

What Can Molecular Markers Tell Us about the Glacial and Postglacial Histories of European Birches?

Katarzyna A. Jadwiszczak

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The last glaciation was one of the most severe of the Pleistocene epoch. The development of the Scandinavian ice sheet forced many species to reduce their ranges to areas with favourable climatic conditions. Most European species survived the Last Glacial Maximum in refugia in southern parts of Europe. Cold-tolerant species, such as birch trees and shrubs, could also inhabit western, eastern or central Europe. After climate warming, Holocene recolonisation began. This paper presents a comparative analysis of the genetic variation of four European *Betula* species to reconstruct their glacial and postglacial histories. Two chloroplast DNA haplotypes dominate within the ranges of all birch species, one haplotype is the most common in western and northwestern Europe, the second haplotype occurs mainly in the eastern and southeastern parts of the continent. This finding suggests that birches have recolonised Europe from the western and the eastern refugia, respectively. Most of Europe was likely populated from higher latitude refugia because there was no evidence of isolation by distance and weak genetic structures were detected. Similar patterns of haplotype distributions within *Betula* ranges indicate that postglacial recolonisation may be disturbed by interspecies hybridisation.

Keywords *Betula*, cpDNA, glacial refugia, microsatellite, suture zone

Addresses Institute of Biology, University of Białystok, Świerkowa 20B, 15-950 Białystok, Poland **E-mail** kszalaj@uwb.edu.pl

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1 Introduction

Although the history of the Earth is rich in events that strongly influenced the abundance and distribution of species, it is probable that the Quaternary epoch was environmentally distinctive and caused extraordinary biotic patterns (Willis and Niklas 2004). For the vast majority of the Quaternary, glacial conditions prevailed on Earth and the ice ages were interspersed with shorter warm periods (Frogley et al. 1999). The Quaternary glacial – interglacial cycles, between 2 580 000 and 11 700 years ago, are called the Pleistocene period. Taking into account both the extent of ice volume and the reduction in tree populations, it is clear that the last glaciation of the Pleistocene was also one of the most severe (Magri 2010). During the Last Glacial Maximum (LGM), climatic conditions across Europe showed strong temperature gradients with temperatures 10–20 °C lower than presently observed in northern Europe, 7–10 °C lower in southern Europe, and 2–4 °C lower in southwestern Europe (Barron and Pollard 2002). Winter and summer precipitations were also differentiated throughout the continent with the highest precipitation occurring in central and southeastern Europe (Barron et al. 2003).

The development of continental ice sheets during the Pleistocene forced large-scale shifts in species distribution. Research presumed that organisms that presently inhabit the area of Europe survived the LGM in refugia situated in the Apennine, Iberian and Balkan Peninsulas as well as near the Caucasus region and the Caspian Sea (Hewitt 1999). Temperature and precipitation simulations confirmed that the southern part of Europe had the highest glacial isolate potential during the LGM (Ohlemüller et al. 2011). However, when the precipitation conditions were relaxed, it became obvious that western France, the northwestern parts of the central European lowlands and the regions south of the Scandinavian ice sheet could also act as glacial refugia (Ohlemüller et al. 2011). Indeed, the increasing number of fossil and genetic data reveals that some cold-tolerant species could survive the LGM in western, eastern or central Europe. Glacial isolates situated at latitudes or longitudes that differ from those that would normally be expected and presented as favourable climatic

islands surrounded by areas with severe climate conditions are referred to as ‘cryptic refugia’ (Stewart and Lister 2001, Stewart et al. 2010) or ‘microrefugia’ (Rull 2009). For example, a Carpathian refugium was proposed for *Fagus sylvatica* L. (Magri et al. 2006) and *Populus tremula* L. (Fussi et al. 2010), while *Fraxinus excelsior* L. could survive in the eastern Alps (Heuertz et al. 2004) and *Saxifraga oppositifolia* L. in northern Russia (Abbott et al. 2000).

Higher latitude refugia were previously deduced from the migration capabilities of some plants, such as oak trees. Reid (1899, Clark et al. 1998) supposed that oak trees with heavy seeds had needed ca. 1 000 000 years to overcome a distance from south European refugia to the northern part of Great Britain. The discrepancy between the time of recolonisation and putative tree migration rates, known as Reid’s paradox, was explained recently when a high latitude refugium for oaks was proposed (Stewart and Lister 2001, Pearson 2006).

Unquestionable information regarding the locations of Pleistocene refugia and Holocene recolonisation is inferred from fossil materials. However, it is not always possible to study the glacial and postglacial histories of certain species solely using fossil data. It was stated that a palaeoecological investigation was not beneficial to discover small glacial populations because of the limited spatial range of remnant localities (Hampe and Petit 2005, Kaltenrieder et al. 2009). Moreover, fossil remains may offer different taxonomic resolution levels, and the preservation of the fossil record may be unsatisfactory (Comes and Kadereit 1998, Maggs et al. 2008). For example, Pragłowski (1966) observed that the surrounding conditions influenced the size of *Betula nana* L. pollen; hence, it is not always possible to distinguish between birch species based on pollen grain size. The lack of or scarcities of fossil data are additional problems regarding a palaeoecological survey, which was revealed for *Prunus spinosa* L. (Mohanty et al. 2002) and *Populus nigra* L. (Cottrell et al. 2005, Fussi et al. 2010). *Betula pendula* Roth. demonstrates an additional problem because the fossil pollen maps do not show a clear chronological sequence of postglacial recolonisation across Europe (Palmé et al. 2003).

The past demographic events often left genetic imprints on contemporary populations (Hewitt 2000), and molecular studies can be used synergistically with palaeoecology to localise potential glacial refugia and possible routes of recolonisation (Hu et al. 2009). It is presumed that ancestral populations have the greatest genetic diversity and correspond to glacial isolates (Bennett and Provan 2008). However, high levels of genetic variations could also result from mixing different genetic lineages (Taberlet et al. 1998). The detection of a high frequency of private haplotypes or alleles (private haplotypes are present in one population only) may be useful in locating putative refugial populations (Maggs et al. 2008). In contrast, newly colonised regions are characterised by low genetic diversity and a lack of private haplotypes. The scientific discipline that describes the geographical pattern of genetic lineages is known as phylogeography (Avise 2000). The most popular molecular marker used in the plant phylogeographic studies is chloroplast DNA (cpDNA). Chloroplast DNA is nonrecombining, maternally inherited in angiosperms, has a smaller effective population size and much higher among-population genetic differentiation than do nuclear markers; thus, it has many advantages in the localisation of glacial refugia and tracking postglacial routes of recolonisation (Palmé et al. 2003). However, biparentally inherited nuclear DNA markers are also used in phylogeographic survey as they provide information from many loci segregating independently (Lascoux et al. 2008, Hu et al. 2009). The aim of present study is to review the recently published molecular data on the distributions of genetic diversity within the ranges of four European *Betula* species in order to compare glacial and postglacial histories of birches.

