ISOZYME VARIABILITY IN CENTRAL ONTARIO JACK PINE

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ABSTRACT

Megagametophyte isozyme analysis of 320 jack pine trees from 32 stands in 8 central Ontario townships indicated a high level of genetic variability as measured by average homozygosity (H=0.15). Of 27 isozyme loci detected in 13 enzyme systems, 20 loci were polymorphic. The proportions of average heterozygosity attributed to differences among townships and differences among stands were 2.5% and 6.7% respectively. Approximately 1.5% and 5.2% of the total genetic variability detected could be attributed to among-township and among-stand differences. The high level of isozyme variability found within a small geographic area of jack pine was similar to that found in other conifer species sampled over a much wider area, and has implications for operational jack pine breeding programs.

INTRODUCTION

The need for genetically improved jack pine (Pinus banksiana Lamb.) seed in Ontario's regeneration effort has lead to the development of two independent jack pine tree improvement programs in the province, with the probability of several more being initiated in the near future. At present, administrative regions and districts are selecting jack pine plus-trees and establishing seed orchards accompanied by open-pollinated progeny tests. Ontario's boreal seed transfer zones are very broad and are based upon Hills' (1960) site regions. Hills (1960) considered these site regions to be areas of relatively uniform microclimate. However, jack pine provenance trials have indicated that genetic variation does exist within site region boundaries (Yeatman 1976; Skeates 1979). Although the ideal situation of having adequate information on population structure is not possible due to the need to initiate selection and testing programs, it is seldom too late to utilize such information in a well-designed, flexible tree improvement program. Specific questions of importance in Ontario are: a) how large are effective breeding populations of jack pine; b) do administrative regions or districts share common jack pine breeding populations; c) can seed orchards be established which service more than one administrative unit without danger of losing broad adaptability, and consequently, any genetic gain? We are attempting to find answers to these questions by assessing isozyme variability in natural jack pine stands from a small area in central Ontario. This area was chosen because of the presence of discontinuous jack pine distribution; major groupings of jack pine interpreted from LANDSAT imagery (Buchert 1979) were used as a first level of sampling in a hierarchial sampling design. Within each of two major jack pine groupings, several townships with heavy jack pine concentrations were chosen.

MATERIALS AND METHODS

Seeds used for electrophoresis were obtained from 320 trees representing 32 collection sites in Ontario. These site were divided among 8 geographic townships (Fig. 1). Within each township 10 trees from each of one to five stands were systematically located at 40 m intervals and current year's cones were collected.

