Theor Appl Genet (1987) 74:554–559

Genetics of the polycross

2. Male fertility variation in Norway spruce, *Picea abies* (L.) Karst.

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Received January 23, 1987; accepted March 27, 1987
Communicated by K. Sittmann

Summary. Seeds from polycrosses with Norway spruce, in which the same sixteen male parents were crossed to a number of female parents in each of two years, were analysed electrophoretically to detect departures from male gamete frequencies expected assuming equal male fertilities, and to detect heterogeneity among female parents in male gamete frequencies in seeds. The data were also used to estimate the fertilities of the polycross trees used as male parents. Significant departures from male gamete frequencies expected assuming equal male fertilities were found in the seed pooled from all crosses. Male fertilities estimated from male gamete frequencies in seed from all crosses also departed significantly from expectation. The results are discussed with respect to assumptions made when estimating general combining abilities and expected response to selection in polycrosses.

Key words: *Picea abies* (L.) Karst. – Polycross – Male fertility variation – Mating system estimation

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Introduction

Variation in male fertility can be an important consideration in plant breeding, especially when the breeding program calls for crossing a number of pollen parents with a number of seed parents (e.g. synthetic varieties, various techniques of recurrent selection) (Allard 1960). Male fertility variation in such breeding populations is likely to increase the average genetic correlation among offspring from single seed parents, and therefore it may potentially bias estimates of general combining ability, expected response to selection, and selfing rate (Squillace 1974; Falconer 1981; Clegg 1980; Griffin 1982; Cheliak et al. 1987). To reduce variation in male fertility among the selected genotypes, breeders sometimes employ a latin square planting design of the clones or inbred lines being crossed. This is intended to maximize gene exchange between individuals and thereby reduce the possibility that the seeds of a given clone or inbred line will be sired disproportionately by a few genotypes (Schaepman 1952; Gutierrez and Sprague 1959; Carlson 1971). Despite these attempts, studies of the mating system in such populations have often revealed significant departures from equal male fertilities among the selected lines (Gutierrez and Sprague 1959; Knowles 1969; Carlson 1971; Müller-Starck and Ziehe 1984; Schoen and Stewart 1986). These departures may be attributable to non-synchronous flowering among lines (Sweet 1975; Griffin 1984), non-random pollen movement due to prevailing winds or directional pollinator movement (Handel 1983), differential pollen viability and pollen tube growth rates (Ottaviano et al. 1980), and non-random embryo abortion (Sorenson 1982).

As an alternative to relying upon open-pollination of genotypes in a latin square planting design, the polycross mating design (sensu Giertych 1975) has been adopted by some breeders (Moran and Griffin 1985; Cheliak et al. 1987). This design involves controlled pollination of separate seed parents with equal quantities of pollen from a number of the different pollen parents. The efficacy of the polycross mating design in reducing male fertility variation, however, can be questioned since some of the above-outlined sources of male fertility variation remain. We report here the results of a study of Norway spruce, *Picea abies* (L.) Karst., aimed at uncovering the extent of male fertility variation following the polycross mating design. Our investigation exploited electrophoretically-detectable variation at four marker loci in this species. It relied upon the ability to discern the marker genotype of the male gamete when both megagametophyte and embryo of the conifer seed are used as separate tissue sources in electrophoresis (Bartels 1971; Conkle 1971). This type of information has often been applied to the estimation of other mating system parameters in open-pollinated populations (Brown et al. 1984). In this paper, we analyse the frequencies of male gametes in seeds from polycrosses conducted over two years to: (1) detect departure from the male gamete frequencies expected assuming equal male fertilities; (2) detect heterogeneity in male gamete frequencies when the same set of paternal parents is crossed with a number of different maternal parents; and (3) estimate the fertilities of the male parents. The analyses reported in the present paper are an extension of those of Cheliak et al. (1987), who primarily analysed single locus data from the same set of crosses, and did not estimate male fertility variation in the crosses.

