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POSTFERTILIZATION CAUSES OF DIFFERENTIAL SUCCESS OF POLLEN DONORS IN *ERYTHRONIUM GRANDIFLORUM* (LILIACEAE): NONRANDOM OVULE ABORTION[1]

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Seed paternity in *Erythronium grandiflorum* does not fully reflect the proportion of pollen on the stigma. When two types of pollen are simultaneously applied to the stigma, outbred seeds are produced over inbred, and seeds from more distant donors are produced over seeds from donors nearby. I looked for postfertilization causes of these previously reported patterns of differential success of pollen donors. I simultaneously pollinated stigmas with pollen from two donors and observed ovule development through a window sliced in the ovary. Pollen donor pairs were self and cross, donors 1 and 100 m from the recipient, and two donors each 100 m from the recipient. Since one donor was always the alternate homozygote from the recipient at the malate dehydrogenase locus, I could determine the paternity of developing seeds. When it appeared that ovules were aborting, I removed them and determined their paternity using starch gel electrophoresis. Ovules fertilized by self pollen were more likely to abort than ovules fertilized by cross pollen, and ovules fertilized by nearby donors were more likely to abort than ovules fertilized by distant donors. Ovules fertilized by donors 100 m from the recipient were equally likely to abort. There was not a significant relationship between the proportion of ovules fertilized by a pollen donor and the probability of those fertilized ovules developing into seeds. There was no relationship between ovule position within a fruit and ovule abortion. I manipulated available resources by removing leaves and by permitting only one fruit to develop per plant. Decreasing the amount of resources increased the proportion of aborted ovules. Abortion of ovules of lesser quality appears to release resources that can then be used to develop other offspring.

Pollen donors may sire a lower percentage of seeds within a fruit than expected based on the number of their pollen grains on the stigma. Within a fruit, outbred seeds are frequently produced in preference to inbred seeds (Pfahler, 1965; Busbice, 1967; Glover and Barrett, 1986; Bowman, 1987; Griffin, Moran, and Fripp, 1987; reviewed in Marshall and Folsom, 1991; Montalvo, 1992; Rigney et al., 1993), and intermorph pollen donors may sire more seeds than self or intramorph donors in heterostylous species (Weller and Ornduff, 1977; Casper, Sayigh, and Lee, 1988; Cruzan and Barrett, 1993).

Greater success of one type of donor over another can be caused by differences in growth rates of pollen tubes (Darwin, 1876; Bateman, 1956; de Nettancourt, 1977; Lewis, 1979). Another potential mechanism for differential donor success is nonrandom ovule abortion. Postfertilization sorting of embryos can be caused by several mechanisms (Marshall and Folsom, 1991). First, embryos of lower genetic quality may abort due to the expression of mutational load (Charlesworth and Charlesworth, 1987; Wiens et al., 1987; Charlesworth, 1989). Second, embryos may abort because of competition for limited maternal resources (Kambal, 1969; Lloyd, 1980; Kress, 1981; Westoby and Rice, 1982; Willson and Burley, 1983; Charlesworth, 1989). Essential resources may not be equally available throughout the fruit, and ovule abortion may be affected by the position of the developing ovule within the fruit (Horovitz, Meiri, and Beiles, 1976; Watson and Casper, 1984; Lee and Bazzaz, 1986; Marshall and Ellstrand, 1988; Nakamura, 1988; Rocha and Stephenson, 1990). Pollen donors may subsequently fertilize eggs at different positions within the ovary (Cooper and Brink, 1940; Horovitz, Meiri, and Beiles, 1976; Marshall and Ellstrand, 1988; Rocha and Stephenson, 1990). The subsequent nonrandom abortion of ovules with respect to position within the fruit may confound direct effects of embryo genotype with genotype-by-position interactions (Marshall and Ellstrand, 1988; Rocha and Stephenson, 1990). Variation in the time of fertilization within an ovary might affect the ability of late-fertilized eggs to garner resources and these eggs could be aborted if maternal resources are limiting (Kambal, 1969; reviewed in Watson and Casper, 1984). In *Cryptantha flava,* a fixed percentage of the developing ovules always abort, suggesting that abortion may be an adaptation for dispersal by wind (Casper and Wiens, 1981).