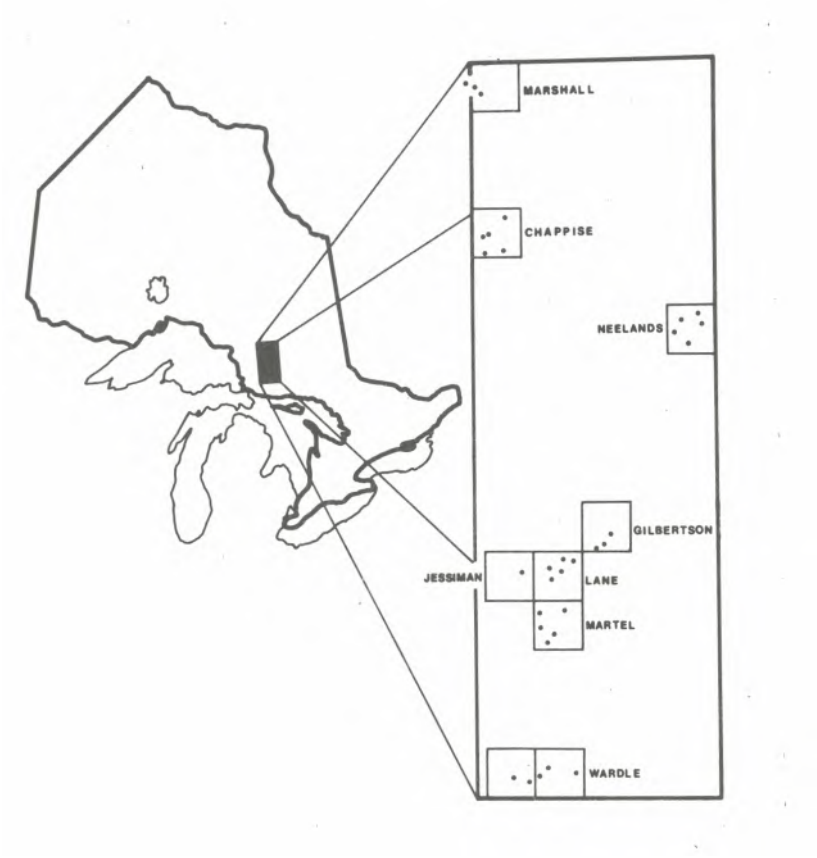

Figure 1: Location map of jack pine isozyme study area in central Ontario. Dots within each township indicate sampled stands.

Seven seeds from each tree were used to determine the tree's genotype. Assuming equal segregation of both alleles in a heterozygous individual, the probability of misclassifying a heterozygote at a locus is $(1/2)\ k^{-1} = 0.02$, where k is the number of megagametophytes analyzed per tree.

Four different buffer systems were used to resolve the various enzymes studied (Table 1). Buffer formulations, as well as running conditions for these four systems are given in Table 2. A total of 27 enzyme loci were resolved from 13 different enzyme systems.

RESULTS

Approximately 47% of the enzyme loci examined are polymorphic in any one stand with each locus averaging 1.59 alleles (Table 3). On a township basis, weighted percent polymorphic loci and average number of alleles per locus are 64.62% and 1.97 respectively, and reflect the effects of an increase in sample size. The total number of alleles detected at each polymorphic locus in this study are as follows: ACPH-2(4); ACPH-3(3); ACO-1(4); ADH-3(3); AAT-1(4); AAT-2,3(3); AAT-4(3); DIA-1(3); DIA-2(4); IDH-2(4); LAP-1(2); MDH-2(4); MDH-3(3); MDH-4(2); PEPT-1(3); PGI-2(3); 6-PGD-1(2); 6-PGD-2(5); and PGM-2(4).

An analysis of gene diversity among subdivided populations was performed according to the procedure outlined by Nei (1973). The three components estimated for each locus were total gene diversity (H_t), the proportion of gene diversity attributable to within population variability (H_s), and the amount of genetic variability due to interpopulation differences (G_{st}). Total gene diversity (H_t) is an estimate of heterozygosity in the species.

The total gene diversity can be given by:

$$H_t = 1 - \sum_{i=1}^{k} \left(\sum_{i=1}^{s} x_i/s \right)^2$$

where x_i is the frequency of the i^{th} of k alleles in the sth subpopulation, and:

$$H_s = 1 - \sum_{i=1}^{k} \left(\sum_{i=1}^{s} x_i^2/s \right)$$

The relative magnitude of interpopulation gene diversity (G_{st}) is measured by:

$$G_{st} = D_{st}/H_t$$

where $D_{st} = H_t - H_s$. D_{st} is the proportion of total heterozygosity attributed to between population differences.

Nei's G - statistics for subdivided populations (Table 4), provide a comparison of variability at the township and stand level, and indicate that levels of genetic variability are fairly high in jack pine as measured by total gene diversity or heterozygosity. The expected value of 15% obtained in this study is very similar to heterozygosity estimates obtained from other conifer species (Table 5). The expected heterozygosity (based on $\Sigma\ h_i/r$ where $h_i = 1 - \Sigma\ xi^2$ for the r^{th} locus, and r = the number of loci) was 0.146 which was similar to the expected heterozygosity based upon the mean H_t.

The largest proportion of this genetic variability or heterozygosity is attributable to within township or within stand gene diversity, and is 97.5% and 93.4% respectively, as measured by (Hs/Ht).

