The structure of genetic diversity in Engelmann spruce and a comparison with blue spruce

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Abstract: Genetic diversity and genetic structure in Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) were interpreted with respect to the effects of glacial and interglacial displacement and compared with patterns in blue spruce (*Picea pungens* Engelm.), which occupies a range well south of the last glacial front. On average, Engelmann spruce populations were polymorphic at 80% of 24 isozyme loci, with 2.4 alleles per locus and expected heterozygosity of 0.255. The respective means for four populations of blue spruce were 42.7%, 1.6 alleles, and 0.138. Of total diversity, 14.7% was among populations in Engelmann spruce and 8.6% in blue spruce. In Engelmann spruce, numbers of alleles increased from south to north. Recent bottlenecks were indicated in the three southernmost populations of Engelmann spruce and in the northernmost population of blue spruce. Cluster analysis divided Engelmann spruce into a northern group and a Southwestern group; however, populations from Utah were distributed among both clusters. Genetic distance was correlated with geographic distance between northern populations but not between Southwestern populations, where drift predominated over gene flow. In two Engelmann spruce populations from Utah, multilocus outcrossing rates were 0.951 (±0.016) and 0.940 (±0.071). At Flys Peak, Arizona, the southernmost location of Engelmann spruce, outcrossing was also high, 0.899 (±0.017). Thus, inbreeding coefficients observed for parental (–0.104 to 0.047) and filial (0.011 to 0.026) generations were low. Although Engelmann spruce seemed genetically robust, the evidence of bottlenecks in the southern extreme of its range suggested future problems in an era of global warming.

Key words: diversity, selfing, genetic drift, bottlenecks, climate change.

Résumé : Les auteurs ont interprété la diversité génétique et la structure génétique de l'épinette d'Engelmann (*Picea engelmannii* Parry ex Engelm.) en relation avec les effets des déplacements glaciaires et interglaciaires, et les ont comparées avec les patrons de l'épinette bleue (*Picea pungens* Engelm.), qui occupe une région bien au sud du dernier front glaciaire. En moyenne, les populations de l'épinette d'Engelmann sont polymorphes chez 80 % de 24 loci isozymiques, avec 2.4 allèles par locus et une hétérozygocité attendue de 0,255. Ces moyennes respectives, chez quatre populations d'épinette bleue, sont de 42,7 %, 1,6 allèles, et 0,138. De la diversité totale, 14,7 % se retrouve parmi les populations de l'épinette d'Engelmann, et 8,6 % parmi celles de l'épinette bleue. Chez l'épinette d'Engelmann, les nombres d'allèles augmentent du sud au nord. On observe des goulots d'étranglements récents dans les trois populations les plus méridionales de l'épinette d'Engelmann et dans la population la plus nordique de l'épinette bleue. L'analyse par regroupement divise l'épinette d'Engelmann en un groupe nordique et un groupe du sud-ouest; cependant les populations de l'Utah sont présentes dans les deux regroupements. La distance génétique est corrélée avec les distances géographiques entre les populations du nord, mais non entre les populations du sud-ouest, où la dérive a prédominé sur le flux génétique. Chez deux populations de l'Utah, les taux de croisements externes à multilocus sont de 0,951 (±0,016) et 0,940 (±0,071). À Flys Peak, en Arizona, localité la plus méridionale de l'epinette d'Engelmann, le croisement externe est également important, 0,899 (±0,017). Par conséquent, les coefficients d'autofécondation observés pour les générations parentales (–0,104 à 0,047) et les générations filiales (0,011 à 0,026) sont faibles . Bien que l'épinette d'Engelmann semble génétiquement robuste, l'évidence de goulots d'étranglement à l'extrémité méridionale de son aire de distribution annonce des problèmes à venir dans une ère de réchauffement global.

Mots-clés : diversité, autofécondation, dérive génétique, goulots d'étranglement, changements climatiques.

[Traduit par la Rédaction]

Introduction

Species of the temperate forest have been displaced during the climatic changes that lead to both glacial and interglacial periods. The effects on their genetic structure are largely unknown. Some regionally distributed species, like pitch pine (*Pinus rigida* Mill.) so recently colonized their current range and have such extensive gene flow that they show little structure (Guries and Ledig 1982). The structure of less vagile species, like Coulter pine (*Pinus coulteri* D. Don), reflects founder events during dispersal northward in interglacial periods (Ledig 2000). However, except for re-

Received 17 February 2006. Published on the NRC Research Press Web site at http://canjbot.nrc.ca on 1 March 2007.

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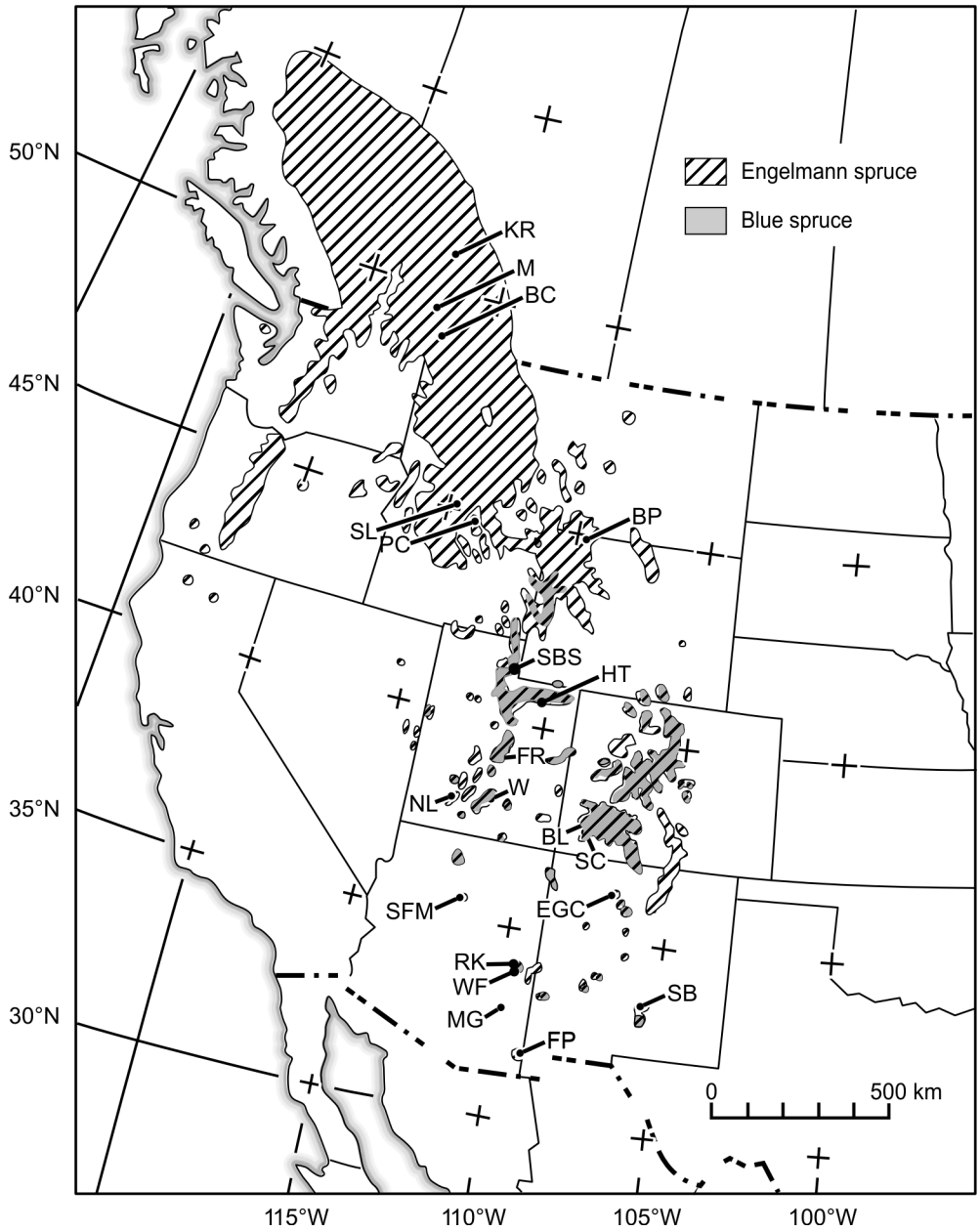
 doi:10.1139/B06-106

Fig. 1. The ranges (after Little, 1971) of Engelmann spruce (*Picea engelmannii*) and blue spruce (*Picea pungens*) and the location of populations included in isozyme analyses. Engelmann spruce: KR, Kootenay River; M, Moyie; BC, Bluebird Creek; SL, Summit Lake; BP, Beartooth Pass; PC, Panther Creek; SBS, Six Bit Spring; HT, Highline Trailhead; FR, Ferron Reservoir; BL, Barlow Lake; NL, Navajo Lake; GC, East Gavilan Canyon; SFM, San Francisco Mountain; SB, Sierra Blanca; MG, Mount Graham; FP, Flys Peak. Blue spruce: W, Wildcat; SC, Scotch Creek; RK, Rudd Knoll; WF, West Fork.



lictual, narrow endemics, and regionally distributed species, few rangewide estimates of genetic diversity or genetic structure exist for any spruce (*Picea* L.).

Engelmann spruce (*Picea engelmannii* Parry ex Engelm.) is a widely distributed species whose range extends over nearly 26 degrees of latitude, from British Columbia almost to Mexico (Fig. 1). It is subalpine in distribution and is a major component of high-elevation Rocky Mountain forests (Alexander and Shepperd 1990). It extends into the eastern Great Basin and down the east slope of the Cascade Range from British Columbia to northern California. It is common in British Columbia and the northern United States, but its distribution becomes increasingly fragmented in the southern ranges of the Central Cordillera.

Engelmann spruce has considerable adaptive variation over its range, as demonstrated in common garden studies (Rehfeldt 1994, 2004), but there are no comparable studies for presumably neutral markers, such as isozymes. Some studies have reported isozyme diversity on local scales (e.g., with relation to microenvironment), but no study approaches a rangewide survey.

Engelmann spruce is sympatric with blue spruce (*Picea pungens* Engelm.) on many of the high mountain ranges from Wyoming southward, and the two species are some-

times difficult to distinguish in the field (Hanover 1975). However, they can be separated by needle anatomy and by isozyme markers (Weng and Jackson 2000; Ledig et al. 2004), and there is no evidence of natural hybridization (Mitton and Andalora 1981; Ernst et al. 1990). Blue spruce's range in southern Idaho, Wyoming, Utah, Arizona, and New Mexico is at least as fragmented as that of Engelmann spruce. However, blue spruce is primarily a montane species and tends to grow at lower elevations than Engelmann spruce. Blue spruce withstands drought and high insolation better than the subalpine Engelmann spruce (Fechner 1990), but is not as rich as Engelmann spruce in adaptive variation (Rehfeldt 2004).

During the last Pleistocene glacial maximum, Engelmann spruce occurred at lower elevations than now and, thus, was less fragmented than is presently the case (Hamrick et al. 1994). We hypothesize that population size would have been reduced during the current interglacial and that the range of Engelmann spruce shrank in the south as the species was forced to disperse to high elevations. Similar fragmentation and isolation might be expected under projections of global warming due to the release of greenhouse gases. With warming temperatures, bottlenecks may occur and, in fact, may already have occurred during the Xerothermic, a period during the present interglacial that was warmer than the current climate.

By contrast, blue spruce can disperse upward in elevation with less danger of being "pushed off the peaks". Although primarily interested in what isozyme markers can tell us about genetic structure in Engelmann spruce, a comparison with blue spruce is of interest with respect to the effects of warming climate. We would expect the subalpine species, Engelmann spruce, to have suffered greater bottlenecks than the montane blue spruce during late Pleistocene and Holocene warming.

