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Diverging patterns of mitochondrial and nuclear DNA diversity in subarctic black spruce: imprint of a founder effect associated with postglacial colonization

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Abstract

High-latitude ecotonal populations at the species margins may exhibit altered patterns of genetic diversity, resulting from more or less recent founder events and from bottleneck effects in response to climate oscillations. Patterns of genetic diversity were investigated in nine populations of the conifer black spruce (*Picea mariana* [Mill.] BSP.) in northwestern Québec, Canada, using seed-dispersed mitochondrial (mt) DNA and nuclear (nc) DNA. mtDNA diversity (mitotypes) was assessed at three loci, and ncDNA diversity was estimated for nine expressed sequence tag polymorphism (ESTP) loci. Sampling included populations from the boreal forest and the southern and northern subzones of the subarctic forest-tundra, a fire-born ecotone. For ncDNA, populations from all three vegetation zones were highly diverse with little population differentiation ($\theta_N = 0.014$); even the northernmost populations showed no loss of rare alleles. Patterns of mitotype diversity were strikingly different: within-population diversity and population differentiation were high for boreal forest populations [expected heterozygosity per locus (H_E) = 0.58 and $\theta_M = 0.529$], but all subarctic populations were fixed for a single mitotype ($H_E = 0$). This lack of variation suggests a founder event caused by long-distance seed establishment during postglacial colonization, consistent with palaeoecological data. The estimated movement of seeds alone (effective number of migrants per generation, $Nm_M < 2$) was much restricted compared to that estimated from nuclear variants, which including pollen movement ($Nm_N > 17$). This could account for the conservation of a founder imprint in the mtDNA of subarctic black spruce. After reduction, presumably in the early Holocene, the diversity in ncDNA would have been replenished rapidly by pollen-mediated gene flow, and maintained subsequently through vegetative layering during the current cooler period covering the last 3000 years.

Keywords: expressed sequence tag polymorphisms, forest-tundra, founder effect, gene flow, mitochondrial DNA haplotypes, *Picea mariana*

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Introduction

Major climatic changes associated with glacial cycles and superimposed low-amplitude oscillations in climate have profoundly shaped the distribution area and population structure of temperate and boreal species. Following drastic warming and ice retreat at the onset of the present interglacial (Holocene), continental-scale areas of suitable habitats have become available for a variety of species confined to glacial refugia. For organisms with long generation times such as trees, the present-day genetic structure may still show the imprint of the pattern and speed of postglacial migration (Newton *et al*. 1999). As shown by computer simulations (Hewitt 1996), gradual expansion should preserve a large proportion of the

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genetic diversity of source population(s) and will cause minor genetic divergence among populations of the newly colonized area. In contrast, a migration process involving leading populations dispersed well ahead of the main front could eventually cause loss of genetic variation in the colonized range, and greater differentiation among founded populations. In addition to founder events, with the populations at the 'leading edge' containing only a subset of the gene diversity present in the main population, further genetic drift in these initially small and isolated populations could cause loss of alleles (Hewitt 1996). Gene diversity reduction through migration-drift does not exclude the possibility that a subsequent merging of expanding populations could increase their genetic variation, especially when they have derived from more than one refugium (e.g. Sinclair *et al*. 1999; Premoli *et al*. 2000; Comps *et al*. 2001; Walter & Epperson 2001).

As a wide ecotonal zone making up the transition between continuous boreal forest and treeless arctic tundra, the subarctic forest-tundra of the eastern coast of Hudson Bay (northwestern Québec, Canada) has proved to be a sensitive ecological tracer of past climatic changes that have occurred at different temporal scales (Payette & Filion 1985; Gajewski *et al*. 1993; Lavoie & Payette 1994). The region was deglaciated around 6000 years BP (Payette 1993), a few thousand years later than its latitudinal equivalent in Europe (Huntley & Birks 1983) and northwestern Canada (Ritchie 1987). Tree species, mainly the wind-pollinated conifer black spruce (*Picea mariana* [Mill.] BSP.), spread northwards from a meridional boreal refugium located south of the Laurentide ice sheet, possibly the Great Plains west of the Appalachian Mountains (Davis 1983). Black spruce reached northernmost sites corresponding to the modern position of the tree line around 4500 years BP, as documented by pollen and macrofossil records (Payette 1993). Following the postglacial climatic optimum, upland forests were depleted progressively into tundra due to late Holocene deterioration of climatic conditions and successive failures of the postfire regeneration process, as shown by the widespread occurrence of charcoal or subfossil trees in present-day open sites (Payette & Gagnon 1985; Arseneault & Payette 1992). At the Holocene time scale, two major climatic periods in the history of forest-tundra forests can thus be distinguished: postglacial spread and forest expansion (before 3000 years BP) followed by fragmentation and regression of the forest cover (from 3000 years BP to present; Payette & Gagnon 1985).

