



Phylogeography of white spruce (*Picea glauca*) in eastern North America reveals contrasting ecological trajectories

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ABSTRACT

Aim The main objective of this study was to investigate the origin of white spruce (*Picea glauca*) range discontinuities in eastern North America. A first analysis aimed at uncovering the glacial origin and post-glacial migration route of white spruce in the eastern part of the range. A second analysis aimed at evaluating whether disjunct white spruce populations in central Québec result from a recent expansion or are remnant stands following a northern range contraction.

Location The first analysis covered the North American transcontinental boreal distribution of white spruce, and the second focused on the eastern part of its range.

Methods A total of 589 *P. glauca* individuals from 51 populations, 24 of which were from a previous published source, were analysed for variation in three chloroplast DNA regions (*trnT/L*, *trnL/F* and *ndhK/C*). At the continental scale, haplotypic diversity, zones of genetic discontinuities, clustering analysis and analysis of molecular variance (AMOVA) were employed. In the eastern part of the range, haplotypic diversity was assessed with a permutation procedure, demographic history with mismatch analyses, and genetic structure with AMOVA.

Results At the continental scale, various lines of evidence (genetic-diversity hotspots, endemic haplotypes, zones of genetic discontinuities, and pattern of molecular variation) show that different lineages migrated from three glacial refugia (Beringian, Mississippian, east Appalachian). Strong genetic discontinuities, low diversity and lack of evidence for demographic expansion were found among stands from isolated high hills in central Québec.

Main conclusions White spruce in the eastern part of its range has an east Appalachian origin, a lineage genetically distinct from populations west of the Great Lakes. Previous studies have shown that the northward migration of white spruce is still ongoing as the northernmost populations expand into the tundra. In contrast, mixed white spruce and balsam fir (*Abies balsamea*) stands from isolated high hills in central Québec have already reached their Holocene maxima and are currently declining. This range contraction is probably a result of increased wildfire disturbance, which gradually creates a pattern of regional white spruce isolation and replacement by fire-prone black spruce (*Picea mariana*) forest.

Keywords

Boreal forest, chloroplast DNA, demographic decline, eastern North America, *Picea glauca*, post-glacial migration, species range.

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INTRODUCTION

Present species ranges are the result of an array of historical contingencies (Lomolino *et al.*, 2006). During the last glacial–

interglacial cycle, temperate and boreal taxa experienced major glacial range contraction into refugia and post-glacial expansion into present-day ranges. For a given tree species, current range boundaries correspond to the maximum Holocene

expansion, whereas a species' regional abundance within its distribution is associated with prevailing ecological regimes since its initial establishment (Payette, 1993). Except for the Beringia refugium (Anderson *et al.*, 2006; Zazula *et al.*, 2006), the entire present-day range of many if not all transcontinental boreal tree species in North America was covered by the Laurentide Ice Sheet during the Last Glacial Maximum (LGM; Ritchie, 1987). In North America, this implies a complete range shift whereby boreal trees migrated from LGM refugia into their current distribution area, and subsequently went extinct in regions south of the ice sheet (Davis & Shaw, 2001). Within the boreal forest, the complex mosaic of dominant forest-cover types is thus the result of demographic processes associated with the different species adaptations to the physical template (prevailing climate, surface deposits, topography), biotic interactions (interspecific competition, insect outbreaks) and historical disturbances (fire) (Larsen, 1980).

In his floristic analysis of the world's biota, Takhtajan (1986) considered white spruce [*Picea glauca* (Moench) Voss] to be the tree most characteristic of the North American boreal forest. However, several lines of evidence show that this widespread species is heterogeneous throughout its transcontinental range. First, pollen diagrams along a putative post-glacial migration route indicate that white spruce migrated faster and earlier in the western than in the eastern part of the range (Ritchie & MacDonald, 1986). Second, white spruce needles have different monoterpene compositions west and east of the Great Lakes, a pattern arguing for the existence of two clines extending from the Great Lakes (Wilkinson *et al.*, 1971). Likewise, a provenance test showed that height growth, diameter at breast height, crown width and form quotient correlate with longitude, another indication of an east–west clinal variation (Khalil, 1985). Finally, chloroplast DNA (cpDNA) variation along the north-western post-glacial migration routes between the Great Lakes and Alaska (Anderson

et al., 2006; Fig. 1) revealed two LGM white spruce refugia, namely in eastern Beringia and south of the Laurentide Ice Sheet. However, the post-glacial origin of white spruce east of the Great Lakes is still unknown.

