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Genetic consequences of glacial survival and postglacial colonization in Norway spruce: combined analysis of mitochondrial DNA and fossil pollen

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Abstract

Norway spruce (*Picea abies* [L.] Karst.) is a broadly distributed European conifer tree whose history has been intensively studied by means of fossil records to infer the location of full-glacial refugia and the main routes of postglacial colonization. Here we use recently compiled fossil pollen data as a template to examine how past demographic events have influenced the species' modern genetic diversity. Variation was assessed in the mitochondrial *nad*1 gene containing two minisatellite regions. Among the 369 populations (4876 trees) assayed, 28 mitochondrial variants were identified. The patterns of population subdivision superimposed on interpolated fossil pollen distributions indicate that survival in separate refugia and postglacial colonization has led to significant structuring of genetic variation in the southern range of the species. The populations in the northern range, on the other hand, showed a shallow genetic structure consistent with the fossil pollen data, suggesting that the vast northern range was colonized from a single refugium. Although the genetic diversity decreased away from the putative refugia, there were large differences between different colonization routes. In the Alps, the diversity decreased over short distances, probably as a result of population bottlenecks caused by the presence of competing tree species. In northern Europe, the diversity was maintained across large areas, corroborating fossil pollen data in suggesting that colonization took place at high population densities. The genetic diversity increased north of the Carpathians, probably as a result of admixture of expanding populations from two separate refugia.

Keywords: fossil pollen, glacial refugia, minisatellite, *Picea abies*, plant mtDNA, range expansion

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Introduction

The climatic oscillations during the last 2 million years have dramatically influenced the distribution of most taxa. In Europe and North America, the repeated glacial and interglacial cycles forced temperate species to retreat into

refugia during periods of advancing ice and permafrost and allowed them to expand from their refugia during interglacial warming (Hewitt 1996). These range changes are generally believed to have played an important role in shaping genetic diversity. Surveys of genetic variation of numerous taxa indeed have revealed patterns of genetic subdivision and diversity which are consistent with isolation in refugia during cold stages and geographic and demographic expansion during the present interglacial (Hewitt 2004). So far, however, only a few studies have tested inferences based on genetic data against independent data from fossil records (e.g. Comps *et al*. 2001; Hugall *et al*. 2002; Petit *et al*. 2003; Cheddadi *et al*. 2006; Magri *et al*. 2006). Inferences based on genetic data alone must be regarded as tentative, also because of inherent limitations of the particular molecular markers applied.

In this study, we combine genetic data with palaeoecological data to analyze the consequences of past demographic events on genetic diversity and structuring in Norway spruce (*Picea abies* [L.] Karst.), a conifer tree widely distributed across Europe (Schmidt-Vogt 1977). Norway spruce is well suited for such an approach for several reasons. Compared to other temperate and boreal forest trees, the fossil record of Norway spruce is particularly detailed. Fossil pollen collected from hundreds of lakes and bogs, and abundant macrofossil findings have allowed the identification of putative refugia and the major routes of postglacial colonization (e.g. Giesecke & Bennett 2004; Terhürne-Berson 2005; Latałowa & van der Knaap 2006). In addition, the patterns of diversity in Norway spruce have not been complicated by interfertile congeners as in many other plant taxa, except possibly in the northernmost part of its range where an introgression zone with Siberian spruce (*Picea obovata* Ledeb.) has been proposed (Krutovskii & Bergmann 1995). However, the morphological distinction between these two taxa is controversial (e.g. Schmidt-Vogt 1977) and there is possibly a smooth morphological gradient across Eurasia rather than a defined zone of introgressive hybridization (Popov 2003). Finally, several molecular markers from the nuclear and organelle genomes have been shown to be informative for reconstructing the recent evolutionary history of *P. abies* (e.g. Vendramin *et al*. 2000; Sperisen *et al*. 2001; Heuertz *et al*. 2006).

The modern range of Norway spruce in Europe is divided into a northern and a southern part (Fig. 1; Schmidt-Vogt 1974). In the north, Norway spruce forms a large continuum covering almost the entire Fennoscandia and European Russia. In the south, the species mainly occurs along the mountain ranges of central and southeastern Europe. The region where the two ranges come together is situated in the middle Polish lowlands (the middle Polish disjunction according to Schmidt-Vogt 1974; see also Latałowa & van der Knaap 2006).

The fossil pollen record suggests that the entire northern range was colonized from a single glacial refugium located in European Russia, and that colonization occurred in westward and northwestward direction following the retreat of the Scandinavian ice sheet (Giesecke & Bennett 2004). This contrasts with central Europe, where fossil pollen and macroremains suggest that several refugia gave rise to the current range of Norway spruce (Terhürne-Berson 2005; Latałowa & van der Knaap 2006). The Alps were colonized from the southeastern Alps and possibly from the adjacent Dinaric Alps. The source for the colonization of the Hercynic area, which comprises the Bohemian Massif, is less clear; the West Carpathians have been proposed (Huntley & Birks 1983), and also the northeastern Alps (Svobodová *et al*. 2001). The former mountain range was certainly the source for the colonization of southern Poland. The East Carpathians and the southwest Bulgarian mountains (Pirin, Rhodope, and Rila Mountains) were probably additional sources of spruce expansion during the Holocene (Latałowa & van der Knaap 2006; Feurdean *et al*. 2007).

The timing and mode of colonization by Norway spruce as revealed by fossil pollen shows both similarities and differences between the northern and southern range. Similarities are that westward colonization was rapid during the early Holocene up to about 9000 BP (9000 years before present, uncalibrated radiocarbon years) and in the middle Holocene after about 6000 BP (Latałowa & van der Knaap 2006). Differences are that westward colonization in the southern range was mostly completed by 5000 BP, whereas it continued in the northern range, and spruce reached the Atlantic Ocean in the north but not in the south (Giesecke & Bennett 2004; Latałowa & van der Knaap 2006). These peculiarities may have been caused by differences in climate, topography, and presence of other, competitive tree species (Terhürne-Berson 2005).

Several genetic surveys of Norway spruce have been performed, but little is still known about the genetic consequences of the history of the species. Studies of nuclear markers and organelle DNA have consistently revealed a clear separation between the northern and southern populations (e.g. Lagercrantz & Ryman 1990; Vendramin *et al*. 2000; Sperisen *et al*. 2001; Collignon *et al*. 2002; Bastien *et al*. 2003; Heuertz *et al*. 2006). Nuclear and chloroplast DNA markers have further shown that the populations in the Alps are distinct from those of the Hercynic-Carpathian area (Bucci & Vendramin 2000; Collignon *et al*. 2002; Acheré *et al*. 2005; Heuertz *et al*. 2006). However, the resolution in these markers was not sufficient to locate genetic boundaries among different refugial expansions nor did they allow assessing genetic change along postglacial colonization routes. An exception is a regional study of mitochondrial DNA (mtDNA) variation in the Alps (Gugerli *et al*. 2001). This study shows highly diverse populations in the eastern Alps and monomorphic populations in the western Alps, a