Wildfire has had a differential impact on forests of the southern (forest subzone) or northern (shrub subzone) part of the forest-tundra ecotone. While southern forests have been able to reestablish themselves on most burned upland sites, northern forests have become progressively fragmented. This seems to be resulting partly from the poor production of viable seeds by shrubby growth forms adopted by black spruce at high latitudes (Sirois 2000). Indeed, black spruce is a morphologically variable species, harbouring arborescent shapes as well as progressively more stunted phenotypes in response to increasingly harsh winter conditions. In a context of recent climate warming and reforestation of local tundra sites from remaining seed-bearing trees (Gamache & Payette, in prep.), the possibility of a loss of genetic variation in marginal black spruce populations, resulting from founder effects and/or subsequent fire-induced fragmentation, deserves to be investigated. As pointed out by Rogers *et al*. (1999), the amount and structure of genetic variation in ecotonal populations is likely to affect the nature and rate of their response to climatic changes.

Black spruce combines many life-history traits that are generally associated with high within-population gene diversity and little population differentiation (Hamrick *et al*. 1992): a widespread distribution, wind-dispersed pollen and seeds and a mixed sexual and asexual mating. An analysis of gene diversity at allozyme loci in island black spruce populations of the middle forest-tundra of northern Québec (Desponts & Simon 1987) previously revealed high within-population variability, with little population structure, similar to the pattern in southern boreal forest populations (e.g. Boyle & Morgenstern 1987; Wang & MacDonald 1992; Isabel *et al*. 1995) and for DNA polymorphisms in boreal black spruce populations (Isabel *et al*. 1995; Perry & Bousquet 2001). Gene flow has thus presumably been effective in spreading nuclear gene diversity into northern populations.

Long-distance transport of wind-dispersed pollen can obscure local migration effects on the genetic structure and make them indiscernible. Due to their maternal mode of inheritance in conifers and in black spruce (Jaramillo-Correa *et al*. 2003), seed-borne mitochondrial DNA (mtDNA) markers are best suited to assess the impact of historical factors on its current genetic diversity, because seeds (even if wind-dispersed) probably migrate over much shorter distances compared to airborne pollen. Hence, a comparison of population genetics parameters estimated with mtDNA markers and biparentally inherited nuclear DNA (ncDNA) markers could help to evaluate the relative contribution of postglacial migration patterns and more recent gene flow to the genetic structure of forest-tundra black spruce. Contrasting population differentiation levels from organelle and nuclear DNA markers have been observed (e.g. Dumolin-Lapègue *et al*. 1997; Newton *et al*. 1999; Gugerli *et al*. 2001), but few studies have compared them directly using the same set of plant populations (McCauley 1994; Sinclair *et al*. 1998; Tomaru *et al*. 1998; Squirrell *et al*. 2001).

In this study, we compared levels of gene diversity and population differentiation for black spruce populations distributed along a latitudinal climatic gradient crossing the subarctic forest-tundra to those of the southern boreal forest, such as estimated with a set of maternally inherited

Table 1 Description of mature black spruce populations sampled in boreal and subarc Québec, Canada

Vegetation zone: population	Latitude (N)	Longitude (W)	*N*	Dominant recruitment mode	Dominant growth form*
Closed-crown boreal forest					
Parc des Grands-Jardins (PGJ)	47°48′	70°54′	32	layering	arborescent
Saguenay River (NS)	48°24′	69°30′	30	postfire sexual reproduction	arborescent
Chibougamau Lake (FV2)	49°12′	73°36′	30	postfire sexual reproduction	arborescent
Open-crown boreal forest					
Great Whale River (GW)	55°09′	75°29′	24	postfire sexual reproduction	arborescent
Subarctic FT†/forest subzone					
Little Whale River (LW)	55°49′	75°31′	24	postfire sexual reproduction	arborescent
Clearwater River (CW)	56°13′	75°29′	24	postfire sexual reproduction	eroded arborescent
Subarctic FT/shrub subzone					
Minto Lake (ML)	57°21′	75°41′	24	layering	whorled > 2.5 m high
Innuksuac River (IR)	58°04′	75°29′	24	layering	shrubby < 2.5 m high
Shrub tundra					
Chavigny Lake (CL)‡	58°11′–58°27′	75°21′–75°30′	28	layering	shrubby < 1 m high

*Nomenclature of growth forms adapted from Lavoie & Payette (1994).
†FT, forest-tundra: the fragmented ecotone between continuous boreal forest and treeless arctic tundra.
‡Isolated clones were pooled to form the population at the species' limit.

sequence-tagged-site (STS) mtDNA markers that were recently shown to be polymorphic in black spruce (Jaramillo-Correa *et al*. 2003), and for which this study represents the first application to a population genetics study, and a set of nuclear STS markers located in transcribed but untranslated regions of coding genes [expressed sequence tag polymorphisms (ESTPs); Perry & Bousquet 1998]. The use of three mtDNA markers in the present study represents an improvement compared to most biogeographical studies on conifers that rely on a single locus (e.g. Isoda *et al*. 2000; Mitton *et al*. 2000; Soranzo *et al*. 2000; Gugerli *et al*. 2001). We studied the following questions: (i) is there any evidence that subarctic black spruce populations, particularly those of the northern forest-tundra, are genetically depauperate compared to boreal forest populations, and (ii) is the strong ecoclimatic latitudinal gradient across the subarctic forest-tundra associated with a hierarchical organization of intraspecific gene diversity, reflecting the differential postglacial histories of the biome subzones? While the former question refers to the genetic consequences of migratory events that took place during the first part of the Holocene (milder climate), the latter refers to more recent fire-induced deforestation events that have occurred during the second part of the Holocene (cooler climate).

