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VARIATION IN POLLEN SIZE, FERTILIZATION ABILITY, AND POSTFERTILIZATION SIRING ABILITY IN *ERYTHRONIUM GRANDIFLORUM*

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Abstract.—The mean volume of pollen grains and total pollen production varied both within and among plants of *Erythronium grandiflorum*. The second flowers of two-flowered plants tended to produce smaller and fewer grains than first flowers, but there was no overall relationship between mean pollen grain size and production per flower. I evaluated the effects of pollen size differences within and among plants on two components of male reproductive success: pollen tube growth and postfertilization siring ability.

Pollen tubes grown in media were longer for second flowers, but were not correlated with the mean size of pollen grains, suggesting that (1) internal resource content of pollen (i.e., carbohydrates plus lipids) was not associated with the hydrated size of pollen, and that (2) pollen from second flowers contained more resources. I analyzed the growth rate and the fertilization ability of pollen growing in styles. Growth rate differed among donors and recipients, but no effects of pollen or donor characters (i.e., pollen production, grain size, and flower position) were detected. In single donor pollinations, pollen size was negatively correlated with fertilization ability across donors, and positively correlated with postfertilization siring ability of donors. A second experiment used pairs of donors; within-plant differences in pollen size and flower position had effects similar to the single donor experiment on fertilization ability, but among-plant differences were not significant. The results corroborate earlier experiments that suggest that the growth of pollen tubes in the style is probably controlled by the recipient, since donor characters had minimal effects on pollen fertilization ability.

Postfertilization siring ability was not affected by within-plant differences in mean grain size and production. For among-donor differences, the number of seeds set for each donor was positively correlated with the mean grain volume, and when a donor producing large pollen fertilized ovules in an ovary, there was increased seed abortion for seeds in the same ovary sired by a second donor. In addition, the total number of seeds produced by a fruit was decreased when both donors had large pollen, apparently due to increased postfertilization abortion. Postfertilization processes appear to be influenced by paternal differences that are expressed through competition among developing seeds for maternal resources.

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Male characters in some species can be related to mating success through an increase in the ability of males to compete for mates (intrasexual selection) (Darwin, 1871). There has been considerable speculation about intrasexually selected characters in plants (Janzen, 1977; Willson and Burley, 1983; Stephenson and Bertin, 1983), and there is some evidence that plant characters can affect pollen delivery (the first stage of reproduction through male function). Characteristics of floral displays have been correlated with direct measures of pollen donation (Willson and Bertin, 1979; Bell, 1985; Stanton et al., 1986; Cruzan et al., 1988) indicating that floral characters can be selected through male function. Delivery of pollen to stigmas does not, however, ensure male reproductive success. Depending on pollinator activity, the number of pollen grains deposited on a stigma can exceed the number of ovules (Snow, 1986; Cruzan, 1989), and usually pollen from several donors is present (i.e., pollen carryover has been found in several species: Thomson and Plowright, 1980; Thomson and Thomson, 1989). It has been suggested that competition for ovules among pollen from different donors would select for high tube growth rates and fertilization abilities (Haldane, 1932; Willson and Burley, 1983; Stephenson and Bertin, 1983). On the other hand, recipient styles may impose incompatibility barriers to certain pollen types, so pollen

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characters that allow the circumvention of these barriers would be advantageous. In either case, the average fertilization ability of pollen donors might differ if there were variation in the characters affecting the *pollen vigor* of donors (the average fertilization ability of pollen when tested across several recipients). Pollen vigor is a statistical attribute of plants; the actual characters conferring an increased fertilization ability on pollen would probably be strictly physiological, making them difficult to measure. Certain more easily measured morphological characters of donors and their pollen, however, represent likely candidates that might be related to pollen vigor. In this context, the average grain volume (if grain size limits resource content) and the total number of grains (if limited resources are divided among grains) produced by a plant may be indicative of the availability of resources in pollen that are potentially limiting to growth. Average pollen size may also be indicative of a plant's overall metabolic vigor if the volume that a grain achieves at maturity is dependent on its growth rate. Either greater resource content or increased metabolic vigor of pollen may increase its fertilization ability, affecting male reproductive success.

In this paper, I first describe variation in pollen grain size and production within and among plants. I then use within-plant differences in these characters to examine their potential impact on pollen tube growth rate, fertilization ability, competitive ability, and postfertilization siring ability of pollen donors.

