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The Mating System of Black Spruce in North-Central Alberta, Canada

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Summary

Mating system parameters for a lowland and an upland black spruce population were simultaneously estimated for the 1983, 1982 and 1981 seed years, and for bulked seed collections from the 1978 to 1976 seed years. Population multilocus outcrossing estimates increased from 0.62 in 1983, to 0.85 in 1978 to 1976. There were no significant differences in outcrossing estimates among populations, or between years, although there was a significant difference between the most recent (1983) and the oldest estimate (1978 to 1976).

Single-locus outcrossing estimates ranged from 0.326 to 1.03 with significant heterogeneity amongst loci in all 3 years in the lowland site, but with only the 1981 population showing significant heterogeneity in the upland site.

Correlation between stand density, assessed from fixed-area plots, and the multilocus outcrossing estimate from each plot was not significant in either population.

Three factors suggested population substructuring: (i) Means of single-locus estimates of outcrossing for each year were consistently lower than multilocus estimates. (ii) There was significant spatial heterogeneity of the outcross pollen pool. (iii) WRIGHT's Index of Fixation was higher than the inbreeding equilibrium coefficient.

Key words: Mating System, Outcrossing Rate, Population Substructuring, *Picea mariana*.

FDC: 165.4; 174.7 *Picea mariana*; (712.3).

Introduction

Black spruce (*Picea mariana* (MILL.) B.S.P.) is the most important pulpwood species in the Canadian boreal forest, due chiefly to the strength derived from its long fibres. It is widely used in reforestation from Manitoba to the Maritime Provinces (MORGENSTERN, 1975). It's importance in the pulp and paper industry has led to genetic improvement programs in many regions (NIENSTAEDT, 1984). Information from these studies indicates that black spruce is highly variable at the provenance level (PARK and FOWLER, 1983). Optimization of tree improvement strategies, however, requires information not only on the genetic variation present in the species, but also on those factors which affect the amount and organization of this variation. One such factor is the degree of outcrossing and selfing, i. e., the type of mating system (ALLARD, 1975). Knowledge of the proportion of selfed seed produced by a species is of practical importance as selfed progeny often exhibit marked inbreeding depression affecting many aspects of growth and survival (SORENSEN and MILES, 1982; YING, 1978). In addition, inbreeding violates a basic assumption of most tree breeding programs – that the parents of wind-pollinated progeny are unrelated. Violation of this assumption causes a bias in estimates of additive genetic variance, heritability and genetic gain (NAMKOONG, 1966; SQUILLACE, 1974).

The technique of gel electrophoresis, used in this study, is widely used to separate polymorphic allozymes of newly germinated tissues as genetic markers in mating system studies (PERRY and DANCIK, 1986; YU *et al.*, 1992).

The semi-serotinous cones of black spruce permit simultaneous collection of seed crops from different years and also allow one to examine variation in the mating system of viable seeds from different seed crops. Estimation of mating system parameters in current seed crops, i. e. soon after the actual mating event, can provide information on the contribution of the mating system to genetic variation in the population. Comparison of mating system parameters in different seeds crops could indicate differential survival of selfed and outcrossed seeds. If survival is random, one would expect mating system parameters to remain relatively constant from year to year, apart from fluctuations due to environmental effects, and assuming no significant temporal variation in selfing events themselves.

The only published reports on the mating system of black spruce have been from studies in central New Brunswick (BOYLE and MORGENSTERN, 1986) and in a seed orchard in Ontario (BARRETT *et al.*, 1987). In the present study mating system parameters were estimated from an isozyme analysis of seed from the 1983, 1982 and 1981 cone crops, plus a bulked seed collection from the 1978 to 1976 cone crops. Results of the isozyme study were also examined for variation in pollen pool allele frequencies, which can affect the amount of gene exchange in the population.