Materials and methods

Study population, crossing design, and electrophoretic methods

The male and female parents used in the crosses are clones from a Norway spruce seed orchard located in the south-eastern part of Norway (latitude 63°31′N, longitude 11°54′E, altitude 155 m) (Skrøppa and Tutturen 1985). The same 16 clones were the male parents in both 1976 and 1983. In each year, branches bearing male strobili were removed from ramets of these clones, and after 3–5 days the pollen was extracted, dried, and cleaned. Equal volumes of pollen from each clone were then combined and mixed. The mixture was divided into samples of 7–8 ml in volume, which were each transferred to separate 15 ml glass vials, sealed, and stored for 3 days prior to the pollinations. Pollen applications to bagged female strobili were carried out by introducing the pollen mixture into the bag through a small hole made with a syringe. One vial of pollen was used for each cross. Seed batches collected from the crosses were cleaned and stored at − 10° to − 20 °C prior to electrophoresis. Cheliak et al. (1987) provide a more detailed description of the crossing, harvest, and seed extraction methods.

Germination and electrophoretic procedures followed those reported by Cheliak et al. (1985). Four unlinked polymorphic marker loci (Cheliak et al. 1987), three of which were diallelic (*Aat-3*, *Aco*, *Gdh*) and one which was triallelic (*Pgi-2*), were assayed from tissue of each embryo and megagametophyte pair. The paternal four-locus gamete contribution to the embryo was determined directly by inspection of the genotypes of the embryo and megagametophyte. Seed samples for electrophoretic analysis consisted of those from 13 clones used as seed parents in each of the two years, as well as an additional sample of 17 clones used as seed parents in 1983 only. Only those crosses yielding 25 or more electrophoretically-assayed seed are included in the analyses below. These represent over 75% of the crosses made.

Calculation of expected frequencies of male gamete types in seed crops from polycrosses assuming equal male fertilities among clones

The expected frequency, φ_i, of each haploid male gamete type *i* in the seed crop under the assumption of equal male fertilities is

$$\varphi_i = 1/N \sum_i \sum_{jk} R_{i,jk}\, n_{jk} \qquad (1)$$

where $R_{i,jk}$ is the probability that diploid genotype *jk* produces haploid gamete *i* by meiosis, n_{jk} is the number of male parents in the polycross with diploid genotype *jk*, and *N* is the total number of male parents used in the polycross.

Eight of the $N = 16$ clones used as pollen parents had unique four-locus diploid genotypes, while among the other eight clones, there were three additional four-locus diploid genotypes, for a total of eleven diploid genotypes (Table 1). These clones produce a total of sixteen four-locus gamete types (Table 1). With one exception, the probabilities that clones produce these gamete types following meiosis reflect normal Mendelian segregation at each locus, as well as the absence of detectable linkage among pairs of loci (Cheliak et al. 1987). One clone, number 5696, showed non-Mendelian segregation ratios at the *Aco* locus (Table 1) (Cheliak et al. 1987).

Estimation of male fertilities of clones used in the polycrosses

Under the assumption of independent union of gametes, it can be shown that p_i, the observed frequency of haploid marker gamete type *i* in the seed sample is

$$p_i = \sum_{jk} R_{i,jk} f_{jk} \qquad (2)$$

or equivalently,

$$\boldsymbol{p} = \boldsymbol{R f} \qquad (3)$$

where $\boldsymbol{p}$ is a column vector of the observed male marker gamete frequencies in the seed sample, $\boldsymbol{R}$ is a matrix containing the probabilities $R_{i,jk}$ (i.e. Table 1), and $\boldsymbol{f}$ is a column vector containing the male fertility parameters, f_{jk}, for all marker gamete types *i* and diploid marker genotypes *jk*. A solution to equation (3), $\boldsymbol{f} = \boldsymbol{G p}$, where G is the generalized inverse of $\boldsymbol{R}$, always exists (Searle 1971). Whenever the elements of $\boldsymbol{G}$ (and $\boldsymbol{R}$) are real constants, then

$$\mathrm{E}(\boldsymbol{f}) = \boldsymbol{G}\,\mathrm{E}(\boldsymbol{p}) \qquad (4)$$

where E( ) denotes expectation (Elandt-Johnson 1971), and so solving equation (4) provides a set of unbiased estimates of the male fertility parameters. Schoen and Stewart (1986) give further details of this estimation model.