Materials and methods

Population sampling

Foliage was collected from 24 to 32 randomly selected mature black spruce trees, spaced at least 10 m apart, in nine populations distributed over a > 1000 km boreal forest–shrub tundra transect in northern Québec (Table 1). The six northernmost populations were selected following the examination of regional aerial photographs and airborne survey along 75°30′ W, allowing the partitioning of the different growth forms (arborescent or shrubby types) and forest cover fragmentation according to latitude, both indicating regional climatic conditions. Three closed-crown boreal populations farther south, for which genetic diversity patterns at ESTP loci have been shown previously to be representative of the boreal transcontinental range of the species (Perry & Bousquet 2001), were included in the analyses. Preliminary tests showed that gene diversity (mtDNA and ncDNA) of the Great Whale River population, located at the mapped limit of open-crown boreal forest and forest-tundra, was essentially indistinguishable from that of populations from the southern forest-tundra. Thus, this population was pooled with the Little Whale River and the Clearwater River populations to form the subarctic/southern forest-tundra region. Similarly, the Chavigny Lake population at the species' limit, a few kilometres north of the mapped limit of forest-tundra, was genetically indistinguishable from northern forest-tundra populations, and was pooled with the Minto Lake and the Innuksuac River populations to form the subarctic/northern forest-tundra region. The three resulting vegetation zones (boreal forest, southern forest-tundra and northern forest-tundra) contained three populations each and were used for regional comparisons of the gene diversity parameters.

In the northern stands with recruitment mainly by vegetative layering, we verified the absence of stem

connections between individuals, to avoid sampling the same genotype twice. At the northern end of the transect, the very last stunted trees forming the species' limit were carefully searched by helicopter. Twenty isolated putative clones were located. Because these clonal shrubs propagate horizontally (occupying up to 520 m^2) and tend to fragment over time (for up to 1800 years; Laberge *et al*. 2000), shoots belonging to the same genotype could not be recognized by visual inspection. Thus, foliage was collected from several ramets at the periphery and centre of each presumed clone, and only unique genotypes ($n = 28$) derived from ESTP markers were retained for the genetic diversity analyses.

DNA isolation and processing

STS mtDNA markers. DNA was isolated from ≈ 50 mg of needles using the CTAB method as described by Bousquet *et al*. (1990), with two additional chloroform–isoamyl alcohol (24:1) extractions. Primer pairs for three different sequence-tagged-site markers of noncoding plant mitochondrial genomic regions, the intron b/c of subunit 1 of NADH dehydrogenase gene (*nad1* intron b/c), the intron 1 of subunit 5 of NADH dehydrogenase gene (*nad5* intron 1) and the V1 region of the small ribosomal subunit of RNA gene (SSU *rRNA* V1 region), all harbouring polymorphisms in black spruce, were used in polymerase chain reactions (PCRs) or digestions following conditions previously described (Jaramillo-Correa *et al*. 2003). Amplified and digestion products were electrophoresed through polyacrylamide gels (8% agarose in TBE) for 4 h at 250 V and visualized under UV light after ethidium bromide staining. Haplotypes at these three mtDNA loci were scored as shown in Jaramillo-Correa *et al*. 2003.

ESTP ncDNA markers. DNA samples were amplified using PCR with primer pairs specific for nine ESTP loci (*Sb06*, *Sb08*, *Sb11*, *Sb24*, *Sb29*, *Sb31*, *Sb62*, *Sb70* and *Sb72*) revealing codominant alleles distinguished by insertion-deletion polymorphisms (Perry & Bousquet 1998). All these markers are located within transcribed but untranslated regions, except for *Sb29*, in a translated region. All primer sequences, putative gene identifications and amplification conditions are reported in Perry & Bousquet (1998). Total information per PCR was increased by duplexing sets of primers that did not have overlapping products (Perry & Bousquet 2001). Without the need for further purification, amplified products were electrophoresed through thin gels (2% agarose in TAE) for 6 h at 130 V and visualized under UV light after ethidium bromide staining. Genotypes at ESTP loci were scored as shown in Perry & Bousquet (1998).

Gene diversity analysis

Because of the circular structure of the mitochondrial genome and its small size (around 1000 kb long in conifers; Kumar *et al*. 1995) compared to its nuclear counterpart, most mtDNA loci should be linked and, consequently, mtDNA data should be treated as composite haplotypes. Thus, for every spruce sampled, haplotypes at the three mtDNA loci were considered simultaneously to delineate a single composite mitochondrial haplotype, refered to as mitotype. For the mitotype data set, diversity measures (except for the percentage of polymorphic loci) and statistics were calculated on mitotype frequencies, rather than allelic frequencies at individual gene loci.