2 Characteristics of the *Betula* Species

The *Betula* genus contains trees and shrubs that inhabit diverse habitats in boreal and temperate climate zones of the Northern Hemisphere, between 79°N and 20°N (Schenk et al. 2008). The exact number of species that belong to this genus

is not known, but it is estimated to be between 30 and 60 (Järvinen et al. 2004). The genus is placed within the Betulaceae family of the order Fagales. In general, four species belonging to the genus *Betula* are described in Europe: *B. pendula*, *B. nana*, *B. pubescens* Ehrh. and *B. humilis* Schrk. However, some authors identify additional tree species: *B. oycoviensis* Bess., *B. szaferi* Jent.-Szaf. ex Stasz., and *B. carpatica* Waldst. and Kit ex Willd. (Staszkiwicz 2001, Ralska-Jasiewiczowa et al. 2004). The present paper compares the distribution of the genetic diversity within the ranges of *B. pendula*, *B. pubescens*, *B. nana* and *B. humilis* because *B. oycoviensis*, *B. szaferi* and *B. carpatica* are locally distributed and have no explicitly defined systematic status (Ralska-Jasiewiczowa et al. 2004).

The relationships among the *Betula* species are difficult to interpret regardless of the marker used in within genus taxonomic analysis. Morphological classification of de Jong (1993) divided genus into five subgenera: *Betula*, *Betulaster*, *Betulenta*, *Chamaebetula* and *Neurobetula*. *Betula pendula* and *B. pubescens* belonged to the subgenus *Betula*, whereas *B. humilis* and *B. nana* to the subgenus *Chamaebetula*. This classification was generally supported by the analysis of secondary metabolites (Keinänen et al. 1999). Based on *ADH* (alcohol dehydrogenase) nuclear gene sequence, Järvinen et al. (2004) distinguished three groups within the genus *Betula*. *Betula humilis* and the *B. pubescens* short allele of *ADH* gene (without indels) were included into Group I. However, when long allele of the *B. pubescens* gene (comprising indels) was considered, then *B. pubescens*, *B. pendula* and *B. nana* were grouped together (Group III). Results of AFLP (amplified fragment length polymorphism) studies suggested that all European birch species should be placed into one group (Group IV. *Betula*) (Schenk et al. 2008). Above taxonomic problems are probably caused by hybridisation/introgression process which has a homogenizing effect on the relationships within the genus; however, a homoplasy being a consequence of the occurrence of major speciation events in a very short time frame cannot be excluded, either (Schenk et al. 2008).

Betula pendula and *B. pubescens* are tall trees and live up to 90–100 years. *B. humilis* and *B. nana* are shrubs with a lifespan of approxi-

mately 20 years. All birches require large amounts of sunlight in all stages of development, possess a broad tolerance of soil and temperature requirements, are fast growing, exist in abundant quantities and fruit annually (Środoń 1979, Ralska-Jasiewiczowa et al. 2004). These features make them effective pioneer species. Birches are monoecious, wind-pollinated and wind-dispersed species. *Betula pendula* and *B. pubescens* are nearly sympatric species, with the exception at high latitudes and altitudes where *B. pubescens* coexists with shrub birches. *Betula humilis* and *B. nana* are distributed in the northern and eastern Europe. *Betula humilis* grows in the former Soviet Union and in Poland, and *B. nana* occurs mainly on the Scandinavian Peninsula and in the northern regions of Russia (Kruszelnicki and Fabiszewski 2001, Załuski et al. 2001). Birch trees are generally the ubiquitous species and occupy a variety of habitats but *B. pubescens* prefers a wetter habitat. *Betula nana* occupies raised bogs, while *B. humilis* populates natural and drained fens, transitional mires and wet meadows. *Betula humilis* and *B. nana* are central European glacial relicts, and the decay of their populations provoked European governments to enforce strict protection.

Sympatric birch populations show frequent hybridisation (Staszkiwicz et al. 1993, Thórsson et al. 2001, 2007, Anamthawat-Jónsson and Thórsson 2003, Palmé et al. 2004). *Betula pendula*, *B. humilis* and *B. nana* are diploids with $2n=28$ chromosomes. *Betula pubescens* is allo-tetraploid ($2n=56$), derived from *B. pendula* and likely from *B. humilis* (Howland et al. 1995, Järvinen et al. 2004). Interspecies birch hybrids can be triploids, as was observed between *B. pubescens* and *B. nana* in Iceland (Anamthawat-Jónsson and Thórsson 2003; Thórsson et al. 2007) or aneuploids, as was suggested for Polish *B. humilis* populations (Jadwiszczak et al. 2011b).

3 Fossil Material

The fossil material does not reveal unequivocally when and where the *Betula* genus initially appeared (Palmé 2003). This is because the initial records of the genus, which date to the Paleocene and Early

Eocene, have been uncertainly assigned (Crane 1981). Molecular estimates predict that the genus *Betula* originated in the Late Eocene (Forest et al. 2005). It is probable that *Betula* was rare in the early Tertiary but became abundant and taxonomically diverse in the late Tertiary (Němejc 1975, Środoń 1979, Huntley and Birks 1983). The changes in the abundance of birch pollen throughout the Quaternary are an excellent indicator of climate transitions. In the early and late phases of the Quaternary glaciations, the highest values of *B. pendula* and *B. pubescens* pollen are noted and *B. nana* pollen dominated in the full glacial stages (Tralau 1963). During the LGM, birches were not confined only to southern European refugia but were also spread in central and eastern Europe (Willis et al. 2000, Willis and van Andel 2004, Markova et al. 2009). From the beginning of the late glacial, birches were dominant species in forest assemblages across different parts of Europe (Ralska-Jasiewiczowa et al. 2004). Birch woodlands were temporarily reduced at the end of the Pleistocene, but *Betula* again became the key taxon in the European landscape at the beginning of the Holocene (Bos 2001). The continuous range of birch forests in 9000 years BP (before present), which spread from the British Isles to Russia, was fragmented in central and western Europe by thermophilous trees (Ralska-Jasiewiczowa et al. 2004). An increase of *Betula* in woodlands started ca. 5 000 years BP as a result of habitat changes induced by anthropogenic and climatic factors (Huntley and Birks 1983).

