

NONRANDOM MATING AND SEXUAL SELECTION IN A DESERT MUSTARD: AN EXPERIMENTAL APPROACH¹

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We report on an experiment to explore the importance of pollinator behavior and postpollination events within flowers in generating nonrandom mating in the desert perennial *Lesquerella fendleri* (Brassicaceae). In this experiment, we crossed four plants with each other in all combinations. We performed these crosses in three different ways, which varied the opportunity for nonrandom mating: single-donor hand pollinations, mixed hand pollinations, and field pollinations. Number of seeds sired by each donor following single-donor pollinations differed only slightly (though significantly) from random, indicating little variation in siring ability. However, we found more dramatic (and significant) departures from random mating for mixed hand pollinations. In the field we found even more nonrandomness, with some donors siring >71% of seeds on some maternal plants, despite equal opportunity to father seeds. The rank ordering of donors was consistent across the four females and across treatments, indicating that there was concordant nonrandom mating, a requirement for sexual selection. The most successful donor in all treatments also had the greatest pollen production per flower, and this trait may therefore be an important cause of the observed patterns. We infer that pollinator behavior and postpollination processes both contribute to the nonrandom mating observed in the field, and discuss the advantages of this new experimental approach.

Key words: Brassicaceae; hand pollination; *Lesquerella fendleri*; mating behavior; pollen production; pollinators; sexual selection.

Plant mating patterns are a source of continuing fascination for evolutionary biologists, since they are of fundamental importance for understanding the evolution of floral traits. An important emerging pattern involves nonrandom mating. Despite the assumptions of many population genetic models, mating in most populations is not random. For plants, this nonrandom mating may be based on such attributes as spatial proximity (Levin and Kerster, 1974; Schaal, 1980; Waser and Price, 1983; Marshall and Ellstrand, 1985; Kohn and Casper, 1992; Karron et al., 1995), genetic relationship or complementarity (Waser et al., 1987; Cruzan, 1990a; Broyles and Wyatt, 1991; Waser and Price, 1991, 1993; Campbell and Dooley, 1992; Montalvo, 1992; Rigney et al., 1993), floral phenotype (Schoen and Clegg, 1985; Galen, 1989; Stanton et al., 1991), pollen production, viability, and fertilization ability (Willson and Burley, 1983; Bertin, 1990; Cruzan, 1990b; Snow and Spira, 1991a, b) or self-incompatibility (de Nettencourt, 1977). If caused by heritable traits, deviations from random mating can have important evolutionary consequences, altering realized mating success and setting the stage for sexual selection. However, the occurrence, importance, and implications of nonrandom mating in plants remain controversial (Stephenson and Bertin, 1983; Willson and Burley, 1983; Charlesworth, Schemske, and Sork, 1988; Lyons et al., 1989; Marshall and Folsom, 1991; Snow, 1994).

A major reason for this controversy is that there is little

information about whether the nonrandom mating demonstrable in controlled pollinations in a greenhouse also occurs in the field, where pollinator behavior as well as plant characteristics can influence mating. It is important to determine whether nonrandom mating is generated by pollinator behavior or postpollination events because the evolutionary and proximate consequences of these two kinds of events differ in at least two ways.

1) Prior to pollination, plants have only indirect control of pollen arrival. Pollen must be moved by vectors whose movements cannot be exactly guided, and a large range of factors extrinsic to the plant can affect vector behavior (e.g., proximity of other plants, weather; we focus on animal-vectored plants). Although specific control of pollen movement is not possible, floral phenotype (e.g., flower size and shape, rewards) still can affect more general attributes of pollinator behavior such as turning angles, movement distances, and number of flowers visited, and can affect the amount of pollen successfully imported and exported (e.g., Galen, 1989; Campbell et al., 1991; Mitchell and Waser, 1992; Mitchell, 1993).

