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Genetics of the polycross

1. Experimental results from Norway spruce

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Summary. Rates and patterns of male gamete incorporation for a polycross mating design were studied for two independent years of pollination in Norway spruce, *Picea abies* (L) Karst. Segregation distortion in a subset of maternal clones was documented for one locus. We have proposed a model, involving the existence of a linked lethal allele, which accounts for these observations. Significant temporal and maternal clonal differences were observed in the rates at which single locus and multilocus gametes were incorporated. Striking differences in apparent fertility existed among four clones which produced unique multilocus gametes. One clone, in particular, was shown to be contributing three times as many gametes to the next generation as predicted by the hypothesis of equal clonal male contribution. These deviations from expectation were also detected in the genotypic distributions of the resultant filial generation. Ramifications of these results on family structures in the filial generation, effective size of the male population, and possible bias in inferences of genetic differences and parameter estimation are discussed.

Key words: *Picea abies* – Polycross – Isozymes – Segregation distortion – Fertility lethal alleles – Paternity analysis

Introduction

Genetic improvement in forest tree crops has relied traditionally on the exploitation of additive genetic variation. Usually, improved seed is produced by open-pollination in seed orchards. This general strategy seems well-founded, as evidence suggests that the majority of genetic variation in commercially important conifers exists as additive variation.

Because of typically long maturation and generation times, and the extensive areas required to accommodate the tests, forest geneticists try to utilize the most efficient breeding schemes possible. This is particularly relevant for the early stages of a domestication program, where it is necessary to quickly, and effectively, estimate the breeding value of a large number of potential candidates. Several types of mating designs are available to produce progenies for estimating breeding values of the parents. Among these is the polycross, in which all parents under test are crossed with a mixture of pollen from a given set of male parents (Burdon and Shelbourne 1971). In a second paper (Skrøppa and Cheliak 1987), we will discuss the statistical and genetical analyses of measurements from this design, and the use of the design in forest tree breeding programs.

In this paper, we will explore some biological assumptions underlying the statistical model for this mating design. In addition, we will test these assumptions by analyzing electrophoretic data from viable progeny derived from a polycross of Norway spruce (*Picea abies* L. Karst).

Before one can make genetic interpretations of statistical analyses of data from a polycross design, it is necessary to make a number of assumptions, particularly about incorporation of the male gamete pool. To avoid additional complications, it is necessary that progeny resulting from a polycross mating design be both in Hardy-Weinberg equilibrium and in linkage equilibrium. Thus, it is necessary to test whether allele frequencies in the male and female gamete pools are equal. If they are not equal, a heterozygote excess will result after random mating (Robertson 1965; Workman 1969). Second, there can be no segregation distortion

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either in the male or female gamete pool. Otherwise, the net effect will be that of an assortative mating system, where one allele is preferentially incorporated, thereby resulting in a change of family structure and expected levels of variation. Finally, male gametes have to be incorporated equally, and at random, over the array of maternal clones. If not, family structures and effective filial population size, and effective male sample size, will be changed from that expected. In particular, non-random incorporation of male gametes can potentially bias estimates of breeding values. This deviation reduces the overall efficiency of the polycross mating design.

Materials and methods

Seed orchard and clonal parental material

The male and female parents for this study are located in an operational Norway spruce seed orchard in the southeastern part of Norway at latitude 63°31'N, longitude 11°54'E, and an altitude of 155 m. The orchard covers a total of 24 ha, and contains ramets (grafted scions) of 211 clones of selected plus trees in a breeding program.

Cross synthesis

Assessments of flowering have been made in the orchard since 1974, and significant amounts of seed have been harvested in 1974, 1976, 1980, and 1983 for use in operational reforestation programs (Skrøppa and Tutturen 1985). In 1976, controlled crosses were made following a polycross design with a pollen mix consisting of equal volumes of pollen from 16 of the clones in the orchard. Seed was obtained from 110 clones, and seedlings from these polycross families were planted in progeny tests on three sites in 1979. In 1983, a year of exceptionally abundant flowering, new controlled crosses were performed following the same procedures and polycross design as in 1976, with the same set of 16 male parents. Additional crosses were made on 42 clones that had not flowered during the original crossings of 1976. Thirteen of the crosses made in 1976 were repeated in 1983.

