J. evol. biol. 10 (1997) 295–314
1010–061X/97/030295–20 $ 1.50 + 0.20/0

Journal of Evolutionary Biology

Effects of pre-dispersal selection on offspring growth and survival in *Erythronium grandiflorum*

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Key words: Abortion; pollen; selection; sporophytic vigor; variance components.

Abstract

Effects of pre-seed-dispersal processes on offspring vigor were examined in *Erythronium grandiflorum* using manipulations of the number of pollen donors contributing to the pollen pool and comparisons of means and variances in offspring growth measurements. There were no effects of the number of donors on measures of pollen-tube growth, ovule abortion, seed set, mean seed weight, or seedling germination. Seeds from pollinations with only one donor produced corms that averaged 5% lighter after one season of growth and had lower overall survival after three years compared to corms from pollinations with either three or ten donors. Patterns of within- and among-family variance estimates for the different treatments were consistent with the hypothesis that less-vigorous offspring were eliminated prior to seed dispersal in the multiple-donor treatments. The difference in the growth of offspring from different treatments was apparently not due to pollen competition because pre-zygotic attrition of pollen tubes led to incomplete fertilization of ovules. Results from this study suggest that post-fertilization abortion of less-vigorous progeny, perhaps as a consequence of early-acting inbreeding depression, is responsible for the increase in the average vigor of offspring from multiply-sired fruits.

A reduction in seed fecundity in plants would be favored if the immediate loss of offspring were offset by expected future gains (Hamilton, 1966; Lloyd, 1980). When

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the number of seeds produced is less than the number of female gametes available, it has generally been assumed that a tradeoff exists between seed quantity and some other aspect of fitness. A low fruit-to-flower ratio in plants that are not pollen-limited may be interpreted as a means of increasing male reproductive success through increased floral display size and the amount of pollen produced (Willson and Price, 1977; Sutherland and Delph, 1984; Bel, 1985; Sutherland, 1986; Devlin et al., 1992). Within a flower, reductions in fecundity can allow the re-allocation of resources from failed ovules to vegetative growth or future reproduction.

Several mechanisms that reduce seed production may increase seed quality. Incompatibility systems may limit fecundity because of the inhibition of self-pollen, thereby avoiding the production of offspring that are likely to suffer from high levels of inbreeding depression (Charlesworth and Charlesworth, 1987; Olmstead, 1989). Selective abortion of fertilized ovules is another mechanism that may limit fecundity. This reduction in fecundity has been attributed to 1) early expression of inbreeding depression (Wiens et al., 1987; Charlesworth, 1989; Savolainen et al., 1992; Rigney et al., 1993), 2) maternal selection for higher quality offspring (Marshall and Ellstrand, 1988), or 3) competition among embryos for limited maternal resources that results in the elimination of weaker genotypes (Lloyd, 1980; Lloyd et al., 1980; Lee and Bazzaz, 1986). Although it is often assumed that post-fertilization selective processes will result in more vigorous offspring, this prediction has rarely been tested (but see Stephenson and Winsor, 1986; Waser and Price, 1989; Rocha and Stephenson, 1991).

The effects of pre-seed-dispersal selection on offspring vigor have more often been studied in the context of pollen competition, which may favorably alter the genetic composition of the resulting offspring. Researchers have found the potential effects of selection on pollen compelling because of the large number of pollen grains that can be present on a stigma relative to the number of ovules (Mulcahy and Mulcahy, 1987). Unfortunately, few experiments on the effects of pollen competition on offspring vigor meet the stringent criteria necessary for an unambiguous interpretation of the results (Charlesworth et al., 1987; Charlesworth, 1988; Walsh and Charlesworth, 1992; but see Schlichting et al., 1990 and Quesada et al., 1993 for notable exceptions). Charlesworth's major objection to interpretations of results from gametophytic selection experiments stems from the lack of an adequate separation of stylar and ovarian processes; the results obtained could be due to either selective fertilization or ovule abortion. A second concern is that different pollination treatments may modify the physiological environment within the flower; if so, results attributed to pollen selection may actually be caused by maternal influences. For example, the auxin production or resource acquisition of a gynoecium may increase because of more pollen tubes present in the style (McKenna, 1986), a longer period of pollen tube growth in the style (Mulcahy and Mulcahy, 1975; McKenna and Mulcahy, 1983), or a difference in the number of developing seeds (Mulcahy and Mulcahy, 1975). Thus, better performance by seeds from heavily-pollinated flowers might be due either to modification of the genetic composition of progeny or to differences in maternal provisioning.

Although it would be impossible to remove every ambiguity regarding genetic versus maternal effects on offspring vigor, we have chosen a design that seeks to clarify the issue by including analyses of differences in means as well as variances in offspring growth (see Charlesworth et al., 1987). We compare single- and multiple-donor pollinations in an effort to assess the effects of pre-dispersal selection, random siring, and maternal effects on differences in offspring vigor (Tab. 1).

If selection were operating to remove the weakest offspring genotypes in multiple-donor crosses, we would expect both an increase in the mean vigor of offspring and a reduction in the total variation among individuals relative to offspring from the single-donor crosses (Tab. 1). Selective removal of weaker offspring in the multiple-donor treatment would also produce within-fruit variance components that were similar between the single- and mixed-donor crosses.

