Genetic diversity and population structure of disjunct Newfoundland and central Ontario populations of eastern white pine (*Pinus strobus*)

Om P. Rajora, Linda DeVerno, Alex Mosseler, and David J. Innes

Abstract: The dramatic decline of eastern white pine (*Pinus strobus* L.) populations in Newfoundland over the past 100 years presents an opportunity to determine and monitor population bottleneck effects on genetic diversity in trees. To provide benchmarks and indicators for monitoring genetic changes due to recent and future bottleneck events and to assist development of conservation strategies, we assessed genetic diversity and structure of six small, isolated white pine populations from two regions at the limits of its geographical range in Newfoundland for comparison with three populations from its central range in Ontario for 20 allozyme loci coding for 12 enzymes. On average, 47.8% of the loci were polymorphic, the number of alleles per locus was 1.75, and the observed and expected heterozygosities were 0.215 and 0.195, respectively. Although most of the alleles were widespread, unique alleles were found in three of the nine populations examined. The Newfoundland populations were as genetically variable as those from Ontario. Generally, all populations exhibited slight excess of heterozygotes at most loci. Only 6.1% of the detected genetic variation was among populations, and the remainder among individuals within populations. The genetic distances among the populations within a province or region were as great as those among populations between the provinces or regions. Canonical discriminant functions and cluster analysis from genetic distances separated nine populations into the same four groups. Neither provincial nor regional or geographic gradient-related patterns of population variation and differentiation were apparent. It appears that 8000 years of postglacial geographic isolation and recent population decline have had little or no detectable effect on genetic diversity or differentiation of disjunct Newfoundland white pine populations from their ancestral mainland populations. Assuming their adaptability, the Ontario seed sources may be acceptable for white pine restoration in Newfoundland.

Key words: *Pinus strobus*, allozymes, gene conservation, genetic diversity and population structure, genetic drift, population bottleneck.

Résumé : À Terre-Neuve, le déclin rapide des populations de pin blanc de l'est (*Pinus strobus* L.), au cours des 100 dernières années, offre une opportunité pour déterminer et suivre les effets d'étranglement des populations sur la diversité génétique des arbres. Afin de définir des repères et des indicateurs pour suivre les changements génétiques dus aux événements d'étranglement récents et passés et pour aider à l'élaboration de stratégies de conservation, les auteurs ont évalué la diversité génétique et la structure de six petites populations isolées de pins blancs, situées dans deux régions à la limite de leur aire géographique de distribution à Terre-Neuve, pour les comparer avec trois populations du centre de l'aire géographique de l'espèce situées en Ontario et en suivant 20 lieux allozymiques codant pour 12 enzymes. En moyenne, 47,8% des lieux sont polymorphes, le nombre d'allèles par lieu est de 1,75, et l'hétérozygocité observée et attendue de 0,215 et 0,195, respectivement. Bien que la majorité des allèles soient largement distribués, les auteurs ont trouvé des allèles uniques dans trois des neuf populations de pin examinées. Les populations de Terre-Neuve montrent autant de variabilité génétique que celles d'Ontario. En général, toutes les populations montrent un léger excès d'hétérozygotes pour la plupart des lieux. Seulement 6,1% de la variation génétique décelée se retrouve entre les populations et le reste se situe entre les individus dans les populations. Les distances génétiques entre les populations, à l'intérieur d'une province ou région, sont aussi grandes que les distances génétiques entre populations, entre les provinces ou régions. Les fonctions discriminantes canoniques ainsi que l'analyse par regroupement des distances génétiques permettent de reconnaître neuf populations dans les mêmes quatre groupes. On n'observe aucun patron provincial, régional ou géographique relié à un gradient dans la variation et la différenciation des populations. Il semble que 8000 ans d'isolation géographique post-glaciaire et un déclin récent de la population n'auraient eu que peu d'effets décelables sur la diversité génétique ou la différenciation des populations disjointes du pin blanc sur l'Île de Terre-Neuve, par rapport aux populations ancestrales du continent. Prenant en compte leur adaptabilité, les sources de semences d'Ontario sont acceptables pour la régénération du pin blanc sur l'Île de Terre-Neuve.

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Introduction

Geographic isolation and the occurrence of large ice-free areas during the last (Wisconsin) glaciation (Rogerson 1983) suggest that the flora of Newfoundland may contain genetically distinct populations from those of the mainland. Although many other plants may have survived the Wisconsin glaciation in ice-free areas in Newfoundland (Brassard 1983, 1984; Belland and Brassard 1988), it seems unlikely, although not inconceivable, that conifers might also have survived glaciation. The current absence of endemic species on the island (Brassard 1983; G.R. Brassard, personal communication, 1994) suggests that most plants probably reentered Newfoundland following glacial retreat. Most conifers probably reentered the island from the adjacent mainland ca. 8400 BP (Critchfield 1987).

Eastern white pine (*Pinus strobus* L.) ranges in Canada from Manitoba to Newfoundland, and in the United States from Minnesota and northeastern Iowa to the Atlantic Ocean, and southward along the Appalachians to northern Georgia and Alabama. It is one of the most important softwood timber species in eastern Canada and northeastern United States, and has been an integral part of the history and economic development of eastern North America. Once a common and widespread species throughout northeastern North America, white pine has undergone fundamental changes in population structure as a result of human interference on the landscape. Harvesting pressures have reduced white pine to small, isolated groups of trees over large portions of its former geographic range (Buchert 1994).