The genetic structure of Engelmann spruce cannot be understood without reference to white spruce (*Picea glauca* (Moench) Voss). Engelmann spruce and white spruce hybridize in British Columbia and Alberta (Roche 1969, 1970; Rajora and Dancik 2000). Most distinguishing morphological characteristics show clinal intergradation along elevational gradients because hybrids occupy the intermediate ecological niche between low-elevation white spruce and high-elevation Engelmann spruce (Roche 1969). The colonization of British Columbia by white spruce is relatively recent; the Cordilleran Ice Sheet covered much of British Columbia as recently as 14 000 years B.P. (Adams and Faure 1997). Because white spruce was pushed almost entirely south of its present distribution during glacial periods, except for scattered relicts (Nienstaedt and Zasada 1990), phantom hybridization (i.e., the occurrence of hybrids where one or both of the parental species are absent) might complicate investigations of Engelmann spruce in areas where white spruce is not currently native. Relict outliers of white spruce remain still in the Black Hills, South Dakota, and in central Montana (Little 1971). S.T. Jackson (personal communication, 2005) observed white spruce in the Big Horn Mountains, Wyoming, and introgressed hybrids in southern Wyoming and northern Colorado, apparently outside the mapped range of white spruce.

Because of hybridization, Engelmann spruce is expected to have much genetic variation (Fowler and Roche 1977), and we tested that hypothesis using putatively neutral isozyme markers. We predicted that the structure of variation would be shaped not only by an increased variation in Canada and the northern United States as a result of current or phantom hybridization and introgression with white spruce, but by loss of diversity because of fragmentation and reduction in population size in the south. For comparison we included blue spruce, which, to a high degree, is reproductively isolated in the genus and, therefore, hybridization is not a complication.

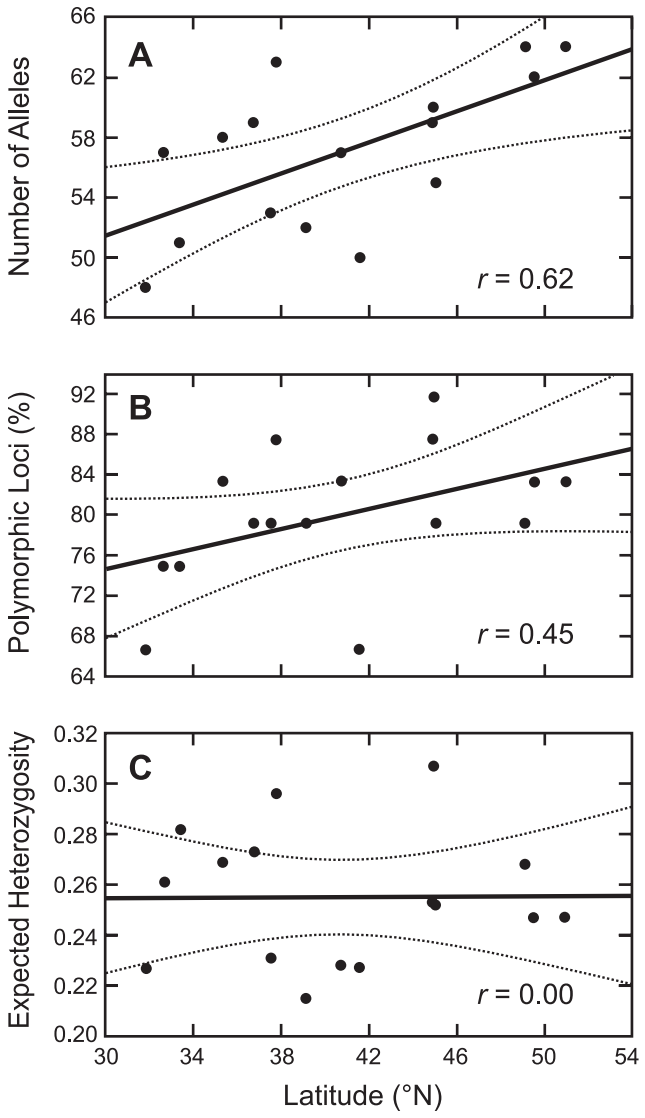


Fig. 2. Relationship between latitude in 16 populations of Engelmann spruce (*Picea engelmannii*) and total number of alleles (A), percentage polymorphic loci, *P* (B), and expected heterozygosity, H_e (C), at 24 isozyme loci. Dashed lines indicate the 95% confidence bands.

Materials and methods

Samples

Sixteen populations of Engelmann spruce, which covered most of the species' range from British Columbia to Ari-

Table 1. Englemann (*Picea engelmannii*) and blue (*Picea pungens*) spruce populations included in isozyme studies and mean number of genomes (*N*) sampled per locus.

Location (locality, state or province)	*N*	Latitude	Longitude	Elevation (m)
Engelmann spruce				
Kootenay River, Nelson Forest Region, British Columbia	52.0	50°58′N	116°16′W	1830
Moyie, Nelson Forest Region, British Columbia	51.9	49°32′N	116°06′W	1700
Bluebird Creek, Nelson Forest Region, British Columbia	51.9	49°08′N	116°53′W	1430
Summit Lake, Payette NF, Idaho	47.3	45°03′N	115°55′W	2075
Beartooth Pass, Shoshone NF, Wyoming	46.9	44°56′N	109°31′W	2985
Panther Creek, Salmon NF, Idaho	52.8	44°53′N	114°18′W	2135
Six Bit Spring, Cache NF, Utah	47.9	41°35′N	111°26′W	2560
Highline Trailhead, Wasatch NF, Utah	44.0	40°44′N	110°52′W	3180
Ferrron Reservoir, Manti-Lasal NF, Utah	40.0	39°08′N	111°27′W	2930
Barlow Lake, San Juan NF, Colorado	48.0	37°45′N	107°59′W	2955
Navajo Lake, Dixie NF, Utah	52.8	37°32′N	112°46′W	2865
East Gavilan Canyon, Carson NF, New Mexico	48.3	36°44′N	106°18′W	2970
San Francisco Mountain, Coconino NF, Arizona	48.0	35°20′N	111°42′W	2775
Sierra Blanca, Lincoln NF, New Mexico	52.2	33°24′N	105°47′W	2925
Mount Graham, Coronado NF, Arizona	156.8	32°40′N	109°52′W	3018
Flys Peak, Coronado NF, Arizona	317.5	31°52′N	109°17′W	2866
Blue spruce				
Wildcat Guard Station, Dixie NF, Utah	48.4	38°07′N	111°20′W	2650
Scotch Creek, San Juan NF, Colorado	48.3	37°39′N	108°03′W	2620
Rudd Knoll, Apache-Sitgreaves NF, Arizona	47.9	33°58′N	109°22′W	2745
West Fork Little Colorado River, Apache-Sitgreaves NF, Arizona	47.8	33°57′N	109°30′W	2745

Note: NF, National Forest (United States).

zona, and four populations of blue spruce were used for isozyme electrophoresis. Barry Jaquish (Kalamalka Forestry Centre, British Columbia Ministry of Forests and Range) supplied bulked seed from three populations of Engelmann spruce from above 1430 m in British Columbia. Below 1300 m, hybridization with white spruce is common, so the populations in our sample represented some of the "purest" Engelmann spruce available in British Columbia. Each lot was a mix of seeds from 15 to 40 trees. Cones were collected and seed was extracted by staff of the British Columbia Ministry of Forests and Range from 1974 to 1980.

Gerald E. Rehfeldt (formerly of the Intermountain Research Station, USDA Forest Service) provided seeds from 13 populations extending from Idaho to the southernmost population of Engelmann spruce near Flys Peak, Chiricahua Mountains, Arizona. Seeds from Flys Peak and from two other locations, Highline Trailhead and Ferron Reservoir, Utah, were maintained separate by mother-tree. The other 10 samples were bulks from at least 25 trees and up to, perhaps, 75 (G.E. Rehfeldt, personal communication). Cones were collected in 1977 for the three northernmost populations in Idaho and Wyoming and from 1985 to 1988 for the populations from Colorado, Utah, Arizona, and New Mexico, except for Flys Peak and Mount Graham, which were sampled in 1990. Rehfeldt also supplied bulked seed collections from four blue spruce populations sampled from 1986 to 1988. Sampling procedures were described in Rehfeldt (1994). Table 1 provides details on location of the sampled populations. All seedlots were stored below freezing until used for this study.

Electrophoresis

Seeds were stratified and germinated in Petri dishes at the Institute of Forest Genetics, Placerville, California. When radicles emerged, megagametophytes and embryos were dissected from the seeds, separated, and extracted in buffer solutions for isozyme electrophoresis. Electrophoresis was conducted over the period 1998 to 2005. Co-electrophoresis of samples ensured that allele nomenclature was consistent throughout the study.

We sampled megagametophytes for most of the analyses. In spruces, the nutritive tissue of the seed is a haploid megagametophyte that gives rise to, and is of the same genotype as, the egg. We analyzed about 50 megagametophytes each for 13 populations of Engelmann spruce and four of blue spruce that were represented by bulk lots. For Highline Trailhead, we deduced genotypes for the parent trees using 50 megagametophytes from each of 22 trees (N = 44 genomes), and for Ferron Reservoir, from five megagametophytes from each of 20 trees (N = 40). Because only six trees were represented in the sample from Flys Peak, we estimated population allele frequencies by running about 60 megagametophyte and embryo pairs from each tree and deducing the pollen genotypes (N = 360) by subtracting the maternal contribution to the embryo, except for the *Got3* and *Mdh6* loci, which did not resolve well in embryos. Allele frequencies for *Got3* and *Mdh6* were estimated from the genotypes for the six trees in the sample (N = 12 genomes).

We used techniques of starch gel electrophoresis based on the laboratory manual of Conkle et al. (1982) to assay enzyme systems. In the megagametophytes, we were able to consistently score 24 presumptive loci in 13 enzyme systems in all 20 populations. We interpreted the number of loci and alleles by drawing on the experience gained in our laboratory from studies of allozymes of other conifer species, including spruces (Conkle 1981; Ledig et al. 1997, 2000,

Fig. 3. Latitudinal variation in selected allele frequencies at 10 loci in 16 Engelmann spruce (*Picea engelmannii*) populations. (A) *Fest*4. (B) *Idh1*1. (C) *Idh2*1. (D) *Mdh1*1. (E) *Mdh2*1. (F) *Pgi1*1. (G) *Pgi2*1. (H) *Skd1*8. (I) *Skd2*1. (J) *Tpi1*1. Dashed lines indicate the 95% confidence bands.