The random siring and maternal effects models would predict contrasting patterns of differences in means and variances in offspring growth. Under random siring there would not be an increase in the vigor of offspring from the multiple-donor treatment and the total variance would be the same for both the single and multiple-donor treatments, as long as donors for each treatment were sampled from the same population (Tab. 1). If there were differential maternal effects in the provisioning of offspring in single- and mixed-donor treatments, then an increase in mean growth of progeny from the multiple-donor crosses might be observed, but the relative magnitude of variance components would be the same as for random siring (Tab. 1). In contrast to the selection model, the random siring and maternal effects models predict that the single-donor treatment would have a lower within-fruit variance. Hence, by comparing variance components as well as the mean responses for the single- and multiple-donor treatments, we can discriminate among the pre-dispersal selection, random siring, and maternal effects hypotheses for differences in offspring growth.

Here we examine the effects of selection in both the style and ovary on offspring vigor and make comparisons of both the means and variances of measures of progeny growth and survival after single- and multiple-donor crosses. Moreover, we attempt to control for the effects of differences in maternal environments by creating treatments that are similar for their pollen load sizes and numbers of

Table 1. Expected differences in mean offspring growth and phenotypic variance components under different models for the effects of single-donor and mixed-donor pollen loads. Note that responses of mean growth alone cannot distinguish the models.

Effect	Random siring	Maternal effects	Selection
Mean growth	single = mixed	single < mixed	single < mixed
Variance components:			
within fruit	single < mixed	single < mixed	single = mixed
among fruit	single > mixed	single > mixed	single > mixed
total	single = mixed	single = mixed	single > mixed

developing seeds. We further control for differences in maternal environments by accounting for the effects of seed size in the statistical analyses. Our measurements of pollen growth traits for each cross allow us to gauge the relative importance of pollen tube competition, pollen-style interactions, and ovarian processes in producing differences in the growth rate and survival of progeny.

Materials and methods

Erythronium grandiflorum Pursh (Liliaceae) is an early-spring perennial that flowers soon after snow-melt in subalpine meadows and aspen forests near the Rocky Mountain Biological Lab in Gothic, Colorado. Flowering plants produce from one to several flowers, with one- and two-flowered plants being the most common. Flowers bear six yellow tepals, 2 to 4 cm in length, and are primarily bumble bee pollinated (Thomson and Thomson, 1989). The number of pollen grains on the stigma commonly exceeds the number of ovules; however, only 20 to 80% of these ever reach the ovary due to attrition of pollen tubes in the style (Cruzan, 1989). Stylar attrition of pollen tubes usually causes incomplete fertilization of ovules, even when sufficient numbers of tubes for full fertilization (30 to 90 ovules per flower) are present in the stigma (Cruzan, 1989). Seed set in this species is often further reduced because of post-fertilization ovule abortion (Cruzan, 1990b; Rigney, 1995).

We pollinated flowers with pollen mixtures representing different numbers of pollen donors to assess the effect of the genetic diversity of the pollen pool on offspring vigor. In June of 1987 we selected one hundred two-flowered plants as pollen recipients in a population located at the upper end of Washington Gulch near Painter Boy Mine, 4 km from Gothic at 3200 m elevation. When flower buds were about to open, they were ‘‘strawed’’ (i.e., had a 2 cm section of dried grass or plastic straw placed over the gynoecium) to prevent pollination (Thomson et al., 1994). We prepared pollen mixtures by combining either one, three, or ten pollen donors by mixing equal numbers of dehiscing anthers from randomly selected donors in glass vials. Fresh pollen was collected each day from plants growing in the same population as the recipients. Pollen mixtures were replaced when the pollen appeared dry and powdery (a sign of reduced viability; pers. observ.; see also Thomson et al., 1994). In the course of the experiment we used approximately 15 different sets of pollen mixtures over a five day period.

Pollinations and analysis of styles and fruits

Flowers were pollinated using a fishing-line applicator (Cruzan, 1989) by evenly coating stigmas with pollen on the second day of anthesis. After pollination, the straws were replaced and left on until the tepals wilted and ovaries began to swell. Treatments were rotated between the first and second flower positions of recipient plants using a balanced incomplete block design. We collected styles five days after pollinating and stored them in 70% ethanol. After staining with acidified aniline blue and aceto-carmine, pollen tube growth was analyzed under a dissecting microscope at 50X by counting the number of pollen tubes in the stigma and at the base of the style (see Cruzan, 1989).

We collected fruits as they matured and counted the seeds and aborted ovules. We weighed the seeds from each fruit as a group after drying them at 35 °C for 48 h. Undeveloped ovules ranged from being small and completely light-colored to larger ovules that were mostly brown. We classified aborted ovules as either unfertilized (small and completely light colored) or fertilized and aborted (having at least some brown color along one side). The presence of a brownish tinge along one side of a small ovule appears to be indicative of post-fertilization expansion of the vascular tissue in the funiculus (unpubl. data). We calculated pre-zygotic attrition as the proportion of pollen tubes present in the stigma that did not produce a seed or aborted ovule, and post-zygotic attrition as the proportion of fertilized ovules that aborted. The measurement of pre-zygotic attrition used here differs from previous definitions of pollen-tube attrition where only failure of pollen tubes during growth in the style was considered (Cruzan, 1989; 1990a,b).

Seed planting and growth measurements

We planted the seeds from the above crosses on 8 August 1987 in a garden at the Rocky Mountain Biological Lab. The garden was prepared by sifting soil into a buried wooden frame (9 cm in depth), which had a metal screen covering its bottom to exclude burrowing rodents. A planting grid was made (122 by 61 cm wide and having 3528 cells 12 mm^2 and 24 mm in depth) by layering two plastic ''eggcrate'' fluorescent light fixture diffusers on top of each other. The planting grid, which had its cells open at the bottom, was positioned in the upper portion of the planting frame so that the cells had 6.5 cm of open soil beneath them. We divided the planting grid into three blocks and then planted up to five seeds from each fruit with a randomly placed group of adjacent cells in each of the three blocks (1511 seeds total with a maximum of 15 seeds from each of 103 fruits). Seeds were planted at a uniform depth by sifting soil level to the top of one grid and placing the second grid on top of the first. After planting seeds we filled the cells with soil level to the top of the second grid and placed wire mesh over the planting frame to exclude rodents.