Methods

General Biology

Erythronium grandiflorum Pursh (Liliaceae) is a perennial herb arising from a buried corm. Plants appear in early spring shortly after snowmelt, and flowering plants produce from one to several flowers. Each flower is relatively large (2.5–4.0 cm), with six anthers occurring in two whorls of three anthers each. On multiflowered plants flowers open sequentially, with approximately 24 hr between the anthesis of each flower on an inflorescence. Anthers in the outer whorl dehisce shortly after anthesis, and the anthers of the inner whorl usually dehisce within 24 hr of the outer anthers. The three lobes of the stigma correspond to three channels within the lumen of the fluid-filled style. The channels are open to each other, but pollen tubes from pollen germinating on each lobe tend to grow along one channel (Cruzan, 1990), allowing me to compare the growth of pollen from different sources in the same style. When pollen from the same source is applied to the two separate lobes the two pollen populations perform similarly (unpubl. data). After pollination, pollen tubes require between 24 and 72 hr to reach the ovary, but no additional tube growth takes place after 72 hr (Cruzan, 1989). When styles were collected 5 days after pollination, there were often over 100 tubes present in the stigmas of flowers, but 20–80% of these never reach the ovary (stylar attrition; Cruzan, 1989). Both stylar and ovarian attrition (failure of pollen tubes entering the ovary to produce seeds) contribute to variation in seed production in naturally pollinated flowers, with stylar attrition having a larger influence than ovarian (Cruzan, 1989). In all of the experiments where tube growth was measured in the style, I used measurements only on tube populations that had at least 10 tubes. I conducted my experiments during the summers of 1985, 1986, and 1987 near the Rocky Mountain Biological Lab, Gothic, Colorado.

Pollen Size Variation

I collected anthers from two-flowered plants to determine within- and among-plant variation in pollen production and grain volume. I collected undehisced anthers from 10 plants within 30 m of each other in each of four populations (Table 1). From each of the two whorls of each flower I removed two anthers, placed them into separate 1.5-ml microcentrifuge tubes, allowed them to dehisce, and stored them in 70% EtOH. I diluted each sample with 100 ml of 0.1% saline, and used a 16 channel Coulter Counter (model TA II; see Harder et al., 1985 for further details) to take three 2-ml subsamples from this solution. I estimated the average grain volume and the total number of grains present for each anther from a size frequency distribution across five

TABLE 1. Mean pollen grain diameter and pollen production for one-flowered *Erythronium grandiflorum* plants growing in four populations in the East River Valley near Gothic, Colorado. Date of collection, elevation, number of plants used in the analysis, mean pollen grain diameter in micrometers, mean total pollen production per plant, and the mean length of the outer tepal of flowers are given. Numbers in parentheses are standard deviations. Within a column, means followed by the same letter are not significantly different from each other (Tukey multiple range test).

Site	Date	Elevation (m)	*N*	Pollen diameter	Pollen production	Tepal length
403 Trail Head	6/21	2,950	8	39.06 a (16.35)	75,762 a (9,414)	29.5 a (1.1)
Bellview	6/21	3,060	10	40.38 b (15.83)	97,338 b (19,230)	31.6 b (1.1)
Baldy	6/24	3,130	9	39.72 c (16.65)	91,962 c (15,450)	28.8 a (1.1)
Schofield Pass	7/2	3,260	9	38.83 a (17.85)	105,006 d (18,807)	31.6 b (1.1)

Coulter Counter size channels that spanned a size range of 3,000–97,000 μm^3. I calculated a total volume for each channel by multiplying the average frequency for a channel by the predetermined midpoint volume of that channel. Before making calculations, I took the natural log of the midpoint volumes to normalize them, and back-transformed the values obtained to give the average volume in cubic micrometers. I analyzed the data in a nested analysis of variance using the Varcomp procedure of SAS (SAS Inst., Inc., 1985).