Heterogeneity is also apparent within the eastern part of the white spruce range (Québec–Labrador peninsula, Atlantic Provinces of Canada and New England), where major range discontinuities are coupled to a variety of species associations (Fig. 1). The bulk of white spruce distribution lies in the moist balsam fir [*Abies balsamea* (L.) Mill.] forest of the southernmost zone of the boreal forest. In this context, white spruce is an associated species occupying *c.* 10% of the forest cover (Jurdant, 1959; Lafond, 1964, 1974). However, noticeable exceptions of high regional abundance exist in the eastern part of the range. For instance, at the northernmost species boundary located outside the balsam fir zone, monospecific forest stands occur along the eastern Hudson Bay coast and the Atlantic coast in Labrador. These coastal populations are of recent origin (300–500 years along Hudson Bay) owing to a delay in post-glacial migration and they are still expanding into the tundra (Payette & Fillion, 1985; Caccianiga & Payette, 2006; Payette, 2007; Laliberté & Payette, 2008; Fig. 2a,b). Other remote northern white spruce populations are found inland, namely on the slopes of isolated high hills (*c.* 1000 m) of the Precambrian plateau located in central Québec (Fig. 2c). In this area, mixed white spruce and balsam fir subalpine stands are located at elevations between those of black spruce [*Picea mariana* (Mill.) B.S.P.] lowland forests and alpine tundra (Fig. 2c). The floristic association between balsam fir and white spruce in the subalpine stands of central Québec is reminiscent of that of the southern balsam fir forest zone (G. de Lafontaine & S. Payette, submitted). The humid altitudinal environment prevailing on high hills is also similar to the moist environment favourable to the balsam fir forest of the southern boreal landscape. However, the subalpine stands

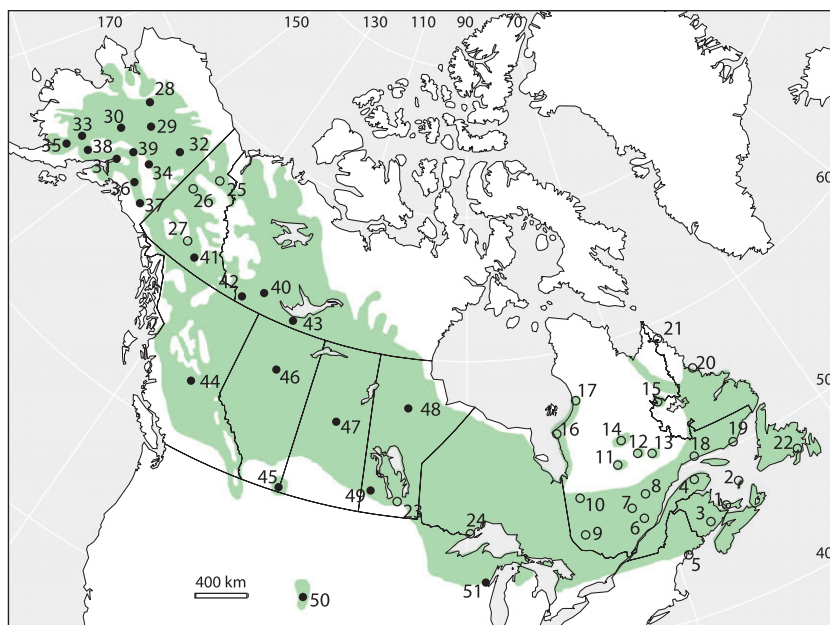


Figure 1 Sampling locations and white spruce (*Picea glauca*) range in North America. The locations of sites sampled for this study (open circles) and by Anderson *et al.* (2006) (closed circles) are shown. The numbers correspond to those in Appendix S1. The distribution range of white spruce is indicated in green.



Figure 2 Remote white spruce (*Picea glauca*) populations. (a) Site 21: northernmost white spruce stand along the Labrador coast – monospecific expanding population. (b) Site 16: ongoing population expansion revealed by white spruce saplings expanding along the eastern coast of Hudson Bay. (c) Site 14: subalpine mixed white spruce and balsam fir old-growth stand forming an elevational belt below a tundra summit and above a lowland black spruce forest.

from central Québec are 300–500 km north of the balsam fir forest zone and are separated from it by the black spruce forest zone. The origin of the disjunct white spruce subalpine stands of central Québec is unknown.

The purpose of this study was to ascertain whether the origin of white spruce range discontinuities in eastern North America is reflected in the patterns of genetic diversity. In order to do this, the first step was to uncover the glacial origin and post-glacial migration route in the eastern part of the range by extending the phylogeographical area analysed by Anderson *et al.* (2006). The second step was to test whether the remote disjunct stands are of recent origin resulting from a delay in migration, as are the stands at the northern range limit along Hudson Bay and Labrador coasts. Alternatively, they could be remnant isolated patches resulting from a northern boundary range contraction probably involving a global demographic decline.