While experiments indirectly suggest that ovule abortion is nonrandom with respect to embryo genotype or quality (Stephenson and Winsor, 1986; Casper, 1988; Marshall and Ellstrand, 1988; Rocha and Stephenson, 1991), the paternity of aborting ovules has never been directly determined. To directly assess whether or not

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ovule abortion is random with respect to an embryo's male parent, the genotype of the aborting ovules needs to be determined. Established techniques for assigning seed paternity (e.g., Meagher, 1986; Devlin, Clegg, and Ellstrand, 1992) cannot answer this question because aborted ovules are, by definition, unavailable for analysis of paternity.

In *Erythronium grandiflorum* var. *grandiflorum* Pursh. (Liliaceae), mating is nonrandom with respect to pollen donor type. When plants are pollinated with a mixture of self and cross pollen, seeds are sired predominantly by cross pollen. Similarly, when pollen from donors 1 m from recipients competes with pollen from donors 100 m away, seeds are mainly sired by the distant donor (Rigney et al., 1993). Some of the variation in success between self and cross pollen is due to differences in the number of pollen tubes that reach the base of the style. Following pure self and cross pollinations, Cruzan (1989) found that self pollen tubes were much less likely to reach the base of the style than cross pollen tubes, when self and cross pollen was applied to the stigma. However, variation in the number of tubes at the base of the style accounted for only a portion of the variation in seed set.

The goals of this study were to determine 1) whether ovules fertilized by self pollen are more likely to be aborted than ovules fertilized by cross donors, 2) whether ovules fertilized by pollen from donors 1 m away are more likely to be aborted than seeds fathered by donors at 100 m, and 3) whether the amount of available maternal resources and position within the fruit affects ovule abortion. My experiments also reflect the use of a surgical technique that allows for the determination of the genotype of aborting ovules.

MATERIALS AND METHODS

Study organism—*E. grandiflorum* is a self-compatible perennial herb common in open meadows and aspen woodlands in the Rocky Mountains. Flower number can vary from 0 to 14 with zero-, one-, two-, and three-flowered plants the most common. Flowers are weakly protandrous; three or more of the six anthers have usually dehisced by the time the stigmas are receptive (Cruzan, 1989). Self pollination may be autogamous or geitonogamous (in plants with two or more flowers). The ovary is a capsule, somewhat triangular in cross-section, and contains approximately 60 ovules in three locules.

Effect of interparent distance on ovule abortion—Experiments were conducted at three sites in Gunnison County, Colorado during three summers: 1) Washington Gulch in 1989 (altitude 3,200 m); 2) Kebler Pass in 1990 (altitude 3,280 m); and 3) Lake Irwin in 1991 (altitude 3,280 m). Experiments were conducted in different years at different sites because capricious spring frosts frequently eliminated all fruit set at some sites.

At each site, two-flowered plants were haphazardly chosen in clusters 10 and 100 m apart and the genotype of each was determined at the malate dehydrogenase (MDH) locus. This enzyme is a neutral marker with each of the two alleles present in equal proportions in the populations studied (Rigney, unpublished data). Only homozygous plants were used as recipients or donors in these experiments. Each recipient received pollen from two pollen donors; one donor was always the alternate homozygote to the recipient. Developing ovules fertilized by this donor would be heterozygous for MDH, while ovules fertilized by the other donor would be homozygous. I used only the first of the two flowers as a recipient.

To prevent contamination of the stigmas by self or other unwanted pollen, I covered the pistil with either a short (2 cm) section of a plastic drinking straw or a grass stem. This technique makes bagging unnecessary and allows self pollen to remain available in the anthers.