Seedlings emerged in late May and early June of 1988, producing a single seedling leaf (cotyledon), and remained green until late July when they died back to a buried corm. Between 20 and 28 June 1988, after the seedling leaves had ceased to elongate, we scored the presence and measured the height of each seedling as the above ground length of its leaf. In early August we exhumed all of the corms, which had buried themselves approximately 4 cm beneath the soil surface. Any corms that could not be located were scored as mortalities. For the corms that were relocated, we obtained their wet weight and replanted them at a depth of 2 cm in one of 24 randomly assigned cells (5.7 by 8.0 cm and 5.5 cm deep) in plastic trays. After planting was completed the trays were covered with an additional 2 cm layer of soil so that corms were approximately 4 cm beneath the soil surface.

In the summer of 1989 and 1990 we measured leaf length and width and scored the presence of all emergent seedlings. In mid-August, once seedlings had senesced, corms were exhumed and weighed as previously described. Corms that could not be found were scored as mortalities. After weighing we returned all corms to their original position in the planting tray and re-buried the tray as described above.

Data analysis – comparison of means

We examined the data for the effects of donor number on several reproductive traits associated with each cross and on the survival and growth of offspring. For most of the response variables (number of pollen tubes present in the stigma and at the base of the style, the numbers of seeds, unfertilized ovules, aborted ovules, and pre- and post-fertilization attrition), we entered the treatment and flower position of the recipient flower and number of donors as fixed factors and the data of pollination as a random factor in analyses of variance using the GLM procedure of SAS (SAS Inst. Inc., 1985). To analyze mean seed weights we included the above factors plus the number of seeds and the degree of pre- and post-fertilization attrition as covariates. We analyzed the arcsine transformed proportion of germinated seeds for each family (Sokal and Rohlf, 1981) using the same model as seed weight with the replacement of mean seed weight for seed number as a covariate. All of the other dependent variables approximated normal distributions.

The ANOVA models used to analyze corm weights and leaf sizes follow the diagram in Figure 1. Flower position was not included in these analyses because only a small number of second flowers set fruit. The mass of corms produced at the end of each season was assumed to depend primarily on their mass in the previous year (or mean seed weight in 1988) and the amount of the photosynthetically active tissue produced at the beginning of the season (seedling leaf length in 1988). The effect of donor number on corm weight was examined as a fixed factor, and the effects of reproductive traits (number of pollen tubes in the stigma, and pre- and post-fertilization attrition) were entered as covariates. Preliminary analyses indicated that there were very few differences among the three blocks of the planting grid, so we eliminated this factor from the final analyses of the 1988 leaf length and corm weight. For the 1989 and 1990 data there were differences among individual trays so this variable was entered as a random factor.

Data analysis – comparison of variances

To evaluate the various hypotheses for differences in offspring growth after pollination with difference numbers of donors (i.e., to compare our results with the patterns in Tab. 1), we compared the variances among treatments for the reproductive traits and within and among fruits for the offspring growth traits. Differences

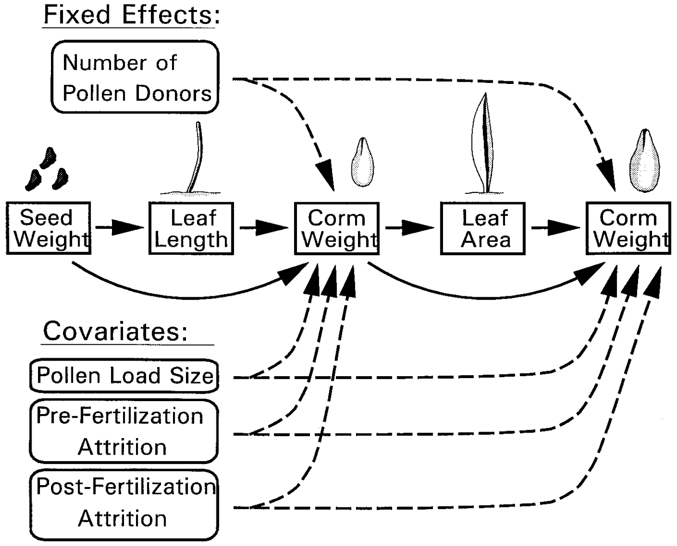

Fig. 1. Diagrammatic representation of the early life-history stages of *Erythronium grandiflorum*. The ANOVA models assume that previous weight and leaf area, which were entered as covariates, are the primary factors affecting the current corm weight of individuals (solid lines).

in the variances among treatments were tested using the log-ANOVA test for homogeneity of variances (Scheffé-Box test; Sokal and Rohlf, 1981). For this test the observations within each treatment group were divided into $\sim\sqrt{n_i}$ (where n_i is the number of observations within group i) nearly-equal subgroups. A weighted analysis of variance (weight = subgroup sample size) on the log_e-transformed variances of the subgroups was then used to test for differences among the treatment variances. The within- and among-fruit variance components were calculated for each treatment group using the VARCOMP procedure of SAS (SAS Inst. Inc., 1985).

Results

Reproductive measures

The type of pollination had little effect on patterns of fertilization and fruit set. The number of pollen donors represented in pollen mixtures did not affect the number of pollen tubes in the stigma or at the base of the style, the degree of pre- or post-fertilization attrition, the number of seeds or ovules of different classes, the mean seed weight per fruit, or the proportion of germinated seeds (Tab. 2). Log-ANOVA tests reveal that the variances for these traits also did not differ among treatments (Tab. 2). Fruit abortion frequencies (38%, 27%, and 33% for the one, three, and ten donors respectively) were homogeneous for the three treatments (2×3 contingency table, $G = 1.71$, $P > 0.426$, 2 df).