Therefore, 2.5% and 6.7% of average heterozygosity detected can be attributed to differences between townships and stands respectively, as measured by (d_{st}).

Further analysis of population substructure was made by comparing observed and expected heterozygosities for the various enzymes investigated (Table 6). Only those loci in which the frequency of the most common allele is 0.95 or less, are included. The fixation index (Fi) is given by $1 - H_o/H_e$, where H_o = heterozygotes observed, and H_e = heterozygotes expected. A total F_i for each locus is given by EwiFi, where F_i is the fixation index of the s^{th} subpopulation, and w_i is the weighting for sample size in the s^{th} subpopulation. This value is similar to Wright's F_{is}, and has been proposed as an estimate of Fis by Workman and Niswander (1970).

Only two of the polymorphic loci investigated (IDH-2 and 6-PGD-2) show an excess of heterozygotes above expectation, as evidenced by negative F_{is} values at the township level. At the stand level however, six loci (AAT-2,3, DIA-2, IDH-2, PGI-2 and 6-PGD-2) show an excess of heterozygotes. When comparisons are made on a stand basis, there is an approximately equal distribution of loci showing excesses and deficiencies of heterozygotes. Such a result is consistent with random fluctuations of alleles in Hardy-Weinberg equilibrium. A comparison of F_{is} values with the fixation index for all 320 trees sampled is also given in Table 6. The total F_i value indicates a deficiency of heterozygotes for six loci, and excesses for three loci, while for two loci observed heterozygote frequencies equal expected frequencies.

Further analysis of population structure was obtained by calculating an outcrossing rate estimator, t, for townships and for stands (Yeh and Layton 1979). This estimator is given by $t = (1-Fi)/(1+Fi)$. An average of t over all loci reveals deviations from Hardy-Weinberg equilibrium. Values of t greater than 1.0000 indicate an excess of heterozygotes over Hardy-Weinberg expectations, while values less than 1.0000 indicate a deficiency. In all the townships investigated except Chappise and Wardle a deficiency of heterozygotes was observed, while 25 of 32 stands had an excess of heterozygotes (Table 7).

Genetic distance estimates between townships were also obtained using Roger's (1973) genetic distance (Table 8). The mean genetic distance between townships was 0.038, with the lowest value existing between Martel and Chappise townships (0.022), and the greatest genetic distance between Jessiman and Marshall (0.060). The product-moment correlation coefficient between genetic distance and geographic distance was not significant (r=-0.191), and suggests that allele frequencies are randomly distributed throughout the area sampled.

DISCUSSION

Most of the genetic variability detected in this study resides within the stand or township sampled. More of the genetic variability detected could be ascribed to interpopulation differences when comparisons were made at the stand level than when made at the

township level. Such a result is anticipated however, since stand sample sizes are smaller, and therefore the probability of omitting certain rare alleles from stand samples becomes greater. Even with the small stand sample size used in this study, approximately 95% of the total genetic variability detected could be attributed to variability within stands.

The levels of within stand variability (as measured by 1-Gst) detected in other conifer studies ranged from a low of 88% in ponderosa pine (O'Malley et al. 1979) to a high of approximately 96-97% in lodgepole pine, pitch pine and douglas-fir (Yeh and Layton 1979; Yeh and O'Malley 1980; Guries and Ledig 1982). This variation in the amount of intrastand variability may be more apparent than real, and may simply reflect differences in the sample sizes used to estimate G_{st}. Our results indicate a trend similar to that of Yeh and Layton (1979), since more intrasite variability can be attributed to the township level than the stand level. Empirical studies on natural populations have shown that the variance in allele frequencies from subdivided populations is inversely correlated with population size (Selander, 1970). Such an effect would tend to increase G_{st} estimates when population sizes are smaller. Therefore, exact comparisons between levels of interpopulation variability cannot be made between different species unless population sample sizes are similar.