To test the goodness of fit of the observed male gamete frequency data to the model expectations, Monte Carlo simulation was employed to generate 300 different random sets of male marker gamete frequencies. Each set of simulated male gamete frequencies was based on the same number of fertilization events as in the observed data. The probabilities of drawing a particular male marker gamete were set as functions both of the male fertility parameters estimated from the observed male marker gamete frequencies and the probabilities that the different haploid gamete types are produced by the different diploid genotypes, i.e. the probability of drawing gamete type *i* in the simulations, Pr_i, was set equal to

$$\mathrm{Pr}_i = \sum_{jk} R_{i,jk} f_{jk}\,. \qquad (5)$$

For each set of simulated data, the likelihood of the eleven fertility estimates was calculated as

$$\mathrm{L} = \mathrm{C} \prod_i \Big(\sum_{jk} R_{i,jk} f_{jk}\Big)^{e_i} \qquad (6)$$

where e_i is the number of the *i*th gamete type present in each simulated data set, and *C* is a constant. The goodness of fit

Table 1. Probability of observing the different four locus marker gametes following meiosis given the 16 clones used as male parents in 1976 and 1983 in a polycross with Norway spruce

Haploid marker gamete[a]	Clone(s) used as male parents										
	2536	2560	5186	5408	5412	5550	5558	5696	2569 2719 5675	2601 2627	5174 5328 5354
	Diploid marker genotypes										
	1211//1212	1111//1222	1111//2212	1111//1112	1111//1111	1111//2113	1112//1212	2111//2221	1111//2112	1111//2121	1111//2111
1111	0	1/8	1/8	1/2	1	1/4	0	0	1/4	1/4	1/2
1112	0	1/8	1/8	1/2	0	0	1/2	0	1/4	0	0
1113	0	0	0	0	0	1/4	0	0	0	0	0
1121	0	1/8	0	0	0	0	0	0	0	1/4	0
1122	0	1/8	0	0	0	0	0	0	0	0	0
1211	1/2	1/8	1/8	0	0	0	0	0	0	0	0
1212	1/2	1/8	1/8	0	0	0	1/2	0	0	0	0
1221	0	1/8	0	0	0	0	0	0	0	0	0
1222	0	1/8	0	0	0	0	0	0	0	0	0
2111	0	0	1/8	0	0	1/4	0	0.347	1/4	1/4	1/2
2112	0	0	1/8	0	0	0	0	0	1/4	0	0
2113	0	0	0	0	0	1/4	0	0	0	0	0
2121	0	0	0	0	0	0	0	0.347	0	1/4	0
2211	0	0	1/8	0	0	0	0	0.153	0	0	0
2212	0	0	1/8	0	0	0	0	0	0	0	0
2221	0	0	0	0	0	0	0	0.153	0	0	0

[a] Loci in order: *Aat-3, Aco, Gdh, Pgi-2*

test was conducted by comparing the likelihood of the eleven fertility estimates calculated from the actual male gamete frequencies to the distribution of likelihoods given the simulated male gamete frequencies (Ritland and Jain 1981). By this procedure, if the likelihood of the estimated fertility parameters given the actual data exceeds at most only 5% of the likelihoods given the simulated data, the model should be rejected.

Results

Observed versus expected frequencies of male gamete types in seed crops from the polycrosses

Male gamete frequencies in the seed of most of the individual polycrosses did not depart significantly from expectation under the assumption of equal male fertilities. There was no detectable heterogeneity in male marker gamete frequencies among individual crosses involving the same maternal parent in different years, or among different maternal parents in the same year (Tables 2 and 3). The male marker gamete frequencies in the seed pooled from all the crosses, however, departed significantly from those expected assuming equal fertilities of the male parents in the polycrosses, and the total of Chi squares for all individual crosses was also significant (Tables 2 and 3). Thus, based on the results of all crosses, the null hypothesis of equal male fertilities is rejected.

