THE POPULATION STRUCTURE OF BLACK SPRUCE IN CENTRAL NEW BRUNSWICK

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ABSTRACT. The inter- and intra-population structure of six populations of black spruce (Picea mariana [Mill.] B.S.P.) in central New Brunswick was studied using allozyme frequencies at 12 loci, of which only seven proved to be independent. Expected heterozygosities varied widely among loci but showed close agreement with observed values. Average heterozygosities were comparable with other coniferous species although possibly slightly inflated due to the small sample of loci.

The amount of population differentiation, as measured by F-statistics, was only 1% of the total genetic diversity. Genetic distances were low and showed no correlation with geographic distances.

Intra-population structure in one stand was studied by relating the number of allele differences between trees to distance of separation. There was no indication of a significant neighbourhood structure.

The implications of these results for black spruce improvement in New Brunswick are that sampling should be concentrated within populations with plus trees being selected by the comparison-tree method.

INTRODUCTION

In forest genetics and tree breeding we are still, to a large extent, dealing with a non-domestic situation. Progress in tree breeding is made by selecting, from wild populations, individuals which are genetically superior for whatever characters are of commercial interest. Thus, the basic problem in tree breeding is one of sampling. The superior individuals that we wish to locate are individual members of a genetic population (Falconer, 1981) and if we wish to maximise genetic gain whilst minimising costs, it is necessary to design an efficient sampling strategy.

The efficiency of a strategy will depend on the distribution of individuals, or more accurately of genotypes, since we are not so much interested in the individual itself as the genes which it carries. The design of an efficient sampling strategy therefore requires knowlege of the population structure of the species - the amount and pattern of genetic diversity within populations and the distribution of genetic differentiation among populations. Although an investigation of population structure should preferably precede the initiation of a tree improvement programme, this is not usually possible. However, the design of flexible programs will allow adjustments as more information becomes available (Danzmann and

Buchert, 1983).

Black spruce (Picea mariana [Mill.] B.S.P.) is one of the most important commercial species in eastern Canada and improvement programs are underway in several provinces. To date however, there has been little investigation of the population structure of the species. In this study, the pattern of genetic differentiation among populations and of genetic diversity within populations was investigated for a small area in central New Brunswick using allozyme frequencies. This forms part of a larger study into the mating system and population structure of black spruce.

MATERIAL AND METHODS

Sampling.

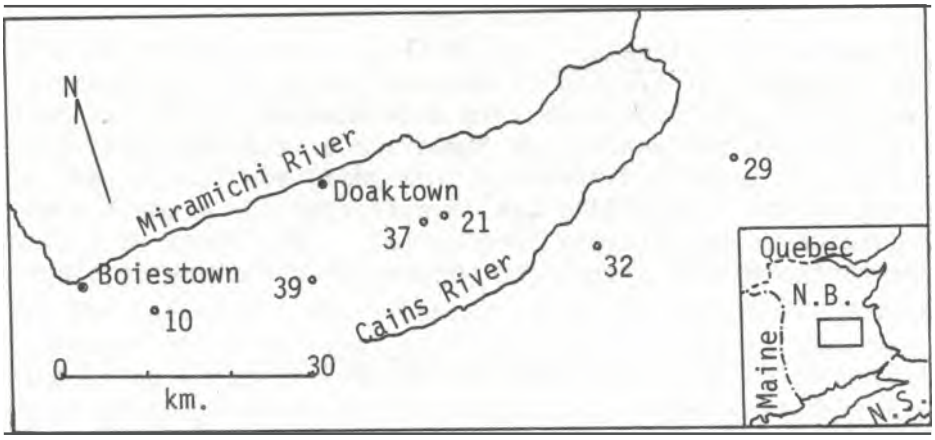
In each of six even-aged stands in central New Brunswick, 40 dominant or co-dominant trees were felled and as many cones as possible collected. The location of the sampled stands is shown on Map 1 and the matrix of distances between them in Table 1. Stand numbers are reference numbers allocated by the New Brunswick Tree Improvement Council. The maximum distance between stands was 52 km and there are no major gradients or differences in soils, climate or elevation within the area. In one population (number 29), each individual in the sample was mapped for the purposes of investigating intra-population structure, as described below. As part of the mating system study, twelve gametophyte-embryo pairs were assayed per tree. The genotype of the mother tree was inferred from the allelic content of the gametophytes. Since the megagametophyte tissue is haploid, the probability of every megagametophyte from a heterozygous mother tree carrying the same allele is given by $0.5^{(k-1)}$, where k is the number of megagametophytes assayed. Thus the probability of a heterozygote being misclassified as a homozygote was 0.5^{11} (<0.0005), or in other words, fewer than one tree in 2000 would be incorrectly genotyped.

