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O. P. Rajora · S. A. Pluhar

Genetic diversity impacts of forest fires, forest harvesting, and alternative reforestation practices in black spruce (Picea mariana)

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Abstract Benchmarks were established for genetic diversity inherent in natural mature populations, and genetic diversity impacts of forest fires, clearcut harvesting and alternative natural and artificial silvicultural regeneration practices were determined in black spruce (*Picea mariana*). Allozymes of 32 loci were used to determine and compare genetic diversity and genetic relationships of adjacent or nearby four stand types: post-fire natural mature (FNM), post-fire natural young (FNR), post-harvest natural young (HNR) and post-harvest plantation (PLT), of black spruce at each of the four study sites located in two ecoregions in Manitoba: Ecoregion 90-Lac Seul Upland (Eastern) and Ecoregion 158 - Mid-Boreal Lowland (Northern). Both allelic- and genotypic-based genetic diversity parameters, as well as latent genetic potential, were determined. Black spruce populations showed typical moderate to high levels of allozyme genetic diversity. The mean genetic diversity parameters over the 16 black spruce populations sampled were as follows: percent loci polymorphic – 67%, mean number of alleles per locus – 2.52, effective number of alleles per locus – 1.70, observed heterozygosity – 0.222, expected heterozygosity – 0.308, mean number of observed genotypes per locus – 3.65, mean number of expected genotypes per locus – 5.03, genotype additivity (observed) – 116.8, genotype additivity (expected) – 161, genotype multiplicity (observed) – 6.16×10^{15}, genotype multiplicity (expected) – 2.06×10^{19} and latent genetic potential – 26.12. The four stand types (FNM, FNR, HNR and PLT) had comparable and statistically similar genetic diversity levels at each of the four study sites as well as overall. No significant differences in black spruce genetic diversity levels were observed between the two ecoregions in Manitoba, as well as between the post-fire and post-harvest regenerated stands. No particular order of genetic relatedness among the four stand types was observed. Black spruce populations showed some sort of site-related differentiation in their genetic constitution. Allelic heterogeneity and genetic distances among populations within stand types and among four stand types suggest that the genetic diversity was maintained at the landscape level in black spruce. The results of our study demonstrate that forest fires and currently used clearcut harvesting, and alternative natural and artificial silvicultural regeneration practices, do not adversely affect genetic diversity in black spruce, and that the genetic diversity effects of clearcut harvesting are not significantly different from those due to forest fires in black spruce.

Keywords Forest management practices · Conservation of forest genetic resources · Genetic biodiversity · Genotypic diversity · Silviculture · Natural regeneration · Plantations

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O. P. Rajora (✉)
Department of Biology, Life Sciences Centre,
Dalhousie University, Halifax, NS B3H 4J1, Canada
e-mail: Om.Rajora@dal.ca
Tel.: (902)-494-2400, Fax: (902)-494-3736

S. A. Pluhar
Department of Renewable Resources, University of Alberta,
Edmonton, AB T6J 2H1, Canada

Present address:
O. P. Rajora, 9 Broadview Avenue, St. Catharines, ON L2T 2H1, Canada

Introduction

Genetic diversity provides the raw material for adaptation, evolution and survival of species and individuals, especially under changed environment and disease conditions. Since trees are normally the keystone species of the forest ecosystems, their genetic diversity has a special significance. Reductions in genetic diversity can predispose forests to the environment-related decline in health and productivity (e.g., Bergmann et al. 1990; Oleksyn et al. 1994; Raddi et al. 1994). Genetic variability is also the basis for tree improvement. Thus, genetic

diversity can be viewed as the foundation for forest sustainability and ecosystem stability. The Canadian Standards Association has identified genetic diversity as one of the criteria/indicators for registration, certification and audit of the sustainable forest management system (CSA 1996a, b). Benchmarking genetic diversity in natural pristine forest tree populations and determining the genetic impacts of forest management practices and natural disturbance on forest tree genetic diversity, and subsequent monitoring, can provide resource managers with an indicator of long-term forest sustainability and ecosystem health (Mosseler and Rajora 1998; Rajora 1999a; Rajora and Mosseler 2001a, b).

Forest management practices based on partial or clear-cut forest harvesting and natural and/or artificial regeneration systems can significantly impact genetic variability in residual or regenerated forest populations (Buchert et al. 1997; Rajora 1999b; Rajora et al. 2000). Natural disturbances, such as forest fires, may also affect genetic diversity. However, genetic implications of forest management practices and natural disturbances are largely unknown for most Canadian species. It has been generally speculated that forest fires can have a significant effect on gene frequencies and genetic drift (e.g., Teich 1970; Perry and Lotan 1979; Chapman and Crow 1981). However, there is little or no information on the genetic effects of natural or prescribed forest fires. Also, it was not known whether the genetic effects of forest fires compare to that due to harvesting practices. Forest fires have been an integral part of the boreal forest ecosystems. It is often proposed that the ecosystem-based natural disturbance regime should be used as a basis for forest management in Canada. However, genetic data either to support or contradict this proposition is generally lacking. Allozymes and a number of DNA markers can assist in determining the genetic diversity and structure of populations, estimating evolutionary genetic processes that maintain genetic diversity, determining genetic impacts of forest management practices and assisting the development of genetically sustainable forest management practices (Rajora 1999a; Rajora and Mosseler 2001a, b).