Approximately the same amount of biochemical genetic variability (H_t), and the same proportion of interstand variability was detected in Ontario jack pine stands sampled within 1° of latitude and longitude as that found in species sampled over a much wider geographic range (Yeh and Layton 1979; O'Malley et al. 1979; O'Malley 1979; Guries and Ledig 1982). This has implications for seed orchard and reforestation programs, since seeds obtained from a narrow geographic range may contain as much genetic variability as widely separated seed sources. However, comparative studies in jack pine over a wide geographic range have not been reported. Clinal variation in allele frequencies at a single locus over a wide geographic range correlated with an environmental gradient may indicate a direct response to selection. In addition, morphological and physiological traits which are influenced to a greater degree by environmental factors, seem to show greater interstand variability than allozymes (Ledig and Fryer 1972; Ledig et al. 1976). Therefore, inferences based upon uniform isozyme variability may be completely unwarranted unless it can be shown that the isozyme markers are linked to growth traits.

The observation of heterozygote deficiencies in townships,(t), is consistent with the Wahlund effect, i.e. reduction in the frequency of heterozygotes (compared to expectations based on average allele frequencies), when the subpopulations being compared differ in their allele frequencies (Wahlund 1928). Conversely the excess heterozygosity observed at the stand level may reflect sampling from closely related family groups. Matings primarily within family groups, but with enough gene flow to maintain a dispersal of rare alleles, will cause an excess of heterozygosity over that expected with complete panmixia (Li 1969; Rasmussen 1978). This breeding structure could account for a "patchy" distribution of allele frequencies around a mean value, resulting in heterozygote

deficiencies at the township level. Selection against homozygous genotypes at the embryonic or seedling stage, however has also been invoked to explain excess hterozygosities at the stand level (Linhart et al. 1981; Shaw, 1981).

Shaw (1981) reported that in douglas fir the effective number of males contributing pollen in an open pollinated cross is between two and four. Similar interpretations have been suggested for observed allelic heterogeneity between adjacent stands in other conifer species (Mitton et al. 1977; Linhart et al. 1981). This indicates that most matings in conifer stands probably occur between adjacent, closely related trees. Inbreeding effects due to selfing would of course decrease excess heterozygosities and may even result in decreased observed heterozygosities at the stand level.

The lack of evidence for significant correlations between genetic distance and geographic distance in jack pine sampling sites examined in this study does not necessarily imply that such correlations do not exist. The geographic range from which these trees were sampled may not have been large enough to produce population substructuring through such forces as random drift or natural selection. Evidence for a correlation between genetic distance and geographic distance between douglas-fir stands has been presented (Yeh and O'Malley, 1979). Stands of jack pine sampled over a wider geographic range may indicate similar effects.

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LITERATURE CITED

Buchert, G.P. 1979. Mapping jack pine distribution by satellite. In: Proc. 26th N.E. For. Tree Improv. Conf. State College, Pa. pp. 86-95.

Cardy, B.J., C.W. Stuber, and M.M. Goodman. 1980. Techniques for starch gel electrophoresis of enzymes from maize (Zea mays L.). Institute of Statistics Mimeograph Series No. 1317, North Carolina State University. 31 p.

Clayton, J.W., and D.N. Tretiak. 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. J. Fish. Res. Bd. Canada 29:1169-1172.

Conkle, M.T. 1979. Isozyme variation and linkage in six conifer species. P. 11-17 in Symp. Isozymes of North American For. Trees and For. Insects. USDA For. Serv. Gen. Tech. Rep. PSW-48.

Guries, R.P. and F.T. Ledig. 1982. Genetic diversity and population structure in pitch pine (Pinus rigida Mill.). Evolution 36: 387-402.

Hills, C.A. 1960. Regional site research. For. Chron. 36:401-423.

Ledig, F.T., and J.H. Fryer. 1972. A pocket of variability in Pinus rigida. Evolution 26:259-266.

Ledig, F.T., C.C. Lambeth, and D.I.H. Linzer. 1976. Nursery evaluation of a pitch pine provenance trial. pp. 93-108 in Proc. 23rd N.E. For Tree Improv. Conf. New Brunswick, N.J. pp. 93-108.

