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Frequency-dependent male reproductive success in a polycross of Douglas fir

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Summary. Frequency dependence of male reproductive success (RS) was investigated in a polycross of Douglas fir. We expect frequency-dependent RS in conifer polycrosses for two reasons. The first is the presence of "pollen chambers" in conifers, which limits the number of competing pollen grains and enhances the reproduction of poor males when at high frequency in the polymix. The second reason is the possibility of competitive interactions among pollen types, which generally gives more complicated patterns of RS. To measure frequency dependence, we performed four separate polycrosses with respective proportions of 1:1:1, 2:1:1, 1:2:1, and 1:1:2 of the same three males. Male RS was measured with isozyme markers in seed offspring, and three different estimation models were applied to the data. A general log-linear model detected frequency-dependent RS, but did not allow any specific characterization of its nature. A comparison of two more specific models, the first incorporating pollen chamber size and the second incorporating competitive interactions, indicated that competitive interactions were predominantly responsible for the observed frequency-dependent RS. The implications of frequency-dependent RS for forest tree breeding are discussed.

Key words: Male reproductive success – Polycross – Frequency-dependent selection – Douglas fir – *Pseudotsuga menziesii*

Introduction

The polycross mating design is the controlled crossing of seed parents with a pollen mix derived from several pollen parents. This mating design has evolved considerably from the original polycross proposed by plant breeders (Frandsen 1940; Tysdal et al. 1942; Wellensiek 1952), wherein selected lines were merely allowed to be open-pollinated for purposes of breeding. In forestry, the polycross has been adopted for several uses, the most important being as an efficient method for estimating the general combining ability of a seed parent (Burdon and Shelbourne 1971; Burdon and van Buijtenen 1990). In addition, Cotterill (1986) has proposed that the polycross be used to maximize genetic gain under index selection. Underlying all these uses is the assumption of equal fertility of the male pollen parents.

Recently, differential male reproductive success (RS) among pollen donors has been reported in polycrosses by several workers. Differential RS has been most pronounced and consistent when the pollen mixture consists of relatively few pollen parents (*Pinus radiata*, Moran and Griffin 1985; *Pseudotsuga menziesii*, Apsit et al. 1989), but bias also occurs with more pollen parents (*Picea abies*, Cheliak et al. 1987; Schoen and Cheliak 1987; *Pinus taeda*, Wiselogel and van Buijtenen 1988). Differential male RS is a problem because it causes a statistical bias of estimates of quantitative genetic variances, as well as of the predicted selection response, and it also reduces the genetic diversity of seed crops produced by polycrosses or supplemental mass pollination. To cope with this, many advocate maximizing the number of males in the polymix (Namkoong 1966; Moran and Griffin 1985; Cheliak et al. 1987; Fowler 1987). However, a consequence of increasing male number in a polycross is a decrease in the frequency of each male, and hence the possibility that frequency-dependent selection may alter our expectations of male RS. There are at least two possible mechanisms of expected frequency-dependent RS.

First, conifers have a unique reproductive structure, the micropylar canal or "pollen chamber," which holds just a few pollen grains. Fowler (1987) pointed out that because only these grains compete for fertilization of eggs, chambers receiving pollen from a single male will buffer reproductive bias, as male-male competition does not occur. In theory, small pollen chambers (holding ca. 2–5 grains) will significantly buffer male reproductive bias (Fowler 1987). Since the probability of filling a chamber with only one pollen type is a function of pollen-type frequencies, we consider the pollen chamber as a potential source of frequency-dependent RS.

Second, in pollen chambers with mixtures of male pollen, the ability of pollen from one male to outcompete pollen of a second male may be specific to that pair of males. In other words, the RS of a given male may be differentially affected by different males; the male is more successful in competition with some males than with other males. Such variation of competitive ability is also a potential source of frequency-dependent RS. The extent of this type of frequency-dependent male RS cannot be determined theoretically, but rather must be measured experimentally.

