sylvestris*, *P. contorta* and *P. thumbergii* from Southern Europe (Spain, Greece, Italy), and screened the chloroplast genome for polymorphism and diversity within population. For all populations, the researchers reported the presence of four variants, with not much higher values (F<sub>st</sub>=0.320 and G<sub>st</sub>=0.22) from what (Kinloch *et al.*, 1986) has reported earlier for monoterpene (0.272-0.378) and isozyme (0.291- 0.311) studies. On the other hand, lack of differentiation (3.24-8.81%) has been reported between Europe's main land populations and Scottish populations based on cpSSR analysis (Provan *et al.*, 1998). In contrast, mtDNA analysis revealed high differentiation F<sub>st</sub>=0.370 between Scottish Scots pine population, which is 13 times higher than that of the equivalent measure of genetic differentiation in the same population using nucle-

Tab. 1. Summary of organelle (mitochondria+chloroplast) genetic variation among European populations of *P. sylvestris*

Population regions	No of population	F <sub>st</sub>	G <sub>st</sub>	Ht/Hs	No of haplotypes mt/cp	References
Scotland	7			Ht=0.991	174(cp)	Provan <i>et al.</i> (1998)
Continental Europe	8			Ht=0.977	133(cp)	
South Italy and Balkans	20		G <sub>st</sub> =0.22	Hs=0.320		Powell <i>et al.</i> (1995)
Scotland, China, Turkey, Russia, Sweden, Germany	25	F <sub>st</sub> =0.370			2(mt); 1(cox,RFLP)	Sinclair <i>et al.</i> (1998)
Scotland, France, Poland, Lithuania, Czech Republic, Spain	23		G <sub>st</sub> =0.59		2(mt)	Soranzo <i>et al.</i> (2000)
West Europe, Scotland, Spain, Germany, France, Poland, Finland, Sweden, Norway, Russia, Scotland	76	F <sub>st</sub> = 0.813(Spain) F <sub>st</sub> = 0.37 (rest Europe)		Ht=0.586 (Spain) Ht=0.120 (rest Europe)		Sinclair <i>et al.</i> (1999)
Finland, Russia, Sweden, United Kingdom, Lithuania, Holland, Germany, Poland, Slovenia, France, Austria, Spain, Turkey	37	F <sub>st</sub> =0.700	G <sub>st</sub> =0.655	Ht=0.583 Hs=0.201	2(mt)	Pyhajarvi <i>et al.</i> (2008)
Iberian Peninsula range	30	F <sub>st</sub> =0.045			98(cp)	Soto <i>et al.</i> (2010)
Spain, isolated pop. in mountains	13		G <sub>st</sub> =0.031	He)= 0.978	139(cp)	J,Robledo Arnuncio <i>et al.</i> (2005)
Spain, Austria, Italy, Germany, Poland, Slovakia, East Europe	141		G <sub>st</sub> >0.80	H <sub>st</sub> =0.600	178(cp)	Cheddadi <i>et al.</i> (2006)
Finland, Russia, Sweden, UK, Lithuania, China, France, Austria, Spain, Turkey, Romania, Czech-Republic, Belarus, Bulgaria, Latvia, Ukraine	54	F <sub>st</sub> =0.685	G <sub>st</sub> =0.657	Hs=0.141	4(mt, nad7) 2(mt, nad1)	Naydenov <i>et al.</i> (2007)
Italy: Alpine	10			0	1(mt)	Labra <i>et al.</i> (2006)
Italy: Northern Apennine						
Sweden, Turkey	22		G <sub>st</sub> =0.58	Ht=0.349		Szmid <i>et al.</i> (1993)

\*F<sub>st</sub>=fixation index; G<sub>st</sub>=gene differentiation; Ht= total genetic diversity; Hs=average genetic diversity

ar isozyme markers  $F_{st}=0.028$  (Sinclair *et al.*, 1998) with two common and one rare variants (mitotypes) detected. Mitotype **a** was present in all sites while mitotype **b** was found in western populations, and mitotype **c** was found in only one population. The presence of mitotype **b** in populations from western Scotland and its absence from the populations of Northern France and Germany (Sinclair *et al.*, 1998) suggest that Scots pine arrived in Scotland probably by following two routes: either from Europe via England or from west refugia, which could be either Ireland or western parts of France. While trying to clarify the origin of Scots pine in the Western part of Europe (Sinclair *et al.*, 1999), a study on mitochondrial genome reported three major mitotypes (**a**, **b**, **d**). All the mitotypes were present in the populations of Spain; mitotype **a** was found in the population of Sierra Nevada, southern Sweden, Poland, Germany, Scotland, northern France, Italy, and Central Europe (Labra *et al.*, 2006), while mitotype **b** was present in Italian populations and in some isolated populations from Scotland. The higher gene diversity ( $H_T = 0.586$ ) reported in Spain as compared to Scotland ( $H_T = 0.120$ ), suggests that Spain might have been the original centre of diversity (Euforgen), while the rest of the populations showed little to no mtDNA diversity within regions, only marking differences between regions. A comparative analysis with pollen data and nuclear genetic markers suggests that present distribution of Scots pine in Western Europe may have derived from more than three different sources after glaciations (Sinclair *et al.*, 1999). Further analysis on ISSRs (inter simple sequence repeats) with respect to the shared alleles between individuals showed the highest degree of differentiation between French and Italian populations, with a divergence between the Alpine and Apennine populations with a higher genetic variability