Specific information on the effects of forest fires and forest management practices on genetic diversity and biological processes affecting genetic diversity is rudimentary, particularly for Canadian forest trees. Rajora and his collaborators have examined and benchmarked genetic diversity inherent in natural populations and determined impacts of silvicultural practices on genetic diversity in white spruce (*Picea glauca*) in Saskatchewan managed under artificial and natural regeneration systems (Rajora 1999b), and in eastern white pine (*Pinus strobus*) managed under shelterwood and seed-tree natural regeneration systems in Ontario (Buchert et al. 1997; Rajora et al. 2000; Rajora et al., in preparation). Artificial regeneration of white spruce and post-harvest residual stands of eastern white pine showed significant reduction in genetic diversity relative to pristine old-growth stands (Buchert et al. 1997; Rajora 1999b; Rajora et al. 2000). However, genetic diversity was maintained in post-harvest natural regeneration in white spruce (Rajora 1999b).

Black spruce (*Picea mariana* (Mill.) B.S.P.) is a widespread transcontinental and important tree species of the Boreal forest and is found in almost all forested regions of Canada with the exception of the Rocky Mountains and the Pacific coast (Hosie 1979). It is one of the most important trees in Canada for the production of pulp and paper and is the most important reforestation species east of the Rocky Mountains in Canada. Black spruce is normally harvested under the clearcut harvesting system and is regenerated naturally or artificially after harvest. Forest fires are natural in the Boreal forest, and black spruce regenerates well after forest fires (Sirois and Payette 1989; St Pierre et al. 1992). A tree improvement program is also in place for this species. For the development of effective strategies for sustainable and ecosystem-based management and conservation of black spruce genetic resources, it is essential to determine and benchmark the amount and pattern of inherent genetic diversity of component populations, and to determine the comparative effects of natural disturbance (forest fires), forest harvesting and different reforestation practices on genetic diversity in black spruce. Genetic diversity of black spruce has been examined for populations from Newfoundland (Yeh et al. 1986), New Brunswick (Boyle and Morgenstern 1987), Quebec (Desponts and Simon 1987) and Ontario (O'Reilly et al. 1985). However, there is no reported information on the genetic diversity of black spruce from Manitoba, as well as on genetic effects of forest fires and alternative harvesting and reforestation practices in this species. Although, no significant allelic heterogeneity was reported among mature and young naturally regenerated and young planted black spruce from Ontario (Knowles 1985), this study was based on such a small number of allozyme loci (five) that may not allow making any meaningful conclusions.

The objectives of the present study were: (1) to determine genetic diversity inherent in post-fire natural mature, post-fire natural young, post-harvest natural young and post-harvest planted stands of black spruce in Manitoba, and (2) to determine and compare the effects of forest fires, forest management practices of clear-cut <harvesting and natural or artificial regeneration on genetic diversity in black spruce. We used 32 allozyme loci coding for 15 enzymes to determine and compare genetic diversity and genetic relationships among pristine post-fire natural mature, post-fire natural young, post-harvest natural young and post-harvest planted stands of black spruce from two different eco-regions and four different locations from Manitoba in Canada.

Materials and methods

Forest harvesting and reforestation practices, and black spruce stands/populations sampled

Black spruce (*Pinus mariana* (Mill.) B.S.P.) is primarily managed under clear cut harvesting followed by natural or artificial regener-

Table 1 Black spruce populations surveyed and their physical and geographical parameters

Region	Site	Stand type	Poulation ID	Longitude W	Latitude N	Altitude (m)	Mean age (years)	Mean height (m)	Mean DBH (cm)
Eastern	Pine Falls (E1)	Post-fire natural mature	E1-FNM	95°54.2′	50°41.0′	274	69	12.6	16.5
		Post-fire natural young regeneration	E1-FNR	95°53.5′	50°40.9′	274	11	2.4	2.0
		Post-harvest natural regeneration	E1-HNR	95°54.2′	50°41.0′	274	13	2.9	2.3
		Plantation	E1-PLT	95°54.5′	50°39.9′	290	12	3.9	6.1
	Bissett (E2)	Post-fire natural mature	E2-FNM	95°16.8′	50°46.7′	320	65	13.3	14.7
		Post-fire natural young regeneration	E2-FNR	95°17.2′	50°47.0′	320	12	1.7	1.0
		Post-harvest natural regeneration	E2-HNR	95°16.8′	50°46.7′	320	10	1.5	0.8
		Plantation	E2-PLT	95°19.0′	50°49.9′	305	11	2.3	1.5
Northern	The Pas (N1)	Post-fire natural mature	N1-FNM	101°23.4′	54°17.5′	294	69	14.5	18.3
		Post-fire natural regeneration	N1-FNR	101°22.8′	54°15.9′	294	5	1.3	0.9
		Post-harvest natural regeneration	N1-HNR	101°28.9′	54°17.7′	290	12	2.8	3.3
		Plantation	N1-PLT	101°26.6′	54°17.6′	290	10	3.5	3.2
	Snow Lake (N2)	Post-fire natural mature	N2-FNM	99°45.5′	54°54.0′	290	72	12.3	14.8
		Post-fire natural young regeneration	N2-FNR	99°58.6′	54°43.3′	274	5	0.5	–
		Post-harvest natural regeneration	N2-HNR	99°45.5′	54°54.0′	290	8	1.4	0.9
		Plantation	N2-PLT	99°45.5′	54°54.0′	290	8	1.1	0.4

ation systems. We included both of these alternative forest reforestation practices in our present study. The study sites were selected in two ecoregions in Manitoba: Ecoregion 90-Lac Seul Upland (Eastern) and Ecoregion 158 - Mid-Boreal Lowland (Northern). In each ecoregion, two separate study sites/areas were selected. At each study site, neighbouring or nearest (within a few km) four stand types (post-fire natural mature, post-fire natural immature, post-harvest natural young and post-harvest planted) were located and sampled (Table 1). The Pine Falls (E1) and Bissett (E2) study sites in the Eastern region are located in the Boreal Shield ecozone in the Manitoba Forest Section 31, a part of the Lake Winnipeg East Forest Management Unit. The Pas (N1) study site (Northern region) is located in the Boreal Plain ecozone in the Forest Management Section 56 of the Saskatchewan River Forest Management Unit. The Snow Lake (N2) study site (Northern region) is located in the Boreal Shield ecozone in the Forest Management Section 61 of the Highrock Forest Management Unit.