2) In contrast, postpollination events, such as pollen germination, pollen tube growth, ovule fertilization, and seed and fruit provisioning and abortion allow maternal plants much more precise control of paternity than do pollination events. There is growing evidence that postpollination events can result in nonrandom mating, with most work to date focusing on the contrast between self and outcross matings (e.g., Schemske and Pautler, 1984; Johnston, 1993; Rigney et al., 1993). More interesting to us is the recent intriguing work indicating that plants make more subtle distinctions among outcross pollen sources (e.g., Waser et al., 1987; Waser and Price, 1989, 1991, 1993; Bertin, 1990; Campbell and Dooley, 1992; Marshall and Ellstrand, 1988; Marshall, 1991; Montalvo, 1992; Snow and Spira, 1991a, b, 1996; Rigney et al.,

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TABLE 1. Attributes of the array plants. Least-squares means \pm 1SE. Pollen available per day is calculated by multiplying pollen production per flower by mean open flowers per day. $N = 8$ flowers/plant for pollen characters, and for seeds mothered and fathered in hand pollinations.

Plant	Genotypes		No. pollen/flower	Pollen diameter (μm)	Hand crosses No. seeds/fruit		Field		
	<i>Pgd1</i>	<i>Pgd2</i>			Mothered	Fathered	No. open flowers/d	No. pollen-plant ⁻¹ ·d ⁻¹	No seeds/fruit mothered
230	33	11	1307 \pm 35	23.5 \pm 0.07	10.6 \pm 1.2	5.9 \pm 1.4	63.4	828 000	10.7 \pm 0.5
235	22	33	3920 \pm 35	22.2 \pm 0.07	5.4 \pm 1.2	8.9 \pm 1.4	59.0	2 312 800	3.1 \pm 0.8
237	33	33	3065 \pm 37	21.9 \pm 0.07	7.9 \pm 1.2	8.4 \pm 1.4	40.6	1 244 390	6.4 \pm 0.6
240	22	22	1984 \pm 35	23.5 \pm 0.07	9.4 \pm 1.2	7.4 \pm 1.4	21.8	432 730	6.7 \pm 0.7

1993). No general conclusions are yet available on exactly how such distinctions are made, but that they occur at all is not in doubt.

Thus, there are two important differences between pollination and postpollination events: the morphological and temporal level of control, and the precision of control of mate identity. These differences may affect evolutionary outcomes. For example, interpollen and intrapetal mechanisms for sorting among mates (such as pollen tube competition, stylar screening, seed and ovule abortion) might evolve under postpollination selection, while petal size or color would not. Our conceptual distinction between pollination and postpollination events does not mean that we think they act in isolation from one another—far from it, since each may alter the opportunity for the other to act. For example, if reproduction is pollen limited, the opportunity to use selective abortion to choose among mates and zygotes is diminished (Mitchell, 1997). However, the relative importance of pollination and postpollination events in causing nonrandom mating and phenotypic selection is largely unknown (but see Stanton et al., 1991; Campbell and Dooley, 1992), and this gap in our knowledge prevents a full understanding of an important component of plant evolution.

The first approach that comes to mind for filling this gap is to measure pollination and postpollination events for many undisturbed plants in their natural habitat. Unfortunately, this is not possible, for at least three reasons. First, even in the most polymorphic plant populations there is ambiguity about field paternity assignments using current techniques (e.g., allozymes, random amplified polymorphic DNAs; see Devlin, Roeder, and Ellstrand, 1988; Snow and Lewis, 1993). Various DNA fingerprinting methods promise to overcome this problem, but even given sufficient variation for unambiguous paternity, high costs per genotype make DNA techniques impractical for generating the large sample sizes required (Ragot and Hoisington, 1993). Second, even with perfect knowledge of paternity, study of undisturbed populations provides only a description of mating patterns, and testing hypotheses generated by those patterns still requires manipulative experiments (e.g., Galen, 1989; Campbell et al., 1991). Third, studying postpollination events necessarily requires hand pollinations, which are difficult and disruptive to conduct in the field. Although the first problem eventually will be solved by technological advances, the second two are unlikely to be completely overcome in the foreseeable future.

Therefore, in the work reported here we used a more tightly controlled experimental approach. In this experiment we constructed a small array of carefully chosen plants, varied the opportunity for nonrandom mating, and

measured the effects of pollination and postpollination events on mating success. We recognize that this substantial increase in experimental control involves some sacrifice in realism, but it allows us to address the following questions. (1) Is there nonrandom mating in *Lesquerella fendleri*? (2) Is nonrandom mating consistent (concordant) across pollen recipients? (3) What mechanisms contribute to nonrandom mating?