MATERIALS AND METHODS

Population sampling

The study was based on a dataset comprising a total of 589 white spruce individuals representing 51 populations throughout the entire range of the species (Fig. 1, see Appendix S1 in Supporting Information). We used data from Anderson *et al.* (2006) collected from 24 sites in the western part of the species range (sites 28–51; Fig. 1). We collected genetic data ourselves for 27 additional populations (sites 1–27; 263 trees, $N = 7–10$ per site, average: 9.7 per site). Five populations were sampled within the western part of the range, three in eastern Beringia (Yukon) and two in the south-central area (Manitoba and Ontario) in order to confirm that our results are coherent with those of Anderson *et al.* (2006). The other 22 sites covered the entire eastern white spruce range (from Québec to Newfoundland; sites 1–22), with samples from the southern boundary on Mount Desert Island, Maine (site 5; Davis, 1966) to the northern limit on the Hudson Bay coast (sites 16 and 17; Caccianiga & Payette, 2006; Laliberté & Payette, 2008) and on the coast of the Labrador Sea (sites 20 and 21; Payette, 2007). In order to test specifically the origin of disjunct stands, subalpine stands from five isolated high hills were sampled, namely at Montagnes Blanches (site 11), Mont Babel (site 12), Monts Groulx (site 13), Monts Otish (site 14) and Schefferville (site 15). To our knowledge these five sites represent all of the eastern disjunct white spruce patches.

DNA extraction, amplification and sequencing

At each sampling site, needles of the current growth season were collected from adult trees spaced ≥ 100 m apart. Upon arrival at the laboratory, the samples were stored at -20 °C prior to DNA extraction. DNA was extracted from 50 to 100 mg of homogenized needles using Nucleospin® Plant II (Macherey-Nagel Inc., Bethlehem, PA, USA) according to the manufacturer's instruction.

The cpDNA regions *trnT/L* and *trnL/F* introns and the pseudogene *ndhK/C* (Wakasugi *et al.*, 1994) were amplified using universal primers (Taberlet *et al.*, 1991) and the *ndhK/C* pseudogene primers (Anderson *et al.*, 2006). For the *trnT/L*

region, however, a more efficient forward primer was designed (*trnA2*: 5'-CAAATGCGATGCTTAACC-3'). Polymerase chain reactions (PCRs) were performed in 50 μ L with 20 ng of DNA, 1 \times PCR buffer (New England Biolabs, Ipswich, MA, USA), 1 \times bovine serum albumin (Promega, Madison, WI, USA), 2 mM MgCl₂, 0.3 μ M of each primer, 0.2 mM concentration of each dNTP, 1 unit (*trnT/L*) or 0.3 unit (*trnL/F* and *ndhK/C*) of *Taq*DNA polymerase (New England Biolabs). PCR profiles comprised: 3 min at 94 °C; eight cycles of 30 s at 94 °C, 45 s at 45 °C, 1 min at 72 °C; 21 cycles of 30 s at 94 °C, 45 s at 48 °C, 1 min at 72 °C; and a final extension of 10 min at 72 °C. PCR products were purified using a QIAquick[®] PCR purification kit (Qiagen, Valencia, CA, USA) and sequenced at the Plate-forme d'Analyses Biomoléculaires (Université Laval, Québec, QC, Canada).

All new chloroplast DNA sequences have been deposited in the GenBank database (accession numbers GQ870265–GQ870273).

Data analysis

Sequences were manually edited with SEQUENCHER 4.7 (Gene Codes, Ann Arbor, MI, USA) and aligned with MEGA 3.1 (Kumar *et al.*, 2004). To perform the transcontinental phylogeographical analysis, the complete dataset (including our dataset and that of Anderson *et al.*, 2006) was used. To test for the presence of phylogeographical structure throughout the entire white spruce range, G_{ST} and N_{ST} were calculated according to Pons & Petit (1996). A test to determine if N_{ST} was significantly larger than G_{ST} was carried out using PERMUTCPSSR 2.0 (<http://www.pierroton.inra.fr/genetics/labo/Software/PermutCpSSR>). N_{ST} takes into account sequence differences between the haplotypes. Thus a N_{ST} higher than G_{ST} indicates that closely related haplotypes are found more often in a given geographical area than would be expected by chance (Pons & Petit, 1996). Intrapopulation haplotype diversity was measured by allelic richness using RAREFAC (<http://www.pierroton.inra.fr/genetics/labo/Software/Rarefac>) to account for the differences in population sample sizes (from 7 to 17 individuals per population; Petit *et al.*, 1998).

