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Differential male reproductive success in Douglas fir

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Summary. Differential male reproductive success was studied in clones at two seed orchards of Douglas fir, *Pseudotsuga menziesii* (Mirb.) Franco. The performance of tester pollen parents was compared in controlled pollinations with two-parent pollen mixes. Marker pollen homozygous for a rare IDH allele was the genetic marker in each pollen mix. The resulting seeds were analyzed electrophoretically. At both seed orchards, the proportion of seeds sired by tester pollen significantly varied among the tester pollen parents. Tester pollen parents did not perform the same across all seed parents. The significant interaction effect was evidence of male-female complementarity. These results suggest a genetic basis to differential male reproductive success in Douglas fir.

Key words: Male reproductive success – Mate choice – Male-female complementarity – Douglas fir – *Pseudotsuga menziesii*

Introduction

Male reproductive success (RS) can vary among pollen parents through a variety of mechanisms (Willson and Burley 1983; Bertin 1988). Although in conifers, incompatibility barriers to fertilization do not appear to play a role in differential male RS (Orr-Ewing 1957; Hagman 1975), clones may differ in the production, size, and viability of their pollen (Adams 1982; Schoen and Stewart 1986), and opportunities for pollen competition among viable grains exist within the developing seed cone when numerous grains germinate in the nucellar chamber (Allen and Owens 1972). In addition to pre-zygotic mechanisms, the presence of a diverse array of genotypes within an ovule due to simple polyembryony (Chamberlain 1957) permits the abortion of embryos with homozygous lethals or with other deleterious allelic combinations (Lindgren 1975; Sorensen 1982). Thus, conifers appear to possess the requisite circumstances for differential male RS to operate during critical stages of the life cycle.

Evidence for differential male RS in conifers comes from several studies conducted in seed orchards; all studies employed allozyme markers to distinguish between pollen parents. In Scots pine, Muller-Starck and Ziehe (1984) found that some clones made primarily male contributions to the seed crop and others primarily female contributions. Variation in phenological characters or pollen production cannot be excluded as factors in these studies, as no controlled crosses were attempted. Indeed, for white spruce, differential male RS was positively related to pollen cone number (Schoen and Stewart 1986). Other studies employed controlled pollinations in a polycross design in which a mix of pollen from several clones was applied to seed cones. In *Pinus radiata* (Moran and Griffin 1985) and *Picea abies* (Cheliak et al. 1987; Schoen and Cheliak 1987), certain pollen parents were more successful than others. However, the nature of these controlled cross experiments, with multiple pollen parents without unique gametic genotypes, may make statistical confirmation of differential male RS difficult (O'Malley and Wheeler, unpublished results).

A thorough understanding of differential male RS in forest trees has practical significance because of its effects on tree breeding and testing programs. Progeny from polycross designs for progeny testing may not represent all pollen parents equally, thereby biasing estimates of genetic parameters and breeding values (Moran and Griffin 1985). In addition, the successful deployment of supplemental mass pollination (SMP) techniques in seed orchards to enhance seed production and genetic gain is

very dependent upon the genetic contribution of pollen parents. Also, if positive correlations exist between male RS and offspring performance, breeding decisions about pollen parents could be made at a much earlier time (Ottaviano et al. 1980; Pfahler 1982).

Douglas fir, *Pseudotsuga menziesii* (Mirb.) Franco, is an excellent model for the study of differential male RS in forest trees. A large body of data exists concerning its reproductive biology (Allen and Owens 1972), mating system (Shaw and Allard 1982), embryonic genetic load (Sorensen 1982), inheritance and linkage of allozymes (El-Kassaby et al. 1982; Wheeler, unpublished results), and suitability for SMP (Daniels 1978; Wheeler and Jech 1985). Preliminary data (O'Malley and Wheeler, unpublished results) indicated that pollen parents could vary in male RS. In this paper we document the presence and extent of differential male RS in an array of Douglas fir individuals.

Materials and methods

Individual trees in Douglas fir seed orchards of Weyerhaeuser at Rochester, Washington and Turner, Oregon were designated as pollen parents or seed parents. Each tree came from a different clone. In 1986 we applied, in controlled pollinations, a series of two-parent pollen mixes to previously bagged, receptive female cones on the seed parent trees. Each pollen mix contained a 1:1 ratio by weight of fresh pollen from a tester pollen parent and a marker pollen parent. The factorial crossing design at the Turner seed orchard involved five seed parents, three tester pollen parents, and one marker pollen parent. At the Rochester seed orchard the factorial crossing design involved five seed parents, four tester pollen parents, and two marker pollen parents. The pairwise combination of tester and marker pollens yielded a total of three pollen mixes for the Turner pollinations and eight pollen mixes for the Rochester pollinations. To minimize confounding pollen parent effects with environmental factors, each mix was replicated three times. Therefore, at Turner each seed parent received a total of 9 randomly assigned, two-parent pollinations, and at Rochester a total of 24 pollinations (1 pollination per bag, 3 replicate pollinations per mix).