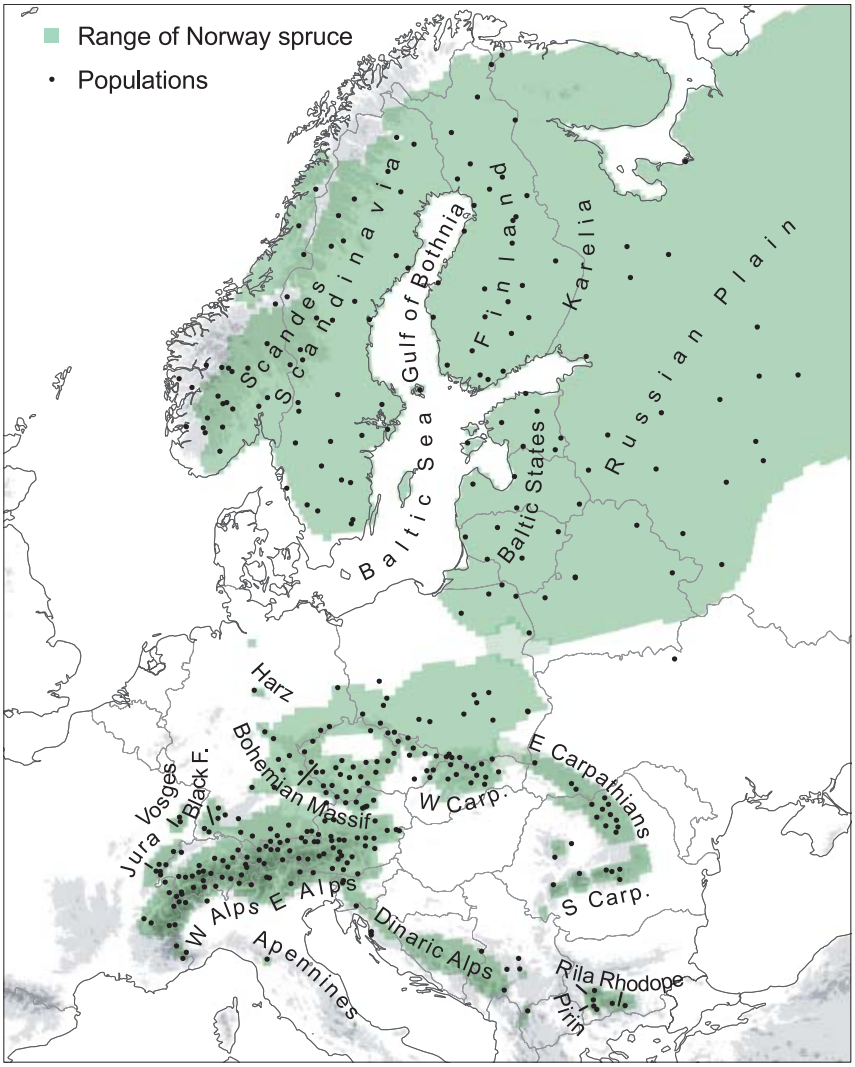

Fig. 1 Populations of *Picea abies* analysed for mitochondrial minisatellite variation. The green shaded areas represent the natural range of Norway spruce as described by Schmidt-Vogt (1974). In addition, the Vosges Mountains were included. The light green areas indicate zones where Norway spruce populations of different origin came into contact during the Holocene (middle Polish lowlands and intra-Carpathian break at the Polish–Slovak border; for discussion see Schmidt-Vogt 1974 and Latałowa & van der Knaap 2006). The grey shading illustrates the topography; the darker the tone, the higher the altitude.

pattern which may be explained by founder effects during westward postglacial colonization.

Here we assess variation in maternally inherited mtDNA across the entire range of Norway spruce and combine these data with recently compiled fossil pollen data (Latałowa & van der Knaap 2006). We apply the same mtDNA marker as used in two earlier, geographically more restricted studies (Gugerli *et al*. 2001; Sperisen *et al*. 2001). This marker comprises a fragment of the second intron of the *nad*1 gene which includes two highly variable minisatellites with repeat sizes of 32 and 34 bp (base pairs) (Sperisen *et al*. 2001). Whereas variation in the minisatellite-flanking sequences separates northern populations from the southern, copy-number variation in the two minisatellites proved useful for assessing diversity and population dynamics on the regional or local scale (Gugerli *et al*. 2001; Scotti *et al*. 2008). We address the following three issues. First, we identify detailed patterns of population subdivision in both geographic ranges of the species by extensive population sampling. Second, we compare the fossil pollen distributions with the genetic patterns observed to evaluate the concordance of these two independent types of evidence and improve inferences on the location of glacial refugia and postglacial colonization routes. Third, in areas where a clear relationship between range expansion inferred from fossil pollen and genetic structure could be established, genetic change in space and time is analysed by relating genetic diversity with the estimated age of first spruce occurrence and with geographic distance to the putative source area of Holocene range expansion.

Materials and methods

Population sampling

This study was based on genetic data from 4876 trees representing 369 populations (Fig. 1; Table S1, Supporting Information). The sampling covered the entire natural

range of Norway spruce as described by Schmidt-Vogt (1974). The sampling also included populations from the western coast of Norway and from the Vosges Mountains, two regions considered to be part of the natural range (Fægri 1950; Kalis *et al*. 2006). Genetic data for 276 populations were obtained particularly for this study; the remaining data were taken from Sperisen *et al*. (2001) and Gugerli *et al*. (2001). Material was sampled from 171 natural populations, from six populations maintained in clonal archives, and from 192 populations maintained in two international provenance trials (International Union of Forest Research Organization of Norway spruce provenance trials 1964/1968 and 1972), and in a Nordic provenance trial. The sampled populations of the provenance trials originate from seed lots harvested from a minimum of 10 trees per stand (Krutzsch 1974; T. Skrøppa unpublished data). Because Norway spruce is one of the most important timber species in Europe, its distribution has been largely expanded by human reforestations (Schmidt-Vogt 1977); thus, special care was taken to sample nonplanted stands. In the Alps, populations on steep slopes and at high elevations were preferred over lowland sites since planting with foreign seed sources was expected to be less pronounced in the former.

DNA extraction, sizing, and sequencing

DNA was extracted from frozen needles using the DNeasy 96 Plant Kit or the DNeasy Plant Mini Kit (QIAGEN) according the manufacturer's instructions. The polymorphic fragment of the second intron of the mitochondrial *nad*1 gene for part of the samples was amplified and sized as described by Sperisen *et al*. (2001) at the Swiss Federal Research Institute for Forest, Snow and Landscape Research laboratory (Birmensdorf, Switzerland). Samples processed at the Norwegian Forest and Landscape Institute (Ås, Norway) were amplified in 20 μL containing 1× PCR buffer (QIAGEN) supplemented with 0.5 mM $MgCl_2$, 0.1 mM of each dNTP, 0.4 μM of each primer, 1.5 U HotStar *Taq* DNA polymerase (QIAGEN) and *c*. 30 ng DNA template using a GeneAmp PCR System 9700 (Applied Biosystems). The cycling profile followed Sperisen *et al*. (2001) except that 31 instead of 26 cycles were run. At both laboratories, two labelled primers were used, followed by restriction digestion with *Eco*RV to increase resolution in fragment sizing. Sizing of the digested PCR products was carried out with ABI 310 genetic analyzers (Applied Biosystems), relative to an internal size standard (ROX500, Applied Biosystems). Reproducibility of amplification and sizing across the two laboratories was verified with 96 samples. To standardize the detection of additional fragments, samples were run at DNA concentrations such that the minimum peak heights for the labelled 5′ and 3′ fragments were ≥ 500 units. Additional fragments were scored if their peak height was ≥ 50 units and ≥ 10% of the height of the main fragment.

Each size variant was sequenced from at least two individuals (except variants which occurred only once) to determine the nature of the polymorphisms and to detect the presence of potential fragment-length homoplasy. The sequences of both strands were assembled and edited with the Seqman program of DNAStar Lasergene software or Autoassembler (Applied Biosystems). Binning of the alleles was based on exact fragment length.

Phylogenetic analysis

The sequences of the identified mitochondrial haplotypes were aligned in BioEdit (Hall 1999) and minor adjustments were done manually. Insertions/deletions (indels) in the region flanking the two minisatellites were coded as presence/absence (1/0), following the simple indel coding of Simmons & Ochoterena (2000). Repeat indels in minisatellites arise through replication slippage and are assumed to evolve according to a stepwise mutation model (see Bastien *et al*. 2003 and Godbout *et al*. 2005 for plant mt minisatellites). We therefore coded the copy numbers of the two minisatellites as two single multistate ordered characters (0–10). To establish phylogenetic relationships among the haplotypes, we applied a parsimony approach implemented with the Tree Analysis Using New Technology (TNT) software 1.0 (Goloboff *et al*. 2004). We used a heuristic search with 1000 random additive replicates. Jackknife support was calculated with 36% of the characters deleted as recommended by Farris *et al*. (1996). To root the phylogenetic tree, we used Siberian spruce (*Picea obovata*), considered as the closest relative of *Picea abies* (Schmidt-Vogt 1977; Ran *et al*. 2006). We sequenced five samples of Siberian spruce, each of them collected from a different location in Siberia.

Population-genetic analyses

Parameters of population diversity and differentiation were estimated according to Pons & Petit (1995, 1996). The programs Permut and Contrib (by R.J. Petit, available at http://www.pierroton.inra.fr/genetics/labo/Software/) were used to calculate average within-population gene diversity (h_S), total gene diversity (h_T), and gene differentiation among populations or groups of populations (G_{ST}).