Seedling growth

Pollination treatment effects did turn up in the long-term performance of the offspring. Seedling leaves produced by germinated seeds did not differ in their mean length among treatments, but by the end of the first season the corms in the single donor treatment were significantly lighter than corms produced by seeds from pollinations with either three or ten donors (Tab. 3). Leaf sizes and corm weights in subsequent seasons did not differ significantly among treatments (Tab. 3). The general decrease in corm weights from 1989 to 1990 appeared to be the result of deposition of a layer of silt over the planting trays that occurred during the early spring snow-melt, hampering emergence of young plants.

The transient nature of corm weight differences among treatments found at the end of the first growing season was apparently due to higher mortality rates for smaller corms. Survival between growing seasons was strongly affected by corm

Table 2. Means and standard deviations (in parentheses) of reproductive parameters for pollinations with pollen loads that consisted of either one donor, or equal mixtures of three or ten donors in *Erythronium grandiflorum*. *F*-tests and probabilities for differences among the treatment means are from ANOVA models that included the position of the recipient flower and the date of pollination (pollen tube, ovule, and seed measurements) with the addition of seed number as a covariate for seed weight and mean seed weight as a covariate for family germination frequency. *F*-tests for standard deviations are from log-ANOVA tests for differences in variance among treatments (see text for details).

Measurement	One	Three	Ten	*F*	Prob.
Flowers (N)	52	56	54	–	–
Number of pollen tubes:					
stigma	132.6	125.4	127.2	0.21	0.814
	(56.4)	(40.8)	(48.3)	1.20	0.322
style base	78.6	77.8	75.5	0.35	0.704
	(29.1)	(29.8)	(31.4)	0.26	0.777
Fruits (N)	27	29	26	–	–
Number of seeds	33.72	30.49	36.72	1.62	0.203
	(14.97)	(13.81)	(15.41)	0.86	0.444
Number of aborted ovules:					
large-brown	24.31	22.72	24.13	0.06	0.940
	(10.68)	(11.73)	(10.12)	1.31	0.300
small-white	8.76	10.33	9.00	0.03	0.967
	(7.52)	(13.94)	(10.75)	0.26	0.771
Attrition:					
pre-fert.	0.58	0.53	0.51	1.50	0.230
	(0.15)	(0.23)	(0.23)	1.19	0.333
post-fert.	0.44	0.42	0.41	1.02	0.366
	(0.17)	(0.16)	(0.16)	0.35	0.709
Mean seed weight	2.80	2.71	2.81	0.76	0.470
(milligrams)	(1.08)	(1.10)	(1.02)	1.41	0.277
Germination frequency	0.606	0.627	0.618	1.60	0.209
	(0.204)	(0.173)	(0.197)	0.60	0.562

Table 3. Means and standard deviations (in parentheses) of offspring growth measurements for seedlings from pollinations with either one, three, or ten donors in *Erythronium grandiflorum*. F-tests and probabilities for differences among the treatment means are from ANOVA models that included the previous year's corm or seed weight and the current year's leaf size (for tests on corm weights). F-tests for standard deviations are from log-ANOVA tests for differences in variance among treatments (see text for details).

Measurement	One	Three	Ten	F	Prob.
Seeds planted	207	289	305	–	–
1988 leaf len. (mm)	52.3	51.2	51.0	1.91	0.150
	(6.4)	(8.1)	(8.1)	6.17	0.005
1988 corms (N)	72	125	116	–	–
weight (mg)	40.5	42.9	42.7	4.47	0.012
	(13.9)	(12.2)	(14.3)	6.73	0.003
1989 leaf area (cm^2)	178.3	184.8	183.5	0.66	0.517
	(61.3)	(61.1)	(66.2)	0.12	0.883
1989 corms (N)	64	109	97	–	–
weight (mg)	78.2	77.2	76.9	1.32	0.270
	(27.3)	(27.5)	(27.9)	0.19	0.830
1990 leaf area (cm^2)	117.2	130.4	119.9	1.24	0.293
	(44.7)	(58.7)	(59.9)	1.05	0.367
1990 corms (N)	23	48	39	–	–
weight (mg)	67.3	67.9	59.7	0.06	0.938
	(31.8)	(37.4)	(31.9)	2.85	0.074

weight in the previous year for both the first (Logistic regression Chi-Square = 13.45, $P < 0.0002$, 1 df) and second winter (Chi-Square = 28.56, $P < 0.0001$, 1 df).

Levels of pre- and post-fertilization were associated with differences in the growth of offspring in the three treatments (Tab. 4). Overall, the corm or seed weight in the previous year and leaf size in the present year explained the majority of the variation in corm weight each season (Tab. 4). Levels of pre- and post-fertil-

Table 4. Analyses of variances for factors affecting corm weight after one, two, and three seasons of growth in *Erythronium grandiflorum*. F-values are given followed by their probabilities in parentheses. Leaf size is for the current season (seed leaf length for 1988) and previous weight is either the mean family seed weight (1988) or the previous season's corm weight (1989 and 1990).