Pollen extraction

Branches with abundant male strobili were removed from ramets of the 16 clones which were used as male parents for the polycross. At anthesis, normally 3 to 5 days after branch removal, the pollen was collected from each clone separately, dried, and cleaned. Equal volumes of pollen from each of the 16 clones were bulked and mixed. Approximately 7–8 mL samples from the pollen mix were placed into 15 mL glass vials and sealed.

Isolation and pollination

Paper bags were used to isolate female flower buds one to two weeks prior to opening. A total of 30 female flowers were isolated for each clone. Pollinations were made by inserting a pollination syringe, 2 mm in diameter, into the bag, without removing the bag itself. Pollinations were performed on each of four days in a five day period in 1976 and on each of five days during a six day period in 1983. At each pollination, one vial of the polymix pollen was applied per clone.

Seed harvest, extraction and storage

Cones were harvested in September, and were stored for two months before processing. The seed was cleaned, and empty seeds determined by floating in alcohol. The cleaned seed was then stored in glass bottles at −10° to −20 °C until required for either the progeny test or for electrophoresis.

Samples for the electrophoretic investigation consisted of polycross seeds from 13 clones which were common between the two years, and an additional sample of 17 clones in 1983. As well, a sample of open-pollinated seeds from each of the male parents was collected for each of the two years.

Electrophoresis and data analyses

Seed pre-treatment, handling, germination, extraction, and electrophoresis were as previously reported for white spruce (*Picea glauca* (Moench) Voss) by Cheliak et al. (1985). Data were recorded by gamete and embryo pairs from a seed for each clone. Up to 100 gamete/embryo pairs were analyzed for each of the 30 clones pollinated by the pollen mix, and up to 24 gametes from clones contributing pollen to the mix.

Because of the way that sexual reproduction occurs in conifers, recording gamete/embryo pairs enables direct determination of the male gamete. Thus, there are several types of data that can be obtained from this survey. Firstly, the haploid female gametophytes enable a direct test be made of the inheritance and linkage of variable allozymes. Second, the diploid genotypic data from embryos allow for characterization of the filial generation. Finally, as previously indicated, combining these data facilitates reconstruction of the pollen pool that was effective in fertilization. Haploid male data can be used to test hypotheses on rates and patterns of incorporation of male gametes based on the genetic composition of the synthetic pollen pool, known from the analysis of haploid female gametophytes from seeds from each pollen parent.

Segregation, linkage, and expected rates of incorporation of male gametes were analyzed by a maximum likelihood G (asymptotic χ^2; Sokal and Rohlf 1969) test statistic. Consistency of the incorporation of male gametes was analyzed by a χ^2 statistic over the array of maternal clones. Expected heterozygosity (h) was calculated for each variable locus in each population by the formula $h = 1 - \Sigma p_i^2$, where p_i represents an estimate of the frequency of the ith allele in either the mature or viable zygotic population. The observed genotypic distribution of the filial generation was compared to that expected under Hardy-Weinberg equilibrium by means of a maximum likelihood G. The proportional decrease or increase in heterozygosity (F) relative to that expected under panmixia was calculated according to the formula $F = 1 - [H/h]$, where H is the observed proportion of heterozygotes and h is as previously defined. This quantity in known as Wright's fixation index.

Results

Inheritance and linkage of allozymes

Representative diagrams of banding patterns observed for the four variable loci are shown in Fig. 1. Two alleles were observed at *Aat-3, Aco, Gdh,* and three alleles at *Pgi-2.* No segregation distortion for the pooled summaries, nor heterogeneity among clones, was observed for *Aat-3* or *Pgi-2.* However, both *Aco*