We estimated genetic diversity for mitotypes, by the percentage of polymorphic loci (P), the number of mitotypes scored (N) and the effective 'heterozygosity' (H_E; Weir 1996). For nuclear ESTPs, we used the percentage of polymorphic loci (P), the mean number of alleles per locus (A), the total frequency of rare alleles (A_R; alleles with frequency ≤ 5% within sampled boreal forest populations being defined as rare), the average observed heterozygosity per locus (H_O), the average expected heterozygosity per locus (H_E, unbiased estimate for small sample sizes; Nei 1978) and the intrapopulation fixation index (f). For ncDNA ESTPs, Hardy–Weinberg equilibrium was tested with Fisher's exact test using Bonferroni correction for multiple tests. ANOVAs (Sokal & Rohlf 1981) with Bonferroni correction were used to test for differences in gene diversity parameters P, A, A_R, H_O and H_E among the three vegetation zones defined above.

Fixation indices were calculated for each ESTP locus following Weir & Cockerham (1984), where f, F and θ are analogous to Wright's (1951) F_{IS}, F_{IT} and F_{ST}, respectively. For haploid mtDNA data, no fixation index f could be computed and, consequently, no distinction was made between F and θ (Weir 1996). Confidence intervals for F-statistics overall values at ESTPs were estimated by bootstrapping over loci to obtain 1000 replicates. Since mitotypes were analysed as a single locus, testing the overall mitotypic θ value using this procedure was not possible, so a variance estimate was obtained by jackknifing over populations (Weir 1996). To test for isolation by distance (divergence due to drift and mutation), unbiased genetic distance measures (Nei 1978) were estimated for all population pairs for both data sets. The relationship between genetic and geographical distances (estimated from the geographical position of sampled populations using the great circle method; Legendre & Vaudor 1991) was estimated using Mantel's tests on both matrices. The significance of the correlation coefficients obtained was assessed using a Monte Carlo simulation with 1000 permutations. Similarly, to test for migration-drift equilibrium (divergence due to drift only), a matrix of coancestry coefficients (θ) for all population pairs was calculated and compared to the geographical distance matrix. Because some population pairs were fixed for identical mitotypes (see Results) and, hence, had undefined coancestry distances, this correlation

could not be estimated for the mitotype data set. For the two distance comparisons computed for ESTPs, Bonferroni correction ($\alpha' = 0.05/2 = 0.025$) was applied.

Finally, populations were regrouped by genetically contrasting regions (see Results) and average θ estimates within and among regions (boreal and subarctic) were calculated for both types of markers (θ_M for mtDNA markers and θ_N for ncDNA markers). Corresponding effective numbers of migrants per generation (*Nm*) were evaluated as indirect estimates of average levels of interpopulation gene flow relative to genetic drift under the infinite island model. For haploid mtDNA markers of maternal inheritance, $Nm_M = {}^1/_2\,(1/\theta_M - 1)$ (Takahata & Palumbi 1985) estimates the seed flow, i.e. the proportion of successfully establishing seeds coming from other populations. For diploid nuclear markers, $Nm_N = {}^1/_4\,(1/\theta_N - 1)$ (Slatkin 1985), and integrates the movement of genes both through successfully establishing seeds and successfully fertilizing pollen coming from other populations. To better assess the relative contribution of seeds and pollen to overall gene flow over time, pollen flow to seed flow ratios within and among regions were estimated from the average θ values for maternally and biparentally inherited markers following Ennos (1994). Statistical analyses were performed using the GDA program (Lewis & Zaykin 2001), except for Mantel's tests which were carried out using ARLEQUIN (Schneider *et al*. 2000).

Results

mtDNA markers

In all boreal and subarctic populations, polymorphism was observed for each mtDNA locus, with two allelic forms at each locus (Table 2). There were four mitotypes of a theoretical maximum of eight if recombination occurred among all the loci (Table 2). There was complete association between haplotypes at the *nad1* intron b/c and *nad5* intron 1 loci. However, association between these loci and SSU *rRNA* V1 region was incomplete. The two most divergent mitotypes (I and IV) could be considered as ancestral, with mitotypes II and III being derived from these after recombination. Sequencing of the three mtDNA markers used here has shown that single-locus haplotypes found in different mitotype configurations are homologous, and that the polymorphisms are caused by a few indels or duplications of small fragments [between 2 and 5 base pairs (bp)] in noncoding regions (Jaramillo-Correa *et al*. 2003).

The frequencies of each mitotype scored in the populations and corresponding vegetation zones sampled are shown in Fig. 1. Overall, mitotype I was the commonest mtDNA variant: Parc des Grands-Jardins and Saguenay River were the sole populations where another mitotype predominated. It is noteworthy that, within populations, mtDNA variation (both at individual loci and in mitotypes) was found exclusively in the southern boreal populations, where all loci were polymorphic and three to four mitotypes were present (Fig. 1 and Table 3). H_E within boreal forest

Table 2 Designation of the mitotypic variants with approximate allelic fragment sizes (bp)* detected at three polymorphic mtDNA loci examined in nine black spruce populations in boreal and subarctic Québec, Canada

	mtDNA locus		
Mitotype	*nad1* intron b/c	*nad5* intron 1	SSU *rRNA* V1 region
I	F	C	B_2
II	F	C	A_2
III	E	B	B_2
IV	E	B	A_2

*For *nad1*: E ≈ 230, 280, 400, 550 and 900 bp and F ≈ 230, 280, 370, 550 and 930 bp; for *nad5*: B ≈ 170, 180, 235, 290, 380 and 490 bp and C ≈ 170, 180, 250, 290, 380 and 490 bp and for SSU *rRNA*: A_2 = 320 bp and B_2 = 328 bp.
Nomenclature of alleles as in Jaramillo-Correa *et al*. (2003).

