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Genetic biodiversity impacts of silvicultural practices and phenotypic selection in white spruce

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Abstract Forest-management practices relying on natural and/or artificial regeneration and domestication can significantly affect genetic diversity. The aim of the present study was to determine and compare the genetic diversity of the pristine old-growth, naturally and artificially regenerated and phenotypically selected white spruce, and to determine the genetic-diversity impacts of silvicultural practices. Genetic diversity was determined and compared for 51 random amplified polymorphic DNA (RAPD) loci for the adjacent natural old-growth, naturally regenerated and planted white spruce stands at each of four sites, one oldest plantation and open-pollinated progeny of 30 phenotypic tree-improvement selections of white spruce from Saskatchewan. Each of the 420 white spruce individuals sampled was genetically unique. The old-growth stands had the highest, and the phenotypic selections the lowest, genetic diversity. The genetic diversity of the natural regeneration was comparable to that of the old-growth, whereas the genetic diversity of the plantations was comparable to that of the selections. On average, the genetic diversity of the old-growth and natural regeneration was significantly higher than that of the plantations and selections. The mean percent of loci polymorphic, the number of alleles per locus, the effective number of alleles per locus, heterozygosity, and Shannon's index was 88.7, 83.8, 72.2 and 66.7; 1.89, 1.84, 1.72 and 1.67; 1.69, 1.62, 1.53 and 1.46; 0.381, 0.349, 0.297 and 0.259; and 0.548, 0.506, 0.431 and 0.381 for the old-growth stands; natural regeneration; plantations; and open-pollinated progeny of selections; respectively. Reduced genetic diversity in the plantations and selections suggest that their genetic base is relatively narrow, and should therefore be broadened in order to maintain genetic diversity, and sustainably manage and conserve white spruce genetic resources.

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Introduction

There are increasing concerns about maintaining biodiversity in our forest ecosystems and about the possible impact of forest practices on biodiversity (e.g., Friedman and Foster 1997). Genetic diversity is the basis of all biodiversity, because it provides raw material for the adaptation, evolution and survival of species and individuals, especially under changed environment and disease conditions. As trees are the keystone species of forest ecosystems, their genetic diversity has a special significance. Recent studies in Europe (e.g., Bergmann et al. 1990; Oleksyn et al. 1994; Raddi et al. 1994) suggest that reductions in genetic diversity predispose forests to an environment-related decline in health and productivity. Genetic variability is also the basis for tree improvement. Thus, genetic diversity is the foundation for forest sustainability and ecosystem stability. The Canadian Standards Association has identified genetic diversity as one of the criteria/indicators for the registration, certification and audit of a sustainable forest-management system (CSA 1996a, b). Benchmarking genetic diversity in natural pristine forest-tree populations, determining the genetic impacts of silvicultural management practices on forest-tree genetic diversity, and subsequent monitoring, provides resource managers with an indicator of long-term forest sustainability and ecosystem health (Buchert 1995; Mosseler and Bowers 1998).

Forest management practices relying on natural and/or artificial regeneration systems, including tree improvement, and natural disturbances such as forest fires, can significantly impact the genetic variability in subsequent forest populations. However, the genetic implications of forest management are largely unknown. Forest managers are faced with the challenge of conserving and maintaining genetic diversity while at the same time

maintaining the economic viability of the forest industry. Land managers need silvicultural guidelines, and indicators for measuring, monitoring, and reporting on genetically sustainable forestry practices. It is well known that domestication in agricultural crops has resulted in great losses of genetic diversity. However, the extent of the genetic effects of domestication in forest trees in not clear.

Properly applied silvicultural treatments need not cause genetic degradation. For example, shelterwood harvesting in old-growth Douglas-fir (*Pseudotsuga menziesii*) appears to have had no negative effects on genetic variation (Neale 1985) or mating systems (Neale and Adams 1985). No significant differences were found between virgin forests and naturally regenerated stands of Norway spruce (*Picea abies*) (Gomory 1992); however, planted stands had a significantly reduced genetic diversity. A few studies on the genetic diversity of natural stands versus seed orchard clones in forest trees have confirmed that expected heterozygosities were similar in natural and managed populations (Savolainen and Karkkainen 1992). Genetic diversity was found to be maintained in advanced-generation breeding populations of the loblolly pine, *Pinus taeda* (Williams et al. 1995). However, in another study of this species, seed orchards were found to have 10–38% reduced genetic diversity as compared to natural populations (Hamrick 1991).

