Relative Success of Self and Outcross Pollen Comparing Mixed- and Single-Donor Pollinations in Aquilegia caerulea

Arlee M. Montalvo

Evolution, Vol. 46, No. 4 (Aug., 1992), 1181-1198.

Stable URL:
http://links.jstor.org/sici?sici=0014-3820%28199208%2946%3A4%3C1181%3ARSOSAO%3E2.0.CO%3B2-M

Evolution is currently published by Society for the Study of Evolution.

Evolution, 46(4), 1992, pp. 1181–1198

RELATIVE SUCCESS OF SELF AND OUTCROSS POLLEN COMPARING MIXED- AND SINGLE-DONOR POLLINATIONS IN *AQUILEGIA CAERULEA*

ARLEE M. MONTALVO[1]
Department of Biology, University of California, Riverside, CA 92521 USA

Abstract.—Flowers frequently receive both self (S) and outcross (OC) pollen, but S pollen often sires proportionally fewer seeds. Failure of S pollen can reflect evolved mechanisms that promote outcrossing and/or inbreeding depression expressed during seed development. The relative importance of these two processes was investigated in *Aquilegia caerulea*, a self-compatible perennial herb. In the field I performed single-donor (S or OC) and mixed-donor (S plus OC) pollinations to compare the relative success of both pollen types at various stages from pollen germination to seed maturity. Single-donor S pollinations produced significantly fewer and lighter seeds ($\bar{x}$ decrease = 12% and 3%, respectively) than OC pollinations. Abortion rates differed by an average of 38% whereas fertilization rates differed by only 5%, indicating that most differences in seed number arose postzygotically. This suggests that inbreeding depression was responsible for most failure of S pollen. One prezygotic effect measured was that 10% fewer S than OC pollen tubes reached ovaries after 42 hr, suggesting S pollen might fertilize proportionately fewer ovules after mixed pollination. Using allozyme markers, I found mixed-donor pollinations produced significantly more and heavier outcrossed than selfed seeds. However, the proportion of selfed seed, fertilized ovules, and aborted seeds for mixed-donor fruits were each predictable from pollen performance in single-donor fruits, suggesting that differential paternity is best explained by inbreeding depression during seed development. Even given these similarities between mixed- and single-donor fruits in the relative performance of S and OC pollen, both individual seed weight and seed set were significantly higher in multiply-sired fruits.

Key words.—*Aquilegia caerulea*, cryptic self-incompatibility, inbreeding depression, paternity analysis, pollen tubes, postpollination selection, seed weight.

Received January 10, 1991. Accepted December 13, 1991.

Cosexual plants frequently receive both self and outcross pollen, but the final genetic representation of these two pollen types in seeds does not necessarily reflect their ratio in stigmatic pollen loads (Weller and Ornduff, 1977; Glover and Barrett, 1986; Bowman, 1987; Bertin and Sullivan, 1988). Self pollen often has reduced success because of inbreeding depression and/or self-discrimination mechanisms that involve specific responses due to interactions between the maternal parent and self pollen, self tubes, or selfed embryos. Distinguishing these two causes is important in understanding the evolution of plant mating systems because self-discrimination mechanisms such as self-incompatibility (SI) can evolve in response to cumulative inbreeding depression expressed between zygote and adult stages (Fisher, 1941; Charlesworth and Charlesworth, 1987; Uyenoyama, 1988). Consequences of non-random paternity caused by inbreeding depression and SI are considered distinct from consequences of non-random paternity caused by sexual selection (Stephenson and Bertin, 1983; Charlesworth et al., 1987; Lyons et al., 1989). In this study, and in the discussion that follows, I examine the postpollination determinants of non-random mating success that are distinct from sexual selection.

Postpollination "selection" (i.e., non-random success) among self (S) and outcross (OC) donors can occur prezygotically via differential pollen germination, tube growth, and fertilization and/or postzygotically via differential seed filling and abortion (Willson and Burley, 1983; Lyons et al., 1989). Prezygotic selection involves specific interaction of pollen grains or haploid tubes with tissues or products of the recipient pistil, whereas postzygotic selection can involve both interaction between developing seeds and pistils as well as inbreeding depression (Seavey and Bawa, 1986). Inbreeding depression classically refers to reductions in

[1] Present address: Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92717 USA.

mean values of traits for individuals with greater homozygosity that result from inbred mating; that is, it occurs after zygote formation (Falconer, 1981). In contrast, incompatibility mechanisms operate primarily before zygote formation (Wiens et al., 1987). It is therefore useful to distinguish prezygotic from postzygotic processes that cause non-random seed paternity.

An extreme example of prezygotic selection is classical self-incompatibility (de Nettancourt, 1977). When SI is well-developed, self-pollen is barred from fertilizing ovules; however, SI is not always absolute. In species with cryptic SI (Bateman, 1956), S and OC pollen produce seeds when applied to separate pistils, but S pollen suffers reduced success when applied together with OC pollen (Levin, 1975; Barrett, 1988; Weller and Ornduff, 1989; Cruzan, 1989). Within this context, I use cryptic SI to refer to processes that cause only differential fertilization by S relative to OC pollen tubes, events distinct from postzygotic inbreeding depression. Pseudo incompatibility (pseudo SI) has been used to describe responses that occur even after single-donor pollination but which are partial, perhaps as a function of the genetic relatedness of pollen to the recipient (Mulcahy and Mulcahy, 1985; Waser, 1992). A cryptic SI system may be very difficult to detect if pseudo incompatibility is also involved. Even though in one survey de Nettancourt (1977) estimated that a slight majority of flowering plants readily produce seeds after self pollination, it is unknown how frequent cryptic SI is in self-fertile species.

It is difficult to distinguish prezygotic self-discrimination mechanisms such as cryptic and pseudo SI, from natural selection occurring postzygotically due to embryo lethality and other manifestations of inbreeding depression (Bertin, 1985; Seavey and Bawa, 1986; Charlesworth et al., 1987; Wiens et al., 1987; Barrett, 1988; Lyons et al., 1989; Bertin et al., 1989; Manasse and Pinney, 1991; Waser, 1992). Distinguishing such factors may be especially difficult if mechanisms are facultative as in cryptic SI, or if inbreeding depression decreases with decreased stress or embryo competition. Progress in distinguishing the relative importance of prezygotic versus postzygotic processes has been made by comparing the performance of single-donor S and OC pollinations at different stages between pollen germination and seed maturity to results after mixed-donor pollinations (Weller and Ornduff, 1977, 1989, 1991; Barrett, 1988; Casper et al., 1988; Bertin et al., 1989).

In this study I performed field experiments to examine whether events following pollination influence paternity of seeds in *Aquilegia caerulea,* a self-fertile species that receives both S and OC pollen when exposed to natural pollinators (Miller, 1978). I examined: 1) the relative success of S versus OC pollen in siring seeds after single- and mixed-donor pollination; 2) relative growth of S and OC pollen tubes, fertilization rates, and abortion rates after single-donor pollinations; 3) relative seed weight of selfed and outcrossed progeny; and 4) variation in these measures among recipients. My goal was to determine: 1) if there is postpollination selection; and 2) if selection is primarily due to pre- versus postzygotic processes.

Materials and Methods

Study System

Aquilegia caerulea James (Ranunculaceae), the blue columbine, is a self-compatible, herbaceous perennial occurring at 2,100–3,700 m in the Rocky Mountains (Miller, 1981). In the study area, populations have a six week flowering period. Flowers have five white petals and five long-spurred petaloid sepals ranging from white to bluish-purple. They are hermaphroditic and protandrous with the male phase lasting 3–5 days followed by a female phase lasting 3–4 days. Despite this protandry, plants have a mixed mating system. Based on seeds genotyped for three allozyme loci ($N = 34$ maternal families; 20 seeds/family), J. Brunet (pers. comm.) estimated an outcrossing rate (t) of 0.403 ± 0.048 SE in 1987 for a population within 300 m of my study site.

Flowers have numerous spirally arranged stamens surrounding an average of 6–7 pistils. Stamens dehisce sequentially over several days. Each pistil produces an average of 37 ovules and is completely separate to the base of the ovary. The linear stigmas become receptive at their tips and sequen-

tially mature receptive papillae toward their bases over several days. Pistils mature into a cluster of individual follicles.

My study site is on a rocky plateau located at 3,500 m in elevation, 6.5 km northwest of the Rocky Mountain Biological Laboratory in western Colorado. All 179 individuals of reproductive age within an 18 × 20 m plot were tagged, mapped, and genotyped (see below). The average inbreeding coefficient (F) was 0.153 (N = 132 plants; SE = 0.0772, unpubl. data based on MPI, MDH, and GDH allozyme loci). Plants (N = 25) produced 6.4 ± 5.52 (mean, SD) inflorescences and 3.4 ± 1.47 flowers per inflorescence. In June, 1987, I randomly selected 20 individuals from those having at least six inflorescences and distinguishable genotypes for use in crosses. Plants were enclosed in wire cages covered with fine nylon netting to exclude flower visitors. To prevent flowers from selfing, I covered the entire gynoecium of each mature bud with lengths of transparent plastic straws, creased at the top. Thirteen caged plants were assigned to experimental groups (see below) according to coincidence of flowering and genotypes appropriate for tracing seed paternity after mixed-donor pollination. Experimental plants were 1–16 m apart, well within the range of interplant flight distances of pollinating hawkmoths and bumblebees.