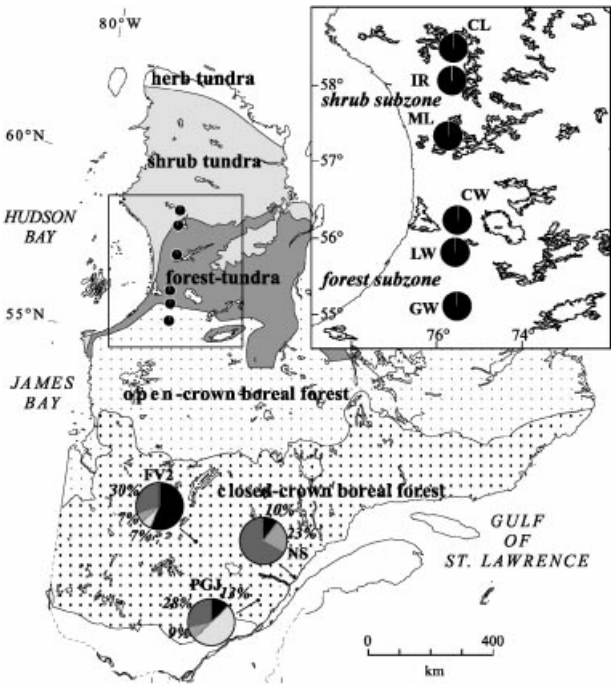

Fig. 1 Frequencies of mitotypes scored within nine black spruce populations sampled in different vegetation zones in Québec, Canada. PGJ, Parc des Grands-Jardins; NS, Saguenay River; FV2, Chibougamau Lake; GW, Great Whale River; LW, Little Whale River; CW, Clearwater River; ML, Minto Lake; IR, Innuksuac River and CL, Chavigny Lake. Portions of pie charts: black, mitotype I; light grey, mitotype II; medium grey, mitotype III; dark grey, mitotype IV. See Table 2 for definition of mitotypes.

Table 3 Summary of genetic diversity estimates obtained for four mitotypes and nine expressed sequence tag polymorphisms (ESTPs) of ncDNA in nine black spruce populations and associated vegetation zones in Québec, Canada

		Mitotypes			ncDNA ESTPs					
								Mean heterozygosity		
Vegetation zone	Population†	*P*	*N*	H_E‡	*P*	*A*	A_R	H_O	H_E	*f*
Boreal forest	PGJ	100.0	4	0.66	100.0	2.44	0.06	0.20	0.22	0.09
	NS	100.0	3	0.50	77.8	2.33	0.10	0.20	0.21	0.08
	FV2	100.0	4	0.59	100.0	2.67	0.24	0.27	0.27	−0.00
	Mean (SE)	100.0 (0)	3.7 (0.4)	0.58 (0.06)	92.6 (7.4)	2.48 (0.10)	0.13 (0.05)	0.22 (0.02)	0.23 (0.02)	0.05 (0.03)
Subarctic										
Southern FT§	GW	0	1	0	100.0	2.33	0.11	0.20	0.19	−0.02
	LW	0	1	0	100.0	2.56	0.23	0.20	0.20	0.00
	CW	0	1	0	77.8	2.22	0.15	0.25	0.21	−0.16*
	Mean (SE)	0 (0)	1 (0)	0 (0)	92.6 (7.4)	2.37 (0.10)	0.16 (0.04)	0.21 (0.02)	0.20 (0.01)	−0.06 (0.05)
Northern FT	ML	0	1	0	88.9	2.22	0.13	0.26	0.23	−0.15*
	IR	0	1	0	88.9	2.33	0.21	0.18	0.17	−0.10
	CL	0	1	0	88.9	2.22	0.11	0.21	0.20	−0.05
	Mean (SE)	0 (0)	1 (0)	0 (0)	88.9 (0)	2.26 (0.06)	0.15 (0.03)	0.22 (0.03)	0.20 (0.02)	−0.09 (0.03)**
Mean, all populations (SE)		33.3 (16.7)	1.89 (0.45)	0.19 (0.10)	91.4 (3.1)	2.37 (0.05)	0.15 (0.02)	0.22 (0.01)	0.21 (0.01)	−0.03 (0.03)

†See Table 1 for population locations and descriptions. *P*, percentage of polymorphic loci; *N*, number of mitotypes; *A*, number of alleles per locus; A_R, total frequency of rare alleles; H_O, observed heterozygosity; H_E, unbiased estimate of expected heterozygosity (Nei 1978); *f*, fixation index.

‡Effective 'heterozygosity' of mitotypes (Weir 1996).