MATERIALS AND METHODS

Experimental organism—We studied wild bladderpod, (*Lesquerella fendleri*; Brassicaceae). This small, short-lived perennial herb (~10–20 cm height, with 1 to >50 flowering branches) is a common native plant of the deserts of southwestern North America. The yellow, 13.7 mm diameter (SD = 2.3, $N = 302$ plants) flowers are self-incompatible (unpublished data), and are pollinated primarily by small bees and bee-flies. There is no temporal separation between pollen presentation and anther dehiscence within flowers. Flowering in the field is mostly from mid-April to late May, sometimes renewing in mid-June or August. Plants produce 1 to >5000 flowers a season (mean \pm SD = 435 \pm 215, $N = 101$), each with 33100 \pm 14700 pollen grains ($N = 108$) and 20.6 \pm 2.8 ovules ($N = 192$). Seed production in the field is not pollen limited (unpublished data).

Lesquerella fendleri exhibits substantial genetic variability at allozyme loci (Cabin, 1996), making it suitable for paternity studies. In other work we have documented nonrandom mating in natural populations of *L. fendleri* (unpublished data), and nonrandom postpollination success in hand pollinations (Mitchell and Marshall, 1995).

Experimental methods—We constructed our array from plants grown from a bulk seed collection (> 50 maternal plants) made from within a 20 \times 100 m area of the Sevilleta LTER (80 km south of Albuquerque, New Mexico, USA). From these seedlings, we chose four plants that had mutually distinguishable homozygous genotypes at the *Pgd1* and *Pgd2* loci (Table 1). Thus, we could unambiguously assign paternity of offspring within the array. In spring 1993, after identifying appropriate plants for this array, we transported them to the field, more than 100 m from the nearest natural *L. fendleri* population. Because flowering in the field was waning at this time, we anticipated that gene flow from plants outside the array would be minimal (see Results). Furthermore, we placed the array at least 20 m from any of the few scattered individuals outside the main population. We arranged the potted plants in a square array with 0.50-m spacing between pot centers. This arrangement minimizes the potential for differences among plants in interplant spacing to affect results for two reasons. First, center-to-center distances are similar for all pairs of plants (0.50 m for rooks' moves vs. 0.71 m for bishops' moves). Second, flowering branches extended beyond the pots by 10–20 cm, so that all plants had some flowers within 15 cm of all other plants in the array. Plants in the field frequently experience the densities used in this experiment.

We left these plants in the field from 30 April to 5 May 1993, watering daily, and allowing native pollinators to visit. We frequently saw

TABLE 2. ANOVA of pollen production per flower and pollen diameter. Type III SS. Plant effect tested over flower(plant).

Source	Pollen production				Modal pollen diameter		
	df	MS	F	P	MS	F	P
Plant	3	23912232	10	0.0001	12.6	22.6	0.0001
Flower (plant)	20	2396287	106	0.0001	0.6	5.9	0.0001
Error	47	22568			0.1		

floral visitors on these plants, primarily small bees, and halictid and andrenid bees, just as is the case for wild populations.

Following field exposure we moved the plants to a greenhouse at the University of New Mexico, and performed eight replicate single- and mixed-donor hand pollinations on each plant. For single-donor pollinations, we crossed each plant with all others in the array. On each day of pollination (11–16 May), we collected anthers from three flowers from each plant, and placed them in four separate petri dishes. We then stirred the anthers with tissue-wrapped forceps, and applied the pollen to receptive stigmas. Before pollination we emasculated recipient flowers to allow access to the stigma. We also performed two self-pollinations on each plant using the same bulk pollen samples and methods, to confirm that these particular plants were self-incompatible—they were (unpublished data).