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Materials and Methods

Collections and electrophoretic assay

Seed collections were made in October 1983 from two 116-year-old black spruce populations in north-central Alberta. The populations were about 20 km apart. Population A was growing on a poorly-drained peaty site while population B was on a well-drained upland site. Forty-nine trees were sampled in each population on a 7 by 7 grid, with the nearest cone-bearing tree being chosen as a sample tree at each grid intersection. Grid points were 30 m apart. The exact location of each sample tree was mapped to provide fixed reference points for comparison of results. Each sample tree was felled and sub-samples of cones were taken from the 1983, 1982 and 1981 crops. Bulked sub-samples were taken from the 1978 to 1976 crops as they were not expected to contain many viable seeds. All subsamples were labelled and kept separate. At each grid intersection a circular fixed-area plot of radius 5 m was established, with the sample tree serving as plot centre. The number of trees in each plot was recorded to provide an estimate of population density at each sample point.

Seeds from each sub-sample were germinated under uniform conditions and the megagametophyte and embryo tissues prepared for electrophoresis following the methods of CONKLE *et al.* (1982). Ten seeds, randomly chosen from each sub-sample, were assayed by horizontal starch gel electrophoresis for the 4 enzymes that could be successfully stained and scored in both megametophyte and embryo tissue: glutamate dehydrogenase (GDH, E. C. 1.4.1.3.), phosphoglucose isomerase (PGI, E. C. 5.3.1.9.), phosphoglucomutase (PGM. E. C. 2.7.5.1.) and 6-phosphogluconate dehydrogenase (6PG, E. C. 1.1.1.44.) (CONKLE *et al.*, 1982). These enzyme systems are coded by 4 polymorphic loci (Gdh, Pgi2, Pgm and 6pg1). All 4 loci have been shown to segregate independently (BOYLE and MORGENSTERN, 1986).

Statistical Analysis

Maternal genotypes were inferred from allozyme segregation of the 4 loci. The number of megametophytes and embryos per maternal tree ranged from 10, where only one seed crop could be germinated, to 40, when all 4 seed crops germinated.

Table 1. – Multilocus, t_m, single locus, t_s, and mean of single-locus outcrossing estimates for 2 black spruce populations in north-central Alberta, Canada.

Pop.	Mating system estimator	Enzyme	Year			
			1983	1982	1981	1978-76
A	t_s	Gdh	0.712 (0.07)[a]	0.831 (0.07)	0.728 (0.07)	-
		Pgi2	0.892 (0.08)	0.848 (0.09)	1.030 (0.07)	-
		Pgm	0.326 (0.04)	0.501 (0.05)	0.543 (0.05)	-
		6Pg1	0.358 (0.07)	0.653 (0.07)	0.613 (0.07)	-
		MEAN	0.565	0.713	0.733	-
	t_m		0.626 (0.04)	0.727 (0.04)	0.731 (0.04)	0.833 (0.09)
B	t_s	Gdh	0.480 (0.06)	0.666 (0.07)	0.638 (0.06)	-
		Pgi2	0.504 (0.09)	0.441 (0.09)	0.353 (0.09)	-
		Pgm	0.587 (0.05)	0.590 (0.05)	0.568 (0.05)	-
		6Pg1	0.535 (0.06)	0.488 (0.06)	0.741 (0.06)	-
		MEAN	0.552	0.598	0.615	-
	t_m		0.616 (0.03)	0.696 (0.03)	0.667 (0.05)	0.856 (0.05)

[a]) Standard errors in paratheses.

Single-locus (t_s) and multilocus (t_m) outcrossing rates and the outcross pollen allele frequencies were estimated by the maximum likelihood procedure of Neale and Adams (1985). This procedure assumes that there is no selection between fertilization and progeny analysis, that the rate of outcrossing is independent of the maternal genotype and that the gene frequency distribution in the pollen pool is identical over maternal plants (Clegg, 1980). The tree count obtained in the fixed-area plot at each sampling point was used as an estimate of population density in the neighbourhood of that sample tree. Sample trees were grouped into density classes, and a multilocus outcrossing rate (t_m) estimated for each class in each population. Since the nature of the distributions was not known, a non-parametric test (Spearsman's coefficient of rank correlation) was used to compare density classes with multilocus outcrossing rates in each population.