4 Molecular Studies on Phylogeography of European Birches

The present comparison of molecular variation distribution of European *Betula* is based on the studies conducted by: Palmé et al. (2003, 2004), Maliouchenko et al. (2007), Thórsson et al. (2010) and Jadwiszczak et al. (2011a, 2012a, b) (Table 1). In general, cpDNA markers were used, and PCR-RFLP (polymerase chain reaction – restriction fragment length polymorphism) method was applied. Three non-coding regions of cpDNA were amplified in PCR reactions with TF, CD and AS primer pairs (Taberlet et al. 1991,

Table 1. Characteristics of phylogeographic studies conducted within the ranges of European *Betula* species.

Birch species	References	Molecular marker used	No of individuals studied	Geographic range of study
<i>Betula pendula</i>	Palmé et al. 2003, 2004	Restriction fragments of cpDNA	439	Whole Europe
	Maliouchenko et al. 2007	Restriction fragments of cpDNA, chloroplast microsatellites	412	Whole Europe
	Thórsson et al. 2010	Restriction fragments of cpDNA	14	Scotland
	Jadwiszczak et al. 2012a	Restriction fragments of cpDNA	36	Poland and Belarus
<i>Betula pubescens</i>	Palmé et al. 2004	Restriction fragments of cpDNA	162	Western, northern and eastern Europe
	Maliouchenko et al. 2007	Restriction fragments of cpDNA, chloroplast microsatellites	213	Western, eastern, northern and southern Europe
	Thórsson et al. 2010	Restriction fragments of cpDNA	235	Iceland, Greenland, Scotland and Scandinavia
	Jadwiszczak et al. 2012a	Restriction fragments of cpDNA	31	Poland and Belarus
<i>Betula nana</i>	Palmé et al. 2004	Restriction fragments of cpDNA	60	Russia and Scandinavia
	Maliouchenko et al. 2007	Restriction fragments of cpDNA, chloroplast microsatellites	69	Iceland, Russia and Scandinavia
	Thórsson et al. 2010	Restriction fragments of cpDNA	171	Iceland and Scandinavia
	Jadwiszczak et al. 2012b	Restriction fragments of cpDNA, nuclear microsatellites	52	Poland
<i>Betula humilis</i>	Jadwiszczak et al. 2011a, b, 2012a	Restriction fragments of cpDNA, nuclear microsatellites	384	Poland and Belarus

Demesure et al. 1995). PCR products were then digested with the restriction enzymes *TaqI* and *HinfI*. The cpDNA-enzyme combination pairs AS-*TaqI*, TF-*TaqI*, TF-*HinfI*, CD-*TaqI* and CD-*HinfI* were chosen because they showed variation in the pilot studies (Palmé et al. 2003). In addition to the non-coding regions of cpDNA, phylogeographic studies of birch trees and shrubs were strengthened by the addition of chloroplast microsatellites and nuclear microsatellites (Maliouchenko et al. 2007, Jadwiszczak et al. 2011a, 2012b). Altogether, 901 *B. pendula* individuals, 641 *B. pubescens*, 352 *B. nana* and 384 *B. humilis* individuals were analysed (Table 1).

5 Patterns of Molecular Variation Distribution

5.1 *Betula pendula*

The range of *B. pendula* is dominated by two haplotypes – haplotype A in western and north-western Europe and haplotype C in eastern and southeastern Europe (Fig. 1A) (Palmé et al. 2003, Maliouchenko et al. 2007). This genetic distribution indicates that the continent was populated by migrations from two distinct refugia. The western and eastern waves of migration mixed and formed