To investigate patterns of genetic subdivision, we applied spatial analysis of molecular variance (SAMOVA; Dupanloup *et al*. 2002) and a clustering approach based on principal component analysis (PCA). SAMOVA is based on a simulated annealing procedure that aims to maximize the proportion of total genetic variance due to differences between groups of populations (F_{CT}). It seeks the composition of a user-defined number of K groups of geographically adjacent populations that maximizes F_{CT}. As input for SAMOVA 1.0 (http://web.unife.it/progetti/genetica/Isabelle/samova.

html), we used aligned sequences which were modified as follows. Indels in the region flanking the two minisatellites were coded as single characters (base for presence/gap for absence). SAMOVA 1.0 does not allow sequence data and multistate-ordered characters to be combined. To achieve a maximum resolution for genetic differentiation, we coded each repeat copy of the two minisatellites as a single character (base for presence/gap for absence). The program was run for 10 000 iterations from each of 500 random initial conditions. The analyses were repeated with increasing values of K (2–12), so that the F_{CT} values reached a plateau. SAMOVA was run for the entire dataset across Europe and separately for data from the northern and southern range of spruce.

PCA in combination with clustering was used to delineate genetically similar populations based solely on population means and variances without using any geographic information. The variable sites in the sequence alignment were coded as numerical characters: indels in the region flanking the two minisatellites as 1 and 0 (present/absent), substitutions as 1 and 0 (since each only had two alternative bases), and the repeat copy numbers of the two minisatellites as 0–10 as described for phylogenetic analysis. PCA was run using the STANDARD option in the PRINCOMP procedure of the SAS software (SAS Institute Inc.) to derive a small number of linear combinations (principal components) that retained as much of the information in the original variables as possible. The PCA was run on the total data set as well as separately for the northern and southern range of spruce. For the two ranges, we calculated population means and within-population variances for the first two principal components, giving four variables as inputs for cluster analyses. We used the FASTCLUS procedure in SAS to run stepwise analyses, increasing the number of clusters by 1 each time (using the MAXCLUSTER statement), until one of the clusters contained only a single population.

The genetic relationship between groups delineated by SAMOVA or PCA was inferred from a minimum-evolution (ME) tree based on pairwise differentiation among groups (F_{CT}) estimated by analysis of molecular variance (AMOVA) with Arlequin 3.01 (Excoffier *et al*. 2005). The ME tree was constructed with MEGA 3.1 (Kumar *et al*. 2004). We used the number of significant pairwise differences between group comparisons to evaluate to which extent the groups were differentiated.

As a template to study genetic change during colonization, a map of fossil pollen was prepared through interpolation of the pollen data described by Latałowa & van der Knaap (2006). Following these authors, all ages are expressed in uncalibrated radiocarbon years BP unless otherwise stated. The pollen threshold was ≥ 2%, interpreted to track the expansion at the front of large populations. Pollen data from 10 000 BP and younger were used to avoid populations that expanded during the Younger Dryas (11th millennium BP) and contracted or even became extinct during the Preboreal (10th millennium BP), a phenomenon observed in the region stretching from eastern Estonia to central Belarus (Latałowa & van der Knaap 2006). Age estimates across the natural range of spruce were obtained through universal kriging using GSTAT library (Pebesma 2004) within R software (R Development Core Team 2007; http://www.r-project.org/). Kriging was applied to the spruce distribution area as described by Schmidt-Vogt (1974). In a first run, the estimated age of spruce in the southwest Bulgarian mountains was considerably younger than that directly inferred from fossil pollen. The reason for this is that this area included only one site with oldest pollen of early Holocene age among several sites of younger, mid-Holocene age (see Figs 4–7 of Latałowa & van der Knaap 2006). This was corrected in a second run by forcing kriging to attain the age of the oldest site as it is inferred from fossil pollen.

To identify trends in genetic diversity and differentiation during colonization, we combined the populations into groups of three to six according to geographic proximity (34 groups in the northern range, 56 in the southern range). The age of each population group was estimated for their centre of mass coordinate. The parameters h_S, h_T, and G_{ST} of each population group were then correlated with the age estimates on different scales: across the entire range and within the northern and southern ranges separately, and within SAMOVA groups assuming that they represent ranges of expansion (see Results for an evaluation of this assumption). For correlation analyses within SAMOVA groups, the grouping of populations was adjusted to only contain populations from a single SAMOVA group. Four populations were excluded due to their geographic isolation. Genetic parameters for the population groups within SAMOVA groups were additionally correlated with geographic distance to the oldest groups of populations.

Results

Mitochondrial DNA variation

Sizing and sequencing of the *nad*1 intron revealed 28 haplotypes in the 4876 trees analysed. These included the 18 haplotypes previously identified by Sperisen *et al*. (2001) and Gugerli *et al*. (2001). The size of the haplotypes ranged from 687 to 1027 bp (Fig. 2). The 1485 bp of aligned sequences included eight substitutions and 26 indels of which 18 were due to copy-number variation of the 32-bp (0–8 copies) and the 34-bp minisatellites (0–8 and 10 copies). Eighteen per cent of our samples showed two or more size variants, presumably reflecting heteroplasmy. However, all of these samples had a dominant variant, which was used for further analysis.

The phylogenetic analysis resulted in a single most parsimonious tree (Fig. 2) consisting of two clades with

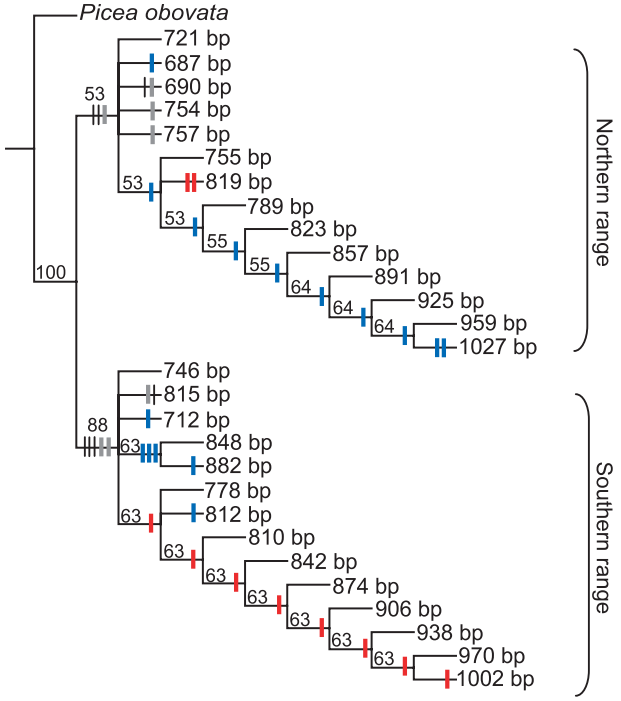

Fig. 2 Phylogenetic tree inferred for the 28 haplotypes of the minisatellite region of the mitochondrial *nad*1 gene in *Picea abies*, assuming a stepwise mutation model. *Picea obovata* was used as outgroup. The haplotypes are indicated according their size in base pairs (bp). Support for the clades are given as jackknifed values. The characters used for the phylogenetic analysis are shown as bars (nucleotide substitutions) and boxes (insertions/deletions); grey boxes, insertions/deletions of 1–69 bp; blue boxes, insertions/deletions of the 34-bp repeated sequence motif; red boxes, insertions/deletions of the 32-bp repeated sequence motif. The two copies of the 32-bp sequence in haplotype 819 bp are located upstream of the two minisatellites and were treated as a normal insertion/deletion.

support of 53% and 88%, respectively, and separated by five substitutions and three indels. Each clade included 14 haplotypes. Corroborating the results of Sperisen *et al.* (2001), the two clades correspond to the two main geographic ranges (southern and northern) of Norway spruce. The only exceptions were a few predominantly southern haplotypes occasionally found in the northern range, usually in or close to the region in Poland where the two ranges came in contact, and vice versa (Fig. S1A, B, Supporting Information). The haplotypes with the 34-bp minisatellite were present in both clades, indicating independent evolution of this minisatellite, whereas the haplotypes with the 32-bp minisatellite were restricted to the southern range. A single northern haplotype (819 bp) contained the same 32-bp sequence motif in two tandemly arranged copies, but these were located upstream of the two minisatellites. With the exception of the predominant and widespread haplotype of 815 bp (0×32 bp, 69 bp indel), all frequent southern haplotypes showed a geographically distinct distribution (Fig. S1A). For example, the haplotype of 746 bp (0×32 bp) was characteristic of the southern Carpathians, 778 bp (1×32 bp) for the mountain ranges around the Hungarian basin, while 842 bp (3×32 bp) and 874 bp (4×32 bp) occurred mainly in the Bohemian Massif. The two southern haplotypes containing 34-bp minisatellites (848 bp, 3×34 bp; 882 bp, 4×34 bp) were predominantly found in two populations in southern Poland. The distribution of the northern haplotypes was less structured (Fig. S1B). The dominant haplotype of 721 bp (0×34 bp) occurred across the entire northern range. The remaining frequent haplotypes (e.g. 789 bp, 823 bp, and 857 bp), all of them containing 34-bp minisatellites, were widespread but absent from western Finland and northern Scandinavia.