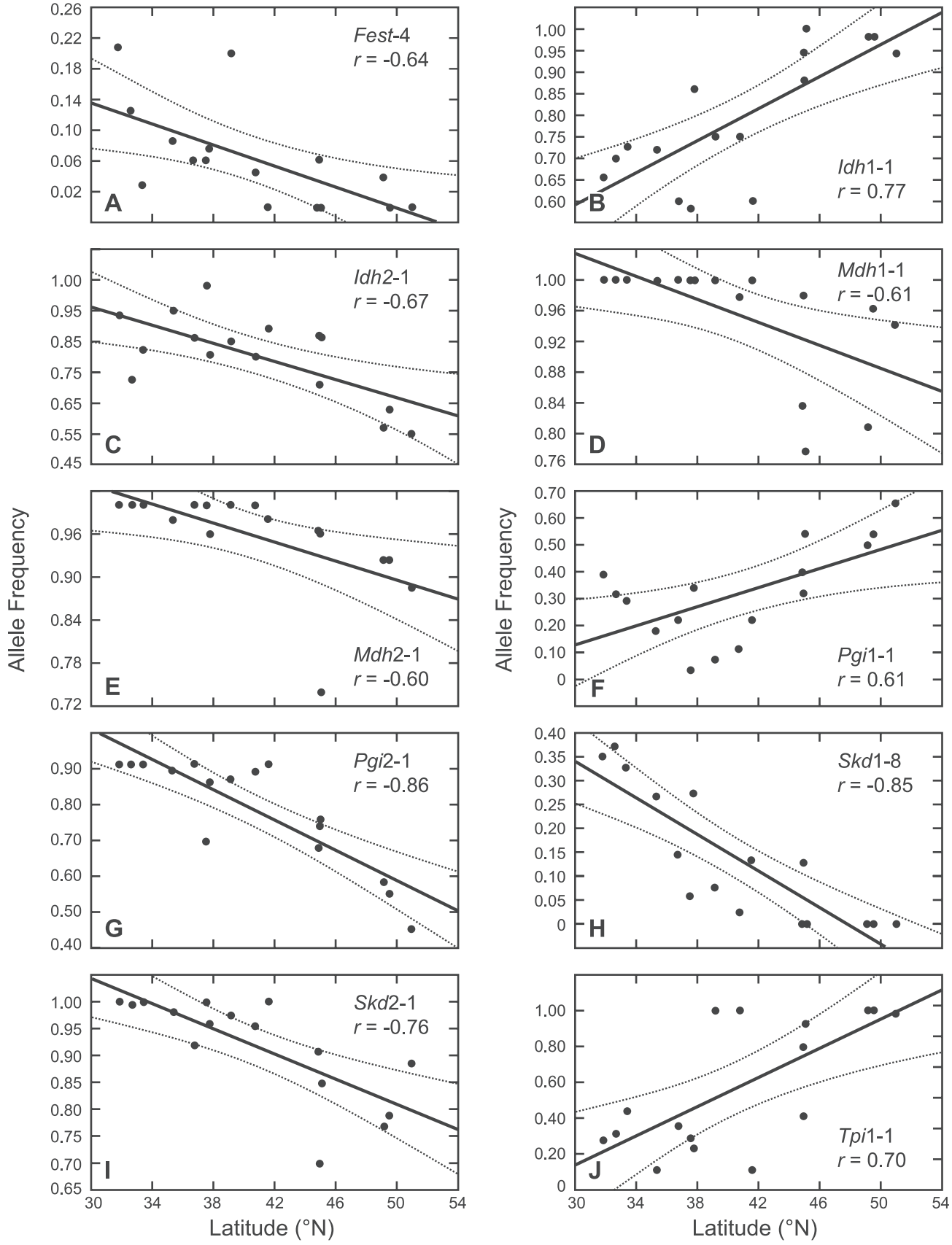


Fig. 4. Relationship between Nei's unbiased genetic distance, *D*, and geographic distance between pairs of 16 Engelmann spruce (*Picea engelmannii*) populations. Dashed lines indicate the 95% confidence band.

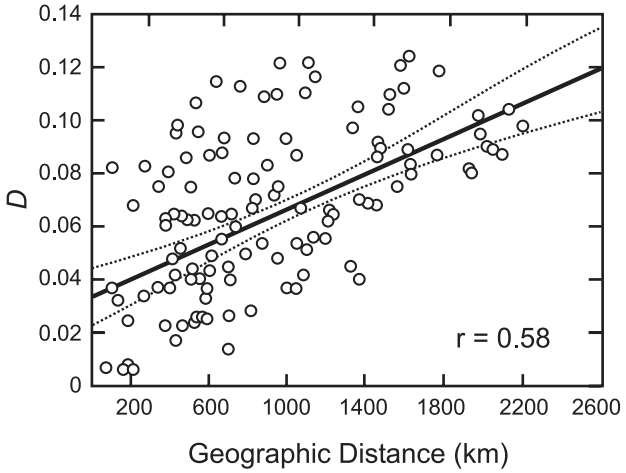


Fig. 5. Cladogram for 16 Engelmann spruce (*Picea engelmannii*) populations constructed from Nei's genetic distance with the UPGMA joining method and rooted with blue spruce (*Picea pungens*). Numbers at the nodes are the number of times a node occurred in 1000 bootstrap-generated data sets.

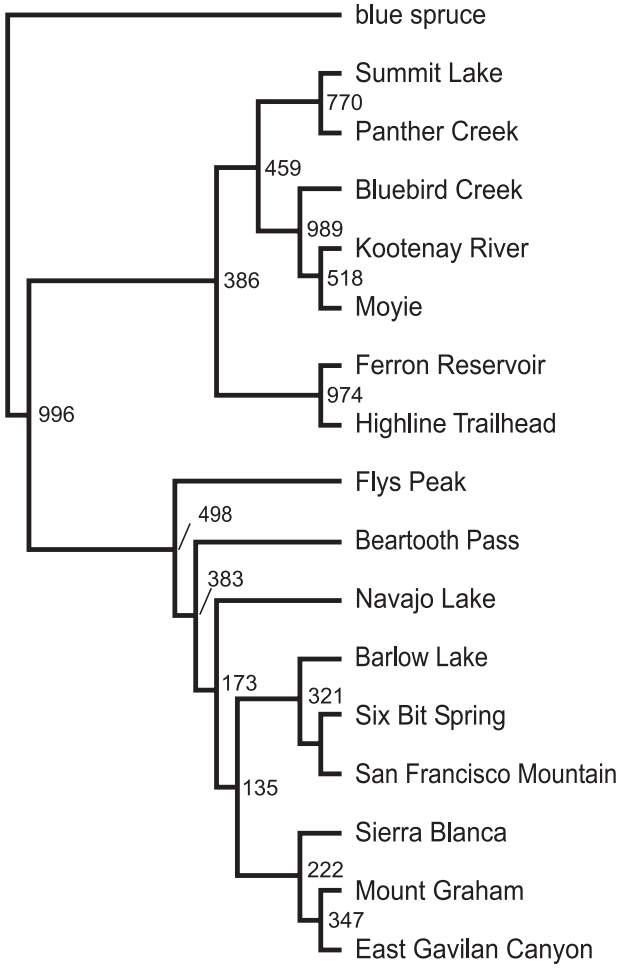
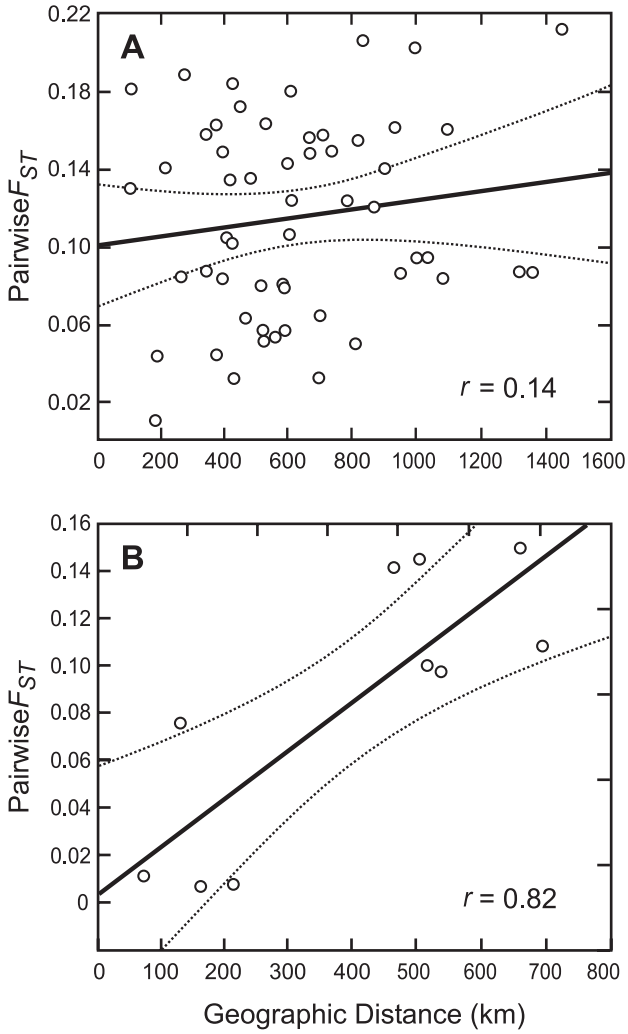


Fig. 6. Relationships between pairwise F_{ST} and geographic distances for 11 populations of Engelmann spruce from the Southwest (including Highline Trailhead and Ferron Reservoir) (A) and for five populations from British Columbia and Idaho (B). Dashed lines indicate the 95% confidence bands.



2002, 2004). Samples of red pine (*Pinus resinosa* Ait.), which are almost invariably homozygous at all loci, were included as standards on each gel to aid interpretation.

Outcrossing rates were estimated for Highline Trailhead, Ferron Reservoir, and Flys Peak. For mating system analysis, we assayed embryos alongside their megagametophytes. When the contribution of the egg (the haploid genotype of the megagametophyte) to the embryo is known, small progeny arrays are adequate to estimate the outcrossing rate with little bias. For Highline Trailhead, we assayed 50 megagametophyte–embryo pairs for each of 20 trees (about 1000 progeny); for Flys Peak, 60 pairs for each of six trees (360 progeny); and for Ferron Reservoir, five pairs from each of 20 trees (100 progeny).

Statistical analysis

We estimated percent polymorphic loci, alleles per locus, expected heterozygosity, and Nei's (1978) unbiased genetic distance with BIOSYS (Swofford and Selander 1981), using population estimates of allele frequencies as input. For small

Table 2. Genic diversity in 16 Engelmann (*Picea engelmannii*) and 4 blue spruce (*Picea pungens*) populations.

Population	n	H_e	P	P_{95}	A
Engelmann spruce					
Kootenay River	51.9	0.247 (0.047)	83.3	66.7	2.7 (0.3)
Moyie	51.9	0.247 (0.044)	83.3	70.8	2.6 (0.3)
Bluebird Creek	51.9	0.268 (0.046)	79.2	66.7	2.7 (0.3)
Summit Lake	47.5	0.252 (0.043)	79.2	75.0	2.3 (0.2)
Beartooth Pass	46.9	0.307 (0.045)	91.7	75.0	2.5 (0.2)
Panther Creek	52.8	0.253 (0.045)	87.5	75.0	2.5 (0.2)
Six Bit Spring	47.9	0.227 (0.045)	66.7	54.2	2.1 (0.2)
Highline Trailhead	43.3	0.228 (0.043)	83.3	58.3	2.4 (0.3)
Ferron Reservoir	40.0	0.215 (0.040)	79.2	62.5	2.2 (0.2)
Barlow Lake	48.0	0.296 (0.046)	87.5	70.8	2.6 (0.3)
Navajo Lake	52.8	0.231 (0.044)	79.2	54.2	2.2 (0.2)
East Gavilan Canyon	48.3	0.273 (0.046)	79.2	75.0	2.5 (0.3)
San Francisco Mts.	47.9	0.269 (0.046)	83.3	66.7	2.4 (0.3)
Sierra Blanca	52.6	0.282 (0.050)	75.0	66.7	2.1 (0.2)
Mount Graham	156.5	0.261 (0.047)	75.0	66.7	2.4 (0.3)
Flys Peak	313.3	0.227 (0.043)	66.7	62.5	2.0 (0.2)
Mean		0.255	80.0	66.7	2.4
Blue spruce					
Wildcat	48.4	0.109 (0.042)	25.0	25.0	1.3 (0.1)
Scotch Creek	48.3	0.140 (0.042)	41.7	37.5	1.7 (0.2)
Rudd Knoll	47.9	0.127 (0.043)	37.5	29.2	1.5 (0.1)
West Fork	47.8	0.177 (0.040)	66.7	54.2	2.0 (0.2)
Mean		0.138	42.7	36.5	1.6

Note: n, mean number of genomes sampled per locus at 24 loci; H_e, expected heterozygosity (unbiased estimate); P, percent polymorphic loci, P_{95}, percent polymorphic loci at 95% criterion; A, number of alleles per locus. SE are shown in parentheses.

samples such as ours, BIOSYS calculates unbiased heterozygosity (Nei 1978). We also used BIOSYS to calculate Wright's (1965) F_{ST}, the proportion of the total genic diversity among populations. Pairwise F_{ST} were calculated using FSTAT2.9.3.2 (Goudet 2001).