Pollen Tube Growth in Vitro

Previous measurements indicated that a considerable pollen size difference could exist between flowers of an individual plant (Cruzan, unpubl. data). I used this attribute of *E. grandiflorum* to determine what effect pollen size might have on germination and pollen tube growth in vitro. I collected undehisced anthers from each flower of 35 two-flowered plants from a single population, and pooled the six anthers from each flower. I stored anthers in air-tight vials to prevent them from dehiscing, and opened all vials at the same time to force the anthers from the two flowers of a plant to dehisce simultaneously. After the anthers had begun to dehisce, I placed a small amount of pollen from each flower in an aqueous germination medium containing 20% sucrose, 15% Knox gelatin, and 5% nutrient solution (containing boric acid, 100 ppm; calcium, 300 ppm; potassium, 100 ppm). I first melted the germination medium and spread a small amount into a thin layer on a microscope slide. I placed the pollen from both flowers of an individual plant at the same time on different areas of the same slide, placed the slide into a petri dish containing moistened tissue paper, and kept the slide in the dark at room temperature for 48 hr. I stained the slides with Alexander's stain (Alexander, 1969), and mounted them by melting the gelatin mixture before adding a cover slip.

I analyzed the slides for grain density, germination, and tube length. I determined grain density and percentage pollen germination by censusing slides under a compound microscope at 100×. I made a transect across the center of each slide and scored all of the grains encountered for germination. I scored abnormally short tubes as ungerminated, because they were much shorter than the median pollen tube length. I calculated grain density for each slide based on the area sampled as the total number of grains encountered and percentage germination for each flower as the fraction of grains encountered that had germinated. I measured tube lengths under a stereoscopic microscope at 12×. Using an optical tracing device in conjunction with a DigitBit digitizer, I recorded the coordinates of points along the length of a tube including its origin and tip, and then added the distances between adjacent pairs of points to give the total pollen tube length. The large number of tubes made tubes toward the center of the pollen aggregation difficult to trace, so I measured tubes extending outward from the main aggregation. I measured the 10 longest traceable pollen tubes present for each flower.

I measured the mean grain volume for each flower as described above. I eliminated two of the plants with unusually large pollen grains from the analysis since they were significantly larger than the mean size of the other plants (based on single observation comparisons to the mean, $P < 0.05$ for both).

Using the GLM procedure of SAS (SAS Inst., Inc., 1985), I compared grain density and percentage germination for the two flower positions in ANOVA models with the identity of each plant entered as a random factor. I analyzed tube length with plant identity as a random factor, flower position as a fixed factor, and mean grain volume, pollen density, and percentage germination as covariates. All data approximated normal distributions, so no transformations were necessary.

Pollen Tube Growth Rate in Vivo

Using the same two-flowered plants used in the in vitro tests described above, I compared the growth of pollen from the two flowers of each donor in the same recipient style. I collected recipient buds, submerged their stems in tubes containing water, and allowed them to open before making pollinations. Using five different recipient flowers for each donor, I pollinated one lobe of each of the three-lobed stigmas with pollen from one flower of a donor plant, and a second lobe of each stigma with pollen from the second flower of the same donor. To provide a point of reference I excised the unpollinated lobe. I fixed styles in 70% EtOH 24 hr after pollinating them and measured pollen tubes under a stereoscopic microscope at 50×. After slitting styles longitudinally and flattening them, I stained them with a 1:1 mixture of acidified 0.1% aniline blue and acetocarmine (40% acetic acid saturated with carmine) for 10 min (Cruzan, 1989), censused tubes in each stylar channel at 1.5 mm intervals along the length of the style, and calculated mean tube lengths from these distributions by assuming that tubes ended halfway between census points (Cruzan, 1986). Using GLM again, I entered the pollen donor identity and the identity of the recipient as random factors, flower position as a fixed factor, and mean grain volume and the number of pollen tubes present in each channel as covariates. Untransformed data were approximately normally distributed.

Fertilization Ability

I measured stylar and ovarian attrition for pollen from different donors after crosses among plants in situ. I selected 35 three-flowered pollen donors and crossed them to 2–7 three-flowered recipients each. To prevent extraneous pollination, I covered the pistils of recipient flowers with grass straws while they were still in bud. I pollinated one or two of the flowers on each recipient with pollen from one of the donors, and in most cases used two donors on each recipient. I collected undehisced anthers from the first flowers of the donors several days before making pollinations and stored them in closed microcentrifuge tubes at 2°C. On each day I allowed anthers to dehisce by opening the vials to expose them to lower humidity and made pollinations by evenly coating recipient stigmas with pollen on the second day after recipient flowers had opened. After completing the pollinations, I preserved the remaining pollen from each donor in 70% EtOH. I collected recipient styles 5 days after pollinating them and stored them in 70% EtOH. Pollen tube growth typically ceases after 2–3 days, and harvesting styles after 5 days has no effect on fruit set (Cruzan, 1989). I collected fruits when they were mature, and counted and weighed the seeds from each fruit. I determined mean grain volume for all donors using the Coulter Counter as described above. After staining styles as described previously, I counted the tubes present in the stigma and at the base of the style, and calculated stylar attrition as the fraction of tubes present in the stigma not reaching the ovary and ovarian attrition as the fraction of the number of tubes present at the base of the style not producing seeds (Cruzan, 1989). Using GLM I determined the effect of mean grain volume on stylar attrition, ovarian attrition, and seed production, in models which included the recipient flower position as a fixed factor, and the mean grain volume of the donor and the number of tubes present in the stigma as covariates. I also examined the effects of pollen tube growth parameters and the mean grain volume of donors on the prob-