Estimation of male fertilities of clones used in the polycrosses

When the estimation model described above is applied to male marker gamete frequencies from the pooled seed of all crosses and years (Table 2), fertility estimates range from 0.028 to 0.205 (Fig. 1). The null male fertility estimate expected when parents have equal fertilities is $0.0625 \times n_{jk}$. Six of the eleven fertility estimates departed significantly from this null value (Fig. 1). The likelihood of these estimates given the actual data is greater than 70% of the likelihoods given the simulated data, indicating a reasonable fit of the observed male gamete frequency data to the model expectations.

Discussion

The comparison of observed with expected frequencies of male gametes from polycrosses with Norway spruce

Table 2. Expected gamete frequencies given equal male fertilities among the 16 parents used in a polycross with Norway spruce compared with observed gamete frequencies

Gamete[a]	Expected frequency	Observed frequency[b]
1111	0.2969	0.3268
1112	0.1250	0.1262
1113	0.0156	0.0185
1121	0.0391	0.0310
1122	0.0078	0.0027
1211	0.0469	0.0392
1212	0.0781	0.0495
1221	0.0078	0.0033
1222	0.0078	0.0016
2111	0.2170	0.2213
2112	0.0547	0.0560
2113	0.0156	0.0141
2121	0.0529	0.0430
2211	0.0174	0.0386
2212	0.0078	0.0152
2221	0.0096	0.0131

[a] Loci in order: *Aat-3*, *Aco*, *Gdh*, *Pgi-2*

[b] Observed frequencies based on 1839 seeds obtained by summing data from all crosses yielding > 25 seeds

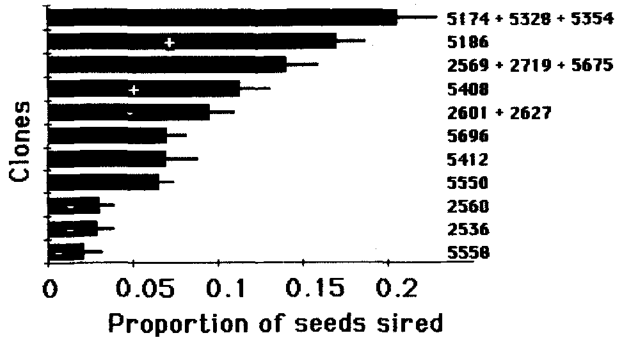

Fig. 1. Male fertilities and standard errors (lines above bars) of 16 clones used as male parents in a polycross with Norway spruce. Plus and minus signs indicate the presence and direction of the significant departures from equal male fertility

Table 3. Test of goodness of fit of observed male gamete frequencies in seeds from polycrosses with Norway spruce to that expected given equal male fertilities among the 16 clones used as male parents

Clone used as seed source	Year	No. of seeds sampled	Degrees of freedom[a]	X^2
2528	1976	99	4	3.18 ns
	1983	50	4	1.95 ns
5174	1976	77	4	7.69 ns
	1983	46	4	1.77 ns
5262	1976	63	4	8.66 ns
5403	1976	77	4	1.72 ns
5408	1976	53	4	1.64 ns
	1983	40	4	1.19 ns
5545	1976	45	4	12.15*
	1983	72	4	12.87*
5547	1976	35	4	6.43 ns
	1983	34	4	1.94 ns
5549	1976	49	4	4.26 ns
5550	1983	34	4	4.49 ns
5617	1976	75	4	7.63 ns
	1983	33	4	10.21*
5651	1983	60	4	2.29 ns
5722	1976	91	4	6.69 ns
	1983	111	4	2.75 ns
5723	1983	98	4	2.87 ns
5724	1976	60	4	6.00 ns
	1983	83	4	7.50 ns
2491	1983	59	4	7.50 ns
5191	1983	52	4	6.29 ns
5193	1983	29	4	3.81 ns
5340	1983	28	4	10.63*
5385	1983	31	4	5.90*
5571	1983	40	4	9.33 ns
5626	1983	36	4	7.43 ns
5677	1983	56	4	9.91*
5696	1983	26	4	2.13 ns
5706	1983	51	4	1.86 ns
5724	1983	46	4	1.31 ns
			Sum = 132	179.23**
		1839	Pooled = 4	41.15***
			Heterogeneity = 128	138.08 ns