The post-fire natural young stands at the Pine Falls and Bissett sites originated following the 1983 forest fires. The post-harvest natural stand at the Pine Falls site originated after the forest harvesting of this area in 1983–1985. The post-harvest natural stand at the Bissett study site originated after the forest harvesting of this area in 1985. The studied post-harvest planted stand at the Pine Falls site originated from the 1985 plantation, whereas the planted stand at the Bissett site originated from the 1984 plantation. The post-fire natural young stand at the Pas site originated after the 1988 forest fire, whereas the post-fire natural young stand at the Snow Lake study site originated after the 1987 forest fire. The post-harvest natural stand at the Pas site regenerated naturally after the forest harvesting in 1985, whereas the planted stand at this site originated from the 1983 plantation. No information was available on the year of fire or plantation for the stands studied at the Snow Lake site.

In each stand at each site, 35 individual trees were sampled randomly and data on height, diameter and age of the sampled trees were recorded. The foliage was collected from each individual sampled tree. The planted stands also had a very small number of naturally regenerated black spruce. Since these black spruce individuals also constituted the gene pool of the post-harvest planted stands, no distinction was made between the planted and natural black spruce individuals for sampling in the plantations.

Enzyme electrophoresis and genotyping

Needle tissues were used for enzyme electrophoresis. Enzymes were extracted by manual grinding of needles in the extraction buffer (Rajora and Dancik 2000) with sterile sand, using a mortar and pestle. The enzyme extracts were adsorbed on to wicks made from Whatmann filter paper. The soaked wicks were stored at –70 °C until used for enzyme electrophoresis.

Horizontal starch-gel electrophoresis was used to resolve allozymes for 15 enzyme systems: acid phosphatase (ACP, EC 3.1.3.2), adenylate kinase (AK, EC 2.7.4.3), aspartate aminotransferase (AAT, EC 2.6.1.1), catalase (CAT, EC 1.11.1.6), colorimetric esterase (CE, EC 3.1.1.1), diaphorase (DIA, EC 1.6.4.3), glucose-6-phosphodehydrogenase (G6PDH, EC 1.1.1.49), glutamate dehydrogenase (GDH, EC 1.4.1.2), isocitrate dehydrogenase (IDH, EC 1.1.1.42), malate dehydrogenase (MDH, EC 1.1.1.37), peroxidase (PER 1.11.1.7), 6-phosphogluconate dehydrogenase (6-PGD, EC 1.1.1.44), phosphoglucose isomerase (PGI, EC 5.3.1.9), phosphoglucomutase (PGM, EC 2.7.5.1) and shikimate dehydrogenase (SKDH, EC 1.1.1.25). Genotypes of individual trees were inferred from allelic constitution and banding pattern in each individual. For enzymes encoded by multiple loci, the loci were numbered from the anodal to cathodal direction. Multiple alleles within a locus were also numbered in this fashion. A total of 32 allozyme loci was scored coding for 15 enzymes as follows: AAT (3), AK (2), ACP (2), CAT (2), CE (3), DIA (3), G6PDH (2), GDH (1) IDH (2), MDH (2), PER (3), PGI (2), PGM (2), 6-PGD (2) and SKDH (1).

Data analysis

Allele frequencies were calculated for each locus in each population. Chi-square goodness-of-fit tests, with and without Levene's (1949) corrections and exact probabilities, were performed to measure deviations from Hardy Weinberg equilibrium. Both allelic-based conventional and genotypic-based unconventional genetic diversity parameters were determined for each population. The conventional genetic-diversity parameters included the following: the percentage of loci polymorphic (P; 95% and 99% criteria), the average number of alleles per locus (A), the average number of effective alleles per locus (Ae), the unbiased estimate of heterozygosity expected under Hardy Weinberg equilibrium (He) (Nei 1978) and the observed heterozygosity (Ho). The number of private alleles in each sampled black spruce population was determined. The latent genetic potential, *LGP*, which is the difference

between the total number of alleles and the effective number of alleles summed over all loci (Bergmann et al. 1990), was calculated. The following genotypic diversity parameters were determined for each population: *G*, the mean number of genotypes (actually observed and that expected under Hardy Weinberg equilibrium) per locus; genotype additivity, G_A which is the sum of the number of single-locus genotypes actually observed or expected under Hardy Weinberg equilibrium over all loci (Rajora 1996; Rajora et al. 2000); and genotype multiplicity (G_M), the multiplication product of all genotype combinations actually observed or expected across all loci (Bergmann et al. 1990).