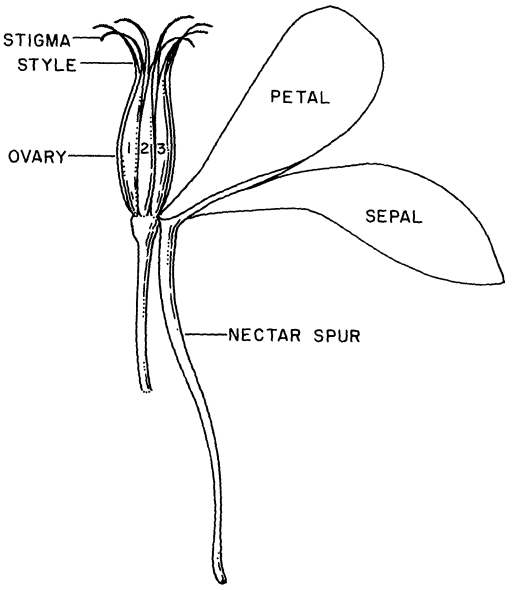

FIG. 1. Diagram of columbine flower with most perianth parts removed to show the multiple separate pistils, each of which received a different pollination treatment.

Electrophoretic Technique

Plants were genotyped for seven loci, but mannose-6-phosphate isomerase (MPI) was the only locus with sufficient variability to use in paternity analysis. At MPI there were four scorable alleles; F, F', I, and S, referring to the fastest through slowest migrating bands respectively.

Leaf extracts were prepared by grinding 2 cm^2 pieces of leaf tissue to a fine powder in liquid nitrogen, then adding 4–5 drops of chilled extraction buffer (0.1 M Tris-HCl, pH 7.5 with 10 mM dithiothreitol) to which 9 mg PVP-40 per 10 ml had been added. For progeny extracts, I ground seeds individually in 20–24 μl of extraction buffer over ice. Extracts were absorbed onto filter paper wicks and run on horizontal 12% starch gels for 5 hr 10 min at 24 mA and 129 V under ice packs. I used 0.065 M L-histidine titrated to pH 6 with citric acid for the electrode buffer and a 1:6 dilution for the gel buffer (Ellstrand, 1984). Gels were stained with a standard assay for MPI and incubated in the dark for 50 min at 35°C prior to scoring.

Experimental Crosses

I performed crosses using three designs. For each design, the multiple pistils (Fig. 1) allowed all pollination treatments to occur within each treated flower, minimizing any effects due to flower position and age. Differences in flower age and/or nutrient allocation within plants can cause significant differences in seed set and seed weight (Brunet, 1990; pers. obs.). General pollination and fruit collection methods follow with differences specific to a design noted later.

Flower Preparation and Pollination: Immediately prior to pollen collection I tagged pedicels of recipient flowers within their first three days of stigma receptivity, removed perianths, spent anthers, and stigma covers, and placed paper dividers between pistils to prevent cross contamination. I also coded each ovary for donor treatment using felt-tip pens (which did not affect fruit devel-

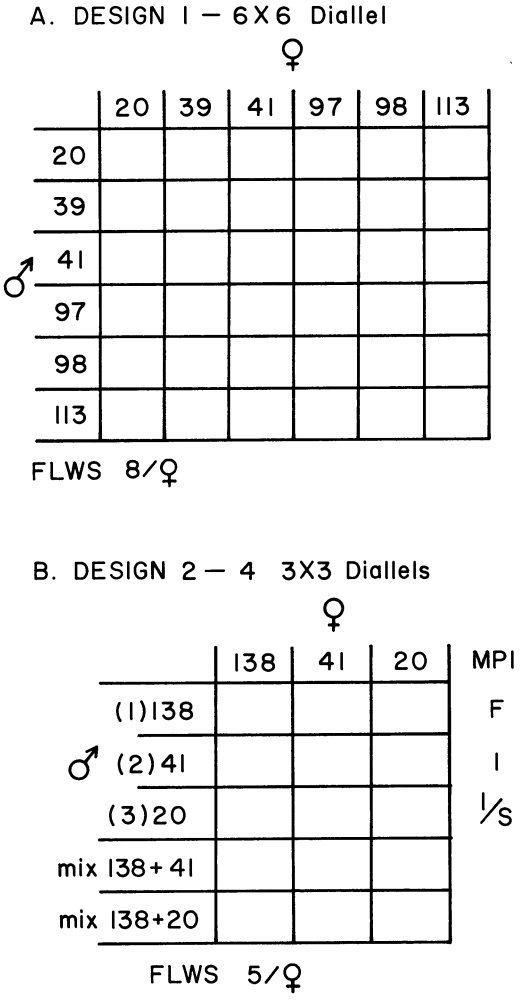

FIG. 2. Breeding designs used to determine crossing success in a natural population of *Aquilegia caerulea*: A = Design 1; B = Design 2. Only one of four breeding groups are shown for D2. Individual 138 represents donor 1, the marker genotype (MPI *F/F*). For both designs, columns represent a single flower with the pollen recipient heading the column, and each cell within a column represents a distinct pistil within the flower. All cells (treatments) occurred within a flower and were replicated across several flowers per pollen recipient.

opment) and collected data in coded form to avoid observer bias.

I collected pollen from donors in random order into separate microcentrifuge tubes. I placed 10 newly dehisced anthers per donor into a given tube and mixed the pollen with a toothpick. All prepared flowers of a given recipient were pollinated in arbitrary sequence, using one donor at a time in random order. I applied pollen to entire stigmas using wooden toothpicks. In a pilot study, usually 400–500 pollen grains germinated when applied in this way. In all experiments, pollinations for each day were completed within 1.5 to 3 hr of pollen collection. Pollen remains viable for several hours (Montalvo, pers. obs.).

Fruit Collection and Processing: I collected mature fruits (follicles) 5–6 weeks after pollination and placed them into separate paper envelopes. Fruits were air dried and stored in an airtight container at 4°C. For each follicle I recorded total seed weight as well as numbers of filled seeds, large and small aborted seeds, and unexpanded ovules. Filled seeds were plump with shiny black seed coats and usually >1.5 mm long. Aborted seeds (large class ≥ 1 mm long; small class ≥ 0.5 mm, <1 mm) were shriveled, and frequently brownish and hollow. Large aborts obviously developed from fertilized ovules, but small aborts could have included some larger unfertilized, unexpanded ovules. I considered unexpanded ovules (<0.5 mm long) as unfertilized although it is possible that a fraction were fertilized and aborted at a very early stage. All filled seeds from each follicle of a multiple fruit were weighed together and mean seed weight was calculated (= mean seed weight/fruit). I also individually weighed a random sample of 10 seeds/single-donor cross (= individual seed weight) to compare with individual weights of S versus OC seeds from mixed-donor fruits.

Design 1 (6 Parents).—The first crossing design (D1) examined the effect of mate type (S and OC) on seed production and seed weight using single-donor pollinations. I performed all possible pollinations among six individuals, including selfs (6 × 6 diallel, Fig. 2A). Outcrossing distances averaged 8.3 m. Within every replicate flower, I pollinated each of six pistils with a different pollen donor. On 2 July I treated 4–5 flowers per recipient and on 7 July an additional 2–5 flowers. Flowers pollinated on the same day on a given recipient were on separate inflorescences.

Design 2 (12 Parents).—For the second design (D2), I assigned 12 individuals (including 5 from D1) into 4 groups of 3 as above (groups A–D). Outcrossing distances averaged 9.3 m. Within each group a mod-

ified diallel cross was used that incorporated both mixed-donor (S plus OC) and single-donor pollinations (Fig. 2B).

I performed all possible single-donor pollinations for each diallel, including selfs. I applied pollen from each of the three individuals separately to a different pistil of a flower and used up to five replicate flowers per recipient. Each diallel consisted of one individual with MPI *F/F* genotype (here designated the "marker donor" or individual 1), and 2 individuals with MPI *I/I* and/or *I/S* genotypes (individuals 2–3) (Fig. 2B).

Concurrently with single-donor treatments, I conducted mixed-donor pollinations. I collected five newly dehisced anthers from each of the two donors, and mixed them together in a microcentrifuge tube before applying pollen to stigmas of the remaining pistils within each flower. As the example in Figure 2B shows, recipient 1 of each 3 × 3 group received two different S/OC mixtures and recipients 2 and 3 received one kind each of S/OC and OC/OC mixture. Lack of individuals homozygous for MPI *S* precluded doing two different S/OC mixtures on recipients 2 and 3. I treated five flowers per recipient except for two plants that had fewer female-phase flowers available. Every pollen mixture was applied to both parents included in the mix.

Pollen Viability and Number: Immediately following pollinations, I determined viabilities of pollen used in single-donor pollinations. I used a nutrient medium in 10% sucrose (Brewbaker and Kwack, 1963) in which 90–100% of columbine pollen germinated in a pilot study. For each pollen sample a sterile drop of nutrient medium was placed on a glass coverslip sitting on moist filter paper within a petri dish. I inoculated nutrient drops with pollen from the pure pollination vials. After 24 hr at room temperature, I inverted the coverslips onto glass microscope slides and viewed them under a microscope. Over 200 pollen grains per sample were assessed for tubes.

Variation in pollen quantity could interfere with obtaining an a priori expectation of equal paternity by each donor of a mix. I determined the average number of pollen grains per anther for a sample of 10 newly dehisced anthers per pollen donor. Anthers were stored in 70% ethanol. Prior to counting, samples were gently shaken and diluted to 100 ml with 0.1% NaCl. Using a Coulter Counter I determined the number of pollen grains in eight replicate 500 μl samples.

Progeny Analysis for Mixed-Donor Fruits: To determine paternity of seeds from mixed-donor fruits (self and outcross), I genotyped a random sample of approximately 20–40 seeds from four flowers per cross per recipient (a total of 551 seeds) and identified sires by progeny testing for MPI. I weighed seeds individually before genotyping. I also ran a sample of seeds from single-donor pistils and detected no illegitimate sires and no deviations from expected Mendelian ratios.