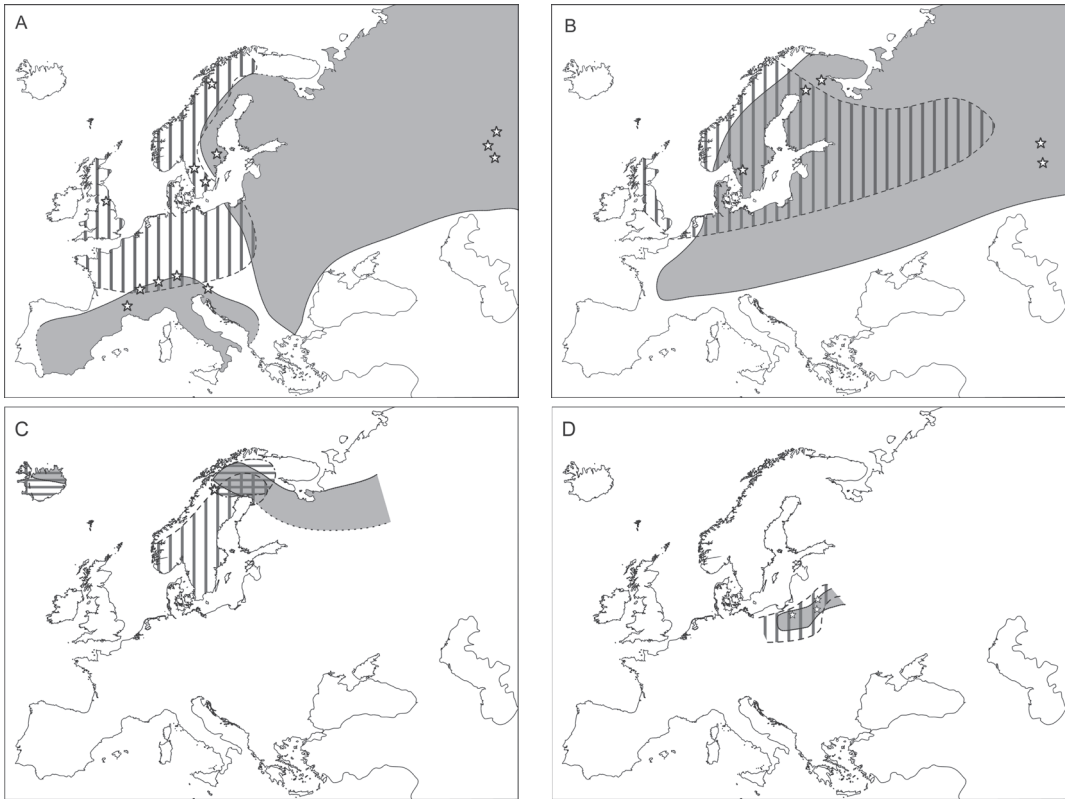


Fig. 1. Geographical distribution of the three most common cpDNA haplotypes and locations of the areas with high genetic diversities in *B. pendula* (A), *B. pubescens* (B), *B. nana* (C) and *B. humilis* (D). Vertical striping indicates the extent of haplotype A (=haplotype I in *B. humilis*). Grey area indicate the extent of haplotype C (=haplotype II in *B. humilis*). Horizontal striping indicates the extent of haplotype T in northern Scandinavia and Iceland. Asterisks indicate areas of high genetic diversities.

a suture zone in the region from northern Sweden into Poland, Slovakia, Hungary, Romania and Greece. Although genetic data did not pinpoint the precise location of the origin of the two most common haplotypes, it could be supposed that one glacial isolate was situated near the Ural Mts. with a second origin north of the Alps. This suggestion was inferred from PCR-RFLP analyses of chloroplast DNA, which revealed three areas of high genetic variation within the *B. pendula* range: near the Ural Mts., north of the Alps and in southern Sweden (Fig. 1A) (Palmé et al. 2003, Maliouchenko et al. 2007). These areas may have acted as glacial refugia during the LGM. Presently, there is no doubt that some species could have survived the LGM at a location in eastern

and central Europe because permafrost did not expand south of 52–53°N and the soil temperatures were not low during this time (Velichko and Zelikson 2005). An eastern refugium was suggested for cold-tolerant plant species such as *Picea abies* (L.) H. Karst. (Vendramin et al. 2000) and *Pinus sylvestris* L. (Pyhäjärvi et al. 2008).

However, was it probable that a glacial isolate of *B. pendula* existed in Scandinavia within the area covered by the ice sheet? There are two possible explanations for the high genetic diversity of *B. pendula* in southern Sweden. First, a population of limited size may have survived on a nunatak (an ice free mountain) and then spread over the Scandinavian Peninsula after the Holocene climate warming. Palmé et al. (2003) presumed

that this explanation was unlikely because the contribution of a small glacial isolate to the present-day species' gene pool would be insignificant and overwhelmed by the fast recolonisation from a southern or eastern refugium. Second, the high genetic variation in the southern part of Scandinavia could be the result of recent mutations, which were subsequently spread locally (Palmé et al. 2003). Although chloroplast microsatellite investigation supported haplotypic diversity of *B. pendula* in southern Scandinavia (Maliouchenko et al. 2007), the issue of a northern glacial refugium could be resolved by determining the in situ existence of *B. pendula* in this area during the LGM.

It is probable that *B. pendula* could exist during the LGM within an area located north of the Alps. Climatic simulations conducted by Ohlemüller et al. (2011) suggest that some species could survive the LGM in the northwestern parts of the central European lowlands. Moreover, macrofossil remains that date to 27 000–18 000 years BP strongly demonstrate that birches existed in the Carpathians and their northern foreland (Ralska-Jasiewiczowa et al. 2004), an area closer to the Scandinavian ice sheet than the foreland of the Alps.

Excluding the refugia situated at higher latitudes, *B. pendula* was also present in southern Europe during the LGM. This was deduced based on private haplotypes noted in populations from Italy, Spain, Corsica and Croatia (Palmé et al. 2003). However, migration waves from these areas were probably hindered by the Alps and Pyrenees and by the presence of other birch populations (Hewitt 2000, Palmé et al. 2003).

5.2 *Betula pubescens*

The highest genetic diversity of *B. pubescens* was observed in the Scandinavian Peninsula and near the Ural Mts. (Fig. 1B) (Palmé et al. 2004, Maliouchenko et al. 2007). It is, however, difficult to say whether those locations could be referred to *B. pubescens* glacial refugia. Macrofossil evidence revealed that *B. pubescens* occurred in western Siberia ca. 33 000 years BP, but no evidence of any *Betula* species was obtained in this region for the late glaciation stage (Binney et al. 2009).

Similar to *B. pendula*, two haplotypes – A and C – are the most common throughout the range of *B. pubescens* (Fig. 1B) (Palmé et al. 2004, Maliouchenko et al. 2007). Relative to *B. pendula*, *B. pubescens* showed much stronger genetic structuring. Seven genetic clusters of populations were distinguished within the range of *B. pubescens* (Maliouchenko et al. 2007). Three major clusters were formed by populations spread from France to Siberia, within Scandinavia and northern Russia, and three other locations in Siberia. Four clusters corresponded to peripheral populations in Iceland, Great Britain, Spain and Siberia. Maliouchenko et al. (2007) explained this phenomenon in two manners. *Betula pubescens* prefers wetter and more acidic soils than *B. pendula*, and it is possible that these two congeneric species had different postglacial dynamics. Moreover, *B. pubescens* is more resistant to low temperatures than *B. pendula* (Ralska-Jasiewiczowa et al. 2004) and could therefore survive the LGM at higher latitudes and could begin recolonisation earlier (Maliouchenko et al. 2007). This hypothesis was supported by macrofossil data that showed that *B. pubescens* had existed on different nunatak sites in the Scandinavian Mts. during the late glacial period (Kullman 2008). The second explanation is hybridisation with *B. nana* (Maliouchenko et al. 2007). *Betula pubescens* and *B. nana* are sympatric in the northern Fennoscandia and in the Arctic where *B. pendula* does not occur (Ralska-Jasiewiczowa et al. 2004). Additionally, *B. nana* is confined to wet habitats in the southern part of the range. Wet habitats are also tolerated by *B. pubescens* but not by *B. pendula*. Favourable conditions for the hybridisation of *B. pubescens* and *B. nana* could cause the formation of genetic structuring within the former species (Maliouchenko et al. 2007).

