The realized effect of postpollination sexual selection in a natural plant population

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The ultimate importance of postpollination sexual selection has remained elusive, largely because of the difficulty of assigning paternity in the field. Here I use a powerful new molecular marker (AFLP) for paternity analysis in a natural population of the outcrossing angiosperm *Persoonia mollis* (Proteaceae) to assess male reproductive success following equal pollination of 15 pollen donors on each of 6310 pistils. These results were contrasted with male reproductive success of these same plants following natural mating. Following equal pollination, there was a significant departure from equal siring success, indicating a potential for postpollination sexual selection. The most successful pollen donor sired more than twice the expected number of seeds, and this was largely consistent across recipient plants. However, siring success following natural mating was significantly different from siring success following artificial pollination and showed that the reproductive gains to be made from superior pollen performance did not translate into increased reproductive success following natural mating. As the ecological context for postpollination sexual selection is strong in *P. mollis*, I suggest that pollen competition may ultimately have only a weak effect on non-random male mating success under natural conditions because the realized opportunities for pollen competition within pistils are limited.

Keywords: pollen competition; mate choice; paternity; DNA fingerprinting; pollination; AFLP

1. INTRODUCTION

Sexual selection is a major evolutionary force in animals (Darwin 1871; Andersson 1994). The use of the term sexual selection for plants, however, remains controversial because of overly strict definitions (Grant 1995), the difficulty in distinguishing between mate choice and sexual competition (Lyons *et al*. 1989), alternation of generations (Delph & Havens 1998) and, consequently, the complex interactions occurring between maternal, paternal and offspring tissues within pistils (Lyons *et al*. 1989; Willson 1994; Snow 1994; Arnold 1994). However, sexual selection, when defined simply and consistently with Darwin's (1871) use of the term as selection arising from differences in mating success, is equally applicable to plants and animals (Arnold 1994; Andersson 1994). As stigmas can often receive surplus pollen from several individuals (Willson 1994; Snow 1994), the pistil provides an arena for 'scrambles' between pollen genotypes (Andersson 1994), leading to differences in male mating success. These pollen scrambles are somewhat analogous to sperm competition in animals, although there are also significant differences (Delph & Havens 1998).

The potential for postpollination selection has been demonstrated in some plants (Snow & Spira 1991; Marshall 1998; Mitchell & Marshall 1998; Pasonen *et al*. 1999) but not in others (Waser *et al*. 1995). For example, in the most powerful study to date, Marshall (1998) found consistent non-random mating for four pollen donors across 16 maternal plants. However, all studies to date have been limited in the number of competing pollen donors on any one stigma and across recipients, with possible confounding effects due to the genetic inter-

actions between mates. For sexual selection to occur, differences in pollen-donor performance must be consistent across recipient plants (Marshall 1998). Inconsistent pollen-donor performance across recipient plants suggests other mate-dependent mechanisms are influencing siring success, such as incompatibility systems and/or genetic relatedness between mates (Lyons *et al*. 1989). Consequently, lower mating success may be due to discrimination against closely related mates (inbreeding depression) or discrimination against distantly related mates (outbreeding depression) (Waser 1993; Waser & Price 1994). Conversely, the most successful pollen donor may have an overall optimal mean genetic distance from all other plants (Bateson 1983; Waser 1993; Waser & Price 1994).

Although the potential for postpollination sexual selection has been demonstrated in some plants, its realized effect following natural mating has remained elusive. This is largely because of the difficulty of assigning paternity in the field (Lewis & Snow 1992; Snow & Lewis 1993; Milligan & McMurray 1993; Willson 1994). Powerful new molecular markers are now available for the routine unambiguous assignment of paternity in natural plant populations (Dow & Ashley 1998; Krauss 1999), enabling these issues to be addressed for the first time. Here I use the polymerase chain reaction (PCR)-based DNA-fingerprinting technique amplified fragment length polymorphism (AFLP; Vos *et al*. 1995) to assign paternity (Krauss 1999), and show that the reproductive gains to be made from superior pollen performance in a natural population of the perennial self-incompatible shrub *Persoonia mollis* (Proteaceae) did not translate into increased reproductive success following natural mating. This is despite the suggestion that postpollination competition among pollen genotypes may be particularly strong in *P. mollis* because the pistils contain relatively long styles and two ovules, only one of which usually develops into a seed.