One individual (#98) was later discovered to be heterozygous *F*/null. Null alleles produce no bands so two ambiguous progeny genotypes (*I/I* and *I*/null) resulted from pollination of the two homozygous *I/I* individuals in the diallel (#s 97 and 110) with a mixture of #98's *F*/null pollen with *I/I* pollen. I estimated frequencies of the two ambiguous genotypes based on the frequency of the unambiguous *I/F* progeny genotype relative to all others represented in the seed sample. This approximation was reasonable because the *F* and null alleles segregated into progeny at equal frequencies when pollen from #98 was used in single-donor pollinations (i.e., no gametic selection). However, because genotypes could not be assigned unambiguously to every seed, #s 97 and 110 are excluded from analyses of individual seed weight.

Design 3 (Tubes).—In 1988, I conducted a 7 × 7 diallel using the six individuals of D1 plus one individual from D2. The purpose was to examine the effect of mate type on success of pollen tubes in reaching the ovary. Under clear field conditions, most pollen tubes that eventually fertilize ovules reach the zone between the basal quarter of the style and the placental region of the ovary by 42 hr and continue to grow for at least another day (Montalvo, pers. obs.).

To obtain a sample size of at least five replicate sets of treatments (i.e., five flowers/plant), I performed pollinations on two days (13 and 15 July). Flowers within the first two days of female anthesis were used, and about 150–200 pollen grains were applied only to the tips of stigmas. Larger loads applied to entire stigmas obscure tube

counts. Pistils were excised and fixed in Carnoy's fluid (Johansen, 1940) in the same sequence as pollination exactly 42 hr, 12 min later, allowing all pollen tubes equal time to germinate and grow.

After fixing, I transferred pistils to 70% ethanol. Pistils were later transferred to distilled water, cleared in 4 M NaOH at 60°C for 1.5 hr, rinsed twice in distilled water, and stained overnight with 0.05% aniline blue in 0.2 M, pH 7.4 phosphate buffer. Stained pistils were placed on microscope slides in a drop of 0.01% ethidium bromide in 0.2 M, pH 7.4 phosphate buffer, gently squashed under a coverslip and viewed under an epiflorescence microscope (Waser et al., 1987). I scored the number of initial tubes (= germinated grains), the number reaching 400 μ and 600 μ down the style measured from the base of the germinating cluster of grains (circa 50% and 75% of the distance from the tip of the stigma to the ovary respectively), and the number entering the ovary. Differences in numbers of initial tubes can be caused by differential pollen adherence and germination, while differences in the proportion of initial tubes reaching the ovary in 42 hr are due to differences in pollen tube attrition and/or growth rates.

Results

Cross Performance for Single-Donor Pollinations

Seed Number and Weight.—I used ANOVA (PROC GLM; SAS Institute, 1985) to compare number of seeds produced and mean seed weight/fruit for S versus OC fruits (mate type) from D1 and D2. Pollen recipient and flowers nested within recipients were random effects, and mate type was a fixed effect in all models. In the analysis of seed number, the square of total ovules/fruit was entered as a covariate to correct for initial differences in ovule number that could influence seed output. Squaring ovule number normalized residuals that were skewed to the left. Assumptions of linearity and slope homogeneity were confirmed. For the later analysis, mean seed weight/fruit was calculated as total seed weight divided by number of seeds in the fruit. Fruits resulting from different flowers provided replicates. Because number of seeds varied among fruits and because sample size affects the reliability of each mean, I weighted the ANOVA of mean seed weight/fruit by number of seeds/fruit (Sokal and Rohlf, 1981; p. 41). F-tests were calculated following Brownlee (1965).

The effect of S versus OC mate type on seed number was significant for D2 (12 parents) and approached significance for D1 (6 parents) (ANOVA, Table 1), with single-donor OC pollinations producing more seeds than S pollinations. D2 produced 51.6% and 46.9% seed set for OC and S pollinations, respectively, while D1 produced 65.1% and 56.0% seed set, representing 9.4% and 14.5% declines for S relative to OC (D1 and D2 respectively). For both designs, seed number significantly covaried with ovule number. The combined probability (Fisher's method, Sokal and Rohlf, 1981; p. 779) shows a significant effect of mate type overall ($X^2 = 12.92$, $df = 4$, $P < 0.025$). Because 5 of 12 parents involved in D2 were also included in D1, it can be argued that the two experiments were not independent tests of the same hypothesis, especially with respect to differences among maternal parents. However, for these five maternal parents in D2, only one of seven S-OC comparisons involved the same maternal and paternal combination as in D1 so that violation of the assumption of independence with respect to mate type is slight. Furthermore, inferences based on test results refer to the population of individuals included in the experiments, not to the total population. Nevertheless, combined probabilities should be interpreted with caution.

Seed weight was also affected by mate type, but most variation was attributable to differences among recipients and flowers (Table 1). Average seed weight per fruit varied significantly among recipients (12% and 35% of variance) and flowers within recipients (26% and 34% of variance for D1 and D2, respectively). There was a significant effect of mate type (S versus OC) for D2, but the effect for D1 was marginally not significant ($P = 0.08$). The combined probability was significant (Table 1). A highly significant mate type × recipient interaction occurred for D1, but not for D2 (Table 1). The significant interaction appears to be caused by two recipients from D2 (65 and 74) having

TABLE 1. Effects of S versus OC pollen (type) on the number of filled seeds and average seed weight/fruit as dependent variables. ANOVAs included pollen recipient and flower (nested within recipient) as main effects (see text), and used SAS GLM Type IV SS (SAS Institute, 1985). Asterisks (*) indicate $P < 0.05$ for combined propabilities (*CP*) of two independent tests. Means shown are pooled over recipients.

Source	Design 1				Design 2				D1-2
	df	*F*	*P*	r^2	*df*	*F*	*P*	r^2	*CP*
Number of filled seeds (ovules^2 as covariate)									
Ovules^2	1	27.97	0.0001	0.029	1	13.20	0.0007	0.033	*
Type	1	4.79	0.0803	0.031	1	7.47	0.0195	0.007	*
Recipient	5	2.78	0.0292	0.081	11	1.58	0.1462	0.184	*
Flowers(Recip)	43	5.56	0.0001	0.251	37	4.19	0.0001	0.392	*
Type × Recipient	5	4.40	0.0025	0.033	11	0.50	0.8907	0.011	*
Type × Fl(Recip)	43	1.42	0.0590	0.064	37	0.81	0.7430	0.076	ns
Model	98	7.75	0.0001	0.796	98	3.55	0.0001	0.879	*
Average seed weight/fruit (weighted by seed number)									
Type	1	27.55	0.0089	0.015	1	3.85	0.0755	0.010	*
Recipient	5	44.25	0.0046	0.120	11	3.20	0.0041	0.345	*
Flowers(Recip)	43	11.08	0.0001	0.258	35	7.63	0.0001	0.344	*
Type × Recipient	5	1.46	0.2223	0.004	11	3.33	0.0041	0.028	*
Type × Fl(Recip)	43	1.09	0.3360	0.025	31	0.59	0.9374	0.023	ns
Model	97	17.01	0.0000	0.894	89	8.25	0.0001	0.945	*

Unweighted means	S (*N*)	OC (*N*)	S (*N*)	OC (*N*)
Seeds/fruit	24.2 (49)	28.1 (244)	17.9 (49)	19.6 (98)
Sd wt/frt (mg)	1.23 (49)	1.27 (244)	1.18 (43)	1.22 (90)

much lighter S than OC average seed weight compared to several recipients having slightly higher average selfed seed weights (Fig. 3). In a regression analysis (not shown), only 2% of the variance in average seed weight per fruit was explained by seed number per fruit.

Ovule Fate.—In the following analyses, filled seeds together with aborted seeds are "fertilized ovules," whereas unexpanded ovules represent "unfertilized ovules." Aborted seeds plus unexpanded ovules are "unsuccessful ovules."

Every recipient in D1 and D2 experienced some seed abortion. Fertilization rates were generally higher for D1 (even comparing only the five individuals that were in both designs; Fig. 4). Seed sets ranged from about 43–78% for D1 and from 20–76% for D2.

To test for differences in abortion and fertilization rates for S versus OC pollinations I performed chi-squared tests on data for every flower of the seven individuals. This provides a within-flower comparison of treatment effects avoiding problems due to heterogeneity among flowers. For abortion rate, a 2 × 2 table consisted of S versus OC pollination × filled seeds versus aborted seeds. For fertilization rate, the tables were S versus OC pollination × fertilized ovules versus unexpanded ovules. The magnitude of the X^2 statistic indicates the degree of difference but not its direction; however, both degree and direction are expressed if the chi-statistic is used, where chi is calculated as the square root of X^2, and given the sign appropriate to the direction of the deviation. Here, negative chi values denote the success of outcross pollen, i.e., fewer abortions or more fertilizations by outcross pollination. Chi from 2 × 2 tables is normally distributed with zero mean and unit variance (Everitt, 1977) and, for example, can be used to combine information from 2 × 2 tables incorporating data from different designs (Lewontin and White, 1960). To determine if outcross pollinations generally result in more fertilizations and fewer abortions, I used a one tailed *t*-test to see if the overall mean chi value for each recipient was significantly less than zero.

Self pollination resulted in significantly higher levels of abortion than OC pollination but there were no significant differences in fertilization rates (Table 2). By themselves, differences in fertilization success

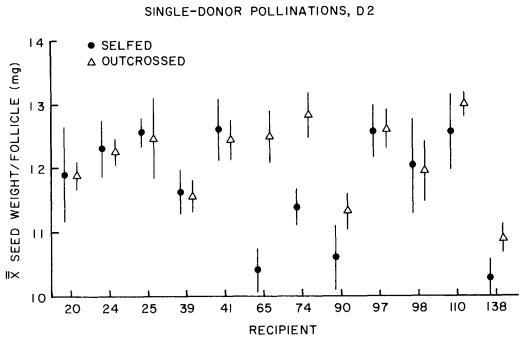

FIG. 3. Means of average seed weight per fruit (follicle) for single-donor self and outcross treatments for recipients of Design 2. Recipients are arbitrarily arranged by ID number. Bars represent one standard error of the mean.

were too small to cause significant differences in seed set (Table 3). These results indicate that elevated seed set for OC relative to S donors after single-donor pollination can mostly be explained by differential post-fertilization abortion. ANOVA on chi values with pollen recipient as a main effect was done to determine if recipients differ in their response to mate type. D1 showed significant differences among recipients for chi values for percent seed set, abortion, and fertilization, while D2 showed no differences among recipients (Table 3).