Consistent with the phylogenetic analysis, SAMOVA with two groups separated the northern from the southern populations (data not shown). The differentiation between the two groups (F_{CT}) was high, explaining 83% of the total variation ($P < 0.0001$). The same clear division was revealed by the first three PCA axes (data not shown), which explained 98% of the total variation ($P < 0.0001$).

The mean within-population gene diversity (h_S) was similar in the two geographic ranges (south: 0.265, SE 0.018; north: 0.241, SE 0.025) and similar to that obtained over all populations (0.257, SE 0.017). Population differentiation (G_{ST}) was higher in the south (0.479, SE 0.028) than in the north (0.277, SE 0.036), and the overall G_{ST} was high (0.638, SE 0.017).

In a separate SAMOVA of the southern populations, F_{CT} generally increased slightly with K and reached a plateau at $K = 9$, suggesting a group structure. In contrast, in the separate SAMOVA of the northern populations, the maximum F_{CT} value (0.767) was reached for $K = 2$, with one group composed of a single population. When more groups were specified, F_{CT} decreased while the number of groups with a single population increased, indicating the absence of a group structure. For the southern populations, we retained the configuration obtained for $K = 6$ as this yielded the highest F_{CT} (0.655) with only one group with a single population (Fig. 3A). This configuration included three large groups: group Ss6 with the populations from the Alps, the southern Dinaric Alps, and the northern Carpathians ($n = 170$); group Ss1 with the populations from and adjacent to the Bohemian Massif ($n = 42$); and group Ss4 from the southern Carpathians and the southwest Bulgarian mountains ($n = 18$). The remaining groups were Ss3 with three populations from the northern Dinaric Alps, Ss5 with two populations from Poland, and Ss2 with one population from the Austrian Alps.

PCA in combination with clustering identified 12 groups in the southern range, largely corresponding to subdivided SAMOVA groups (Fig. 3C, D). In southeastern Europe, PCA

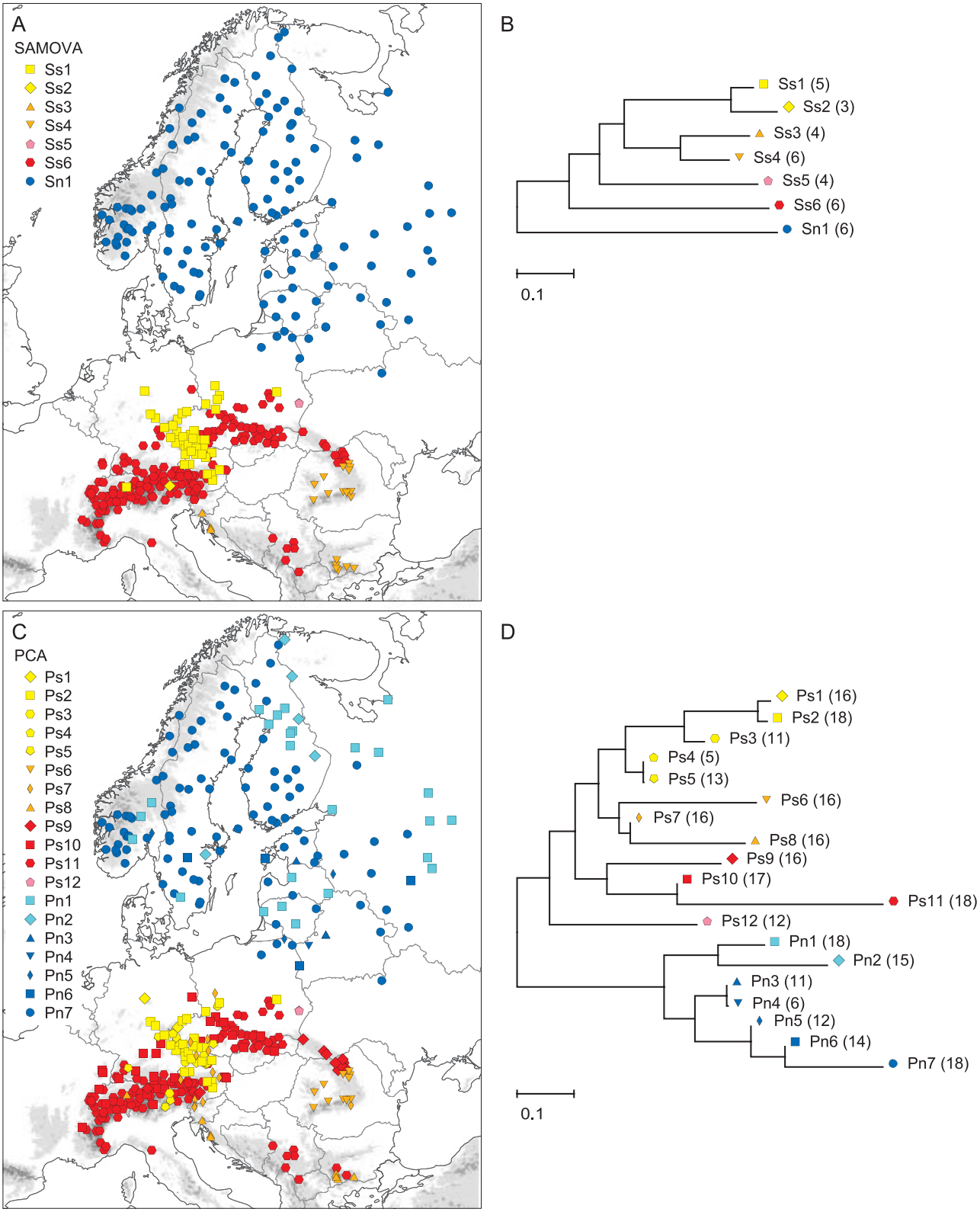

Fig. 3 Geographic distribution of groups of *Picea abies* populations delineated by SAMOVA (A) and PCA clustering (C), and their relationships inferred as minimum-evolution trees based on pairwise F_{CT} (B and D, respectively). The numbers of pairwise comparisons where P (random value ≥ observed value) was < 0.05 are given in parentheses. The total number of pairwise comparisons was six for SAMOVA groups and 18 for PCA groups. For example, Sn1 (6) on the SAMOVA tree means that group Sn1 was significantly differentiated from all the other SAMOVA groups. The grey shaded areas on the maps illustrate the topography.

and clustering refined the resolution and separated the northern part of the East Carpathians (group Ps9) from the West Carpathians (mainly group Ps11), as well as the southern Carpathians (group Ps6) from the southwest Bulgarian mountains (group Ps8). The only main discrepancy between SAMOVA and PCA was that group Ps7 comprised 13 populations from both Ss6 from the eastern Alps and Ss1 from the Bohemian Massif. Seven northern PCA groups were identified, of which Pn7 and Pn1 were large (Fig. 3C). Group Pn1 (n = 25) extended from the Russian Plain in the north to central Finland and in the west to the Baltic States and southern Scandinavia. Group Pn7 (n = 92) occurred across the entire northern range and co-occurred with Pn1 in the Baltic States and southern Scandinavia.

The two minimum-evolution trees (Fig. 3B, D) associated the Bohemian populations with the southern Carpathian, southwest Bulgarian, and northern Dinaric Alpine populations. The populations of the Alps and the northern Carpathians formed a separate cluster. Interestingly, the two populations from southern Poland (group Ps12) appeared closest to the main split between the southern and northern populations in PCA (Fig. 3D). The groups delineated by PCA in the northern range formed two clusters, each of them containing one of the two large groups of populations.

