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Estimates of Outcrossing Rates in Six Populations of Black Spruce in Central New Brunswick

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Abstract

Single-locus outcrossing rates in six populations of black spruce *(Picea mariana* [Mill.] B. S. P.) were estimated based on allozyme data from six polymorphic loci using maximum likelihood methods. Multi-locus estimates were also calculated.

An average single-locus estimate of 0.932 was obtained for the outcrossing rate compared with a multi-locus average of 0.924. There was little evidence of a downward bias in single-locus estimates resulting from non-random forms of mating other than selfing, indicating a lack of development of family clustering.

In four of the populations the observed inbreeding coefficient was negative and significantly less than the expected equilibrium inbreeding coefficient. Gametic and post-zygotic selection favouring heterozygotes is proposed as an explanation. In one population the observed inbreeding coefficient was greater than that expected under equilibrium, possibly suggesting an inbred maternal population.

Key words: *Picea mariana*, isozymes, mating system, heterozygosity, inbreeding.

Zusammenfassung

Fremdbefruchtungsraten wurden durch Untersuchungen einzelner Loci in 6 Populationen von *Picea mariana* ermittelt, wobei 6 polymorphe Loci und die Methode der größten Wahrscheinlichkeiten verwendet wurden. Schätzungen, die auf mehreren Loci beruhten, wurden ebenfalls durchgeführt.

Die durchschnittliche Fremdbefruchtungsrate betrug 0.932 auf der Basis einzelner Loci und 0.924 auf der Basis mehrerer. Es gab wenig Anzeichen für eine Verringerung der Einzel-Locus Schätzung durch Abweichung von zufälliger Paarung außer Selbstbefruchtung, sodaß eine Familienstruktur der Bestände kaum anzunehmen ist.

In 4 Populationen war der Inzuchtkoeffizient negativ und signifikant geringer als der erwartete Gleichgewichtswert. Es wird vorgeschlagen, daß die Erklärung in gametischer und post-zygotischer Auslese zu suchen ist, wobei die Heterozygoten bevorteilt werden. In einer Population war der Inzuchtkoeffizient größer als der erwartete Gleichgewichtswert, was womöglich auf Inzucht in der mütterlichen Population zurückzuführen ist.

Introduction

The pattern by which gametes are united to pass genes from one generation to the next is termed the mating system (Stern and Roche 1974). In the past, species were often characterised as "inbreeders" or "outbreeders", "self-fertile" or "self-sterile". However, it is now recognised that the mating system is far from being a fixed, species-specific constant, but is rather a dynamic entity, varying in time and space and affected by a large number of biotic and abiotic factors. These include the degree of selfcompatability, population density, variation in phenology and climatic variables (Stern and Roche 1974).

The mating system of a species affects the genetic structure of populations - the distribution of genotypes and the dynamics of this structure in relation to evolutionary forces (Jain 1975, Brown 1978, Ritland and Jain 1981). In addition, the estimation of many genetic parameters on which breeding strategies are based makes use of an assumption of random mating which may not be valid (Squillace 1974).

Most models used in describing the mating system consider the production of progeny from an outcross event with probability t to a pollen pool with allele frequency p. Since both of these parameters are unknown, they must be estimated simultaneously (Ritland and Jain 1981). The estimates of t obtained by the observation of progeny genotypes will be affected by other forms of non-random mating

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such as mating between closely related individuals, variation in self-compatability and gametic selection (Brown *et al.*, 1975). Some of these effects can be isolated (see below), but the estimates of t should be considered as "effective" outcrossing rates (Brown and Allard 1970).

Various methods for estimating mating system parameters have been reviewed by Jain (1979). The use of maximum likelihood methods, as developed by Fyfe and Bailey (1951), enable the simultaneous estimation of t and p independently of the genotypic distribution of maternal trees, which means that the assumption of inbreeding equilibrium in the population is not required (Brown *et al.* 1975).

The use of enzyme polymorphisms is highly advantageous since isozymes are usually co-dominantly expressed and many indepent loci can be assayed simultaneously on the same individual allowing a large amount of information on the mating system to be obtained (Jain 1979).

Morgenstern (1972) obtained estimates of inbreeding coefficients in southern and northern stands of black spruce (*Picea mariana* [Mill.] B. S. P.) in Ontario using variance components from a hierarchical series of progeny tests. In the larger northern stands, inbreeding coefficients corresponding to an outcrossing rate of 0.94 were obtained, whilst in the smaller, more isolated southern stands, the estimated outcrossing rate was 0.85. Park and Fowler (1984) used the precentage of full seed produced following open pollination and controlled pollination with unrelated polymix pollen to estimate the level of inbreeding in a New Brunswick black spruce population. Results suggested high levels of outcrossing.