Pollen Tube Performance.—I tested whether S and OC pollen have different growth and/or attrition rates. If OC tubes reach ovules faster or in higher proportions than S tubes, then they may have a fertilization advantage after mixed-donor pollination even when there are no significant differences in percent fertilization by S and OC pollen in separate pistils. Preliminary analysis showed that there were fewer (sometimes zero) pollen tubes reaching the ovary for the second day of the pollen tube experiment (D3), possibly due to cool and rainy conditions. Because of such differences, and because zero counts made residuals strongly non-normal, I used 2 × 2 chi-square tests to look for differences in the proportion of initial tubes that reached the ovary for the two mating types for each flower separately (excluding seven flowers that had no pollen tubes reaching the ovary). I did a sign test to determine if chi values were less than zero (i.e., if OC tubes generally outperformed S tubes).

In all seven recipients in D3 ($P = 0.008$, sign test), and in 34 of 45 flowers (pooling over recipients, $P < 0.005$, sign test), the average number of pollen tubes reaching the ovary after 42 hr was higher for OC pollinations than in S (Fig. 5). Furthermore, the proportion of initial pollen tubes that reached the ovary in 42 hr was higher for OC pollinations in 32 of 44 experimental flowers (pooling over days and recipients, $P < 0.005$, sign test). Pooling is appropriate because the proportion of initial S versus OC tubes reaching the ovary showed no differences among pollen recipients, day of experiment, or their interaction (two-way ANOVA on chi values: recipient: $F_{[6, 30]} = 0.89$, $P = 0.515$; day: $F_{[1, 6]} = 0.00$, $P = 0.966$; r × d: $F_{[6, 30]} = 0.50$, $P = 0.805$). Furthermore, the number of S relative to OC tubes declined continuously between the zone of germination and the ovary, resulting in 10% fewer S tubes reaching the ovary (Table 4).

Cross Performance for Mixed-Donor Pollinations

Seed Paternity.—Outcrossed progeny exceeded selfed for 11 of 15 treatment × recipient combinations, excluding one tied result ($P = 0.059$, sign test). The mean proportion of S seeds was 0.458. Observations on pollen viability and number showed that there was heterogeneity in number of viable grains per donor in many pollen mixtures, indicating that a 1:1 ratio of S:OC pollen could not be assumed. For example, individual 39 had more than twice the pollen viability of donor 65 as well as 22% more pollen per anther.

The reciprocal nature of the crossing design eliminates such bias when one examines the relative success of a particular donor for a mix when applied to self compared to the outcross individual. For example, donor 39 of the 39/65 mix sired 71.8% of the seeds on shelf and 78% on recipient 65 representing an 8% decrease in success upon selfing. The proportion of progeny sired by the marker donor (individual 1) of each pollen mixture was higher when applied to the outcross recipient than to the self recipient from the mix for seven of the eight recipient pairs ($P < 0.035$, sign test; Fig. 6) with an average 12.9% decline in performance of S relative to OC paternity.

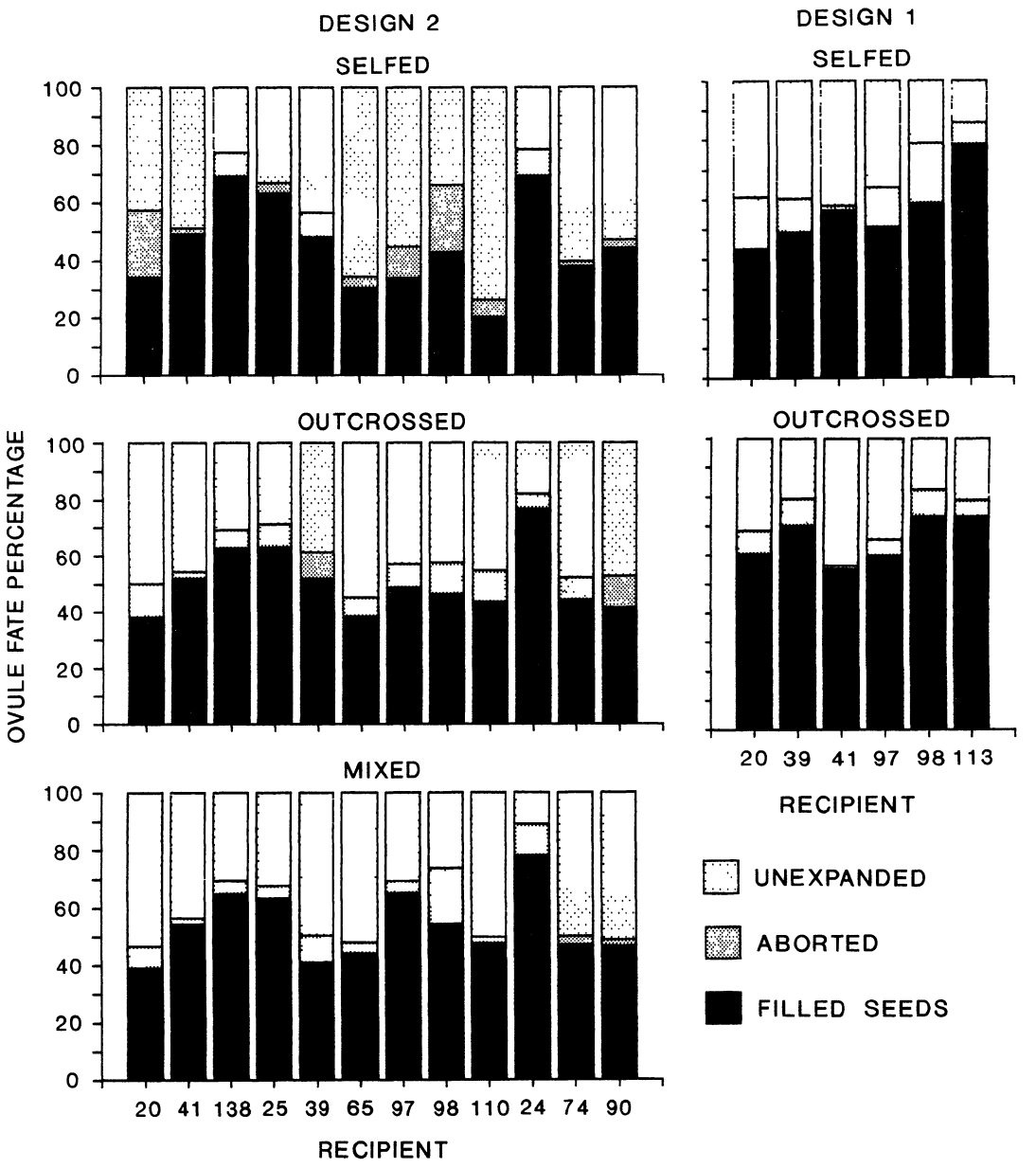

FIG. 4. Cumulative percentages for three classes of ovule fate. Data are pooled across flowers for each recipient. Mixed-donor results represent self + outcross mixtures only. Average sample sizes (fruits/recipient): D1, 8.2, 40.7 for S and OC fruits respectively; D2, 4.7, 8.2, 13.3 for S, OC, and mixed fruits respectively. Average total ovules/flower: 43 and 37 for D1 and D2, respectively.

Expected Success of Self versus Outcross Pollen.—To determine if mixed-donor results were additive and predictable from results of single-donor pollinations, I used paired-comparison ANOVA (Sokal and Rohlf, 1987; p. 354) in a series of tests examining differences between observed and expected angular transformed proportions of selfed seeds, as well as filled seeds, fertilized ovules, and aborted seeds within mixed-donor fruits. There were 16 S plus OC mixed-pollination treatments. The expected proportion of selfed seeds in each treatment was the proportion of selfed seeds in all single-donor fruits [i.e., S seeds/(S + OC seeds)], averaged over replicate flowers.

TABLE 2. Results of one-tailed *t*-tests on chi values for proportion of ovules fertilized (fert. chi) and proportion of fertilized seeds that aborted (abort. chi) after single-donor S versus OC pollination. There was no significant difference in fertilization by S versus OC pollen, but there were significantly more selfed than outcrossed abortions. The null hypothesis is that mean chi is not significantly less than zero. *CP* is the combined probability for two tests.

	Fert. chi				Abort. chi			
	$\bar{x}$	*t*	*df*	*P*	$\bar{x}$	*t*	*df*	*P*
Design 1	−0.500	−0.99	5	ns	−1.383	−2.90	5	**
Design 2	−0.388	−1.36	11	ns	−0.846	−3.20	11	***
CP			4	ns			4	***

* = $P < 0.05$; ** = $P < 0.025$; *** = $P < 0.005$.

For each of the 12 pollen recipients, the expected proportion of ovules that developed into filled seeds in mixed-donor fruits was the average from all single-donor S and OC pollinations for the recipient in question. The expected and observed proportion of fertilized ovules (relative to unexpanded ovules), and of seeds that aborted (relative to filled seeds) in mixed-donor fruits were calculated in the same way.