Combination of genetic and fossil pollen data

To evaluate whether groups of populations delineated by SAMOVA and PCA correspond to different ranges of Holocene expansion, the genetic maps were superimposed on interpolated fossil pollen data (Fig. 4). All three large SAMOVA groups of the southern range could be traced back to regions of early Holocene pollen, suggesting that they represent separate ranges of expansion (Fig. 4B). The disjunct group Ss6 was mapped to two separate regions of early Holocene pollen (the eastern Alps and the northern Carpathians), from which expansion occurred during the Holocene. The oldest pollen of group Ss1 was found in the southern part of the Bohemian Massif. Similarly to group Ss6, group Ss4 was mapped to two separate regions of early Holocene pollen, located in the southern Carpathians and in the southwest Bulgarian mountains. The northernmost population of the small group Ss3 was mapped to a region of early Holocene pollen in the northern Dinaric Alps. The finer resolution obtained by the PCA separating populations in the northern part of the East Carpathians (group Ps9) from those of the West Carpathians (mainly group Ps11) coincided with regions of early Holocene pollen (Fig. 4C). PCA further separated populations of the southern Carpathians (group Ps6) from those of the southwest Bulgarian mountains (mainly group Ps8). In the northern range, both large PCA groups (Pn1 and Pn7) could be traced back to the oldest pollen regions in the Russian Plain, suggesting that they represent the same range of expansion (Fig. 4D). All the remaining, smaller PCA groups identified in northern Europe were restricted to younger pollen regions.

Genetic change in space and time

Based on the above results, genetic change in space and time was analyzed within the frame of the SAMOVA groups, as these groups appeared to represent the overall ranges of Holocene expansion. The population-genetic parameters h_S, h_T, and G_{ST} were calculated for groups of three to six neighbouring populations within each SAMOVA group and superimposed on the pollen map (Fig. 5). Most regions of early Holocene spruce expansion were characterized by high gene diversity (h_S). Exceptions were parts of the West Carpathians and of the eastern Alps, and the southwest Bulgarian mountains (Fig. 5A). The pattern for total gene diversity (h_T) (data not shown) was largely congruent with that for h_S. In contrast, genetic differentiation (G_{ST}) was low in most regions with early Holocene pollen. A high G_{ST} was found in the southwest Bulgarian mountains (Fig. 5B).

When analysed across Europe, h_S and h_T correlated significantly with the estimated age of spruce, decreasing from old to young groups of populations, while G_{ST} showed no trend (Table 1). No significant trends were observed in the analysis of the entire southern range. However, significant correlations existed within the different regions of range expansion. The Alps and the West Carpathians (group Ss6) were analyzed separately because expansion occurred from both regions. Along the Alps, the three population-genetic parameters significantly deceased from east to west (Table 1). h_S remained high in some younger pollen regions northwest of the Alps, such as the Black Forest and the Vosges Mountains (Fig. 5A). Conversely, both h_S and h_T increased in a pronounced and significant way with increasing geographic distance from the oldest group in the West Carpathians. Within the Bohemian Massif (group Ss1), there was no overall significant trend. But when the northeasternmost group in Poland was excluded, h_S and h_T decreased significantly with increasing geographic distance. G_{ST} in this region increased, although not significantly. Group Ss4 was not considered because expansion from the southern Carpathians and the southwest Bulgarian mountains was limited.

In the northern European range of spruce, h_S and h_T significantly decreased with decreasing age as well as with increasing distance from the oldest population group (Table 1). G_{ST} did not show any significant trend (Table 1). East of the Baltic Sea, h_S was high across a vast area, but there was a conspicuous lack of diversity in western Finland (Fig. 5A). G_{ST} significantly increased with decreasing age in this area (Table 1, Fig. 5B). We found no significant trends for the area west of the Baltic Sea (results not shown), but

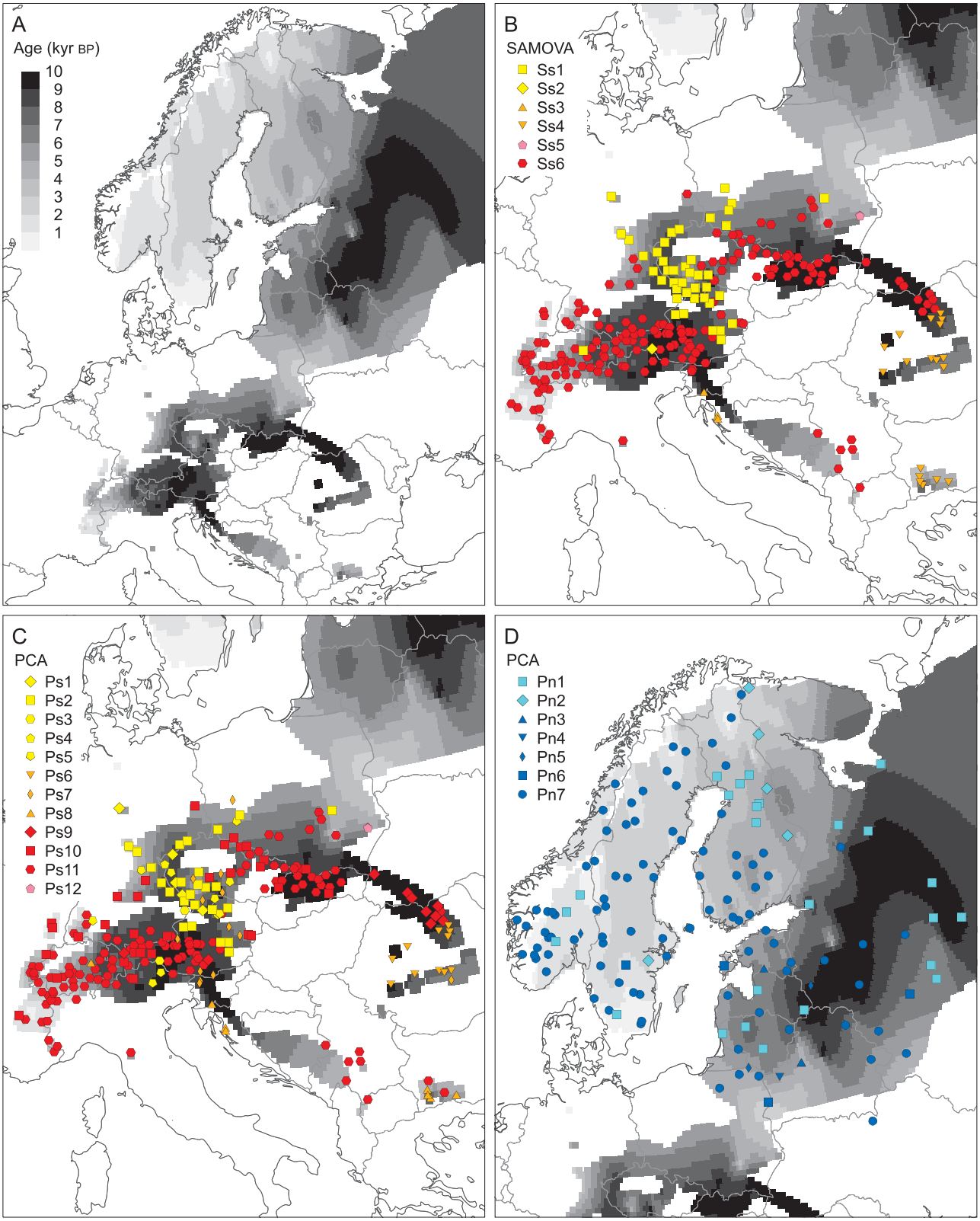

Fig. 4 Combined maps of genetic groups and fossil pollen for the inference of Holocene expansion regions in *Picea abies*. (A) Interpolated age (in time intervals of 1000 BP) of *Picea abies* fossil pollen (threshold ≥ 2%); fossil pollen data were retrieved from Latałowa & van der Knaap (2006). (B) SAMOVA population groups for the southern range shown on the fossil pollen map. (C) PCA population groups for the southern range shown on the fossil pollen map. (d) PCA groups for the northern range shown on the fossil pollen map.