In this study, we investigate these two sources of frequency-dependent male RS in a polycross of Douglas fir. A total of three pollen donors and eight seed parents with unique multilocus isozyme genotypes was used in four separate polycrosses. The four polycrosses used pollen mixes of equal (1 : 1 : 1) and unequal (2 : 1 : 1, 1 : 2 : 1, 1 : 1 : 2) proportions, applied in a factorial mating design. These varying proportions allow frequency-dependent male RS to be measured. Three models of frequency dependence were applied to the data: a log-linear model, a model incorporating pollen chamber size, and a model incorporating variation of competitive abilities among pairs of males. The implications of frequency-dependent male RS for forest tree breeding are also discussed.

Materials and methods

Crossing design and electrophoretic methods

The study was conducted at the Douglas fir seed orchard of Canadian Pacific Forest Products, Saanichton, BC (latitude 48°35′, longitude 123°24′, elevation 50 m). In 1987, controlled crosses were performed on eight seed parents using four pollen mixes from three unrelated pollen parents. Seed and pollen parents were chosen that had multilocus allozyme genotypes giving unambiguous determination of paternity (Table 1). The four pollen mixes consisted of pollen from the three males in different ratios, by volume, as follows: (1) 1 : 1 : 1, (2) 2 : 1 : 1, (3) 1 : 2 : 1, and (4) 1 : 1 : 2.

Isolated pollen buds were collected from the three pollen parents and processed following standard pollen extraction methods (25–30 °C for 48 h). Pollen samples from each pollen parent were used to determine their respiration level. Respiration is considered to be the best laboratory method of predicting pollen fertility (Webber and Bonnet-Masimbert, unpublished results). A value of 20 µl O_2/min/g is considered to provide good seed yield. The respiration values of the three samples were 19.4, 18.4, and 22.7 for hydrated pollen of males 1, 2, and 3, respectively.

Table 1. Multilocus isozyme genotypes of trees in this study

Parent	Locus[a]			
	Pgi2	*G6pd*	*6Pgd1*	*Idh*
Male 1	11	11	11	11
Male 2	22	12	12	12
Male 3	11	23	11	11
Female 1	11	22	11	15
Females 2–5, 7, 8	11	22	11	11
Female 6	11	22	11	13

[a] See El-Kassaby et al. 1982a for allelic designation

On each seed parent tree, approximately 200 seed cones were isolated and 50 cones per tree were pollinated by each pollen mix. Receptivity of cones was determined by daily monitoring of reproductive phenology, and individual cones were pollinated from two to three times. The recovery rate of pollinated seed cones was high ($\bar{x} = 84\%$, range 81–87%). Cones were harvested and air dried at room temperature. Seeds were extracted, dewinged, and cleaned by hand, and stored at 3 °C until further use.

A total of 5,208 seeds, averaging 160 seeds per tree × cross combination, were electrophoretically assayed for both megagametophytic and embryonic tissues. Four loci were scored (*Pgi2*, *G6pd*, *6Pgd1*, and *Idh*); these loci corresponded to the isozymes phosphoglucoisomerase, glucose-6-phosphate dehydrogenase, 6-phosphogluconic dehydrogenase, and isocitrate dehydrogenase. The electrophoretic procedures, staining recipes, enzyme nomenclature, and inheritance and lack of linkage of these loci are reported elsewhere (El-Kassaby et al. 1982a, b).

Log-linear model for frequency-dependent RS

To test for frequency-dependent RS, a general approach is to formulate a log-linear model whose interaction terms measure frequency dependence. For this model, the expected ratios at zygotic seed assay for the 1 : 1 : 1 polymix ratio are specified by main terms:

$$w_1 : 1 : w_3 \,.$$

The w_i measure the relative ability of pollen of parent i to fertilize eggs when pollen is applied at a uniform ratio. They are relative to male parent 2, frequency independent, and analogous to "fitness" in population genetics. For the 2 : 1 : 1 polymix, the expected ratio without frequency dependence is $2\,w_1 : 1 : w_3$. With frequency dependence, this 2 : 1 : 1 polymix has an expected ratio specified by main terms plus two interaction terms:

$$2\,w_1 : w_{12} : w_3\,w_{13} \,.$$

The interaction terms, w_{12} and w_{13}, describe the proportional change of parentage by males 2 and 3, respectively, when the frequency of male 1 is doubled. If w_{12} or w_{13} differ from one, frequency dependence is present. For the polymix ratio of 1 : 2 : 1 the expected ratio is

$$w_1\,w_{21} : 2 : w_3\,w_{23}$$

and for the polymix ratio of 1 : 1 : 2, the expected ratio is

$$w_1\,w_{31} : w_{32} : 2\,w_3 \,,$$

where w_{ij} is the porportional change of parentage by male j when the frequency of male i is doubled. In this model, the number of parameters (eight) equals the degrees of freedom in the data, so data exactly fits the model.

The likelihood-ratio test was used to determine statistical significance of deviations of w_i or w_{ij} from the null value of one. In this test, the likelihood of the data under the complete model (L_0) and the likelihood under the constrained model (L_1, where one w is constrained to unity) are compared by computing the likelihood ratio, $-2\ln(L_0/L_1)$, which is distributed as Chi-square with one degree of freedom.

Model for frequency-dependent RS via the pollen chamber

Specific models for frequency-dependent RS can allow inferences about the underlying mechanisms of frequency dependence. In conifers, the most obvious mechanism is the pollen chamber, which effectively restricts the "level of selection" to a few individuals. Generally, the effect of the pollen chamber is to increase the RS of weak males when at high frequency in the pollen mix, because males at higher frequencies are more likely to compete only with themselves in chambers with few pollen.

To model this, let the representation of n pollen types in a polymix be $p_1, p_2 \ldots p_n$, with $\sum p_i = 1$. Each pollen type has "intrinsic" RS w_i (a function of germination percentage and pollen tube growth rate) independent of other pollen types and relative to some $w_j = 1$. For a chamber of size m, let a particular sample of pollen be represented by the integers $k_1, k_2 \ldots k_n$ (the number of pollen of type 1, 2 … n) with $\sum k_i = m$. The expected, "realized" RS of pollen type i in this sample is $\left(\dfrac{k_i w_i}{\sum_i k_i w_i}\right)$. Over all chambers receiving pollen in a polycross, the "realized" RS of pollen type i, denoted f_i, equals the weighted average over all combinations $\mathbf{k}$ of pollen types:

$$f_i = \sum_{\mathbf{k}} \binom{k_i}{k_1\, k_2 \ldots k_n} p^{k_1}\, p^{k_2} \ldots p^{k_n} \left(\frac{k_i w_i}{\sum_i k_i w_i}\right) \tag{1}$$

(the weighting factor is a multinomial probability). If chamber size m equals unity, the frequencies in the polymix cannot be altered by differences of "intrinsic" male RS.

Maximum likelihood can be used to estimate the innate male RS parameters w_i under this model. If the chamber size m is known, it is sufficient to perform just one polycross to estimate these w_i. If chamber size is unknown, it can be estimated as follows. Several polycrosses, each with different input ratios (as in this study), are performed. Then, for each putative chamber size, estimates of the w_i and the likelihood are calculated. The chamber size is inferred as that giving the highest likelihood. In our study, intrinsic RS was estimated by maximizing the likelihood L of the data over all four polycrosses.

$$L = \prod_{i,l} f_{il}^{x_{il}} \tag{2}$$

for x_{il} and f_{il}, the observed and expected (i.e., realized, Eq. 1) frequencies of paternity by male i in polycross l, respectively. Two intrinsic RS parameters, w_1 and w_3, relative to $w_2 = 1$, were estimated. The bisection method (Phillips and Taylor 1973) found values of w_1 and w_3 maximizing Eq. 2. Estimates of w_i and likelihoods were found for chamber sizes 2 through 8.