The rapidly declining numbers and sizes of eastern white pine populations in Newfoundland have become an urgent conservation issue because of threats to its survival as a naturally occurring species on the island (Newfoundland Forest Service 1997). This decline resulted from forest harvesting for sawlogs at the turn of this century (Munro 1978), followed by the introduction of the white pine blister rust disease (*Cronartium ribicola*) in the 1930s from Europe to mainland North America and then to Newfoundland (Carroll 1990). Prior to these events, white pine was widely, albeit diffusely, spread across central and western Newfoundland largely as a minor component of a mixedwood, fire-origin forest dominated (number of stems per hectare) by species such as aspen (*Populus tremuloides* Michx.), black spruce (*Picea mariana* (Mill.) BSP.), balsam fir (*Abies balsamea* (L.) Mill.), tamarack (*Larix laricina* (DuRoi) K. Koch), and white spruce (*Picea glauca* (Moench) Voss). Healthy "old growth" white pine is no longer a common feature of the island's landscape. The extant white pine now consists of a few small surviving patches of younger stands ranging in age from 40 to 70 years. The remaining isolated patches often consist of less than 30–50 individuals, creating a situation where inbreeding and genetic drift could begin to affect genetic diversity, structure, and reproductive success.

Geographical patterns of genetic variation in eastern white pine for phenotypic traits, survival, and disease and insect resistance have been examined through several range-wide seed source studies (reviews in Genys 1991; Buchert 1994). These studies have suggested that eastern white pine is highly variable genetically, as can be expected of a species with wide distribution and adaptation over very diverse environments and site conditions. However, relatively little is known in terms of biochemical and molecular genetic variation and structure of natural populations of this species. Eckert et al. (1981) determined the variability and inheritance of allozymes in 35 clones from New Hampshire and Maine. Ryu (1982) studied allozyme genetic variation and structure of 27 provenances from a range-wide provenance test of eastern white pine, and reported substantial genetic differentiation within and among provenances. Brym and Eckert (1987) examined spatial distribution of allozyme genetic variability within an unmanaged white pine stand from New Hampshire and found strong spatial genetic structure within this stand. Genetic diversity and structure of 10 natural white pine populations from two regions in Quebec were determined by Beaulieu and Simon (1994*a*), and weak among-population genetic differentiation and regional differences in genetic diversity levels were reported. In a recent study of old-growth eastern white pine from the northern margin of its range in Ontario, Buchert et al. (1997) and O.P. Rajora and G.P. Buchert (unpublished data) found moderate to high genetic diversity and within-stand spatial genetic structure in preharvest populations, and reduced genetic diversity in postharvest residual populations. Nevertheless, there is no information on genetic variability and population structure of natural populations from its range in Newfoundland or central Ontario. Also, it is not known whether the genetic diversity of the disjunct Newfoundland populations is comparable to that of populations from the centre of the geographic range in Ontario.

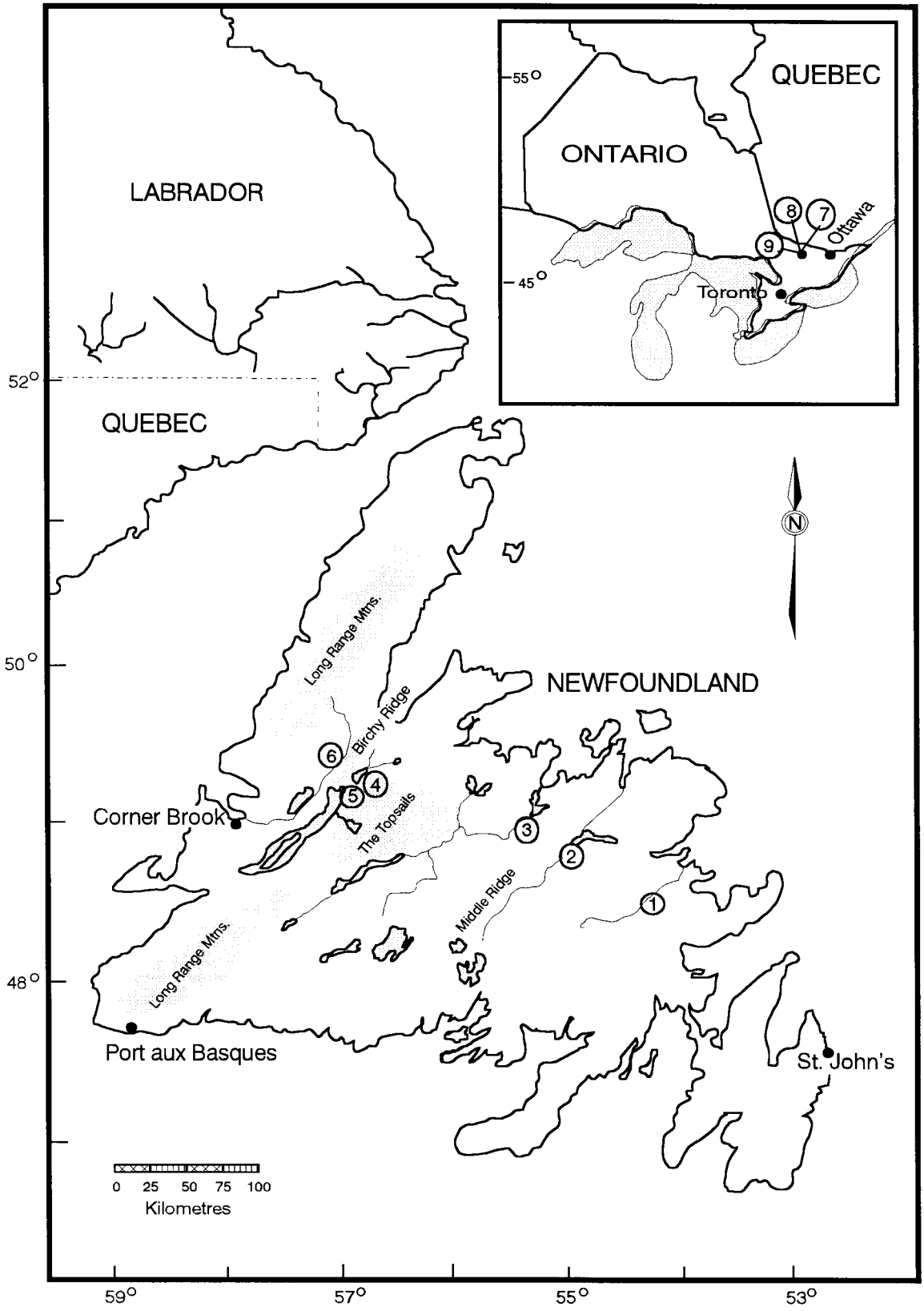
The dramatic and recent decline of Newfoundland's white pine population has focussed attention on conservation and development of a conservation strategy based on ecological restoration (Newfoundland Forest Service 1997). The objectives of this study were to develop benchmark information on genetic diversity and structure of disjunct eastern white pine populations from Newfoundland and to determine the extent of their genetic differentiation that may have arisen following geographic isolation from ancestral mainland populations, in order to monitor the genetic effects of population decline and to plan restoration efforts. We used isozyme analysis to determine genetic diversity and structure of six small isolated eastern white pine populations from two different regions in Newfoundland (Newfoundland east and west) and of three populations from the Ottawa River Valley region in Ontario. We also compared the levels of genetic diversity and differentiation within and among populations from the two provinces.