The effects of forest fires, clearcut harvesting and alternative reforestation practices on genetic diversity in black spruce were determined by comparing the genetic diversity of the post-fire natural young, post-harvest natural and post-harvest planted stands, with the genetic diversity of the post-fire natural mature stands. The significance of differences in genetic diversity levels among the four stand types, between two regions and among four study locations, was tested by the Analysis of Variance and Duncan's Multiple Range Test, using SAS (Windows version 8.2; SAS 2001). Correlations were determined between genetic diversity parameters and age, height and diameter of the sampled individuals and populations.

Genetic differentiation among all 16 black spruce populations, among four stand types and among populations within the same stand types, was determined by calculating allele frequency heterogeneity, genetic distances (Nei 1978) and hierarchical *F*-statistics (Wright 1978). Heterogeneity of allelic frequencies over all 16 populations and among four stand types at each location was examined using a contingency chi-square test (Workman and Niswander 1970). The unbiased estimates of genetic identities and distances among all populations, and among populations within and between stand types, were determined (Nei 1978). A cluster analysis of all 16 populations, as well as of four populations at each study site, was done based on the matrix of Nei's (1978) unbiased estimates of genetic identities, using the unweighted pair-group method with arithmetic averages (UPGMA).

Results

Allele frequencies and allelic differentiation

Two (*Cat2* and *Pgm2*) of the 32 allozyme loci were invariant in all 16 black spruce populations. Two to five alleles were detected at a polymorphic locus, with a total of 129 alleles detected in all 16 populations at the 30 polymorphic loci (data not shown). Most of the alleles were well spread over the populations; a few of them were found only in one population (Table 2). The number of private alleles detected within the regions, study sites and stand types were as follows: Eastern region – 13, Northern region – 15; Pine Falls – 5, Bissett – 5, the Pas – 8, Snow Lake – 4; post-fire natural mature stands – 5, post-fire natural young stands – 4, post-harvest natural

Table 2 Genetic diversity parameters (SE) for individual black spruce stands/populations and means for different stand types, sites and regions. FNM, post-fire natural mature; FNR, post-fire young natural regeneration; HNR, post-harvest natural regeneration; PLT, post-harvest plantation. Means for each genetic diversity parameter for the stand types, regions or sites followed by the same letter are not significantly different at $P < 0.05$ according to Duncan's multiple range test

Population	Percent loci polymorphic		Total no. alleles	Mean no. alleles/locus	Effective no. alleles/locus	Private no. alleles	Observed heterozygosity	Expected heterozygosity
	95%	99%						
E1-FNM	65.6	81.3	83	2.59 (0.18)	1.65	2	0.215 (0.037)	0.296 (0.045)
E1-FNR	62.5	75.0	74	2.31 (0.18)	1.61	1	0.176 (0.033)	0.265 (0.045)
E1-HNR	62.5	75.0	81	2.53 (0.20)	1.67	1	0.222 (0.040)	0.293 (0.047)
E1-PLT	65.6	71.9	81	2.53 (0.22)	1.62	1	0.198 (0.037)	0.279 (0.045)
E2-FNM	75.0	81.3	83	2.59 (0.19)	1.67	0	0.223 (0.035)	0.317 (0.042)
E2-FNR	75.0	81.3	86	2.69 (0.20)	1.72	1	0.263 (0.041)	0.335 (0.042)
E2-HNR	59.4	78.1	80	2.50 (0.19)	1.79	1	0.267 (0.047)	0.322 (0.05)
E2-PLT	71.9	75.0	85	2.66 (0.21)	1.75	2	0.240 (0.039)	0.328 (0.045)
N1-FNM	59.4	71.9	75	2.34 (0.19)	1.60	1	0.201 (0.044)	0.272 (0.046)
N1-FNR	62.5	71.9	75	2.34 (0.19)	1.65	2	0.193 (0.042)	0.280 (0.046)
N1-HNR	71.9	78.1	82	2.56 (0.20)	1.77	5	0.219 (0.043)	0.335 (0.045)
N1-PLT	59.4	65.6	69	2.16 (0.18)	1.63	0	0.203 (0.041)	0.291 (0.045)
N2-FNM	68.8	68.8	84	2.63 (0.22)	1.84	2	0.242 (0.045)	0.341 (0.048)
N2-FNR	68.8	71.9	79	2.47 (0.19)	1.59	0	0.169 (0.030)	0.279 (0.043)
N2-HNR	71.9	78.1	85	2.66 (0.19)	1.78	1	0.239 (0.044)	0.328 (0.047)
N2-PLT	68.8	78.1	86	2.69 (0.20)	1.86	1	0.279 (0.048)	0.362 (0.047)
Mean – Overall	66.8	75.2	80.5	2.52	1.70	1.31	0.222	0.308
Mean – Stand Type								
FNM	67.2 A	75.8 A	81.3 A	2.54 A	1.69 A	1.25 A	0.220 A	0.307 A
FNR	67.2 A	75.0 A	78.5 A	2.45 A	1.64 A	1.00 A	0.200 A	0.290 A
HNR	66.4 A	77.3 A	82.0 A	2.56 A	1.75 A	2.00 A	0.237 A	0.320 A
PLT	66.4 A	72.7 A	80.3 A	2.51 A	1.72 A	1.00 A	0.230 A	0.315 A
Mean – Region								
Eastern	67.2 A	77.4 A	81.6 A	2.55 A	1.69 A	1.13 A	0.226 A	0.304 A
Northern	66.4 A	73.1 A	79.4 A	2.48 A	1.72 A	1.50 A	0.218 A	0.311 A
Mean – Site								
Pine Falls	64.1 A	75.8 A	79.8 AB	2.49 AB	1.64 B	1.25 A	0.203 A	0.283 B
Bissett	70.3 A	78.9 AB	83.5 A	2.61 A	1.73 AB	1.00 A	0.248 A	0.326 A
The Pas	63.3 A	71.9 B	75.3 B	2.35 B	1.66 AB	2.00 A	0.204 A	0.295 AB
Snow Lake	69.6 A	74.2 AB	83.5 A	2.61 A	1.77 A	1.00 A	0.232 A	0.328 A