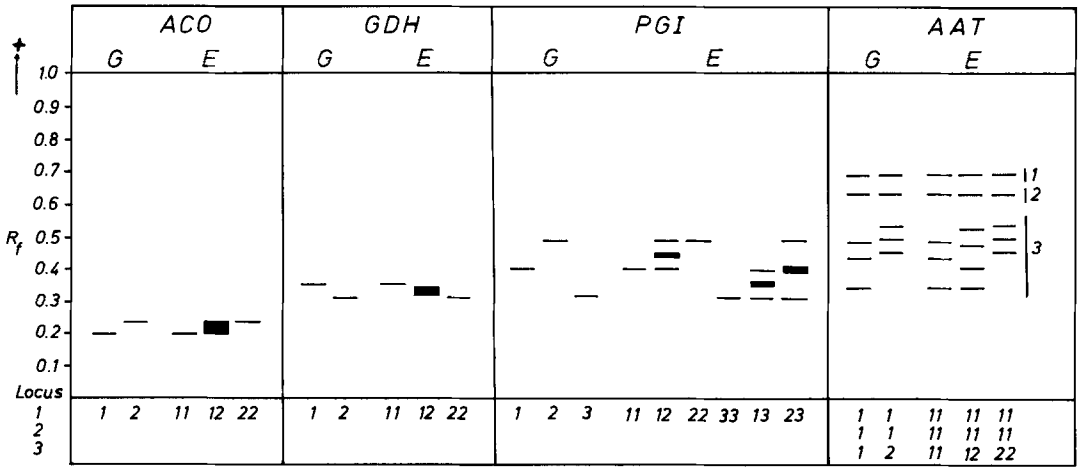

Fig. 1. Representative diagrams of banding patterns for haploid gametophytes (G) and diploid embryos (E) for four variable enzyme systems in Norway spruce

Maternal Genotype	Pollen Pool Gametes
1L	1L
2l	2L
	2l

Zygote Array Gamete Contribution	
Maternal	*Paternal*
1L	1L
1L	2L
1L	2l
2l	1L
2l	2L
2l	2l (inviable)

Fig. 2. Model for the existence of a lethal allele (l) linked to an allozyme marker (number 2 in this case). Note that because the zygote 2L/2l is inviable, the segregation parameter for the maternal gametes changes from 1 : 1 to 3 : 2

and *Gdh* showed significant departures from the distribution of gametes expected for 1 : 1 segregation. Significant heterogeneity was also evident for the segregation parameter among clones heterozygous for *Aco*.

In the case of *Aco* the significant heterogeneity among clones, in addition to inspection of the data, indicates that there are two distinct groups. One group, the majority of clones (10 of 14), seem to segregate as per Mendelian expectations. The other four clones deviate from simple Mendelian expectations, and have a consistent deficiency of the number 2 allozyme. If we separate these two groups, and test the expected segregation parameter among the group of 10 clones that seem to segregate as per Mendelian expectations, the G test for deviation is 0.592 with no heterogeneity ($G_h = 6.32$, 6 df).

A possible explanation for the aberrant segregation ratio of the second group of four clones could be the existence of a lethal allele linked to the number '2' allozyme at *Aco*. Consider the scheme as presented in Fig. 2. Suppose that at meiosis, segregation occurs at random to produce two types of female gametes, 1L and 2l, in a 1 : 1 ratio. To simplify matters, we will assume that the lethal allele, l, is completely linked to the 2 allozyme. As well, we will assume that 1 is a recessive lethal, and does not affect fitness in the heterozygous form. In the synthetic pollen pool, suppose that three types of gametes are possible, 1L, 2L, and 2l. Although six zygotes can be formed when mating occurs at random, only five are viable (Fig. 2). The result of the loss of this class of zygotes will be a change in the apparent segregation ratio of haploid maternal gametes from a 1 : 1 to a 3 : 2.

If we test the hypothesis of a 3 : 2 segregation ratio against the data observed for the aberrant segregating group, no significant departures are detected ($G = 2.65$). Furthermore, there is no heterogeneity among clones for this revised segregation parameter ($G_h = 2.73$, 3 df). Additional evidence to be presented later will lend further support to the hypothesis that there could be a lethal allele associated with the 2 allozyme at *Aco*.

Linkage analyses

From the available data, all potential pairs of heterozygous loci were tested for linkage by means of an appropriate maximum likelihood G test. Only one family indicated weak linkage between *Aco* and *Pgi-2*. However, this was likely due to chance. Thus, in subsequent calculations, we can conclude that we are dealing with an independent set of loci.

Genetic structure of the parental and filial generations

Parental generation. Unlike the classical Wahlund effect, a variance in allele frequency during the haploid phase (i.e., between the male and female gamete pools) can result in a heterozygote excess after random mating (Robertson 1965; Workman 1969). To test the homogeneity of allele frequency between the male and female gamete pools at the various loci, we used a t-test for the equality of two percentages (Sokal and Rohlf 1969). There were significant differences between the allele frequency of the male gamete pool and the 1976/83 female gamete pools for *Aat-3* and *Gdh* (Table 1). However, significant differences at these loci did not lead to significant excesses of heterozygotes in the filial generation.