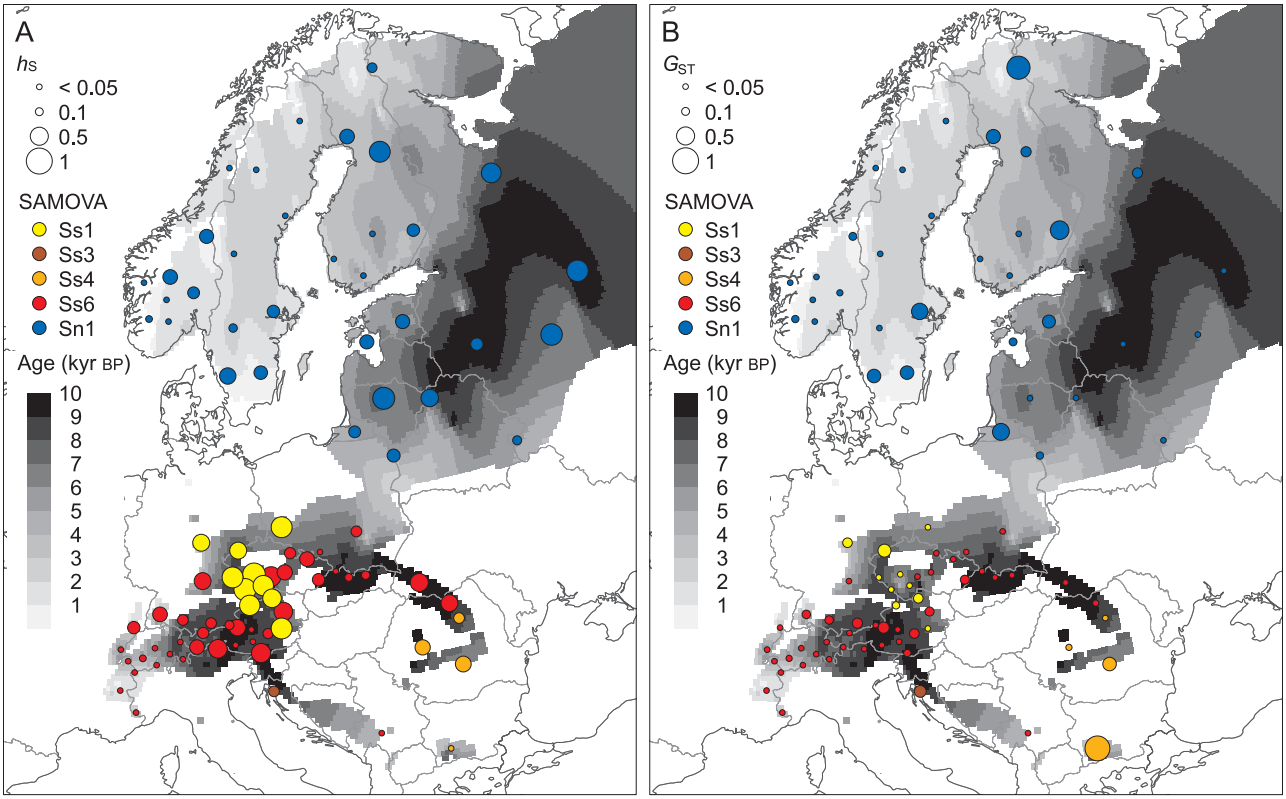

Fig. 5 Combined maps of fossil pollen and average within-population gene diversity (h_S; A) and genetic differentiation (G_{ST}; B) for groups of neighbouring *Picea abies* populations within SAMOVA groups. SAMOVA groups Ss2 and Ss5 were excluded because they contain less than three populations. Circle sizes are proportional to the values of h_S and G_{ST}, respectively. Values < 0.05 are shown as 0.05.

several population groups in southern Scandinavia had a high diversity compared to those in northern Scandinavia (Fig. 5A).

Discussion

Our combined analyses of the mitochondrial *nad*1 mini-satellite region and fossil pollen data of Norway spruce strongly suggest that demographic changes associated with Quaternary climatic fluctuations have played an important role in shaping the genetic diversity of extant populations. Our results not only allow us to reconstruct detailed pathways of population divergence, but also to infer genetic change during colonization at a resolution that exceeds that of any previous study of the species.

Glacial refugia

Fossil records indicate that Norway spruce was widespread in Europe during the Eemian Interglacial (Frenzel 1968) and the early Weichselian Interstadials (Ravazzi 2002). Climate cooling associated with the formation of extensive ice sheets across Fennoscandia and the Alps forced Norway spruce to retreat into refugia of suitable environment. Fossil macroremains clearly show that spruce survived in northern as well as in central and southeastern Europe during the last glacial maximum (LGM) (Terhürne-Berson 2005). The deep divergence observed between the mitochondrial lineages of the northern and the southern range (Figs 2 and 3) demonstrates that surviving populations in the two regions were separated for a substantial period of time possibly over several glacial–interglacial cycles. The existence of two or more genetic lineages is in concordance with the pattern observed in other European plant and animal species (Taberlet *et al.* 1998; Hewitt 2004). But in contrast to most of these other taxa, one lineage of spruce lies entirely in northern Europe and the other to the south of it. The pollen data show that the northern and southern ranges of spruce came in contact one or several millennia ago in the middle Polish lowlands (Latałowa & van der Knaap 2006). The occasional occurrence of southern haplotypes in the Baltic States and that of the most frequent northern haplotype in central Europe is in support of such a secondary contact zone.

Our combined data suggest that isolation in several distinct refugia played an important role in the southern

Table 1 Correlations between the genetic parameters h_S, h_T, and G_{ST} for groups of neighbouring populations and population age estimated from pollen data (cf. Fig. 4A) on a European scale and within the northern and southern range separately. SAMOVA groups are regarded to represent regions of range expansions (cf. Fig. 4) and correlations between genetic parameters for groups of neighbouring populations and population age and distance to the oldest groups of populations within the SAMOVA groups Ss1 and Ss6 are presented. Correlations between the regressors' age and distance are also shown. The correlations within Ss1 in the Bohemian Massif are shown both with and without the northernmost group of neighbouring populations located in Poland as the inclusion of this group in the correlations seemed to mask a significant trend between h_S, h_T and distance from the oldest group. The two groups belonging to SAMOVA group Ss6 in the northern part of the East Carpathians were not included in the correlations as this area probably experienced a separate expansion (cf. Fig. 4c). $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P \leq 0.05$.

Region	No. of groups	Correlation between age of groups and			Correlation between distance to oldest group and			Correlation between age and distance
		h_S	h_T	G_{ST}	h_S	h_T	G_{ST}	
Europe, overall	90	0.351**	0.292**	–0.027				
Northern range	34	0.596***	0.467**	–0.088	–0.425*	–0.442*	–0.176	–0.847***
East of the Baltic Sea	18	0.564*	0.195	–0.552*	–0.060	–0.137	0.353	–0.585*
Southern range	56	0.237	0.219	0.024				
Ss1, Bohemian Massif (without Poland)	9	0.355	0.568	–0.035	–0.680*	–0.737*	0.447	–0.730*
Ss1, Bohemian Massif (with Poland)	10	0.308	0.555	0.038	–0.498	–0.629	0.214	–0.727*
Ss6, Alps	26	0.306	0.325	0.420*	–0.403*	–0.416*	–0.400*	–0.929***
Ss6, West Carpathians	11	–0.421	–0.429	–0.112	0.853***	0.848***	0.336	–0.677*

range of the species. All three major southern groups of populations delineated by SAMOVA could be traced back to regions of early Holocene pollen, consistent with the presence of separate refugia and independent postglacial expansions from the refugial populations (Fig. 4B). Somewhat surprisingly, however, the first of these three large groups (Ss6) covered both the Alps and the northern Carpathians, although both macrofossil and pollen data have demonstrated refugia in both mountain ranges. The palaeo-data suggest that Norway spruce survived at the slopes of the southeastern Alps, from where it expanded northward and westward (Sercelj 1996; Terhürne-Berson 2005; Latałowa & van der Knaap 2006), and in the northern Carpathians, with expansion westwards (Ralska-Jasiewiczowa 1980; Huntley & Birks 1983; Latałowa & van der Knaap 2006). The northern Carpathians have most likely even harboured two separate refugia, located in the West Carpathians and the northern part of the East Carpathians, as both are old pollen regions with genetically divergent populations according to our PCA analysis (Fig. 4C). The genetic association of the Alps and the northern Carpathians implies, however, a common origin. It is possible that the populations now inhabiting these areas are disjunct relics of a larger population present in the early and middle Weichselian (110 000–34 000 BP), when spruce was widely distributed in central and southeastern Europe (Ravazzi 2002). Relicts of this population may have survived the LGM on the Hungarian Plain where several macrofossils dated to 32 000–17 000 BP (calibrated BP) have been found (Willis *et al*. 2000; Willis & van Andel 2004). Alternatively, some mtDNA haplotypes may have been lost in the Alps and/or the Carpathians, confounding the reconstruction of the refugial history. The second main SAMOVA group (Ss1) may have its origin in the area extending from the northernmost slopes of the Alps to the southern Bohemian Massif, from where Norway spruce expanded northwards towards the Harz Mountains. Colonization of the Bohemian Massif in a northward direction has previously been proposed by Svobodová *et al*. (2001) based on pollen data. Macrofossil data indicate that this refugium may have included the plain between the northern slopes of the Alps and the Bohemian Massif (Willis & van Andel 2004). Colonization of this region may also have been supplemented by populations from a refugium in the southeastern Alps, as indicated by the PCA group Ps7 (Fig. 4C). Magri *et al*. (2006) suggested a similar scenario for *Fagus*; the southern Bohemia–southern Moravia region was considered as a possible source area from which this species colonized central and northern Europe. Our data add further support to the proposal of this region as an important refugium for trees located north of the Alps. The third main SAMOVA group (Ss4) was subdivided by PCA into two major population groups (Ps6 and Ps8), a pattern consistent with the pollen data that suggest spruce survival and expansion in both the southern