Specific information on the effects of alternative silvicultural practices on genetic diversity and biological processes affecting genetic diversity is lacking for almost all Canadian forest-tree species, with a few exceptions. No significant heterogeneity was detected in allele frequencies at five allozyme loci among the mature stands, young natural regeneration and young plantation or seed orchard clones in jack pine (*Pinus banksiana*) and black spruce (*Picea mariana*) (Knowles 1985). The seed orchard clones of sitka spruce (*Picea sitchensis*), western red cedar (*Thuja plicata*) and Douglas-fir were found to have a similar or higher genetic diversity to the natural populations (review in El-Kassaby 1995). Genetic diversity was reduced by 25–50% in old-growth eastern white pine (*Pinus strobus*) in the post-harvest residual gene pool (Buchert et al. 1997).

White spruce [*Picea glauca* (Moench) Voss] is a widespread and important tree species of the boreal forest and is found in almost all forested regions of Canada with the exception of the Pacific coast (Hosie 1979). It is one of the most important trees in Canada for the production of wood pulp and lumber. White spruce is naturally, as well as artificially, regenerated in Canada. The genetic diversity of white spruce has been examined for populations from Newfoundland (Innes and Ringius 1990), Quebec (Tremblay and Simon 1989), Ontario (Cheliak et al. 1985), Alberta (King et al. 1984; Rajora and Dancik, Manuscript in preparation), and Alaska (Alden and Loopstra 1987). There is no reported information on either the genetic diversity of white spruce or any other forest tree species from Saskatchewan or on the genetic effects of alternative harvesting and regeneration silvicultural practices in white spruce. Also, very little is known about the genetic effects of phenotypic selection and domestication in white spruce. In a study of white spruce phenotypic selections from Ontario, allelic richness was found to be reduced by 25% as compared to natural populations (Cheliak et al. 1988); however, heterozygosity was not significantlly different in the selected versus random populations. Reduced observed heterozygosity and allelic richness were identified in the seed orchard clones of interior spruce [a complex consisting of white spruce, Engelmann spruce (*Picea engelmanii*) and their hybrids] as compared to their breeding zone populations (Stoehr and El-Kassaby 1997). However, allelic richness was comparable and heterozygosities were higher in white spruce selections from Quebec (Desponts et al. 1993).

The objectives of the present study were to: (1) benchmark and compare the genetic diversity of the pristine old-growth, post-harvest naturally regenerated, post-harvest planted, and phenotypically selected populations of white spruce from Saskatchewan, (2) determine the impact of silvicultural management system on genetic diversity in white spruce, and (3) provide benchmark information for developing genetic-biodiversity criteria and indicators for sustainable forest management and the conservation of white spruce genetic resources. Fifty one random amplified polymorphic DNA (RAPD) loci were used to determine and compare genetic diversity and genetic relationships among pristine old-growth, natural regeneration, plantations and open-pollinated progeny of phenotypic selections of white spruce from Saskatchewan.

Materials and methods

White spruce populations/stands and trees, and controlled crosses

The study sites were located in two geographically distant areas in Saskatchewan: the Prince Albert Model Forest area in the north and the Hudson Bay-Maloneck Creek area in the southeast (Table 1). In the Prince Albert Model Forest area, adjacent natural old-growth, naturally regenerated and planted white spruce stands/populations located at each of three sites were studied (Table 1). In the Hudson Bay-Maloneck Creek area, natural old-growth and natural regeneration at the Prairie River and the nearest plantation at Tee Pee Creek, and the oldest planted stand at Maloneck Creek, were sampled (Table 1). Thirty individual trees were sampled randomly from each of the 13 white spruce populations. Additionally, one open-pollinated progeny each of 30 individual phenotypic selections, made by Weyerhaeuser Canada in different parts of Saskatchewan for tree-improvement porposes, were sampled (Table 1). Although the open-pollinated (OP) progeny sampled, represent only maternal genetic contributions of the phenotypic selections, these, for simplicity, are referred to as tree improvement selections, phenotypic selections or selections at places in this paper. White spruce occurs as a high-density mixed-wood species along with trembling aspen (*Populus tremuloides*), white birch (*Betula papyrifera*) and balsam fir (*Abies balsamea*) in the four natural old-growth stands sampled. The natural regeneration sampled at each site was from the same stands as old-growth, which appeared on the edges or cut lines of the stand after harvested for road and power-line constructions. At the Prairie River site, there was some advanced natural regeneration within the matured stand that was sampled. At the Scales Lake site, the sampled natural regeneration was about 50–100 m away from the old-growth stand. Foliage samples were collected from each sampled tree, stored on ice and brought to the University of Alberta.