For mixed hand pollinations, we used similar methods to apply a mixture of pollen from all four plants. To do so, we placed six freshly dehiscid anthers from each of the four plants in a single petri dish, mixed the sample thoroughly, and applied pollen as described above. We performed these crosses at the same time as the single-donor pollinations, so that a single replicate of the hand pollinations for any one plant included three single-donor pollinations and a mixed pollination. Although the hand pollinations occurred 1 wk after the field pollinations, we do not think this short temporal separation for these unstressed plants affected our results, based on results in other experiments with *L. fendleri* (Mitchell and Marshall, 1995, and unpublished data), and results from related species (Marshall, unpublished data).

To measure pollen production per flower, we sampled eight freshly opened flowers from each of the four plants, and stored them individually in microfuge tubes with 70% ethanol. Later we used an Elzone 280PC particle counter to count three subsamples from each flower, and to measure modal pollen diameter for each count. We collected fruits and counted seeds as they matured.

To determine paternity for seeds from field and mixed hand pollinations, we placed the seeds in pots of damp sand and sprayed with 1g/L gibberellic acid to encourage germination (Evans, Mitchell, and Cabin, 1996), watering as needed. When seedlings reached 3–4 wk of age, we performed starch gel electrophoresis to assess paternity, using a morpholine-citrate (pH 8.0) gel buffer and the 6-phosphoglucohydrolase (PGD) staining recipe in Soltis and Soltis (1989; see also Mitchell and Marshall, 1995). The two PGD loci in *L. fendleri* exhibit Mendelian segregation (personal observation). Accidents in the laboratory meant that some gels were unreadable. This resulted in loss of information from 30% of the 435 seedlings genotyped, but because the unreadable gels contained a haphazard sample of available seedlings, this should only reduce statistical power, not bias results.

Statistical analysis—To analyze plant traits (e.g., pollen and seed production), we used fixed-effects ANOVA. We considered plant a fixed effect because we had a small sample of nonrandomly chosen genets. Accordingly, our conclusions are specific to the particular plants in this experiment. For pollen production and diameter, we used a nested design, with samples nested within flowers nested within plants, and used Type III sums of squares. For seed production, we looked for effects of maternal plant, paternal plant, and replicate. Because this plant is self-incompatible, not all combinations of maternal and paternal plants were represented in the seed samples. Therefore we used Type IV sums of

TABLE 3. ANOVA for seed production following hand single-donor pollinations.

Source	df	MS	F	P
Maternal plant	3*	75.4	2.97	0.045
Donor plant	3*	124.1	4.89	0.006
Replicate	7	21.3	0.84	0.5
Maternal plant × donor plant	6	49.0	1.93	0.1
Maternal plant × replicate	21	26.7	1.05	0.4
Donor plant × replicate	21	29.5	1.16	0.3
Error	35	25.4		

* Other Type IV testable hypotheses exist for these effects.

squares in testing for significance, which are appropriate when there are missing cells (Shaw and Mitchell-Olds, 1993).

To analyze paternal success (as number of seeds sired), we used χ^2 goodness-of-fit tests (Steel and Torrie, 1980). We generated expected values for these tests under several different hypotheses (e.g., the hypothesis of random mating predicts that one-third of the seeds produced on each plant will be fathered by each nonself donor), as described in the Results section. We compared those expectations to observed values in a two-way goodness-of-fit table, with one row for each maternal plant, and one column for each paternal plant; 12 of the 16 cells in this table were cross pollinations, and therefore had nonzero expected values. This generated a total of 11 df, 8 df for testing for overall deviations from expected values (2 df for each maternal plant) and 3 to test for heterogeneity in response across maternal plants (Steel and Torrie, 1980).

RESULTS

The four plants in the array differed significantly in pollen production per flower, and in modal pollen grain size (Tables 1, 2). In hand pollinations, seed set per fruit not only differed among maternal plants, but also among pollen donors (Tables 1, 3). There was no evidence of selective provisioning of fruits from mixed pollinations, as indicated by seed set per fruit (a priori comparison of mixed- and single-donor fruits: $F_{1,54} = 0.17$, $P = 0.6$). Proportion fruit set for hand pollinations did not differ significantly among maternal plants or pollen donors ($F_{3,5} < 1.5$, $P > 0.3$ for both; per plant fruit set ranged from 83 to 98%). We found no significant difference between maternal or paternal plants in seed abortion rate ($F_{3,55} < 0.8$, $P > 0.5$; mean aborts/fruit = 0.5). In field pollinations, seed set per fruit differed significantly among maternal plants ($F_{3,249} = 24$, $P = 0.0001$; Table 1).