Li, C.C. 1969. Population subdivision with respect to multiple alleles. Ann. Hum. Genet. Lond. 33:23-29

Linhart, Y.B., J.B. Mitton, K.B. Sturgeon and M.L. Davis. 1981. Genetic variation in space and time in a population of ponderosa pine. Heredity 46: 407-426.

Mitton, J.B., Y.B. Linhart, J.L. Hamrick and J.S. Beckman. 1977. Population differentiation and mating system in ponderosa pine of the Colorado front range. Theor. Appl. Genet. 51:5-13.

O'Malley, D.M., F.W. Allendorf and G.M. Blake. 1979. Inheritance of isozyme variation and heterozygosity in Pinus ponderosa. Biochem. Genet. 17:223-250.

Rasmussen, D.I. 1978. Sibling clusters and genotypic frequencies. Amer. Natur. 113:948-951.

Rogers, J.S. 1972. Measures of genetic similarity and genetic distance. Univ. Tex. Publ. No. 7213:145-154.

Selander, R.K. 1970. Behavior and genetic variation in natural populations. Am. Zool. 10:53-66.

Selander, R.K., M.H. Smith, S.Y. Yang, W.E, Johnson, and J.B. Gentry. 1971. Biochemical polymorphism and systematics in the genus Peromyscus. I. Variation in the old-field mouse (Peromyscus polionotus). Univ. Tex. Stud. Genet. 6:49-90.

Shaw, C.R., and R. Prasad. 1970. Starch gel electrophoresis of enzymes - a compilation of recipes. Biochem. Genet. 4:297-320.

Shaw, D.V. 1981. The mating system and breeding structure of douglas-fir (Pseudotsuga menziesii var. menziesii). Ph.D. thesis. University of California, Davis, Ca.

Skeates, D.A. 1979. Discontinuity in growth potential of jack pine in Ontario, a 20 year provenance assessment. For. Chron. 55:137-141.

Wahlund, S. 1928. Zusammensetzung von populationen and korrelationsercheinungen vom standpunkt der vererbungslehre aus betrachtet. Hereditas 11:65-106.

Workman, P.L. and J.D. Niswander. 1970. Population studies on southwestern Indian tribes. II. Local differentiation in the Papago. Amer. J. Human Genet. 22:24-49.

Yeatman, C.W. 1976. Seed origin, first, last and always. Can. For. Serv. Chalk River, Ont. Inf. Rep. PS-X-64. 12 pp.

Yeh, F.C. and C. Layton. 1979. The organization of genetic variability in central and marginal populations of lodgepole pine Pinus contorta spp. latifolia. Can. J. Genet. Cytol. 21:487-503.

Yeh, F.C. and D. O'Malley. 1980. Enzyme variations in natural populations of douglas-fir, Pseudotsuga menziesii (Mirb.) Franco, from British Columbia I. Genetic variation patterns in coastal populations. Silv. Genet. 29:83-92.

Table 1 Enzyme systems used which provided the best resolution of isozymes in jack pine.

Enzyme	Abbreviation	Buffer System[1]	Number of loci scored
Acid phosphatase	ACPH	B	2[a]
Aconitase	ACO	A	1
Alcohol dehydrogenase	ADH	D	1[b]
Aspartate aminotransforase	AAT	C	4
Diaphorase	DIA	D	3
Glucose-6-phosphate dehydrogenase	G-6-PDH	A	1
Isocitrate dehydrogenase	IDH	A	2
Leucine aminopeptidose	LAP	C	1
Malate dehydrogenase	MDH	B	4
Peptidases	PEPT	C	2c
6-Phosphogluconic dehydrogenase	6-PGD	B	2
Phosphoglucose isomerase	PG1	B	2
Phosphoglucomutase	PGM	D	2

[1] The buffer systems used and running conditions are described in Table 2.

[a] Five loci were resolved for ACPH activity. All five loci were polymorphic but only ACPH-2 and ACPH-3 were scored since allelic variation at the other three loci could not be accurately determined.