ability of fruit set using logistic regression with the CATMOD procedure of SAS.

Pollen Competitive Ability

Using genetic markers to assign paternity to seeds, I examined the effect of variation in pollen production and pollen size both between and within pollen donors on the competitive ability of pollen after double pollinations. I used pollen from both flowers of pairs of donors to pollinate two lobes on each recipient stigma. In this design, each recipient flower always received pollen from two different donors and from two different donor flower positions. I electrophoretically screened 240 two-flowered plants from a population at the upper end of Washington Gulch, about 5 km from the RMBL, for the MDH locus. From this group I chose 110 homozygous individuals to act as pollen donors and recipients. I used 18 pairs of donors, with one donor of each pair being homozygous fast and the other being homozygous slow, to pollinate groups of five homozygous recipients (either fast or slow) by doing single donor pollinations to each of two stigma lobes of each flower. For each recipient flower I pollinated one stigma lobe with pollen from the first flower of one donor, and pollinated the second lobe with pollen from the second flower of the other donor. For the second flower of the same recipient I reversed the donor flower positions, and switched the flower positions for the pair of donors between the recipient flower positions for successive recipients. Five days after pollinating them, I collected the recipient styles and stored them in 70% EtOH and determined the amount of stylar and ovarian attrition for each donor's pollen in each recipient flower as described above. I stored and analyzed the pollen from each donor flower using the Coulter Counter as described previously. I measured the length of an undehisced anther from each donor flower as a measure of total pollen production ($r^2 = 0.814$, $P < 0.0001$ for the pollen production vs. anther length relationship; J. Thomson, pers. comm.). After counting and air drying the seeds from fruits, I electrophoretically determined the genotype of a sample of 15 seeds from each fruit, or all of the seeds for fruits having less than 15 seeds, to estimate the proportion of seeds sired by each donor. I analyzed the effects of both donors' characters on each donor's stylar and ovarian attrition and the proportion of seeds each sired by alternately treating each donor as either the focal donor (measurements were treated as response variables), or as the nonfocal donor (measurements were treated as independent variables). I used different models to analyze both within- and among-plant effects of grain size and production. For within-plant effects, I held the identity of the focal donor statistically constant using GLM and analyzed the effects of grain size and production and flower position of both the focal donor and the nonfocal donor on the focal donor's pollen tube growth, ovarian attrition, and fertilization success. For the among-plant analysis, I used path models that included the number of tubes present in the style and grain size and production for both donors with the focal donor's degree of stylar attrition and fertilization success as dependent variables using the REG procedure of SAS. Prior to analysis I square root-arcsin transformed the proportion of seeds sired and left all other variables untransformed.

TABLE 2. Components of variation in pollen size and pollen production in *Erythronium grandiflorum*. Two anthers were collected from each anther whorl of each flower of 10 two-flowered plants in four populations. Numbers represent the percentage of total variation explained by each component. Variance components and significance levels are from a completely nested model using type IV sums of squares from the GLM procedure of SAS.

Component	*d.f.*	Volume	Production
Population	3	12.73***	16.93***
Plant	32	48.06***	18.72***
Flower	36	22.27***	50.62***
Whorl	72	3.80**	0.38 ns
Error	144	13.14	13.35

** $P < 0.01$.
*** $P < 0.001$.
ns = $P > 0.10$.

RESULTS

Pollen Size Variation

Mean grain volume and production varied within and among plants. The size distributions of pollen from plants of the four populations censused were unimodal for 36 of the 40 plants tested and bimodal for the others. The latter individuals had large

TABLE 3. Mean pollen diameter (μm), in vitro mean length of the longest 10 pollen tubes (mm), the percentage of grains germinating and the grain density on the slide, and in vivo mean pollen tube length and number of pollen tubes present for pollen from the first and second flowers of *Erythronium grandiflorum*. Pollen diameters are in micrometers and pollen tube lengths are in millimeters. Means followed by the same letter are not significantly different from each other. Standard deviations are in parentheses.

