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

Valentina FLORAN\(^1\),*\, Radu E. SESTRAS\(^1\), Maria Rosario GARCÍA GIL\(^2\)

\(^1\)University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, 3-5 Mănăștur, 400372, Romania; Floran.Valentina@slu.se (corresponding author), riestras@yahoo.co.uk

\(^2\)Department of Forest Genetics and Plant Physiology, SLU, SE-901 83 Umeå, Sweden; M.Rosario.Garcia@slu.se

**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 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 (İbrahim 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; Brewet et al., 2002; Hewitt, 2000). During this period, a large proportion of the European tree flora was lost due the onset of

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**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).
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 southernmost 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 hardness 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; Mikkola, 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 refugia areas scattered throughout Europe (Birks, 1989; Tautau 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 (Tautau 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 (Bennet 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 nonrecombining 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) as compared to nuclear markers (biparentally inherited) in outcrossing hermaphrodites, and (2) presence of restricted recombination. A reduced effective population size (N) results in higher rate of genetic drift for organelle DNA (Bucci et al., 2007; Nasr et al., 2008; Vendramin et al., 2008), which is twice than that of the rate for nuclear DNA (Palumbl 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 Gst 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 Gst is always larger for mtDNA markers than for the cpDNA. For example, in Scots pine Fst 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 Fst 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 consideration 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 Table 1.

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

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

*Fst=fixation index; Gst=gene differentiation; Ht= total genetic diversity; Hs=average genetic diversity.*
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 ar isozyme markers \(Fst=0.028\) (Sinclair et al., 1998) with two common and one rare variants (mitotypes) detected.

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 colonisation 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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