Table 1. Geographic distances between populations (kilometres).

	10	21	29	32	37	39
10		26.2	52.1	39.3	24.4	13.4
21			26.2	14.3	1.45	12.8
29				13.7	27.8	38.7
32					15.7	25.9
37						11.2
39						

Electrophoretic methods.

Horizontal starch gel electrophoresis was used to separate the isozymes at eleven enzyme systems. These are listed in Table 2. Details of the procedure and staining recipes used will be given elsewhere (Boyle and Morgenstern, in preparation).



Map 1. Location of the six sampled stands.

Table 2. Enzyme systems assayed.

Enzyme system	Enzyme commission #	Abbr.	# of loci
Aldolase	E.C.4.1.2.13	ALD	1
Aspartate amino-transferase	E.C.2.6.1.1	AAT	1
Fumarase	E.C.4.2.1.2	FUM	1
Glucose-6-phosphate dehydrogenase	E.C.1.1.1.49	G6P	1
Glutamate dehydrogenase	E.C.1.4.1.3	GDH	1
Isocitrate dehydrogenase	E.C.1.1.1.42	IDH	1
Malate dehydrogenase	E.C.1.1.1.37		3
Phosphoglucomutase	E.C.2.7.5.1	PG•	1
6-Phosphogluconate dehydrogenase	E.C.1.1.1.44	6PGD	2
Phosphoglucose isomerase	E.C.5.3.1.9	PGI	1
Shikimic acid dehydrogenase	E.C.1.1.1.25	SDH	1

Statistical analysis.

Measures of gene diversity within populations.

A measure of the amount of genetic diversity within populations should take into account both the number of alleles at a locus and their relative frequencies. Although several methods have been developed that take both these factors into account, the most commonly reported measures of genetic diversity are expected and average heterozygosity (Nei, 1975). When possible, it is of interest to use the same statistics as have been used previously since this enables comparisons to be made among studies of the same and different species (Linhart et al., 1977). Therefore expected and average heterozygosity were used as measures of genetic diversity in this study together with the average number of alleles per locus and the effective number of alleles per locus (Crow and Kimura, 1970).

Genetic differentiation among populations.

Again, several methods have been developed to quantify the amount of genetic differentiation among populations. Of these, the most commonly used methods are the contingency X^2 test of Workman and Niswander (1969), the adaptation by Nei (1973) of Wright's (1965) F-statistics and Nei's (1972) genetic distance. All three methods were used in this study.

Distribution of genetic diversity within stands.

There have been far fewer studies on the intra-population structure of forest trees than on inter-population structure. Sakai and Miyazaki (1972) counted the number of allele differences between individuals in a natural population of Thujopsis dolobrata (L.f.) Sieb. and Zucc. They then fixed an arbitrary number of differences below which individuals were considered to be related at some given level of relationship. Guries and Ledig (1977) also counted the number of allele differences, but weighted them according to allele frequencies. However, they did not attempt to equate the number of allele differences to some coefficient of relationship.