Geographical patterns of genetic variation throughout the distribution range were estimated using two different but complementary approaches, and results were compared. First, a Bayesian spatial clustering algorithm implemented in BAPS 5.2 (Corander *et al.*, 2008) was used to yield the optimal grouping of populations. Second, Monmonier's maximum difference algorithm implemented in BARRIER 2.2 (Manni *et al.*, 2004) was used to detect zones of maximum genetic discontinuity (F_{ST}) along the network of connecting populations. Although the two approaches are suitable for defining spatially explicit genetic structure within the range, the rationales behind these approaches are markedly different. The Bayesian algorithm tends to find clusters of genetically similar populations (lumping process), whereas Monmonier's algorithm tends to uncover maximal genetic differences between populations (splitting process). As a complement,

analyses of molecular variance (AMOVAs; Excoffier *et al.*, 1992) were used to confirm the uncovered spatial pattern of genetic differentiation using ARLEQUIN 3.11 (Excoffier *et al.*, 2005) with significance tests based on 1000 permutations.

The second step was to investigate the origin of the remote subalpine populations from central Québec. Analyses were limited to the populations associated with the eastern lineage (see Results; sites 1–22). If remote stands are of recent origin, as are the other better studied and expanding northern populations (Payette & Filion, 1985; Caccianiga & Payette, 2006; Payette, 2007; Laliberté & Payette, 2008), they should share genetic attributes indicative of continuing demographic expansion. This was assessed with a mismatch analysis (Rogers & Harpending, 1992) implemented in ARLEQUIN version 3.11 (Excoffier *et al.*, 2005). Alternatively, if they are remnant patches undergoing a demographic decline, they should display some of the genetic signatures expected from bottlenecks, namely reduced diversity and increased differentiation. Intrapopulation haplotype diversity of the subalpine populations (sites 11–15) was compared with that of the other eastern populations. Sample size being uniform in the east (8–10 individuals per population; average \pm standard deviation: 9.8 ± 0.5), Nei's (1973) gene diversity (H) was used to estimate intrapopulation haplotype diversity directly in this analysis. A permutation procedure was used to test whether the gene diversity of the five subalpine populations was significantly lower than that of groups of five artificial populations generated by randomly reassigning the haplotype pool of the eastern range (100,000 permutations; see Appendix S2). Second, genetic structure was examined among all eastern populations as well as among the five central Québec isolated populations by AMOVA (Excoffier *et al.*, 1992) using ARLEQUIN version 3.11 (Excoffier *et al.*, 2005).

RESULTS

Transcontinental analysis

The 263 individuals sampled in 27 sites added nine new haplotypes (N1–N9; see GenBank accession numbers GQ870265–GQ870273) to the dataset of Anderson *et al.* (2006) (Fig. 3; see Appendix S3). A phylogeographical structure was detected on the basis of haplotype-identity permutations (Pons & Petit, 1996) [N_{ST} (0.132) > G_{ST} (0.104), $P < 0.05$]. This is mostly a result of the high frequency of haplotypes II and Ia and their derivatives in the western and eastern parts of the species' range, respectively (Figs 3 & 4a). Relative to the data of Anderson *et al.* (2006), sampling in the eastern part increased haplotype Ia occurrence by 335%, whereas haplotype II (the most abundant haplotype in the western part) was increased by only 26%.

Three areas with endemic haplotypes also harboured populations showing higher than average genetic diversity (Fig. 4b). Indeed, seven new haplotypes were unique to the eastern part (N1–N7; Fig. 4b). Likewise, three haplotypes were endemic to the south-central region (Anderson *et al.*, 2006) and 10