The number of seeds sired varied among both donors and pollination methods (Fig. 1, Table 4). In single-donor hand pollinations, paternal success was significantly nonrandom (Fig. 1, Tables 2, 4), but the deviations from random mating were relatively small. The mean absolute deviation from the 33% expectation was only 3.7 percentage points, or 11.1% of the expectation. Note that the null expectation was that one-third of the seeds on each maternal plant will be sired by each of the nonself donors, because of self-incompatibility. Male success was concordant across females, as indicated by: (1) the non-significant interaction of maternal and paternal plant in the ANOVA for seed production in Table 2, (2) the non-significant heterogeneity χ^2 s in Table 4, and (3) the fact that on all maternal plants (excluding self), donor 230 sired less than one-third of the seeds, and donor 235 sired more than one-third.

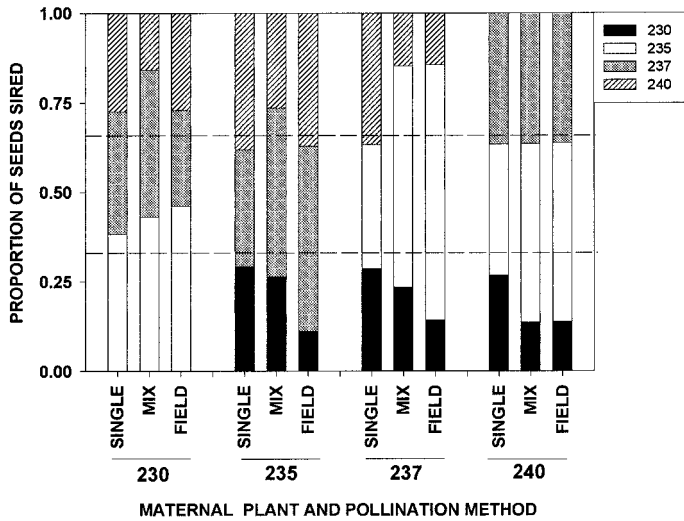


Fig. 1. Paternal success following different pollination methods for the four plants in the array. Dashed lines indicate the null expectation if all donors were equally successful (siring 33.3% of offspring each).

In mixed hand pollinations, the number of seeds sired again differed significantly from the 33% expectation (Fig. 1, Table 4). There was again strong concordance among results on different female plants; in all cases, donor 230 did worse than expected, and 235 did better. The magnitude of nonrandom mating was considerably larger than for single-donor pollinations (mean absolute deviation = 13.3 percentage points, or 39.9% of expectations). However, when expectations were adjusted to account for differences among donors in pollen production, there was no significant deviation from expectations (Table 4; for each maternal plant, we calculated the expected values based on relative pollen production per flower for the three nonself pollen donors). This suggests that most of the nonrandom mating in mixed hand pollinations was due to differences in pollen production. Note that sample sizes were small ($N = 19-54$ offspring/plant/treatment), and this limited our power to conclude that pollen production completely explains the inequality. Results from mixed hand pollinations and predictions based on success in single-donor hand pollinations differed significantly (Table 4), although the rank order of males was identical for both (Fig. 1).

Field pollinations resulted in even more dramatic deviations from random mating (Fig. 1, Table 4). We compared these observed mating patterns to several different hypotheses. First, we tested the hypothesis that all non-