Model for frequency-dependent RS via competitive interactions

Competitive interactions among pollen types can be a second source of frequency dependence. To describe competitive interactions, we adopted the "mass action" model (Huang et al. 1971; Cockerham et al. 1972), common in chemistry. Under this model, the RS of a pollen type is determined by the probabilities of encountering other pollen types during competition for fertilization of female ovules; these probabilities equal the frequencies of other pollen types in the polymix. If there are n pollen types with frequencies $\mathbf{p} = (p_1, p_2 \ldots p_n)$, the "marginal" RS of pollen type i is

$$\bar{w}_i(\mathbf{p}) = p_1 w_{i1} + p_2 w_{i2} + \ldots p_n w_{in}\,, \tag{3}$$

where w_{ij} is the RS of pollen type i when in association with pollen type j ($0 \leq w_{ij} \leq 1$ and $\sum w_{ij} = 1$). They are termed "associative" RS values. Although individual w_{ij} values are constant, variation of w_{ij} across j causes the marginal RS to be frequency dependent. In this model, it does not matter whether a pollen grain competes with only one other pollen (of type i with probability p_i) for the entire period of pollen tube growth, or if a pollen competes with many different pollen (each with probability p_i) during pollen tube growth.

With n pollen types, there are $n^2 - 1$ independent w_{ij}. In any particular polycross, there are $n-1$ degrees of freedom in the data. Thus, to fully estimate the w_{ij}, we must perform at least $n+1$ separate polycrosses, each with *different* pollen input frequencies $\mathbf{p}$. In our case, with three male parents, the minimum of four polycrosses was available to estimate the w_{ij}.

Maximum likelihood can be used to estimate these RS values as follows. In polycross k, let p_{il} be the frequency of male i and let the vector $\mathbf{p}_l$ represent the frequencies of all males. The likelihood of the data is

$$L = \prod_{i,l} \left(p_{il}\, \bar{w}_i(\mathbf{p}_l) \Big/ \sum_j p_{jl}\, \bar{w}_j(\mathbf{p}_l) \right)^{x_{il}}. \tag{4}$$

An expectation-maximization recursion that finds maximum-likelihood estimates, subject to the constraints $0 \leq w_{ij} \leq 1$ and $\sum w_{ij} = 1$, is

$$w'_{ij} \propto \sum_l x_{il} \left(\frac{p_{il}\, p_{jl}\, w_{ij}}{p_{il}\, \bar{w}_i(\mathbf{p}_l)} \right) \Bigg/ \sum_{i,j} p_{il}\, p_{jl}\,, \tag{5}$$

where the w'_{ij} are the unnormalized new values. For successive iterations, these values are normalized and substituted into the right side of Eq. 5. This process is repeated until convergence (50–100 iterations).

The statistical error of estimates was determined by bootstrapping. This method involves the formation of replicate data sets by randomly sampling, with replacement, observations from the original data set. The distribution of estimates performed on these replicate data sets represents the sampling distribution of the original estimate. Standard errors of estimates were based upon the distribution of 100 bootstraps.

A model incorporating both pollen chamber size and competitive interactions was not found, as it was determined to be too complex, with the number of independent parameters depending upon pollen chamber size in an unknown way. For example, for $m = 2$ there are three parameters and for $m = \infty$ there are eight parameters; for intermediate chamber sizes the number of parameters is between three and eight. The effect of a pollen chamber upon the mass-action model is to increase the diagonal elements of the matrix of associative RS parameters. At the limit, a chamber size of two causes these diagonal elements to all equal unity.

Results

The data are presented in Table 2. Based upon these data, Table 3 gives the estimates of male RS parameters under the log-linear model. Under the assumption of equal male RS, crosses of the 1 : 1 : 1 pollen mix are expected to yield