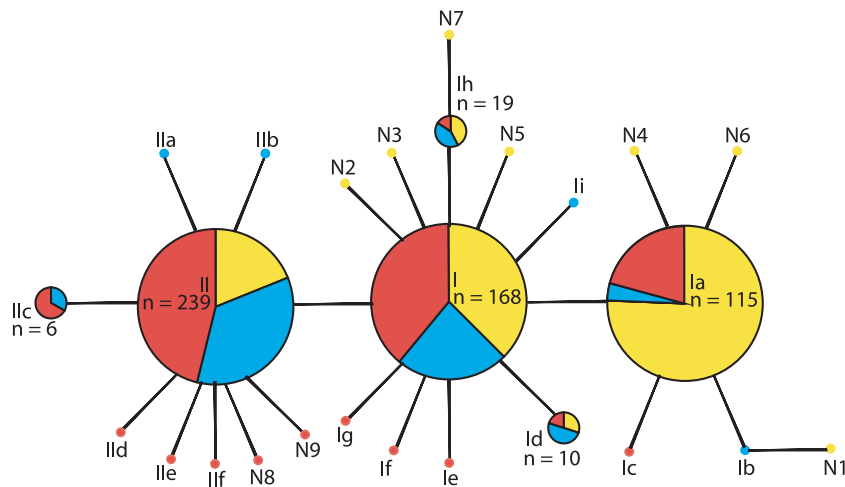


Figure 3 White spruce (*Picea glauca*) chloroplast DNA haplotype network ($n = 589$). Haplotypes unique to either the western, the central or the eastern parts of the distribution range are coloured red, blue or yellow, respectively. Haplotypes found in more than one region are represented as a pie chart in which each colour (red, blue and yellow) represents the relative contribution of each region defined by Monmonier's algorithm (west, central and east, respectively) to the total haplotype abundance. Circle size indicates haplotype frequency in the 51 populations (large, intermediate and small circles correspond to frequencies $\geq 20\%$, $\geq 1\%$ and $< 1\%$, respectively). Haplotype nomenclature follows Anderson *et al.* (2006), with new haplotype codes starting with N (N1–N9).

haplotypes were unique to the north-west area (Alaska, Yukon, Northwest Territory), two of which were new (N8 and N9), from two Yukon sites sampled for the present study (Fig. 4b).

The Bayesian spatial analysis found an optimal grouping distinguishing two clusters of populations, west and east of the Great Lakes (natural logarithm of the marginal likelihood ($\log ml$) = -851.404 ; data not shown). However, when fixing the number of clusters to three, the Bayesian spatial analysis grouped all Alaskan populations as the third cluster ($\log ml$ = -856.552 ; Fig. 4c; Appendix S4). Zones of maximum genetic discontinuity identified by Monmonier's algorithm further supported the existence of these three regions (Fig. 4c; Appendix S5). However, the two-first and strongest barriers ('a' and 'b'; Fig. 4c) revealed maximal differentiation among the subalpine populations from central Québec. Barrier 'a' completely circumscribed the central subalpine population (site 12), and barrier 'b' isolated two other nearby subalpine populations (sites 11 and 14). The third barrier ('c'; Fig. 4c) stood between the north-western and central regions of the species distribution, separating British Columbia, Yukon and Alaska from the rest of the range. The fourth and fifth barriers ('d' and 'e'; Fig. 4c) isolated a population in Ontario, but most importantly uncovered a discontinuity between the central and eastern regions that corresponded exactly to the west–east clusters independently found by the Bayesian spatial analysis.

Analyses of molecular variance confirmed the spatial genetic structures defined by the Bayesian and Monmonier's algorithms (Table 1). Indeed, a significant proportion of genetic variance was accounted for by differences among the three regions (north-west, south central and east) as defined by the Bayesian and Monmonier's algorithms (16.42% and 16.38%, respectively, both $P < 0.001$), whereas the total variance assigned to the difference among populations within these regions was not significant ($P = 0.255$ and 0.231 , respectively).

Eastern range analysis

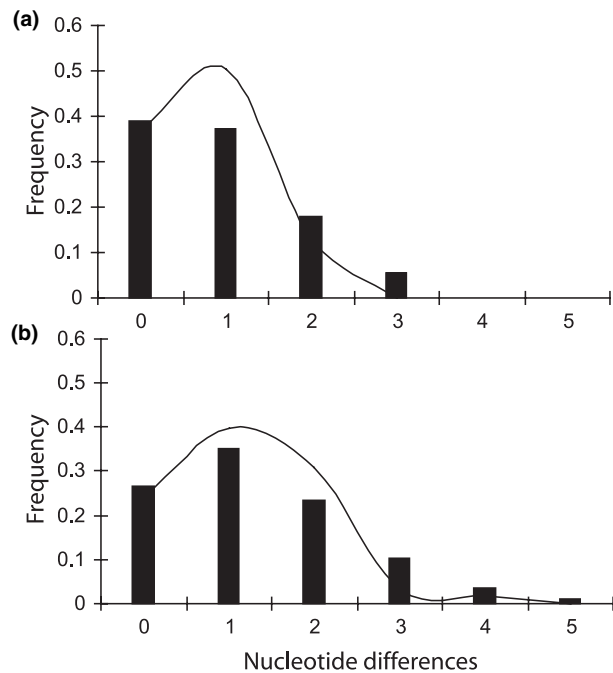
Remote populations from central Québec displayed demographic signatures different from those of other northern, expanding populations. Globally, the eastern white spruce component did not fit the expectations of expansion ($P = 0.02$), nor did the isolated populations from central Québec ($P = 0.03$; Fig. 5a). By contrast, the northernmost populations (Labrador: sites 20 and 21; eastern Hudson Bay coast: sites 16 and 17) were the only eastern components fitting the demographic expansion model, either globally ($\tau = 1.3$; $P = 0.09$; Fig. 5b) or separately (Labrador: $\tau = 1.7$; $P = 0.07$; Hudson Bay: $\tau = 1.1$; $P = 0.08$). Moreover, each of the five subalpine populations in central Québec had lower intrapopulation gene diversity than average in the eastern part of the range (average $H = 0.688$; Fig. 6). This resulted in the gene diversity within the five subalpine populations ($H = 0.630$) being significantly lower than that of groups of five artificial populations generated by reassigning the haplotype pool of the eastern part of the range ($H = 0.724$; $P = 0.013$).