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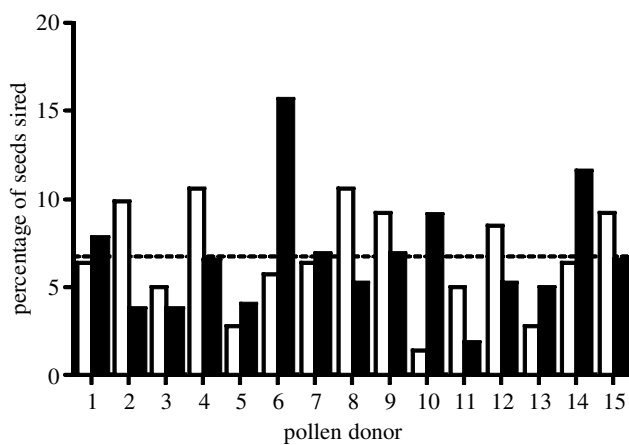
Figure 1. The percentage of seeds sired by each of 15 plants of *Persoonia mollis* (Proteaceae) in a natural population. Solid bars indicate siring success following artificial pollination with a pollen mix comprising an equal pollen contribution from all 15 donors competing directly on each of 6310 pistils on the same 15 plants (total number of seeds = 320). Open bars indicate siring success following natural mating (total number of seeds = 180). The dashed line indicates the expected percentage of seeds sired given equal siring success. Solid bars depart significantly from the expected value ($\chi^2 = 24.6$, d.f. = 14, $p < 0.05$) and from the natural mating values ($\chi^2 = 43.5$, d.f. = 14, $p < 0.0001$). Successful sires were identified by Amplified Fragment Length Polymorphism (AFLP) DNA-fingerprinting of seeds. A total of 125 polymorphic loci were generated for paternity exclusion analysis.

Consequently, of the many pollen grains that are typically deposited on stigmas by bees (Krauss 1994; Bernhardt & Weston 1996), there is usually only one winner.

2. METHODS

Fifteen even-aged plants (10–14 years old, each producing 10 000–40 000 flowers per year) in an isolated natural population near Sydney, Australia, contributed equally to a pollen mix (see below and §3) through the collection of an equal number of fresh pre-dehisced anthers from each plant. This pollen was mixed thoroughly in a 2 ml eppendorf and applied within 3 h with a metal pointer to the receptive stigmas of a similar number of flowers on each of these same 15 plants once a week over the course of the flowering season (January–April). While 15 pollen donors competing directly with each other on each pistil may be an unnatural scenario, my objective was to provide the most intensive test of competitive pollen performance yet achieved in a natural population. Pollinated flowers were bagged with insect mesh to exclude natural pollinators during the course of the experiment. In total, 6310 flowers were pollinated.

Mature fruits were collected in December, and 320 seeds (typically one per fruit) were assigned paternity using the PCR-based multi-locus DNA-fingerprinting technique AFLP. DNA-extraction techniques, AFLP protocols and paternity-assignment procedures were as set out previously (Krauss 1999, 2000). Similarly, paternity was also assigned to 180 naturally pollinated seeds (12 from each of the same 15 plants) to assess male reproductive success following natural mating.