Table 2. Number of seeds sired by male parents, by polymix × female combination

Poly-mix	Male	Female 1	2	3	4	5	6	7	8	Total
1 : 1 : 1	1	29	36	44	38	36	45	37	48	313
1 : 1 : 1	2	85	72	71	77	86	83	77	71	622
1 : 1 : 1	3	46	52	45	45	38	33	46	41	346
2 : 1 : 1	1	47	60	70	62	56	65	74	54	488
2 : 1 : 1	2	82	61	50	65	73	71	53	69	524
2 : 1 : 1	3	32	39	40	33	31	25	34	37	271
1 : 2 : 1	1	31	26	24	23	27	31	15	30	207
1 : 2 : 1	2	129	102	101	82	100	124	81	107	826
1 : 2 : 1	3	40	33	35	56	33	45	24	23	289
1 : 1 : 2	1	22	44	75	20	30	25	30	36	282
1 : 1 : 2	2	79	50	58	63	50	67	74	58	499
1 : 1 : 2	3	59	67	27	77	81	68	96	66	541

Table 3. Estimates for the log-linear model of frequency-dependent RS, with likelihood-ratio tests for parameter values equal to the neutral value of one

Pollen mix	w^a	Estimated value of w	Log-likelihood under $w=1$	Likelihood ratio[b]
1 : 1 : 1	w_1	0.503	−5384.46	123.74*
	w_3	0.556	−5369.61	94.04*
2 : 1 : 1	w_{12}	1.081	−5323.52	1.86
	w_{13}	1.005	−5322.59	0.01
1 : 2 : 1	w_{21}	0.996	−5322.59	0.03
	w_{23}	1.258	−5328.28	11.38*
1 : 1 : 2	w_{31}	1.152	−5324.76	4.34*
	w_{32}	1.026	−5322.69	0.21

[a] w_i = RS at 1 : 1 : 1 ratio (relative to $w_2=1$); w_{ij} = proportional change of RS of male j when frequency of male i is doubled (relative to $w_{ii}=1$; note $w_{ij}=1$ with no frequency dependence)
[b] $-2\ln(L_0/L_1)$, for $L_0=-5322.59$ = log-likelihood under unconstrained model and L_1 = log-likelihood under $w=1$
* $P<0.05$

Table 4. Estimates of intrinsic reproductive success (RS) of males 1 and 3, relative to male 2, given pollen chamber size (Eq. 1); log-likelihoods of estimates

Chamber size	w_1	w_3	Log-likelihood
2	0.206	0.262	−4.1000
3	0.342	0.405	−4.0979
4	0.393	0.453	−4.0973
5	0.420	0.483	−4.0970
6	0.433	0.496	−4.0969
7	0.461	0.513	−4.0969
8	0.459	0.518	−4.0967
Infinite	0.508	0.577	−4.0586

Table 5. Observed and expected reproductive success under the frequency-independent model with no pollen chamber (total $\chi^2=17.17$, 6 *df*; $P<0.05$)

Male ratio		Male 1	Male 2	Male 3
1 : 1 : 1	observed	313	622	346
	expected	312.2	614.6	354.2
2 : 1 : 1	observed	488	524	271
	expected	502.9	494.8	285.3
1 : 2 : 1	observed	207	826	289
	expected	217.8	857.1	247.1
1 : 1 : 2	observed	282	499	541
	expected	252.5	496.8	572.8

offspring of equal proportions. The observed proportions deviated far from these expectations, as the estimates of relative male RS were $w_1=0.503$ and $w_3=0.556$, relative to $w_2=1$ (Table 3). The likelihood ratio test shows that these estimates of w_1 and w_3 both differ significantly from unity (Table 3).

When the polymix contained male proportions of 1 : 1 : 2, no significant frequency dependence was detected (Table 3). In other words, doubling the frequency of male parent 3 did not detectably change the original RS ratio of 0.503 : 1.0 : 0.556. However, when the polymix was altered to either 2 : 1 : 1 or 1 : 2 : 1, frequency dependence was detected: estimates of w_{23} and w_{31} were both significantly greater than unity (likelihood ratio, Table 3).

Table 4 gives estimates of relative male RS under the pollen chamber model of frequency dependence, for chambers of size 2–8 and infinite size. Differences between males were most pronounced when a chamber size of 2 was assumed, and these differences decreased as larger chambers sizes were assumed. Likelihoods increased with increasing chamber size; the highest likelihood was observed for the infinite chamber size. In this case, male 2 was nearly twice as successful as males 1 and 3 (Table 4).