Table 1 White spruce stands/populations and selections studied

Area/location	Latitude (N)	Longitude (W)	Altitude (m)	Stand type	Age (years)
(1) Northern Saskatchewan: Prince Albert Model Forest					
Timber Cove	53°58′	105°54′	531	Natural old-growth Natural regeneration Plantation	100–130 15–20 13
Scales Lake	54°03′	105°28′	598	Natural old-growth Natural regeneration Plantation	100–150 15–25 17
Snowfield Road	53°42′	106°03′	605	Natural old-growth Natural regeneration Plantation	100–150 10–20 6
(2) Southeastern Saskatchewan: Hudson Bay–Maloneck Creek					
Prairie River	52°50′	102°40′	440	Natural old-growth Natural regeneration	100–130 20–40
Tee Pee Creek	52°58′	102°37′	420	Plantation	40
Maloneck Creek	52°08′	101°51′	500	Plantation	54
(3) Various Saskatchewan Locations					
Tree-improvement selections		53°–55°	104°–108°	Open-pollinated progeny	1

Table 2 RAPD primers, their sequences and the RAPD loci amplified and scored

Primer	Sequence	RAPD loci
UBC289	ATC AAG CTG C	*U289–2257, U289–2005, U289–1731, U289–1455, U289–1340, U289–1087, U289–1002, U289–926, U289–772, U289–699, U289–644, U289–438*
UBC295	CGC GTT CCT G	*U295–1765, U295–1516, U295–1384, U295–1262, U295–1160, U295–1063, U295–988, U295–887, U295–785, U295–672, U295–594, U295–555*
UBC296	CCG CTG GGA G	*U296–1456, U296–1088, U296–914, U296–795, U296–661, U296–520, U296–448, U296–294*
UBC299	TGT CAG CGG T	*U299–2041, U299–1784, U299–1524, U299–1403, U299–1260, U299–1015, U299–856, U299–737, U299–670, U299–634*
UBC300	GGC TAG GGC G	*U300–1444, U300–1205, U300–1090, U300–989, U300–903, U300–829, U300–705, U300–639, U300–586*

For valid use and correct interpretation of genetic markers, their genetic control must be determined by inheritance studies. Parents and F_1 progeny of four white spruce controlled crosses were used to determine the genetic control of random amplified polymorphic DNA (RAPD) markers. F_1 hybrid seeds and foliage of the parents of the four controlled crosses made by Weyerhaeuser Canada were procured. Seeds were germinated in a growth chamber, and 45 F_1 individuals per cross were analyzed.

Genetic analysis

Genetic-diversity analysis was conducted by using RAPD molecular markers. DNA was extracted from each of the 420 individuals sampled from the 13 white spruce populations and open-pollinated progeny of phenotypic selections according to a protocol modified from Murray and Thompson (1980). DNA was also extracted from the foliage of the four parents and 180 individual progeny of their four controlled crosses. RAPD techniques for white spruce genetic-fingerprinting and genetic-diversity analysis were developed. After the pre-screening of many primers from a set of 100 decamer RAPD primers procured from the University of British Columbia, five primers (Table 2) were selected for use in the genetic-diversity analysis. Genetic variation within and among white spruce populations was examined for 30 individual trees per population (Table 2).

A PCR reaction of 20 µl contained 30 ng of white spruce template DNA, dNTPs at a final concentration of 200 µM, 8 mM of Tris-HCl-KCl buffer, pH 8.3, 2 mM of magnesium chloride, 0.378 µM of primer and 1 unit of *Taq* polymerase. PCR reactions were conducted in a Perkin Elmer 480 or 9600 Thermal Cycler. The amplification cycle consisted of 40 cycles of 94°C for 1 min, 37°C for 1 min and 72°C for 2 min, with an initial denaturing at 94°C for 2 min and a final extension at 72°C for 5 min. The amplified DNA products were separated by electrophoresis on 1% agarose gels, with 0.33 µg of ethidium bromide in 1 x TBE buffer at 200 V. A DNA-mass ladder and/or a 1-kb DNA ladder (Gibco BRL, Life Technologies, Gaithersburg, Md., USA) were used as DNA size markers.