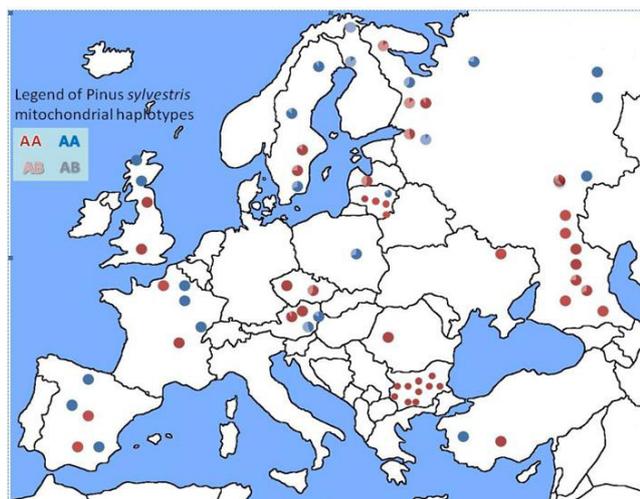


Fig. 1. Distribution of mitochondrial haplotypes for *P. sylvestris* in Europe\*

\*haplotype AA = most common allele for *nad1* and most common allele for *nad7*; haplotype AB = most common allele for *nad1* and less common allele for *nad7*; colours represent different studies as: haplotypes in blue revealed by Pyhäjärvi *et al.* (2008), and haplotypes in red revealed by Naydenov *et al.* (2007)

ity on Alpine populations (GD  $0.310 \pm 0.0252$ ) than Apennine samples (GD  $0.217 \pm 0.09$ ) (Labra *et al.*, 2006). A clear view over the distribution of the most common mitotypes are presented on Figure 1, reported by Naydenov *et al.* (2007) (red pie charts) and Pyhäjärvi *et al.* (2008) (blue pie charts).

Given the fact that Europe has been considered as having the largest potential distribution area for Scots pine during the LGM, studies carried out here reached the same conclusions regarding the impact of glacial refugia on migration pathways as the modern genetic diversity investigations on Scots pine. Genetic analysis carried out on mtDNA and cpDNA (Cheddadi *et al.*, 2006) combined with the paleodata, indicates that the potential refugia were located between  $40^\circ\text{N}$  and  $50^\circ\text{N}$  i.e. in the Iberian Peninsula, Italy and in the eastern part of the Alps. Altitudinal migration of Scots pine was followed using cpSSR analysis on mountain populations from northern Meseta and Iberian Peninsula, showing little differentiation among populations (Cheddadi *et al.*, 2006). However, while comparing within the species, a significant positive correlation was reported between genetic diversity and summer precipitation for some of the mountain pines (*P. uncinata*, *P. sylvestris* and *P. nigra*) (Soto *et al.*, 2010). Interestingly, isolated populations of Scots pine growing on disjoint mountain massifs (on slopes flowing to the same basin) are genetically closer than those growing on different slopes of the same mountain chain, but flowing to different basins (Robledo-Arnuncio *et al.*, 2005). Investigations on its Eurasian natural range revealed four mitochondrial haplotypes which were geographically highly structured, suggesting at least four genetically distinct ancestral lineages (Naydenov *et al.*, 2007). A new geographically restricted lineage was found confined to Asia Minor, and yet another was restricted to further northern latitudes in north-eastern Europe and the Baltic region, confirming recent observations for cold tolerant species about the possible existence of refugial populations at mid-northern latitudes contributing significantly to the re-colonization of northern Europe (Pyhäjärvi *et al.*, 2008).

## Conclusions and future research

All the studies to-date clearly point to Southern Europe (Iberian Peninsula, Italy and the Balkans) as being the most important area with glacial refugia for Scots pine. Several postglacial colonisations routes of Scots pine in Europe originated from these southern refugia. Similarly, other 'hot spots' were mentioned in central, western and even in northern parts of Europe, where Scots pine might have survived during the last ice age. More populations sampled in these regions may offer new insights into the evolutionary history of this cold-tolerant tree species. The analysis should focus on natural, relict populations since there are numerous plantations of Scots pine, which were established in the last two centuries with material of

unknown origin. Artificial long-distance seed transfer is assumed in many instances and should be kept in mind when sampling new populations for molecular study.

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