5.3 *Betula nana*

Sampling conducted by Palmé et al. (2004), Maliouchenko et al. (2007) and Jadwiszczak et al. (2012b) distinguished only one population with high genetic diversity. This population was Salfjellet in northern Norway (Fig. 1C). Could *B. nana* have survived the last glaciation at high latitude? Macrofossils discovered in northern

Russia dated to ca. 22 000 cal. years (radiocarbon calibrated years) BP showed that *B. nana* was located near the Scandinavian ice sheet (Binney et al. 2009). Could it have existed in the Arctic? Only a palaeoecological survey demonstrating a continuous in situ existence of the *B. nana* species in northern Norway during the LGM could answer this question. Glacial in situ survival within the area of Norway was previously suggested for other plants: *Sagina caespitosa* (J. Vahl) Lange and *Arenaria humifusa* Wahlenb. (Westergaard et al. 2011). Molecular markers revealed that conifer trees could also exist in northwestern Norway as early as 22 000–17 000 years ago (Parducci et al. 2012). *Betula nana* is a micro-thermal plant; therefore, it is possible that this species could occupy the ice-free areas of the Scandinavian Peninsula. However, if *B. nana* survived the last glaciation in northern Norway, that shrub population did not spread to the south. The distribution of cpDNA haplotypes throughout Scandinavia showed that the peninsula was recolonised by haplotype A and C from the southwestern and southeastern directions (Fig. 1C) (Palmé et al. 2004, Maliouchenko et al. 2007). The same waves of migrations could have populated central Europe as the two most common cpDNA haplotypes were also found in Poland (Jadwiszczak et al. 2012b).

A detailed analysis of chloroplast DNA of Icelandic *B. nana* populations revealed high haplotypic diversity in the eastern part of Iceland (Thórsson et al. 2010). According to Thórsson et al. (2010), this result may strongly suggest that the populations from the eastern part of Iceland are the oldest. Iceland was probably colonised from northern Scandinavia in the early Holocene. Genetic evidence supporting this scenario is haplotype T, which occurred with high frequency in Iceland and with much lower frequency in the northern part of the Scandinavian Peninsula (Palmé et al. 2004, Maliouchenko et al. 2007, Thórsson et al. 2010).

5.4 *Betula humilis*

Only 19 populations located in the southwestern and subcentral regions of the species range have been studied. Similar to other birch species, two

haplotypes dominate the *B. humilis* study region, which may suggest a recolonisation from two distinct refugia (Fig. 1D) (Jadwiszczak et al. 2012a). However, based on genetic data, it is not possible to indicate the exact location of these refugia. Taking into account the high genetic diversity and relatively high frequency of one private haplotype in the central Belarusian populations, Jadwiszczak et al. (2012a) proposed that one glacial isolate could be located in eastern Europe. This agrees with genetic data presented for other birch species (Palmé et al. 2003, 2004, Maliouchenko et al. 2007). The second *B. humilis* glacial isolate may be situated either in the Carpathians or in the western Europe. The hypothesis of the Carpathian refuge can be supported by macrofossils identified in the Weichselian sediments in the Ukrainian part of the Carpathians (Stachowicz-Rybka et al. 2009). In turn, *B. humilis* fossil fruits that were discovered in northern Germany and dated to the LGM (Freund et al. 2001) could suggest that a refugium had existed in western Europe. The present existence of remnant *B. humilis* populations in Germany, Austria and Romania may indicate that this species populated the western and central parts of Europe during the Weichselian (Załoski et al. 2001).

An interesting result of the cpDNA studies was the high genetic variation in northeastern Poland because the area was in close proximity to the ice sheet during the LGM (Fig. 1D) (Jadwiszczak et al. 2012a). According to the authors, the most probable explanation is a contact zone between two phylogenetic lineages. An unknown cryptic refugium located in northeastern Poland could be a second explanation. However, it is less likely due to the low frequency of private cpDNA haplotypes and private nuclear microsatellite alleles (Jadwiszczak et al. 2011a, 2012a).

6 Concluding Remarks

Although the response of every species to the Pleistocene climatic changes was varied in direction and scale, which consequently resulted in the origin of new biomes with no modern equivalents (Markova et al. 2009), European birches show some similarities in phylogeographical patterns.

The range of every birch species is predominated by two of the same haplotypes. These haplotypes have a central position in the birch phylogenetic trees and differ from one another by three mutations. It was proposed that these haplotypes appeared before the LGM and could reflect an ancestral polymorphism (Palmé et al. 2004, Thórsson et al. 2010, Jadwiszczak et al. 2012a). The clear dominance of haplotype A (=haplotype I in *B. humilis*; Jadwiszczak et al. 2012a) in western and northwestern Europe and haplotype C (=haplotype II in *B. humilis*) in eastern and northeastern Europe could be explained by recolonisation from two distinct refugia. Second, it is likely that most of Europe was recolonised by waves of migrations from glacial refugia at higher latitudes because no isolation by distance (adjacent populations are more genetically similar than distant ones) was observed in *B. pendula*, *B. pubescens* and *B. humilis* (Maliouchenko et al. 2007, Jadwiszczak et al. 2012a). The genetic evidence supporting high latitude glacial refugia of the *Betula* species is a weak genetic structure compared to other broadleaved trees (Palmé et al. 2003, Maliouchenko et al. 2007, Thórsson et al. 2010, Jadwiszczak et al. 2012a). Finally, similar patterns of haplotype distribution within the ranges of the studied birches clearly suggest considerable interspecies hybridisation. Unfortunately, this hybridisation makes the picture of postglacial recolonisation difficult to interpret (Palmé et al. 2004, Maliouchenko et al. 2007, Jadwiszczak et al. 2012a).