Materials and methods

White pine populations and sampling

Six natural eastern white pine populations–stands from Newfoundland and three populations–stands from Ontario were sampled (Fig. 1). In Newfoundland, remnants of the once extensive eastern white pine population are in evidence as large, old, standing dead or

Fig. 1. Map of the island of Newfoundland and the province of Ontario, showing the location of the white pine populations investigated in this study. 1, Terra Nova Lake (TN); 2, Northwest Gander River (NW); 3, Grand Falls (GF); 4, Birchy Lake (BL); 5, Sandy Lake (SL); 6, Adies Stream (AS); 7, Corry Lake (CL); 8, Cartier Lake (CT); 9, Wylie Lake (WL). These nine populations were grouped into three regions as follows: Newfoundland east, including Terra Nova Lake, Northwest Gander River, and Grand Falls; Newfoundland west, including Birchy Lake, Sandy Lake, and Adies Stream; and Ottawa River Valley, including Corry Lake, Cartier Lake, and Wylie Lake.



dying trees scattered throughout the forests of central and western Newfoundland. Presently, white pine exists here primarily as small, isolated stands of younger age classes. These stands are quite small in size, often consisting of less than 50 trees. The six sampled populations were from these remnant stands, which ranged from 30 to 70 years in age and were grouped into two regions, Newfoundland east and Newfoundland west (Fig. 1). The distances between any two Newfoundland east populations ranged from 40 to 115 km, whereas between any two Newfoundland west populations distances ranged from 10 to 34 km. The Newfoundland west populations are separated (103–243 km) from the Newfoundland east populations by the Long Range Mountains, treeless barrens with an average altitude of 500 m. The three Ontario white pine stands are located within 5 km of each other within the Petawawa Research Forest in the Ottawa River Valley near Chalk River. This area contains the largest and most extensive population of eastern white pine in North America and represents the centre of the present geographical range of the species. Trees ranged in age from 60 to 80 years, and were grouped into a single Ontario (Ottawa River Valley) region.

In 1991 and 1992, cones were collected from 8–24 trees from each of the six Newfoundland (Terra Nova Lake, 14; Northwest Gander River, 20; Grand Falls, 8; Birchy Lake, 20; Sandy Lake, 18; and Adies Stream, 15) and three Ontario (Corry Lake, 22; Cartier Lake, 23; and Wylie Lake, 24) stands–populations of white pine. From each Newfoundland population, cones were collected from every tree with a cone crop. Thus, our sample can more accurately be described as a reproductive "census" of these stands, representing ≥90% of the total white pine trees in a stand. In the Ontario populations, trees were sampled randomly. Although additional trees could have been sampled from the Ontario populations, the sample size was kept similar to that of the Newfoundland populations. Cones were collected approximately 1 week prior to natural cone maturation from at least four main branches in the upper crown. Seeds were extracted, cleaned, and then stored at 4°C until required for isozyme analysis.

Enzyme electrophoresis and genotyping

Tissues of haploid megagametophytes were used for enzyme electrophoresis. Seeds were imbibed in Petri dishes on filter paper moistened with distilled water at 4°C for a minimum of 16 h prior to seed dissection and enzyme extraction. Individual megagametophytes were placed in separate wells in a microtitre plate containing 30 μL of cold extraction buffer (Khasa et al. 1993) and homogenized manually with a plastic rod. Electrophoresis was performed on freshly homogenized samples. Enzymes in at least eight megagametophytes from each of the 164 trees were examined for genotyping.