The controlled pollinations were performed using standard tree-breeding protocols. Seed cone buds were isolated 1–3 weeks prior to receptivity, using paper breeding bags and removing pollen buds prior to bagging. At the time of pollen shed, pollen buds were picked from desired pollen parents and processed in a warm-air extraction facility for 24 h. We tested the moisture content of all pollen lots to insure no bias was introduced during pollen mixing due to inherent weight. After 24 h of processing, the moisture content of all pollen lots tested between 6% and 10%. Pollen lots were subsequently placed in a desiccator, over Drierite, for 24 h to further equilibrate moisture content. Pollen mixes were performed independently for each replication. Seed bud phenology was monitored daily during the period of peak receptivity, and strobili were pollinated by brush at the appropriate time. On occasion, receptivity varied among strobili within a bag; these bags received pollen twice.

The marker pollen parents in the pollen mixes were homozygous for a rare isocitrate dehydrogenase (IDH) allele while the tester pollen parents and the seed parents were all homozygous for a common IDH allele. The method of two-parent pollen mixes allowed for the unambiguous determination of seed paternity without limiting tester pollen parents to those with unique combinations of electromorphs. Seeds sired by the tester pollen were homozygous and those by the marker pollen heterozygous. We determined seed genotype through cellulose acetate electrophoresis (Yeh and O'Malley 1980) of material from germinated seeds. The mean sample size was 140 seeds per pollen mix per seed parent for the Turner crosses and 151 seeds for the Rochester crosses. Differences in filled seed production relative to the marker would document the presence of differential male RS among the tester pollen parents.

The CATMOD procedure of SAS 5.16 (SAS Institute 1985) was performed with the weighted-least-squares method log-linear analyses of the proportions of seed sired by the tester pollen parents. The main effects were tester pollen parent, marker pollen parent, and seed parent. For the analyses, the data from the three replicates per pollen mix were pooled. When interactions with seed parent were statistically significant, pollen parent effects were tested within seed parent.

Results

Comparisons across seed parents

At the Turner seed orchard, both tester pollen parent and seed parent had highly significant effects on the proportion of seeds sired (Table 1). Among seed parents the proportion of seeds sired by tester pollen ranged from 0.42 to 0.61. The tester pollen parent × seed parent interaction was also significant.

For clones at the Rochester seed orchard, tester pollen parent, marker pollen parent, and seed parent all had significant effects on the proportion of seeds sired by tester pollen (Table 1). This proportion ranged from 0.48 to 0.56 among seed parents. Seed parent had significant two-way interactions with marker pollen parent and with tester pollen parent, again showing that the identity of the seed parent influenced pollen parent performance. But the three-way interaction of the main effects was not significant, nor in the overall test was the tester pollen parent × marker pollen parent interaction.

Table 1. Log-linear analyses of the proportion of seeds sired by tester pollen parents at the Turner and Rochester seed orchards

Source	DF	X^2	*P*
Turner seed orchard			
Tester pollen parent	2	17.78	0.0001
Seed parent	4	39.95	0.0001
Tester pollen parent × seed parent	8	20.93	0.0073
Rochester seed orchard			
Tester pollen parent	3	123.93	0.0001
Marker pollen parent	1	6.91	0.0086
Marker × tester	3	4.42	0.2195
Seed parent	4	26.80	0.0001
Marker × seed parent	4	10.67	0.0305
Tester × seed parent	12	30.65	0.0022
Marker × tester × seed parent	12	17.79	0.1212

Table 2. Proportions of seeds sired by tester pollen parents at the Turner and Rochester seed orchards. At the bottoms of columns are results of log-linear tests within seed parent for the effects of marker and tester pollen

Pollen mix		Seed parent				
Turner seed orchard						
Marker pollen	Tester pollen	20	21	22	23	24
1	10	0.57	0.50	0.55	0.54	0.30
1	11	0.63	0.76	0.44	0.67	0.56
1	12	0.47	0.55	0.36	0.44	0.39
Tester $\underline{X}^2$		6.4*	24.2***	10.4**	13.8***	22.8***
Rochester seed orchard						
Marker pollen	Tester pollen	25	26	27	28	29
1	13	0.44	0.51	0.46	0.41	0.51
1	14	0.47	0.53	0.53	0.51	0.54
1	15	0.39	0.70	0.65	0.63	0.70
1	16	0.60	0.56	0.71	0.62	0.64
2	13	0.44	0.39	0.42	0.47	0.40
2	14	0.41	0.45	0.48	0.50	0.51
2	15	0.60	0.65	0.66	0.60	0.65
2	16	0.47	0.48	0.62	0.70	0.54
Tester $\underline{X}^2$		8.0*	36.2***	45.2***	29.1***	32.5***
Marker $\underline{X}^2$		0.1	9.4**	2.4	0.5	6.9**

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

Comparisons within seed parents

Log-linear tests within seed parents showed that for all the seed parents at the Turner seed orchard there were significant differences among tester pollen parents in the proportion of seed sired (Table 2). In one case, the proportion of seed sired ranged from 0.50 to 0.76. Thus differential male RS did exist among the tester pollen parents. However, as expected from the significant interaction effect, the relative success of tester pollen parents was not the same over all seed parents (Table 2). For example, tester pollen parent 10 when crossed with seed parent 22 sired the highest proportion of seeds among the 3 tester clones, while on seed parent 24 it sired the lowest proportion.