[b] Three loci were detected for ADH activity, but only ADH-3 was clearly resolved.

[c] Allelic variation was detected at PEPT-3, but not all samples were scored for activity at this locus. Therefore, variation at this locus is excluded during the present study, as only PEPT-1 and PEPT-2 were scored.

Table 2 Buffers and running conditions used to survey isozyme variation in jack pine.

Buffer designation/	Electrode buffer formulation	Gel buffer formulation	Running Conditions
A2	0.040 M Citric acid monohydrate adjust to pH 6.7 with N-(3-aminopropyl) morpholine	1:20 dilution of electrode buffer	approximately 200 V at 75 m.a.
B	0.065 M L-histidine[3] and 0.020 M Citric acid monohydrate buffer pH=5.7	1:7 dilution of electrode buffer	approximately 230 V at 50 m.a.
C	0.190 M Boric acid and 0.028 M Lithium hydroxide monohydrate buffer pH=8.1	0.0076 M Citric acid monohydrate and 0.051 M Tris4 buffer pH=8.3 gels were made using a 9:1 dilution of gel:electrode buffer	adjusted to 75 m.a.5
D	0.223 Tris and 0.08615 M Citric acid monohydrate adjust to pH 6.2 with 1N NaOH	1:35 dilution of electrode buffer	approximately 150 V at 75 m.a.

[/] Buffer systems were originally described by:
(A) Clayton and Tretiak (1972); (B) Cardy et al., (1980); (C) Selander et al., (1)71); and (D) Shaw and Prasad (1970).

[2] (A) gel system was composed of a 2:1 mixture of Connaught starch: Electrostarch, while all other gel types were made using a 1:1 mixture.

[3] (L- -Amino-B-imidazolepropionic Acid)

[4] Tris (hydroxymethyl) aminomethane.

[5] Voltage is increased throughout the run to maintain a constant amperage.

Table 3 Average number of alleles detected, heterozygosities, and percentage of polymorphic loci in 32 collection sites of jack pine.

Township	Stands	Percent of polymorphic loci	Average number of alleles per locus	Expected heterozygosities based on allele frequencies	Observed heterozygosities
Martel	01	52	1.59	0.1346	0.1296
	02	41	1.59	0.1190	0.1037
	03	44	1.52	0.1113	0.1111
	04	48	1.67	0.1463	0.1741
	05	55	1.70	0.1587	0.1645
Average		48±2.2	1.6±0.028	0.1339±0.0077	
Total		67	2.07	0.1436	0.1371
Jessiman	01	41	1.52	0.1392	0.1185
Lane	01	44	1.67	0.1576	0.1481
	02	52	1.63	0.1446	0.1333
	03	55	1.67	0.1667	0.1481
	04	52	1.55	0.1670	0.1481
	05	44	1.52	0.1353	0.1304
Average		49±2.0	1.61±0.027	0.1542±0.0056	
Total		70	1.96	0.1595	0.1441
Gilbertson	01	33	1.52	0.1152	0.1111
	02	44	1.59	0.1433	0.1407
	03	44	1.67	0.1436	0.1243
Average		40±3.0	1.59±0.035	0.1340±0.0077	
Total		55	1.85	0.1407	0.1253
Wardle	01	48	1.63	0.1337	0.1407
	02	37	1.41	0.1216	0.1091
	03	52	1.74	0.1503	0.1488
	04	44	1.59	0.1178	0.1185
	05	44	1.52	0.1311	0.1519
Average		45±2.2	1.58±0.049	0.1309±0.0050	
Total		59	1.96	0.1349	0.1349

(cont'd)

Table 3 (Cont'd) Average number of alleles detected, heterozygosities, and percentage of polymorp c loci in 32 collection sites of jack pine