Filial generation. No significant differences were detected when allele frequencies of the filial generation were compared between the two years on a common set of maternal parents for *Aat-3* and *Pgi-2.* For *Aco,* significantly more of the number 2 allozyme was recovered in the 1983 pollinations than in 1976 ($t = 2.87$). For *Gdh,* significantly fewer of the number 2 allozyme were recovered in the 1983 pollinations ($t = -3.45$). Although significant differences between the years in recovered pollen allele frequencies were detected for *Pgi-2,* these differences did not manifest themselves in the filial generation.

No deviations from Hardy-Weinberg equilibrium were observed for the filial generation at *Aat-3* or *Gdh* (Table 1). However, for *Aco* there was an excess of heterozygotes detected from the complete set of parents for 1983, and the pooled distribution.

For *Aco,* additional support for the possible existence of a lethal allele associated with the number 2 allozyme at this locus derives from an analysis of the distribution of heterozygous progeny from the maternal parents in question. Irrespective of the allele frequencies in the pollen pool, a heterozygous maternal plant should produce homozygous and heterozygous offspring in a 1 : 1 ratio, if segregation is according to Mendelian expectations. However, in the case of the several suspect clones, which demonstrate the apparent segregation distortion, the ratio of homozygous to heterozygous progeny should be 3 : 2 or 60% : 40%. There were four clones which showed an apparent segregation distortion. A test of homozygous : heterozygous progeny in these four clones indicates that the distribution is not different from a 3 : 2 ratio. There was, however, a significant departure from a 1 : 1 distribution. We believe that these observations support the hypothesis of a lethal allele associated with the number 2 allozyme at this locus.

A constant, and significant, deviation from Hardy-Weinberg equilibrium was observed at *Pgi-2.* In 1976, there was a large excess of homozygotes recovered after mating. In 1983, however, the reverse was true; significant excesses of heterozygotes were detected, both in the 1983 common and pooled samples of maternal parents. Again, differences between allele frequency in the male and female gamete pools were not the factor contributing to this excess of heterozygotes.

Inter-year differences in the recovered pollen pool

There were more significant differences between allele frequencies in the recovered pollen pool between the two years of pollination, using the same set of maternal parents, than could be expected by chance (Table 2). Only locus *Aat-3* conformed to expectations that the pollen pool was being similarly incorporated in the two years of pollination. Although the one significant deviation observed at *Gdh* could be expected by chance, the directional component of deviation suggested that allele 2 was being incorporated at a higher rate in the 1976 pollination. This result is further confirmed by the significant difference observed in allele frequencies from pooled samples for the two years. Significantly more common alleles were recovered for both *Aco* and *Pgi-2* in the crosses made in 1976. Ten positive differences (i.e., where the common allele was recovered more frequently), of a possible 13, were observed for both of these loci.

Inter-clonal homogeneity

Consistency of incorporation. In this set of tests, we were interested in determining if, irrespective of the expected allele frequency in the synthetic pollen pool, all of the clones were incorporating male gametes at the same rate. No significant differences were observed between different maternal clones for 1976 or 1983 at *Gdh* or *Aco* (Table 3). Similar homogeneity was observed among clones at *Aat-3* for 1976, but significant heterogeneity was observed among the entire set of maternal clones for 1983. Heterogeneity was detected among the maternal clones for both years of pollination for locus *Pgi-2.* Although both years of pollination had significant heterogeneity, these differences were observed at different loci. This inter-locus heterogeneity in the rate at which male gametes are incorporated into the next generation demonstrates the complexity of biological forces which can act between pollination and the production of a viable embryo.

Consistency relative to expected frequencies. The expected genetic composition of the synthetic pollen pool

Table 1. Summary of allele frequencies and genotypic distributions of males, females, and progeny after a polycross