In this study, enzyme polymorphisms were used to obtain both single-locus and multi-locus estimates of t and p in six populations of black spruce in central New Brunswick.

Materials and Methods

In each of six stands in central New Brunswick, cones were collected from 40 dominant or co-dominant trees within an area of about 1—2 ha. The location of these stands has been described by Boyle and Morgenstern (1985b). All stands were fully stocked and composed almost entirely of black spruce.

Twelve embryo-gametophyte pairs were assayed at six enzyme loci. Details of electrophoresis procedures and staining recipes are given in Boyle and Morgenstern (1985b).

Several maximum likelihood methods are available in order to obtain single-locus estimates of t and p. The expectation-maximization (EM) alrgorithm of Cheliak *et al.* (1983) has certain attractive features, namely the restriction of $\hat{t}$ and $\hat{p}$ within the biologically possible limits of zero and one and the determination of the proportion of selfed and outcrossed embryos in phenotypically confounded classes. The required assumptions of the EM algorithm are common to all mixed mating models:

(i) the frequency of alleles in the pollen pool is homogeneous;
(ii) the probability of an outcross is independent of genotype; and
(iii) all embryos have equal fitness, i. e. there is no gametic or postzygotic selection (Cheliak *et al.* 1983).

Although all maximum likelihood methods are capable of handling multi-allelic loci, if some alleles are infrequent, many observational classes are empty which leads to problems with estimation and tests of significance (Shaw and Allard 1981). Therefore all alleles other than the most frequent were pooled to produce a diallelic situation.

Failure of any of the assumptions listed above will result in a bias in the estimates of t and p. Multi-locus methods are less sensitive to failure of these assumptions (Shaw and Allard 1981) and can therefore produce less biased estimates and at the same time give some indication of the degree to which the assumptions fail. Again, several multi-locus methods have been developed and for this study the method of Shaw *et al.* (1981) was used. Shaw and Brown (1982) considered the optimum number of loci and progeny to sample for multi-locus estimation. They concluded that for expected outcrossing rates close to one, all convenient independent polymorphic loci and as many seedlings as possible should be assayed.

From the maternal genotype frequencies, the inbreeding coefficient of Wright (1965) can be calculated as:

$$F = 1 - (h/[1-\sum_i p_i^2]),$$

where h is the observed proportion of heterozygotes and p_i is the frequency of the i^{th} allele. This can then be compared with the expected coefficient of inbreeding at equilibrium, calculated from the mean outcrossing rates by the formula:

$$F_e = (1 - t)/(1 + t)$$)Fyfe and Bailey 1951.

Results

Single-locus outcrossing rates, estimated for each of the six independently segregating, polymorphic loci (ALD, IDH, PGM, 6PGD-1, 6PGD-2 and PGI), range from 0.591 to 0.999 (*Table 1*), with standard errors as calculated by the method

Table 1. — Single-locus and multi-locus estimates of outcrossing rates. Standard errors are in parentheses.

	Population					
Locus	10	21	29	32	37	39
ALD	0.992 (0.063)	0.992 (0.116)		0.921 (0.118)	0.992 (0.108)	0.982 (0.050)
IDH	0.936 (0.111)	0.996 (0.112)	0.992 (0.106)		0.992 (0.119)	0.953 (0.108)
PGM	0.889 (0.068)	0.591**(0.067)	0.882**(0.067)	0.782**(0.064)	0.813**(0.062)	0.887 (0.058)
6PGD-1	0.931 (0.062)	0.977 (0.065)	0.970 (0.068)	0.829**(0.065)	0.959 (0.070)	0.954 (0.065)
6PGD-2	0.943 (0.119)	0.963 (0.110)	0.999 (0.058)	0.949 (0.109)	0.999 (0.066)	0.999 (0.074)
PGI	0.984 (0.087)	0.964 (0.112)	0.915 (0.068)	0.998 (0.080)	0.993 (0.071)	0.998 (0.057)
Mean	0.938**(0.015)	0.908 (0.063)	0.946* (0.022)	0.888**(0.038)	0.952 (0.029)	0.958*(0.017)
Multi	0.952 (0.044)	0.891* (0.046)	0.933 (0.040)	0.891* (0.043)	0.902* (0.044)	0.976 (0.037)

* Significantly different form t = 1 at the 5% level.
** Significantly different form t = 1 at the 1% level.