Mendelian inheritance of the RAPD markers was determined by examining their segregation in the F_1 progeny of the four controlled crosses. There were 65 consistent RAPD loci resolved by five primers. However, only 51 of these markers showed single-locus Mendelian inheritance (Table 2), and were therefore included in the study. The RAPD loci were designated by the primer name and size of the amplified DNA product (Table 2).

Data analysis

The RAPD fingerprint of individual trees were scored in terms of positive or null phenotypes of each for the 51 RAPD markers which showed single-locus Mendelian inheritance (Table 2).

RAPD markers are dominant markers with a two-allele system. These markers show positive and null phenotypes on the gels. It is difficult to differentiate between homozygotes and heterozygotes for the positive phenotypes. The null homozygotes can be identified by the absence of any amplified product (null phenotypes). White spruce has been shown to conform to random mating in earlier studies (King et al. 1984; Cheliak et al. 1985). Therefore, the frequency of the two alleles at a RAPD locus could be calculated from the frequency of null phenotypes (homozygous recessive), q^2, as follows: $q=\sqrt{q^2}$; and $p=1-q$.

The data on allele frequencies and on the frequency and individual absence and presence of RAPD fragments were analyzed by using Biosys-1 (Swofford and Selander 1989) and PopGene (Yeh and Boyle 1997) softwares. The following genetic-diversity parameters were calculated: proportions of loci polymorphic, average number of alleles per locus, effective number of alleles per locus, mean heterozygosity, the number of unique genotypes, and Shannon's index. The Shannon's index was calculated from both RAPD fragment and allele frequencies. The neutrality of the 51 RAPD markers was tested according to Manly (1985), using PopGene. Most of the 51 RAPD markers conformed to neutrality. The number of marginally significant depatures observed ranged from 2 (Scales Lake plantation) to 9 (Snowfield Road old-growth), with an average of 4.64 per population. These departures could be expected by chance. Only three RAPD loci showed a significant departure in selections. Therefore, the 51 RAPD markers used could at best be considered as neutral markers. Unbiased estimates of genetic distances among old-growth, natural regeneration, plantation at each site and phenotypic selections, and among all the 14 individual populations were calculated (Nei 1978) and cluster plots of the populations were constructed based on the unweighted pair group method (UPGMA) analysis of genetic distances. Heterogeneity of allelic frequencies at RAPD loci over all populations, and over the three population types at each site and the phenotypic selections, was examined by using χ^2 and G^2 tests. The differences in genetic-diversity parameters of the three population types at each location and phenotypic selections, and of overall old-growth, natural regeneration, plantation and selections were tested by the Analysis of Variance and Duncan's Multiple Range Test, using SAS (Windows version 6.11; SAS 1995).

Results

Fifty of the fifty one RAPD markers were polymorphic in at least one population; only one locus (*U300–1205*) was monomorphic in all the populations. Each of the 420 individuals from the 13 stands and 30 progeny of the 30 phenotypic selections sampled (Table 1) was found to have a unique multilocus genotype. There were significant differences in primers for revealing genetic diversity in the white spruce populations examined. Generally the primer UBC295 revealed the highest, and the primer UBC300 the lowest, genetic diversity. Representative RAPD profiles (fingerprints) revealed by UBC299 are given in Fig. 1. Significant heterogeneity was observed in allele frequencies at a number of RAPD loci over different population types at each location (Table 3). In a pair-wise comparison of any two of the old-growth, natural regeneration and plantation at each location, the lowest number of RAPD loci showing significant allele-frequency heterogeneity was between old-growth and natural regeneration, as expected; whereas the highest number was between natural regeneration and plantation (Table 3). The number of RAPD loci that showed significant allele-frequency heterogeneity among different populations of the same stand types was as follows: 27 in the four old-growth populations, 40 in the four naturally regenerated populations, and 40 in the five plantations.