§FT, forest-tundra: the fragmented ecotone between continuous boreal forest and treeless arctic tundra.

f values designated with * = 95% and ** = 99% significant, based on 1000 randomizations.

populations was 58% (Table 3). Further north, all the subarctic populations were fixed for mitotype I (Fig. 1 and $H_E = 0$; Table 3), and the majority of trees in the nearest boreal forest population (Chibougamau Lake; Fig. 1) also had this mitotype.

Levels of population genetic differentiation (Weir's θ) varied widely among mitotypes, with a high overall value of θ = 0.529 (Table 4). The estimated Nei's pairwise genetic distances derived from mitotypes varied extensively, ranging from 0 (among all forest-tundra populations) to 1.957 (among the Saguenay River boreal forest population and any forest-tundra population) with a mean value of 0.691. A significant positive association was detected between genetic and geographical distances (Mantel's test: $r = 0.748$, $P = 0.006$), suggesting strong isolation by distance between black spruce populations for mtDNA.

ncDNA markers

Between 77.8 and 100.0% of ESTP ncDNA loci were polymorphic within populations. The overall *P* value was 91.4%, and the mean number of alleles per locus (*A*) ranged from 2.2 to 2.7 (average = 2.4; Table 3). Compared to previous studies (Perry & Bousquet 1998, 2001), no new alleles were found in the populations investigated. The percentage of polymorphic loci and mean number of alleles per locus did not differ between the three vegetation zones (ANOVAs: $P = 0.885$ and 0.252, respectively). Five ncDNA alleles had frequencies lower than 5% in the boreal forest populations (*Sb06–609*, *Sb08–634*, *Sb29–580*, *Sb70–404* and *Sb72–515*). The total frequency of such rare alleles (A_R) per population was not significantly reduced in the northern forest-tundra populations compared to that of the southern forest-tundra or the boreal forest populations (Table 3; ANOVA: $P = 0.895$). Within-population observed heterozygosities (H_O) were generally slightly higher than expected heterozygosities (H_E), with average values of 0.22 and 0.21, respectively. Neither heterozygosity estimates differed between vegetation zones (ANOVAs: $P = 0.971$ and 0.257, respectively). By Fisher's exact tests with Bonferroni correction, all individual ESTP loci in all populations were found to be in Hardy–Weinberg equilibrium. However, most within-population fixation indices (*f*) were slightly negative, with a range of –0.16–0.09, indicating a slight excess of heterozygotes in some forest-tundra populations (Table 3).

Intrapopulation fixation indices (*f*) for individual loci ranged from –0.083 to 0.241 with an overall nonsignificant

Table 4 *F*-statistics showing gene diversity and population structure estimated for four mitotypes and nine expressed sequence tag polymorphisms (ESTPs) of ncDNA surveyed in nine black spruce populations in boreal and subarctic Québec, Canada

DNA markers	*f*	*F*	θ
Mitotypes*	—	—	0.529
SE†	—	—	0.035
ncDNA ESTPs			
Sb06	0.027	0.026	−0.001
Sb08	−0.029	−0.010	0.018
Sb11	−0.020	0.038	0.057
Sb24	−0.024	0.004	0.027
Sb29	−0.062	−0.057	0.004
Sb31	−0.083	−0.076	0.006
Sb62	−0.033	−0.021	0.012
Sb70	0.035	0.037	0.002
Sb72	0.241	0.239	−0.003
Overall	−0.025	−0.011	0.014
Upper bound‡	0.004	0.018	0.022
Lower bound	−0.043	−0.032	0.007

f, fixation index within populations; *F*, fixation index in the total population; θ, coancestry among populations, estimated as described by Weir & Cockerham (1984).
*For haploid data, no distinction is made between *F* and θ (Weir 1996).
†Estimated by a jackknife resampling over populations.
‡Upper and lower bounds are of 95% confidence intervals based on 1000 bootstrap resamplings over loci.

value of −0.025, indicating no apparent inbreeding (Table 4). Coancestry coefficients among populations (θ) varied from −0.003 to 0.057 and indicated that, overall, only 1.4% (and no more than 2.2%) of the total nuclear gene diversity observed was among populations. Although small, this amount of among-population differentiation was statistically significant.

Nei's pairwise population multilocus genetic distances were low, ranging from 0 to 0.011 with a mean value of 0.003. Based on Mantel's test, a significant positive relationship was detected between genetic and geographical distances ($r = 0.416$, $P = 0.013$). An identical positive association was detected between pairwise population multilocus θ values and geographical distances (Mantel's test: $r = 0.417$, $P = 0.013$), thus confirming the evidence for isolation suggested by the overall θ value for nuclear genes.

Regional population structure and gene flow: mtDNA vs. ncDNA markers

Mean levels of regional population structure revealed by mtDNA and ncDNA markers (θ_M and θ_N) were compared for the same nine populations regrouped in two genetically contrasting regions: boreal and subarctic, regardless of the forest-tundra subzone (which had no effect on any genetic parameter, see above). The overall among-population differentiation estimated was more than one order of magnitude higher for mitotypes than for ncDNA ESTPs (Table 5). For mitotypes, regional differences contributed more to the overall population structure than did within-region differences, presumably because all the subarctic populations were fixed for a single mitotype. In contrast, for ncDNA markers, various levels of comparisons (overall, within the boreal region or between boreal and subarctic regions) led to almost identical amounts of population structure ($\theta_N = 0.014$, 0.013 and 0.012, respectively; Table 5).