To check the assumption of equal pollen contribution by each pollen donor, I counted pollen grains for all anthers from a random sample of flowers. Each of 12 anthers from three flowers

Table 1. *Siring success rank for each pollen donor against each maternal plant following artificial equal pollination*

(Numbers indicate the rank siring success of each pollen donor on each recipient, with 1 indicating the most successful pollen donor. Mean rank varied significantly (ANOVA, $F = 3.07$, $p < 0.0003$). Post-hoc Student–Newman–Keuls tests show that plant 6 < 2, 3, 5, 11, 12 and that plant 14 < 11. All other means were not significant. The column in bold highlights the most successful sire. Recipient plants 1 and 2 are not included here as they produced very few seeds.)

recipient plant	pollen donor														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
3	4	13	—	8	11	**1**	8	7	3	2	11	8	13	4	4
4	1	8	8	—	8	**3**	8	3	3	3	8	1	8	8	3
5	10	10	4	1	—	**4**	10	3	4	8	8	10	4	1	14
6	2	10	6	2	10	—	2	2	10	6	10	10	6	1	6
7	7	3	5	5	11	**2**	—	7	11	1	11	11	10	3	7
8	10	10	10	3	6	**1**	1	—	6	3	10	10	6	3	6
9	11	7	7	2	7	**4**	4	7	—	11	11	2	4	1	11
10	13	11	11	3	3	**1**	3	7	7	—	13	3	7	1	7
11	5	5	11	11	2	**1**	3	5	5	3	—	11	5	11	5
12	1	9	11	11	3	**2**	11	11	5	5	5	—	3	9	5
13	3	7	10	14	10	**1**	3	10	6	7	10	7	—	3	2
14	5	5	5	5	5	**1**	2	5	2	2	5	5	5	—	5
15	6	6	2	6	6	**6**	6	6	4	6	6	1	2	4	—
mean	6.0	8.0	7.5	5.9	6.8	**2.2**	5.1	6.1	5.5	4.8	9.0	6.6	6.1	4.1	6.3
overall rank	7	14	13	6	12	1	4	8	5	3	15	11	9	2	10

from each pollen donor was dried in an eppendorf tube with silica gel. The pollen and anther were then vortexed with 1 ml of staining solution (70% ethanol, methylene blue and detergent). Four samples, each of 5 µl, were placed on a microscope slide and all viable pollen grains counted.

To determine whether siring success was related to the genetic distance between mates, I calculated the genetic dissimilarity as a simple count of the total number of presence–absence differences (Manhatten distance) between all pairs of plants from AFLP fingerprints (Krauss 2000). I also calculated the mean genetic dissimilarity for each plant to all other plants, as well as the variance around this mean, to determine whether there was a relationship between the genetic properties of pollen donors relative to the rest of the population and their reproductive success.

3. RESULTS

Following equal pollination, where all pollen donors had equal access to all stigmas, there was a significant departure from equal siring success (figure 1; $\chi^2 = 24.6$, d.f. = 14, $p < 0.05$). The most successful pollen donor sired more than twice the expected number of seeds and more than eight times as many seeds as the least successful pollen donor. Following natural mating, however, individual siring success departed significantly from siring success following artificial equal pollination (figure 1; $\chi^2 = 43.5$, d.f. = 14, $p < 0.0001$).

Siring success of the most successful pollen donor was weakly consistent across recipient plants following equal pollination (table 1). Mean rank differed significantly
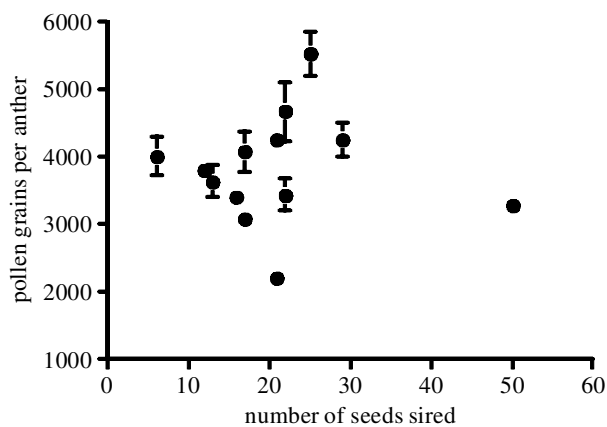
Figure 2. Mean number of pollen grains per anther (± s.e.m.) plotted against the number of seeds sired by each pollen donor in the artificial pollination experiment.