I applied approximately equal amounts of pollen from each of two donors to the points of a pair of fine watchmaker's forceps, and applied the mixed pollen load to stigmas of the first flower of a plant. Pollen combinations were: self and cross pollen from donors at various distances from the recipient; pollen from donors 1 and 100 m from the recipient; and pollen from two donors, each 100 m from the recipient. The second flower was removed to increase the probability of fruit set in the first flower.

Ovary window technique—Approximately 4 weeks after pollination, when fruits had almost achieved their maximal length (approximately 45 mm), I sliced a longitudinal section in one locule wall, approximately 20 mm long by 5–8 mm wide, using a sharp razor blade. This "window" allowed me to observe the developing ovules within one locule. Surprisingly, the ovules within that locule, and ovules in the locules with intact ovary walls, developed normally without any additional protection. Fully mature fruits contain the following three categories of ovules (in descending order of size class): plump, fully developed (green) seeds, brown, flattened and shriveled ovules, and very small, white ovules (usually 1–2 mm long). Fully developed seeds are 5–6 mm in length and 1–2 mm wide. The brown, flattened ovules range in size from 3 mm to 6 mm. I interpret the brown and shriveled category as ovules with fertilized eggs that started to develop and then aborted, since they have increased in size over virgin ovules. The small, white structures appear to be ovules with eggs that were never fertilized (Motten, 1983) because they are no larger than virgin ovules in unpollinated flowers.

Seeds were fully matured about 8 weeks after pollination. Growth of ovules ceased as early as 22 days after pollination with some ovules ceasing to expand within 1 week of complete fruit maturity. I inferred that these ovules were aborting and that they would eventually shrivel and turn brown. Beginning 6 weeks after pollination and continuing almost to complete fruit maturation, I removed the presumptively aborting ovules with fine forceps from the locule with the window. The ovules were placed immediately in 50 μl of extraction buffer containing Tris-HCl, polyvinyl pyrrolidine (pH 8.0), and beta-mercaptoethanol, placed on ice, and taken back to the lab where they were immediately ground using a frozen mortar and pestle. Genotypes at the MDH locus were determined by starch gel electrophoresis (Rigney et al., 1993).

In addition to determining the paternity of aborting ovules, I determined the paternity of 15 mature seeds from each of the fruits from which I had removed developing ovules. Electrophoresis of mature seeds was performed in the same manner as described above, except that the seeds were soaked for 1 to 2 weeks in distilled water prior to grinding in the extraction buffer.

If two types of pollen present on the stigma are equal in their abundance, ability to germinate, grow pollen tubes, fertilize ovules, and develop seeds, then the proportions of aborted ovules should be equal. Although the hand-pollination procedure did not assure *exact* equality of pollen loads, it should not have introduced a directional bias. *G*-tests of heterogeneity were used to determine whether certain classes of pollen donors were more likely to be aborted than others (Sokal and Rohlf, 1981). A *G*-test (Sokal and Rohlf, 1981, pp. 744–747) was used to test whether the status of an ovule (matured into a seed or aborted) was independent of paternity type for pollen combinations of self and cross pollen and for 1 and 100 m. I combined all of the aborted ovules and matured seeds from all fruits in both pollen combinations for this analysis. Since the assignment of donors in each pair of 100-m donors was arbitrary, I tested the independence of ovule status by paternity type by analyzing 2 × 2 tables on each fruit separately. I used Fisher's Exact Test (Statxact, 1989) for the analysis of the 100-m crosses, because sample sizes of aborted ovules were low in some fruits (Sokal and Rohlf, 1981).

The hypothesis that successful fertilization equals paternity was tested by examining the relationship between the proportion of ovules fertilized by one donor in a fruit (number of ovules fertilized by donor X/total number of ovules per fruit) and the probability of those fertilized ovules developing into seeds (number of seeds sired by donor X/number of ovules fertilized by donor X). I used the number of aborted ovules plus the number of mature seeds from all of the above crosses combined as an estimate of the number of fertilized ovules.