Data on the level and distribution of genetic diversity are very useful for various aspects of the conservation of endangered birch species and forest management. Based on results of molecular studies, it is possible to indicate appropriate populations for conservation, and then the most proper protection activities can be applied. For example, northeastern Polish populations of the endangered *B. humilis* are most valuable units for conservation as they represent the highest level of genetic variation, thus they constitute the bulk of genetic resources of the species in central Europe (Jadwiszczak et al. 2011a, 2012a). To protect *B. humilis* populations in declining southwestern margin of the species range, it is necessary to conduct regular coppicing because the overgrowing of fens by brushwood and forest competitors is one

of the greatest threat for the existence of this birch shrub (Zaluski et al. 2001). However, there are also situations when populations with low genetic diversity should be protected. These are populations with private cpDNA haplotypes or other private markers because the unique markers give a special feature for populations. For example, three isolated and genetically depauperated localities of *B. nana* in Poland should be protected as they comprise many private microsatellite alleles and belong to the two distinct phylogeographic lineages (Jadwiszczak et al. 2012b).

Another practical application of genetic studies is an avoidance of germplasm transfer between different phylogenetic lineages as well as between populations adapted to different climatic conditions. Analysis of the distribution of genetic variation within birch species allows also to track their population histories and to predict future range shifts induced by the climate change.

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References

- Abbott, R.J., Smith, L.C., Milne, R.I., Crawford, R.M.M., Wolff, K. & Balfour, J. 2000. Molecular analysis of plant migration and refugia in the Arctic. *Science* 289: 1343–1346.
- Anamthawat-Jónsson, K. & Thórsson, A.T. 2003. Natural hybridisation in birch: triploid hybrids between *Betula nana* and *B. pubescens*. *Plant Cell, Tissue and Organ Culture* 75: 99–107.
- Avise, J.C. 2000. *Phylogeography: the history and formation of species*. Harvard University Press, Cambridge.
- Barron, E. & Pollard, D. 2002. High-resolution climate simulations of oxygen isotope stage 3 in Europe. *Quaternary Research* 58: 296–309.

- , van Andel, T.H. & Pollard, D. 2003. Glacial environments. II. Reconstructing the climate of Europe in the last glaciation. Neanderthals and modern humans in the European landscape during the last glaciation. In: van Andel, T.H. & Davies, S.W. (eds.). McDonald Institute for Archaeological Research, Cambridge. p. 57–78.
- Bennett, K.D. & Provan, J. 2008. What do we mean by 'refugia'? *Quaternary Science Reviews* 27: 2449–2455.
- Binney, H.A., Willis, K.J., Edwards, M.E., Bhagwat, S.A., Anderson, P.M., Andreev, A.A., Blaauw, M., Damblon, F., Haesaerts, P., Kienast, F., Kremenetski, K.V., Krivonogov, S.K., Lozhkin, A.V., MacDonald, G.M., Novenko, E.Y., Oksanen, P., Sapelko, T.V., Väliranta, M. & Vazhenina, L. 2009. The distribution of late-Quaternary woody taxa in northern Eurasia: evidence from a new macrofossil database. *Quaternary Science Review* 28: 2445–2464.
- Bos, J.A.A. 2001. Lateglacial and Holocene vegetation history of the northern Wetterau and the Amöneburger Basin (Hessen), central-west Germany. *Review of Palaeobotany and Palynology* 115: 177–212.
- Clark, J.S., Fastie, Ch., Hurr, G., Jackson, S.T., Johnson, C., King, G.A., Lewis, M., Lynch, J., Pacala, S., Prentice, C., Schupp, E.W., Webb, III T. & Wyckoff, P. 1998. Reid's paradox of rapid plant migration. *Bioscience* 48: 13–24.
- Comes, H.P. & Kadereit, J.W. 1998. The effect of Quaternary climatic changes on plant distribution and evolution. *Trends in Plant Science* 3: 432–438.
- Cottrell, J.E., Krystufek, V., Tabbener, H.E., Milner, A.D., Connolly, T., Sing, L., Fluch, S., Burg, K., Lefèvre, F., Achard, P., Bordács, S., Gebhardt, K., Vornam, B., Smulders, M.J.M., Vanden Broeck, A.H., Slycken, J.V., Storme, V., Boerjan, W., Castiglione, S., Fossati, T., Alba, N., Agúndez, D., Maestro, C., Notivol, E., Bovenschen, J. & van Dam, B.C. 2005. Postglacial migration of *Populus nigra* L.: lessons learnt from chloroplast DNA. *Forest Ecology and Management* 206: 71–90.
- Crane, P.R. 1981. Betulaceous leaves and fruits from the British Upper Paleocene. *Botanical Journal of the Linnean Society* 83: 103–136.
- De Jong, P.C. 1993. An introduction to *Betula*: its morphology, evolution, classification and distribution with a survey of recent work. The IDS *Betula* Symposium. International Dendrology Society, Sussex, U.K.
- Demesure, B., Sodji, N. & Petit, R.J. 1995. A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Molecular Ecology* 4: 129–131.
- Forest, F., Savolainen, V., Chase, M.W., Lupia, R., Bruneau, A. & Crane, P.R. 2005. Teasing apart molecular- versus fossil-based error estimates when dating phylogenetic trees: a case study in the birch family (Betulaceae). *Systematic Botany* 30: 118–133.
- Freund, H., Birks, H.H. & Birks, H.J.B. 2001. The identification of wingless *Betula* in Weichselian sediments in Gross Todtshorn borehole (Lower Saxony, Germany) – the occurrence of *Betula humilis* Schrank. *Vegetation History and Archaeobotany* 10: 107–115.
- Frogley, M.R., Tzedakis, P.C. & Heaton, T.H.E. 1999. Climatic variability in northwest Greece during the last interglacial. *Science* 285: 1886–1889.
- Fussi, B., Lexer, C. & Heinze, B. 2010. Phylogeography of *Populus alba* (L.) and *Populus tremula* (L.) in central Europe: secondary contact and hybridization during recolonisation from disconnected refugia. *Tree Genetics and Genomes* 6: 439–450.
- Hampe, A. & Petit, R. 2005. Conserving biodiversity under climate change: the rear edge matters. *Ecology Letters* 8: 461–467.
- Heuertz, M., Hausman, J.F., Hardy, O.J., Vendramin, G.G., Frascaria-Lacoste, N. & Vekemans, X. 2004. Nuclear microsatellites reveal contrasting patterns of genetic structure between western and south-eastern European populations of the common ash (*Fraxinus excelsior* L.). *Evolution* 58: 976–988.
- Hewitt, G.M. 1999. Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society* 68: 87–112.
- 2000. The genetic legacy of the Quaternary ice ages. *Nature* 405: 907–913.
- Howland, D.E., Oliver, R.P. & Davy, A.J. 1995. Morphological and molecular variation in natural population of *Betula*. *New Phytologist* 130: 117–124.
- Hu, F.S., Hampe, A. & Petit, R.J. 2009. Palaeoecology meets genetics: deciphering past vegetational dynamics. *Frontiers in Ecology and the Environment* 7: 371–379.
- Huntley, B. & Birks, H.J.B. 1983. An atlas of past and present pollen maps for Europe: 0–13000 years ago. Cambridge University Press, Cambridge.