In this study, the expected number of allele differences, weighted by allele frequency, were calculated for various levels of relationship (selfs, full-sibs and half-sibs) for each of the observed multilocus genotypes in population 29. An equation of the form:

y = ax(b + cxf + dxg + ex") was then fitted through the calculated points for the regression of coefficient of relationship on number of allele differences. The weighted number of allele differences between each individual and each other tree in the stand was then summed over all loci and the coefficient of relationship calculated for every pair from the regression equation. Another regression equation, of the form y = axb, was then calculated for the regression of coefficient of relationship on distance of separation using all possible pairwise combinations of the 40 trees. This method is described in greater detail in Boyle (in preparation).

The variation of allele frequencies in population 29 was also examined by means of a X test. The observed distribution of alleles was compared with the expected distribution by dividing the stand into seven quadrats each of size 40 m x 40 m. The proportions of alleles in each quadrat should then equal the overall observed proportions if no neighbourhood structure exists.

RESULTS

Fourteen zones of activity were scored for the eleven enzyme systems. However, for two of these (SDH and MDH-1) the genetic origin of the observed variation could not be confirmed. Many of the other loci were involved in linkage or gametic associations and MDH-2 was monomorphic (Boyle and Morgenstern, in preparation). This left a sub-sample of seven loci (including MDH-2) which could be considered to be independent, namely ALD, IDH, MOH-2, PGM, 6PGD-1, 6PGD-2 and PGI. Several of the methods described above require an assumption of independence, for example the contingency X^2 tests, F-statistics and genetic distance. For these methods therefore, only the seven independent loci were used whilst for other methods

results for all twelve loci and for the seven independent loci are given.

Since loci with high levels of variability are the most useful for the estimation of mating system parameters, there may be a slight bias in the selection of loci scored in favour of more variable loci. Thus the sample of loci may not be completely random (for example only MDH-2 was monomorphic in all populations). However, since to a large extent comparisons of the relative variability are considered, the estimates of genetic differentiation should not be greatly affected (Shaw, 1982).

Genetic diversity.

(a) Allele frequencies and heterozygosities. Table 3 shows the observed allele frequencies at all the loci for each population, along with the overall mean allele frequencies, observed and expected heterozygosities and average heterozygosity based on the seven independent loci. Two loci (PGM and 6 PGD-1) had a mean expected heterozygosity over all populations of greater than 0.5, two more loci (G6P and PGI) produced heterozygosities between 0.25 and 0.5 whilst all the remaining loci gave mean heterozygosities of less than 0.25.

Comparison of the observed and expected heterozygosities shows that in most cases there is very close agreement, especially, as would be expected, at the less variable loci. Looking at the mean heterozygosities over all populations, again there is very good agreement at most loci. Only at four loci, namely PGM, AAT, G6P and MDH-3, is there any apparent deficiency in observed heterozygosities. Interestingly, in all these cases, the major culprit is population 39 where far fewer heterozygotes were observed compared with the expected numbers.

(b) Average number of alleles per locus. Table 4 shows the average number of alleles per locus, for all twelve loci and for the sub-sample of seven independent loci. There appears to be little relationship between the average number of alleles per locus and expected heterozygosity, reflecting the observation of Crow and Kimura (1970) that often alleles may be present at very low frequencies which contribute very little to the heterozygosity.

(c) Effective number of alleles per locus. The effective number of alleles for all loci in each population are given in Table 5, with the geometric means in Table 4. Since both expected heterozygosity and effective number of alleles are functions of the squared observed allele frequencies and both are maximised when allele frequencies are equal, there is obviously a very close relationship between the two.

Genetic differentiation.

(a) Contingency X^2 tests. Results of the contingency X^2 tests for differences in allele frequencies are given in Table 6. The values should be compared with a Xdistribution with (s-1) (k-1) degrees of freedom, where s is the number of populations and k is the total number of alleles recorded at the locus. When considered individually, there are only two loci at which allele frequencies differ significantly between populations. Differences in allele frequencies at G6P are significant at the 5% level, whilst at GDH the differences are significant at the 1% level. The X^2 values

Table 3. Allele frequencies, expected and observed heterozygosities and overall population means for all 11 polymorphic loci. Average heterozygosities are also given, based on the seven independent loci, with standard errors.