Within seed parents at Rochester, two-way log-linear tests of tester and marker pollen effects always showed a significant effect of tester pollen parent, again demonstrating differential male RS (Table 2). Within seed parent 26 the proportion of seeds sired by tester clones ranged from 0.70 down to 0.39. Marker pollen parent had an effect in two of the seed parents. The tester pollen parent × marker pollen parent interaction was significant in only one of the five seed parents (clone 25: $X^2 = 18.8$, $P=0.0003$). An extreme case of the tester pollen parent × seed parent interaction is the actual reversal of rank seen in the seed production of tester pollen 15 when mixed with marker pollen 1 (Table 2). On seed parents 26 and 29 it had the greatest male RS of the tester pollen parents, but on seed parent 25 its success was the least.

Discussion

Differential male RS was consistently found among Douglas fir clones at two production seed orchards. Variation in male RS under outcrossed, controlled pollinations apparently exists in other conifers (Moran and Griffin 1985; Schoen and Cheliak 1987) and in flowering plants (see review by Lee 1988). In our study, the RS of a pollen parent was not the same across all seed parents. The significant tester pollen parent × seed parent and marker pollen parent × seed parent interactions are evidence of male-female complementarity. Similar interactions were found in another study of Douglas fir (Daintith and Dancik, unpublished results) but not for Norway spruce (Schoen and Cheliak 1987).

Many factors or mechanisms could have contributed to the observed differences in male RS. These can be broadly classified as non-genetic, genetic-prezygotic, and genetic-postzygotic. Although a single ramet represented each clone, considerable effort was made to reduce environmental/experimental sources of error. Pollens were all collected and processed in a standard manner, with emphasis on controlling extraction time (period of exposure to dry air) and moisture content. Pollen mixes were replicated. Environmentally induced clonal variation in pollen size could not explain the large magnitude of differential male RS found in this study, according to data for the same clones in 1988 (Nakamura and Wheeler, unpublished results). If non-genetic factors had affected moisture content or pollen size and were responsible for differences among tester pollen parents, a particular pollen mix should have behaved uniformly across all seed parents. Consequently, we interpret our results to mean predominantly genetically influenced male RS.

This study was not designed to determine at what stage in the life cycle and through what particular mechanisms differential male RS and male-female complementarity occurred. Pre-zygotic pollen selection among tester pollen clones could have occurred as a result of differences in the rates of germination and pollen tube growth or through biochemical recognition of pollen genotypes by nucellar or gametophytic tissues. Both mechanisms are well-recognized in flowering plants (de Nettancourt 1977; Ottaviano et al. 1980; Pfahler 1982). There is virtually no published evidence that either of these mechanisms are active in gymnosperms. However, pollen viability differences exist among Douglas fir clones (Wheeler, unpublished results; Webber, unpublished results). Colangeli (1988) notes that developmental and cytological studies with western hemlock (*Tsuga heterophylla*) indicate that some form of incompatibility may operate during pollen tube growth.

The presence of polyembryony in Douglas fir (and most other conifers) provides a likely mechanism for post-zygotic selection (Sorensen 1982). As many as four or five embryos per ovule may be fertilized, with only one surviving (Allen and Owens 1972). By what process mortality occurs is unknown. Abortion because of embryo competition or maternal choice could have favored embryos sired by some tester pollen parents over others. Potential causes of male-female complementarity include inbreeding or outbreeding depression (Charlesworth et al. 1987).

Efforts are presently underway to repeat the crosses from 1986 and to study pre- and post-zygotic mechanisms of male success. Diluting pollen mixes with dead pollen will show whether reducing pollen competition affects differential male RS. Data from electrophoretic surveys and controlled self-pollinations will relate the genetic similarity and genetic load between pollen and seed parents to male-female complementarity. In addition, tests in the nursery will allow us to contrast success in male RS with seedling growth.

The apparent presence of genetically determined differential male RS and of male-female complementarity in Douglas fir is of value to tree improvement programs, particularly those which rely on selected pollen and seed-cone clones for full-sib seed production via SMP. Specific pollen parents could yield significantly greater successful pollinations when coupled with the proper seed parent. In addition, estimates of combining ability assume in a polycross design unbiased representation of the pollen parents in the offspring. Our results question that assumption. Male-female complementarity may be of lesser consequence to male RS in natural populations. Other factors, such as parental fecundity, phenology of pollen shed and seed strobilus receptivity, and distance to mates, may overshadow male-female interactions within seed cones.

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