Table 2. — Estimates of the frequency of allele 1 in the outcross pollen pool. Standard errors are in parentheses.

Locus	Population 10		21		29		32		37		39	
ALD	0.998	(0.002)	0.983	(0.007)	0.991	(0.000)	0.995	(0.003)	0.983	(0.006)	0.990	(0.005)
IDH	0.965	(0.011)	0.960	(0.011)	0.950	(0.014)	0.988	(0.000)	0.975	(0.008)	0.977	(0.008)
PGM	0.573	(1.324)	0.462	(7.573)	0.546	(3.751)	0.583	(1.217)	0.548	(3.380)	0.547	(3.207)
6PGD-1	0.652	(0.293)	0.693	(0.164)	0.612	(0.574)	0.607	(0.676)	0.628	(0.406)	0.522	(14.628)
6PGD-2	0.939	(0.017)	0.971	(0.009)	0.942	(0.005)	0.959	(0.012)	0.962	(0.011)	0.958	(0.011)
PGI	0.840	(0.041)	0.842	(0.046)	0.779	(0.073)	0.823	(0.048)	0.842	(0.040)	0.806	(0.053)

of Brown *et al.* (1975). Estimates of the outcross pollen allele frequencies are given in *Table 2*. Aldolase in stand 29 and IDH in stand 32 were both monomorphic, and although heterozygous progeny were observed, since so many observational classes were empty, estimates of outcrossing rates were not calculated.

Only five of the estimates showed significant deviations from the null hypothesis value of t = 1 and of these, four were accounted for by PGM.

Estimates of outcrossing rates significantly less than one indicate either a significant level of inbreeding or the inadvertant sampling of sub-populations with differing allele frequencies, termed "Wahlund's effect" (Shaw and Allard 1981). A heterogeneity G-test (Sokal and Rohlf 1969) can test for variability in the number of heterozygous progeny observed from homozygous mother trees. Significant levels of heterogeneity may indicate varying allele frequencies in the pollen pool, suggesting that Wahlund's effect was in fact encountered. Again, most of the values are non-significant and of the five that are significant, PGM accounts for three *(Table 3)*.

The mean estimates of the outcrossing rate over all the loci for each stand *(Table 1)* range from 0.888 for stand 32 to 0.958 for stand 39 with an average of 0.932. The precision of single-locus estimates depends on the frequencies of alleles in the pollen pool and of maternal genotypes, with loci having little allelic variation yielding large standard errors (Shaw and Allard 1982). In these black spruce populations, PGM, 6PGD-1 and PGI were the most variable loci and these do indeed generally provide the smallest standard errors. To take account of this, the contribution of each locus to the calculated mean was weighted inversely by the variance of the estimate.

Table 3. — Heterogeneity G values for allele frequencies in outcross pollen pools for each locus in each stand..

Locus	Population 10	21	29	32	37	39
ALD	7.33	35.92	18.64	11.86	28.77	20.53
IDH	33.03	52.42*	46.59	18.64	28.93	27.26
PGM	39.14**	35.21**	35.28**	16.60	12.08	8.70
6PGD-1	26.02	16.41	18.97	14.46	15.20	20.52
6PGD-2	40.78	31.51	38.64	35.09	35.40	40.80
PGI	41.00*	24.49	15.72	27.79	19.71	28.93

* Significant at the 5% level.
** Significant at the 1% level.

Table 4. — Observed and expected equilibrium inbreeding coefficients. The observed coefficients (F) are calculated from the six independent, polymorphic loci. The expected, equilibrium coefficients are calculated from the single-locus outcrossing estimates [F_e(s)] and the multi-locus estimates [F_e(m)].

Type of F	Population 10	21	29	32	37	39	Mean
F	-0.032**	-0.042**	0.033	-0.029**	-0.041*	0.054	0.002
F_e(s)	0.032	0.048	0.028	0.059	0.025	0.021	0.035
F_e(m)	0.025	0.058	0.035	0.058	0.052	0.012	0.040

* Significant at the 5 % level.
** Significant at the 1% level.

The estimated allele frequencies in the outcross pollen pool can be compared with those in the maternal population by means of a t-test based on percentages (Sokal and Rohlf 1969). None of the comparisons were significant.

Multi-locus estimates

The multi-locus estimates of outcrossing rates for each stand range from 0.891 in stands 21 and 32 to 0.976 for stand 39 with an average of 0.924. Three of these estimates were significantly different from t = 1 at the 5% level *(Table 1)*.