To test whether the opening in the ovary wall affected the number of ovules developed into seeds and/or aborted, I applied cross pollen to the stigmas of 20 flowers, one per plant, at Washington Gulch in 1990, cut windows in one locule, and removed aborted ovules from one locule as described above. When these fruits were mature, I determined the percentage of ovules developed and aborted in the locule with the ovary window (cut) and in the unmanipulated (uncut) locule.

To test the hypothesis that there was a difference in the percentages of aborted ovules between the cut and uncut locules, I used the GLM procedure in SAS (SAS Institute, Inc., 1985). Plant identity was designated a random factor, and locule treatment (cut vs. uncut locule), a fixed factor, was tested over the interaction (Sokal and Rohlf, 1981). The percentages were transformed to achieve normality using the arcsine-square transformation.

Time of expression of MDH—To determine whether a homozygous embryo was inbred or outbred with only the maternal allele being expressed, it was essential to determine the developmental stage when *both* the maternal and paternal alleles were expressed. I determined this by applying outcross pollen from one donor to the stigmas of 37 first flowers. The second flower was removed, as above. The donor and the recipient were reciprocal homozygotes at the MDH locus; therefore, all ovules were heterozygous, but would appear to be homozygous until the paternal allele was expressed. Twenty-two days after pollination, I cut the ovary windows and collected developing ovules every 3–5 days, until 47 days after pollination. It is difficult to distinguish aborting ovules from healthy ones until about 3 weeks after pollination. The ovules were assayed and scored for MDH expression.

The hypothesis that the percentage of ovules that were homozygous remained equal over time was tested using Cochran's Q (Sokal and Rohlf, 1981). Cochran's test requires two attributes; samples of two ovules that included one or two heterozygotes were assigned a value of 1 and samples without any heterozygotes were assigned a value of 0. Most ovules electrophoresed after 29 days, and all ovules after 47 days expressed both the maternal and paternal genes (Cochran's $Q = 1070$, $P < 0.001$, $N = 21$). Because of this delay in expression of MDH, all results presented in this paper concern only aborted ovules that had been pollinated at least 47 days previously.

Effect of maternal resources on ovule abortion—In 1988, 24 groups of six two-flowered plants were chosen at Kebler Pass. To determine whether limited resources affected production of seeds, I manipulated resource levels by removing leaves and by allowing only one fruit to develop per plant. Leaves were kept trimmed as near to the ground as possible throughout the summer. I used the GLM procedure in SAS (SAS Institute, Inc., 1985) to determine whether there was an effect of these treatments on the number of ovules fertilized and aborted, and the number of mature seeds.

Effect of position within the fruit on ovule abortion—To determine if position of an ovule in an ovary had an effect on the abortion, I divided each exposed locule latitudinally into four quarters and counted the total number of ovules and aborted ovules in each quarter. I collected fruits from eight plants that received natural pollination and from 56 plants that were hand pollinated with equal amounts of self and cross pollen and recorded the position of aborted ovules. I also determined the genotype of aborted ovules from a subset of the hand-pollinated plants.

To test the null hypothesis that abortion was equal in all four quarters of the fruit, I used a Goodness-of-Fit test (Sokal and Rohlf, 1981). To determine whether abortion in different regions of the fruit was independent of the genotype of the embryo, I used Fisher's Exact Test in a 2 × 2 table with genotype and region as the two factors (Statxact, 1989).

RESULTS

Effect of interparent distance on ovule abortion—When both self and cross pollen were present on the stigma, inbred ovules aborted more frequently than outbred ovules (Tables 1, 2). Following simultaneous application of pollen from donors 1 and 100 m from the recipient, ovules fertilized by nearby pollen donors were significantly more likely to abort than those fertilized by distant donors (Table 2). There was no difference in ovule abortion when both donors were 100 m from the recipient ($G_{\text{heterogeneity}}$, Table 2).