Table 5 gives observed and expected male RS for the most likely pollen chamber size of Table 4 (infinite size). Generally, doubling the frequency of a given male in the polymix resulted in lower relative RS of that male; for example, in the 2 : 1 : 1 polycross, male 1 sired fewer offspring than expected. This is the opposite trend expected from the pollen chamber effect. Other deviations from expectations included: male 2 in the 2 : 1 : 1 cross, males 2 and 3 in the 1 : 2 : 1 cross, and males 1 and 3 in the 1 : 1 : 2 cross.

Table 6 gives the estimates of associative RS, based on the mass-action model of frequency dependence. Estimates of these values ranged from 0.029 (male 1 in association with male 2) to 0.180 (male 2 in association with male 3). The associative RS of males with themselves (diagonal of Table 6) was generally lower and less variable than the associative RS of males with other males (off-diagonal of Table 6). Many associative RS values

Table 6. Estimates of associative reproductive success under the mass-action model of frequency-dependence (*SE* in parenthesis)

RS of	When in association with		
	Male 1	Male 2	Male 3
Male 1	0.088 (0.011)	0.029 (0.013)	0.127 (0.016)
Male 2	0.234 (0.017)	0.067 (0.010)	0.180 (0.017)
Male 3	0.090 (0.016)	0.103 (0.015)	0.081 (0.011)

significantly differed from each other, and male 2 was a superior competitor both in combination with male 1 and with male 3. Note that the standard errors of associative RS values were lowest for males associated with themselves, as this combination was most frequent.

A Chi-square test for heterogeneity among the eight seed parents, applied to the data of Table 2, detected no male-female complementarity at the 1:1:1 ratio ($\chi^2 = 15.7$; 95% critical value for 14 df is 23.7). Male-female complementarity was only significant at the 2:1:1 ratio ($\chi^2 = 25.7$) and the 1:2:1 ratio ($\chi^2 = 23.9$). Complementarity was strongly present at the 1:1:2 ratio ($\chi^2 = 110.6$). The presence of such interactions suggests that the above conclusions are restricted to the particular combinations of males and females used in the experiment.

Discussion

This study detected frequency-dependent male reproductive success (RS) in a polycross, and investigated two possible causes of frequency dependence. The first is the "pollen chamber" in conifers, which tends to buffer differential male RS by increasing the representation of weak males when more common. On the basis of morphology, a chamber size of 3–4 in Douglas fir has been observed (Allens and Owens 1972). This study found no evidence for this buffering effect, as we generally found that less successful males did not show higher RS when represented at higher frequency in the polymix.

A second type of frequency-dependent male RS occurs when the success of a particular male depends on competitive interactions, and hence upon the composition of males in the polymix. This type of frequency dependence has been commonly studied in *Drosophila* (Huang et al. 1971) and annual plants (Allard and Adams 1969). In measuring competitive interactions, we have assumed a "mass action model," wherein the RS of a male is the weighted mean of several "associative" RS values. Each associative RS is specific to a certain male, and is weighted by the frequency of that male in the polymix. In the first study of frequency dependence in a polycross, we have found significant variation of associative RS among pairs of males. We conclude that variation of male-male competitive ability, and not the presence of the pollen chamber, is the source of frequency-dependent RS in our Douglas fir polycross.

Irrespective of frequency dependence, our data also demonstrate reproductive bias towards specific males, and support results of earlier work with conifer polycrosses involving either small and large number of males (Moran and Griffin 1985; Cheliak et al. 1987; Schoen and Cheliak 1987; Apsit et al. 1989). However, our detection of male-female complementarity limits our conclusions to the particular sample of trees used in our study.