	Corm weight		
Source	1988	1989	1990
Number of donors	4.47 (0.012)	1.32 (0.270)	0.06 (0.938)
Leaf size	29.89 (0.001)	188.9 (0.001)	27.0 (0.001)
Previous weight	24.23 (0.001)	13.32 (0.001)	29.4 (0.001)
Grains on stigma	1.46 (0.228)	0.14 (0.710)	1.44 (0.233)
Attrition:			
pre-fertilization	8.38 (0.004)	1.45 (0.229)	1.54 (0.218)
post-fertilization	8.83 (0.003)	0.96 (0.328)	0.47 (0.497)

Table 5. Variance components for seedling families after pollinations with mixtures of pollen from one, three, or ten donors in *Erythronium grandiflorum*. Estimates for among (S_a^2) and within (S_e^2) seed family variances were obtained using the Type I method for the VARCOMP procedure of SAS (SAS Inst., 1985). The per cent variance explained for each component is given in parentheses. Negative variance estimates were assumed to be zero (Sokal and Rohlf, 1981).

	Treatment		
Measurement	One	Three	Ten
1988 leaf length:			
S_a^2	15.5 (37.3)	20.8 (31.2)	17.8 (27.0)
S_e^2	25.9 (62.7)	45.8 (68.8)	48.1 (73.0)
1988 corm weight:			
S_a^2	48.0 (25.9)	28.4 (19.1)	79.6 (38.7)
S_e^2	137.5 (74.1)	120.5 (80.9)	126.4 (61.3)
1989 corm weight:			
S_a^2	158.2 (21.2)	125.1 (16.5)	130.2 (16.7)
S_e^2	590.0 (78.8)	634.0 (83.5)	649.3 (83.3)
1990 corm weight:			
S_a^2	83.9 (8.3)	−11.0 (0.0)	−6.7 (0.0)
S_e^2	928.3 (91.7)	1406.9 (100.0)	1026.0 (100.0)

ization attrition were associated with higher corm weight in the first year only, with corm weight increasing with greater amounts of attrition for both (slope = 0.015 and 0.016 for pre- and post-fertilization attrition respectively). When the analysis was restricted to the ten-donor treatment, the effect of pre-fertilization attrition on corm weight was no longer apparent (slope = 0.003, $P > 0.728$), but there was still a significant effect of post-fertilization attrition (slope = 0.033, $P < 0.004$). In the second and third season there were no apparent effects of reproductive traits on the size of corms (Tab. 4).

Analysis of variance components

There were significant differences in the variance among treatments for seedling leaf lengths and corm weights in the first season, but not for any of the offspring growth measurements in subsequent years (Tab. 3). Examination of the within-family components reveals higher variation in seedling leaf length for the ten-donor treatment, followed by the three- and one-donor treatments (Tab. 5). By the end of the first season the rankings of the within-family variances for corm weight among treatments had nearly reversed, with the ten-donor treatment having the lowest variance, followed by the one- and three-donor treatments (Tab. 5). The within-family variance components for corm weight were very similar among the three treatments in the second and third seasons.

The among-family variance components declined in the second and third seasons for all three treatments (Tab. 5). The decrease in variation among families was apparently due to a reduction in the differences among seed families in the weight of surviving offspring and was more rapid in the mixed-donor treatments (Tab. 5).

Seedling survival

Survival of seedlings during the first season of growth (from seedling leaf to corm) was significantly lower for the single-donor treatment (83.1%) than for the three- and ten-donor treatments (90.3 and 89.5% respectively; test for heterogeneity $G = 6.48$, $P < 0.039$, 2 df). There was no significant difference among treatments in the survival of offspring from the first to the second season ($G = 3.80$, $P > 0.149$, 2 df) or from the second to third season ($G = 0.42$, $P > 0.812$, 2 df; Fig. 2).

Seed families from the three donor-number treatments displayed different distributions of offspring survival frequencies after three years of growth (Fig. 3). Overall, the families in the ten-donor treatment had the highest survival frequencies (mean across families = 19.8%, ±standard error = 12.6%, $N = 34$ families), followed by the three-donor treatment (16.7%, ±9.7%, $N = 38$ families), and least for the single-donor pollinations (11.5%, ±11.9%, $N = 31$ families; Fig. 3). The number of seed families with no surviving offspring was much higher in the single-donor treatment (25.8%) than in either of the multiple-donor treatments (5.13% and 5.71% for the three- and ten-donor treatments, respectively; $G = 8.12$, $P < 0.017$, 2 df). The ten-donor treatment also had a greater proportion of seed families with greater

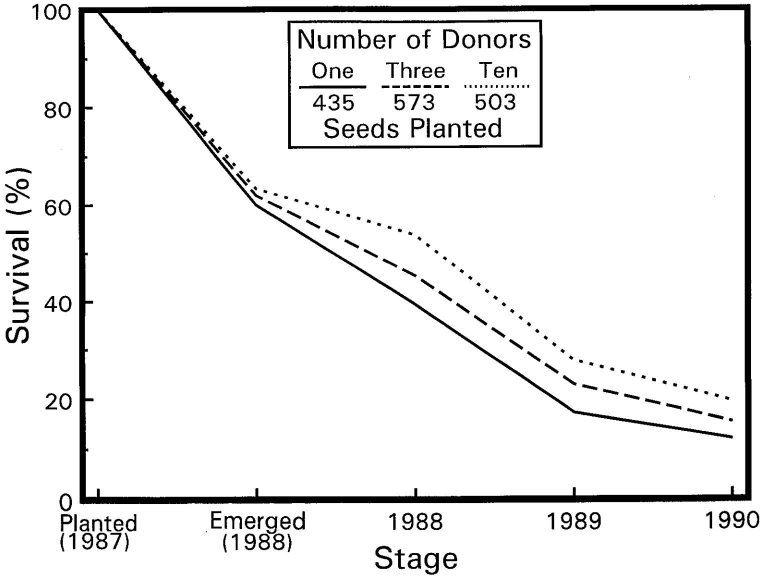

Fig. 2. Survival of progeny from three pollination treatments with different numbers of donors over the first three years of growth in *Erythronium grandiflorum*.