- Jadwiszczak, K.A., Banaszek, A., Jabłońska, E. & Sozinov, O.V. 2011a. Could *Betula humilis* Schrk. have survived the last glaciation at a current margin of its distribution? - testing the hypothesis of glacial refugium using nuclear microsatellites. *Plant Systematics and Evolution* 297: 147–156.
- , Banaszek, A., Jabłońska, E. & Sozinov, O.V. 2012a. Chloroplast DNA variation of *Betula humilis* Schrk. in Poland and Belarus. *Tree Genetics and Genomes* 8: 1017–1030.
- , Drzymulska, D., Banaszek, A., Jadwiszczak, P. 2012b. Population history, genetic variation and conservation status of the endangered birch species *Betula nana* L. in Poland. *Silva Fennica* 46(4): 465–477.
- , Jabłońska, E., Kłosowski, S. & Banaszek, A. 2011b. Aneuploids in the shrub birch *Betula humilis* populations in Poland. *Acta Societatis Botanicorum Poloniae* 80: 233–235.
- Järvinen, P., Palmé, A., Morales, L.O., Länneppää, M., Keinänen, M., Sopanen, T. & Lascoux, M. 2004. Phylogenetic relationships of *Betula* species (Betulaceae) based on nuclear ADH and chloroplast matK sequences. *American Journal of Botany* 9: 1834–1845.
- Kaltenrieder, P., Belis, C.A., Hofstetter, S., Ammann, B., Ravazzi, C. & Tinner, W. 2009. Environmental and climatic conditions at a potential Glacial refugial site of tree species near the Southern Alpine glaciers. New insights from multiproxy sedimentary studies at Lago della Costa (Euganean Hills, Northeastern Italy). *Quaternary Science Reviews* 28: 2647–2662.
- Keinänen, M., Julkunen-Tiitto, R., Rousi, M. & Tahvanainen, J. 1999. Taxonomic implications of phenolic variation in leaves of birch (*Betula* L.) species. *Biochemical Systematics and Ecology* 27: 243–254.
- Kruszelnicki, J. & Fabiszewski, J. 2001. *Betula nana* L. In: Kaźmierczakowa, R. & Zarzycki, K. (eds.). *Polska czerwona księga roślin* (Polish plant red book). Instytut Botaniki im. W. Szafera, PAN, Kraków: 82–83.
- Kullman, L. 2008. Early postglacial appearance of tree species in northern Scandinavia: review and perspective. *Quaternary Science Reviews* 27: 2467–2472.
- Lascoux, M., Pyhäjärvi, T., Källman, T. & Savolainen, O. 2008. Past demography of forest trees: what can we learn from nuclear DNA sequences that we do not already know? *Plant Ecology and Diversity* 1: 209–215.
- Maggs, C.A., Castilho, R., Foltz, D., HENZLER, C., Taimour, J.M., Kelly, J., Olsen, J., Perez, K.E., Stam, W., Väinölä, R., Viard, F. & Wares, J. 2008. Evaluating signatures of glacial refugia for north Atlantic benthic marine taxa. *Ecology* 89 (Supplement): 108–122.
- Magri, D. 2010. Persistence of tree taxa in Europe and Quaternary climate changes. *Quaternary International* 219: 145–151.
- , Vendramin, G.G., Comps, B., Dupanloup, I., Geburek, T., Gömöry, D., Latałowa, M., Litt, T., Paule, L., Roure, J.M., Tantau, I., van der Knaap, W.O., Petit, R.J. & de Beaulieu, J-L. 2006. A new scenario for the Quaternary history of European beech populations: palaeobotanical evidence and genetic consequences. *New Phytologist* 171: 199–221.
- Maliouchenko, O., Palmé, A.E., Buonamici, A., Vendramin, G.G. & Lascoux, M. 2007. Comparative phylogeography and population structure of European *Betula* species, with particular focus on *B. pendula* and *B. pubescens*. *Journal of Biogeography* 34: 1601–1610.
- Markova, A.K., Simakova, A.N. & Puzachenko, A.Yu. 2009. Ecosystems of Eastern Europe at the time of maximum cooling of the Valdai glaciation (24–18 kyr BP) inferred from data on plant communities and mammal assemblages. *Quaternary International* 201: 53–59.
- Mohanty, A., Martín, J.P. & Aguinalde, I. 2002. Population genetic analysis of European *Prunus spinosa* (Rosaceae) using chloroplast DNA markers. *American Journal of Botany* 89: 1223–1228.
- Němejc, F. 1975. *Paleobotanica*, T. IV, Praha.
- Ohlemüller, R., Huntley, B., Normand, S. & Svenning, J-C. 2011. Potential source and sink locations for climate-driven species range shifts in Europe since the Last Glacial Maximum. *Global Ecology and Biogeography* 21: 152–163.
- Palmé, A. 2003. Evolutionary history and chloroplast DNA variation in three plant genera: *Betula*, *Corylus* and *Salix*. Diss. thesis, University of Uppsala, Uppsala.
- , Su, Q., Palsson, S. & Lascoux, M. 2004. Extensive sharing of chloroplast haplotypes among European birches indicates hybridization among *Betula pendula*, *B. pubescens* and *B. nana*. *Molecular Ecology* 13: 167–178.