Observed inbreeding coefficients, and those expected from both the single-and the multi-locus outcrossing estimates for each population varied among stands *(Table 4)*. Tests of significance for the null hypothesis: F = 0 were calculated by the method of Brown *et al.* (1975). In two stands (29 and 39) there were excesses of homozygotes compared with the frequencies expected from random mating, as shown by positive F values. Stand 39 also produced a greater proportion of homozygotes than predicted by the estimated outcrossing rates, whilst for stand 29 the observed coefficient lay between the expected equilibrium coefficients for the single- and multi-locus estimates.

Discussion

A large majority of the single-locus estimates of "effective" outcrossing rates are not significantly different from the null hypothesis value of t = 1. In all stands except stand 10, there is also little apparent heterogeneity among the estimates from different loci, especially if PGM and 6PGD-1 are ignored. This is in contrast to results obtained in several other studies where considerable interlocus heterogeneity was observed (eg. Brown *et al.* 1975, El-Kassaby *et al.* 1981, Mitton *et al.* 1981, Shaw and Allard 1982). This heterogeneity arises since there are several factors which influence the effective outcrossing rate and which may vary between loci. For example, Wahlund's effect and family clustering will bias the estimates down, while gametic or post-zygotic selection following fertilization will bias them up (Shaw *et al.* 1981).

If Wahlund's effect was important, it would be expected that there would be some relationship between the level of heterogeneity in the frequencies of outcrossed embryos as measured by the G-tests and the estimates of outcrossing. However, although as noted above, PGM and 6PGD-1 provide the majority of significant values in both cases, the agreement is not particularly good, with significant selfing rates occurring in stands with non-significant heterogeneity and vice versa. For example, at PGM, selfing rates are significant in stands 32 and 37 but levels of heterogeneity are non-significant. Significant heterogeneity was recorded in stands 10, 21 and 29 and although the selfing rate in stand 10 was non-significant, significant rates were obtained for the other two. It is interesting to note that in stand 21, where heterogeneity was significant, the estimated outcrossing rate was abnormally low (0.591).

Significant heterogeneity G values may indicate either spatial variability in the allele frequencies in the pollen pool or variability in outcrossing rates (Mitton *et al.* 1981). In stand 29 the location of the trees was recorded and an analysis of maternal allele frequencies by quadrats demonstrated very little variation (Boyle and Morgenstern 1985a). Since there was no significant difference between maternal and outcross pollen pool allele frequencies, there is some evidence to suggest that the significant levels of heterogeneity may be due to variation in outcrossing rates between trees. Park and Fowler (1984) found that self-compatibility was highly variable among individuals in a New Brunswick black spruce population.

Whatever other influences affect the estimated outcrossing rate, since the estimates were obtained using the same progeny for each locus, the true outcrossing rate must be constant between loci (El-Kassaby *et al.* 1981). The weighted mean of all independent loci therefore gives a better estimate.

Since multi-locus estimates are less severely affected by the failure of assumptions in the mixed mating model, comparison of single- and multi-locus estimates can indicate the severity of any failure (Shaw and Allard 1981). As discussed above, if Wahlund's effect and family clustering were significant, the single-locus estimates of outcrossing rates would be biased downwards. In fact, the populations are equally split with three producing lower single-locus than multi-locus estimates and three higher. However, in stand 32 the difference is negligible (single-locus: 0.888 cf. multi-locus: 0.891) and the mean over all populations is greater for the single-locus estimates (0.932 cf. 0.924). This suggests that generally Wahlund's effect and family clustering is not significant but gametic and/or post-zygotic selection may be effective in biasing single-locus estimates upwards. Based on the coefficient of relationship and distance of separation of the sampled trees in stand 29, Boyle and Morgenstern (1985a) concluded that family clustering was poorly developed.

On upland sites such as those sampled in this study, black spruce usually regenerates following a natural catastrophe such as fire (Heinselman 1957). Regeneration occurs predominantly with seed from the surrounding surviving stands, resulting in large areas of even-aged forest. Under such conditions, a strong family structure is unlikely to develop. On the other hand, species which tend to regenerate *in situ*, or after local disturbance, are more likely to develop family clustering with microgeographic genetic differentiation. For example, ponderosa pine (*Pinus ponderosa* Laws.) has been shown to form strongly differentiated family clusters (Mitton *et al.* 1977).