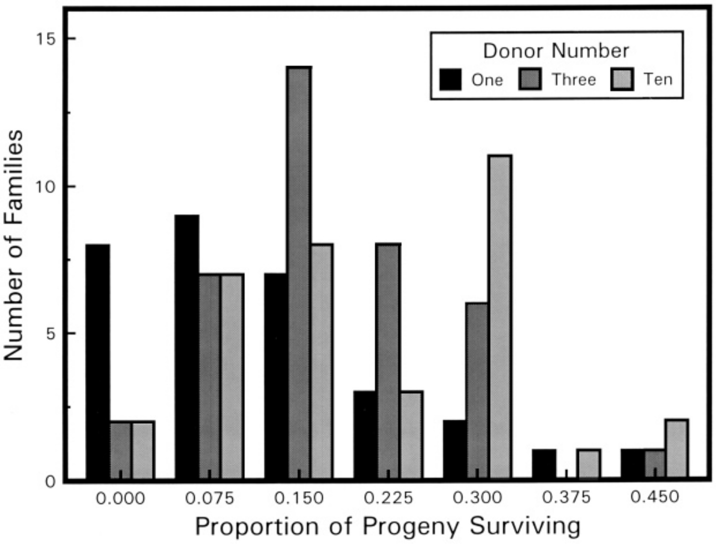

Fig. 3. Distributions of the proportion of progeny surviving after three years of growth for seed families from pollination treatments with different numbers of donors in *Erythronium grandiflorum*.

than 25% survival after three years of growth (40.0%), than did the three-donor treatment (17.0%), or the single-donor treatment (12.9%; $G = 7.60$, $P < 0.022$, 2 df).

Survival frequencies after three years of growth were associated with several pre-dispersal reproductive traits. Survival across treatments was not affected by either stigma load size ($P > 0.483$) or pre-fertilization attrition ($P > 0.251$), but higher survival frequencies were associated with lower levels of post-fertilization attrition (slope $= -0.324$, $P < 0.012$). Examination within individual treatments reveals significant effects only with ten donors, where higher rates of survival were associated with larger pollen loads (slope $= 0.0043$, $P < 0.0003$) and lower levels of pre-fertilization attrition (slope $= -0.0444$, $P < 0.0001$). Post-fertilization attrition did not have a strong effect on offspring survival in the ten-donor treatment ($P > 0.793$).

Discussion

Fecundity reductions in *Erythronium grandiflorum* were substantial at both the pre-fertilization (55.5% pollen-tube attrition and 14.2% unfertilized ovules) and post-fertilization (41.4% ovule abortion) stages of reproduction. Within-flower fecundity reductions have been reported in a number of other species (Cooper and Brink, 1940; Sayers and Murphy, 1966; Wiens, 1984; Wiens et al., 1987; Casper, 1988; Manasse and Pinney, 1991; Waser and Prince, 1991; Montalvo, 1992). If these reductions represent selection that effectively eliminates the poorest quality male gametes and zygotes, then we would expect a greater effect on offspring vigor when the variance in gamete quality was the greatest. In *E. grandiflorum* the increased growth and survival of progeny from pollinations with ten donors is consistent with the hypothesis that pre-dispersal selection is modifying offspring quality. It has been suggested that differences in maternal environments could be responsible for apparent increases in sporophytic vigor (Walsh and Charlesworth, 1992), but it is unlikely that this was a factor in the current study (see below). By examining the patterns of growth and survival for offspring from the different pollination treatments, and the within- and among-family variance components for the growth measurements, we can discriminate among the alternative explanatory hypotheses for the among-treatment differences in progeny vigor in *E. grandiflorum*.

Patterns of offspring growth and survival

Offspring produced from crosses with pollen from single donors had slower growth and lower rates of survival than offspring produced from crosses with mixtures of pollen from several donors. The lower first-season corm weights and the reduced survival observed in the single-donor treatment suggest that pre-dispersal selection elimates weaker genotypes in the multiple-donor treatments. This interpretation assumes that 1) there is variation in the average vigor of offspring from different crosses, and 2) pre-dispersal selection eliminates the weaker genotypes from the array of pollen or embryos that are present. The second statement reflects a broader assumption that any maternal influences on the siring of pollen or the maturation of zygotes, or any among-pollen or among-zygote interactions, are context-dependent (i.e., that they reflect the outcome of competition and are not based on an absolute scale). This implies that the probability of abortion would depend on the relative vigor of other zygotes present in the same developing fruit. In contrast, specific pollen or zygote genotypes may be aborted regardless of other genotypes present (e.g., due to self-incompatibility reactions: Nettancourt, 1977; or lethal mutations: Savolainen et al., 1992). Context-dependent elimination of pollen or zygotes can occur as a result of both pre-fertilization processes (due to differential pollen-tube growth rates: Bowman, 1987; Snow and Spira, 1991; Cruzan and Barrett, 1993; Quesada et al., 1993) or post-fertilization processes (context-dependent fruit or ovule abortion: Lee and Bazzaz, 1986; Casper, 1988; Krebs and Hancock, 1990; Becerra and Lloyd, 1992; Martin and Lee, 1993; Niesenbaum and Casper, 1994). We can assess whether predispersal removal of gametes or zygotes is acting in a context-dependent manner to affect progeny vigor in *E. grandiflorum* by examining patterns of offspring growth and survival.

Before we can examine the data for effects of pre-dispersal selection we need to establish whether there were differences among crosses in progeny vigor. This can be accomplished by examining offspring performance for the single-donor treatment. Mortality was highest in this treatment with 25% of the single-donor families having no offspring surviving after 3 years of growth. The presence of seed families that had relatively high survival rates, as well as those with high rates of mortality in the single-donor treatment indicates that crosses differed substantially in the quality of offspring they produced. These differences in progeny vigor could be genetically determined or may be the consequence of differences in maternal provisioning of seeds.