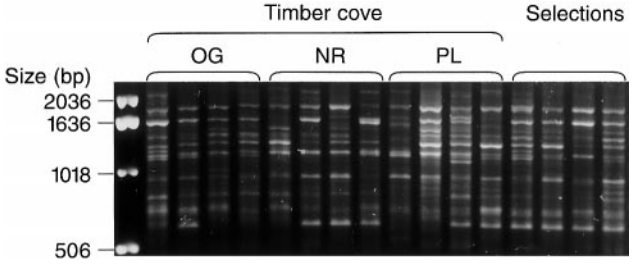

Fig. 1 Representative RAPD fingerprints/profiles of select white spruce trees from the old-growth (*OG*) stand, natural regeneration (*NR*), and plantation (*PL*) at Timber Cove, and from the OP progeny of phenotypic selections (*Selections*) produced by primer UBC299

The old-growth populations/stands at each site consistently had the highest genetic diversity as compared to the natural regeneration and plantations from the same sites (Table 4). The genetic diversity of the natural regeneration was comparable to that of the old-growth populations but substantially higher than that of the plantations and selections (Table 4). Among the old-growth stands as well as natural regeneration, Snowfield Road populations were the most and the Scales Lake populations the least genetically variable. However, there were no significant differences among the old-growth stands for genetic-diversity parameters. Among the plantations, the Timber Cove population was the most and the Scales Lake population the least genetically diverse. However, genetic-diversity levels of four of the five plantations studied

Table 3 Number of RAPD loci showing significant (5%) allele-frequency heterogeneity among different stand types. OG, old-growth; NR, natural regeneration; PL, plantation; SEL, OP progeny of phenotypic selections

Location	Four populations	Three populations	Two populations		
	OG-NR-PL-SEL	OG-NR-PL	OG-NR	OG-PL	NR-PL
Timber Cove	36	29	16	20	25
Scales Lake	26	22	10	19	15
Snowfield Road	42	30	22	23	23
Prairie River	37	32	17	20	26
Mean	35.25	28.25	16.25	20.50	22.25

Table 4 RAPD genetic-diversity parameters for individual white spruce stands/populations and over all different stand types. P, percentage of loci polymorphic; A, mean number of alleles per locus; Ae, mean effective number of alleles per locus; H, mean expected heterozygosity; SIF, Shannon's index based on the RAPD fragment frequency; SIA, Shannon's index based on the RAPD allele frequency

Location/stand type	Mean genetic-diversity parameters[a]					
	P	A	Ae	H	SIF	SIA
(1) Individual populatios						
Timber Cove						
Old-growth	90.2	1.90	1.68	0.387	0.421	0.551
Natural regeneration	86.3	1.86	1.64	0.359	0.382	0.514
Plantation	76.5	1.80	1.57	0.322	0.399	0.459
Scales Lake						
Old-growth	84.3	1.84	1.65	0.366	0.382	0.520
Natural regeneration	72.6	1.73	1.55	0.312	0.331	0.445
Plantation	60.8	1.61	1.49	0.270	0.306	0.382
Snowfield Road						
Old-growth	94.1	1.94	1.74	0.412	0.429	0.588
Natural regeneration	94.1	1.94	1.73	0.409	0.491	0.579
Plantation	76.5	1.76	1.55	0.315	0.380	0.452
Prairie River						
Old-growth	86.3	1.86	1.68	0.375	0.433	0.531
Natural regeneration	84.3	1.84	1.60	0.343	0.412	0.494
Tee Pee Creek Plantation	74.5	1.75	1.56	0.317	0.345	0.451
Maloneck Creek						
Plantation	72.6	1.73	1.50	0.290	0.326	0.419
Tree-improvement selections	66.7	16.7	1.46	0.264	0.272	0.381
(2) Stand types (overall)						
Old-growth	88.7A	1.89A	1.69A	0.381A	0.416A	0.548A
Natural regeneration	83.8A	1.84A	1.62A	0.349A	0.404A	0.506A
Plantation	72.2B	1.72B	1.53B	0.297B	0.351A	0.431B
Tree-improvement selections	66.7B	1.67B	1.46B	0.259B	0.272B	0.381B

[a] Means for each genetic-diversity parameter for the stand types followed by the same letter are not significantly different at $P<0.05$ according to Duncan's multiple range test

were comparable (Table 4). At each of the four sites, genetic diversity of the natural regeneration was not significantly different from that of the old-growth population. Whereas, the plantations and selections generally had substantially lower genetic diversity than the old-growth or natural regeneration at each location (Table 4). However, the differences were statistically significant only for the Scales Lake and Snowfield Road populations. The genetic diversity of the plantations from each site was not significantly different from that of the selections. Nevertheless, the selections showed significantly reduced genetic diversity when compared to the old-growth and natural regeneration at each of the four sites studied.