In the transcontinental analysis presented above, the strongest discontinuities revealed by Monmonier's algorithm throughout the white spruce range were among the five subalpine populations from central Québec (barriers 'a' and 'b'; Fig. 4c), and these five isolated stands were significantly differentiated (Table 2; $F_{ST} = 0.152$, $P = 0.014$). The eastern genetic structure suggested by barriers 'a' and 'b' in Monmonier's algorithm (three groups: site 12; sites 11 and 14; all other eastern sites) was tested using AMOVA (Table 2). A significant proportion of genetic variance was accounted for by differences among the three groups (6.88%, $P = 0.01$; Table 2), but the total variance assigned to the difference among populations within these groups was not significant ($P = 0.73$).

Table 1 Transcontinental analyses of molecular variance (AMOVAs) for haplotypes of white spruce (*Picea glauca*) in North America.

Source of variation	d.f.	Var (%)	Fixation index	P-value
North-west, south central and east regions defined by the Bayesian algorithm (see Fig. 4c)				
Among regions	2	16.42	0.164	< 0.001
Among populations, within regions	48	0.72	0.009	0.255
Within populations	538	82.86	0.171	< 0.001
Total	588			
North-west, south central and east regions defined by Monmonier's algorithm (see Fig. 4c)				
Among regions	2	16.38	0.164	< 0.001
Among populations, within regions	48	0.81	0.010	0.231
Within populations	538	82.81	0.172	< 0.001
Total	588			

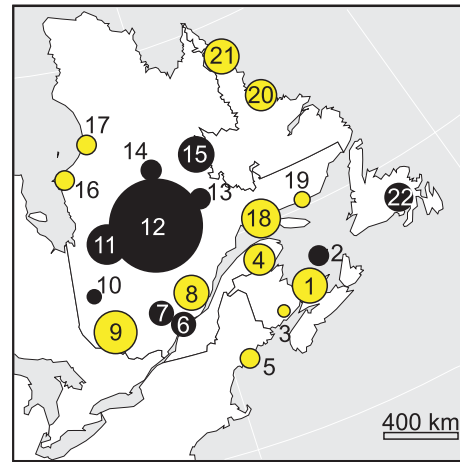
d.f., degrees of freedom; Var (%), percentage of variation.

**Figure 5** Mismatch distributions of (a) remote white spruce (*Picea glauca*) populations in central Québec, and (b) northernmost white spruce populations. Bars indicate observed mismatch differences, and lines represent the expected distribution under a demographic expansion model.

DISCUSSION

Glacial refugia and post-glacial migration

We found evidence for three white spruce glacial refugia. Indeed, throughout the white spruce range, three regions

**Figure 6** Distribution of intrapopulation chloroplast DNA genetic diversity in the eastern part of the range of white spruce (*Picea glauca*) in North America. Yellow and black circles indicate diversity above and below the mean ($H = 0.688$) for 22 eastern populations, respectively. Circle sizes are proportional to deviations from the mean, with the largest yellow circle representing the highest diversity, the largest black circle representing the lowest diversity, and the smallest circles having values closest to the mean. Site numbers correspond to those in Fig. 1 and Appendix S1.**Table 2** Analyses of molecular variance (AMOVAs) for haplotypes from the eastern part of the range of white spruce (*Picea glauca*).

Source of variation	d.f.	Var (%)	Fixation index	P-value
Subalpine populations from central Québec (sites 11–15)				
Among subalpine populations	4	15.15	0.152	0.014
Within populations	43	84.85		
Groups in the eastern part of the range defined by Monmonier's algorithm (see Fig. 4c)				
Among groups	2	6.88	0.0688	0.012
Among populations, within groups	19	-0.85	-0.009	0.730
Within populations	194	93.97	0.060	0.303
Total	215			

d.f., degrees of freedom; Var (%), percentage of variation.

displayed high genetic diversity and endemic haplotypes. These regions corresponded closely to the three clusters determined by the Bayesian algorithm and to the three main areas defined by major genetic discontinuities determined by Monmonier's algorithm. These regions are thus considered as evidence for the proximity of three LGM refugia from which white spruce migrated.

The analysis of the entire range of white spruce confirms the existence of a north-west (Beringia) refugium, as previously discussed by Anderson *et al.* (2006). This is not surprising, given the fact that most of the data analysed in this area came

from Anderson *et al.* (2006). However, the three Yukon populations sampled for the present study (sites 25, 26 and 27) also showed high intrapopulation genetic diversity and added two unique haplotypes, providing further support for a Beringian refugium.