Locus	Allele	Population 10	21	29	32	37	39	Mean
ALD	1	0.013	0.013	0.000	0.013	0.013	0.025	0.013
	2	0.987	0.987	1.000	0.975	0.987	0.937	0.979
	3	0.000	0.000	0.000	0.012	0.000	0.038	0.008
Expected h		0.025	0.025	0.000	0.049	0.025	0.119	0.041
Observed h		0.025	0.025	0.000	0.050	0.025	0.125	0.042
1DH	1	0.050	0.025	0.050	0.000	0.013	0.025	0.027
	2	0.950	0.975	0.950	1.000	0.987	0.975	0.973
Expected h		0.095	0.049	0.095	0.000	0.025	0.049	0.053
Observed h		0.100	0.050	0.100	0.000	0.025	0.050	0.054
PGM	1	,0.587	0.550	0.500	0.462	0.512	0.500	0.519
	2	0.063	0.125	0.138	0.138	0.138	0.163	0.128
	3	0.350	0.325	0.362	0.400	0.350	0.337	0.353
Expected h		0.528	0.576	0.600	0.607	0.596	0.610	0.590
Observed h		0.525	0.625	0.575	0.625	0.550	0.450	0.558
6PG0-1	1	0.400	0.363	0.475	0.450	0.387	0.413	0.415
	2	0.562	0.637	0.487	0.537	0.600	0.587	0.568
	3	0.038	0.000	0.038	0.013	0.013	0.000	0.017
Expected h		0.522	0.462	0.535	0.508	0.490	0.485	0.505
Observed h		0.475	0.475	0.575	0.525	0.550	0.475	0.513
6PGD-2	1	0.037	0.025	0.088	0.050	0.062	0.050	0.052
	2	0.950	0.975	0.912	0.950	0.925	0.937	0.942
	3	0.013	0.000	0.000	0.000	0.013	0.013	0.006
Expected h		0.096	0.049	0.160	0.095	0.140	0.118	0.110
Observed h		0.100	0.050	0.125	0.100	0.150	0.125	0.108
PGI	1	0.013	0.012	0.000	0.000	0.013	0.013	0.009
	2	0.825	0.900	0.750	0.825	0.775	0.737	0.802
	4	0.162	0.088	0.250	0.175	0.212	0.250	0.189
Expected h		0.293	0.182	0.375	0.289	0.354	0.393	0.321
Observed h		0.350	0.200	0.350	0.300	0.400	0.325	0.321
AAT	1	0.000	0.000	0.000	0.013	0.000	0.000	0.002
	2	0.987	0.937	0.912	0.912	0.975	0.912	0.939
	3	0.013	0.063	0.088	0.075	0.025	0.088	0.059
Expected h		0.025	0.117	0.160	0.162	0.049	0.160	0.114
Observed h		0.025	0.125	0.175	0.175	0.050	0.075	0.104
FUM	1	0.038	0.013	0.050	0.025	0.025	0.038	0.032
	2	0.962	0.987	0.950	0.975	0.975	0.962	0.968
Expected h		0.072	0.025	0.095	0.049	0.049	0.072	0.062
Observed h		0.075	0.025	0.100	0.050	0.050	0.075	0.063
G6P	1	0.063	0.175	0.187	0.138	0.188	0.162	0.152
	2	0.100	0.013	0.088	0.100	0.050	0.088	0.073
	3	0.000	0.000	0.000	0.000	0.050	0.025	0.013
	4	0.837	0.812	0.725	0.762	0.712	0.725	0.762
Expected h		0.285	0.309	0.432	0.390	0.452	0.440	0.391
Observed h		0.250	0.325	0.350	0.400	0.500	0.375	0.367
GDH	1	0.013	0.025	0.163	0.050	0.150	0.138	0.090
	2	0.987	0.975	0.837	0.950	0.850	0.862	0.910
Expected h		0.025	0.049	0.272	0.095	0.255	0.237	0.163
Observed h		0.025	0.050	0.325	0.050	0.300	0.275	0.171
MDH-3	1	0.000	0.000	0.013	0.000	0.000	0.013	0.004
	2	0.950	0.912	0.862	0.937	0.962	0.837	0.910
	4	0.050	0.088	0.125	0.063	0.038	0.150	0.086
Expected h		0.095	0.160	0.240	0.117	0.072	0.276	0.164
Observed h		0.100	0.125	0.275	0.125	0.075	0.175	0.146
Average h		0.223 +0.086	0.192 +0.088	0.252 +0.095	0.221 +0.095	0.233 +0.093	0.253 +0.090	