The ratio of S:OC seeds from the two types of single-donor fruits (S versus OC) averaged 0.473 selfed seeds, while the ratio in multiple-donor fruits (S + OC) averaged 0.458 selfed seeds. Single- and mixed-donor fruits (fruit type) did not differ significantly in the proportions of seeds sired by S relative to OC pollen, nor was there a significant difference among recipients (Fig. 7, paired-comparison ANOVA: fruit type $F_{[1, 15]} = 0.155$, $P > 0.5$; recipient $F_{[15, 15]} = 0.599$, $P > 0.75$). Four of the 12 pollen recipients each received two S plus OC mixtures, but each mixture incorporated a different OC donor. I therefore considered the 16 different mixed-donor by recipient combinations to be independent in this analysis. There was also no significant correlation in proportion of selfed seeds for mixed- and single-donor pollinations ($r = -0.302$, $P > 0.05$) revealing a lack of consistency in which treatment yielded the lower proportion of selfed seeds. These results indicate that OC pollen does not enjoy a further increase in success relative to S pollen when both pollen types share a common pistil.

TABLE 3. Recipient by treatment interaction determined by ANOVA on chi statistics measuring effect of self (S) versus outcross (OC) pollination on seed set, fertilization, and abortion. Top: overall percent seed set, fertilization, and abortion for single-donor S and OC pollinations. Relative differences (%) between S and OC results are shown in parentheses. Data were pooled over flowers and recipients. Bottom: ANOVA results on chi statistics from contingency table analyses of relative seed set (seed chi), ovule fertilization (fert. chi), and seed abortion (abort. chi) for S versus OC single-donor pollinations. *CP* is the combined probability.

	% Seed set		% Fertilization		% Abortion	
	S	OC	S	OC	S	OC
D1:	55.9	65.4 (14.5)	68.0	71.9 (5.4)	17.8	9.1 (48.9)
D2:	47.5	52.4 (9.4)	56.4	59.1 (4.6)	15.8	11.4 (27.8)

	Seed chi			Fert. chi			Abort. chi		
Source	*df*	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Design 1:									
Recipient	5	4.66	**	5	2.98	*	5	2.87	*
Error	43			43			43		
Design 2:									
Recipient	11	0.50	ns	11	0.66	ns	11	1.19	ns
Error	36			36			26		
CP	4		*	4		ns	4		*

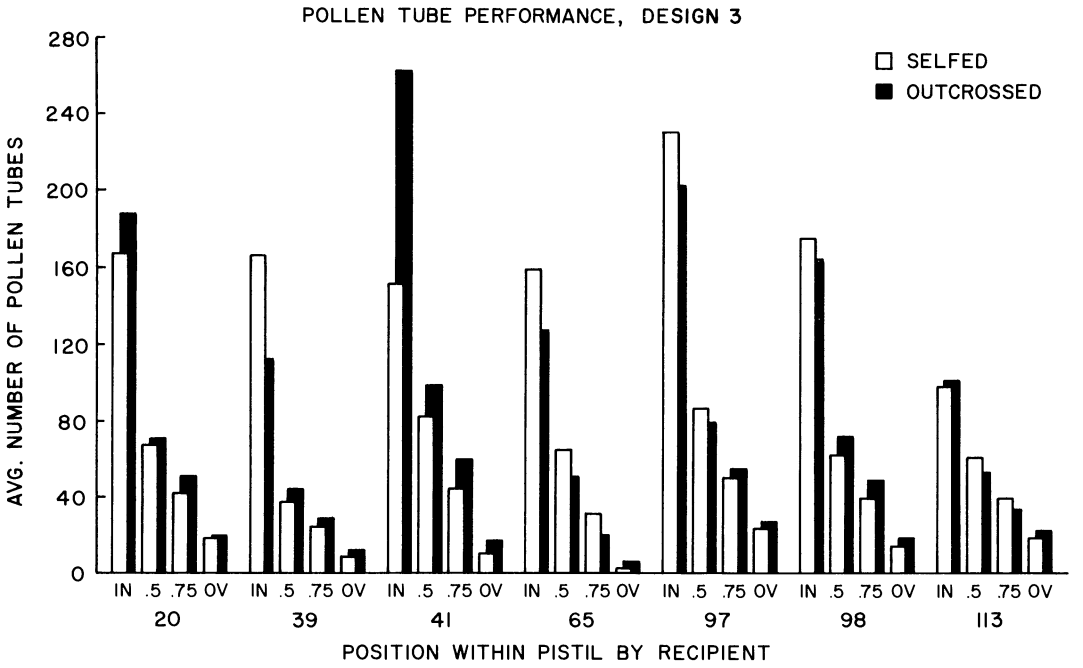

FIG. 5. Average number of pollen tubes at four levels along the style 42 hr after pollination by self versus outcross pollen donors. Initial number of tubes = IN; number at 400 μ (½ distance to ovary) = 0.5; number at 600 μ (¾ distance to ovary) = 0.75; and number reaching the ovary = OV.

The proportion of filled seeds relative to unsuccessful ovules for mixed-donor fruits exceeded the expectations based on single-donor fruits and there were significant differences among recipients (paired ANOVA: fruit type $F_{[1,\,11]} = 8.462$, $P < 0.025$; recipient $F_{[11,\,11]} = 7.842$, $P < 0.001$). Eleven of 12 recipients produced mixed fruits with higher than the expected proportion of filled seeds ($P = 0.003$, sign test). Thus, even though mixed- and single-donor fruits produced similar ratios of S:OC seeds, a larger proportion of ovules developed into filled seeds in mixed fruits.

Neither the proportion of ovules that were fertilized nor the proportion of seeds that aborted differs significantly from the expectations based on single-donor fruits, however in both cases there was significant variation among recipient plants (paired-comparisons ANOVA, ferts: recipient $F_{[11,\,11]} = 9.09$, $P < 0.001$; treatment $F_{[1,\,11]} = 2.70$, $P > 0.10$; aborts: recipient $F_{[11,\,11]} = 5.54$, $P < 0.005$; treatment $F_{[1,\,11]} = 2.07$, $P > 0.10$). For mixed pollinations, 11 of 12 recipients had either a lower expected proportion of abortions ($\bar{x}$ difference = 22%) and/or higher proportion of fertilizations ($\bar{x}$

TABLE 4. Mean number of pollen tubes (±SE) at different levels within the pistil 42 hr following pollination with S versus OC pollen. Initial tubes are near the tip of the stigma and "percent to ovary" is the percentage of initial tubes that reached the ovary. Data are averaged over 49 flowers and 7 pollen recipients ($N = 304$ outcrossed pistils and 49 selfed pistils). The ratio of S:OC tubes indicates the relative performance of S tubes at each level.

Mate type	Mean number of pollen tubes				Percent to ovary
	Initial	400 μ	600 μ	Ovary	
Self	163.84	65.35	38.96	15.00	9.16
	(12.23)	(4.71)	(4.04)	(2.51)	
Outcross	163.80	66.15	42.14	16.63	10.15
	(5.81)	(1.94)	(1.66)	(0.95)	
S:OC	1.000	0.988	0.925	0.902	0.902

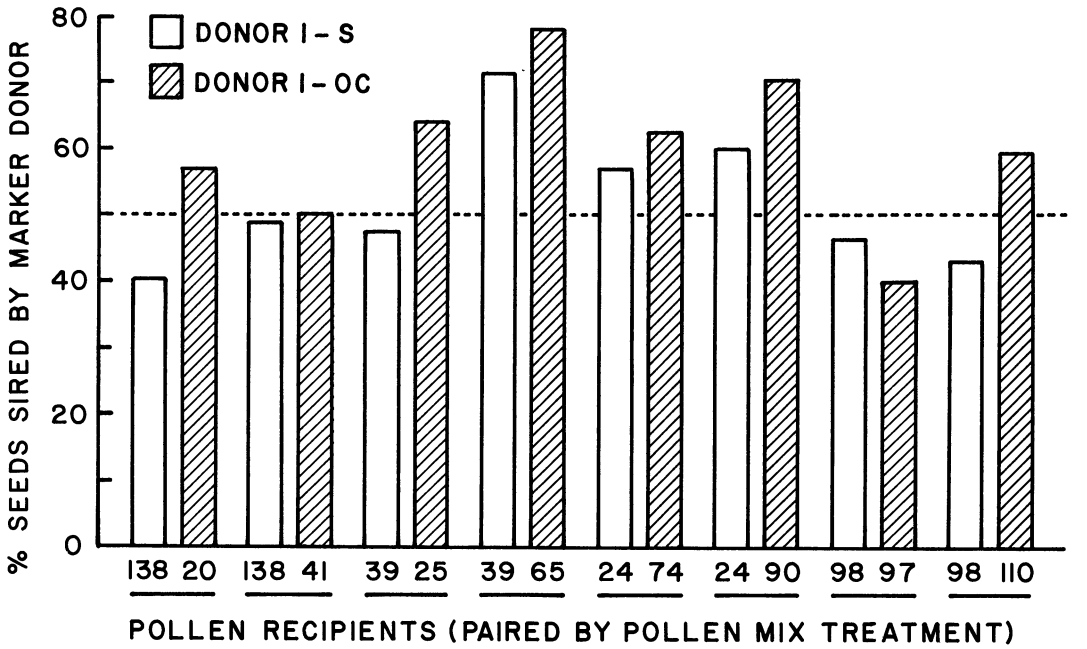

FIG. 6. Proportion of progeny sired by the marker donor (individual 1, see text) of each two-donor mixture in Design 2. Individuals represented in each mixture also acted as pollen recipients and are grouped in pairs along the horizontal axis. The first individual of each pair served as the marker donor. For each pair, the hatched bar represents success of the marker donor as outcross pollen, and the open bar represents its success as self pollen. The dashed horizontal line represents unity in the ratio of S:OC seeds.

difference = 7%). While the relative importance of factors influencing increased seed set differs among individuals, differences in seed number demonstrate a general trend toward reduced fertility for single relative to mixed crosses.