South of the ice sheet, two distinct refugia were defined, corresponding to areas west and east of the Appalachian mountains (Mississippian and east Appalachian refugia, respectively). Indeed, spruce pollen and macrofossils dating back to the LGM were found both west and east of the Appalachian mountains (Davis, 1983; Watts, 1983; Ritchie, 1987; Jackson *et al.*, 1997). Our analysis clearly showed that the latter fossil evidence corresponded to two distinct LGM refugia from which two post-glacial migration routes are inferred to explain contemporary lineages west and east of the Great Lakes. White spruce from the Mississippian refugium probably migrated northwards west of the Great Lakes, whereas those from the east Appalachian refugium migrated into New England and northwards into Québec–Labrador and the Atlantic Provinces. This pattern east of the Rocky Mountains is similar to that revealed by mitochondrial DNA in two other widespread North American boreal species, black spruce (Jaramillo-Correa *et al.*, 2004) and jack pine (*Pinus banksiana* Lamb.) (Godbout *et al.*, 2005). The clear distinction between western and eastern white spruce lineages is also congruent with previous biochemical and growth-pattern studies suggesting two clines west and east of the Great Lakes (Wilkinson *et al.*, 1971; Khalil, 1985).

Origin of the eastern range discontinuities

The genetic signatures detected for the five subalpine populations sampled in central Québec support the hypothesis of white spruce decline in this region. The low intrapopulation gene diversity in central Québec populations coupled with the genetic differentiation across these five isolated populations is a strong indication of a demographic decline in this area. These populations were also readily delineated by Monmonier's algorithm, which adds to the evidence that they form a distinct group. Moreover, the mismatch distribution of these populations does not fit the expansion model corroborated for those other northern populations for which continuing post-glacial expansion is documented.

This genetic pattern in central Québec could equally result from a founder effect during the post-glacial migration of white spruce (Hewitt, 2000). Ecological evidence shows that monospecific white spruce populations at their northern limits along the coasts of Hudson Bay and Labrador have a recent origin and are still undergoing a northward migration process (Payette & Filion, 1985; Caccianiga & Payette, 2006; Payette, 2007). After such a migration delay, a founder effect should be expected at the northern range boundary. However, the northernmost coastal populations (sites 16, 17, 20 and 21) do not display reduced diversity or differentiation from other populations of the eastern lineage. If the recently established populations at the northern range limit in Québec and

Labrador do not show these signs of founder effects after such a migration lag, it is unlikely that a founder effect scenario explains the current pattern of genetically depauperate populations in central Québec. A long-distance migration could also have resulted in the observed pattern of white spruce genetic diversity in these remote stands (Ibrahim *et al.*, 1996). Although this possibility cannot be formally ruled out, it would be very unlikely that the floristic composition of these high-hill stands resulted from the independent long-distance migration of each species to these remote and relatively small suitable habitat patches. In contrast, the demographic decline and range contraction scenario has ecological support.

Indeed, the white spruce populations of central Québec are isolated, subalpine, mixed white spruce and balsam fir stands surrounded by black spruce forests in the lowlands. The flora in the isolated subalpine stands resembles that of the southern balsam fir forest located 300–500 km to the south (G. de Lafontaine and S. Payette, submitted) more than the surrounding flora typical of the black spruce forest zone (La Roi, 1967; Pollock, 2008). The regional abundance of white spruce and balsam fir, typical of the southern boreal flora, suggests that their range once extended to this region earlier in the Holocene. Balsam fir is underrepresented in fossil pollen assemblages, and white spruce pollen is hardly distinguishable from black spruce pollen, but there is palaeoecological evidence supporting the hypothesis of a previous northern extension of the balsam fir forest into the present-day black spruce forest zone. We reinterpreted pollen diagrams around central Québec using dates calibrated with CALIB (version 5.01) software (Stuiver *et al.*, 2005). For each diagram an age/depth model was computed from which the time period of fir presence could be interpolated. Pollen diagrams from the Caniapiscou Reservoir area (250 km north of site 14) suggested the presence of balsam fir in central Québec between 5600 and 3700 cal. yr BP (Richard *et al.*, 1982). A pollen diagram from Gravel Ridge, Labrador (250 km east of site 15) showed high white spruce and balsam fir abundance between 6500 and 4900 cal. yr BP, followed by black spruce (Lamb, 1985). Pollen diagrams from Churchill Falls, central Labrador (250 km south-east of site 15) showed a peak of balsam fir pollen starting at 6000 cal. yr BP followed by a sudden decrease (Morrison, 1970). Pollen diagrams from south-east Labrador indicated balsam fir and white spruce between 8800 and 6500 cal. yr BP (Engstrom & Hansen, 1985) and 7400 and 6200 cal. yr BP (Lamb, 1980), subsequently replaced by black spruce. Balsam fir remains dated between 5120 and 4690 cal. yr BP were found buried under a live jack pine and black spruce stand located 100 km east of James Bay coast (Arseneault & Sirois, 2004).