Most inferences apply to the population of mature, cone-bearing trees. However, we were able to determine observed heterozygosity and, thus, calculate the inbreeding coefficient, F, for both the parental and progeny generations from Highline Trailhead, Ferron Reservoir, and Flys Peak because seeds were maintained separately by trees in these three populations.

The degree of genetic isolation among populations was estimated by Nm. Nm is the number of migrants per generation. Nm was calculated from the number and mean frequency of private alleles, the unique alleles found in only one population (Slatkin 1985; Barton and Slatkin 1986) and from the relationship (Wright 1951): $Nm = (1 - F_{ST})/4F_{ST}$.

We used the computer program BOTTLENECK (Cornuet and Luikart 1996) with 1000 bootstraps to determine whether effective population numbers had been restricted in the recent past. We employed the infinite allele model (Kimura and Crow1964) because empirically it tends to fit allozyme data better than alternatives (Luikart and Cornuet 1998). The Wilcoxon sign-rank test was preferred to the sign test because the former has higher power and can be used with as few as four polymorphic loci (Piry et al. 1999). However, we had at least 14 polymorphic loci available for every Engelmann spruce population, and in most cases, 18 or more. For blue spruce, at least six polymorphic loci were available.

The associations of pairwise F_{ST} and genetic distances with geographic distances between populations were checked using Mantel's (1967) generalized regression procedure and Cavalcanti's (1988) program MANTEL FOR WINDOWS with 10 000 permutations. Geographic distances between populations were calculated from latitude and longitude using Kindred's (1997) distance calculator.

Correlations of diversity measures and allele frequencies with latitude, longitude, and elevation were tested using linear regression and multiple regression modules in STATISTICA (StatSoft 1995).

We investigated phylogeographic relationships among the populations with cluster analysis, using the SEQBOOT, GENDIST, NEIGHBOR, and CONSENSE programs in the PHYLIP package (Felsenstein 1995). Bootstrapping was performed via random resampling of our allele frequency data set with the SEQBOOT program to generate 1000 data sets. Nei's (1972) genetic distances were calculated for these 1000 sets with GENDIST. Unweighted pair group (UPGMA) and neighbor-joining (NJ) methods were used to produce cladograms using the NEIGHBOR clustering program, and majority-rule consensus trees were generated from bootstrap trees using the program CONSENSE. The goal of bootstrapping was to test the consistency with which our data set supported phenetic relationships among popula-

Table 3. Wilcoxon sign-rank test for recent bottlenecks in 16 Engelmann spruce (*Picea engelmannii*) populations and four blue spruce (*Picea pungens*) populations under the infinite allele model of mutation-drift equilibrium.

Population	No. of loci	n^a	P^b
Engelmann spruce			
Kootenay River	20	51.9	0.899
Moyie	20	51.9	0.715
Bluebird Creek	19	51.8	0.675
Summit Lake	19	48.2	0.284
Beartooth Pass	22	47.5	0.194
Panther Creek	21	53.9	0.514
Six Bit Spring	16	48.1	0.188
Highline Trailhead	20	44.8	0.774
Ferron Reservoir	19	40.0	0.779
Barlow Lake	21	48.5	0.317
Navajo Lake	19	53.9	0.430
East Gavilan Cyn.	19	47.8	0.258
San Francisco Mts.	20	47.5	0.261
Sierra Blanca	18	53.0	0.015
Mount Graham	18	155.9	0.033
Flys Peak	14	329.9	0.002
Blue spruce			
Wildcat	6	45.7	0.016
Scotch Creek	10	47.5	0.161
Rudd Knoll	9	46.1	0.102
West Fork	16	47.0	0.719

[a]Number of genomes sampled.

[b]Wilcoxon rank-sign test one-tailed *P* value of heterozygote excess under the infinite allele model of mutation-drift equilibrium (Kimura and Crow 1964).

tions. High bootstrap scores (>70%) suggest strong support for a particular cluster, whereas lower levels of support suggest a lower order of differentiation. The phylogenetic trees were drawn using TREEVIEW (Page 1996). Trees were rooted with blue spruce from Scotch Creek, Colorado, as the outgroup.

We used Ritland's (1986, 1990, 1994) MLTR program with 1000 bootstraps to estimate outcrossing for individual loci, mean single-locus outcrossing rate ($\bar{t}_s$), multiple-locus outcrossing rate (t_m), and the correlation of outcrossed paternity (r_p) among progeny within families at the population level. Allele frequency and *t* were always estimated jointly, and the Newton–Raphson method was used for iteration.

Results

Genetic diversity

Of the 24 loci, all were polymorphic in at least one population of Engelmann spruce (Appendix Table A1). For some loci, notably *Fest*, *Lap2*, *Pgi2*, and *Skd1*, six and even seven alleles were observed in highly variable populations. By contrast, blue spruce was completely monomorphic at eight loci (*Idh1*, *Mdh1*, *Mdh2*, *Mdh4*, *Skd2*, *Tpi1*, and *Tpi2*) and had fewer alleles than Engelmann spruce at several of the polymorphic loci (Appendix Table A2). Allele frequencies varied considerably among populations of both species.

Percent polymorphic loci (*P*) ranged from 62.5% to 91.7% in Engelmann spruce populations, with a mean of 80% (Table 2). In contrast, *P* was 25.0%–66.7%, averaging 42.7% in blue spruce. The mean number of alleles per locus (*A*) was 2.0–2.7, averaging 2.4, in Engelmann spruce and only 1.3–2.0, averaging 1.6, in blue spruce. Expected heterozygosity (H_e) in Engelmann spruce ranged from 0.215 to 0.307, with a mean of 0.255, and in blue spruce, H_e ranged from 0.109 to 0.177, with a mean of 0.138 (Table 2).

Of the 16 Engelmann spruce populations, bottleneck analysis indicated significant heterozygosity excess for the three southernmost populations sampled, Flys Peak, Mount Graham, and Sierra Blanca and, therefore, recent bottlenecks. For the other 13 populations, the Wilcoxon sign-rank test indicated no greater heterozygosity than that expected for populations at mutation-drift equilibrium (Table 3). Among the four blue spruce populations, the northernmost, Wildcat, had significant heterozygosity excess, indicating a recent bottleneck (Table 3).

Genetic structure

A was related to latitude in Engelmann spruce, but *P* and H_e were not (Fig. 2). The geographic range and number of populations of blue spruce were too small for meaningful regressions and no relationship was obvious from the data. For example, blue spruce populations from West Fork and Rudd Knoll, which are only 12 km apart, had, respectively, the highest and next to the lowest estimates for *A*, *P*, and H_e.

Allele frequencies at 12 of the 24 loci were significantly correlated with latitude in Engelmann spruce (Table 4, Fig. 3). Slopes were significantly different from zero at about the 0.01 probability level or less for at least some alleles at seven loci, which was many more than would be expected by chance (0.24 at the 0.01 probability level or 1.2 at the 0.05 probability level in a sample of 24). By contrast, alleles at only two of the 24 loci were significantly correlated with longitude, and stepwise multiple regression on latitude and longitude added no significant improvement in prediction over that associated with latitude alone for either of the two loci.

Nei's unbiased genetic distance among Engelmann spruce populations ranged from a low of 0.006 between Moyie and Bluebird Creek on the one hand and Kootenay River on the other, up to a high of 0.125 between Summit Lake and Flys Peak (Table 5). Geographic distance and genetic distance were positively related (Fig. 4). Mantel's test matrix correlation was 0.58 ($P = 0.0001$). Genetic distances between blue spruce populations ranged only from 0.014 to 0.033.

Wright's F_{ST} also indicated significant genetic structure for Engelmann spruce (Table 6). Of the total genetic diversity, 14.7% was among populations in Engelmann spruce. Despite the limited range sampled for blue spruce, it too was characterized by a moderately high level of differentiation; 8.6% of the total genetic diversity was among populations.

The number of migrants per generation, *Nm*, was estimated from private alleles as 5.0 and from F_{ST} as 1.5 in Engelmann spruce. In blue spruce, *Nm* was only 1.6 based on private alleles and 2.7 from F_{ST}.

Cluster analysis using the UPGMA method linked proximal populations in several cases: for example, Highline Trailhead and Ferron Reservoir, from Utah, joined with a high level of certainty; all three populations from British

Table 4. Significant correlations, regression slopes and intercepts, and probability values for the relationships of allele frequencies to latitude.

Allele	Intercept	Slope	*r*	*P*
Fest 1	0.645	0.0067	0.54	0.0309
Fest 4	0.340	–0.0068	–0.64	0.0082
Idh1 1	0.034	0.0186	0.76	0.0004
Idh1 2	0.532	–0.0106	–0.66	0.0056
Idh2 1	1.355	–0.0148	–0.67	0.0044
Idh2 3	–0.317	0.0096	0.78	0.0004
Idh2 5	–0.158	0.0043	0.63	0.0088
Mdh1 1[a]	1.261	–0.0075	–0.61	0.0125
Mdh2 1[a]	1.225	–0.0066	–0.60	0.0142
Pgi1 1	–0.399	0.0177	0.61	0.0117
Pgi1 2	1.393	–0.0186	–0.60	0.0135
Pgi2 1	1.853	–0.0247	–0.86	0.0000
Pgi2 9	–0.359	0.0098	0.73	0.0013
Skd1 1	1.131	–0.0198	–0.52	0.0409
Skd1 4	–1.126	0.0346	0.80	0.0002
Skd1 8	0.909	–0.0190	–0.85	0.0000
Skd2 1	1.394	–0.0117	–0.76	0.0006
Tpi1 1	–1.074	0.0406	0.70	0.0027

Note: *r*, correlation coefficient; *P*, *P* value, or probability of observing a more extreme *r* when the true *r* is zero.

[a]Only two alleles were observed at both *Mdh1* and *Mdh2*, so a significant correlation for the frequency of one allele at these loci necessarily implies a significant correlation for the frequency of the other allele.

Columbia, which are within 209 km of each other, formed another cluster; and Summit Lake and Panther Creek, the two populations from Idaho, also formed a terminal cluster. These seven populations, in turn, formed one branch of the cladogram and the remaining nine populations joined a loose Southwestern group from Arizona, New Mexico, southern Utah, and southern Colorado (Fig. 5). Less parsimonious was the inclusion of Beartooth Creek from Wyoming and Six Bit Spring from northern Utah within the Southwestern group. The cladogram constructed with the NJ method (not shown) was less well supported by bootstraps. It differed from the UPGMA cladogram in that Highline Trailhead and Ferron Reservoir formed a third branch; they did not join the tree until after the five populations from British Columbia and Idaho and the nine populations in the southwestern group were clustered. Also, in the NJ tree, Beartooth Pass rather than Flys Peak was closest to the base of the southwestern cluster.