Locus	Parameter	Male pool	Female pool			Progeny			
		(Common)	1976/83	1983	Pooled	1976	1983[a]	1983[b]	Pooled
Aat-3	Allele 1	0.625	0.800*	0.588	0.688	0.690	0.671	0.634	0.654
	Allele 2	0.375	0.200	0.412	0.312	0.310	0.329	0.366	0.346
	h	0.469	0.320	0.484	0.430	0.428	0.441	0.464	0.452
	F	−0.333	0.170	0.510	0.418	0.008	0.055	0.023	0.021
	G	1.90	0.38	4.65**	5.43**	0.066	3.23	0.97	1.26
	n	16	13	17	30	1,050	1,081	1,868	2,918
Aco	Allele 1	0.813	0.830	0.735	0.781	0.868	0.846	0.820	0.838
	Allele 2	0.187	0.170	0.265	0.219	0.132	0.154	0.180	0.162
	h	0.305	0.280	0.389	0.342	0.230	0.261	0.295	0.272
	F	−0.179	−0.200	−0.360	−0.280	−0.056	−0.029	−0.057	−0.053
	G	0.461	0.600	3.31	2.51	3.47	0.93	6.04**	8.14**
	n	16	13	17	30	994	1,094	1,760	2,754
Gdh	Allele 1	0.875	0.970*	0.794	0.875	0.918	0.935	0.922	0.920
	Allele 2	0.125	0.030	0.206	0.125	0.082	0.065	0.078	0.080
	h	0.219	0.060	0.327	0.219	0.150	0.120	0.144	0.147
	F	−0.143	−0.030	−0.259	−0.143	−0.019	−0.001	−0.020	−0.021
	G	0.57	0.03	1.84	0.65	0.50	0.002	0.96	1.61
	n	16	13	17	30	1,326	1,464	2,159	3,489
Pgi-2	Allele 1	0.688	0.700	0.794	0.750	0.697	0.712	0.721	0.712
	Allele 2	0.281	0.200	0.147	0.172	0.236	0.222	0.219	0.226
	Allele 3	0.031	0.100	0.059	0.078	0.067	0.066	0.060	0.062
	h	0.447	0.460	0.344	0.402	0.454	0.440	0.428	0.438
	F	−0.118	0.130	0.175	0.145	0.091	−0.037	−0.048	0.007
	G	0.923	0.18	1.80	2.05	18.28**	11.99**	13.18**	10.23**
	n	16	13	17	30	1,265	1,320	2,084	3,349

[a] Based on 13 clones common between 1976 and 1983
[b] Based on 30 clones pollinated in 1983
* Significant difference in the allele frequency between male and female gamete pools, based on the genotypic structure of the parents
** Significant departure in expected genotypic distribution, based on Hardy-Weinberg equilibrium

Table 2. Number of significant differences observed at the 5% level, and the directional component of difference for two years of crossing a synthetic pollen pool for the same set of 13 maternal clones

Parameter	*Aat-3*	*Aco*	*Gdh*	*Pgi-2*
Significant "*t*" tests	1	4	1	5
Direction				
Positive	8	10	3	10
Negative	4	3	10	2
No change	1	–	–	1
Common allele frequency				
1976	0.631	0.875	0.877	0.749
1983	0.602	0.832	0.910	0.692
"*t*" value	13	2.6**	−2.8**	3.2**

** See Table 1

Table 3. Summary of chi-square tests of inter-clonal homogeneity for rates of pollen gamete incorporation from a synthetic pollen pool

Locus	Year		
	1976	1983[a]	1983[b]
Aat-3	11.39 (11)	16.85 (12)	43.92 (29)**
Aco	16.55 (12)	15.08 (11)	31.71 (28)
Gdh	8.29 (12)	9.51 (12)	40.75 (29)
Pgi-2	43.38 (12)**	40.99 (12)**	67.70 (29)**

[a] 13 clones common between the two years
[b] 30 clones which were pollinated in 1983
** See Table 1

has been determined from an independent sample of open-pollinated seeds from the male parents. In this series of tests, we were interested in determining whether all clones were incorporating male gametes at rates predicted from the known composition of the synthetic pollen pool. Tests for inter-clonal homogeneity against the expected frequencies indicated, similar to previous results, that different sets of loci deviated in the different years (Table 4).

Significant heterogeneity was detected among the complete set of maternal parents for 1983 at *Aat-3*. This result is similar to that observed in the previous tests. However, there was also a significant deviation from the rate that male gametes should have been incorporated, given the genetic composition of the pollen pool. In this case, the deviation from expected frequencies was due to a significant excess of the 2 allozyme. No heterogeneity, or deviation from expectations, was noted for the set of common maternal parents.

For *Aco*, no heterogeneity was detected in the rate at which male gametes were being incorporated among the maternal parents. However, in 1976 there was a significant deviation from the rate predicted by the composition of the synthetic pollen pool for this locus. This lack of fit was due to a significant deficiency of the number 2 allozyme.