Cellulose acetate gels (Helena Laboratories, Beaumont, Tex.) were used for electrophoresis and resolution of allozymes (Hebert and Beaton 1989) for 12 enzyme systems: acid phosphatase (ACP, EC 3.1.3.2), aspartate aminotransferase (AAT, EC 2.6.1.1), diaphorase (DIA, EC 1.6.4.3), fumarase (FUM, EC 4.2.1.2), isocitrate dehydrogenase (IDH, EC 1.1.1.42), leucine aminopeptidase (LAP, EC 3.4.11.1), malate dehydrogenase (MDH, EC 1.1.1.37), malic enzyme (ME, EC 1.1.1.82), 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 (SDH, EC 1.1.1.25). Due to a lack of good quality seeds from four of the 15 trees from Adies Stream, only four enzymes (MDH, PGI, PGM, and SDH) could be assayed in these four trees. All 12 enzymes were assayed in the remaining 11 trees from this population.

Genotypes of individual trees were inferred from allelic constitution and banding pattern in individual megagametophytes. For enzymes encoded by multiple loci, the loci were numbered from anodal to cathodal direction as described in Buchert et al. (1997). The inheritance and linkage of the allozymes of the 14 polymorphic loci used in our study have been determined earlier (Beaulieu and Simon 1994*b*).

Statistical analysis

Allele frequencies were calculated for each locus in each population. The correlations between allele frequencies and latitude, longitude, and altitude of the populations were determined. The following genetic diversity parameters were determined for each population: percentage of loci polymorphic (P; 95% criterion), average number of alleles per locus (A), average number of alleles per polymorphic locus (A_p), unbiased estimate of heterozygosity expected under Hardy–Weinberg equilibrium (H_e) (Nei 1978), observed heterozygosity (H_o), and average number of effective alleles per locus ($A_e = 1/(1–H_e)$). Chi-square goodness of fit tests, with Levene's (1949) corrections for small samples, were performed to measure deviation from Hardy–Weinberg equilibrium. Fixation index ($F = 1–Ho/He$) was calculated for each polymorphic locus. The BIOSYS-1 version 1.7 software (Swofford and Selander 1989) was used to compute population genetic parameters, with the exception of latent genetic potential and multivariate analysis. The latent genetic potential of individual populations was calculated as described in Bergmann et al. (1990).

Mdh2 and *Me1* were found to be perfectly linked in all megagametophytes of the 160 trees analyzed for MDH and ME enzymes. Both of these loci were included for determining the genetic diversity parameters. However, due to linkage disequilibrium between *Mdh2* and *Me1*, only one *Mdh2* was used for all other analyses.

Heterogeneity of allelic frequencies over all populations, over populations within each of the two provinces, and over three populations within each of the three regions was examined using a χ^2 test. The genetic structure and differentiation of the complete set of populations, populations within each of the two provinces, and populations within each of the three regions were determined by Wright's F statistics (Wright 1965). Gene flow estimates among the populations were calculated from the F_{ST} (correlations between uniting gametes among subpopulations or inbreeding in subpopulations relative to the total population) estimates (Crow and Aoki 1984). The genetic distances among all populations, and among populations within and between the provinces, and the regions were determined (Nei 1972). A cluster analysis of all the populations was done based on the matrix of Nei's (1972) standard estimates of genetic distances using the unweighted pair-group method with arithmetic averages (UPGMA). The analysis of population structure and differentiation at different hierarchical levels (regions, provinces, and total) was performed using Wright's hierarchical F statistics (Wright 1978).

The genotypes of individual trees at each of the 13 polymorphic loci (*Me1* excluded) were coded according to the method of Smouse and Neel (1977). Canonical discriminant analysis was done using the CANDISC procedure of SAS (Windows version 6.11; SAS Institute Inc. 1995). The centroids of the populations were plotted for the first three significant canonical discriminant functions (CAN) in a three-dimensional plot.

Results

Allele frequency and genetic diversity

A total of 20 loci coding for 12 enzymes were studied. Of these 20 loci, six loci (*Aat1*, *Acp2*, *Dia2*, *Mdh3*, *Me2*, and *6Pgd1*) were invariant in all nine populations. Two to four alleles were detected at a polymorphic locus, with a total of 37 alleles in all nine populations at the 14 polymorphic loci (Table 1). Most of the alleles were well spread over the populations, and a few of them were found only in one, two, or a few populations (Table 1). The alleles that were unique to a single population were as follows: *Pgi1–1* in Sandy Lake, *Pgm1–1* and *Pgm2–4* in Corry Lake, and *Sdh1–1* in Birchy Lake. All of these alleles were rare (frequency ≤ 0.05). Certain alleles were rare (frequency ≤ 0.05) in certain populations but common (frequency > 0.95) in the others (Table 1). The number of such

Table 1. Allele frequencies for polymorphic loci in Newfoundland and Ontario *Pinus strobus* populations.