Nonrandom ovule abortion is most interesting if the ratio of paternity among mature ovules (seeds) is different

TABLE 1. Numbers of aborted ovules removed from one locule per fruit. Stigmas were simultaneously hand pollinated with two pollen donors. Pollen donors were self and outcross, 1 and 100 m, and two donors each 100 m from the recipient.

Self	Outcross	1 m	100 m	100 m	100 m
1	6	8	3	6	1
6	0	3	0	3	2
0	1	1	5	0	2
6	1	4	0	0	4
4	0	2	0	2	1
1	0	5	0	1	0
1	0	3	0	3	0
1	0	1	0	4	1
2	0			0	1
1	0			1	0
5	2				
0	3				
4	0				
0	1				
5	2				
3	7				
2	0				
2	0				
11	0				
3	0				
3	0				
3	0				

than the ratio of paternity in aborted ovules. For example, if 80% of the aborted *and* developed ovules are from one donor, then the ratio of paternity in the aborted ovules only reflects a bias in fertilization (or unequal pollen mixtures), not in ovule abortion. In this experiment, the ratio of aborted to matured ovules depended on the paternity of the ovule in both the self/cross ($G = 56.978$, df $= 1$, $P < 0.001$) and 1-m/100-m ($G = 10.488$, df $= 1$, $P < 0.005$) combinations. Twenty-seven selfed ovules were developed and 66 were aborted, while 96 outbred ovules were developed and 23 were aborted. For the 1-m/100-m combination, donors at 1 m developed and aborted almost equal numbers of ovules (28 developed, 27 aborted), but 100-m donors matured many more ovules (36) than they aborted (eight). There was no difference in the ratio of aborted to developed ovules when both donors were 100 m from the recipient.

TABLE 2. Ovule abortion following simultaneous pollinations with three different combinations of pollen types. Pollen combinations were self and cross, 1 and 100 m, and two donors that were each 100 m from the recipient.

Pollen combinations	G values		
	Total[a]	Pooled[b]	Heterogeneity[c]
Self/Cross	80.2***	20.1***	60.0***
1 m/100 m	30.2***	10.9***	19.3**
100 m/100 m[d]	—	—	2.0

[a] G_{total} tests the probability of obtaining the entire set of patterns of abortion for all the plants. The degrees of freedom equal the number of plants.

[b] G_{pooled} compares the observed ratios of abortion to 50:50 ignoring individual plants. In this case, the degrees of freedom = 1 (comparing two groups).

[c] $G_{\text{heterogeneity}}$ indicates differences among plants in their deviation from equal abortion of each distance class. The degrees of freedom equal the number of plants − 1.

[d] I did not calculate G_{pooled} or G_{total} for this pollen combination because the assignment of a donor to be donor X is arbitrary and a different arrangement of donors could give different G_{total} and G_{pooled} results.

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.0001$.

TABLE 3. Average proportion of ovules fertilized, aborted, and matured into seeds following cutting the ovary window in *E. grandiflorum*.[a]

Ovule status	Intact ovary wall $N = 24$	Ovary window $N = 12$
Fertilized	0.62b (0.053)	0.63b (0.460)
Aborted	0.08b (0.021)	0.02c (0.007)
Matured seed	0.54b (0.046)	0.63b (0.059)

[a] Means followed by different lowercase letters differ significantly ($P < 0.05$). Standard errors are in parentheses.

For all of the pollination combinations combined, there was not a significant relationship between the proportion of ovules fertilized by a pollen donor and the probability of those fertilized ovules developing into seeds ($r_s = 0.05$, $P = 0.7577$, $N = 37$).

Cutting a window in the ovary and removing aborting ovules from one locule did not significantly affect the percentage of seeds per fruit ($F = 1.48$, df $= 1$, $P = 0.254$), but there was a small and significant decrease in the percentage of ovules that aborted (Table 3, $F = 6.55$, df $= 1$, $P = 0.031$) in other uncut locules.