Table 3 Latent genetic potential, and genotypic diversity parameters for individual black spruce stands/populations and means for different stand types, sites and regions. FNM, post-fire natural mature; FNR, post-fire natural young regeneration; HNR, post-harvest natural regeneration; PLT, post-harvest plantation. Means for each genetic diversity parameter for the stand types, regions or sites followed by the same letter are not significantly different at $P < 0.05$ according to Duncan's multiple range test

Population	Latent genetic potential	Mean no. genotypes per locus		Genotype additivity		Genotype multiplicity	
		Observed	Expected	Observed	Expected	Observed	Expected
E1-FNM	39.15	3.72	5.19	119	166	5.29×10^{15}	2.57×10^{19}
E1-FNR	22.49	3.25	4.34	104	139	2.03×10^{13}	2.98×10^{16}
E1-HNR	27.50	3.69	5.06	118	162	9.52×10^{14}	2.65×10^{18}
E1-PLT	29.23	3.66	5.19	117	166	3.76×10^{14}	1.10×10^{18}
E2-FNM	29.50	3.75	5.22	120	167	4.37×10^{15}	1.98×10^{19}
E2-FNR	30.91	3.78	5.59	121	179	5.29×10^{15}	9.92×10^{19}
E2-HNR	22.66	3.87	4.94	124	158	3.32×10^{15}	2.14×10^{18}
E2-PLT	28.89	3.84	5.53	123	177	3.41×10^{15}	2.65×10^{19}
N1-FNM	23.97	3.28	4.50	105	144	3.01×10^{13}	3.57×10^{16}
N1-FNR	22.33	3.25	4.50	104	144	1.78×10^{13}	3.31×10^{16}
N1-HNR	25.50	3.63	5.16	116	165	1.72×10^{15}	7.14×10^{18}
N1-PLT	16.95	3.03	3.91	97	125	2.65×10^{12}	3.97×10^{14}
N2-FNM	25.22	3.78	5.50	121	176	9.36×10^{14}	5.88×10^{18}
N2-FNR	28.0	3.78	4.84	121	155	1.08×10^{15}	6.35×10^{17}
N2-HNR	28.01	4.00	5.44	128	174	2.37×10^{16}	5.29×10^{19}
N2-PLT	26.47	4.09	5.59	131	179	4.80×10^{16}	8.57×10^{19}
Mean – Overall	26.12	3.65	5.03	116.8	161	6.16×10^{15}	2.06×10^{19}
Mean – Stand Type							
FNM	27.21 A	3.63 A	5.10 A	116.3 A	163.3 A	2.66×10^{15} A	1.29×10^{19} A
FNR	25.93 A	3.52 A	4.82 A	112.5 A	154.3 A	1.60×10^{15} A	2.50×10^{19} A
HNR	25.94 A	3.80 A	5.15 A	121.5 A	164.8 A	7.42×10^{15} A	1.62×10^{19} A
PLT	25.39 A	3.66 A	5.06 A	117.0 A	161.8 A	1.30×10^{16} A	2.83×10^{19} A
Mean – Region							
Eastern	27.67 A	3.70 A	5.13 A	118.3 A	164.3 A	2.88×10^{15} A	2.21×10^{19} A
Northern	24.57 A	3.61 A	4.93 A	115.4 A	157.8 A	9.44×10^{15} A	1.90×10^{19} A
Mean – Site							
Pine Falls	27.34 A	3.58 BC	4.95 AB	114.5 BC	158.3 AB	1.66×10^{15} A	7.37×10^{18} A
Bissett	27.99 A	3.81 AB	5.32 A	122.0 AB	170.3 A	4.10×10^{15} A	3.69×10^{19} A
The Pas	22.19 B	3.30 C	4.52 B	105.5 C	144.5 B	4.41×10^{14} A	1.80×10^{18} A
Snow Lake	26.95 AB	3.91 A	5.34 A	125.3 A	171.0 A	1.84×10^{16} A	3.63×10^{19} A

stands – 8 and post-harvest planted stands – 4. Most of the loci conformed to the Hardy Weinberg equilibrium in most of the populations. However, significant ($P < 0.05$) departures from the Hardy Weinberg equilibrium were observed in some cases, which were primarily due to a deficiency of heterozygotes. Nevertheless all of the polymorphic loci conformed to Hardy Weinberg equilibrium in at least one population.