Locus[a]	Allele	Rf[b]	Terra Nova Lake	Northwest Gander River	Grand Falls	Birchy Lake	Sandy Lake	Adies Stream	Corry Lake	Cartier Lake	Wylie Lake
*Aat3***	*1*	0.19	0.54	0.45	0.38	0.30	0.22	0.46	0.52	0.37	0.62
	2	0.15	0.46	0.55	0.62	0.70	0.78	0.54	0.48	0.63	0.38
*Acp1**	*1*	0.40	0.32	0.40	0.50	0.62	0.33	0.50	0.39	0.33	0.56
	2	0.34	0.68	0.60	0.50	0.38	0.67	0.50	0.61	0.67	0.44
*Dia1**	*1*	0.53	0.57	0.55	0.94	0.68	0.61	0.82	0.73	0.50	0.62
	2	0.46	0.43	0.45	0.06	0.32	0.39	0.18	0.27	0.50	0.38
Fum1[ns]	*1*	0.37	0.00	0.05	0.00	0.00	0.00	0.09	0.00	0.02	0.04
	2	0.32	1.00	0.95	1.00	1.00	1.00	0.91	1.00	0.98	0.96
*Idh1**	*1*	0.38	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.09	0.00
	2	0.34	1.00	1.00	1.00	1.00	1.00	1.00	0.98	0.91	1.00
*Lap1***	*1*	0.66	0.11	0.02	0.00	0.02	0.03	0.00	0.02	0.04	0.02
	2	0.63	0.89	0.98	0.94	0.88	0.61	0.91	0.91	0.89	0.88
	3	0.57	0.00	0.00	0.06	0.10	0.36	0.09	0.07	0.06	0.10
*Mdh2***	*1*	0.44	0.39	0.52	0.69	0.62	0.25	0.43	0.48	0.63	0.77
	2	0.39	0.57	0.38	0.19	0.30	0.67	0.47	0.43	0.22	0.13
	3	0.34	0.04	0.10	0.12	0.08	0.08	0.10	0.09	0.15	0.10
*Me1***	*1*	0.40	0.39	0.52	0.69	0.62	0.25	0.54	0.48	0.63	0.77
	2	0.34	0.57	0.38	0.19	0.30	0.67	0.32	0.43	0.22	0.13
	3	0.31	0.04	0.10	0.12	0.08	0.08	0.14	0.09	0.15	0.10
Pgi1[ns]	*1*	0.75	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00
	2	0.71	0.00	0.08	0.00	0.02	0.00	0.00	0.00	0.00	0.00
	3	0.63	1.00	0.92	1.00	0.98	0.97	1.00	1.00	1.00	1.00
*Pgi2***	*1*	0.41	0.04	0.05	0.06	0.02	0.00	0.23	0.02	0.00	0.00
	2	0.35	0.96	0.95	0.94	0.98	1.00	0.77	0.98	1.00	1.00
Pgm1[ns]	*1*	0.82	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00
	2	0.75	0.04	0.05	0.06	0.00	0.00	0.03	0.07	0.06	0.02
	3	0.72	0.96	0.95	0.94	1.00	1.00	0.97	0.89	0.94	0.98
Pgm2[ns]	*1*	0.52	0.18	0.20	0.19	0.18	0.25	0.17	0.14	0.20	0.17
	2	0.50	0.79	0.78	0.62	0.75	0.75	0.77	0.77	0.80	0.75
	3	0.44	0.03	0.02	0.19	0.07	0.00	0.06	0.07	0.00	0.08
	4	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00
*Sdh1**	*1*	0.50	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00
	2	0.47	0.07	0.10	0.00	0.03	0.00	0.03	0.09	0.02	0.00
	3	0.45	0.93	0.90	1.00	0.92	1.00	0.97	0.91	0.98	1.00
*Sdh2***	*1*	0.34	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.17
	2	0.29	0.36	0.50	0.56	0.38	0.30	0.47	0.34	0.26	0.52
	3	0.26	0.64	0.50	0.44	0.62	0.67	0.53	0.66	0.74	0.31

[a] Significance of heterogeneity of allele frequencies among populations: *, significant at $P < 0.05$; **, significant at $P < 0.01$; ns, not significant.
[b] Migration of allozyme variant relative to that of buffer front migration.

alleles ranged from two (Adies Stream) to five (Northwest Gander River) (Table 1). The frequencies of three alleles at *Mdh2* and *Me1* were identical in all populations, except for Adies Stream, where a different number of trees were analyzed for these loci, i.e., 15 for *Mdh2* and 11 for *Me1*. None of the allele frequencies showed significant correlation with latitude, longitude, or altitude.

The estimates of genetic diversity parameters and latent genetic potential (LGP) for the white pine populations examined are presented in Table 2. The Northwest Gander River, Adies Stream, and Corry Lake populations representing the three regions exhibited higher genetic diversity than the others (Table 2). Neither populations from Newfoundland and Ontario, nor those of Newfoundland east and west differed substantially in their genetic diversity parameters. The Corry Lake population had the highest and the Sandy Lake population the lowest LGP. The Ontario populations, on average, had higher LGP (9.29) than the Newfoundland populations (7.48). The average LGP of the Newfoundland east populations (7.32) was similar to that of the Newfoundland west (7.63) populations.

Most loci in most populations conformed to the Hardy–Weinberg equilibrium. Only a few significant ($P < 0.05$) departures from the panmictic situation were observed: *Dia1* in the Terra Nova Lake and Sandy Lake, *Fum1* in Adies Stream, *Pgm2* in Sandy Lake and Wylie Lake, *Sdh1* in Terra Nova Lake, and *Sdh2* in Northwest Gander River and Grand Falls populations. This number of significant departures would be expected by chance.

In all populations, the observed proportion of heterozygotes was higher than the expected heterozygosity under the Hardy–Weinberg equilibrium (Table 2). In general, all populations exhibited slight excess of heterozygotes at most loci

Table 2. Genetic variability estimates and their mean fixation index (*F*) values for eastern white pine populations.