					In vivo	
Flower position	Mean diameter	Length	In vitro density	Germination	Length	Number
First	56.12 a (21.38)	4.18 a (1.20)	90.68 a (43.01)	47.47% a (16.97)	3.17 a (0.86)	19.1 a (5.8)
Second	49.53 b (23.75)	5.17 b (1.27)	84.45 a (33.82)	56.38% b (14.46)	3.07 a (0.82)	18.4 a (8.0)

numbers of small, apparently aborted pollen grains and were removed from the analysis. The mean pollen diameter ranged from 36.2 to 41.8 μm (mean = 39.5, standard deviation = 17.1, $N = 36$). Pollen production varied among plants much more than pollen volume (mean = 15,519.6 grains/anther; standard deviation = 446.6, $N = 288$), and there was no relationship between mean pollen production and mean pollen volume among plants ($r = 0.12$, $P > 0.40$, $N = 36$).

Pollen size and production varied among the four populations censused (Table 1), although a greater proportion of the total variation in these characters was among plants than among populations (Table 2). Pollen size and production also varied within plants. Pollen volume varied between flowers of plants to a much greater extent than within flowers (between or within whorls), but not as much as among plants (Table 2). Pollen production varied between the two flowers of plants to a much greater extent than either within flowers or between individual plants (Table 2). Overall, the second flowers of plants in these populations produced less pollen (mean = 82,470 grains per flower) than their first flowers (mean = 103,620 grains per flower), and the pollen from second flowers tended to be smaller in diameter (mean = 39.2 μm) than pollen of their first flowers (mean = 39.9 μm).

Pollen Tube Growth in Vitro

Second flowers of the plants used in this experiment and the in vivo experiment had smaller grains than first flowers (Table 3). The difference between the mean pollen size of first and second flowers was not consistent for all plants, so effects of pollen size on pollen vigor can be determined separately from effects due to flower position.

The mean length of the longest 10 pollen tubes on germination slides was affected by both the identity of the donor, and the position of the source flower (Tables 3 and 4). The proportion of grains germinating was positively correlated with their density on

TABLE 4. Analysis of in vitro pollen tube length of pollen from first and second flowers of *Erythronium grandiflorum*. The identity of the donor was entered as a random factor, donor flower position as a fixed factor, pollen tubes measured nested within the flower position, and mean pollen grain volume, grain density on the slide, and percentage germination entered as covariates. Parameters are based on type III sums of squares from the GLM procedure of SAS.

Source	*d.f.*	*F* value	Probability *P*
Donor identity	26	4.84	0.0001
Donor flower position	1	14.18	0.0002
Pollen tube (flower)	18	0.82	0.6813
Mean pollen grain volume	1	0.17	0.6786
Grain density	1	0.64	0.4253
Percentage germination	1	1.41	0.2357

TABLE 5. Analysis of in vivo pollen tube length of pollen from the first and second flowers of *Erythronium grandiflorum*. Donor identity and recipient identity were entered as random factors, flower position as a fixed factor, and the mean grain volume and number of pollen tubes present were entered as covariates. Parameters are based on type III sums of squares from the GLM procedure of SAS.

Source	*d.f.*	*F* value	Probability *P*
Donor identity	19	4.04	0.0004
Donor flower position	1	0.10	0.7554
Recipient identity	3	7.33	0.0008
Donor × recipient interaction	24	4.76	0.0001
Mean pollen grain volume	1	0.40	0.5325
Number of tubes present	1	0.71	0.4070

TABLE 6. Analyses of the number of pollen tubes in the stigma and at the base of the style, the amount of stylar and ovarian attrition, and the number of seeds set in *Erythronium grandiflorum*. Independent variables are the position of the recipient flower (fixed factor), mean pollen grain volume of the donor, and number of pollen tubes in the stigma and at the base of the style (covariates). Numbers are F values calculated from the type III sums of squares from the GLM procedure of SAS (SAS Inst., 1985).