- , Su, Q., Rautenberg, A., Manni, F. & Lascoux, M. 2003. Postglacial recolonization and cpDNA variation of silver birch, *Betula pendula*. *Molecular Ecology* 12: 201–212.
- Parducci, L., Jørgensen, T., Tollefsrud, M.M., Elverland, E., Alm, T., Fontana, S.L., Bennett, K.D., Haile, J., Matetovici, I., Suyama, Y., Edwards, M.E., Andersen, K., Rasmussen, M., Boessenkool, S., Coissac, E., Brochmann, C., Taberlet, P., Houmark-Nielsen, M., Larsen, N.K., Orlando, L., Gilbert, M.T.P., Kjær, K.H., Alsos, I.G. & Willerslev, E. 2012. Glacial survival of boreal trees in northern Scandinavia. *Science* 335: 1083–1086.
- Pearson, R.G. 2006. Climate change and the migration capacity of species. *Trends in Ecology and Evolution* 21: 111–113.
- Praglowski, J. 1966. On pollen size variations and the occurrence of *Betula nana* in different layers of a bog. *Grana Palynologica* 6: 528–543.
- Pyhäjärvi, T., Salmela, M.J. & Savolainen, O. 2008. Colonization routes of *Pinus sylvestris* inferred from distribution of mitochondrial DNA variation. *Tree Genetics and Genomes* 4: 247–254.
- Ralska-Jasiewiczowa, M., Wacnik, A., Mamakowa, K. & Nalepka, D. 2004. *Betula L.* –Birch. In: Ralska-Jasiewiczowa, M., Latałowa, M., Wasylkowa, K., Tobolski, K., Madeyska, E., Wright, Jr. H.E. & Turner, C. (eds.). *Late Glacial and Holocene history of vegetation in Poland based on isopollen maps*. Instytut Botaniki im. W. Szafera, PAN, Kraków. p. 57–68.
- Reid, C. 1899. *The origin of the British flora*. Dulau, London.
- Rull, V. 2009. Microrefugia. *Journal of Biogeography* 36: 481–484.
- Schenk, M.F., Thienpont, C.N., Koopman, W.J.M., Gilissen, L.J.W.J. & Smulders, M.J.M. 2008. Phylogenetic relationships in *Betula* (Betulaceae) based on AFLP markers. *Tree Genetics and Genomes* 4: 911–924.
- Środoń, A. 1979. Brzoza w minionych czasach (Birch in the past). In: Białobok, S. (ed.). *Brzozy. Betula L. (Birches. Betula L.)*. PWN, Warszawa-Poznań. p. 9–23.
- Stachowicz-Rybka, R., Granoszewski, W. & Hrynowiecka-Czmielewska, A. 2009. Quaternary environmental changes at Starunia palaeontological site and vicinity (Carpathian region, Ukraine) based on palaeobotanical studies. *Annales Societatis Geologorum Poloniae* 79: 279–288.
- Staszkiewicz, J. 2001. *Betula szaferi* Jent.-szaf. ex Stasz. In: Kaźmierczakowa, R. & Zarzycki, K. (eds.). *Polska czerwona księga roślin (Polish plant red book)*. Instytut Botaniki im. W. Szafera, PAN, Kraków. p. 79–81.
- , Białobrzaska, M., Truchanowicz, J. & Wójcicki, J.J. 1993. Variability of *Betula humilis* (Betulaceae) in Poland. 4. Hybrid and introgressive forms. *Fragmenta Floristica et Geobotanica Polonica* 38: 475–488.
- Stewart, J.R. & Lister, A.M. 2001. Cryptic northern refugia and the origins of modern biota. *Trends in Ecology and Evolution* 16: 608–613.
- , Lister, A.M., Barnes, I. & Dalén, L. 2010. Refugia revisited: individualistic responses of species in space and time. *Proceedings of the Royal Society B* 277: 661–671.
- Taberlet, P., Fumagali, L., Wust-Saucy, A-G. & Cosson, J-F. 1998. Comparative phylogeography and post-glacial colonization routes in Europe. *Molecular Ecology* 7: 453–464.
- , Gielly, L., Patou, G. & Bouvet, J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.
- Thórsson, Æ.Th., Salmela, E. & Anamthawat-Jónsson, K. 2001. Morphological, cytogenetic, and molecular evidence for introgressive hybridization in birch. *Journal of Heredity* 92: 404–408.
- , Pálsson, S., Lascoux, M. & Anamthawat-Jónsson, K. 2010. Introgression and phylogeography of *Betula nana* (diploid), *B. pubescens* (tetraploid) and their triploid hybrids in Iceland inferred from cpDNA haplotype variation. *Journal of Biogeography* 37: 2098–2110.
- , Pálsson, S., Sigurgeirsson, A. & Anamthawat-Jónsson, K. 2007. Morphological variation among *Betula nana* (diploid), *B. pubescens* (tetraploid) and their triploid hybrids in Iceland. *Annals of Botany* 99: 1183–1193.
- Tralau, H. 1963. The recent and fossil distribution of some boreal and arctic montane plants in Europe. *Arkiv för Botanik* 5: 533–881.
- Velichko, A.A. & Zelikson, E.M. 2005. Landscape, climate and mammoth food resources in the East European Plain during the Late Paleolithic epoch. *Quaternary International* 126–128: 137–151.
- Vendramin, G.G., Anzidei, M., Madaghiale, A., Sperisen, C. & Bucci, G. 2000. Chloroplast microsatellite analysis reveals the presence of population

- subdivision in Norway spruce (*Picea abies* K.).
Genome 43: 68–78.
- Westergaard, K.B., Alsos, I.G., Popp, M., Engelskjøn, T., Flatberg, K.I. & Brochmann, C. 2011. Glacial survival may matter after all: nunatak signatures in the rare European populations of two west-arctic species. *Molecular Ecology* 20: 376–393.
- Willis, K.J. & Niklas, K.J. 2004. The role of Quaternary environmental change in plant macroevolution: the exception or the rule? *Philosophical Transactions of the Royal Society London B* 359: 159–172.
- & van Andel, T.H. 2004. Trees or no trees? The environments of central and eastern Europe during the Last Glaciation. *Quaternary Science Review* 23: 2369–2387.
- , Rudner, E. & Sümegei, P. 2000. The full-glacial forests of central and south-eastern Europe. *Quaternary Research* 53: 203–213.
- Załoski, T., Pisarek, W., Kucharczyk, M. & Kamińska, A.M. 2001. *Betula humilis* Schrank. In: Kaźmierczakowa, R. & Zarzycki, K. (eds.). *Polska czerwona księga roślin* (Polish plant red book). Instytut Botaniki im. W. Szafera, PAN, Kraków. p. 79–81.

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