self donors had equal success (33%). Male success differed significantly from this expectation (Table 4). The mean absolute deviation from the expectation of 33% was 15.4 percentage points (46.2% of the expectation). These deviations are again concordant across females, with donor 230 doing worse than expected, and donor 235 better on all maternal plants (excluding self). Second, we tested the hypothesis that donor success was determined by pollen production per plant (as opposed to production per flower, described earlier). We did this by adjusting expectations to reflect the proportional representation of pollen in the pollen pool, with each plants' contribution estimated by the product of pollen production/flower and the number of open flowers. Paternal success was not fully explained by this hypothesis (total $\chi^2_3 = 24.3$, $P < 00001$. Heterogeneity $\chi^2_5 = 2.8$, $P > 0.7$; mean deviation from total pollen expectations = 9.1 percentage points, or 27% of the mean). This suggests pollen-level mechanisms beyond pollen production are important in generating concordant nonrandom mating. Third, we tested the hypothesis that paternity in the field was predictable from success in single-donor hand pollinations. This hypothesis was not quantitatively consistent with our results (Table 4). Fourth, we tested the hypothesis that paternity in the field was predictable from that in mixed hand pollinations. In contrast to the tests of the previous hypotheses, observed paternity did not differ significantly from these expectations (total $\chi^2_3 = 5.5$, $P = 0.13$; heterogeneity: $\chi^2_5 = 7.2$, $P = 0.2$), or from expectations based on pollen production per flower (Table 4), implying that much of the variation in male success in the field may have arisen from the same causes as those responsible for the mixed hand pollinations.

We also tested the hypothesis that interplant spacing was responsible for the nonrandom mating observed in the field. We did this by adjusting expectations to 38% for the two near plants, and to 22% for the far plant, assuming that because the far plant was 40% farther away, it would father 40% fewer offspring. Fit to this hypothesis was not good (total $\chi^2_3 = 45.8$, $P < 0.0001$; heterogeneity: $\chi^2_5 = 34.4$, $P > 0.0001$), indicating that variation in interplant distance was not responsible for the observed nonrandom mating.

Despite the isolation of these arrays and the low abundance of wild flowering plants in the field at this time, gene flow into the array was substantial, with an average of 13.7% of seeds having genotypes that could not occur if mating were completely within the array (Table 5). We detected no cryptic self-compatibility (e.g., Bowman, 1987).

TABLE 4. χ^2 tests for fit of observed paternity to that expected under various hypotheses: the hypothesis that each donor should sire one-third of the offspring on each maternal plant, the hypothesis that each donor should sire offspring in proportion to the representation of pollen in the nonself pollen pool based on pollen production per flower, and the hypothesis that each donor should sire offspring in proportion to results from the single donor pollinations. Values shown are χ^2 values (P). Heterogeneity is abbreviated "het." and refers to variation among maternal plants in their response. Significant values are in boldface.

Pollination treatment	vs. 33% expectation		vs. pollen/flower expectation		vs. singles expectations	
	Total (3 df)	Het. (5 df)	Total (3 df)	Het. (5 df)	Total (3 df)	Het. (5 df)
Single donor	26.2 (0.001)	-6.3 (0.3)	65.1 (0.0001)	33.5 (0.0001)	—	—
Mixed donors	30.8 (0.0001)	-4.8 (0.5)	2.4 (0.5)	2.8 (0.7)	15.2 (0.002)	4.6 (0.4)
Field	45.8 (0.0001)	-0.5 (0.9)	5.0 (0.2)	3.4 (0.6)	36.4 (0.0001)	0.2 (0.9)

TABLE 5. Number of seeds genotyped and estimates of gene flow in the field for each maternal plant.

Maternal plant	No. seeds single donor	No. seeds genotyped		
		Mix	Field	% Gene flow (field)
230	311	51	54	3.7
235	171	19	39	30.8
237	227	34	48	12.5
240	288	22	38	5.3
Total	997	126	179	13.7

DISCUSSION

We found significant and concordant nonrandom mating in an experimental field array of *L. fendleri*. In addition, by comparing field pollinations to hand mixed and single pollinations, we are able to suggest which mechanisms may produce nonrandom mating.

First, we have evidence for nonrandom mating among compatible donors of *L. fendleri*. Pollen donors fathered unequal numbers of seeds in single-donor pollinations, mixed hand pollinations, and field pollinations. Consequently, it is possible for nonrandom mating to play a role in the evolution of reproductive characters, as is true for some other plant species (Bertin, 1990; Cruzan, 1990b; Marshall, 1991; Snow and Spira, 1991a, b; Stanton et al., 1991; Rigney et al., 1993; Jones, 1994; Björkman, Samimy, and Pearson, 1995). The fact that we detected such effects in this relatively small study indicates that nonrandom mating might be common and strong in natural populations.