Genetic diversity and effects of forest fires, harvesting and alternative reforestation practices

The sampled black spruce populations/stands showed typical moderate to high levels of allozyme genetic diversity as measured by conventional genetic diversity parameters (Table 2). Observed heterozygosity was consistently lower than the expected heterozygosity in all populations, suggesting an excess of homozygotes. Genetic diversity parameters of the four stand types (post-fire natural mature, post-fire natural young, post-harvest natural young and post-harvest planted) were comparable at each study site as well as over the four study sites (Table 2). No significant differences were observed due to stand types ($P = 0.06$–0.79) or regions ($P = 0.06$–0.79) in the percent loci polymorphic, the total number of alleles (A_T), the mean number of alleles per locus (A), the effective number of alleles per locus (Ae), the number of private alleles (Ap), the observed heterozygostiy (Ho) and the expected heterozygosity (He) (Table 2). However, significant differences ($P = 0.03$–0.05) due to study sites were observed for A_T, A, Ae and He. On average, the black spruce populations from the Pas (N1) study site generally showed the lowest genetic diversity (Table 2) except that Ae and He, on average, were the lowest in black spruce populations from the Pine Falls (E1) site (Table 2). The Pas black spruce populations on average had significantly lower A_T and A, whereas the Pine Fall black spruce populations had significantly lower Ae and He. No significant differences were observed between the post-fire and post-harvest regenerated stands for any of the genetic diversity parameters.

The latent genetic potential (LGP) and various genotypic genetic diversity parameters for individual populations and means over stand types, regions and study sites are given in Table 3. As observed for the conventional genetic diversity parameters (Table 2), the latent genetic

Table 4 Number of allozyme loci showing significant ($P < 0.05$) allele frequency heterogeneity (A) among different stand types (post-fire natural mature, post-fire natural young, post-harvest natural and planted) at four study sites, (B) among populations within stand types and (C) among individual populations

Region	Location/ stand type	No. populations	No. loci c polymorphi	No. and (%) allozyme loci showing allele-frequency heterogeneity
(A) Among stand types				
Eastern	Pine Falls	4	29	20 (69.0)
	Bissett	4	29	22 (75.9)
Northern	The Pas	4	29	25 (86.2)
	Snow Lake	4	27	23 (85.2)
(B) Among populations within stands types				
Stand type				
Post-fire natural mature		4	29	25 (86.2)
Post-fire natural young		4	27	25 (92.6)
Post-harvest natural young		4	30	25 (83.3)
Post-harvest planted		4	27	23 (85.2)
(C) Among individual populations		16	30	29 (96.7)

potential and genotypic diversity parameters of the four stand types were comparable at each study site as well as over the four study sites (Table 3). ANOVA indicated no significant differences among stand types ($P = 0.6$–0.93) or regions ($P = 0.1$–0.85) for the latent genetic potential, the mean number of genotypes per locus, genotype additivity and genotype multiplicity. However, significant differences ($P = 0.02$–0.04) due to study sites were observed in the mean number of genotypes per locus and genotype additivity. On average, the black spruce populations from the Pas (N1) study site had significantly lower latent genetic potential, genotype numbers and genotypic additivity than the populations from the other three study sites (Table 3). No significant differences in genotypic multiplicity parameters due to study sites, regions or stand types, were observed (Table 3). Also, there were no significant differences between post-fire and post-harvest regenerated stands for genotypic diversity parameters and latent genetic potential.

No significant correlations between age, height or diameter and genetic diversity parameters, either based on population means or individual trees, were observed.

Genetic differentiation and relationships among populations, stand types, study sites and regions

Significant ($P < 0.05$) allele-frequency heterogeneity was observed at 29 of the 30 polymorphic loci among the 16 individual black spruce populations. Only *Dia3* did not show significant allele-frequency heterogeneity over the populations. The number and proportion of loci showing allele-frequency heterogeneity among populations within individual stand types was higher than that among different stand types at individual study sites (Table 4). The number (and %) of loci showing significant allele-frequency heterogeneity among four stand types at a study site ranged from 20 (69%) at Pine Falls to 25 (86%) at the Pas (Table 4). The number and percent loci showing significant allele-frequency heterogeneity among four populations within the same stand type ranged from 83.3% for the post-harvest natural young stands to 92.6% for the post-fire natural young stands. The hierarchical *F*-statistics indicated no differentiation among stand types within the total sample (data not shown).

Genetic distances (Nei 1978) among populations ranged from 0.019 between the post-fire natural mature and post-harvest planted stands at Pine Falls to 0.189 between the post-fire natural young stand at Bissett and the post-harvest natural young stand at the Pas sites (data not shown), with an average of 0.094 among all 16 stands. On average, the post-harvest natural stand at Snow Lake showed the lowest (0.076), and the post-harvest plantation at the Pas the highest (0.116), genetic distances from other populations. Among the four stand types, the lowest mean genetic distance was between the post-fire natural mature and post-harvest natural young stands, and the highest between the post-fire natural young and planted stands (Table 5). Within stand types, the lowest genetic distances were among the four post-fire natural mature populations and the highest among the four post-fire natural young populations (Table 5). On average, genetic distances among different stand types (0.091) were lower than among different populations within the same stand types (0.105) (Table 5). The mean genetic distance among populations from the Eastern region was 0.073, and among populations from the Northern region was 0.084. The mean genetic distance between Eastern and Northern region populations was 0.108.