Table 4. Average number of alleles per locus and effective number of alleles per locus for each population. Figures based on all 12 loci are given first, with those based on the seven independent loci below.

	Population					
	10	21		32	37	39
Average number of alleles/locus	2.33±0.65 2.43±0.79	2.17±0.58 2.14±0.69	2.17±0.72 2.00±0.82	2.25±0.75 2.14±0.90	2.42±0.79 2.43±0.79	2.50±0.80 2.43±0.79
Effective number of alleles/locus	1.25 1.34	1.24 1.29	1.38 1.39	1.29 1.36	1.32 1.38	1.38 1.41

Table 5. Effective number of alleles for each locus in each population.

	Population					
	10	21	29	32	37	39
ALD	1.03	1.03	1.00	1.05	1.03	1.14
IDH	1.10	1.05	1.10	1.00	1.03	1.05
PGM	2.12	2.36	2.50	2.55	2.48	2.56
6PGD-1	2.10	1.86	2.15	2.04	1.96	1.94
6PGD-2	1.11	1.05	1.19	1.10	1.16	1.14
PGI	1.41	1.22	1.60	1.41	1.55	1.65
AAT	1.03	1.13	1.19	1.19	1.05	1.19
FUM	1.08	1.03	1.10	1.05	1.05	1.08
G6P	1.40	1.45	1.76	1.64	1.83	1.78
GDH	1.03	1.05	1.38	1.10	1.34	1.31
MDH-3	1.10	1.19	1.32	1.13	1.08	1.38

Table 6. Results of the X^2 contingency tests and F-statistics for each locus. The total values are for the seven independent loci.

	X^2d	df	HT	Hs	GST
ALD	13.20	10	0.041	0.040	0.016
IDH	6.09	5	0.053	0.052	0.013
PGM	5.86	10	0.589	0.586	0.005
6PGD-1	10.58	10	0.504	0.500	0.008
6PGD-2	6.76	10	0.111	0.110	0.007
PGI	11.92	10	0.321	0.314	0.020
AAT	12.63	10	0.114	0.112	0.016
FUM	2.27	5	0.061	0.060	0.005
G6P	28.05*	15	0.390	0.384	0.014
GDH	22.50**	5	0.163	0.155	0.047
MDH-3	14.31	10	0.164	0.160	0.023
TOTAL	54.41	55	0.231	0.229	0.010**

from independent loci can be summed and compared with a X^2 distribution with degrees of freedom equal to the sum of the individual degrees of freedom (Workman and Niswander, 1970). The total X^2 over all independent loci is 54.41, with 55 d.f., which is not significant.

(b) F-statistics. The values of HT, Hs and GS_T as defined by Nei (1973) are given in Table 6 for individual loci, with the overall value for the seven independent loci. The value of GST gives an indication of the relative amount of variation between populations compared with the total observed variation. Thus between population variation accounts for only 1% of the total overall, whilst at individual loci this varies between 0.5% at FUM to 4.7% at GDH. The sample standard error of total G_{ST} calculated using the expression derived by Chakraborty (1974) was only 0.0028. The total gene diversity is measured by the overall HT value. The figure of 0.231 obtained here is very similar to the mean value of average heterozygosity over all populations (0.229).