The basis and timing of male RS

A multitude of factors can affect RS. In our experiment, these include differential male fertility among pollen grains, the number of pollen grains, pollen viability, and male-female complementarity. First-pollination primacy in conifers (Franklin 1974) cannot affect our results, since pollen mixes of different males were applied simultaneously. In addition, non-genetic factors, such as maternal effects (i.e. tree vigor) or location of pollinated seed cones on branches, can be ruled out, as our crosses were allocated randomly to pollination bags on a given tree. The importance of careful experimental designs in selective fertilization trials has been emphasized by Squillace and Bingham (1958).

Differences of male RS can arise during pollen tube growth and fertilization (the prezygotic stage), or during maturation of the embryo (the postzygotic stage). If prezygotic selection occurred, our measurements of pollen respiration should correlate with male RS. No correlation was evident; in fact, the opposite trend was evident, as pollen with the lowest rate showed highest male RS. Alternatively, prezygotic selection could arise from differential pollen number or size. Because we measured pollen inputs by volume, any advantage of higher pollen number would be largely offset by disadvantages of smaller size. Finally, a third line of evidence against prezygotic selection is the aforementioned lack of a pollen chamber effect.

All these lines of evidence indicate that selective embryo abortion rather than pollen competition was the major mechanism of differential male RS. Zavada and Taylor (1986) and Mulcahy and Mulcahy (1987) have also concluded that postzygotic selection, rather than competition and selection among pollen grains, is most important. Recently, in a study with Douglas fir, Nakamura and Wheeler (1992) found that male bias in polycrosses diluted with dead pollen was the same as that in undiluted crosses. Since it has been postulated that dilution with dead pollen reduces the effective chamber size and hence reduces pollen competition (Fowler 1987), Nakamura and Wheeler conclude that differential male RS in Douglas fir is due to selective embryo abortion, rather than pollen competition.

Douglas fir is polyembryonic, with four to six eggs per ovule (Allen and Owens 1972). Most of these eggs are fertilized, but only one embryo per ovule survives to maturity. Furthermore, its polyembryony is "simple": eggs all arise from the same gametophyte and possess identical maternal haploid genomes (Willson and Burley 1983). Because of this uniform maternal background, male pollen effectively compete directly, and on even footing, at the postzygotic stage. In this light, the detection of variation of associative RS in this study is not surprising.

The potential for frequency-dependent RS via the pollen chamber

For chamber size m and a polycross containing n equally frequent males, the probability that a chamber is filled with pollen from a single male is $\left(\frac{1}{n}\right)^m$. This event prevents male-male competition and hence buffers reproductive bias. Therefore, since $\left(\frac{1}{n}\right)^m$ decreases with m, reproductive bias should increase with larger pollen chamber size (Fowler 1987).

One also expects relative reproductive bias to increase with the number of males in a polymix, as $\left(\frac{1}{n}\right)^m$ also decreases with n. In other words, the chance that a poor male fills a pollen chamber with its own pollen, ensuring reproduction, is reduced as the number of males in a polymix is increased. We emphasize this is *relative* RS: the ratio of the input (polymix) to output (seed) frequency of a given male in a polycross.

By contrast, one expects, on average, the diversity of males in a polycross to increase with the number of males in a polymix. To show this, let diversity be measured by $1-\sum p_i^2$ (analogous to expected heterozygosity), for p_i, the seed frequency of male i. The expected diversity in the seed population produced by an equal-male polymix is $1-n\,\mathrm{E}\,[p_i^2]=1-\left(\frac{1}{n}\right)-n\,\mathrm{Var}\,[p_i]$, where E [ ] denotes expectation, and Var [ ] denotes variance. For pollen chamber size m, since $z=\left(\frac{1}{n}\right)^{m-1}$ is the probability that a pollen type occurs in a chamber with only others of its own, then $\mathrm{Var}\,[p_i]=\mathrm{Var}\,[(z+(1-z)\,w_i)/\bar{w}]=(1-z)^2\,\mathrm{Var}\,[w_i/\bar{w}]$, where w_i is the RS of male i. Putting together these relations, the expected diversity is $1-\left(\frac{1}{n}\right)-n\left(1-\left(\frac{1}{n}\right)^{m-1}\right)^2 \cdot \mathrm{Var}\,[w_i/\bar{w}]$ which, for constant variation of male RS, increases with the number of males n.