Effect of Pollen Donor Number

To determine if number of sires represented in a fruit potentially influences seed provisioning among fruits (e.g., Marshall and Ellstrand, 1986; Marshall, 1988), I compared individual seed weights resulting from mixed- versus single-donor S and OC pollinations of D2 (Table 5, Fig. 8). Outcrossed seeds weighed more than S seeds, and both S and OC seeds from mixed-donor fruits weighed more than those from single-donor fruits. Seed weights also varied significantly among recipients. Significant recipient × donor, recipient × mate type, and three-way interactions show that there was variation among recipients in response to mate type and donor number. The significant three-way and donor × recipient interactions are likely the result of very low S:OC seed weight for single-donor fruits in recipient 65 (Fig. 8).

Higher than expected percent seed set and seed weight for S/OC mixed-donor fruits suggests that number of sires affects seed set and seed provisioning. I further examined if there was an overall effect of one versus two donors on seed set, percent abortion, and fertilization. Results for S/OC and OC/OC mixtures were combined into a two-donor group, and single-donor S and OC into the single-donor group. Single-donor fruits produced an average of 5.3% fewer seeds, and experienced 4.7% more abortions and 4.4% fewer fertilizations than mixed-donor fruits despite the fact that the single-donor group was overrepresented by outcrossed matings (2:1 versus about 7:5 for multiple donors). Effects of donor number were significant only for seed number. Two-donor fruits, in general, produced significantly more seeds than single-donor fruits, and seed set varied among recipients (ANCOVA where variation in initial ovule number removed as covariate: donors $F_{[1, 11]} = 7.14$, $P = 0.022$; recipient $F_{[11, 311]}$

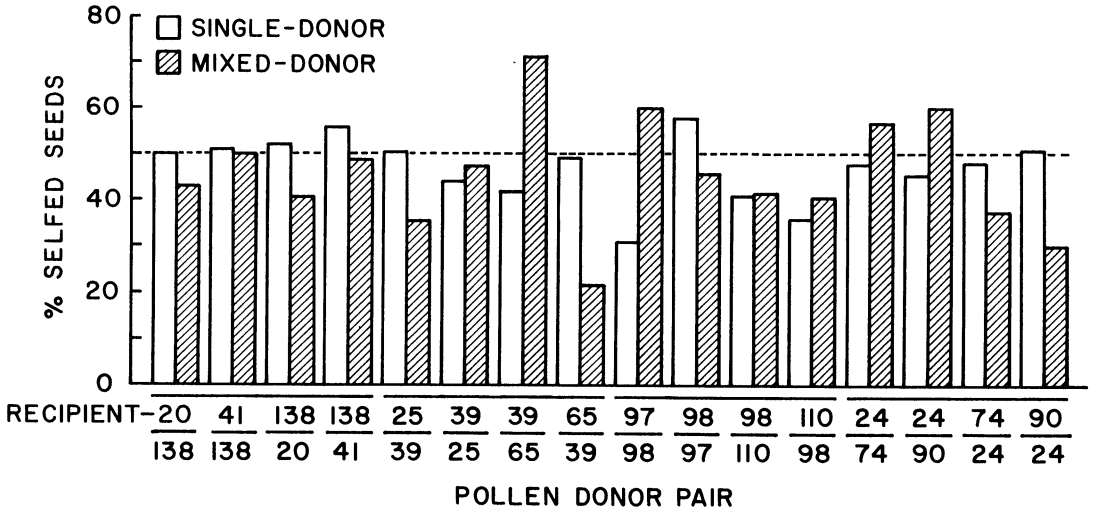

FIG. 7. Comparison of the percentage of seeds sired by self pollen for single-donor (S or OC) and mixed-donor (S plus OC) pollinations for every recipient and pollen pair combination (Design 2). Self donors, and therefore pollen recipients, are represented by the upper number of each pair of pollen donors. Members of a diallel are connected by a continuous line parallel to the horizontal axis. The horizontal dashed line represents unity in S relative to OC seed number.

= 7.33, $P = 0.0001$). Other response variables also differed significantly among recipients (ANOVA on angular transformed data: % aborted, recipient $F_{[11, 314]} = 5.96$, $P = 0.0001$; % fertilized, recipient $F_{[11, 335]} = 7.14$, $P = 0.0001$). There were no significant interactions.

DISCUSSION

Cross Performance for Single-Donor Pollinations

Self pollination resulted in 12% average decreases in percent seed set and 3.3% decreases in mean seed weight/fruit compared to single-donor outcross pollination. Most selection was postzygotic: depressed seed number for selfs was best explained by the significant 38% higher seed abortion rate after selfing, while the 5% reductions in fertilization were not significant, even given the poorer performance of S pollen tubes. Ten percent fewer S than OC pollen tubes reached ovaries in the same amount of time when in separate pistils. Given that tubes were fixed before completing growth, and there was no difference in initial tubes (Table 4), this difference reflects slower growth and/or higher attrition of S tubes, suggesting the presence of weak pseudo SI. Because I applied many more pollen grains to stigmas than there were ovules to fertilize, 10% differences in growth or attrition would not necessarily result in fewer fertilizations by S tubes after single-donor pollination. For such crosses, differential fertilization may only be detectable under conditions of pollen limitation.

Although lower weight of S relative to OC seeds can be explained by inbreeding depression, differences in fertilization sequence generate dominance hierarchies of embryos in fruits of other species (Ganeshaiah and Shaanker, 1988). Brunet (1990) has shown that earlier opening flowers of *A. caerulea* produce more and heavier seeds than later opening flowers, suggesting earlier fertilized ovules may also experience an allocation advantage. Superior growth of OC pollen tubes potentially results in earlier fertilization of ovules. Could OC embryos gain a head start on garnering limited resources if ovules in OC pistils were fertilized earlier than those in S pistils within the same flower? If fertilization sequence of ovules among pistils affects seed provisioning, then earlier pollinated pistils should produce larger, and perhaps more, seeds. In a greenhouse pilot experiment I found no significant difference in either percent seed set or average seed weight per fruit for pistils of single flowers pollinated with a single OC donor at time

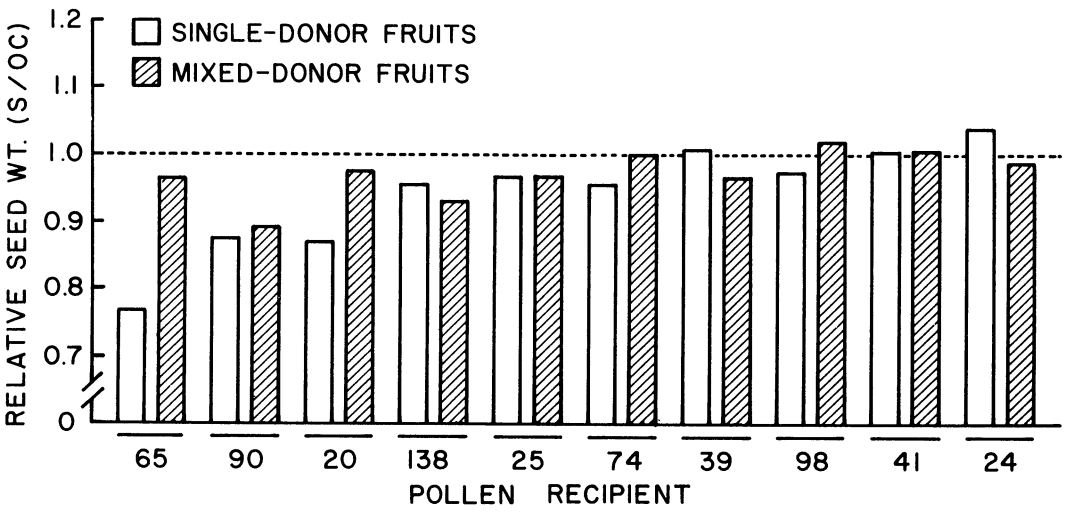

Fig. 8. Selfed relative to outcrossed individual seed weights for singly and multiply sired fruits. Pollen recipients are ordered by increasing mean relative seed weight. The horizontal dashed line represents unity in S relative to OC seed weight. All recipients except for #65 showed small differences in seed weight among single- and mixed-donor fruits and 7 of 12 had heavier seed weights for mixed-donor fruits.

0, 8, 24, or 32 hours ($N = 8$ flowers; ANOVA, angular transformed % seed set $F_{[3, 28]} = 0.05$, $P = 0.99$; average seed weight $F_{[3, 28]} = 0.04$, $P = 0.99$). Because resource allocation among pistils within flowers is independent of pollination time (and likely fertilization sequence), these results indicate that lower selfed seed weights were not due to indirect effects of poorer pollen tube growth.

Evidence suggests seed abortion after selfing in both single- and mixed-donor fruits was a result of inbreeding depression rather than rejection based on interaction of recipient pistil and embryo genotypes. First, aborted seeds were variable in size. Abortions due to late-acting SI are expected to occur at a uniform stage (Seavey and Bawa, 1986). Second, depressions in selfed relative to outcrossed seed weights and seed sets were no larger than the magnitude of inbreeding depression measured for seedling emer-

TABLE 5. ANOVA of individual seed weights (mg^2) resulting from single- versus mixed-donor (donors) and self versus outcross (mate type) pollinations for Design 2 using SAS GLM Type IV sums of squares (see text). Recipients 97 and 110 are excluded because a null allele prohibited matching of seed weights to donors for seeds from those mixed-donor fruits.