Mating system and inbreeding

Multilocus outcrossing estimates (t_m) were 0.899 for Flys Peak and 0.951 for Highline Trailhead, both of which differed significantly from 1.000, indicating statistically significant selfing. For Ferron Reservoir, t_m was 0.940, which was within a standard error of 1.000 and, therefore, indistinguishable from complete outcrossing (Table 7). The mean of the single-locus estimates of outcrossing were all lower than the corresponding multilocus estimates, which may suggest some inbreeding by biparental crosses among relatives in addition to inbreeding by selfing. However, the difference was statistically significant only for Highline Trailhead and not for Ferron Reservoir or Flys Peak. The lack of significance for Ferron Reservoir and Flys Peak was largely the result of higher standard errors, probably because the number or size of progeny arrays in those samples were much smaller than in Highline Trailhead. The observed correlations of outcrossed paternity, r_p, among progeny within families was moderately high (Table 7), which is consistent with pollination by a limited number of pollen donors. However, standard errors were large, so any conclusions are weak.

It was possible to calculate observed heterozygosity and estimate the inbreeding coefficients for both the parental and the progeny generations for the three populations in which seed was separated by tree. Little or no inbreeding was indicated in any of the three populations for either parents or progeny, and Highline Trailhead even had a heterozygote excess (Table 8). Despite the lack of significant inbreeding, observed heterozygosity in the progeny was always lower than in the seed parents. The progeny, however, always had a larger number of rare alleles because they sampled pollen from more than the 6–22 seed parents represented in the samples.

Discussion

Genetic diversity

Engelmann spruce was much more genetically diverse than blue spruce, perhaps reflecting Engelmann spruce's wider distribution and larger populations; blue spruce is often restricted to narrow, linear populations along streams. The higher genetic diversity in Engelmann spruce may also be the result of introgressive hybridization with the transcontinental white spruce. At the eastern periphery of Engelmann spruce's range in Alberta and at its lower elevational limits in British Columbia, hybridization with white spruce is common (Roche1969; Rajora and Dancik 2000). Based on a comparison of isozymes of Engelmann and white spruces in Alberta, Rajora and Dancik (2000) felt that Engelmann spruce should be considered a subspecies of white spruce, in agreement with Daubenmire (1974). In our samples, estimates of genetic diversity are, indeed, among the highest where Engelmann spruce was neighboringly allopatric with white spruce, such as in interior British Columbia (Roche 1969), and in Wyoming, where hybrids between Engelmann and white spruces have been observed (S.T. Jackson, personal communication, 2005).

The estimates of *P*, *A*, and H_e that we found in Engelmann spruce were slightly higher than the average for widespread woody species, 74.3%, 2.56, and 0.228, respectively, in Hamrick et al. (1992). Our estimates are high compared even with some other reports for Engelmann spruce itself. H_e for five Engelmann spruce populations in Alberta was 0.182 (Rajora and Dancik 2000). In a single population in Colorado, H_e was estimated at 0.203 (Ernst et al. 1990), which is considerably lower than our estimate of 0.296 ± 0.046 for Barlow Lake in southern Colorado. At the western edge of the Engelmann spruce distribution in Utah and Nevada, Hamrick et al. (1994) found H_e of 0.181, *P* of 56.5%, and *A* of 2.5 for 10 populations surveyed at 25 loci. Estimates from our four samples from Utah averaged 0.224, 77.1%, and 2.2, for these parameters, respectively. The differences may be due to differences in technique, in the par-

Table 5. Half-matrices of Nei's unbiased genetic distance above diagonal and distances (km) between populations below diagonal for 16 populations of Engelmann spruce (*Picea engelmannii*).

	KR	M	BC	SL	BP	PC	SBS	HT	FR	BL	NL	EGC	SFM	SB	MG	FP
KR		0.006	0.006	0.064	0.070	0.045	0.122	0.057	0.070	0.089	0.112	0.085	0.119	0.104	0.081	0.094
M	160		0.007	0.062	0.065	0.040	0.122	0.048	0.062	0.092	0.107	0.078	0.124	0.095	0.075	0.085
BC	209	72		0.064	0.060	0.044	0.110	0.041	0.052	0.087	0.099	0.082	0.112	0.102	0.076	0.089
SL	658	499	460		0.075	0.032	0.112	0.099	0.109	0.087	0.112	0.064	0.116	0.075	0.085	0.125
BP	838	714	728	505		0.062	0.046	0.062	0.067	0.028	0.054	0.043	0.042	0.045	0.038	0.083
PC	692	535	512	129	378		0.102	0.071	0.093	0.075	0.096	0.055	0.111	0.090	0.065	0.104
SBS	1107	955	941	530	403	434		0.072	0.073	0.024	0.023	0.026	0.014	0.039	0.038	0.057
HT	1211	1060	1047	632	479	540	106		0.006	0.061	0.057	0.061	0.081	0.073	0.059	0.074
FR	1368	1213	1192	754	664	681	272	185		0.071	0.052	0.069	0.080	0.068	0.062	0.074
BL	1608	1462	1453	1046	808	952	518	414	339		0.044	0.022	0.017	0.026	0.025	0.057
NL	1518	1360	1331	875	866	826	464	391	212	423		0.033	0.034	0.053	0.043	0.053
EGC	1769	1627	1622	1227	950	1128	697	595	525	187	581		0.037	0.022	0.023	0.042
SFM	1775	1618	1590	1137	1082	1083	694	604	422	427	262	511		0.037	0.037	0.064
SB	2130	1984	1975	1559	1320	1470	1036	931	814	522	782	373	585		0.036	0.059
MG	2099	1943	1920	1470	1362	1409	999	900	732	589	601	557	341	390		0.045
FP	2198	2043	2020	1572	1451	1509	1095	994	830	663	705	606	445	370	104	

Note: KR, Kootenay River; M, Moyie; BC, Bluebird Creek; SL, Summit Lake; BP, Beartooth Pass; PC, Panther Creek; SBS, Six Bit Spring; HT, Highline Trailhead; FR, Ferron Reservoir; BL, Barlow Lake; NL, Navajo Lake; EGC, East Gavilan Canyon; SFM, San Francisco Mountain; SB, Sierra Blanca; MG, Mount Graham; FP, Flys Peak.

Table 6. Estimates of Wright's (1965) F_{ST} for 24 polymorphic loci in Engelmann spruce (*Picea engelmannii*) and 17 in blue spruce (*Picea pungens*).

Locus	Engelmann spruce	Blue spruce
Fest	0.060	0.000
Gdh	0.084	0.006
Got-3	0.259	0.005
Idh1	0.114	—
Idh2	0.076	0.100
Lap1	0.269	0.068
Lap2	0.150	0.039
Mdh1	0.119	—
Mdh2	0.096	—
Mdh3	0.075	0.238
Mdh4	0.056	—
Mdh5	0.054	0.103
Mdh6	0.041	0.036
Mpi1	0.037	0.068
6Pg1	0.117	0.143
6Pg2	0.044	0.064
Pgi1	0.131	0.032
Pgi2	0.159	0.036
Pgm	0.098	0.100
Skd1	0.191	0.047
Skd2	0.099	—
Tpi1	0.489	—
Tpi2	0.041	—
Ugp1	0.057	0.053
Mean	0.147	0.086

ticular isozymes surveyed, or in tissue type; for example, Hamrick et al. (1994) and Rajora and Dancik (2000) sampled foliage, whereas we sampled megagametophytes in which isozyme patterns are easier to interpret. Note also that these other studies sampled only small portions of the Engelmann spruce range and often at the margins of its distribution (Hamrick et al. 1994; Rajora and Dancik 2000).

Engelmann spruce was much more diverse than the four endemic spruces, Brewer spruce, Chihuahua spruce, Martínez spruce, and Mexican spruce, the latter of which is a close relative of Engelmann spruce. All these endemics have small, fragmented populations in North America. Among them, means varied only from 27.0% to 44.2% for P, from 1.4 to 1.5 for A, and from 0.093 to 0.129 for H_e (Ledig et al. 1997, 2000, 2002, 2005). Even the three Engelmann spruce populations in which recent bottlenecks were indicated (Table 3), had higher estimates of diversity than the endemic spruces, averaging 70.8% for P, 2.1 for A, and 0.251 for H_e. On the other hand, diversity in blue spruce was similar to and only slightly higher than that in these endemic spruces (Table 2).

The regionally distributed red spruce and the widespread Norway spruce are the only other nonendemic spruces sampled across their entire range, or most of it, and their diversity was roughly half that reported here for Engelmann spruce. In the regionally distributed red spruce P_{99} was 37.1% and H_e was 0.079 based on a rangewide sample of 19 populations and 36 loci (Hawley and DeHayes 1994). Another study of five populations of red spruce from the center of the species' distribution in the Canadian maritime

Table 7. Single-locus (t_s) outcrossing estimates and their standard errors and means of single-locus ($\bar{t}_s$) estimates, multilocus (t_m) outcrossing estimates, and correlation of outcrossed paternity (rp) in three populations of Engelmann spruce (*Picea engelmannii*).

Locus	Highline Trailhead	Ferron Reservoir	Flys Peak
Fest	—	0.990 (0.224)	1.314 (0.096)
Gdh	1.027 (0.081)	0.932 (0.182)	0.048 (0.018)
Idh1	1.048 (0.046)	1.362 (0.294)	—
Idh2	—	—	0.938 (0.097)
Lap2	—	—	0.749 (0.157)
Mdh3	0.922 (0.090)	0.940 (0.132)	0.905 (0.246)
Mdh4	0.890 (0.069)	—	1.120 (0.165)
Mdh5	—	—	0.557 (0.217)
6Pg1	—	—	1.829 (0.351)
6Pg2	0.901 (0.104)	—	—
Pgi2	0.557 (0.167)	0.831 (0.733)	—
Pgm	—	—	1.349 (0.201)
Skd1	0.732 (0.035)	0.967 (0.393)	—
Tpi1	—	—	0.892 (0.070)
Ugp1	1.136 (0.058)	0.679 (0.260)	0.865 (0.108)
$\bar{t}_s$	0.898 (0.017)	0.907 (0.055)	0.872 (0.034)
t_m	0.951 (0.016)	0.940 (0.071)	0.899 (0.017)
rp	0.468 (0.110)	0.235 (0.107)	0.231 (0.207)

Note: SE are in parentheses.

provinces and from five disjuncts in Ontario was in good agreement; P_{99} was 42.7% and H_e was 0.100 (Rajora et al. 2000), close to Hawley and DeHayes' (1994) estimate of 0.090 for the less fragmented, northeastern portion of red spruce's range. Norway spruce, a species with a very wide distribution across the boreal forests of Europe and Asia, exhibited relatively little variation at isozyme loci compared to Engelmann spruce. For 70 populations spread over a distance of about 4000 km, H_e ranged from only 0.076 to 0.174, averaging 0.115 (Lagercrantz and Ryman 1990).

Although no rangewide study exists for the transcontinental white spruce, which is Engelmann spruce's closest relative, the combined evidence from regional studies indicates that it is as variable as Engelmann spruce. In Quebec, H_e was estimated as 0.319 (Tremblay and Simon 1989); in Ontario as 0.183 for the parental population and 0.298 in the progeny (Cheliak et al. 1985); and in Alaska as 0.270 (Alden and Loopstra 1987). Our population mean of H_e = 0.255 for Engelmann spruce fell within the range of estimates for white spruce and was much higher than estimates for the widespread Norway spruce, the regionally distributed red and blue spruces, or the endemics, Brewer spruce, Chihuahua spruce, Martínez spruce, and Mexican spruce.