The overall means for RAPD genetic-diversity parameters for the old-growth, natural regeneration and plantation types of white spruce populations are given in Table 4. As for the individual sites, overall the old-growth stands had the highest, and the open-pollinated progeny of the tree-improvement selections the lowest, genetic diversity (Table 4). Also, as was the case for individual sites, overall genetic diversity of the natural regeneration was comparable and not significantly different from that of the old-growth. The plantations and phenotypic selections had significantly reduced genetic diversity as compared to the old-growth and natural regeneration. Although overall genetic diversity of the phenotypic selections was the lowest, it was not significantly different from that of the plantations. The mean and range of genetic-diversity reductions in natural regeneration, plantations and phenotypic selections in comparison to old-growth stands can be calculated from the data in Table 4. As compared to the old-growth, the mean (and range) for reductions in different genetic-diversity parameters were as follows: 2.3–7.8% (gain of 14.4% to a reduction of 14.8%) in the natural regeneration, 8.6–20.5% (5.2–27.9%) in the plantations, and 11.4–34.5% (9.2–37.2%) in the OP progeny of the phenotypic selections. Most of the reduction in mean genetic-diversity parameters for the natural regeneration was contributed by the Scales Lake population. The highest reductions were for heterozygosity in the natural regeneration and plantations, and for the RAPD-fragment frequency based Shannon's index, followed by heterozygosity in the selections.

Within each stand type, the lowest genetic distances were among the old-growth stands and the largest among the plantations (Table 5). On average, among different population types, the lowest genetic distances were between the old-growth and natural regeneration, with the largest between the plantations and natural regeneration (Table 5). Overall, the old-growth showed higher genetic distances from the plantations and phenotypic selections than from the natural regeneration. When genetic differentiation from the F_{ST} estimates among the four population types was compared, the least differentiation was observed between the old-growth and natural regenera-

Table 5 Means and (range) of genetic distances (Nei 1978) among (below the diagonal) and within (at the diagonal) stand types of white spruce

Stand type	No. of populations	Old-growth	Natural regeneration	Plantation
Old-growth	4	0.043 (0.014–0.069)		
Natural regneration	4	0.069 (0.023–0.102)	0.087 (0.054–0.117)	
Plantation	5	0.073 (0.043–0.118)	0.091 (0.033–0.169)	0.103 (0.068–0.171)
Selections (OP progeny)	1	0.076 (0.044–0.099)	0.088 (0.029–0.155)	0.087 (0.032–0.126)

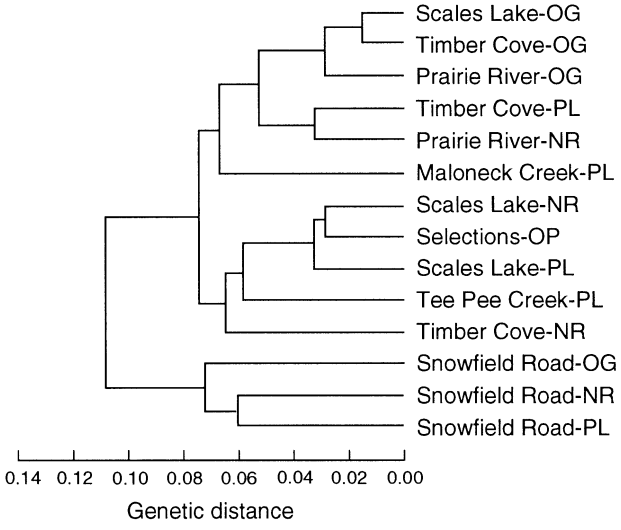

Fig. 2 UPGMA cluster plot of all 14 white spruce populations based on genetic distances (Nei 1978). *OG*, old-growth; *NR*, natural regeneration; *PL*, plantation; and *OP*, open-pollinated progeny of the phenotypic selections