Carpathians and the southwest Bulgarian mountains (Fig. 4). A southern Carpathian refugium including Transylvania is also supported by a recent compilation of fossil pollen and macroremains (Feurdean *et al*. 2007). The small SAMOVA group Ss3 and PCA group Ps8 further support a refugium in the northern Dinaric Alps, an area in which Late Glacial pollen has been found (Latałowa & van der Knaap 2006). However, our combined data suggest that northward expansion from this area was limited. Spruce may have expanded southwards into the Balkan Peninsula, but since sampling was limited in this area, this remains an open question. The small SAMOVA group in the more recently colonized area in Poland (Ss5) probably does not reflect a separate refugium, but rather an independent evolution of the 34-bp repeat region or recombination among mitochondrial molecules, which may occur at the intra- and intermolecular level (Kmiec *et al*. 2006). Since we have indications of heteroplasmy in as much as 18% of our samples, possibilities for recombination among co-occurring haplotypes do exist. For example, recombination among haplotype 746 bp and a haplotype from the northern range could explain the rare variants of 848 and 882 bp in Poland (see Fig. S1A).

Interestingly, the populations from the refugia located in the southern Carpathians and the southwest Bulgarian mountains did not expand northwards. The dry plains of central and southeast Europe, including the Hungarian Plain, probably formed an ecological barrier, whereas humid mountain ranges elsewhere in central Europe facilitated northward spread of other spruce groups. The same pattern has been observed in *Fagus*; populations from the southern refugia failed to expand northwards, whereas more northern refugia gave rise to most present-day populations (Magri *et al*. 2006). Similarly, the genetic structure in *Betula* seems to have been influenced more by northern than by southern refugia (Palme *et al*. 2003).

Unlike in the south, our SAMOVA test did not detect any genetic subdivision of the northern spruce populations, although haplotype diversity in the northern lineage is similar to that in the southern. This finding is consistent with the pollen record, suggesting that the entire northern European range of spruce was colonized from a single glacial refugium located in the southern part of the Russian Plain (Damblon *et al*. 1996). In PCA, however, we detected two major groups of populations, both in early colonized regions of the Russian Plain (Figs 3 and 4). This does not contradict the possibility of a single major refugium, but may reflect older polymorphisms maintained in a large refugial population. The Russian Plain populations may also have experienced introgression from Siberian spruce (*Picea obovata*) prior to its Holocene expansion. There are indications that Siberian spruce expanded in this area during the Late Glacial, after which it rapidly declined and became extinct (Latałowa & van der Knaap 2006). However, sizing and sequence analysis of the minisatellite region of spruce samples collected in Siberia and eastern European Russia showed a distinct difference between Norway spruce and Siberian spruce which follows the Ural Mountains; a 9-bp sequence motif is duplicated in the samples west of this mountain range but not to the east (C. Sperisen, unpublished data). This result strongly argues against any influence of Siberian spruce on the current mtDNA structure of spruce west of the Ural Mountains.

Genetic structure and diversity in spruce refugia

Populations in refugial regions often show high allelic diversity due to refugial persistence and accumulation of variation (Hewitt 2001). In accordance with this prediction, we observed relatively high within-population gene diversity (h_S) in most of the refugial regions inferred for Norway spruce (Fig. 5A). Exceptions were parts of the eastern Alps, the West Carpathians, and the southwest Bulgarian mountains. The low genetic diversity in these areas may be explained by genetic drift induced by small size of the refugial populations and/or by reduced population sizes during the cold and dry Younger Dryas episode (11th millennium BP).

The mountain regions of central and southeast Europe are assumed to have provided particularly suitable habitats for species survival during the last glacial period, because they allowed species to respond to the climatic oscillations using the altitudinal gradient. Such movements are expected to be rather slow and may have promoted population divergence at the regional scale (Hewitt 1999). Indeed, a high G_{ST} was found among populations located in the refugium of the southwest Bulgarian mountains (Fig. 5B). On the other hand, relatively low levels of G_{ST} were evident in population groups of the refugia located in the eastern Alps, the Carpathians, and the Bohemian Massif. The rapid climate fluctuations at the end of the last glacial period may have caused the populations to expand and contract at high speed, thus promoting frequent secondary contact among populations, a process leading to decreased population differentiation and increased within-population diversity (cf. Petit *et al*. 2003). Mixing of local refugial populations may have been further facilitated by the fact that spruce was actually widely distributed in central Europe during the LGM, especially if a pollen threshold of 1% is considered to represent actual occurrences (cf. Latałowa & van der Knaap 2006). The lowest levels of G_{ST} were observed in the oldest pollen regions of the Russian Plain. Even if there were several refugial pockets also here during the LGM, this region facilitates extensive wind dispersal of winter-released seeds over the snow-crusted plains. In this region, Norway spruce probably already existed as a large and continuous population in the early Holocene, and large areas were colonized before 9000 BP (Fig. 4A).

Genetic changes during colonization

The observed general decrease in within-population genetic diversity from the earliest colonized areas to the later as defined by pollen, overall as well as on a regional scale (Fig. 5A), is according to expectation, resulting from successive founder events during colonization (Hewitt 1996; Comps *et al*. 2001; Petit *et al*. 2002). We found, however, somewhat different patterns of diversity among range expansion regions, which may be explained by differences in colonization history.

A particularly pronounced decrease in within-population diversity is evident in the central Alps (Fig. 5A), a finding first described by Gugerli *et al*. (2001), and substantiated by Maghuly *et al*. (2007) who found higher levels of diversity in the eastern than in the western Austrian Alps as revealed by three mtDNA markers. The results of the present study indicate that genetic diversity decreased within a relatively short distance; populations throughout large parts of the Austrian Alps showed a relatively high level of genetic diversity, while populations of the westernmost Austrian Alps showed low levels of diversity. With few exceptions, populations of the Swiss and French Alps were monomorphic. According to pollen data, spruce colonized the eastern part of the Alps before the arrival of broad-leaved trees such as *Ulmus*, *Tilia*, and *Corylus* (Ravazzi 2002), so it could expand and migrate with little competition. The colonization occurred at rapid rates, possibly facilitated by pre-existing populations. Between 9000 and 6000 BP, however, there was no more than minor westward expansion of Norway spruce in the eastern part of the western Alps (Latałowa & van der Knaap 2006). This can be explained by the presence of *Fagus* that had immigrated from the north, of *Abies* immigrated from the south (van der Knaap *et al*. 2005), and of *Pinus cembra*, forming more closed forests that slowed down the population expansion of spruce. These events correspond well to the pronounced decrease in genetic diversity in spruce at the transition between the eastern and western Alps. Population bottlenecks and/or founder events accompanied by genetic drift may thus explain the dominance of a single mtDNA haplotype (815 bp) in the western Alps. This process may have been reinforced by the numerous mountain ridges and dry valleys which were unsuitable for Norway spruce (van der Knaap *et al*. 2005), thus acting as barriers to range expansion. Colonization of the northern Alps and the area north of it including the Black Forest and the Vosges Mountains were clearly less affected by population bottlenecks.