A test of local heterogeneity of gene frequencies in the outcross pollen pool was conducted by comparing the number of detectable outcrosses (heterozygotes) with the number of homozygotes for each homozygous mother. Values were entered in a 2 x m contingency table, where m was the number of maternal trees of one homozygous genotype in the sample (Brown *et al.,* 1975). In Gdh, Pgm and 6pgl, which had more than one maternal tree homozygous for more than one genotype, a chi-square value was obtained for each genotype and the population value was obtained by summation.

As a measure of the deviation of observed heterozygosity from that expected under Hardy-Weinberg equilibrium, Wright's fixation index (F_{is}) was calculated for the adult population and also for each of the embryo populations.

Results and Discussion

Multilocus outcrossing estimates

Estimates of the outcrossing rate, t_m, increased significantly from the 1983 seed crop (0.626 in population A, 0.616 in population B) to the bulked sample from the 1978 to 1976 seed crops (0.82 in population A, 0.856 in population B) (*Table 1*, *Figure 1*). Year to year estimates of t_m in both populations show a large, through not significant, increase from the 1983 to the 1982 seed crop, going from 0.626 to 0.727 in population A and from 0.616 to 0.696 in population B. From 1982 to 1981, however, the t_m of population A only increased from 0.727 to 0.731, while that of population B showed a slight decrease from 0.696 to 0.667. The estimate of t_m then increased in population A to 0.833 in the bulked seed from 1978 to 1976. The estimate in population B showed a parallel increase to 0.856 in the bulked seed from 1978 to 1976. Although the estimates of t_m were consistently lower in population A, the differences between the 2 populations were small and not significant for any seed year.

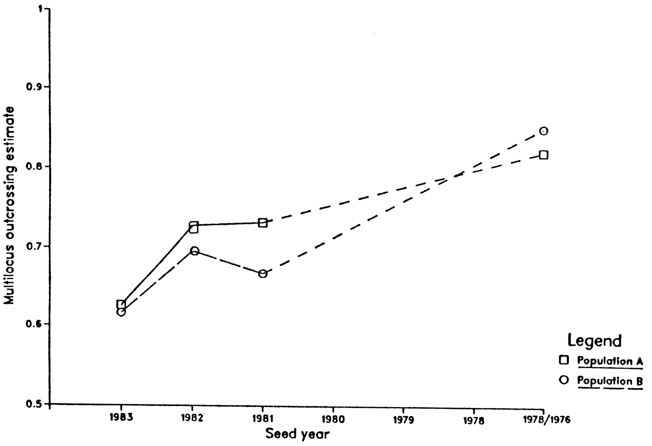

Figure 1. – Multilocus outcrossing estimates by seed-year in 2 black spuce populations from north-central Alberta.

While t_m in both populations increased from 1983 to 1978/1976, the most rapid increase was from 1983 to 1982. This is probably because so many selfed seeds were eliminated during the 1983 to 1982 year that t_m would naturally then increase at a slower, more consistent rate. The slope of the line for population A is consistent with this hypothesis as is population B, except for the slight anomaly of the 1981 seed year which could have been due to some local environmental circumstance. A similar trend of increasing t_m with increasing age of seed crop was observed in jack pine (*Pinus banksiana* Lamb.), a serotinous-coned species, by Cheliak *et al.* (1985), from central Alberta and by Snyder *et al.* (1985) in a Minnesota population. The most likely explanation is that the decrease in proportion of selfs was due to pre-germination selection against selfed seeds, so that a greater proportion of viable seeds from older samples arose from outcrossing events. Pregermination selection would eliminate some of those embryos that were homozygous for recessive lethal and deleterious alleles, a situation that can arise after inbreeding in natural stands of wind-pollinated species (Sorensen, 1982).