Maternal effects

Variation in the gynoecial environment during seed development has been reported to affect early seedling growth in a number of species (reviewed in Roach and Wulff, 1987). In the current study the reproductive traits measured did not differ among the donor-number treatments, suggesting that developmental environments were similar. Alternatively, the increased genetic diversity of pollen tubes in the multiple-donor treatments might have stimulated increased resource allocation to the seeds produced. This is unlikely since it would require that resource allocation be based on differences in the levels of genetic variation among zygotes within a fruit. This assumption contradicts current models of resource allocation within plants, which are based on the relative strength of ‘‘sinks’’ (i.e., fruits and growing meristems: Wardlaw, 1968; Watson and Casper, 1984). The sink-strength model predicts that allocation of resources to individual fruits would follow the total vigor and not the variance in vigor of the embryos present.

The patterns of variation in seed and early seedling sizes within and among treatments is consistent with the hypothesis that differential allocation of resources was based on the vigor of developing embryos. Average seed weights among treatments were similar, suggesting that other aspects of the environments of maternal plants may have had greater influences on the total amount of resources allocated to fruits. A similar result for single- and multiple-donor pollinations was observed in *Raphanus sativus* (Marshall and Ellstrand, 1986). Within *Erythronium* fruits there appeared to be greater variation in the maternal provisioning of seeds for the multiple-donor treatments, which affected early seedling size. This is apparent from the observation that the ten-donor treatment had the highest within-family variance for the size of seedling leaves. The large variance in leaf lengths in the multiple-donor treatments probably reflect a larger variance in seed weights, since these leaves are a direct product of the seed (Foster and Gifford, 1974), and cotyledon size tends to be associated with seed mass in other species (Morse and Schmitt, 1985; Ellison and Thompson, 1987; Roach and Wulff, 1987).

The higher variance in seed weights for the multiple-donor treatments suggests that resource allocation in multiply-sired fruits was dependent on the relative vigor of individual zygotes. A higher variance in embryo vigor in these fruits may have contributed to greater differences in seed weight. Seeds in these fruits are likely to be sired by more than one donor since Thomson et al. (1994) found that multiply-sired fruits predominated in *E. grandiflorum* even when only two donors were applied. Compared to the single-donor treatment, the fruits with multiple paternity would be expected to have greater genetic diversity of zygotes. This could create greater differences in the relative sink strength of zygotes and produce larger differences in the amount of resources allocated to individual seeds for fruits with seeds sired by more than one donor (see also Marshall and Ellstrand, 1986).

The effects of differences in seed size within multiply-sired fruits were apparently transient and did not contribute to the observed differences in the growth and survival of offspring among treatments. Seedlings in the ten-donor treatment, which initially had the highest variance in cotyledon length, had achieved a relatively consistent size by the end of the first season. Such transient effects of seed size on seedling size have been observed previously in *E. grandiflorum* (Rigney, 1993), and may be expected under non-competitive conditions (Stratton, 1989). This interpretation reflects conditions in the present study, where the spacing of seedlings among containers would have left little opportunity for competitive interactions among individuals.

Comparison of variance components

Comparisons of the within- and among-family variance components reveals a more complicated pattern than those outlined in the introduction; the expectations of the distribution of variances (Tab. 1) assume that the progeny produced by a single-donor cross are homogeneous. In reality, genetic segregation, recombination, and differences in the environments of individual offspring will produce variation within single-donor families. The expectation of no among-family variation in the ten-donor treatment is also unrealistic; differences in the composition of donors that sired seeds and in maternal environments would introduce differences among seed families. These complications make the two variance components difficult to compare; however, there are still clear expectations for comparisons of the relative magnitude of each type of variance component among treatments. Specifically, we would expect that, in the absence of pre-dispersal selection, the within-family variance for the multiple-donor treatments should be substantially larger than the within-family variance for the single-donor treatment.

Recognition of the effects of seed size variation on early offspring growth allows a clearer interpretation of the variance components within and among treatments. The primary effect of maternal provisioning patterns was to obscure differences in the variance components for corm weight in the first year (Tab. 1). By the second and third year the observation of a higher among-family variance in corm weight for single-donor crosses was consistent with the expectation of greater variation among families in this treatment. This pattern held in spite of the higher rate of mortality for smaller corms from single-donor crosses. More importantly, the magnitudes of within-family variance components for the three treatments were similar all three years. The low within-family variance for the multiple donor treatments contradicts the expectations from the random-siring model, and indicates that less vigorous offspring were being eliminated from these fruits prior to seed maturation.

The differences in the measures of offspring growth and survival and the patterns of variance in these measures indicate that weaker genotypes produced by some crosses in the single-donor treatment were not present in the multiple-donor treatments. Results from previous studies of the effects of multiple-donor pollinations on progeny vigor have been mixed; offspring growth was increased for the multiple-donor treatments in *Costus allenii* (Schemske and Pautler, 1984) and in *Raphanus sativus*

(Marshall and Ellstrand, 1988), but not in *Raphanus raphanistrum* (Snow, 1990) or *Chamaecrista fasciculata* (Sork and Schemske, 1992). Our data suggest that in muliple-donor pollinations, the less vigorous genotypes are either not being produced because of pre-fertilization selection on pollen, or are being eliminated after fertilization through differential ovule abortion. Furthermore, the elimination of these genotypes is occurring in a context-dependent fashion, because they were produced in the single-donor crosses.