Of all the widespread boreal tree species, white spruce and especially balsam fir are the least adapted to fire, whereas jack pine and black spruce are fire-adapted species in the drier, northern parts of the closed-crown boreal forest (Halliday & Brown, 1943; Rowe, 1972; Rowe & Scotter, 1973; Ritchie, 1987; Payette, 1993). The presence of a black spruce forest zone between the moist southern balsam fir forest zone and the

disjunct mixed white spruce and balsam fir stands in central Québec suggests fire as the major ecological forcing promoting the development of black spruce forest over balsam fir forest. A change in the fire regime during the Holocene would have constrained balsam fir forests to the moister areas of the boreal landscape, mostly in the south. As a result, balsam fir and white spruce in central Québec would be relict stands maintained in humid, fire-protected environments of the high plateau. The role of fire as the primary ecological factor structuring the boreal landscape has already been documented for eastern North America (Payette, 1992). Empirical testing of black spruce decline in the forest-tundra landscape showed that deforestation by fire began around 3000 cal. yr BP (Asselin & Payette, 2005). The transformation of old-growth, closed-crown black spruce forests into lichen-black spruce woodlands has occurred over the last 100 years (Girard *et al.*, 2008, 2009). This process, triggered by high fire activity, is most active in lowland forests surrounding the subalpine stands (Girard *et al.*, 2008, 2009). An ongoing analysis of charcoal particles buried in soils of the subalpine stands and in the southern balsam fir forest zone will provide dates for the onset of increased fire activity in central Québec.

The present study focused on the range discontinuities observed in the eastern part of the range, and sampling sites were chosen accordingly. By contrast, Anderson *et al.* (2006) sampled west of the Great Lakes with the objective of uncovering glacial refugia and post-glacial migration routes in the western part of the range. If the white spruce decline hypothesis was to be tested in other parts of the range, disjunct populations located in a matrix of fire-prone species [black spruce, jack pine, lodgepole pine (*Pinus contorta* Dougl.)] should be sampled. It is possible that the same process is at work in other parts of the range of white spruce. Based on pollen and macrofossil stratigraphies, Liu (1990) inferred an initial post-glacial colonization by white spruce and balsam fir in northern Ontario with a subsequent replacement by black spruce. This latter pattern resembles the one in the east in the present study and could imply the same ecological processes.

CONCLUSIONS

Our transcontinental analysis has revealed the existence of a third, Appalachian, white spruce refugium that is highly compatible with the long-documented longitudinal heterogeneity between western and eastern populations (Wilkinson *et al.*, 1971; Khalil, 1985; Ritchie, 1987). In eastern North America, white spruce shows contrasting demographic trajectories. Along the Hudson Bay and Labrador coasts, the species is still migrating into tundra. This northern range boundary corresponds to the maximum Holocene expansion. By contrast, in central Québec, genetically distinct and depauperate populations are strongly indicative of a demographic decline, probably a consequence of an ecological sorting process resulting in disjunct stands surrounded by other species having different adaptations to fire.

These distinct demographic trajectories within the white spruce distribution exemplify the two processes shaping the range of a species. Ultimately, the geographical range of species is the result of both their maximum post-glacial expansion and their fate after initial colonization. 'Phylogeography is a field of study concerned with the principles and processes governing the geographic distributions of genealogical lineages' (Avice, 2000, p. 3). As such, the discipline should focus on both of these historical processes leading to the present-day range of species. However, most phylogeography studies on temperate and boreal trees have focused on one of these processes, that is, the post-glacial migration of species. The present study has shown that with an accurate knowledge of a species' range and a detailed understanding of its ecology, biogeographical hypotheses regarding the fate of the species after initial colonization can also be tested using genealogical lineages.

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SUPPORTING INFORMATION

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

Appendix S1 Details of the white spruce populations sampled.

Appendix S2 Permutation procedure.

Appendix S3 Variable cpDNA sites and GenBank accession numbers.

Appendix S4 Clustering of white spruce populations defined by BAPS 5.2.

Appendix S5 Zones of maximal genetic discontinuity defined by BARRIER 2.2.

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This work was conducted as part of **Guillaume de Lafontaine's** PhD thesis, a multidisciplinary work integrating phylogeography, palaeoecology and population ecology focusing on the origin and dynamics of subalpine white spruce populations.

Julie Turgeon is a professor of evolution at Université Laval and is interested in the phylogeography and hybridization of aquatic organisms.

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