Population	Mean no. of alleles per locus	Mean no. of alleles per polymorphic locus	Effective no. of alleles per locus	Percentage of loci polymorphic[a]	Latent genetic potential	Mean heterozygosity		
						Observed	Expected[b]	Mean *F*
Terra Nova Lake	1.70 (0.16)	2.27	1.24	45.0	7.18	0.218 (0.063)	0.193 (0.051)	–0.082
Northwest Gander River	1.80 (0.16)	2.23	1.27	60.0	8.15	0.265 (0.069)	0.212 (0.052)	–0.200
Grand Falls	1.65 (0.17)	2.30	1.22	50.0	6.63	0.213 (0.065)	0.182 (0.053)	–0.176
Birchy Lake	1.80 (0.19)	2.45	1.23	45.0	9.37	0.195 (0.052)	0.189 (0.050)	–0.057
Sandy Lake	1.65 (0.18)	2.44	1.23	40.0	6.42	0.206 (0.062)	0.186 (0.052)	–0.115
Adies Stream	1.75 (0.16)	2.25	1.28	50.0	7.10	0.225 (0.065)	0.216 (0.053)	+0.053
Corry Lake	1.90 (0.20)	2.50	1.26	50.0	10.56	0.225 (0.057)	0.204 (0.051)	–0.129
Cartier Lake	1.75 (0.16)	2.25	1.24	50.0	8.30	0.204 (0.052)	0.191 (0.049)	–0.089
Wylie Lake	1.75 (0.19)	2.50	1.22	40.0	9.02	0.185 (0.054)	0.181 (0.050)	–0.013
Average	1.75	2.35	1.24	47.8	8.08	0.215	0.195	–0.090

Note: Standard errors are given in parentheses.

[a] A locus was considered polymorphic if the frequency of the most common allele did not exceed 0.95.

[b] Unbiased estimate (Nei 1978).

Table 3. Average of genetic distances (Nei 1972) among populations within (on the diagonal) and among (below the diagonal) regions.

Region	No. of populations	Newfoundland east	Newfoundland west	Ottawa River Valley
Newfoundland east	3	0.015 (0.005–0.025)		
Newfoundland west	3	0.015 (0.009–0.036)	0.017 (0.010–0.022)	
Ottawa River Valley	3	0.012 (0.004–0.023)	0.018 (0.007–0.043)	0.015 (0.009–0.020)

Note: Ranges are given in parentheses.

and, with the exception of Adies Stream, on average over all loci (Table 2). However, a number of loci had homozygote excesses in certain populations, namely seven cases in the six Newfoundland populations and three cases in the three Ontario populations.

Heterogeneity of allele frequencies among the nine populations was significant for 10 of the 14 polymorphic loci (Table 1). Among the six Newfoundland populations, only five loci (*Dia1*, *Lap1*, *Mdh2*, *Me1*, and *Pgi2*), and among the three Ontario populations only four loci (*Aat3*, *Mdh2*, *Me1,* and *Sdh2*) showed significant ($P < 0.05$) allele frequency heterogeneity. The heterogeneity of allele frequencies was significant for six loci (*Acp1, Fum1*, *Lap1*, *Mdh2*, *Me1*, and *Pgi2*) among the three populations of Newfoundland west and for one locus (*Dia1*) among the three populations of Newfoundland east.

Population genetic structure and gene flow

The *F* statistics consistently indicated an excess of heterozygotes both for within populations and for the total sample relative to Hardy–Weinberg expectations. For the complete set of nine populations, the mean and range of F_{IS} (correlations between uniting gametes within subpopulations or inbreeding in individuals relative to subpopulations to which they belong), F_{IT} (correlations between uniting gametes for the total population or inbreeding in subpopulations relative to the total population), and F_{ST} values over the loci were –0.139 (–0.273–0.407), –0.069 (–0.202–0.432), and 0.061 (0.016–0.105), respectively. The mean values for F_{IS}, F_{IT}, and F_{ST}, respectively, were –0.160, –0.091, and 0.059 for the six Newfoundland populations; –0.224, –0.185, and 0.047 for the three Newfoundland east populations; –0.076, –0.021, and 0.052 for the three Newfoundland west populations; and –0.098, –0.048, and 0.046 for the three Ontario populations.

The hierarchical *F* statistics analysis suggested that the population differentiations at the regional, provincial, and total sample levels are comparable with each other, with 3.5% (total set), 4.1% (populations within provinces), and 4.5% (populations within regions) of population differentiation. No differentiation of the regions within provinces or regions or provinces within the total sample was detected.

Gene flow rates estimated from F_{ST} values were 3.9 migrants per generation among the complete set of nine populations, 4.0 among the six populations from Newfoundland, 5.1 among the three populations from Newfoundland east, 4.6 among the populations from Newfoundland west, and 5.2 among the three Ontario populations.

Genetic distances and relationships among populations

Genetic distances (Nei 1972) among populations were small and ranged from 0.004 between Terra Nova Lake and Corry Lake to 0.043 between Sandy Lake and Wylie Lake (data not shown), with an average of 0.0153 among all stands. On average, Corry Lake showed the lowest (0.0103) and Sandy Lake the highest (0.0244) genetic distances from other stands. The genetic distances among populations within a region were as great as genetic distances among populations between regions (Table 3). The average and (range) of genetic distances among populations within Newfoundland and Ontario were 0.016 (0.005–0.036) and 0.015 (0.009–0.020), respectively. The average and range of genetic distances between Newfoundland and Ontario were 0.015 and 0.004–0.043. A UPGMA cluster analysis based on genetic distances revealed four groupings of

Fig. 2. UPGMA cluster plot of eastern white pine populations based on Nei's (1972) standard estimates of genetic distances. The numbers in parentheses after the population names correspond to population numbers in Fig. 1.