The estimates of t_m from this study are comparable to the estimates of $t_m = 0.83$ for black spruce in a seed orchard in Ontario (Barrett *et al.,* 1987). However, they are lower than the estimate of $t_m = 0.924$, averaged over 6 populations in central New Brunswick (Boyle and Morgenstern, 1986). Neither of these studies specified the age of the seed used. The only published reports of temporal trends in outcrossing estimates for serotinous-coned species are for jack pine (Cheliak *et al.* 1985; Snyder *et al.* 1985). Although the estimates of t_m for the black spruce in this study show a similar trend, they are generally lower than those reported for jack pine. In addition, the likelihood ratio test showed all estimates of t_m for black spruce in this study to be significantly lower than the null hypothesis of $t_m = 1$, the estimate for a population in Hardy-Weinberg equilibrium.

Low estimates of t_m could be due to a number of factors. We would expect black spruce to be reasonably self-compatible because it is a pioneer species (Stebbins, 1957; Lande and Schemske, 1985). This allows new habitats to be colonised by isolated individuals. Park and Fowler (1983) concluded that black spruce had a relatively high self-fertility (47.2%) and carried a lower genetic load than late successional species. Outcrossing estimates can also be depressed by spatial variation in the population due to wide spacing between trees or clustering of related trees (i.e. the substructuring of the population). Stand density has been shown to be positively related to outcrossing levels in ponderosa pine (*Pinus ponderosa* Laws.) (Farris and Mitton, 1984) and has been suggested as a possible explanation for low estimates of t in tamarack (*Larix laricina* (Du Roi) K. Koch) (Knowles *et al.,* 1987). In the present study the fixed area plots in population A were classed into 19 density classes with tree numbers ranging from 7 to 38. Population B had 17 density classes with tree numbers ranging from 5 to 26. However, Spearman's coefficient of rank correlation (*Table 2*) did not indicate any correlation between stand density and the multilocus outcrossing estimate, t_m, with rs values of −0.332 and 0.127 for populations A and B, respectively.

Single-locus outcrossing estimates

The single-locus estimates of outcrossing range from 0.326 (Pgm) to 1.03 (Pgi2) (*Table 1*). There was significant heterogeneity amongst loci in all 3 years in population A, while in

Table 2. – Values of the paired variables, stand density (tree count from fixed area plots), and multilocus outcrossing rate, t_m, in 2 black spruce populations in north-central Alberta, Canada.

Population A		Population B	
S.D.[a]	t_m[b]	S.D.[a]	t_m[b]
7 (1)	0.721 (13)	5 (1)	0.879 (16)
8 (2)	0.416 (2)	6 (2)	0.725 (11)
9 (3)	0.824 (16)	7 (3)	0.492 (4)
10 (4)	0.542 (7)	9 (4)	0.527 (5)
11 (5)	0.557 (8)	10 (5)	0.865 (15)
12 (6)	0.855 (17)	11 (6)	0.538 (6)
15 (7)	0.937 (19)	12 (7)	0.661 (9)
16 (8)	0.618 (9)	13 (8)	0.423 (2)
18 (9)	0.744 (14)	14 (9)	0.636 (8)
19 (10)	0.637 (10)	16 (10)	0.431 (3)
20 (11)	0.684 (11)	17 (11)	0.781 (13)
21 (12)	0.875 (18)	18 (12)	0.221 (1)
22 (13)	0.801 (15)	20 (13)	0.560 (7)
23 (14)	0.430 (4)	21 (14)	0.698 (10)
24 (15)	0.510 (5)	22 (15)	0.796 (14)
26 (16)	0.512 (6)	23 (16)	0.969 (17)
28 (17)	0.418 (3)	26 (17)	0.753 (12)
30 (18)	0.698 (12)		
38 (19)	0.415 (1)		