Figure 3. Genetic dissimilarity (Manhatten distance) between all mate pairs plotted against the percentage of seeds sired per recipient plant. Analysis of progeny with known levels of relatedness indicated that genetic dissimilarities of about 32 and 23 equate to half-sib and full-sib levels of relatedness, respectively, while the population mean was 44 (Krauss 2000).

(Student–Newman–Keuls tests, $p < 0.05$) between the most successful sire and the five least successful sires. All other donors showed inconsistent rank siring success across recipient plants.

Siring success was not correlated with the number of pollen grains produced per anther, although there was a weak positive relationship when the most successful pollen donor was removed from this comparison (figure 2). Consequently, some differential siring success may be explained by pollen production. However, pollen production does not explain the siring success of the most successful pollen donor.

The most successful mate pairs possessed an intermediate genetic dissimilarity based on 125 polymorphic AFLP loci. In contrast, reduced reproductive success was found for mates at the extremes of genetic dissimilarity, suggesting an optimal intermediate outcrossing distance between mates (figure 3). To investigate this further, I calculated the mean genetic dissimilarity for each plant to all other plants, as well as the variance around the mean genetic dissimilarity. However, there was no correlation between siring success of each pollen donor and mean genetic dissimilarity or its variance, a result that was most clearly demonstrated by the nearly identical genetic properties of the most and least successful sires (not shown). That is, the most and least successful pollen donors were, on average, equally genetically distant from all other plants.

Following natural mating, however, the relative success of the most compared to the least successful pollen donor was negated (figure 1). Apparently, the most vigorous pollen is rarely presented with the opportunity to out-compete other pollen within pistils because stigmas typically receive single-donor pollen loads. To investigate further, I analysed paternity for rare fruits producing two seeds to assess multiple siring as an indirect measure of mixed pollen loads. Following the artificial pollination experiment, 21 fruits produced two seeds, out of which 16 pairs (76%) had different sires and five pairs (24%) had the same sire. In contrast, following natural pollination, nine fruits produced two seeds, out of which only three pairs (33%) had different sires and six pairs (67%) had the same sire; the difference between results for the two pollination methods was significant (Fisher's exact test,
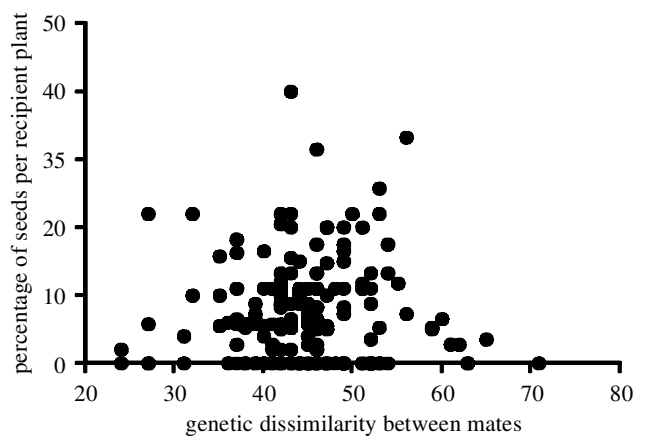
$p < 0.05$). Apparently, there was significantly less mixing of pollen genotypes on naturally pollinated stigmas than on artificially pollinated stigmas.

4. DISCUSSION

An assessment of the realized effect of postpollination selection in a natural plant population has been elusive (Snow 1994). By applying a powerful new molecular marker for the unambiguous assignment of paternity, I have shown that the reproductive advantages to be made from superior pollen were not translated into increased reproductive success following natural mating. Following natural mating, the pollen donor with the most successful pollen sired no more seeds than the pollen donor with the least successful pollen. Indirect results suggested that there was significantly less mixing of pollen genotypes on naturally pollinated stigmas than on artificially pollinated stigmas. Consequently, in contrast to the suggestion that mixed pollen loads and multiple paternity appear to be common in nature (Willson 1994; Marshall & Ellstrand 1985; Waser & Price 1985), the opportunities for post-pollination 'scrambles' among pollen genotypes appear to be limited in *P. mollis*. Consequently, postpollination sexual selection played only a minor role in the reproductive success of the plants in this population.