Since Engelmann spruce colonized British Columbia and northern Idaho only after the recession of the Cordilleran Ice Sheet, fewer alleles might be expected there than in refugial origins south of the glacial front because of founder events (Cwynar and MacDonald 1987). Instead, the number of alleles increased from south to north. Therefore, the high variability of Engelmann spruce in British Columbia and northern Idaho reflects lower levels of genetic drift than experienced by populations in the Southwest and, perhaps, introgressive hybridization with white spruce.

Genetic structure

Engelmann spruce was highly structured in comparison to many other forest tree species. The level of differentiation among Engelmann spruce populations (F_{ST} = 0.147) was moderately high for conifers and approached or exceeded that in the highly fragmented Mexican spruces (between 0.024 for Martínez spruce and 0.248 for Chihuahua spruce) and Brewer spruce (0.157). Nei's genetic distance between some pairs of populations approached the level that characterizes subspecies (Gottlieb 1977); e.g., 0.125 between Summit Lake and Flys Peak (Table 5). Cluster analysis indicated a deep split between Southwestern populations and northern populations (Fig. 5). D averaged 0.051 within the group of seven northern populations from British Columbia, Idaho, and northern Utah, and 0.039 within the nine Southwestern populations, excluding Highline Trailhead and Ferron Reservoir. Between populations belonging to the two groups, D averaged 0.085, about twice as large as estimates within groups.

Mantel's matrix correlation between geographic distance and D was significant and positive, indicating that proximal populations tended to be more similar than distant ones (Fig. 4). However, closer analysis suggested that the relationship reflected the difference between the northern and Southwestern groups. Among Southwestern populations, neither D nor F_{ST} were related to geographic distance and the scatter was large (Fig. 6A). Figure 6A corresponds to case III of Hutchison and Templeton (1999) in which a region is subjected to climatic changes adverse to the organism, fragmenting and isolating populations to the point where random genetic drift becomes more influential than gene flow. The recent bottlenecks revealed in the three southernmost populations of Engelmann spruce bolstered this argument.

Table 8. Percent polymorphic loci (P), number of alleles per locus (A), observed heterozygosity (H_o), inbreeding coefficients (F), equilibrium inbreeding coefficient calculated from t_m (F_e), number of loci (L), and mean number of individuals sampled per locus (N) in a comparison of parental (mature trees) and progeny (embryos) generations in three Engelmann spruce (*Picea engelmannii*) populations.

Population and generation	L	N	P	A	H_o	F	F_e
Highline Trailhead	8						0.025
Parents		20.0	100.0	2.8 (0.5)	0.425 (0.095)	–0.104	
Progeny		975.4	100.0	3.8 (0.6)	0.371 (0.069)	0.026	
Ferron Reservoir	7						0.031
Parents		20.0	100.0	3.0 (0.5)	0.407 (0.069)	–0.002	
Progeny		95.4	100.0	3.3 (0.7)	0.373 (0.068)	0.011	
Flys Peak	11						0.053
Parents		6.0	90.9	2.2 (0.2)	0.348 (0.057)	0.047	
Progeny		357.0	100.0	2.5 (0.2)	0.327 (0.051)	0.021	

Among the northern populations, the relationship between F_{ST} or D and geographic distance was positive and monotonic, suggesting case I of Hutchison and Templeton (1999), in which a regional equilibrium exists between genetic drift and gene flow (Fig. 6B). However, the scatter does not become progressively greater with distance from the graph's origin, as expected for case I; a plot of the residuals from the regression against geographic distance (not shown) was perfectly flat. Therefore, the positive relationship between F_{ST} and geographic distance may reflect recent proximity to white spruce and introgressive hybridization for the northernmost populations and not an equilibrium between drift and gene flow.

Despite their limited usefulness in nonequilibrium conditions, estimates of Nm are valuable as a basis for comparisons. Nm for Engelmann spruce was low relative to values in many conifers; for example, as reviewed for pine in Ledig (1998) and for spruce in Ledig et al. (1997). Engelmann spruce from the western Rocky Mountains and eastern Great Basin had an Nm of 2.04 (Hamrick et al. 1994) similar to our estimates. In local studies within single sites or sites separated by 100 m, Shea (1990) and Cheliak et al. (1985) concluded that gene flow in Engelmann and white spruces, respectively, should be measured in tens of metres, not kilometres. This may be true of spruce in general; pollen dispersal is more limited in Engelmann and blue spruces than in pines (Jackson and Smith 1994). The positive, albeit weak, relationship between genetic and geographic distance that we detected in Engelmann spruce may occur because of gene exchange during the Pleistocene rather than gene flow in the present.

Allele number decreased from north to south, although H_e did not. Such a pattern might be expected in a subalpine species that was more abundant during the Pleistocene when it occupied lower elevations in the Rocky Mountains and Great Basin than now (Hamrick et al. 1994). As climate warmed after the glacial maximum, and particularly during the Xerothermic Period (Adams and Faure 1997), populations would have shrunk in size at the southern extremes of the range and become more fragmented. Fluctuations in tree line occurred throughout the Holocene but, generally, subalpine species were pushed to higher elevations than present during the period from 9000 to 5000 B.P. (Rochefort et al. 1994). Recent bottlenecks occurred in the southernmost populations in our sample, supporting this scenario.

In contrast, diversity in blue spruce was highest in the south. Blue spruce is a species adapted to lower elevations and latitudes than Engelmann spruce, judging by its distribution. It is the most drought tolerant of the spruces native to Canada and the United States (Fechner 1990). Only the northernmost blue spruce population sampled had significantly greater heterozygosity than that expected for populations at mutation-drift equilibrium, therefore suggesting a bottleneck.

Phylogeography

The dendrogram indicated a major division between northern and southern Engelmann spruce. Rehfeldt (1994, 2004) called attention to a similar break based on adaptive traits measured in common garden studies. He (Rehfeldt 2004) drew the line between northern and southern Engelmann spruce at approximately the southern borders of Utah and Colorado, although he suggested that it was difficult to draw a boundary. Isozyme markers suggest a complex pattern; neutral markers may reflect differences in refugial and dispersal history whereas quantitative traits tell more about adaptation to current conditions. All populations from Arizona, New Mexico, extreme southern Colorado, and southern Utah formed a cluster. However, Beartooth Pass from northern Wyoming and Six Bit Spring from northern Utah were, anomalously, also members of this cluster. Other populations from Utah (i.e., Highline Trailhead and Ferron Reservoir) either joined the northern cluster of Idaho and British Columbia or attached loosely and erratically to the cladogram, depending on joining method and the distance measure used. More intensive sampling and the use of other markers would help to clarify the phylogeographic structure of Engelmann spruce.

Frequencies of several alleles in Engelmann spruce were correlated with latitude, which may reflect one of the following: (1) past dispersal patterns as in Coulter pine (Ledig 2000) and many other organisms (Gooch and Glazier 1986; Cwynar and MacDonald 1987; Moran et al. 1989; Hewitt 2000; Davis and Shaw 2001); (2) linkage to adaptive gene sequences; or (3) hybridization and gene flow from white spruce. Clinal patterns of adaptive variation were amply demonstrated in extensive studies of Engelmann spruce that included the populations sampled here (Rehfeldt 1994, 2004). Most isozymes are probably selectively neutral; however, they may mark sections of DNA that are under selec-

tion pressure. Differences in *Pgm* allele frequencies and *Ugp* genotype frequencies have been found between wet and dry sites, and selection at these loci was suggested by Stutz and Mitton (1988) and Mitton et al. (1989). However, we found no significant relationships between allele frequencies at *Pgm* or *Ugp* and latitude, even though correlations might be expected because populations from British Columbia seemingly grow under more mesic conditions than those from Arizona.

The significant relationships between latitude and allele frequencies for several alleles seemed to be the result of step clines, or differences between the northernmost populations from British Columbia and Idaho vs. the extreme Southwestern populations, rather than a true linear trend (e.g., see especially *Idh1*1, *Mdh1*1, *Pgi*2*1*, and *Tpi1*1 in Fig. 3). More than half of the loci in which we observed correlations of latitude versus allele frequencies may reflect this division into northern and southern Engelmann spruce. The extreme northern populations may differ from the southern ones because of gene flow from white spruce at northern latitudes or may reflect genetic drift during past isolation in different refugia. Clines in allele frequency might be established as a result of secondary intergradation.

Mating system and inbreeding

Estimates of t_m and $\bar{t}_s$ indicated that Engelmann spruce was predominantly outcrossing, even in the isolated, southernmost population at Flys Peak. A small but statistically significant amount of effective selfing occurs, perhaps 5%–10%, in two of the three populations surveyed. In the third population, about 94% outcrossing (6% selfing) was indicated, but standard errors were high and the estimate of t_m was not significantly different from 100%.

The presence of linkage disequilibrium would violate the assumptions of the mating system model. If gametic disequilibrium existed, the likely result would be low estimates of t_m (Hedrick and Ritland 1990). However, estimates of $\bar{t}_s$ are not affected by disequilibrium and our estimates of $\bar{t}_s$ were similar to, but slightly lower than, estimates of t_m. The difference between t_m and $\bar{t}_s$ measures biparental inbreeding; that is, crosses among related individuals. In fact, $\bar{t}_s$ was significantly lower than t_m for Highline Trailhead, suggesting biparental inbreeding in that population at least.

Shea's (1987) estimate of outcrossing in Engelmann spruce from the Front Range of Colorado, 0.87, was similar and only slightly lower than ours. Estimates for the related white spruce were generally high: $\bar{t}_s$ in Ontario was 0.98 (Cheliak et al. 1985) and in Alberta was 0.893 to–0.894, depending on year (King et al. 1984). An exception was Innes and Ringius' (1990) estimate of 0.76 for white spruce in Newfoundland. In Sitka spruce, another member of the white spruce complex, t_m was 0.923 (Chaisurisri et al. 1994). Thus, it seems that outcrossing is relatively high in the white–Engelmann–Sitka complex, usually with 10% or less effective selfing.

Rates of outcrossing estimated in the endemic spruces of Mexico were generally lower than 70%, and as low as 0% and 15% in two small populations of Chihuahua spruce (Ledig et al. 1997, 2000, 2002). In contrast to the Mexican endemics, the rare Serbian spruce (*Picea omorika* (Panc.) Purk.), whose populations occupy, in total, only 60 ha in its native range and which is almost entirely self-fertile, had an outcrossing rate of 84% (Kuittinen and Savolainen 1992), and rates of outcrossing in the endemic Brewer spruce were also similar to those for Engelmann spruce (Ledig et al. 2005). Outcrossing estimates were somewhat low for the transcontinental black spruce (*Picea mariana* (Mill.) B. S. P.), ranging from 62% in seed stored for a few years to 85% in older seed, the difference resulting, perhaps, from a difference in longevity between selfed and outcrossed embryos in storage (Sproule and Dancik 1996).

Inbreeding coefficients for both the parental and progeny generations at Flys Peak, Ferron Reservoir, and Highline Trailhead, like the estimates of outcrossing rate, also suggested that levels of inbreeding were low. In fact, F in the mature trees at Highline Trailhead was negative, which indicates excess heterozygosity above expectations from random mating. Similar situations have been reported in several conifers (e.g., Plessas and Strauss 1986; Ledig et al. 2002), which suggests that selfed progeny were weeded out during stand development and that heterozygotes had a selective advantage. In all three Engelmann spruce populations for which we could measure observed heterozygosity, H_o, it was greater in the mature trees than in the embryos (Table 8), although the differences were small and not statistically significant.