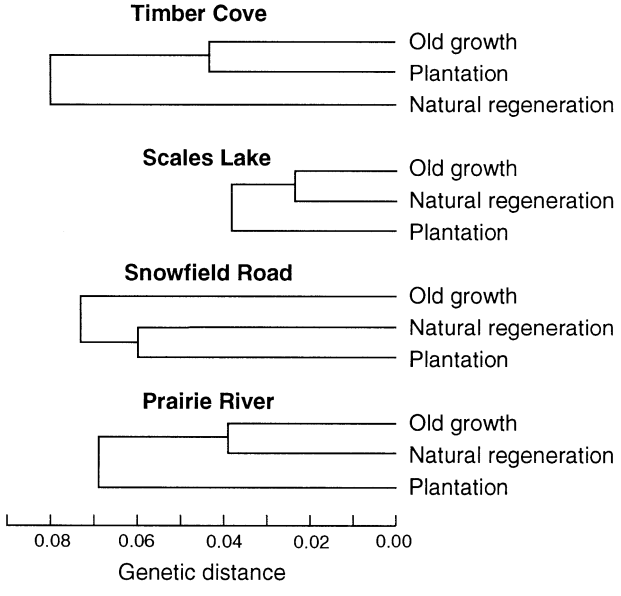

Fig. 3 UPGMA cluster plot of the old-growth, natural regeneration and plantation at each of the four sites based on genetic distances (Nei 1978)

tion at each of the four sites. The 14 populations clustered into three groups based on their genetic distances (Fig. 2). Three of the four old-growth populations clustered closely together in the same group, whereas the Snowfield Road old-growth population was in a separate group, consisting of all different stand types from that location (Fig. 2). The natural regeneration, plantations and selections clustered in two other, or all three, groups (Fig. 2). From the UPGMA cluster based on genetic distances (Nei 1978) among the old-growth, natural regeneration and plantation for each site, the old-growth and natural regeneration clustered closer together than either with the plantations for the Scales Lake and Prairie River sites (Fig. 3). At the Timber Cove site, the old-growth was closer to the plantation than to the natural regeneration, and at the Snowfield Road site the natural regeneration was closer to the plantation than to the old-growth (Fig. 3). When selections were added for clustering with the other three population types at each site, they either clustered with the plantations or else formed a separate group (data not shown).

Discussion

The study indicates that the plantations and phenotypic tree-improvement selections have significantly reduced genetic diversity as compared to the old-growth and natural regeneration, suggesting their narrower genetic base than that of the old-growth and natural regeneration. This is consistent with similar results reported for the comparison of old-growth, natural regeneration and plantations of Norway spruce (Gomory 1992) but contrast with the fact that no significant allelic heterogeneity was observed between the natural stands and plantations of jack pine and black spruce (Knowles 1985). However, the jack pine and black spruce study was based on only five allozyme loci.

The plantations studied have been established from nursery raised seedlings originating from bulk seeds. The origin and details of the seed collections, and the nursery and seedling-handling conditions are unknown. It may be possible that the seeds for these plantations may have come from a relatively small numbers of seed trees, which would narrow their genetic base. Nursery raised

seedlings are subject to a number of selective factors that may also affect genetic diversity. The nursery environment is quite different from, and more homogeneous than, that of the natural forest. Selection most likely operates differently in the nursery than in the forest. In addition to a relatively narrow genetic base of the plantations, nursery environmental factors may also have contributed towards reduced genetic diversity in the plantations.

Genetic diversity, especially heterozygosity, is expected to manifest better in heterogeneous environments (Lerner 1954). Also, genetic diversity is expected to be increased with age. Therefore, the highest genetic diversity observed for the old-growth stands was no surprise. The old-growth stands studied represent a well-adapted climax, or almost-climax, white spruce gene pool. Nevertheless, genetic diversity of the sampled natural regeneration and plantations was not related to age. The five plantations sampled in the present study were of 6–54 years of age. Irrespective of their age, different plantations have very similar levels of genetic diversity. The only exception was the Scales Lake plantation, which had the lowest genetic diversity of all plantations. Although the genetic diversity of the five plantations was comparable, their genetic base seems to be different because the plantations had the highest genetic distances and showed allele-frequency heterogeneity at the highest number of loci among themselves, and different plantations grouped separately based on genetic distances. This is one of the positive aspects for maintaining genetic diversity at the landscape level. Nonetheless, the results suggest that, in order to maintain genetic diversity in plantations, their genetic base needs to be broadened, and seed collections need to be monitored so that the seed is a mix from a large numbers of seed parents. Despite the fact that the age of the natural regeneration was similar to, and in some cases lower than, that of the plantations surveyed, the natural regeneration had a higher genetic diversity than the plantations. The natural regeneration also showed higher genetic similarities to the old-growth stands as was evident from the genetic-distance and allele-frequency heterogeneity analyses. Therefore, the present results suggest that the genetic diversity and integrity of the white spruce forest could be maintained by the natural regeneration system.