Table 5 Population differentiation* and gene flow† estimated from mitotypes and expressed sequence tag polymorphisms (ESTPs) of ncDNA surveyed in nine black spruce populations and associated regions in Québec, Canada

	Mitotypes		ncDNA ESTPs		
Grouping of populations	θ_M	Nm_M	θ_N	Nm_N	Pollen flow/ seed flow
Among populations (overall)	0.529 (0.035)‡	0.4	0.014 (0.007; 0.022)§	17.6	77.1
Among boreal populations	0.256 (0.011)	1.5	0.013 (0.005; 0.025)	19.0	24.1
Among subarctic populations	—¶	—	0.007 (–0.002; 0.021)	35.5	—
Between regions	0.573 (—)	0.4	0.012 (–0.002; 0.025)	20.6	108.5

*θ, coancestry among populations as described by Weir & Cockerham (1984).
†*Nm*, effective number of migrants per generation (Slatkin 1985; Takahata & Palumbi 1985) and pollen flow/seed flow ratio (Ennos 1994).
‡SE based on a jackknife resampling over populations (could not be estimated from less than three groups; Lewis & Zaykin 2001).
§Lower and upper limits of 95% confidence intervals based on 1000 bootstrap resamplings over loci.
¶Undefined coancestry distance due to the fixation of one mitotype.

Because most of the nuclear gene diversity was present within each population, such a hierarchical analysis with regional structuring did not provide much more valuable information than a population-based analysis.

The low dispersal ability of black spruce seeds compared to pollen was clearly reflected in the effective numbers of migrants per generation estimated from the θ_M and θ_N values derived from mtDNA and ncDNA markers, respectively (Nm_M vs. Nm_N; Table 5). Seed-borne mtDNA markers were characterized by low levels of interpopulation gene flow ($Nm_M < 2$), whereas very large levels of gene exchange ($Nm_N > 17$) were obtained for ncDNA markers dispersed through both seeds and pollen. Although seemingly small, gene flow by seed movement was more important among the nearby boreal forest populations than among boreal and subarctic regions ($Nm_M = 1.5$ vs. 0.4, respectively). In contrast, nuclear alleles seemed to have dispersed as efficiently among the nearby boreal forest populations than among remote regions ($Nm_N = 19.0$ vs. 20.6, respectively). Correspondingly, among boreal forest populations, the contribution of pollen to gene flow over time was 24 times more important than the contribution of seeds, while the same pollen flow/seed flow ratio reached 109 among the boreal and subarctic regions (Table 5).

Discussion

Pattern of postglacial migration

Because a relatively short time span has elapsed since the late deglaciation of the Hudson Bay region (≈ 6000 years), it was expected that subarctic black spruce forests would have retained the genetic imprint of a rapid postglacial spread. Contrary to ncDNA markers, our survey of three mtDNA STS markers revealed a striking south–north gene discontinuity between boreal forest (polymorphic) and subarctic (monomorphic) spruce populations, a high level of population differentiation (θ_M) and a strong isolation by distance, indicative of a presumed founder event. The transition zone from a polymorphic to a monomorphic mitochondrial pattern must occur somewhere between the two main regions surveyed, in the open-crown boreal forest or lichen–woodland. Owing to the availability of recently tested mtDNA markers, this is the first time that such a reduction in gene diversity has been recorded in black spruce, for which intrapopulation gene diversity is typically very high (Isabel *et al*. 1995; Perry & Bousquet 2001). South–north genetic disjunctions with fixation of an organelle haplotype in northernmost populations have been reported for various plant species of the American Pacific Northwest (Soltis *et al*. 1997) and for *Pinus sylvestris* in Europe (Sinclair *et al*. 1999). Similar east–west genetic discontinuities have been found in coniferous populations across the mountain ranges of the Alps (Gugerli *et al*. 2001) and the Andes (Marchelli *et al*. 1998).

A migration process involving 'leading edge' expanding populations, in addition to an advancing main front allowing short-distance step-by-step diffusion, would be compatible with the conservation of a single mitotypic variant (mitotype I) in the subartic forest-tundra populations. Based on tree macrofossil evidence, average tree migration rates of 0.2–0.4 km/year were obtained for *Picea* taxa in eastern Canada (Payette *et al*. 2002). These represent mean annual apparent rates of spread between two widely distant sampling sites (one in a source area south of the late Laurentidian ice border and one at the tree line). Since trees must reach the age of sexual maturity (≈ 25 years old; Morneau & Payette 1989) before they could disperse seeds, minimal dispersal distances should reach 5–10 km per tree generation, which seems too high for a step-by-step colonization (see also Petit *et al*. 1997; Walter & Epperson 2001). Outliers established from long-distance seed transport must be invoked to account for both these high migration rates as well as the observed mitochondrial differentiation of subarctic black spruce populations. Black spruce seeds could presumably be dispersed by strong south winds, particularly over the snowpack during the autumn–winter season (Ritchie 1987), and redistributed by snow melting in the spring. Furthermore, the presence of isolated, unevenly aged black spruce clones a few kilometres north of the tree line, and the absence of charred remains of former forests in surrounding open sites (Payette & Gagnon 1985; Lavoie & Payette 1996) suggest that long-distance seed dispersal events, with tree line trees as potential maternal sources, have continued to occur during mild periods of the Holocene. Consistent with these ecological data, the mitochondrial pattern documented here gives further support to theoretical simulations showing that rare long-distance dispersal events, occurring at frequencies $\approx 10^{-5}$ times lower than short-distance dispersal events (Shigesada *et al*. 1995), might disproportionately influence many aspects of population dynamics, including rates of geographical spread (Clark *et al*. 1998) and genetic structure (Hewitt 1996).