Effect of maternal resources on ovule abortion—Clipping leaves caused a significant drop in the percentage of seeds per fruit ($F = 7.35$, $P = 0.01$, Table 4), while allowing only one fruit to develop per plant (style removal treatment) caused the percentage of seeds per fruit to increase ($F = 10.7$, $P = 0.002$, Table 4). These changes in the amount of seeds produced per fruit were due to differences in ovule abortion; fruits on plants with clipped leaves had significantly more ovule abortion than fruits on plants with intact leaves ($F = 6.78$, $P = 0.01$), and plants where only one fruit was allowed to develop had significantly less ovule abortion than plants with two developing fruits ($F = 8.38$, $P = 0.007$, Table 4). Differences in the percentage of fertilized ovules between this experiment and the experiment that determined whether there was an effect of cutting the ovary window (above and Table 3) may be due to differences in location of the experiments and to differences in the weather in the different years the experiments were conducted.

Effect of position within the fruit on ovule abortion—An equal number of ovules was aborted in each quarter of the fruit in both the naturally pollinated flowers ($G = 0.727$, df $= 3$, $P > 0.05$) and in the flowers hand pollinated simultaneously with self and cross pollen ($G = 2.504$, df $= 3$, $P > 0.05$). The frequency of ovule abortion in the four quarters was independent of the genotype of the aborting ovule (FI = 2.418, df $= 3$, $P = 0.49$) (Table 5).

DISCUSSION

This is the first study to demonstrate directly that variation in paternity within fruits is caused, in part, by nonrandom ovule abortion. In addition, it corroborates pre-

TABLE 4. The effect of clipping leaves and of preventing fruit development in the other fruit (by removing styles) of *E. grandiflorum* on the mean proportion of ovules fertilized, aborted, and developed into seeds.[a]

	Treatments			
Ovule status	Leaves clipped $N = 68$	Leaves intact $N = 12$	Style intact $N = 56$	Style removed $N = 33$
Fertilized	0.91b	0.85b	0.88b	0.93b
	(0.014)	(0.040)	(0.021)	(0.013)
Aborted	0.35b	0.42c	0.41b	0.47c
	(0.022)	(0.031)	(0.023)	(0.028)
Developed	0.42b	0.56c	0.47b	0.64c

[a] Means followed by different lowercase letters are significantly different from each other ($P < 0.05$). Standard errors are given in parentheses.

TABLE 5. Total number of aborted ovules in each quarter of all fruits sampled following natural and hand self and cross pollinations.

Type of pollination	Aborted ovules 1[a]	2	3	4	Number of fruits
Natural	23	18	19	21	8
Selfed and crossed seeds	33	43	32	41	56
Selfed seeds only[b]	6	6	6	10	12
Crossed seeds only[c]	0	2	2	1	12

[a] Quarter 1 is at the stylar end of the capsule.

[b] Subset of selfed and outbred category. This category includes only selfed aborted seeds.

[c] Subset of aborted selfed and outbred seeds. This category includes only outbred aborted seeds.

vious evidence that cross and more distant donors are more likely to sire seeds than self and near donors in *Erythronium grandiflorum* (Cruzan, 1990; Rigney et al., 1993). Following simultaneous deposition of pollen from two types of donors, the proportion of paternity of aborted ovules did not reflect the proportion of pollen applied to the stigma. On most, but not all, of the plants, ovules fertilized by self and near pollen donors were more likely to abort than were ovules fertilized by cross and more distant donors.

The pattern of nonrandom ovule abortion was not influenced by the position of the developing ovule within the ovary. If certain donors are more likely to fertilize ovules within sections of the ovary, and if abortion is more likely in those sections, then patterns of nonrandom abortion might be a result of the position of the ovule within the ovary. In *E. grandiflorum,* abortion was not more frequent in any quarter of the ovary, and inbred ovules aborted with equal frequency in every quarter.