	Mean seed weight (mg) using backtransformed means			
Sire	Mixed	*N*	Single	*N*
Self	1.187	(206)	1.135	(98)
Outcross	1.219	(253)	1.209	(189)

Source	*df*	MS	*F*	*P*	r^2
Donors	1	1.3557	6.84	0.028	0.016
Mate type	1	1.9275	7.34	0.024	0.023
Recipient	9	1.3880	16.16	0.0001	0.148
Donors × type	1	0.2063	1.21	0.299	0.002
Donors × recipient	9	0.1982	2.31	0.0147	0.021
Type × recipient	9	0.2627	3.06	0.0013	0.028
Donors × type × recip	9	0.1698	1.98	0.0393	0.018
Model	39	0.6078	7.08	0.0001	0.281
Error	705	0.0859			

gence or survival to three years (Montalvo, 1991). Although Seavey and Bawa (1986) have argued that evidence for late-acting SI operating after fertilization may be rare because it has rarely been considered, Wiens et al. (1987) argue that a combination of inbreeding depression and interovarian competition could explain most differential abortion. In populations of predominantly self-fertile species such as *A. caerulea,* where >80% of selfed ovules successfully matured seeds after single-donor pollination, invoking late-acting SI mechanisms is warranted only if unusually high mutation rates to deleterious recessives are required to account for seed abortions, which was not the case.

Cross Performance for Mixed-Donor Pollinations

Except for differences in pollen tube growth, performance of S and OC single-donor pollinations predicted outcomes after mixed-donor pollination. There were no significant differences in proportions of selfed seeds, fertilized ovules, or aborted seeds for mixed-donor fruits compared to expected values based on averages for single-donor S and OC fruits. If paternity after mixed-donor pollination had been affected predominantly by cryptic SI, then abortion rates should have been lower than expected values due to fewer S fertilizations, and the deficit of selfed seeds should have been even more pronounced than after single-donor pollination. In contrast, if after mixed-donor pollination differential abortion was the most important determinant of seed paternity, then both observed fertilization and abortion rates should have equaled expected values and S:OC seed ratios would have remained the same, unless competition within fruits increased abortion. Competition between developing S and OC seeds could result in an increase in abortion rates of S seeds relative to that in single-donor fruits, decreasing the ratio of S:OC seeds. Lack of significant changes in S:OC seed ratios, fertilization and abortion rates argues that differential abortion due to a constant effect of inbreeding depression best explains the deficit of S seeds observed in mixed-donor fruits, with only a weak influence of pseudo SI.

Studies on other species have detected cryptic SI in vivo where S pollen tubes grew more slowly, had higher attrition rates, or fewer ovule penetrations than OC tubes (Weller and Ornduff, 1977, 1989; Cruzan, 1989; Hessing, 1989), suggesting that when S and OC pollen share a pistil, this may result in fewer fertilizations by S tubes. In *Aquilegia caerulea,* even though differences in S and OC tube growth were small, they were consistent across recipients and were expected to result in increased fertilization success of OC pollen when sharing a common pistil with S pollen; however, there was no evidence that differential tube growth significantly influenced seed paternity after mixed pollination. Thus, cryptic SI does not occur.

Recent studies on two other self-fertile species also indicate that predicting paternity from pollen tube performance after single-donor pollinations may not be appropriate without verifying results with paternity analysis. Aizen et al. (1990) found that when S and OC pollen were placed on separate stigmas of a common pistil in *Dianthus chinensis,* differences in growth rate for S and OC pollen were even greater than when growing in styles of separate flowers. In contrast, Cruzan (1990*a*) found inhibition of OC tubes from focal donors when sharing a style with S pollen. Such results suggest that the differences observed in tube growth after single-donor pollinations in *A. caerulea,* or lack of differences observed in species such as *Chamaecrista fasciculata* (Fenster and Sork, 1988), do not necessarily predict success of S tubes after mixed-donor pollination.

Effect of Pollen Donor Number

Mixed-donor fruits received a higher allocation of resources per seed: both S and OC seeds from mixed-donor fruits weighed more than S and OC seeds from single-donor fruits, and mixed-donor pollinations produced a significantly higher percentage of filled seeds than single-donor pollinations. Increased seed sets could be explained by a slight increase in fertilization combined with a slight decrease in abortion for most recipients, but not by any one factor independently. Marshall (1988) argued that similar increases in seed number and weight in wild radish were more likely due to selective

provisioning by the maternal parent than by changes in competition among embryos. Seeds accompanied by diverse siblings in the same fruit may be each capable of sequestering more resources because of lessened competition such as occurs when seedlings of different genotypes are grown together rather than alone (Allard and Adams, 1969; Price and Waser, 1982). It was not possible to distinguish between these alternatives for *A. caerulea* based on present data.

Postpollination Selection: Potential in Nature

In my study, all data were from pollinations and seeds matured on plants in their native population allowing observation of the extent to which postpollination events influence paternity under natural environmental conditions. Even though the effect of inbreeding on seed production was small relative to differences among maternal parents, the effect on cumulative inbreeding depression was considerable (Montalvo, 1991). In the field, non-random success of pollen in mixtures was also documented with self-sterile *Campsis radicans* (Bertin and Sullivan, 1988; Bertin et al., 1989) and with self-fertile *Erythronium grandiflorum* (Cruzan, 1990*b*). As in the present study, both found that selection was primarily postzygotic, but the lack of inbreeding depression could be only addressed for *C. radicans* because the latter study used only OC/OC mixtures. Other studies using progeny testing have shown that postpollination factors can affect seed paternity after mixed-donor pollination in self-fertile plants (for wild collected genotypes under controlled conditions: Weller and Ornduff, 1977; Glover and Barrett, 1986; Bowman, 1987; Epperson and Clegg, 1987; Casper et al., 1988; for cultivars: Bateman, 1959; Currah, 1981; Levin, 1975). However, the relative effects of inbreeding depression and SI have been rarely documented (e.g., Glover and Barrett, 1986; Casper et al., 1988; Weller and Ornduff, 1989, 1991; Manasse and Pinney, 1991), and the importance of such factors in nature is largely unknown. Variation in stress and nutrient status of parents is known to have a potentially large influence on seed weight and seed set of pollen recipients (Smith-Huerta and Vasek, 1987; Roach and Wulff, 1988), and on competitive ability of pollen (Young and Stanton, 1990). My study exposed significant genetic effects on pollen success despite natural random effects of environment and large maternal effects.

There are other reasons for variation among pollen recipients in terms of the success of self relative to outcross pollen. For example, I did not control for inbreeding histories of parents, so variation in individual heterozygosity and relatedness among parents was likely. Differences among individuals in seed provisioning and abortion due to inbreeding depression are therefore possible. Also, S relative to OC pollen success on a single maternal parent may change with relatedness to the OC donor.

Inbreeding depression in seed development and size could have important evolutionary consequences if these traits influence total fitness (e.g., Kalisz, 1989). Large cumulative inbreeding depression from seed development to progeny reproduction is expected to promote evolution of specific mechanisms that reduce self-fertilization, leading to increases in outcrossing rate and possibly biasing seed provisioning in favor of outcrossed seeds if seed weight influences seedling success. However, with cumulative inbreeding depression of >0.5 measured through the third year of progeny growth (Montalvo, 1991), outcrossing rates in *A. caerulea* can be low. This may mean that factors in addition to the transmission bias of selfing genes are important in promoting selfing (e.g., reproductive assurance, see Jain, 1976; Piper et al., 1986; Charlesworth and Charlesworth, 1990), or that genetic factors beside inbreeding depression per se are involved (Holsinger, 1988).

The large potential influence of both environmental factors and inbreeding histories on inbreeding depression may indirectly influence the evolution of self-discrimination mechanisms. These factors can be expected to vary among populations. Future investigations of the effect of environmentally induced stress on inbreeding depression, pseudo and cryptic SI, and maternal control over seed provisioning are warranted.

ACKNOWLEDGMENTS

I thank L. Nunney, R. Shaw, and D. Reznick for statistical advice, J. Clegg and C. Sassaman for advice on electrophoresis, B. Devlin, N. Ellstrand, R. Mitchell, M. Price, R. Shaw, and N. Waser for many fruitful discussions, and J. Ackerman, K. Bill, and D. Noble for field assistance. I especially thank S. Barrett, C. Eckert, N. Ellstrand, B. Epperson, R. Goldingay, R. Mitchell, L. Nunney, R. Shaw, N. Waser, A. Weis, and several anonymous reviewers for constructive criticism of the manuscript. L. Nunney suggested the analysis of chi-values. The Rocky Mountain Biological Laboratory provided valuable research facilities. This work was funded by an NSF Dissertation Improvement Grant (BSR-8700870), the University of California, Riverside (UCR) Chancellor's Patent Fund, a UCR, Department of Biology I. M. Newell Graduate Research Award, a UCR Academic Senate Intercampus Research Opportunity Grant, a Sigma Xi Grant-in-Aid, and Harriet Barclay and Lee Snyder Memorial Grants for Graduate Student Research at the RMBL. During this study I was supported by a Ford Foundation Dissertation Fellowship and a UCR Graduate Council Predoctoral Research Fellowship.

LITERATURE CITED

AIZEN, M. A., K. B. SEARCY, AND D. L. MULCAHY. 1990. Among- and within-flower comparisons of pollen tube growth following self- and cross-pollinations in *Dianthus chinensis* (Caryophyllaceae). Am. J. Bot. 77:671–676.

ALLARD, R. W., AND J. ADAMS. 1969. The role of intergenotypic interactions in plant breeding. Proc. XII Intern. Congr. Genetics 3:349–370.

BARRETT, S. C. H. 1988. The evolution, maintenance, and loss of self-incompatibility systems, pp. 98–124. *In* J. Lovett Doust and L. Lovett Doust (eds.), Plant Reproductive Ecology. Oxford University Press, NY USA.

BATEMAN, A. J. 1956. Cryptic self-incompatibility in the wallflower: *Cheiranthus cheiri* L. Heredity 10: 257–267.

BERTIN, R. I. 1985. Nonrandom fruit production in *Campsis radicans*: Between-year consistency and effects of prior pollination. Am. Nat. 126:750–759.