A UPGMA cluster analysis based on genetic distances revealed four major groups among the 16 black spruce populations corresponding almost to four study sites (Fig. 1). With the exception of three populations (E2-

Table 5 Mean and (range) of Nei's (1978) unbiased estimates of genetic distances within (on the diagonal) and among (below the diagonal) different stand types. FNM, post-fire natural mature; FNR, post-fire natural young regeneration; HNR, post-harvest natural regeneration; PLT, post-harvest plantation

Stand type	No. populations	FNM	FNR	HNR	PLT
FNM	4	0.085 (0.049–0.107)			
FNR	4	0.091 (0.026–0.149)	0.120 (0.088–0.171)		
HNR	4	0.084 (0.034–0.141)	0.097 (0.050–0.189)	0.101 (0.048–0.150)	
PLT	4	0.087 (0.019–0.149)	0.100 (0.028–0.162)	0.089 (0.027–0.146)	0.113 (0.077–0.144)

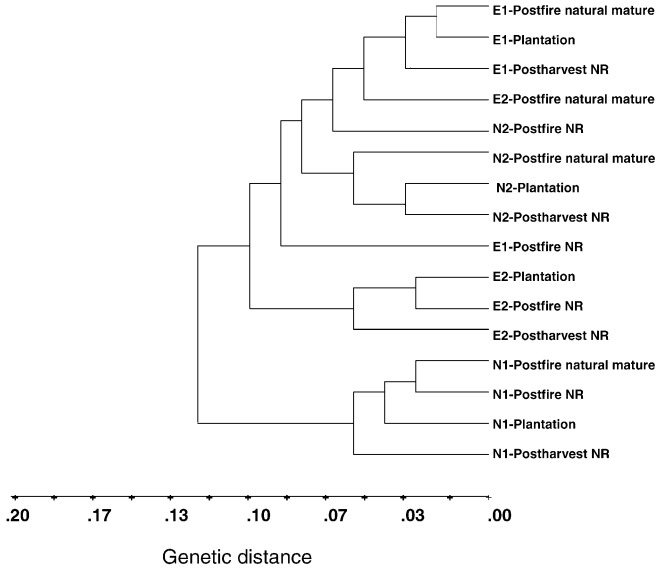

Fig. 1 UPGMA cluster plot of black spruce populations based on their unbiased estimates of genetic distances (Nei 1978). *NR* = natural regeneration

Post-fire natural mature, N2-Post-fire natural young and E1-Post-fire natural young), all populations/stands, irrespective of their types, from the same study site/area clustered together. The black spruce stands from the Pas (N1) area were genetically the most differentiated from the other stands. A UPGMA cluster analysis based on genetic distances among four stands from individual study sites did not reveal any particular clustering order of the stand types (data not shown).

Discussion

The project results suggest that black spruce populations sampled in Manitoba have typical moderate to high levels of allozyme genetic diversity. The allozyme genetic diversity observed in our present study is comparable to that reported for black spruce from Quebec (P = 80%, A = 2.0, Ho = 0.466, He = 0.351; Desponts and Simon 1987), but higher than that reported for black spruce from Newfoundland (P = 38%, A = 1.44, He = 0.107, Ho = 0.120; Yeh et al. 1986), Ontario (P = 47.5/57.3, He = 0.21/0.23; O'Reilly et al. 1985) and New Brunswick (A = 2.17–2.50, He = 0.192–0.253; Boyle and Morgenstern 1987). This is the first report of Canadian black spruce genetic diversity employing over 30 allozyme loci.

Our data indicate that there are no significant differences in black spruce genetic diversity levels between the eastern and northern regions in Manitoba. This suggests that there are no regional patterns/differences in genetic diversity levels in black spruce. However, our study demonstrates that the black spruce populations from the Pas (N1) study site have lower genetic diversity than the other three study sites. The Pine Falls (E1), Bissett (E2) and Snow Lake (N2) sites are located in the Boreal Shield ecozone, whereas the Pas sites are located in the Boreal Plain ecozone. The four black spruce populations from the Pas site were also genetically differentiated from the 12 black spruce populations from the three other study sites. The differences in genetic diversity levels and the genetic constitution of the Pas black spruce populations may be related to the ecozone, which these populations come from. The Boreal Plains and Boreal Shield ecozones differ in soil and several other ecological characteristics (information on file). The Boreal Shield ecozone most likely has more heterogeneous microsites and environments than the Boreal Plains ecozone. It has been hypothesized that heterogeneous environments promote the maintenance of higher genetic diversity due to developmental homeostasis (Lerner 1954). Genetic constitution and diversity in black spruce may be related to the site conditions as was the case for white spruce from dry and wet sites (Rajora, unpublished).

It has been generally assumed that genetic diversity, particularly heterozygosity, should increase with the age of plants. The classical concept of developmental homoeostasis (Lerner 1954) supports this assumption. However, we did not observe a significant positive correlation between genetic diversity and age, height or diameter in the black spruce populations examined. This contrasts with higher genetic diversity observed in old-growth populations of red spruce, *Picea rubens* (Mosseler et al. 2002), and white spruce (Rajora, unpublished). However, in white spruce, with the exception of the old-growth populations, genetic diversity was not related to the age of trees (Rajora 1999b).

Our study clearly demonstrates that the four stand types (post-fire natural mature, post-fire natural young, post-harvest natural young and post-harvest planted) have comparable and statistically similar allozyme genetic diversity at each of the four study sites and in two ecoregions. This was the case with both allelic-based conventional (Table 2) and genotypic-based unconventional (Table 3) genetic diversity parameters, as well as latent genetic potential (Table 3). These results clearly suggest that forest fires, and currently used clearcut harvesting and natural or artificial regeneration silvicultural practices, do not adversely affect genetic diversity in black spruce. Also, the number of allozyme loci showing significant allele-frequency heterogeneity among four populations within each stand type and among four stand types at individual study sites was similar (Table 4). This suggests that forest fires, clearcut harvesting and alternative natural and artificial regeneration practices apparently did not cause any directional changes in allele frequencies. Any effect on allele-frequency heterogeneity was rather random.