Significant deviations from expected rates of incorporation of male gametes at *Gdh* were observed for the maternal parents in 1983. In addition, significant heterogeneity was detected among the complete set of clones for that year. In both cases, the lack of fit to the expected distribution was attributable to a significant deficiency of the number 2 allozyme.

In agreement with previous observations, there was a constant, and significant heterogeneity of incorporation of male gametes observed among clones for *Pgi-2*. In addition, the observed distribution in 1976 differed from that expected as significantly more of the number 1 allozyme was incorporated in this sample.

Paternity and fertility analyses

Given the genotypic structure of the paternal population, 50% of the male parents had unique diploid genotypes (Table 5). The other 50% occur as three groups with clones 2,569, 2,719, 5,675 sharing a common diploid genotypes, clones 2,601 and 2,627 forming a second group and clones 5,174, 5,328, and 5,354 forming the final group sharing a common diploid genotype. From these 16 plants a total of 16 different multilocus gametes can be formed, and of these, seven are unique, in that their origin can be ascribed to a single male parent. The seven unique gametes are produced by four different male parents.

Table 4. Summary of log-likelihood G tests for inter-clonal homogeneity of pollen gamete incorporation, at the rate expected, based on genetic composition of the synthetic pollen pool

Parameter	*Aat-3*	*Aco*	*Gdh*	*Pgi-2*
Significant differences				
1976[a]	1	5	2	6
1983[a]	1	1	2	3
1983[b]	5	2	8	4
Pooled deviations				
1976[a]	0.09	28.54**	0.04	21.12**
1983[a]	2.89	1.17	15.25**	0.80
1983[b]	12.92**	0.62	40.23**	0.95
Heterogeneity				
1976[a]	14.75	17.83	13.47	49.01**
1983[a]	16.99	19.62	8.95	43.39**
1983[b]	44.15**	36.63	53.10**	71.03**

1 df is associated with the pooled deviation

[a] Results are based on 13 different maternal parents. The degrees of freedom for the heterogeneity test is 12

[b] Results are based on 30 different maternal parents, 13 of which were common to the two years. The degrees of freedom for the heterogeneity test is 29

** See Table 1

Table 5. Diploid genotypes of the male parents contributing to the polycross pollen pool

Clone no.	Locus			
	Aat-3	*Aco*	*Gdh*	*Pgi-2*
2,536	11	22	11	12
2,560	11	12	12	12
5,186	12	12	11	12
5,408	11	11	11	12
5,412	11	11	11	11
5,550	12	11	11	13
5,558	11	12	11	22
5,696	22	12	12	11
2,569	12	11	11	12
2,719	12	11	11	12
5,675	12	11	11	12
2,601	12	11	12	11
2,627	12	11	12	11
5,174	12	11	11	11
5,328	12	11	11	11
5,354	12	11	11	11

With the genetic composition of the paternal haploid pool we can derive the expected proportion of various gamete classes that should be recovered under the simple hypothesis of random mating. Significant differences were detected for 1976 (G_1 115.24), 1983 (G_1 95.92), and the pooled samples (G_1 250.51) for the rate at which multilocus male gametes were incorporated, compared to the rate expected (Fig. 3). Although there was significant heterogeneity between the

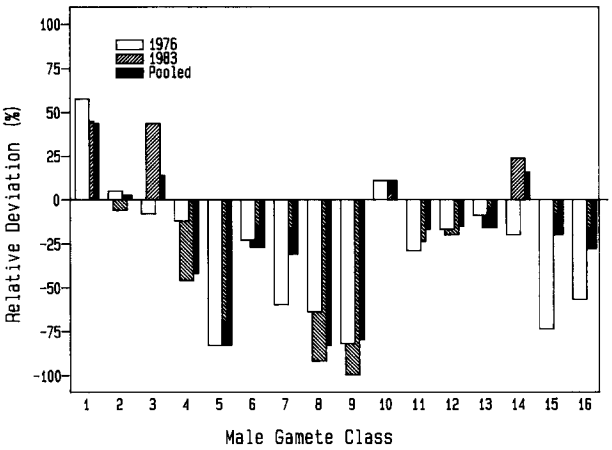

Fig. 3. Relative deviation from expected rates of incorporation for multilocus male gametes involved in a polycross of Norway spruce. Relative deviation is defined as [(obs−exp)/exp] · 100. Gamete classes correspond to the following multilocus gametes, with the gene order *Aat-2, Aco, Gdh, Pgi-2,* 1=1111, 2=1112, 3=1113, 4=1121, 5=1122, 6=1211, 7=1212, 8=1221, 9=1222, 10=2111, 11=2112, 12=2113, 13=2121, 14=2211, 15=2212, 16=2221

two years (G_h 15 df 27.94), this was primarily due the degree, rather than direction of deviation. There were only three changes in the sign of the deviation between the two years (gametes 2, 3, 14), with gamete 3, produced by clone 5,550, accounting for the majority of the difference between the two years. This inter-year heterogeneity has also been observed for the rate at which single loci were incorporated, as well as in the genotypic structure of the filial generation.