[a]) Stand density, represented by tree counts in fixed area plots.
[b]) Multilocus outcrossing rate, t_m.
[c]) Rankings of paired variables in parentheses.

population B only the 1981 population showed significant heterogeneity. Heterogeneity of single-locus estimates has also been observed in Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (El-Khassaby *et at.,* 1981; Shaw and Allard, 1982; Yeh and Morgan, 1987) and tamarack (Knowles *et al.,* 1987). Variation amongst loci in single-locus outcrossing estimates could be due to spatial changes of genotypic frequencies as would result from clustering of related individuals (Shaw and Allard, 1982). Single-locus estimates of t are more sensitive to violations of the assumptions of the mixed mating model, so they would be expected to be lower than estimates of t_m (Shaw and Allard, 1982). Estimates of t_s in experimental populations of morning glory and sorghum were lower where clusters of related individuals occurred, than in populations without clustering (Ennos and Clegg, 1982; Ellstrand and Forster, 1983). Shaw and Allard (1982) suggested comparison of t_s and t_m as a possible indicator of family structure. To make the comparison in this study, means for the single-locus estimates for each year were calculated by weighting individual estimates of t_s by the inverse of their standard errors. This adjustment compensated for the difference in precision among estimates made with different marker loci. The results (*Table 1*) show that, with the exception of the 1981 estimate in population A, the mean values of t_s are consistently lower than the t_m estimates.

Further evidence of spatial variation in the genetic composition of the populations is shown by the results of the chi-square test for spatial heterogeneity in the pollen pool (*Table 3*). Spatial changes in the genetic composition of populations have been suggested as the most likely cause of pollen pool heterogeneity (Cheliak *et al.*, 1985). Given that low estimates of t_s can be due to spatial variation of gene frequencies, one could expect that loci with low estimates of t_s might also shown significant spatial heterogeneity in the pollen pool. In the present study Pgm and 6pg1, which had low estimates of t_s in population A and Pgm, which had a low estimate in population B, all showed significant spatial heterogeneity in the pollen pool. One qualification that must be made, however, is that maintenance of such heterogeneity would imply limited gene flow within the populations, i.e. limited dispersal distances of both pollen and seed.

Table 3. – Chi-square test for spatial heterogeneity in the pollen pools of 2 black spruce populations in north-central Alberta, Canada.

Enzyme	Population A	Population B
Gdh	34.05[a] (n=22)[b]	31.58 (n=24)
Pgi2	27.45 (n=31)	32.50 (n=22)
Pgm	54.47[a] (n=19)	74.41[a] (n=18)
6pg1	44.67[a] (n=26)	55.24[a] (n=20)

[a]) Significant heterogeneity at the 0.05 level.
[b]) n = the number of inferred heterozygous trees at the locus.

Homogeneity of the pollen pool is one of the assumptions of the method used to estimate outcrossing rates (Neale and Adams, 1985). The results from *table 3* suggest that this assumption may have been violated in this study. The problem of pollen pool heterogeneity and estimation of outcrossing rates

Table 4. – WRIGHT's fixation indices, F_{is}, by enzyme and year, for 2 black spruce populations in north-central Alberta, Canada.

Population	Enzyme	Filial populations			Maternal population
		1983	1982	1981	
A	Gdh	0.380[a]	0.296[a]	0.383[a]	-0.048
	Pgi2	0.084[a]	0.088[a]	0.040[a]	0.082
	Pgm	0.455[a]	0.278[a]	0.241[a]	0.157
	6pgl	0.591[a]	0.345[a]	0.432[a]	-0.045
	Mean	0.377	0.252	0.274	0.0365
A	Gdh	0.525[a]	0.312[a]	0.415[a]	0.297
	Pgi2	0.422[a]	0.320[a]	0.346[a]	-0.064
	Pgm	0.285[a]	0.290[a]	0.250[a]	0.238
	6pgl	0.436[a]	0.424[a]	0.252[a]	0.169
	Mean	0.417[a]	0.336	0.315	0.160