However, these calculations assume a pollen chamber effect, which we did not find in our experimental study. More studies of Douglas fir, as well as other conifers, are needed to document the potential for pollen chambers to buffer male reproductive bias. To detect a pollen chamber effect, it is sufficient to use two males of unequal RS in three polycrosses, with input ratios of 1:1, 2:1, and 1:2 (or any other pair of skewed mixtures). Replication over different male pairs would be highly desirable.

Frequency-dependent RS via differential competitive abilities

The three males of this study showed wide variation in their ability to compete against each other, as indicated by the estimates of associative RS values (Table 6). These estimates are in partial agreement with the frequency-independent estimates (Tables 3 and 4), as male 2 was superior in competition with either males 1 or 3. The agreement breaks down when comparing males 1 and 3; although male 3 showed greater mean RS than male 1 (Table 3), it was inferior in competition with male 1 (Table 6). Thus, underlying average RS is a complex web of competitive interactions.

The total reproduction of a pair of interacting males i and j is the average of w_{ij} and w_{ji}, or if the males are the same, simply w_{ii}. If we compare total reproduction of interacting pairs, we find that, interestingly, the RS of males in association with themselves is significantly lower than that of males in association with other males (0.077 versus 0.127). The RS of males in association with themselves also showed a much lower coefficient of variation (0.019 versus 0.267). These results suggest that increasing the number of males in a polycross, and hence the level of male-male competition, will tend to increase the variability of average RS among males.

Compensation of pollen mix ratios for known male biases

In theory, if one has measured male reproductive bias of a specific set of males in a polycross, then using this information, the polymix ratios can be altered to produce approximately equal representation of males in the seed. Without frequency dependence, these altered ratios would be in proportion $1/w_1, 1/w_2 \ldots 1/w_n$, where the w_i are the estimates of relative male RS. From Table 4, using the estimates that assume no chamber, the altered male ratios in our study would be 0.42:0.21:0.37. With frequency dependence, the altered ratios (r) that give equal male representation satisfy $\sum_k r_i\, r_k\, w_{ik}=\sum_k r_j\, r_k\, w_{jk}$ for all pairs i and j. The solution cannot be obtained explicitly but can be found numerically. From the estimates of Table 6, the altered male ratios would be 0.39:0.20:0.41. These ratios do not substantially differ from the altered ratios predicted on the basis of no frequency dependence.

Implications for tree breeding

The presence of differential male fertility has several applied implications. Differential fertility increases the proportion of sibs sharing the same father. Consequently, estimates of quantitative genetic variances (heritability) will be biased upwards, and estimates of general combining ability will suffer greater variability. Male-female complementarity introduces the added complication that any estimated bias may not predict bias in polycrosses with other female parents. Generally, we conclude that the possibility of frequency dependence warrants consideration in proposals to increase male number.

Supplemental mass pollination (SMP), or the broadcast of viable pollen to nonisolated receptive strobili, is a common crop-management practice in conifer seed orchards. With differential male fertility, seed crops produced by SMP will suffer reduced genetic diversity. In addition, estimates of a crop's genetic value will be inaccurate due to the unknown relationship between male fertility and breeding value. To this, we add that frequency-dependent male RS will augment variability of male ratios in the broadcast pollen and further exacerbate differences of male RS.

In contrast with the idea that many males in a polymix are most desirable (Namkoong 1966; Moran and Griffin 1985; Cheliak et al. 1987; Fowler 1987), polycrosses with few males do have some merit. In view of the unpredictable male RS in polycrosses, few or single males may be advised for estimating general combining ability when ranking parents for selection. However, due to the increased co-ancestry among offspring, using fewer males prevents concurrent testing and selecting. A combination of polycross and half-diallel mating (i.e., a complementary mating design) will provide an ideal system for general combining ability evaluation and the production of advanced generation selections (van Buijtenen 1984).

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