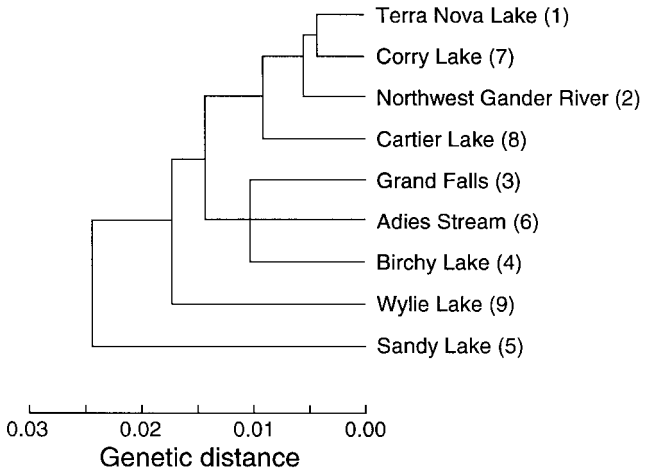
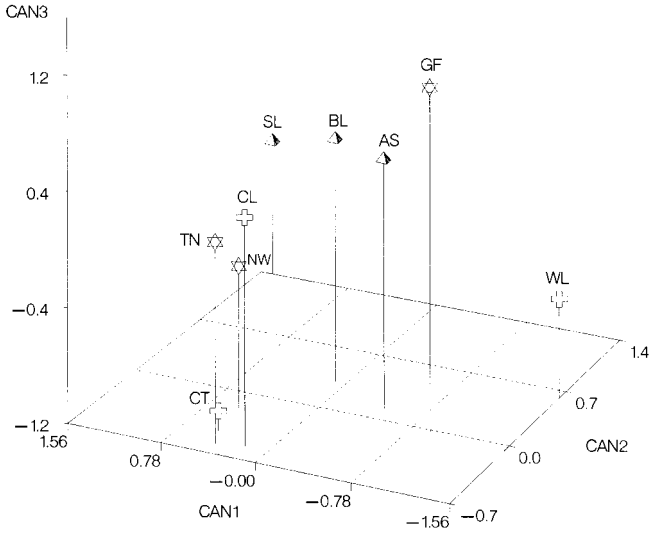


Fig. 3. Scatter of centroids of eastern white pine populations on canonical discriminant functions 1 (CAN1), 2 (CAN2), and 3 (CAN3). Full population names are given in Fig. 1. ✹, Newfoundland east; ◮, Newfoundland west; ✶, Ontario.



the nine populations that were unrelated either to province or region (Fig. 2).

Discriminant analysis of interpopulation differentiation

Three significant ($P < 0.05$) canonical discriminant functions (CAN) accounted for 71.4% of the total variance in 13 polymorphic loci. The first CAN accounted for 34.1% of total variance, 43% of which (i.e., r^2, canonical correlation squared; $r = 0.65$) was explained by the populations' contributions. The second CAN accounted for 22.6% of the total variance, 33% of which was explained by the populations' contribution ($r = 0.58$). The third CAN accounted for 14.7% of the total variation, about 24% of which ($r = 0.49$) was explained by the populations' contributions.

The first three discriminant functions separated the nine populations into four groups (Fig. 3), similar to that obtained from genetic distance cluster analysis. The Sandy Lake population was the most differentiated from the others (Fig. 3). The first discriminant function (CAN1) separated the populations in a random pattern, whereas the second discriminant function (CAN2) separated the three Newfoundland west populations and Grand Falls and Wylie Lake from the remaining four populations. Surprisingly, the Corry Lake and Cartier Lake populations from Ontario showed the least differentiation from the geographically most distant Terra Nova Lake and Northwest Gander River from the Newfoundland east populations (Fig. 3), as was also observed from the cluster analysis of the genetic distances (Fig. 2). The third CAN separated the two Ontario populations, Cartier Lake and Wylie Lake, from the others (Fig. 3).

Discussion

The results of our study suggest that both Newfoundland and Ontario white pine populations harbour moderate to high genetic diversity. The genetic diversity levels observed are generally comparable to that reported for eastern white pine from other parts of the range (Eckert et al. 1981; Ryu 1982; Beaulieu and Simon 1994*a*; Buchert et al. 1997), and are consistent with data for wind-pollinated, winged-seeded, late successional, primarily outcrossed gymnosperms with regional distribution (Hamrick and Godt 1990). Our results demonstrate that despite their marginal location and 8000 years of postglacial geographic isolation, extant disjunct Newfoundland white pine populations are as genetically diverse as the populations from the species' central range in Ontario. White pine reaches the northeastern limits of its natural range in Newfoundland. Marginal populations in other pine species have shown decreased genetic diversity compared with central populations (Yeh and Layton 1979; Guries and Ledig 1982). Given the geographic isolation and small size of Newfoundland white pine stands–populations, one might expect reduced genetic diversity in these populations due to genetic drift and inbreeding. Contrary to our expectations, we found the genetic diversity levels of the marginal Newfoundland populations similar to those of central populations from Ontario. These results are consistent with those reported for white pine from Quebec, where two marginal populations showed genetic diversity levels that were comparable to or higher than those of the central populations (Beaulieu and Simon 1994*a*). Our data do not indicate any substantial regional or provincial differences in genetic diversity levels. In fact, one population each from Newfoundland east, Newfoundland west, and Ontario showed similarly high genetic diversity. In contrast, regional differences in genetic diversity levels were observed in eastern white pine in Quebec (Beaulieu and Simon 1994*a*), where reduced genetic diversity was observed in populations from the St. Lawrence River Valley in comparison to those from the Ottawa River Valley, largely due to heavy logging of white pine in the region. The populations sampled in our study have also experienced at least one cycle of intensive harvesting. However, the Ontario populations generally have higher latent genetic potential than Newfoundland populations, a genetic potential that is a function of allelic richness and allele frequencies (Bergmann et al. 1990). This latent genetic potential

could be an important factor in adaptation to new or changing environments.