BERTIN, R. I., C. BARNES, AND S. I. GUTTMAN. 1989. Self-sterility and cryptic self-fertility in *Campsis radicans* (Bignoniaceae). Bot. Gaz. 150:397–403.

BERTIN, R. I., AND M. SULLIVAN. 1988. Pollen interference and cryptic self-fertility in *Campsis radicans*. Am. J. Bot. 75:1140–1147.

BOWMAN, R. N. 1987. Cryptic self-incompatibility and the breeding system of *Clarkia unguiculata* (Onagraceae). Am. J. Bot. 74:471–476.

BREWBAKER, J. L., AND B. H. KWACK. 1963. The essential role of calcium ion in pollen germination and pollen tube growth. Am. J. Bot. 50:859–865.

BROWNLEE, K. A. 1965. Statistical Theory and Methodology in Science and Engineering, Second Edition. John Wiley and Sons, Inc., N.Y., USA.

BRUNET, J. 1990. Gender specialization of flowers within inflorescences of hermaphroditic plants. Ph.D. Diss. State University of New York at Stony Brook, NY USA.

CASPER, B. B., L. S. SAYIGH, AND S. S. LEE. 1988. Demonstration of cryptic incompatibility in distylous *Amsinckia douglasiana*. Evolution 42:248–253.

CHARLESWORTH, D., AND B. CHARLESWORTH. 1987. Inbreeding depression and its evolutionary consequences. Annu. Rev. Ecol. Syst. 18:237–268.

———. 1990. Inbreeding depression with heterozygote advantage and its effect on selection for modifiers changing the outcrossing rate. Evolution 44: 870–888.

CHARLESWORTH, D., D. W. SCHEMSKE, AND V. L. SORK. 1987. The evolution of plant reproductive characters: Sexual versus natural selection, pp. 317–336. *In* S. Stearns (ed.), Evolution of Sex and Its Consequences. Birkhauser Verlag, Basel, Switzerland.

CRUZAN, M. B. 1989. Pollen tube attrition in *Erythronium grandiflorum*. Am. J. Bot. 76:562–570.

———. 1990*a*. Pollen-pollen and pollen-style interactions during pollen tube growth in *Erythronium grandiflorum* (Liliaceae). Am. J. Bot. 77:116–122.

———. 1990*b*. Variation in pollen size, fertilization ability, and postfertilization siring ability in *Erythronium grandiflorum*. Evolution 44:843–856.

CURRAH, L. 1981. Pollen competition in onion (*Allium cepa* L.). Euphytica 30:687–696.

ELLSTRAND, N. C. 1984. Multiple paternity within the fruits of the wild radish, *Raphanus sativus*. Am. Nat. 123:819–828.

EPPERSON, B. K., AND M. T. CLEGG. 1987. First-pollination primacy and pollen selection in the morning glory, *Ipomoea purpurea*. Heredity 58:5–14.

EVERITT, B. S. 1977. The Analysis of Contingency Tables. Chapman and Hall, John Wiley and Sons Inc., N.Y., USA.

FALCONER, D. S. 1981. Introduction to Quantitative Genetics. Second edition. Longman Group, Essex, UK.

FENSTER, C. B., AND V. L. SORK. 1988. Effect of crossing distance and male parent on in vivo pollen tube growth in *Chamaecrista fasciculata*. Am. J. Bot. 75: 1898–1903.

FISHER, R. A. 1941. Average excess and average effect of a gene substitution. Ann. Eugen. 11:53–63.

GANESHAIAH, K. N., AND R. U. SHAANKER. 1988. Seed abortion in wind-dispersed pods of *Dalbergia sissoo*: Maternal regulation or sibling rivalry? Oecologia 77:135–139.

GLOVER, D. E., AND S. C. H. BARRETT. 1986. Variation in the mating system of *Eichhornia paniculata* (Spreng.) Solms. (Pontederiaceae). Evolution 40: 1122–1131.

HESSING, M. B. 1989. Differential pollen tube success in *Geranium caespitosum*. Bot. Gaz. 150:404–410.

HOLSINGER, K. E. 1988. Inbreeding depression doesn't matter: The genetic basis of mating-system evolution. Evolution 42:1235–1244.

JAIN, S. K. 1976. The evolution of inbreeding in plants. Annu. Rev. Ecol. Syst. 7:469–495.

JOHANSEN, D. A. 1940. Plant Microtechnique. McGraw-Hill, N.Y., USA.

KALISZ, S. 1989. Fitness consequences of mating system, seed weight, and emergence date in a winter annual, *Collinsia verna*. Evolution 43:1263–1272.

LEVIN, D. A. 1975. Gametophytic selection in *Phlox*, pp. 207–217. *In* D. L. Mulcahy (ed.), Gamete Competition in Plants and Animals. North-Holland Publ. Co., Amsterdam, The Netherlands.

LEWONTIN, R. C., AND M. J. D. WHITE. 1960. Interaction between inversion polymorphisms of two chromosome pairs in the grasshopper, *Moraba scurra*. Evolution 14:116–129.

LYONS, E. E., N. M. WASER, M. V. PRICE, J. ANTONOVICS, AND A. MOTTEN. 1989. Sources of variation in plant reproductive success and implications for concepts of sexual selection. Am. Nat. 134:409–433.

MANASSE, R. S., AND K. PINNEY. 1991. Limits to reproductive success in a partially self-incompatible herb: Fecundity depression at serial life-cycle stages. Evolution 45:712–720.

MARSHALL, D. L. 1988. Postpollination effects on seed paternity: Mechanisms in addition to microgametophyte competition operate in wild radish. Evolution 42:1256–1266.

MARSHALL, D. L., AND N. C. ELLSTRAND. 1986. Sexual selection in *Raphanus sativus*: Experimental data on nonrandom fertilization, maternal choice, and consequences of multiple paternity. Am. Nat. 127: 446–461.

MILLER, R. B. 1978. The pollination ecology of *Aquilegia elegantula* and *A. caerulea* (Ranunculaceae) in Colorado. Am. J. Bot. 65:406–414.

———. 1981. Hawkmoths and the geographic patterns of floral variation in *Aquilegia caerulea*. Evolution 35:763–774.

MONTALVO, A. M. 1991. Postpollination selection, progeny performance, and the genetic basis of fitness traits in *Aquilegia caerulea* (Ranunculaceae). Ph.D. Diss. University of California, Riverside CA USA.

MULCAHY, D., AND G. B. MULCAHY. 1985. Gametophytic self-incompatibility or the more things change . . . Heredity 54:139–144.

DE NETTANCOURT, D. 1977. Incompatibility in Angiosperms. Springer-Verlag, N.Y., USA.

PIPER, J. G., B. CHARLESWORTH, AND D. CHARLESWORTH. 1986. Breeding system evolution in *Primula vulgaris* and the role of reproductive assurance. Heredity 56:207–217.

PRICE, M. V., AND N. M. WASER. 1982. Population structure, frequency-dependent selection, and the maintenance of sexual reproduction. Evolution 36: 35–43.

ROACH, D. A., AND R. WULFF. 1987. Maternal effects in plants. Annu. Rev. Ecol. Syst. 18:209–235.

SAS INSTITUTE. 1985. SAS User's Guide: Statistics, Version 5 Edition. SAS Institute, Inc., Cary, NC USA.

SEAVEY, S. R., AND K. S. BAWA. 1986. Late-acting self-incompatibility in angiosperms. Bot. Rev. 52: 195–219.

SMITH-HUERTA, N. L., AND F. C. VASEK. 1987. Effects of environmental stress on components of reproduction in *Clarkia unguiculata*. Am. J. Bot. 74:1–8.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry, Second Edition. W. H. Freeman and Company, N.Y., USA.

STEPHENSON, A. G., AND R. I. BERTIN. 1983. Male competition, female choice, and sexual selection in plants, pp. 109–149. *In* L. Real (ed.), Pollination Biology. Academic Press, Orlando, FL USA.

UYENOYAMA, M. K. 1988. On the evolution of genetic incompatibility systems. III. Introduction of weak gametophytic self-incompatibility under partial inbreeding. Theor. Popul. Biol. 34:47–91.

WASER, N. M. 1992. Population structure, optimal outbreeding, and assortative mating in angiosperms. *In* N. W. Thornhill (ed.), The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives. University of Chicago Press, Chicago, IL USA. *In press.*

WASER, N. M., M. V. PRICE, A. M. MONTALVO, AND R. N. GRAY. 1987. Female mate choice in a perennial herbaceous wildflower, *Delphinium nelsonii*. Evol. Trends. Plants 1:29–33.

WELLER, S. G., AND R. ORNDUFF. 1977. Cryptic self-incompatibility in *Amsinckia grandiflora*. Evolution 31:47–51.

———. 1989. Incompatibility in *Amsinckia grandiflora* (Boraginaceae): Distribution of callose plugs and pollen tubes following inter- and intramorph crosses. Am. J. Bot. 76:277–282.

———. 1991. Pollen tube growth and inbreeding depression in *Amsinckia grandiflora* (Boraginaceae). Am. J. Bot. 78:801–804.

WIENS, D., C. L. CALVIN, C. A. WILSON, C. I. DAVERN, D. FRANK, AND S. R. SEAVEY. 1987. Reproductive success, spontaneous embryo abortion, and genetic load in flowering plants. Oecologia 71:501–509.

WILLSON, M. F., AND N. BURLEY. 1983. Mate Choice in Plants: Tactics, Mechanisms, and Consequences. Princeton University Press, Princeton, NJ USA.

YOUNG, H. J., AND M. L. STANTON. 1990. Influence of environmental quality on pollen competitive ability in wild radish. Science 24:1631–1633.

Corresponding Editor: S. C. H. Barrett