Second, nonrandom mating was concordant across pollination methods and across maternal plants. That the rank order of pollen donor performance was consistent across maternal plants implies an opportunity for sexual selection. Other researchers have reported such consistency of male success across females (e.g., Snow and Spira, 1991b; Marshall et al., 1996), although there are many exceptions (e.g., Bertin, 1986; Waser et al., 1987; Schlichting and Devlin, 1989; Waser and Price, 1989, 1991, 1993; Bertin and Peters, 1992; Björkman, Samimy, and Pearson, 1995).

Which traits might be under sexual selection is not completely clear from these data. One trait that appears to be important is pollen production per flower, suggesting that it (or some closely related trait) is under sexual

selection. The simplest explanation for the importance of this trait would be that plants with greater pollen production constitute a larger fraction of the pollen pool available to fertilize seeds. Yet, in the field exposure, representation in the pollen pool (pollen per plant) was not as good a predictor of male success as was pollen production per flower. This conundrum may be explained by pollinator behavior. Many pollinators of *L. fendleri* are pollen foragers, which might prefer to visit plants producing more pollen per flower. If plants producing more pollen per flower are visited more often, this would generate results similar to those from mixed hand pollinations, but through a different mechanism. Preliminary evidence indicates that this is possible, since pollen production per flower tends (nonsignificantly) to be positively correlated with per flower visitation rate for small bees ($r = 0.38$, $P = 0.09$, $N = 21$; unpublished data). These small bees are the most abundant visitors to *L. fendleri* (unpublished data).

Third, comparison of performance among pollination methods gives some clues about the mechanisms of nonrandom mating in this species. Across these pollination methods there was perfect qualitative agreement in the relative ranking of males (ranks for 230, 235, 237, and 240 = 4, 1, 2, 3 in all cases), although the magnitude of the advantage varies among methods. There is thus no evidence that nonrandom mating caused by pollination is opposed by postpollination events—in fact it seems to be strengthened, since the minor advantage in single-donor pollinations becomes progressively stronger with hand mixed and field pollinations.

We think that the general scheme outlined in Table 6 helps to organize thinking about mechanisms (see also Lyons et al., 1989). Consider single-donor pollinations. When such pollinations generate nonrandom mating (e.g., some fathers sire more seeds than others), one can conclude that there is some difference among fathers in whatever general characters affect the ability to sire seeds, such as pollen viability, pollen germination, pollen tube growth, and ovule fertilization (referred to as “intrinsic ability”), or in compatibility relationships, or in seed or fruit abortion rates (interactions between specific male and female plants). Since we applied saturating pollen loads in this study, pollen production could not be responsible for nonrandom mating in single-donor crosses. Similarly, pollinator behavior (e.g., visitation rate and in-

TABLE 6. Conclusions to be drawn from different outcomes of an array experiment. Each row and column represent one of the pollination methods. Each entry indicates our interpretation of the meaning of a significant difference between results from the methods in the corresponding row and column. Entries on the diagonal are for comparison of that experiment to random mating expectations. Entries off the diagonal indicate comparisons among results of different methods. In each case where pollen number might be important, the expectation can be adjusted for pollen production to test that hypothesis as necessary. Note that it is readily possible to adjust expectations for differences in pollen production or intrinsic ability during analysis, allowing more definitive conclusions than depicted here.

Pollination method	Pollination method		
	Single	Hand mix	Field mix
Single	Differences in intrinsic ability, compatibility, or fruit abortion		
Hand mix	Postpollination sorting or pollen production	Differences in intrinsic ability, Postpollination sorting or pollen production	
Field mix	Postpollination sorting or Pollinators	Pollinators	Differences in intrinsic ability, Postpollination sorting or pollen production or pollinators

tensity) did not vary among plants for these hand pollinations. Because we found significant deviations from random mating even in single-donor pollinations, and no significant difference in fruit set, we infer that there was some inequality in intrinsic ability/compatibility within the array. However, the small magnitude of that deviation may indicate that this source of variation is not important in natural situations. Note that since we found no significant difference in seed or fruit abortion rates among pollen donors, those mechanisms are not likely to be important in generating nonrandom mating in the present study.