Possible explanations for deficiencies or excesses in the proportions of observed homozygotes compared with those expected from the mating system are discussed by Brown (1978). The most commonly suggested reasons for a deficiency of homozygotes (as found in populations 10, 21, 32 and 37) in predominant outbreeders are heterozygous advantage for chromosomal segments containing the marker loci, associative overdominance, negative assortative mating and differences in allele frequencies between the male and female gamete pools (Cheliak 1983). Negative assortative mating in the form of differences in male and female phenology on the same individual has been observed in conifers (Sarvas 1962) and gametic selection, for example by retardation of pollen tube growth of pollen with a similar genotype to the female gamete would further emphasize this effect. Since polyembryony occurs in conifers (Sarvas 1962), post-zygotic selection, for example through the action of embryonic lethal genes is a distinct possibility and would effectively result in heterozygous advantage or associative overdominance which would take effect prior to the assay of the embryos. Strobeck (1979) has shown that heterozygotic advantage at a locus affects the whole genome, not just those loci in association with it. Park and Fowler (1984) have estimated 1.8 archegonia per ovule and five to seven embryonic lethals for a black spruce in New Brunswick. Thus selection against homozygotes in the form of gametic and post-zygotic selection seems to be the most likely explanation for the deficiency of observed homozygotes.

Excesses of homozygotes (as found only in stand 39) could result from a variety of causes (Brown 1978). Wahlund's effect, positive assortative mating, restricted neighbourhood size and an inbred parental generation (Squillace 1974) could all contribute to this excess. Wahlund's effect does not appear to be a factor in stand 39 and although the variance effective population size for stand 39 (and 29) is lower than most of the other populations (Boyle, in preparation), the difference is not great, so restricted neighbourhood size is unlikely to be the cause. Since positive assortative mating is unlikely to apply, the most likely explanation is an inbred parental population.

Conclusions

Based on the multilocus estimates, which are less sensitive to failures in the assumptions of the mixed mating model, outcrossing rates in these six populations range between 0.891 and 0.976. These figures compare well with Morgenstern's (1972) earlier estimates of outcrossing rates in northern Ontario (0.94).

The absence of an overall downward bias of single-locus estimates in most of the populations suggests that family clustering is not significant, although for one locus (PGM) there is some evidence of downward bias.

Comparison of the observed inbreeding coefficients and those expected at inbreeding equilibrium shows that in four stands there is a significant deficit in the number of observed compared with expected homozygotes. Although there are several possible explanations for this, the most likely cause in predominant outbreeders is some form of selection favouring heterozygotes, perhaps as a result of selection against homozygotes through gametic and post-zygotic selection. Of the possible causes for an excess of homozygotes, positive assortative mating is therefore unlikely to apply in this case. Wahlund's effect and restricted neighbourhood size may play some role but an inbred maternal population is likely to be the dominant cause. Only stand 39 produced an excess of homozygotes and the reason for a more highly inbred maternal population in this stand than in the others is not apparent.

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Genetic Parameters of Blue Spruce (Picea pungens) at Two Locations in Michigan[1]

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Abstract

One hundred sixty three open-pollinated families from 42 stands from throughout the natural range of blue spruce were grown at two test sites in Michigan. Within and between stand variance components, family heritabilities, and genetic correlations were calculated for height, foliage color and time of bud-burst. Results at both sites were, for the most part, similar. Except that no geographic variation was detected for height in the northern test (probably because cold damage reduced the growth of trees from southern origins there), family variation for height and foliage color was approximately equally divided among geographic regions, stands within regions and families within stands. For bud burst, measured only in the southern Michigan test, family within stand variation was twice as great as the regional or stand within region components. Estimates of genetic components of variation derived from these results indicate that within stand genetic variation is approximately twice as great as between stand genetic variation for height and foliage color, while within stand genetic variation is eight times greater than between stand genetic variation for bud-burst. Family heritabilities for height and foliage color were about 0.5, and for bud-burst about 0.8. Genetic correlations were small, but significant for foliage color with height and bud-burst.

These results were used to develop a possible first generation selection strategy for blue spruce which utilizes within and between stand genetic variation.

Key words: selection, tree breeding, genetic correlations, heritability.

Zusammenfassung

Auf zwei Teststandorten in Michigan wurden 163 Familien aus freier Abblüte von 42 *Picea pungens* Beständen aus dem gesamten natürlichen Verbreitungsgebiet angebaut. Nach Messung der Höhe und Feststellung der Nadelfarbe und des Knospenaustriebes nach 4 Jahren wurden die Variationskomponenten zwischen und innerhalb der Bestände, die Familien-Heritabilitäten und die genetische Korrelation berechnet. Für beide Anbaustandorte waren die Ergebnisse größtenteils ähnlich. Außer daß im nörd-

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