The east–west decrease of within-population diversity in the Alps is in contrast with the patterns observed in the region of the Bohemian Massif and the West Carpathians (Fig. 5A). In the latter mountain range, diversity increased from the oldest spruce pollen area towards the younger. The highest within-population diversity was found towards the west, where the West Carpathian lineage probably met the Bohemian lineage. Genetic differentiation in this area was low (Fig. 5B), as expected in an admixture zone with high levels of gene flow (Hampe & Petit 2005). The diversity in the Bohemian Massif decreased significantly in northern direction, but was still high throughout the region (Fig. 5). The entire Bohemian region was colonized before 8000 BP at high speed (Latałowa & van der Knaap 2006) and at high population density. The maintenance of genetic diversity in this area may also be explained by admixture from the West Carpathians and the eastern Alps, and possibly also from the southeastern Alps as suggested by the distribution of PCA group Ps7 (Fig. 4C). Population differentiation apparently increased towards the younger areas, but mainly due to the divergent Harz Mountains populations (Fig. 5B).

From the Russian Plain to the Baltic States and Karelia, within-population diversity was relatively high in many population groups (Fig. 5A). This is consistent with the pollen data, as they suggest that colonization westwards and northwestwards during the mid-Holocene occurred as a front-like spread at high population density (Giesecke & Bennett 2004). Genetic differentiation among populations clearly increased in this area (Fig. 5B). As shown by computer simulations, genetic differentiation increases in a colonization process involving rare long-distance dispersal events (Le Corre *et al*. 1997). A front-like spread interspersed with long-distance dispersal events would explain the high migration rates observed in northern Europe (Latałowa & van der Knaap 2006).

A marked decrease in within-population diversity was observed in the northern part of Fennoscandia (Fig. 5A). As the pollen data show that colonization in this area was slow (Fig. 4A), it is reasonable to suggest that repeated population bottlenecking during colonization caused the low diversity. This colonization pattern may reflect that northern Fennoscandia represents the climatic limit of the species. Also southwestern Finland (colonized 6000–3000 BP) showed a marked decrease in diversity. This region was already forested at the time of spruce arrival (Huntley & Birks 1983), making it more difficult for spruce to colonize. The colonizing populations may thus have been prone to drift induced by founder events or subsequent bottlenecks reducing genetic diversity.

The pollen record suggests that Norway spruce colonized Scandinavia both along a northern route by crossing over land in the north, and over the narrowest part of the Baltic Sea, the Gulf of Bothnia (Giesecke & Bennett 2004), reaching central Scandinavia 3000–2000 BP. The high within-population diversity observed in several parts of central and southern Scandinavia (Fig. 5A) is not consistent with a single colonization route from the north, where the populations were much less diverse. An alternative hypothesis of local glacial refugia in Scandinavia has been

proposed based on several old macrofossils (11 000–8000 BP) from the Scandes Mountains in central Sweden (e.g. Kullman 2001). The proposed refugia may have been located on the exposed coastal shelves in northwestern Norway (Kullman 2002), as the Scandes Mountains were covered by ice until approximately 9500 BP (Svendsen *et al*. 2004). However, Birks *et al*. (2005) present abundant evidence against this hypothesis, and also the genetic pattern does not support any Scandinavian refugia, as no unique mtDNA haplotypes were found and all haplotypes detected belong to the single northern European SAMOVA group and to widespread PCA groups (Fig. 4). Rather, our genetic data suggest that spruce also arrived via a second, southern colonization route across the Baltic Sea. The presence of several haplotypes (Fig. S1B) and one of the PCA groups (Pn2) in central and southern Scandinavia and east of the Baltic Sea, but absence from northern Scandinavia, supports this hypothesis. A second, southern migration route can also account for the high within-population diversity in several parts of central and southern Scandinavia. Colonization of this area from diverse sources has previously been proposed by Vendramin *et al*. (2000), who found high levels of variation in chloroplast microsatellites in southern Scandinavia.

Long-distance dispersal of spruce across the Baltic Sea has previously been considered improbable. However, spruce sheds its seeds in winter, and there is ample evidence that the seeds can be blown hundreds of kilometres over snow and ice surfaces (Kuusela 1990). Thus, dispersal may have occurred across the Baltic Sea 3000–2000 BP during winter via strong winds over sea ice. It has recently been shown that extremely long-distance seed dispersal has occurred frequently for several species to the isolated arctic archipelago of Svalbard, probably with wind across frozen sea ice or with drifting ice (Alsos *et al*. 2007). Earlier dispersal of spruce seed over the Baltic may also have happened in the period 9000–8000 BP when there was a mainland connection between southern Sweden and the Baltic coast, following the *Ancylus* regression of the Baltic Sea (Björck 1995).

We cannot, however, exclude the possibility of human influence on genetic structure. Planting of foreign provenances of spruce has been common in northern Europe. Provenances from the Baltic states/Belarus were frequently planted in Sweden in the period 1950–1994 (Laikre *et al*. 2006). However, provenances from this region have not been imported to Norway. Because of this fact and because our sampling was carefully designed to only include old forests with known history, we consider spontaneous dispersal across the Baltic Sea as the much more likely explanation.

Concluding remarks

Combined genetic and pollen data suggest that during the last glaciation, Norway spruce (*Picea abies*) survived in at least seven refugial areas from which it expanded during the Holocene. These were the Russian Plain, southeastern Alps, southern Bohemian Massif including its southern foreland, northern Dinaric Alps, northern Carpathians, southern Carpathians, and southwest Bulgarian mountains. The northern Carpathians have probably harboured two separate refugia. The Russian Plain represents the single, but large refugium for the northern lineage. The southern lineage expanded mainly out of refugia located in the southeastern Alps, the southern Bohemian Massif, and the West Carpathians. Climatic factors associated with latitude, altitude, proximity to the ice sheets, and topography were very different between the northern and the southern refugia, and probably crucial in determining the distribution and demography that shaped the genetic structure of the surviving populations. We demonstrate that, in contrast to general expectation, within-population diversity is low in some refugial areas (West Carpathians and southwest Bulgarian mountains). We further demonstrate that Norway spruce experienced conspicuously different genetic changes along the different colonization routes. Colonization across high mountain ridges (the Alps) induced a more pronounced loss of within-population diversity than colonization of large plains (northern Europe) and even across large water bodies (Baltic Sea). The maintenance of within-population diversity observed in parts of the southern range (Bohemian Massif and north of the West Carpathians) is probably the consequence of the mixing of colonization routes. The maintenance of diversity across large areas in the northern range (east of the Baltic Sea), on the other hand, may be explained by colonization at high population density.

There is considerable evidence that population isolation in spruce during the last glaciation and the largely separate history of the various ranges during the Late Glacial and the Holocene have left marks in the nuclear genome. Studies carried out with various marker types have shown that populations of the northern range are divergent from those in the southern, but also that within the southern range, the populations of the Alps are divergent from those of the Hercynic-Carpathian area, although less strongly so (Collignon *et al*. 2002; Acheré *et al*. 2005; Heuertz *et al*. 2006). The latter subdivision is most clearly seen in quantitative traits, which in addition separate East Carpathian populations from those of the West Carpathians and Bohemian Massif (Collignon *et al*. 2002). Natural selection is one of the factors shaping nuclear variation, both in refugial and in expanding populations. An example is the strong latitudinal trend in bud burst (Collignon *et al*. 2002), which is explained by adaptation to the local environment. The historical information gained in our study is thus useful to better understand the impact of these different evolutionary forces on Norway spruce.

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This collaborative work reflects the common interest of the authors in evolutionary and biogeographical processes in trees and plants. Mari Mette Tollefsrud is a PhD candidate studying the genetic diversity and structure of Norway spruce using different types of molecular markers. The focus of the research of Christian Brochmann is the phylogeography and evolutionary history of arctic and arctic-alpine plants. Felix Gugerli, Roy Kissling, and Christoph Sperisen develop and apply molecular markers for the study of evolutionary processes in trees and alpine plants. The work of Øystein Johnsen is focused on the epigenetics of Norway spruce. Thomas Geburek and Tore Skrøppa develop and implement concepts for the conservation of genetic resources in forests. Rachid Cheddadi, Thomas Litt, Małgorzata Latałowa, Ruth Terhürne-Berson, and Pim van der Knaap are palaeoecologists. They investigate the glacial and postglacial history of forest trees and develop models for predicting the impact of global warming on the distribution of forest trees.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 Geographic distribution of the 28 haplotypes of the minisatellite region of the mitochondrial *nad*1 gene in *Picea abies*. (A) southern lineage. (B) northern lineage.

Table S1 Location of analysed *Picea abies* populations and distribution of the 28 haplotypes of the minisatellite region of the mitochondrial *nad*1 gene.

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