Now consider mixed-donor hand pollinations. If results from such pollinations differ from those from single-donor pollinations, pollen number, as well as postpollination events (such as pollen tube competition, stylar screening, or ovule and seed abortion) might generate deviations from equal success. Note that gross differences in the intrinsic ability of donors would show up in single-donor pollinations, and comparison of single and mixed pollination results should factor out differences in intrinsic ability. In mixed-donor pollinations, the interaction between simultaneously arriving pollen grains can also be important (e.g., Mitchell and Marshall, 1995; Marshall et al., 1996). Our results showed strong deviations from equality of pollen donors in mixed pollinations, implicating postpollination events and/or pollen production as important in generating nonrandom mating. Because we found no significant deviation from expectations when differences in pollen production were accounted for, we infer that most or all of the nonrandom mating in mixed hand pollinations was generated by differences in pollen production.

Finally, consider field pollinations. Deviations from equal siring success in the field might be caused by pollination or postpollination events. However, a deviation between results from mixed hand pollinations and field pollinations is probably due solely to the effects of pollinators. Such effects might occur through differences in visitation rates to some plants, but more subtle effects of the way pollen is deposited on flowers might also occur. For example in the field, pollen might be deposited in small amounts at widely spaced intervals, while in mixed hand pollinations large mixed loads of pollen are deposited at once. The former situation reduces the potential for pollen tube competition to be important (Mulcahy, Curtis, and Snow, 1983; Snow, 1986). We found unequal male success in the field, but no difference between results in field and mixed hand pollinations. At first glance this seems to negate the importance of pollination-level events in generating nonrandom mating, but pollinator preference for plants with greater pollen production per flower could in principle generate a pattern similar to that following from a simple numerical advantage (see discussion above). Thus, information on the natural history of pollinator-plant relationships can help guide and alter the interpretations listed in Table 6.

One confounding factor may also play a role in the field results—gene flow. The amount of detected gene flow from field populations was rather high, but in the same range as for other small populations (Ellstrand and Marshall, 1985; Ellstrand, Devlin, and Marshall, 1990; Kohn and Casper, 1992). Incoming pollen alleles should

be representative of the surrounding population, so that the array plant containing the population's most common alleles would tend to be erroneously assigned paternity for many of the progeny resulting from gene flow. This could mislead us about the importance of pollination events. In fact, the most successful father in the field was plant 235, which had the alleles that were most common in the surrounding population (Cabin, Mitchell, and Marshall, unpublished data). However, this cannot completely explain the field results for the following reasons. (a) The rank order of pollen donor performance is equal across treatments. (b) On plant 235, where gene flow from the most common genotype is most accurately detected, the performance of the other three donors is exactly in the rank order of the two hand pollination portions of the study. (c) Rare alleles from the surrounding population (e.g., allele 1 at the *Pgd1* locus) were not detected in the array progeny. Thus, even in the face of competition from other donors outside the population, the performance of our target donors was concordant across treatments and plants.

Results from our array experiment provide a more complete view of plant mating patterns and mechanisms than would be achieved from observations in unmanipulated populations (where proximity to other plants and other factors would be uncontrolled) or experimental study of greenhouse pollinations (where the complications of the field must be ignored). Combining hand pollinations to test for compatibility and mate choice, with field pollinations to test for effects of pollinators, allowed us to more directly determine the mechanisms of nonrandom mating in the field than would either approach by itself. Paternity information from greenhouse or field alone cannot provide as satisfactory or complete a picture of the interactions among plants as a combination of the two.

It is not our intention, however, to discount other studies using other methods. We have studied paternity resulting from controlled hand pollinations and from unmanipulated wild populations in the past, and we hope to do more of both in the future. Such work is necessary to generate useful hypotheses about plant mating biology. But to progress beyond mere description to an understanding of how and why some individuals in field populations are more successful than others at siring offspring, manipulative experiments are necessary, at least as an adjunct to observational studies (see also Lyons et al., 1989; Galen, 1992). We think that a careful blend of observational and experimental work provides new and useful answers to questions about the mating behavior of plants, and that the array experiments we describe here are an especially useful new approach to that ideal.

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