Of the four stand types studied, two (FNM, FNR) were of fire origin (post-fire regenerated) and the other two (HNR, PLT) were of post-harvest origin (post-harvest regenerated). Our study demonstrates that the genetic diversity of the post-fire regenerated stands was comparable and not significantly different from the genetic diversity of the post-harvest regenerated stands at each and over all study sites. These results clearly suggest that the genetic diversity effects of currently used clearcut harvesting followed by natural or artificial regeneration, are not significantly different from those due to forest fires in black spruce. Therefore, a carefully implemented natural or artificial regeneration system should be able to maintain genetic diversity in black spruce. This, in turn, should ensure sustainable management of black spruce genetic resources.

The results of the present study demonstrating comparable genetic diversity in four stand-types and between post-fire and post-harvest regenerated stands, are consistent with the reproductive biology and regeneration processes in black spruce. Black spruce is an early successional pioneering species that begins producing seeds when as young as 10 years old, and stands that are of 30 years age or older generally always have a continuous supply of seeds (Viereck and Johnston 1990). The cones of black spruce are semi-serotinous and trees can retain cones from several preceding seed years, providing a genetically diverse pool of seeds. The semi-serotinous cones of black spruce remain partially closed and disperse seeds over a period of several years, providing an adequate supply of seeds to reproduce the stand after fire or harvest (Viereck and Johnston 1990; St Pierre et al. 1992; Fleming and Mossa 1996). The seeds of black spruce remain viable in fallen cones for over 10 years (Schooley et al. 1979). Black spruce typically seeds promptly, and regenerates well after both forest fires and clearcut harvesting (Brumelis and Carleton 1988; Sirois and Payette 1989; St Pierre et al. 1992; Fleming and Mossa 1996). All of these features would favor maintenance of high levels of genetic diversity in post-fire and post-harvest naturally regenerated stands in black spruce. Vegetative reproduction through layering also occurs in black spruce, when lower branches become covered with mosses or litter. However, this mode of reproduction is common only in swamps, bogs, muskegs and tree line (Viereck and Johnston 1990). In the post-fire and post-harvest stands that we sampled, the incidence of layering was absent or negligible. Thus, layering did not have any impact on genetic diversity of the sampled stands.

Genetic diversity of the plantations was comparable to the post-fire or post-harvest naturally regenerated stands at each and over the four study sites. This suggests that the plantations sampled in our study have a broad genetic base, which is not significantly different from the genetic base of the naturally regenerated stands. The seed used for plantations originated from local seed sources. These plantations also had some naturally regenerated black spruce seedlings, which were not excluded from sampling and may have also enriched the plantations' gene pool. These results are in contrast with those reported for sympatric white spruce where plantations were found to have significantly reduced genetic diversity as compared to natural old growth and young natural regeneration (Rajora 1999b). This may be related to the differences in the genetic base of the plantation stocks, reproductive biology and regeneration processes of these spruce species. In addition to a broad genetic base of the seed used for the sampled plantations, a seed source from semi-serotinous black spruce cones may most likely contribute to a more diverse seed source for plantations, as well as for the post-harvest natural regeneration. Nevertheless, plantations should use a local seed source collected from a large number of diverse seed trees in order to maintain genetic diversity and locally adapted gene pools. Also, it would be prudent to periodically monitor the genetic diversity of new plantations to ensure that their wide genetic base is maintained.

The results of our study for comparable genetic diversity between the post-fire natural mature and post-harvest naturally regenerated stands are consistent with those reported for white spruce (Rajora 1999b), Norway spruce, *Picea abies* (Gomory 1992) and another early succesional semi-serotinous conifer lodgepole pine, *Pinus contorta* (Thomas et al. 1999).

Significant allele frequency heterogeneity at 83–93% of the polymorphic loci among different populations from different study areas and regions within the same stand type, and at 69–86% of the polymorphic loci among different stand types at individual study sites, suggests that the genetic constitution of black spruce is variable, and high genetic diversity is maintained in black spruce at the landscape level in Manitoba. This is further supported by the genetic-distance data among populations within stand types and among stand types. Therefore, forest fires and the currently used clearcut harvesting and alternative-natural and artificial-regeneration practices do not adversely affect the maintenance of

high levels of genetic diversity in black spruce at the landscape level.

The results of our study suggest that black spruce from the four different study sites/areas sampled in Manitoba has a somewhat different genetic constitution, with the populations from the Pas study site being quite genetically differentiated from the others (Fig. 1). Therefore, silviculture regeneration practices should take this genetic distinctiveness into account, in order to maintain overall as well as regional and area-specific genetic diversity. For plantations, this may be taken care of by implementing existing seed-zone guidelines.

Our study presents the first published data of its own kind, comparing the genetic effects of forest fires, clearcut harvesting and alternative natural and artificial silvicultural regeneration practices in boreal forest trees.

Conclusions

Black spruce in Manitoba has typical moderate to high levels of allozyme genetic diversity, and black spruce from each of the four study sites has a somewhat distinct genetic constitution. Forest fires and currently used clearcut harvesting, and alternative natural and artificial silvicultural regeneration practices, do not adversely affect genetic diversity in black spruce. The genetic diversity effects of clearcut harvesting are not significantly different from those due to forest fires in black spruce. Therefore, carefully implemented natural or artificial regeneration systems should be able to maintain genetic diversity in black spruce. Nevertheless, silvicultural practices should ensure that the regional and area–specific genetic diversity and the black spruce gene pools are maintained. This study has provided benchmarks and framework for future monitoring of genetic diversity and genetic effects of natural disturbance and forest management practices in black spruce.

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