Observed inbreeding coefficients were very close to equilibrium expectations estimated from outcrossing rates (Table 8). The equilibrium inbreeding coefficient, F_e, was calculated from Allard et al. (1968) as follows: $F_e = (1 - t_m)/(1 + t_m)$.

r_p is the probability that a randomly chosen pair of embryos from the same seed tree were full siblings. For Highline Trailhead and Ferron Reservoir, r_p differed significantly from zero. Values around 0.25 (Ferron Reservoir in Table 7) suggest at least four pollen parents in the progeny array if all were equally represented, a fairly diverse pollen pool. Higher values would suggest fewer pollen parents per progeny array. An r_p of about 0.5, as in Highline Trailhead, would indicate two pollen parents if each was equally represented. Thus, each seed-tree at Highline Trailhead sampled only a limited pollen pool, composed, perhaps, of a few neighbors. However, standard errors for r_p were high, so these inferences are weak.

Conclusions

All our estimates of diversity, inbreeding, and outcrossing indicate a genetically robust Engelmann spruce with greater diversity and higher levels of effective outcrossing than might be expected based on the literature for many other spruces of North America. Even the recently bottlenecked, southernmost population at Flys Peak had high rates of outcrossing, and inbreeding coefficients were close to those expected at equilibrium.

However, the hypothesis that Engelmann spruce would be vulnerable to bottlenecks in the southern portions of its range was confirmed. The genetic evidence indicated that the three southernmost populations in our sample suffered bottlenecks in the recent past. One population of blue spruce also showed evidence of bottlenecking, but it was the north-

ernmost of the four in our sample and bordered the Great Basin near the westernmost limits for this species.

Also as predicted, genetic diversity in Engelmann spruce was highest in the north, perhaps partly as a result of current introgression with white spruce in British Columbia and phantom hybridization in Idaho and as far south as northern and central Utah. Gene flow as estimated by *Nm* or as implied by the weak relationship between genetic and geographic distance seems too low for the effects of Holocene introgression in British Columbia and Alberta to have extended far south of the area of white spruce – Engelmann spruce sympatry. The sharp break in the cladogram (Fig. 5) further supports this. Extensive introgression may connect white and Engelmann spruces across elevational gradients in Canada, but as suggested by the ranges and distinct ecologic niches of the two species and by this and previous estimates of gene flow (Shea 1990), this is probably a case of secondary intergradation, not primary.

Blue spruce was not as rich in isozyme diversity as Engelmann spruce, which parallels their comparative levels of variance in adaptive traits as revealed by uniform garden studies (Rehfeldt 2004). Higher levels of genetic diversity might be expected in Engelmann spruce because it has a broad range and is a common species with historically large populations, particularly during glacial periods. In contrast, blue spruce is less widely distributed and its values for percentage polymorphic alleles, number of alleles per locus, and heterozygosity were only a little more than half as great as those in Engelmann spruce.

Diversity in Engelmann spruce was highly structured across its geographic range as indicated by an F_{ST} of 0.147, and this might be expected in a subalpine species that now occupies isolated sky islands. Many alleles had latitudinal gradients which may, in part, reflect past and present introgression from white spruce, but also reflects the loss of alleles through random genetic drift in the south as populations became smaller and more isolated in the Holocene. Thus, the relationship between either F_{ST} or D and geographic distance suggests a species whose structure is influenced by introgressive hybridization in the north, as previously demonstrated by others, and by fragmentation, isolation, and genetic drift in the southwest. Selection may also play a role; some alleles seem to show real clinal variation from north to south (e.g., *Fest*1, *Idh1*1, *Idh2*1, and *Skd1*8 in Fig. 3).

Variation in adaptive, quantitative traits generally follow clinal patterns in Engelmann spruce (Rehfeldt 2004). However, Rehfeldt (1994) noted a break between northern and southern Engelmann spruce, and our results with largely neutral markers confirmed this. We are not sure where the break occurs because we did not sample intensively. Rehfeldt (2004) suggested a break at about or just north of the 37th parallel along the border that separates Utah and Colorado from Arizona and New Mexico. We suggest that the pattern is complex, perhaps reflecting past, local hybridization with white spruce during the glacial maximum or dispersal from multiple refugia at the end of the Pleistocene. Further study using more definitive markers would improve our understanding of the effects of past climates on Engelmann spruce and aid in predicting its response to future global warming.

Acknowledgments

This study was an undertaking of the Forest Genetic Resources Working Group/North American Forestry Commission/Food and Agricultural Organization of the United Nations. The authors thank B. Jaquish and G.E. Rehfeldt for seed collections; S.T. Jackson for insights into the location of spruce during the last glacial maximum; J.A. Baldwin for statistical review; and G.E. Rehfeldt and two anonymous reviewers for helpful comments on the manuscript.

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Appendix A.

Appendix tables A1 and A2 appear on the following pages.

Table A1. Allele frequencies for 24 polymorphic loci in 16 populations of Engelmann spruce (*Picea engelmannii*).

Locus and allele[a]	Population[b] (sample size[c])															
	KR (52.0)	M (51.9)	BC (51.9)	SL (47.3)	BP (46.9)	PC (52.8)	SBS (47.9)	HT (44.0)	FR (40.0)	BL (48.0)	NL (52.8)	EGC (48.3)	SFM (48.0)	SB (52.2)	MG (156.8)	FP (317.5)
Fest																
1	1.000	0.942	0.962	1.000	0.940	1.000	1.000	0.796	0.775	0.925	0.900	0.898	0.914	0.971	0.862	0.792
2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.091	0.000	0.000	0.040	0.041	0.000	0.000	0.000	0.000
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.013	0.000
4	0.000	0.000	0.038	0.000	0.060	0.000	0.000	0.045	0.200	0.075	0.060	0.061	0.086	0.029	0.125	0.208
5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6	0.000	0.058	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Gdh																
1	0.769	0.865	0.865	0.933	0.750	0.740	0.775	0.545	0.625	0.535	0.880	0.867	0.698	0.860	0.824	0.994
2	0.231	0.135	0.135	0.067	0.250	0.260	0.225	0.455	0.350	0.465	0.120	0.133	0.302	0.140	0.176	0.006
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Got3																
1	1.000	1.000	1.000	1.000	0.980	0.982	1.000	0.977	0.975	0.960	0.982	1.000	1.000	1.000	0.994	0.594
2	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.023	0.025	0.040	0.000	0.000	0.000	0.000	0.000	0.406
3	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.006	0.000
Idh3																
1	0.942	0.981	0.981	1.000	0.880	0.945	0.600	0.750	0.750	0.860	0.582	0.600	0.720	0.727	0.700	0.656
2	0.000	0.000	0.000	0.000	0.060	0.055	0.140	0.159	0.075	0.120	0.036	0.340	0.100	0.200	0.261	0.072
3	0.000	0.000	0.000	0.000	0.060	0.000	0.260	0.091	0.175	0.020	0.382	0.040	0.180	0.073	0.039	0.239
4	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.033
6	0.058	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Idh2																
1	0.499	0.578	0.519	0.812	0.659	0.818	0.841	0.750	0.800	0.756	0.982	0.812	0.900	0.773	0.676	0.885
2	0.173	0.173	0.231	0.000	0.250	0.000	0.045	0.250	0.200	0.224	0.000	0.104	0.080	0.170	0.324	0.115
3	0.135	0.096	0.231	0.188	0.091	0.182	0.114	0.000	0.000	0.020	0.018	0.063	0.020	0.038	0.000	0.000
4	0.058	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000	0.000
5	0.135	0.115	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000
Lap1																
1	0.962	1.000	1.000	0.180	0.520	0.382	0.420	0.932	0.975	0.460	0.582	0.327	0.560	0.444	0.456	1.000
2	0.038	0.000	0.000	0.220	0.040	0.218	0.140	0.045	0.025	0.080	0.127	0.265	0.140	0.000	0.082	0.000

Table A1. (*continued*).

Locus and allele[a]	Population[b] (sample size[c])															
	KR (52.0)	M (51.9)	BC (51.9)	SL (47.3)	BP (46.9)	PC (52.8)	SBS (47.9)	HT (44.0)	FR (40.0)	BL (48.0)	NL (52.8)	EGC (48.3)	SFM (48.0)	SB (52.2)	MG (156.8)	FP (317.5)
Mdh6																
1	0.962	0.865	0.923	0.939	0.977	1.000	0.740	0.932	0.950	0.960	0.963	0.940	0.837	0.909	0.944	0.917
2	0.038	0.135	0.077	0.061	0.023	0.000	0.260	0.068	0.050	0.040	0.037	0.060	0.163	0.091	0.056	0.083
Mpi1																
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.975	1.000	1.000	0.920	0.971	1.000	1.000	1.000
2	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.080	0.000	0.000	0.000	0.000
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.029	0.000	0.000	0.000
4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6Pg1																
1	0.942	0.846	1.000	0.920	0.860	0.982	0.880	0.977	0.925	0.800	0.964	0.840	0.680	0.473	0.850	0.922
2	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3	0.058	0.154	0.000	0.060	0.140	0.018	0.040	0.000	0.075	0.200	0.036	0.160	0.320	0.382	0.150	0.075
4	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.145	0.000	0.003
5	0.000	0.000	0.000	0.000	0.000	0.000	0.060	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6Pg2																
1	0.962	0.943	0.904	0.900	0.920	0.927	0.980	0.955	1.000	0.860	0.964	0.960	1.000	0.836	1.000	1.000
2	0.000	0.000	0.058	0.020	0.020	0.000	0.000	0.045	0.000	0.020	0.036	0.000	0.000	0.000	0.000	0.000
3	0.038	0.038	0.038	0.000	0.040	0.073	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
4	0.000	0.000	0.000	0.080	0.000	0.000	0.020	0.000	0.000	0.120	0.000	0.040	0.000	0.164	0.000	0.000
5	0.000	0.019	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pgi1																
1	0.655	0.539	0.500	0.540	0.320	0.400	0.220	0.114	0.075	0.340	0.036	0.220	0.180	0.291	0.314	0.390
2	0.288	0.423	0.481	0.460	0.460	0.582	0.760	0.864	0.925	0.660	0.964	0.780	0.580	0.673	0.680	0.610
3	0.000	0.000	0.000	0.000	0.220	0.018	0.020	0.000	0.000	0.000	0.000	0.000	0.140	0.036	0.006	0.000
4	0.038	0.038	0.019	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000	0.000	0.100	0.000	0.000	0.000
5	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pgi2																
1	0.463	0.577	0.616	0.820	0.800	0.727	1.000	0.977	0.950	0.940	0.745	1.000	0.980	1.000	1.000	1.000
2	0.096	0.077	0.038	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000
3	0.115	0.077	0.096	0.000	0.200	0.255	0.000	0.000	0.050	0.000	0.055	0.000	0.000	0.000	0.000	0.000

Table A1. (*continued*).