Despite the heterogeneity between the two years, there were some consistent patterns of gamete incorporation across the set of years and clones. There were three distinct patterns of male gamete incorporation. The first pattern, represented by gamete classes 2, 3, 10, 11, 12, 13, 14, and 15 was where the gametes seem to be incorporated at about the rate expected, across years and sets of maternal parents. The second pattern, represented by gametes 4, 6, 7, and 16, was where there was a net deviation of more than 25%, but less than 40%, from the distribution expected in the pooled sample. In addition, this group is further characterized by considerable variation in the degree of deviation between the two years for these gamete classes. However, apart from a consistent under-representation of these multilocus gamete classes, there were no additional obvious patterns, involving either sets of loci or alleles over the two years, or in the pooled sample. Finally, there was a group of gametes, represented by 1, 5, 8, and 9 which had a consistent, and large, deviation from the expected distribution based on random mating. All of these gamete classes deviate by more than 40% from their expected representation, and can deviate by as much as 100%. Within these classes of gametes there were, in general, excellent agreement between the two years of pollination on a common set of maternal parents. Of the four gametes which demonstrated a large deviation, 5, 8, and 9, all contributed by clone 2,560, were consistently under-represented in the filial generation by about 80%. Similar results have recently been reported using a type of polycross in *Raphanus sativus* by Marshall and Ellstrand (1986).

Table 6. Rate of incorporation of unique male gametes for a polycross of Norway spruce

Clone	% deviation	
	Combined	Pooled
5,550	29.0	28.3
2,560	−52.4	−58.3
5,186	64.8	90.8
5,696	−12.1	−14.5
G test	15.6	34.9

Four clones produce unique gametes. These include numbers 2,560, which produces three unique gametes, 5,550, which produces two unique gametes, and 5,186 and 5,696, each of which produces one unique gamete. As a rough measure of fertility, we tested whether these four clones were contributing unique gametes to the next generation as expected, based on the genetic composition of the pollen pool.

As with previous results, there were marked departures from the expected rates at which unique gametes were recovered for both the 1976 and 1983 pollinations, as well as the pooled sample (Table 6). However, unlike the previous results, no heterogeneity was detected between the two years of pollination on a common set of maternal parents. Thus, to increase the sample size, only the combined data will be presented. Only clone 5,696 seemed to be contributing gametes to the next generation at approximately the rate expected, based on the genetic composition of the pollen pool. The most significant factor influencing the departure from expected distribution was the over-representation of clone 5,186, and the under-representation of clone 2,560 in the next generation.

One of the most obvious reasons could simply be a difference in the competitive abilities of the pollen from the two clones. Because the pollen of all clones was applied simultaneously as a mix, there would be likely be little scope for a 'first-come, first-served' type of competitive advantage that can be a factor in open-pollination systems (Franklin 1971). Thus, we propose that the apparent competitive advantage of clone 5,186 could be due to more rapid pollen germination and/or elongation, relative to the other clones. Similarly, the

disadvantage of clone 2,560 could be due to slower germination of elongation, as well as an inability to withstand the pollen extraction protocols. However, further experimentation would be necessary to confirm these preliminary hypotheses.

Discussion

Results from this set of materials clearly demonstrate that several of the assumptions necessary for interpretation of the statistical analysis of data from the polycross design can be violated in experimental material. Segregation distortion, and the possible effects of a linked lethal allele, were documented for a subset of maternal clones for one locus. Effects of this distortion were further detected in the genotypic distribution of the filial generation at this locus.

Typically, reduced yield of filled seed after self-pollinations, when compared to yields after cross-pollinations on the same maternal plant, have been interpreted as evidence of the effect of lethal or sublethal alleles (Koski 1973; Lindgren 1975; Griffin and Lindgren 1985). In Norway spruce, Koski (1973) found an average number of 9.6 so-called embryonic lethals per tree based on a sample of 87 trees from Finnish provenances. This estimate demonstrates the high apparent genetic load in Norway spruce.