Pre- vs. postzygotic control of pollen donor success—Pre- and postfertilization mechanisms have been suggested to act in concert, creating a kind of "genetic sieve" that could produce preferential development of certain embryo genotypes (Bertin, 1982; Stephenson and Bertin, 1983). A combination of both pre- and postzygotic mechanisms could cause patterns of nonrandom ovule abortion through variation in pollen tube growth (Mulcahy and Mulcahy, 1973; Lee, 1984). Pollen tubes from certain donors could grow more slowly and fertilize ovules later (if faster tubes were insufficient in number to preempt all ovules). These later fertilized ovules might be weaker sinks for resources and subsequently might abort. In such cases, ovule abortion may only be the result of variation in pollen tube growth rates.

This scenario was suggested by the work of Cooper and Brink on *Medicago sativa.* They examined both patterns of pollen tube growth (Brink and Cooper, 1938) and abortion of fertilized ovules (Cooper and Brink, 1940). The slow pollen tube growth of self pollen combined with the abortion of inbred ovules accounted for most of the difference in seed production following selfing and outcrossing.

If variation in pollen tube growth was foreshadowing subsequent ovule abortion in *E. grandiflorum,* I would expect a correlation between the number of ovules fertilized by one pollen donor and the number of seeds sired by that donor. But success of pollen donors at these two stages was not correlated in *E. grandiflorum.* I did not find a significant relationship between the number of ovules fertilized by a pollen donor and the number of mature seeds. While my estimate of the proportion of ovules fertilized by each donor may be an underestimate since some ovules aborted before I could unambiguously determine their paternity, the lack of a relationship between these two stages of reproduction suggests that qualities that favor fertilization may not be the same as qualities that favor embryo development; different selective mechanisms may be operating at each stage of reproduction. Since success at the pollen tube and fertilization stages depends partly on the pollen genotype (de Nettancourt, 1977), and successfully developing into a seed is dependent on the genome of the zygote (Queller, 1987), it is not too surprising that different mechanisms may act at these different stages.

Another piece of evidence that different selective mechanisms may be acting at various stages in reproduction is the discrepancy between the pattern of ovule abortion in the 1-m/100-m pollen combination found here and the variation in pollen tube length between nearby and more distant donors found by Cruzan (1990). Cruzan found that pollen tubes from nearby donors, in this case pollen from donors within 3 m, were more likely to reach the base of the style than were pollen tubes from more distant donors (30 m, 100 m, and 300 m) when both types of pollen were simultaneously applied to the stigma. Nearby donors may be more successful at growing pollen tubes to the base of the style, and may subsequently affect more fertilizations; however, the resulting ovules abort in greater frequency than do ovules fertilized by more distant donors. The combined results of this study and Cruzan's work suggest that while both pre- and postfertilization mechanisms are important in controlling patterns of ovule development in *E. grandiflorum,* they may not be acting to promote successful paternity of the same type of pollen donor.

Postfertilization causes of nonrandom abortion—Differential abortion of ovules fertilized by pollen from self and nearby donors could be partially due to inbreeding depression. *E. grandiflorum* pollen and seed dispersal distances are short; average dispersal distances are approximately 1.5 m for pollen (Thomson and Thomson, 1989) and 33 cm for primary dispersal of seeds (Wieblen and Thomson, unpublished data). As a result, plants growing nearby may be closely related and mating between such plants may result in the expression of inbreeding depression. Inbreeding depression is expressed following pure hand-pollinations of self and cross pollen. Fruits pollinated with self pollen generally contain fewer mature seeds and have a greater proportion of aborted ovules (Rigney and Thomson, unpublished data). However, after pure pollinations of pollen from donors growing 1 and 100 m from the recipient, there were no detectable differences in the number of fruits or seeds matured. Differences in seed viability alone will not explain the observed patterns of nonrandom ovule abortion.