Locus and allele[a]	Population[b] (sample size[c])															
	KR (52.0)	M (51.9)	BC (51.9)	SL (47.3)	BP (46.9)	PC (52.8)	SBS (47.9)	HT (44.0)	FR (40.0)	BL (48.0)	NL (52.8)	EGC (48.3)	SFM (48.0)	SB (52.2)	MG (156.8)	FP (317.5)
3	0.000	0.000	0.000	0.600	0.440	0.255	0.440	0.000	0.000	0.400	0.291	0.408	0.300	0.556	0.000	0.000
4	0.000	0.000	0.000	0.000	0.000	0.145	0.000	0.023	0.000	0.060	0.000	0.000	0.000	0.000	0.462	0.000
Lap 2																
1	0.490	0.510	0.431	0.140	0.160	0.185	0.300	0.300	0.250	0.380	0.164	0.541	0.160	0.365	0.268	0.725
2	0.490	0.490	0.549	0.300	0.840	0.167	0.700	0.650	0.750	0.500	0.836	0.313	0.700	0.327	0.598	0.275
3	0.020	0.000	0.000	0.560	0.000	0.314	0.000	0.000	0.000	0.040	0.000	0.083	0.140	0.154	0.102	0.000
4	0.000	0.000	0.020	0.000	0.000	0.019	0.000	0.050	0.000	0.060	0.000	0.021	0.000	0.000	0.000	0.000
5	0.000	0.000	0.000	0.000	0.000	0.185	0.000	0.000	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000
6	0.000	0.000	0.000	0.000	0.000	0.130	0.000	0.000	0.000	0.020	0.000	0.021	0.000	0.154	0.032	0.000
Mdh 1																
1	0.942	0.962	0.808	0.776	0.980	0.836	1.000	0.977	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
2	0.058	0.038	0.192	0.224	0.020	0.164	0.000	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh 2																
1	0.885	0.923	0.923	0.740	0.960	0.964	0.980	1.000	1.000	0.960	1.000	1.000	0.980	1.000	1.000	1.000
2	0.115	0.077	0.077	0.260	0.040	0.036	0.020	0.000	0.000	0.040	0.000	0.000	0.020	0.000	0.000	0.000
Mdh 3																
1	0.904	0.808	0.789	0.880	0.455	0.836	0.700	0.591	0.500	0.720	0.667	0.800	0.720	0.527	0.681	0.695
2	0.000	0.000	0.038	0.000	0.000	0.000	0.000	0.023	0.025	0.000	0.074	0.000	0.120	0.000	0.075	0.168
3	0.096	0.192	0.173	0.120	0.522	0.164	0.300	0.386	0.475	0.260	0.259	0.200	0.160	0.473	0.244	0.137
4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000
5	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh 4																
1	0.942	0.885	0.885	0.911	0.733	0.927	0.978	0.841	0.800	0.800	0.943	0.880	0.940	0.855	0.888	0.876
2	0.000	0.000	0.000	0.089	0.267	0.055	0.000	0.000	0.000	0.200	0.057	0.120	0.060	0.145	0.112	0.124
3	0.000	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
4	0.058	0.115	0.115	0.000	0.000	0.000	0.022	0.159	0.200	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh 5																
1	0.824	0.943	0.789	1.000	0.909	0.891	1.000	0.932	0.875	0.900	0.648	0.880	0.820	0.982	0.928	0.877
2	0.059	0.000	0.019	0.000	0.023	0.073	0.000	0.000	0.000	0.060	0.000	0.020	0.000	0.000	0.000	0.053
5	0.039	0.019	0.115	0.000	0.000	0.018	0.000	0.000	0.075	0.000	0.148	0.020	0.040	0.000	0.046	0.000
6	0.000	0.000	0.019	0.000	0.068	0.018	0.000	0.000	0.050	0.040	0.185	0.080	0.060	0.000	0.013	0.000
7	0.078	0.038	0.058	0.000	0.000	0.000	0.000	0.068	0.000	0.000	0.019	0.000	0.080	0.018	0.013	0.070

Table A1. (*continued*).

Locus and allele[a]	Population[b] (sample size[c])															
	KR (52.0)	M (51.9)	BC (51.9)	SL (47.3)	BP (46.9)	PC (52.8)	SBS (47.9)	HT (44.0)	FR (40.0)	BL (48.0)	NL (52.8)	EGC (48.3)	SFM (48.0)	SB (52.2)	MG (156.8)	FP (317.5)
4	0.019	0.019	0.038	0.100	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
5	0.000	0.000	0.000	0.060	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.020	0.000	0.000	0.000
6	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000	0.000
7	0.038	0.058	0.058	0.020	0.000	0.018	0.000	0.000	0.000	0.020	0.182	0.000	0.000	0.000	0.000	0.000
8	0.000	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
9	0.269	0.173	0.154	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Pgm																
1	0.346	0.157	0.340	0.327	0.460	0.036	0.500	0.295	0.450	0.540	0.327	0.340	0.720	0.564	0.344	0.234
2	0.635	0.843	0.640	0.673	0.540	0.946	0.500	0.705	0.550	0.460	0.673	0.660	0.280	0.436	0.656	0.766
3	0.019	0.000	0.020	0.000	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Skd1																
1	0.154	0.077	0.212	0.022	0.106	0.000	0.690	0.522	0.725	0.182	0.615	0.348	0.400	0.400	0.236	0.515
2	0.096	0.096	0.000	0.022	0.128	0.067	0.067	0.250	0.000	0.205	0.269	0.204	0.178	0.182	0.007	0.115
3	0.058	0.038	0.077	0.222	0.106	0.000	0.000	0.023	0.000	0.023	0.000	0.020	0.044	0.000	0.007	0.006
4	0.654	0.635	0.576	0.445	0.446	0.777	0.044	0.114	0.100	0.068	0.000	0.265	0.022	0.091	0.270	0.018
5	0.019	0.058	0.058	0.222	0.043	0.156	0.022	0.000	0.025	0.000	0.000	0.000	0.067	0.000	0.000	0.000
6	0.000	0.000	0.000	0.067	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.000	0.000	0.000
7	0.000	0.038	0.019	0.000	0.043	0.000	0.044	0.023	0.050	0.205	0.058	0.000	0.000	0.000	0.081	0.000
8	0.000	0.000	0.000	0.000	0.128	0.000	0.133	0.023	0.075	0.272	0.058	0.143	0.267	0.327	0.372	0.345
9	0.019	0.058	0.058	0.000	0.000	0.000	0.000	0.045	0.025	0.045	0.000	0.020	0.000	0.000	0.027	0.000
Skd2																
1	0.885	0.788	0.769	0.848	0.700	0.907	1.000	0.955	0.975	0.959	1.000	0.920	0.980	1.000	0.994	1.000
2	0.077	0.212	0.154	0.087	0.300	0.093	0.000	0.045	0.000	0.000	0.000	0.080	0.020	0.000	0.000	0.000
3	0.038	0.000	0.077	0.065	0.000	0.000	0.000	0.000	0.000	0.041	0.000	0.000	0.000	0.000	0.006	0.000
6	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Tpi1																
1	0.981	1.000	1.000	0.925	0.410	0.796	0.111	1.000	1.000	0.233	0.288	0.356	0.111	0.440	0.314	0.278
2	0.019	0.000	0.000	0.075	0.590	0.204	0.889	0.000	0.000	0.744	0.712	0.644	0.889	0.560	0.686	0.722
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.000	0.000

Table A1. (*concluded*).

Locus and allele[a]	Population[b] (sample size[c])													
	KR (52.0)	M (51.9)	BC (51.9)	SL (47.3)	BP (46.9)	PC (52.8)	SBS (47.9)	HT (44.0)	FR (40.0)	BL (48.0)	NL (52.8)	EGC (48.3)	SFM (48.0)	SB (52.2)	MG (156.8)	FP (317.5)
Tpi2																
1	1.000	0.962	0.962	0.960	1.000	0.909	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.945	1.000	1.000
2	0.000	0.038	0.000	0.000	0.000	0.091	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.055	0.000	0.000
4	0.000	0.000	0.038	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ugp1																
1	0.539	0.577	0.404	0.720	0.460	0.455	0.380	0.455	0.625	0.460	0.618	0.660	0.380	0.782	0.656	0.685
2	0.404	0.346	0.519	0.180	0.520	0.509	0.620	0.545	0.375	0.540	0.382	0.340	0.620	0.218	0.338	0.315
3	0.000	0.000	0.000	0.100	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
4	0.038	0.019	0.019	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
5	0.019	0.058	0.058	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000

[a]Allele numbers may not be consecutive here, but are numbered to coincide with isozymes that co-electrophorese to the same position in Brewer spruce and various Mexican spruces and follow the nomenclature in Ledig et al. (2004).

[b]KR, Kootenay River; M, Moyie; BC, Bluebird Creek; SL, Summit Lake; BP, Beartooth Pass; PC, Panther Creek; SBS, Six Bit Spring; HT, Highline Trailhead; FR, Ferron Reservoir; BL, Barlow Lake; NL, Navajo Lake; EGC, East Gavilan Canyon; SFM, San Francisco Mountain; SB, Sierra Blanca; MG, Mount Graham; FP, Flys Peak.

[c]The mean number of genomes per locus.

Table A2. Allele frequencies for 17 polymorphic loci in four populations of blue spruce (*Picea pungens*).

	Population[b] (sample size)[c]			
Locus and allele[a]	W (48.4)	SC (48.3)	RK (47.9)	WF (47.8)
Fest				
1	0.360	0.240	0.263	0.286
2	0.640	0.760	0.737	0.714
Gdh				
1	0.000	0.000	0.000	0.023
2	1.000	1.000	1.000	0.977
Got3				
1	0.000	0.000	0.000	0.020
2	1.000	1.000	1.000	0.980
Idh2				
1	1.000	1.000	1.000	0.860
2	0.000	0.000	0.000	0.140
Lap1				
1	0.640	0.720	0.959	0.720
2	0.360	0.220	0.041	0.280
4	0.000	0.060	0.000	0.000
Lap2				
1	1.000	0.980	0.898	0.900
2	0.000	0.000	0.000	0.020
3	0.000	0.000	0.102	0.000
6	0.000	0.020	0.000	0.080
Mdh3				
1	0.082	0.140	0.531	0.620
2	0.918	0.860	0.469	0.380
Mdh5				
1	1.000	0.900	1.000	0.720
3	0.000	0.040	0.000	0.120
4	0.000	0.060	0.000	0.160
Mdh6				
1	1.000	1.000	1.000	0.940
2	0.000	0.000	0.000	0.060
Mpi1				
1	0.000	0.000	0.000	0.100
2	1.000	1.000	1.000	0.867
4	0.000	0.000	0.000	0.033
6Pg1				
1	0.400	0.720	0.660	0.900
2	0.420	0.000	0.340	0.060
3	0.180	0.260	0.000	0.040
4	0.000	0.020	0.000	0.000
6Pg2				
1	1.000	1.000	0.880	0.980
2	0.000	0.000	0.120	0.020
Pgi1				
1	0.000	0.000	0.000	0.020
2	1.000	0.900	0.980	0.880
4	0.000	0.100	0.020	0.100
Pg-2				
1	0.000	0.000	0.000	0.060
2	1.000	1.000	1.000	0.940
Pgm				
1	1.000	0.860	1.000	1.000
3	0.000	0.140	0.000	0.000
Skd1				
1	0.600	0.420	0.367	0.320
2	0.340	0.400	0.347	0.600

Table A2 (*concluded*).

Locus and allele[a]	Population[b] (sample size)[c]			
	W (48.4)	SC (48.3)	RK (47.9)	WF (47.8)
3	0.000	0.140	0.265	0.000
7	0.060	0.000	0.020	0.040
8	0.000	0.040	0.000	0.000
9	0.000	0.000	0.000	0.040
Ugp1				
1	0.760	0.460	0.460	0.560
2	0.240	0.540	0.540	0.380
4	0.000	0.000	0.000	0.060

[a]Allele numbers may not be consecutive here, but they are numbered to coincide with isozymes that co-electrophorese to the same position in Brewer spruce and various Mexican spruces and follow the nomenclature in Ledig et al. (2004).

[b]W, Wildcat; SC, Scotch Creek; RK, Rudd Knoll; WF, West Fork.

[c]The mean number of genomes per locus