The tree-improvement selections, the progeny of which we sampled, were made for the same desired phenotypic characteristics: height, diameter branch angle, branch diameter, stem form, taper, crown shape, pruning and bark characteristics, and volume. Thus, their genetic base is expected to be narrower. However, given that, with a few marginal departures, the RAPD markers that we used conformed to neutrality, we expected higher genetic diversity in tree-improvement selections because of their origin from widely distant areas. Nevertheless, in contrast, we found their genetic base to be the narrowest as compared to the old-growth, natural regeneration and plantations. The results for reduced genetic diversity in the phenotypic tree-improvement selections reported here are consistent with the reduced allelic richness in white spruce phenotypic selections from Ontario (Cheliak et al. 1988), and reduced allelic richness and heterozygosity in seed-orchard clones of interior spruce from British Columbia (Stoehr and El-Kassaby 1997), but contrast with the increased variability in a selected white spruce population from Quebec (Desponts et al. 1993). All of these earlier studies were based on 12–17 allozyme loci, whereas the present study was based on 51 RAPD loci. Enzyme-coding (allozyme) genes belong to a particular class of genes, and represent only a very small portion of the conifer genome and, therefore, may not represent a random sample of the entire genome. By contrast RAPD markers may sample the genome randomly. In other conifers, seed-orchard clones or advanced-generation breeding stocks have been found to have a genetic diversity that is comparable to, or even higher than, that of the natural populations (reviewed in Savolainen and Karkkainen 1992; El-Kassaby 1995; Williams et al. 1995; El-Kassaby and Ritland 1996). However, all these studies were based upon isozyme markers and used expected heterozygosity as a primary measure of genetic diversity. Heterozygosity is a measure of allelic evenness, which is not as sensitive to reflect changes due to bottlenecks as allelic-richness measures. Drift depletes genetic variance (heterozygosity) very slowly at the rate of $1/2N_e$ per generation, where N_e is the effective population size. Theoretically two individuals could maintain 50% of the original heterozygosity. Therefore, populations with a moderate number of individuals could loose little of their expected heterozygosity in one generation. However, in the study reported here most reductions were for heterozygosity, suggesting a real loss of genetic diversity in the plantations and selections. It should be noted that some of the significant genetic variation, e.g., disease and insect resistance, could be in the form of allelic richness and that RAPD markers, due to their dominant and diallelic nature, underestimate allelic richness. Nevertheless, the present study unequivocally suggests that the genetic base of the phenotypic tree-improvement selections is narrow, and needs to be broadened in order to maintain genetic diversity.

The phenotypic tree-improvement selections, the open-pollinated progeny of which were surveyed, have been used by Weyerhaeuser Canada to establish a clonal seed orchard in Saskatchewan. Seeds from this orchard will soon be used for artificial regeneration. Thus the narrower genetic base of these selections could be passed on to the plantations arising from the seed-orchard seeds. White spruce is primarily regenerated artificially through plantations in Saskatchewan. Thus, for sustainable management and conservation of white spruce genetic resources, the genetic base of the tree-improvement selections needs to be broadened. One way to broaden the genetic base in a tree-improvement program is to maintain multiple breeding populations (Namkoong 1984).

Conclusions

The plantations and phenotypic tree-improvement selections sampled have significantly reduced genetic diversity as compared to the old-growth and naturally regenerated white spruce. The old-growth stands have the highest, and the tree-improvement selections the lowest, genetic diversity. Therefore, the genetic base of the plantations and selections is much narrower than that of the natural old-growth and natural regeneration, which needs to be broadened to ensure the conservation and sustainable management of white spruce genetic resources. As the genetic diversity of the natural regeneration was comparable to, and not significant different from, that of the old-growth stands, it could be concluded that genetic diversity in white spruce could be maintained by a prober natural regeneration silvicultural system. It is generally known that white spruce does not regenerate well after clearcuts. Therefore, it is primarily regenerated by plantations after clearcuts. Natural regeneration under properly designed shelterwood or seed-tree systems may not only help in the regeneration of white spruce but may also assist in maintaining genetic diversity.

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