Effects of pre- and post-fertilization selection

The high rates of pre- and post-fertilization attrition provide ample opportunity for selection to remove weaker genotypes, and the observation of increased progeny fitness for the multiple-donor treatments suggests that the culling of pollen and zygotes was not random. Although it is apparent that pre-dispersal selection must have occurred in these crosses, the relative contribution of pre- and post-fertilization processes to the removal of weaker genotypes is less clear. We might expect that higher rates of attrition would produce more vigorous progeny; however, if there were a lack of sufficient variation in selection intensity (i.e., level of attrition) then this relationship may be difficult to detect. However, we can examine patterns of pollen-tube growth and ovule abortion to infer the potential for different pre- and post-fertilization processes to contribute to variation in offspring vigor.

The elimination of weaker genotypes could occur at the pre-fertilization stage through pollen competition or non-random attrition of pollen genotypes. Post-fertilization mechanisms that may contribute to elimination of weaker genotypes include post-zygotic incompatibility and early acting inbreeding depression. Post-zygotic incompatibility is apparently not acting in this species because there is not a consistent elimination of selfed ovules (Rigney et al., 1993). Moreover, incompatibility reactions are not known to act in a context dependent manner (Nettancourt, 1977; Williams et al., 1994), so pre- and post-fertilization incompatibility responses are not likely to be responsible for the elimination of weaker genotypes from the mixed-donor treatments.

Previous studies of pre-fertilization selection have examined the effects of pollen competition that results from variation in pollen tube growth rates (Snow, 1986; Mulcahy and Mulcahy, 1987; Quesada et al., 1993). The pollen competition hypothesis assumes that there is exploitation of a limited number of ovules by the fastest growing pollen genotypes. By definition this is a context-dependent process; however, in this study with *E. grandiflorum* there was incomplete fertilization so pollen competition *sensu stricto* was not possible.

Pre-fertilization attrition of pollen could increase the vigor of the resulting offspring if this process resulted in the elimination of intrinsically weak pollen genotypes. In the current study selection on pollen vigor could occur if stringent stylar environments provided only limited stylar resources. Under this model we would expect weaker pollen genotypes to suffer higher levels of attrition when growing in the same style as stronger genotypes. However, previous experiments with *E. grandiflorum* indicate that pollen genotypes having high attrition in single-donor pollinations will have reduced attrition when growing in the same style as low-attrition genotypes (Cruzan, 1990a), implying that there is not competition for stylar resources. Additional studies (e.g., disabling of stylar selection mechanisms; Cruzan, 1993) would be needed before any conclusions could be made about the role of pre-fertilization selection on sporophytic vigor in *E. grandiflorum*.

Post-fertilization selection on developing zygotes is more likly to have eliminated weaker genotypes from the multiple-donor families in *E. grandiflorum*. Abortion of embryos in *E. grandiflorum* appears to depend on the relative level of inbreeding expressed by developing seeds within fruits (Rigney et al., 1993; Rigney, 1993, 1995). The high outcrossing frequency for *E. grandiflorum* ($t = 0.83$; Rigney et al., 1993) is consistent with the conditions necessary for the maintenance of high levels of inbreeding depression (Charlesworth and Charlesworth, 1987). Furthermore, the abortion of selfed embryos depends on the presence of outcrossed zygotes (Rigney, 1995), so embryo abortion in *E. grandiflorum* appears to occur in a context-dependent manner. Patterns of embryo abortion consistent with a context-dependent response have been observed in other species (Lee and Bazzaz, 1986; Casper, 1988; Krebs and Hancock, 1990; Becerra and Lloyd, 1992; Martin and Lee, 1993).

High levels of genetic load results in a skewed distribution of zygote abortion frequencies among recipients, with a minority of individuals experiencing higher rates of abortion (Krebs and Hancock, 1990). For *E. grandiflorum* the observed variation in the level of post-fertilization attrition may represent a response to the inbreeding history of different plants. This early-acting inbreeding depression could produce patterns of embryo abortion that reflects a response to inbreeding load rather than the intensity of selection on zygotes. Hence, context-dependent abortion of inbred zygotes could increase the average growth rate of offspring produced, but we would not necessarily expect to see a correlation between abortion frequency and average progeny vigor when comparisons are made across recipients. Further studies, including the manipulation of maternal resources, will be useful in elucidating the effects of post-fertilization selection on the average vigor of offspring.

Conclusions

Within-flower fecundity reductions were severe in *E. grandiflorum* at both the pre- and post-zygotic stages. Analysis of components of variance for offspring growth parameters indicates that pre-dispersal selection eliminates weaker progeny genotypes in multiply-sired fruits. Observations of pollen-tube growth suggest that pre-fertilization selection is unlikely to be contributing to the observed differences in progeny vigor. The selective removal of weaker genotypes appears to occur through embryo abortion, probably as a result of inbreeding depression expressed at early developmental stages. Although these processes act to reduce seed production, the opportunity for the selective removal of weaker genotypes would avoid investment in progeny that have reduced viability. Further studies of pollen-style interactions and embryo abortion patterns will continue to contribute to our understanding of the early expression of inbreeding depression and the effects of pre-seed-dispersal selection on offspring growth and survival.

Acknowledgements

We are indebted to J. Brunet, L. Rigney, N. O'Connor, A. Seidel, and J. Sigadel for assistance in the field and the lab, and to L. Rigney and M. V. Price for comments on the manuscript. This research was funded by NSF dissertation improvement grant number BSR-8601104 to MBC and NSF grant number BSR-8614207 to JDT. This is contribution number 984 from the Graduate Program in Ecology and Evolution, State University of New York at Stony Brook.

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Received 1 April 1996;
revised 20 June 1996;
accepted 17 October 1996.