Developing embryos fertilized by nearby donors may be somewhat weaker than embryos fertilized by more distant donors, since seedlings grown from these seeds develop smaller corms and have lower rates of survival (Rigney and Thomson, unpublished data). Perhaps competition within the ovary intensifies subtle viability differences between ovules fertilized by 1- and 100-m donors and results in increased abortion of ovules fertilized by donors at 1 m.

A similar scenario was proposed by Westoby and Rice (1982). In their model, less vigorous offspring abort when maternal resources are limited. As an extension, Seavey and Bawa (1986) suggested that the threshold for abortion of an ovule is genetically determined and that inbred offspring will always fall below the threshold and will always be aborted. The abortion of *E. grandiflorum* ovules fits this pattern, except that inbred offspring do not always automatically fall below the threshold. Approximately 20% of the plants in the self/cross pollination combination had more outbred then inbred ovules abort, and approximately 10% of the plants in the 1-m/100-m combination had more abortion of ovules fertilized by distant donors than fertilized by near donors.

There are several possible explanations for these anomalous patterns. First, I could not genotype all of the aborting ovules because MDH was not detectable with starch gel electrophoresis early in seed development. Some of these early aborted ovules may have been fertilized by self and near pollen. Second, because plants may have different histories of inbreeding (Wright, 1977), the amount of inbreeding depression resulting from different types of crosses may vary among maternal plants and as a consequence, the percentage of inbred seeds matured may vary among plants. While not ruling out the first explanation, there is evidence from other experiments on *E. grandiflorum* that suggests there is variation in the amount of inbreeding expressed. In an experiment designed to measure inbreeding depression, some mothers developed heavier inbred seeds and others heavier outbred seeds, independent of seed number (Rigney and Thomson, unpublished data). There was also variation in the percentage of inbred seeds matured following simultaneous application of self and cross pollen (Rigney et al., 1993). While most plants developed outbred seeds over inbred, about 20% of the fruits contained only seeds sired by self pollen. Variation in the percentage of aborted inbred offspring may be another manifestation of variation in maternal inbreeding history.

***Effect of nonrandom abortion on offspring quality*—** Maternal plants may incur a cost by allowing pollen donors of lower quality to fertilize ovules and thus prohibit the fertilization of these ovules by donors of better quality (Lloyd, 1980; Waser and Price, 1991). Nonrandom abortion of ovules could be a mechanism that might increase the average quality of the remaining offspring of a plant, in spite of the cost of an ovule (Darwin, 1876; Charnov, 1979; Lloyd, 1980; Westoby and Rice, 1982; Stephenson and Bertin, 1983; Stearns, 1987). The average quality of offspring can be increased in two ways: 1) removal of lower quality offspring will increase the average genetic quality of the remaining offspring; and 2) removal of some offspring may free resources for investment in other offspring (Hamilton, 1966). The average quality of offspring is increased in both of these ways in *E. grandiflorum.* Abortion of ovules fertilized by self and near donors affects the average quality of offspring by removing developing seeds that might be less fit. The cumulative probability of survival of selfed offspring (the probability of fruit maturation × the probability of seed development × the probability of emergence × the probability of survival for 3 years) is only 31% that of outcrossed offspring (Rigney and Thomson, unpublished data).

Abortion of ovules fertilized by self and near donors also frees resources for investing in other offspring. In ovaries where aborting ovules were removed from one locule, there was significantly less abortion in the unmanipulated locules than in ovaries where no ovules were removed. The abortion of ovules of lesser quality appears to release resources that can then be used to develop other offspring. Allowing pollen donors of lower quality to fertilize ovules and thus prohibit the fertilization of these ovules by donors of better quality may cost plants an ovule (Lloyd, 1980; Waser and Price, 1991), but if the resources can be used by other embryos of higher quality, then abortion of these lower quality seeds may result in increased fitness of the maternal plant.

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