(c) Genetic distance. The matrix of genetic distances between populations, based on the gene identities of the seven independently segregating loci, and standard errors as calculated by the method of Nei and Roychoudhury (1974), are given in Table 7. As explained above, since monomorphic loci are probably under-represented, these estimates of genetic distance are probably upper limits. If the observed genetic distances between populations are due to isolation by distance, it would be expected that a strong relationship should exist between genetic and geographic distance (Nei, 1975). Such a relationship can be examined by use of a product-moment correlation (Yeh and O'Malley, 1980). The correlation coefficient in this case is 0.064, which is non-significant.

Table 7. Genetic distance between populations.

	21	29	32	37	39
10	.0023 ±.0011	.0038 ±.0018	.0031 ±.0024	.0021 +.0011	.0036 +.0020
21		.0084 ±.0049	.0041 ±.0022	.0033 ±.0024	.0042 ±.0025
29			.0023 +.0009	.0025 +.0020	.0025 +.0015
32				.0017 ±.0009	.0023 ±.0013
37					.0008 ±.0003

Genetic diversity within stands.

(a) Regression analysis. The overall weighted expected number of allele differences between selfs, full-sibs and half-sibs in stand 29 were 2.54, 3.09 and 4.19 respectively. These compare with an overall weighted average

number of allele differences between two random trees of 6.24, corresponding to a coefficient of relationship of 0.059. However, the correlation coefficient between distance of separation and coefficient of relationship was only 0.004.

(b) The X^2 analysis. In analysing the distribution of allele frequencies by quadrats, the size of quadrat is important since if they are too small a large number of quadrats will have very small expected proportions, while if they are too large the method is insensitive to small neighbourhood sizes. The sensitivity of the method also depends on the relative allele frequencies as variations in allele frequencies are most easy to detect for loci with two or more alleles at approximately equal frequencies. Table 8 shows results for seven quadrats of size 30 m x 30 m, each containing between three and seven trees, for the PGM locus at which the frequency of allele 1 was 0.5. The total X^2 value of 4.17, with six degrees of freedom, is nonsignificant.

Table 8. Analysis of the distribution of PGM allele 1 in stand 29 by a X^2 test. The frequency of allele 1 was 0.5.

Quadrat	Observed	Expected	X2
A	7	7	0.00
B	8	6	0.67
C	5	7	0.57
D	7	7	0.00
E	4	3	0.33
F	2	5	1.80
G	7	5	0.80
		40	4.17 (6 d.f., ns)

DISCUSSION

In this study average heterozygosities ranged from 0.192 to 0.253 for the six populations of black spruce with a mean of 0.229. These estimates are similar to those obtained for other conifer species. For example, in Douglas fir (Pseudotsuga menziesii) [Mirb.] Franco), estimates of 0.155 (Yeh and O'Malley, 1980) and 0.388 (Yang et al., 1977) have been reported. In Sitka spruce (Picea sitchensis [Bong.] Carr.), Yeh and El-Kassaby (1980) found an average T Tozygosity of 0.147, whilst in eleven populations of Norway spruce (P. abies Karst.) in Sweden, Lundkvist and Rudin (1977) obtained estimates ranging from 0.32 to 0.41.

In most of these studies considerable inter-locus heterogeneity has been noted in expected heterozygosities, though characteristically with a modal value approaching zero (Yeh and O'Malley, 1980). This heterogeneity results in large standard errors of average heterozygosity, as indeed was the case in this study, with standard errors ranging from 36% to 46% of the

estimates. For this reason Yeh (1979) recommended a large sample of loci. Yeh and O'Malley (1980) considered that the much higher estimate of average heterozygosity obtained for Douglas fir by Yang et al. (1977) was in fact an artifact due to the much smaller number of 167 used in the latter study. This theory would appear to be reasonable, since the high estimates reported for Norway spruce by Lundkvist and Rudin (1977) were also based on a small number of loci (four). Although twelve loci were assayed in this study, only six proved to be both polymorphic and to fulfil the required assumption of independence (Nei and Roychoudhury, 1974). The estimates reported here are therefore likely to be somewhat biased upwards.