At some time in the past, the reduction of gene diversity through migration-induced bottlenecks must have affected the entire genome of black spruce. However, a lack of genetic diversity was observed in mtDNA only in the subarctic forest-tundra, and not for ncDNA ESTPs markers. All populations, including the northernmost site, had about the same allelic richness (A) and frequency of rare alleles (A_R; Table 3), which are usually very sensitive to bottlenecks (Widmer & Lexer 2001).

Seed vs. pollen-mediated gene flow

The overall structure of black spruce mtDNA diversity between the southern and northern components of the

natural range emphasizes the inertia of maternally inherited mtDNA to be quickly homogenized, hence retaining effectively the imprint of postglacial colonization. The limited amount of maternal gene flow during the Holocene, connecting marginal black spruce populations and boreal forest populations, can explain why mtDNA diversity still reflects past dissemination patterns. With only around one effective migrant per generation ($Nm_M < 2$; Table 5), seed-mediated gene flow has not been high enough to overcome the substantial regional differentiation through founder events.

In contrast, dispersal through both seeds and pollen ($Nm_N \approx 20$ effective migrants per generation between boreal and subarctic regions; Table 5) has probably blurred the effects of an original founder event by carrying missing nuclear alleles from the southern boreal forest pool to the newly colonized area, up into the northernmost populations. In those populations currently maintained by layering, high within-population gene diversity is observed at ncDNA loci, with essentially no population differentiation. Similar to the present study, black spruce clones from the tree line (Laberge *et al*. 2000) showed no loss of infrequent random amplified polymorphic DNA (RAPD) fingerprints relative to boreal forest trees (Isabel *et al*. 1995). Such effective gene flow towards the north must have been favoured by the mild climatic conditions prevailing during the mid-Holocene (before 3000 years BP), a period more favourable to sexual reproduction than current conditions (Payette & Gagnon 1985). Thus, between the time of arrival of black spruce at the tree line (4500 years BP) and the end of the early Holocene milder climatic conditions (3000 years BP), 1500 years appear to have been sufficient for replenishing the diversity of nuclear DNA of black spruce populations from the northern forest-tundra to levels equivalent to those currently observed in populations from the boreal forest. In terms of number of tree generations, this represents a remarkably short period. In spite of reduced reproductive success induced by subsequently harsher climatic conditions, the northernmost black spruce populations appear to have maintained their genetic diversity by means of individual survival through repeated cycles of layering, for up to 1800 years in some cases (Laberge *et al*. 2000). As such, the capacity of lateral expansion through layering constitutes a remarkable adaptation process allowing long-term maintenance of populations and genetic diversity, even in absence of any significant recruitment since the initial postfire seedling establishment (see also Jelinski & Cheliak 1992 for *Populus tremuloides*).

Black spruce pollen seems to be characterized by nearly unlimited dispersion distances at the spatial and temporal scales considered here, similarly to ponderosa pine (Latta *et al*. 1998) and other wind-pollinated conifers. High nuclear gene flow among populations could be inferred from other studies of black spruce from the boreal forest, using allozymes ($Nm_N \approx 25$, Boyle & Morgenstern 1987; $Nm_N \approx 30$, Isabel *et al*. 1995) and DNA markers such as RAPDs ($Nm_N \approx 10$; Isabel *et al*. 1995) or ESTPs ($Nm_N \approx 40$; Perry & Bousquet 2001). The pollen flow/seed flow ratio of 24 estimated in this study among black spruce populations from the boreal forest is also comparable to the values obtained for wind-pollinated *Pinus* taxa (Ennos 1994). However, the pollen flow/seed flow of 109 estimated between boreal and subarctic black spruce greatly exceeds these values and is more comparable to the ratio estimated for barochorous species such as oaks (Ennos 1994).

If it is not accompanied by a substantial modification of fire activity leading to greater forest fragmentation, the persistence of the 20th-century warming trend in the decades to come should allow shrubby spruce stands of the northern forest-tundra (i) to recover an arborescent growth form by developping a main bole and (ii) to produce viable seeds (Payette *et al*. 2001). Present findings predict that populations that will presumably expand into adjacent tundra sites (Gamache & Payette, in prep.) will be genetically well diversified, harbouring almost all nuclear allelic forms present throughout the circumboreal range of the species. Given a sufficiently long period of mild climatic conditions, more mitotypic variants from the boreal forest should move northwards and incorporate more or less rapidly into the subarctic black spruce cover, depending on the frequency of chance long-distance dispersion events and competition from already established forest or tundra vegetation.

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