[a]) Significant deviation from HARDY-WEINBERG at the 0.05 level.

was addressed by FU *et al.* (1992) in a study of 2 jack pine populations from northwest Ontario. From their field investigations and related computer simulations they concluded that random pollen pool heterogeneity alone did not appear to influence the estimate of t. However, pollen pool heterogeneity combined with consanguineous mating did exert a downward bias on t. It seems likely therefore, that the estimates of t_s, and to a lesser extent t_m, in this study were depressed by population substructure with consequent heterogeneity of the pollen pool and consanguineous mating. Multilocus outcrossing estimates in a Douglas-fir seed orchard were felt to have been depressed by matings between relatives (RITLAND and EL-KASSABY, 1985).

WRIGHT's index of fixation

Estimates of WRIGHT's index of fixation (WRIGHT, 1951) (*Table 4*) showed a significant deviation from HARDY-WEINBERG equilibrium at all loci in the embryo populations. This was due to an excess of homozygotes in the embryos of both populations which decreases with increasing age of the seed crop. In the adult populations there is a slight, non-significant, excess of homozygotes similar to values obtained by BOYLE *et al.* (1990) and KNOWLES (1991) in Ontario lowland black spruce populations. Reduced homozygote numbers in the maternal populations suggests that natural selection during the life of the stand may be acting to remove the excess homozygotes. Since inbred lines are more likely to be homozygous, this could possibly be interpreted as selection against selfs and other inbreds. A significantly lower survival rate has been documented for selfed seedlings of Douglas-fir and ponderosa pine (SORENSEN and MILES, 1974), white spruce (*Picea glauca* (MOENCH) VOSS) (YING, 1978) and black spruce (PARK and FOWLER, 1983).

An excess of homozygotes can result from (i) inbreeding, (ii) selection against heterozygotes, or (iii) the WAHLUND effect – subdivision of the population into groups, each forming a breeding unit by itself, with allelic frequencies differing between groups (LI, 1955). If the population is in inbreeding equilibrium, an equilibrium inbreeding coefficient, Fe, can be calculated from the outcrossing estimate t_m (SPIESS, 1977):

$$Fe = \frac{1-t_m}{1+t_m}$$

Given the situation that any deficiency of heterozygotes was due solely to the mating system, Fe for any seed year would then be equal to the mean of F_{is}, over the 4 loci, for that seed year. Calculation of Fe for the most recent seed crop (1983) minimizes the effects of selection, but still shows F_{is} to be much larger than Fe in both populations (*Table 5*). This could be explained by variations in local gene frequency that would have biased F_{is} upwards and t_m downwards, i.e. the WAHLUND effect.

The combined evidence of t_s being consistently lower than t_m, loci with the lowest t_s estimates showing significant spatial heterogeneity of outcross pollen pool allele frequencies, and F_{is}

Table 5. – Comparison of F_{is} (arithmetic mean of the 4 loci) and F_e for the 1983 seed from 2 black spruce populations in north-central Alberta, Canada.

Year	Population	Mean F_{is}	F_e
1983	A	0.377	0.230
	B	0.417	0.238

being larger than Fe suggests that there may be some substructuring in these populations.

The results of this study show that in natural stands of black spruce the excess of selfed seedlings in the embryo generation is probably reduced by natural selection as the stand matures. However, the practise of collecting the most recent cones as a seed source for nursery production of seedlings may lead to germination of large numbers of selfed seed. To some extent this increase in the number of selfed seedlings may be counterbalanced by the nursery practise of culling the poorer seedlings, many of which are presumably selfed, from the seedbed. The time and effort spent in germinating selfed seedlings (which will later be culled from the seedbed) could be reduced by collecting only one-year-old cones which would mean that some of the selfed seeds had already been eliminated, while little seed viability would have been lost. However, since collection of new cones is the accepted practise, it would be instructive to examine directly the effects of different methods of storage of cones or seed on the estimate of apparent outcrossing.

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