Township	Stands	Percent of polymorphic loci	Average number of alleles per locus	Expected heterozygosities based on allele frequencies	Observed heterozygosities
Chappise	01	52	1.67	0.1791	0.1778
	02	44	1.63	0.1457	0.1333
	03	63	1.74	0.1578	0.1852
	04	52	1.63	0.1439	0.1778
	05	48	1.55	0.1530	0.1418
Average		522.8	1.644±0.027	0.1559±0.0056	
Total		74	2.18	0.1641	0.1638
Marshall	01	44	1.55	0.1098	0.0778
	02	52	1.63	0.1477	0.1667
	03	48	1.59	0.1413	0.1407
Average		48±1.9	1.59±0.019	0.1329±0.0096	
Total		59	1.92	0.1470	0.1321
Neelands	01	44	1.59	0.1513	0.1370
	02	44	1.59	0.1487	0.1391
	03	44	1.34	0.1248	0.1111
	04	44	1.55	0.1098	0.1210
	05	52	1.67	0.1589	0.1823
Average		46+1.4	1.550.049	0.1387±0.0082	
Total		67	1.89	0.1429	0.1466
Grand Average		47±10.23	1.59±0.015	0.1409±0.0031	
Grand Total		74	2.70	0.1466	0.1400

Table 4 Analysis of gene diversity among 32 stands of jack pine from 8 geographic townships.

Locus	Total Gene Diversity (Ht)		Gene diversity within sites (Hs)		Proportion of gene diversity resulting from intersite differences (Gst)	
	Township	Stand	Township	Stand	Township	Stand
ACPH-2	0.3203	0.3148	0.3159	0.2961	0.0136	0.0596
ACPH-3	0.4440	0.4381	0.4265	0.3970	0.0393	0.0938
AC0-1	0.4193	0.4227	0.4145	0.3945	0.0114	0.0667
ADH-3	0.0198	0.0124	0.0193	0.0119	0.0263	0.0451
AAT-1	0.0491	0.0581	0.0482	0.0550	0.0181	0.0539
AAT-2,3	0.3762	0.3756	0.3647	0.3533	0.0305	0.0592
AAT-4	0.0149	0.0151	0.0147	0.0145	0.0102	0.0408
DIA-1	0.0344	0.0398	0.0339	0.0376	0.0145	0.0552
DIA-2	0.4672	0.4593	0.4581	0.4363	0.0194	0.0499
DIA-3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
G-6-PDH-1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
IDH-1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
IDH-2	0.2879	0.2824	0.2838	0.2695	0.0143	0.0457
LAP-1	0.0367	0.0409	0.0362	0.0394	0.0155	0.0394
MDH-1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
MDH-2	0.0418	0.0339	0.0412	0.0314	0.0131	0.0745
MDH-3	0.0906	0.1007	0.0890	0.0948	0.0177	0.0588
MDH-4	0.0246	0.0277	0.0244	0.0258	0.0096	0.0702
PEPT-1	0.0198	0.0216	0.0196	0.0205	0.0100	0.0552
PEPT-2	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
PGI-1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
PGI-2	0.1433	0.1659	0.1401	0.1577	0.0222	0.0490
6-PGD-1	0.5000	0.4999	0.4816	0.4746	0.0367	0.0505
6-PGD-2	0.3298	0.3138	0.3211	0.2955	0.263	0.0585
PGM-1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
PGM-2	0.0634	0.0963	0.0614	0.0648	0.0321	0.3272
MEAN	0.1503	0.1516	0.1466	0.1416	0.0152	0.0523

Table 5 Expected heterozygosities reported for various conifer species in studies

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Species	Number of sites sampled	Number of trees sampled	Number of loci	Expected Ht[1]	Heterozygosity[2]	Reference
Pinus banksiana	32	320	27	0.151	0.146	Present study
Pinus rigida	11	694	21	0.152	0.146	Guries and Ledig,1982
Pseudotsuga menziesii	11	NA	21	0.158	0.155	Yeh and O'Malley,1980
Pinus contorta	9	135	25	0.167	0.160	Yeh and Layton,1979
Pinus contorta	1	40	39	NA	0.185	Conkle, 1979
Pinus ponderosa	10	45	23	0.123	0.127	O'Malley et al. 1979
Pinus attenuata	10	49	22	NA	0.125	Conkle, 1979
Pinus jeffreyi	4	75	43	NA	0.261	Conkle, 1979

[1] determined by aaveraging Nei's H_t value for each locus.