Prepollination factors, such as the ability of a plant to attract and reward pollinators because of plant size, number of flowers, nectar production, etc. (Andersson 1994; Snow 1994; Stanton 1994), as well as the immediate density of plants around a donor (Levin 1988; Karron *et al.* 1995), affect the realized potential for postpollination processes to influence non-random male mating success. For example, more isolated plants of *P. mollis* have more sires than clumped plants, which are predominantly sired by the nearest pollen donor (Krauss 2000). While it remains unclear how density might affect the mixing of pollen on receptive stigmas, it does suggest that the ultimate importance of postpollination effects may vary within a population. Rapid stigma closing after the first pollination (Fetscher & Kohn 1999) may also restrict the

opportunities for pollen scrambles, as may the timing of pollen deposition. In general it appears that there is a strong first-male advantage in plants, so that subsequent pollen depositions are at a disadvantage, even if of higher quality (Epperson & Clegg 1987). This is contrary to the situation in insects and birds, where second- and last-male sperm precedence, respectively, is common (Simmons & Siva-Jothy 1998; Birkhead & Møller 1992).

In contrast to some previous artificial-pollination experiments involving direct competition between pollen donors, which have shown a highly consistent performance for all donors across recipient plants (Snow & Spira 1991; Marshall 1998; Mitchell & Marshall 1998; Pasonen *et al*. 1999), the success of the best pollen donor was only weakly consistent across recipients in the current study. Inconsistencies between some earlier studies and my study may be explained by differences between taxa in pollen-donor performance due to postpollination competitive effects, or by methodological differences whereby the number and source of pollen donors in previous experiments may have exaggerated differences from more natural mating conditions. While I contrasted 15 pollen donors within a single natural population, earlier studies have compared just a small number (two to four) of directly competing pollen donors, and have sometimes included donors mixed from different populations, which may exaggerate the competitive effects found in natural populations. The results of the current study support the suggestion that natural plant populations harbour only a weak potential for postpollination sexual selection (Waser *et al*. 1995). Conversely, the conclusion of weak concordance of pollen-donor performance may be due to insufficient statistical power due to small average sample sizes (21 seeds per recipient on average). More seeds (at least four times the number of pollen donors per recipient) are required in future experiments to detect consistent pollen-donor performance (if present) across recipients.

Genetic relatedness and pollen production were found to be only weak influences on differential reproductive success in the current study. In one of the most detailed analyses of the genetic relatedness of plants in a natural population, I sampled 147 loci, out of which 125 were polymorphic. There was no relationship between the reproductive success of sires and their overall genetic relatedness to other plants in the population, nor was there any relationship between geographical and genetic structure (Krauss 2000). Similarly, there was no relationship between pollen production and siring by the most successful pollen donor following equal pollination. This contrasts to a similar study on *Lesquerella fendleri* that found that the most successful donor also had the greatest pollen production (Mitchell & Marshall 1998). Therefore, neither mean genetic relatedness to all other plants nor pollen production explain the difference in siring success of pollen donors in the current study. Consequently, the siring success of the most successful pollen donor following artificial pollination was apparently due to postpollination sexual selection.

I have shown that the potential for postpollination sexual selection was present in a natural population of *P. mollis*, and that genetic relatedness and pollen production were only weak influences on non-random

mating due to postpollination events. However, as yet unidentified prepollination factors negated the reproductive gains to be made from superior pollen in this study. Given that the ecological context for postpollination sexual selection is strong in *P. mollis*, this study provides the best evidence to date that postpollination selection may ultimately have only a weak effect on male mating success under natural conditions.

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