Instead of the expected heterozygote deficiency, a heterozygote excess was observed for the white pine populations examined. The mean F_{IS} values suggest an excess of heterozygotes within populations of 13.9% for the complete set of nine populations, 16.0% for the six populations from Newfoundland, 22.4% for the three populations from Newfoundland east, 7.6% for the three populations from Newfoundland west, and 9.8% for the three populations from Ontario. A similar trend of heterozygote excess was evident for the total population. Therefore, our observations indicate that the effects of inbreeding and genetic drift on genetic diversity are not yet evident in the surviving remnants of white pine following its dramatic population decline in Newfoundland. This is probably due to the fact that the Newfoundland gene pool that we sampled was constituted prior to the more serious population collapse resulting from the white pine blister rust infection that has devastated white pine since the 1930s. The effects of inbreeding and genetic drift may become evident in subsequent generations, as shown by a significant deficit of heterozygotes and changes in allele frequencies in the filial generation (seed crop) of the studied populations (data not shown). Thus, our data can serve as a genetic benchmark for future monitoring.

As F_{ST} is equivalent to Nei's (1977) G_{ST} (coefficient of gene differentiation among subpopulations), the F_{ST} values could indicate the extent of population differentiation. Our F_{ST} values indicate that most of the genetic variation resides among individuals within stands–populations, and the estimates for among-population genetic differentiation are comparable to that reported for white pine in other studies (Beaulieu and Simon 1994*a*; O.P. Rajora and G.P. Buchert, unpublished data) and for other conifers (Hamrick and Godt 1990). Genetic studies in other pines suggest that mutation rates may be too low to produce much detectable interpopulation genetic differentiation at the biochemical and molecular level over geological periods such as the interglacials (Mosseler et al. 1991, 1992; Deverno and Mosseler 1997).

The results from genetic distance, *F* statistics, hierarchical *F* statistics, and multivariate canonical discriminant analyses, which take both allele frequency and genotype differences into account, were parallel for interpopulation differentiation at the total sample and various hierarchical levels. These results suggest similar levels of population differentiation at different hierarchical levels and confirm very little or no detectable among-population differentiation within either Newfoundland and Ontario or between these two provinces. The interpopulation gene flow estimates were high (3.9–5.2 migrants per generation), suggesting either that periodical gene exchange among the sampled populations is high or that very little genetic change has occurred since white pine reentered Newfoundland from the mainland following glaciation. Such gene flow rates prevent population genetic differentiation and maintain genetic diversity (Slatkin 1985). However, genetic distances or F_{ST} estimates did not increase with geographic distances, and no regional or provincial patterns of differentiation were apparent. The populations within the two provinces and three regions, on average, were as similar to each other as they were to the populations chosen at random from the other province or regions. Despite 8000 years of postglacial geographic isolation, white pine on the island of Newfoundland shows no evidence of substantial genetic differentiation from white pine in Ontario, suggesting a common origin and that isolation by distance has not played a major role in genetic differentiation at the allozyme loci studied.

A similar pattern of genetic relationship among the nine populations was evident from both canonical discriminant and genetic distance cluster analyses, which were not related to province, region, longitude, latitude, or altitude. Also, there was no significant correlation between allele frequencies and longitude, latitude, or altitude. Thus, our study does not suggest any clinal allozyme variation in eastern white pine in Newfoundland and Ontario related to geographic gradients. However, our population sampling strategy was not targeted to assess adequately the presence of such variation. It should be noted that eastern white pine has shown clinal variation for morphological and adaptive traits (Mergen 1963; Wright 1970).

In summary, it appears that geographic isolation and 8000 years of postglacial evolution since white pine reentered the island of Newfoundland have had little detectable effect on genetic differentiation at the biochemical level between Newfoundland and ancestral mainland populations. Newfoundland white pine is as genetically variable as that from central Ontario. Population decline and associated processes of inbreeding and genetic drift apparently have had little or no detectable effect on genetic variability at the biochemical level in the remnant white pine population of Newfoundland. Furthermore, neither clinal nor provincial, regional, or marginal versus central patterns of genetic variability and differentiation were evident, and isolation by distance has not played a detectable role in population differentiation. Our results provide some genetic benchmarks for monitoring the genetic effects of small population size in eastern white pine following a dramatic decline in both population sizes and numbers. Genetic information from our study may be important for conservation and restoration of white pine, and perhaps other rare and (or) marginal species, in Newfoundland by providing a basis for developing conservation strategies. However, it should be noted that allozymes are often considered as selectively neutral genetic markers, and the adaptive nature of allozyme variation is controversial in forest trees (Bush and Smouse 1992). Moreover, white pine in Quebec has shown substantial provenance variation for adaptive traits (Li et al. 1997).

The vulnerability of white pine's existence as a naturally occurring species in Newfoundland has recently been recognized through the development and implementation of a conservation strategy that focuses on in situ protection of white pine and artificial regeneration (Newfoundland Forest Service 1997). The implementation of this strategy has been hindered by poor seed crops over a succession of recent good cone crops in native pine stands. This poor seed quality may be related either to a deterioration in local climate or to the effects of inbreeding depression related to small population size. In the absence of any information on adaptive trait variation for the Newfoundland white pine, seeds introduced from mainland seed sources as far away as the centre of the geographic range in Ontario may be genetically acceptable for the restoration of white pine in Newfoundland, considering their high genetic similarities and common origin suggested from our study and assuming their adaptability to Newfoundland conditions. However, this assumption will be tested through future analy-

sis of adaptive trait variation in field tests containing the seed sources–populations described here. These seed sources have already been established at locations in central Newfoundland (Grand Falls) and at the Petawawa Research Forest, near Petawawa, Ontario.

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