As noted previously, the effective number of alleles per locus is correlated very closely with expected heterozygosity since there response to relative allele frequencies is so similar. However, since the effective number of alleles does give an indication of the number of alleles at a locus, it should be included in any description of genetic diversity.

Only two loci out of eleven showed any significant differentiation in allele frequencies between the six populations. In contrast, in ponderosa pine (Pinus ponderosa Laws.), differentiation among six clusters within a single stand of about 2 ha was detectable at two out of seven loci (Linhart et al., 1977). Guries and Ledig (1977) studied four populations of pitch pine (Pinus rigida Mill.) in New Jersey which were separated by distances comparable to those in this study. They found significant differentiation at nine out of 15 loci, together with considerable variation in X^2 values which they attributed to individual loci responding independently to different factors causing the variation. They therefore concluded that environmental heterogeneity was at least partly responsible. Similar results were obtained from a larger study of the same species involving eleven populations from North Carolina to Quebec (Guries and Ledig, 1981).

Values of G_{ST} in most conifer species studied have tended to be less than 0.1 (summaries in Guries and Ledig, 1981 and Yeh, 1979). This is interpreted as meaning that more than 90% of the total variation observed is attributable to variation within populations and less than 10% to variation between populations. The results obtained for black spruce suggest that as much as 99% of the variation is contained within populations with 1% between populations, at least within distances of up to 52 km. The low value for the standard error of GST suggests however that the amount of variation contained within populations is significantly different from zero. These figures are larger than for most species, but comparable with balsam fir (Abies balsamea [L.] Mill.) in New Hampshire (Neale, 1978). Yeh (1979) has attributed low GST values to the ecological amplitude of the species investigated, their breeding system and to the lack of effective barriers to gene flow. The obvious implications of low GST values are that intensive efforts to sample a large number of populations within a region are not worthwhile; it would be far more efficient to concentrate on intra-population sampling. Since local differentiation of black spruce is apparently negligible (at least in central New Brunswick), the conclusions of Morgenstern (1969, 1978) that variation in black spruce is predominantly clinal, are substantiated. This also suggests that variation patterns detected in the present series of range-wide provenance tests to a large

extent summarise the complete pattern of population variation in black spruce.

Comparisons of genetic distance should be made with caution since the absolute values are affected by the number of monomorphic loci included (Linhart et al., 1977). Also, as Nei (1972) points out, the method is not reliable Tr values near zero and the large standard errors calculated indicate that many of the distances are not significantly different from zero. Nevertheless, these results are clearly typical of many conifer species. Lundkvist (1979), using only polymorphic loci, obtained distances between four Swedish populations of Norway spruce ranging from 0.011 to 0.042. On the other hand, Yeh and O'Malley (1980), with three monomorphic loci out of 21 assayed, found genetic distances among eleven populations of Douglas fir ranging from 0.0002 to 0.0082. As noted earlier, a strong correlation between genetic and geographic distance is expected if isolation by distance is responsible for the observed differentiation. The near-zero correlation coefficient found here suggests that some other factor is responsible for the differentiation. Of course, due to the large standard errors of genetic distances, the correlation coefficient may merely be reflecting the fact that many of the distances are approximately zero.

Although black spruce appears to be typical of many conifer species in terms of the amount and distribution of genetic diversity, it is perhaps surprising that larger values were not obtained for GST and D. Black spruce readily hybridizes with red spruce (Picea rubens Sarg.), which forms a major component of the natural forest in New Brunswick. It might be expected therefore that differences in the degree of introgression of the two species would inflate the estimates of between-population variation. However, the populations sampled have been selected as phenotypically superior stands and may therefore be purer black spruce than the average New Brunswick population.