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

[a] Degrees of freedom reflect pooling of gamete classes required to fulfill minimum expected frequency criteria

suggests that male parents did not contribute gametes equally to the seed crop. Estimates of male fertilities of individual clones (or combinations of clones), more than half of which depart significantly from the values expected if all parents in the polycrosses contributed male gametes equally to the seed crops, support this finding. This strengthens earlier results which showed that at least some of the male parents did not contribute gametes equally to the seed crop (Cheliak et al. 1987). Evidence of male fertility variation has also been reported by Moran and Griffin (1985) for *Pinus radiata*.

A number of potential causes underlie the variation in male fertilities observed here. These include: (1) pollen grains from paternal parents differ in size, so that the attempted equalization of pollen contributions carried out on the basis of mixing equal volumes from the different parents does not result in equal quantities of pollen from each parent (Adams 1982); (2) there are differences among parents in pollen viability and/or pollen tube growth rate; and (3) there are differences in post-zygotic abortion which depend on the genotypes of the paternal and maternal parents, and may be due to inbreeding depression or to specific combining ability effects. Prezygotic interactions between maternal and paternal genotypes could also produce variation in male fertilities, but these are generally believed to be of lesser importance in gymnosperms (Sarvas 1968; Koski 1973). Which of these non-mutually exclusive causes is responsible for the observed departure from equal male fertilities can not be determined with certainty from the results at hand.

In estimating a number of quantitative genetic parameters from traits expressed in families derived from pollination of maternal parents with a number of paternal parents, plant breeders often assume for simplicity that variation in male fertility is minimal or non-existant. In the open-pollinated populations studied by Gutierrez and Sprague (1959), Carlson (1971), Müller-Starck and Ziehe (1984), and Schoen and Stewart (1986), it is clear that this was not the case. For example, in the latter study, the estimated male fertilities of single clones in a population with 33 potential clonal male parents ranged from 0 to 0.50. While generally less variable than the range of male fertilities in the open-pollinated populations referred to above, the observed range of male fertilities in the Norway spruce polycrosses reported here is, nevertheless, substantial. The distribution of male gamete contributions is skewed, with 5 of the 16 parents contributing close to half of the male gametes to the total seed crop. This is expected to increase the average genetic correlation among offspring to a value greater than 0.25, resulting in overestimates of heritability (Squillace 1974), and inaccurate estimates of general combining abilities and expected response to selection. The latter estimates may be either lower or higher than those expected on the basis of equal fertilities, depending on the correlation between general combining abilities for the traits of interest and fertilities (Griffin 1982).

Given that male fertility variation has also been reported in *Pinus radiata* (Moran and Griffin 1985), breeders of forest trees, and perhaps other plant species, should exercise caution in interpreting results based on the polycross mating design. Studies of male fertility variation, such as the one reported here, could perhaps be carried out in conjunction with breeding programs utilizing the polycross, and the male fertility estimates obtained for the different polycross parents could be used to achieve more accurate estimates of quantitative genetic parameters. Griffin (1982), for example, shows how estimates of the expected response to selection can be modified when male and female fertility estimates are available. While such information may be moderately costly to obtain, it may prove worthwhile if it can be used to improve mating designs which fall short of the breeder's expectations.

Acknowledgements. This work was supported in part by grants from the Natural Science and Engineering Research Council to DJS. We thank the Norwegian Institute of Forest Research for supplying us with the data from these crosses, and Dr. Steven C. Stewart for his advice and criticism of an earlier version of this paper.

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