[2] determined by E hi/r,where h_i =1-E xi^2 for the rth locus, k=the number of alleles, and x_i = the frequency of the kth locus.

Table 6 Comparison of Township and Stand Fis values with total population fixation index, for 11 polymorphic loci in jack pinel.

Enzyme Locus	F_{is}		F_i
	Township	Stand	
ACPH-2	0.077	0.027	0.082
ACPH-3	0.222	0.148	0.233
ACO-1	0.041	0.022	0.066
AAT-2,3	0.012	-0.020	0.067
DIA-2	0.016	-0.024	0.030
IDH-2	-0.011	-0.052	0.028
MDH-3	0.005	0.000	0.100
PG1-2	0.029	-0.083	-0.018
6-PGD-1	0.044	0.023	0.058
6-PGD-2	-0.021	-0.061	-0.019

[1] frequency of most common allele 0.95 or less.

Table 7 Comparison of estimated outcrossing rates (t) among townships and stands based upon 11 polymorphic loci[2].

Stand[1]	Township	t	s.e.	t	s.e.
01	Martel	0.9289	±0.1342	0.9487	±0.0582
02		1.0149	±0.1171		
03		1.0373	±0.1037		
04		1.3345	±0.1675		
05		1.4584	±0.2099		
01	Jessiman	0.8877	±0.1549	0.8877	±0.1549
01	Lane	1.0600	±0.1229	0.9330	±0.0671
02		1.0251	±0.1447		
03		1.1547	±0.1172		
04		0.9630	±0.1088		
05		1.0514	±0.1290		
01	Gilbertson	1.0450	±0.1557	0.7951	±0.1240
02		1.1570	±0.1841		
03		0.8494	±0.1803		
01	Wardle	1.5305	±0.4265	1.0330	±0.0954
02		1.1718	±0.1577		
03		1.0965	±0.1352		
04		1.1378	±0.1787		
05		1.5820	±0.3865		
01	Chappise	1.1108	±0.1513	1.0311	±0.0841
02		0.9974	±0.1069		
03		2.0433	±0.7503		
04		1.5994	±0.1717		
05		0.9045	±0.1190		
01	Marshall	0.8566	±0.1283	0.9367	±0.0841
02		1.2011	±0.1051		
03		1.0365	±0.1476		
01	Neelands	1.1593	±0.1748	0.9852	±0.0713
02		1.0472	±0.1242		
03		1.0355	±0.1316		
04		1.2346	±0.0997		
05		1.8439	±0.4424		

[1] stands indicated correspond to townships listed in the central column.
[2] frequency of most common allele 0.95 or less.

Table 8 Roger's genetic distance between townships sites of jack pine is above the diagonal and geographic distance[1] is below the diagonal, with average heterozygosity (H) for the collection site, on the diagonal.

	Martel	Jessiman	Lane	Gilbertson	Wardle	Chappise	Marshall	Neelands
Martel	(0.144)	0.051	0.032	0.036	0.031	0.022	0.037	0.029
Jessiman	10.4	(0.139)	0.057	0.050	0.053	0.048	0.060	0.046
Lane	10.4	8.0	(0.160)	0.042	0.034	0.033	0.026	0.034
Gilbertson	17.6	16.8	8.8	(0.141)	0.033	0.036	0.044	0.026
Wardle	24.0	33.6	34.4	39.2	(0.135)	0.034	0.042	0.026
Chappise	75.2	65.6	65.6	64.8	99.2	(0.164)	0.043	0.029
Marshall	104.8	96.0	95.2	93.6	128.8	30.4	(0.148)	0.037
Neelands	64	58.4	52.8	47.2	86.4	42.4	60.8	(0.143)

[1] geographic distances are in km.