In stand 29, two trees taken at random can be expected to have a coefficient of relationship of about 0.059. Since there appears to be no association of trees showing high coefficients of relationships, neighbourhoods do not appear to be well developed. Evidence from the X^2 test seems to confirm the absence of neighbourhoods. This contrasts with the findings of Mitton et al. (1977) who detected a well developed neighbourhood structure in ponderosa pine over fairly small distances, which they attributed to the effects of diversifying selection, since there was no apparent barrier to pollen flow.

The effectiveness of wind-borne pollen as a means of gene flow is a subject of much debate (Levin and Kerster, 1974) since although pollen may be blown long distances, differences in phenology may nullify the effects of such gene transfer. This has led some authors (e.g. Bradshaw, 1972) to the generalization that outcrossing plants tend to form rather small neighbourhoods. The other aspect of gene flow, namely seed dispersal, is often ignored in forest trees. However, in black spruce the seed is very small and has been observed to travel for a considerable distance over crusted snow (Heinselman, 1957). It appears likely therefore that simultaneous receptivity over a quite large area, combined with more effective seed

dispersal than in many species and an absence of diversifying selection intensities in a relatively homogeneous environment, all combine to prevent the formation of a significant neighbourhood structure in black spruce. Black spruce is an early successional species, and on upland sites does not regenerate itself in the absence of fire. This means that it will usually occupy a site for one generation only. The even-aged structure of these stands implies that they are indeed most likely fire-originated. Regeneration of an area following fire by an influx of seed from surrounding unburnt areas will prevent the development of a neighbourhood structure.

The existence of family clustering in other stands has not been investigated, but there is no reason to believe that stand 29 is not typical.

Ledig (1974) reviewed the efficiency of the comparison-tree and baseline methods of plus tree selection. He showed that in even-aged stands where the coefficient of relationship between the candidate and comparison trees is low, the comparison-tree method is most efficient. Such is the case for black spruce in central New Brunswick, where the stands are often fire-originated and usually even-aged and, as demonstrated by the lack of a significant correlation between distance of separation and coefficient of relationship, neighbouring trees are not necessarily more closely related than two random trees.

CONCLUSIONS

Estimates of genetic diversity should take into account both the number of alleles at a locus and their relative frequency. Therefore both average heterozygosity and the average number of alleles per locus, or the effective number, are required to characterise the diversity. Since there is a large amount of inter-locus heterogeneity, a large, random sample of loci is required in order to provide accurate estimates. Unfortunately, since five of the twelve loci assayed in this study were either linked to or formed gametic associations with other loci, there were only six independent, polymorphic loci and a single monomorphic locus. Standard errors of the various measures of diversity were therefore large. Despite this, black spruce appears to be typical of many conifer species in terms of levels of genetic diversity, especially when the upward bias due to the small number of loci is considered.

Similarly, although the various estimates of population differentiation suggest that somewhat more of the total variation in black spruce resides within populations than in some other species, the figures obtained are not atypical. An intensive within-population sampling strategy is therefore recommended for black spruce tree improvement in New Brunswick.

The degree of differentiation between populations proved to be non-significant based on the contingency X^2 analysis. Thus the present range-wide provenance tests probably adequately sample the overall pattern of variation in the species. What differentiation does exist between populations is apparently not the result of isolation by distance since genetic

and geographical distances are not correlated. However, this interpretation can only be tentative since the standard errors of genetic distance were so large.

The lack of effective barriers to gene flow proposed by Yeh (1979) to explain the low levels of GST in many conifer species is probably also partially responsible for the lack of a well-defined neighbourhood structure in black spruce in central New Brunswick. The general similarity in the environment, in terms of soil, climate and elevation, over a considerable area is also likely to contribute since differences in selection intensities are unlikely to be great. Since neighbouring trees are apparently not more closely related than two random individuals, the currently used method of comparison-tree plus tree selection is justified.

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