By far the most striking aspect of these results were deviations from expected rates, and patterns of male gamete incorporation. Differences were detected at the level of single genes, multilocus gametes, and clonal contributions, as indexed by unique multilocus gametes. These deviations from expected rates seriously effect the assumption of random, and equitable contribution of male gametes to the next generation by clones involved in the pollen mix. Moran and Griffin (1985) have observed similar problems in a polycross of *Pinus radiata* D. Don.

The processes of pollination, fertilization, and embryo and seed development in conifers offer several possibilities for violation of the assumptions of random and equal contribution of genes from the males in the pollen mix. In Norway spruce, the number of pollen grains that can be accommodated in the pollen chamber varies, but is normally four or more (Sarvas 1968). On germination, pollen tubes grow rapidly through the nucellus and toward the megaspore mother cell, in which fertilization occurs four to six weeks after pollination. Normally, embryo systems from several archegonia will develop, but only one embryo ordinarily remains in a fully developed, germinable seed. Thus, opportunities for selection can occur during these stages of the life cycle. Evidence of male gametophytic selection is scarce in conifers, but has been found in maize (Ottaviano et al. 1982). Polyembryony has been interpreted as one mechanism which allows a considerable genetic load to be carried in the population (Sorensen 1982).

Forcing of male strobili and handling of the pollen (collection, drying, and cleaning) may influence its viability. Such effects may be different for males in the mix, and may, if present, violate the assumption of potential random and equal contribution from all males. However, if the polycross is to be used as a breeding strategy, these "technique-induced" violations become part of the overall biology of the system. Since only fresh pollen was used, and the same techniques were applied to all males, it is reasonable to believe that such effects were minimized. The persistence of deviations from expected rates across the two independent years of pollination seems to confirm this.

There were two major, but related, effects of these departures from random and equitable male contribution in the filial generation. The first concerns effective population size. This includes the filial generation, and the size and representativeness of the haploid male population. The second effect, related to the first, was the change in family structure from random expectations.

Suppose that there were N_m males, each contributing gametes at random and equitably to the next generation. In a sample of N_p progeny from a maternal parent, we can expect a mixture of full- and half-sib families. In general, there will be N_m full-sib families, each with N_p/N_m individuals within the family. The family structure of the filial generation obviously depends on the sample size of the filial generation drawn from each maternal parent. In particular, when $N_p \ll N_m$, full-sibling family structure poses no real problem. However, usually $N_p \gg N_m$, and some family structure greater than half-siblings can be expected.

Nevertheless, as the variance of individual male contribution rates increases, the effective number of individuals are reduced. As a result, genetic structure of the families is not simply N_m full-sib families, each with N_p/N_m members within the family, but has to be changed to reflect an increase in variance of the size of the full-sib families represented in the sample of N_p progeny from a maternal parent. For example, suppose that there were three males represented in a pollen mix. Instead of equal contributions to the next generation, suppose they contributed gametes in a 1 : 3 : 1 proportion. In a sample of five progeny from a maternal parent, there will be, on average, three full-sib families, of sizes one, three, and one individuals, respectively.

Obviously, families obtained from the more fertile males will contribute proportionally more information for evaluation. Thus, inferences of genetic differences among individuals (i.e., estimation of general combining ability) can be compromised as a result of the potential bias caused by non-additive effects. These non-additive gene effects, or specific combining ability, can also compromise any estimates of variance components made from the structured families. Finally, the effective size of the male population which has been sampled for the progeny test is not simply given by

N_m, but has to be weighted by the variance in gamete contribution by various males. Thus, the number of males that have been sampled will be given by the harmonic mean, which, if the variance in contribution is large, can be substantially less than the census number of individuals. Using our previous example of a census size of three males, but proportional contributions of 1 : 3 : 1 gametes to the next generation, we have an effective male sample size of 1.29. Thus, the representativeness of the male population which is acting as the tester could be radically different from the expected.

In order to minimize the impact of these effects, it is advisable to keep the number of males in the polymix as large as is feasible within a program. The final size of the male pool should also take into consideration the anticipated number of progeny that will be sampled from any particular maternal parent. It should then be possible to account, in a limited way, for the possible effects of structured families in the estimation of variance components.

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