Source	Pollen tubes		Attrition		Seeds sired
	Stigma	Base	Stylar	Ovarian	
Recipient flower position	3.53*	1.73 ns	2.82†	3.73*	6.47**
Donor pollen grain volume	0.38 ns	7.44**	15.26***	2.36 ns	9.17**
Pollen tubes in stigma	—	80.05***	10.44**	3.02†	5.41*
Pollen tubes at style base	—	—	—	0.15 ns	14.21***

† $P < 0.10$.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.
ns = $P > 0.10$.

the slide ($r = 0.21$, $P < 0.05$, $N = 56$). Pollen tubes from second flowers were significantly longer than pollen tubes of pollen from first flowers (Table 3). Pollen density, percentage germination, and mean volume had no significant effects on tube length in vitro (Table 4).

Pollen Tube Growth Rate in Vivo

After 24 hr, tubes usually extended from one-half to three-quarters the length of the style. Mean tube lengths after 24 hr ranged from 1.69 to 5.32 mm. Tube lengths in vivo were not correlated with pollen tube lengths for the same plants obtained in growth medium ($r = 0.084$, $P > 0.5$). Pollen from the two flower positions growing in the same style produced similar numbers of tubes, which grew at similar rates (Table 3). Mean tube lengths for these pollinations were most strongly affected by the identity of the pollen donor, the identity of the pollen recipient, and their interaction (Table 5). Mean grain volume and the number of tubes present had no effect on mean tube length in vivo (Table 5).

Fertilization Ability

The mean grain diameter of the donors (first flowers) ranged from 24.4 to 33.0 μm (mean = 29.5, standard deviation = 1.76). Approximately 25% of the pollinations failed due to loss of pollen viability. This was probably due to prolonged exposure to dry air since *Erythronium* pollen is sensitive to desiccation (unpubl. data). Previous work indicates that storage of *Erythronium* pollen in air-tight vials does not affect pollen tube growth (unpubl. data). The number of tubes present in stigmas was not affected by either the mean grain volume of the donor or the position of the recipient flower (Table 6). The number of tubes at the base of the style and stylar attrition were significantly affected by the position of the recipient flower, the number of tubes present in the stigma, and the mean grain volume of the donor (Table 7). Plants that produced small pollen tended to have less stylar attrition than plants that produced large pollen grains ($r = 0.48$, $P = 0.0016$).

Only 26 of the 57 flowers pollinated produced a fruit, apparently because many ovules were left unfertilized. The amount of stylar attrition was a fairly good indicator of the probability of fruit retention ($P < 0.008$; from a logistic regression model). Pollinations resulting in a small degree of stylar attrition had a greater probability of producing a fruit (holding the number of pollen tubes at the base of the style constant by entering this variable into the model as a covariate). For flowers that set a fruit, postfertilization ovule abortion was most strongly affected by the recipient flower position and the number of tubes present in the stigma (Table 6). Seed set was affected by the recipient flower position, the number of tubes at the base of the style, and the average pollen grain volume of the donor (Table 6). Overall, plants producing large pollen grains sired more seeds than plants producing small grains ($r = 0.49$, $P = 0.035$ for the correlation between pollen size and number of seeds sired).

TABLE 7. Variance components from analyses of covariance for within-donor variation with donor identity held constant for several measures of donor performance after two-donor pollinations in *Erythronium grandiflorum*. The donor identity was treated as a random factor and recipient identity was nested within donor identity. Effects listed include recipient and sire's flower positions, and the number of pollen tubes present in the stigma, pollen production, and mean pollen grain volume for both donors. The percentage of the variance explained by each parameter from type IV expected mean squares from the GLM procedure of SAS is given (SAS Inst., 1985).

Source	Pollen tubes at style base	Attrition		Seeds sired
		Stylar	Ovarian	
Recipient identity	0.0*	1.2†	60.0	24.7*
flower position	0.0	0.6	0.0	5.7
Sire identity	0.0	0.1†	40.0†	40.0*
Flower position	2.3*	25.1***	0.0	4.8
Tubes in stigma	92.4***	18.6**	0.0	4.8
Grain volume	3.3**	24.9**	0.0	4.3
Pollen production	0.0	0.0	0.0	2.5
Nonsire				
Tubes in stigma	0.6	7.7*	0.0	6.5
Grain volume	0.0	0.8	0.0	3.2
Pollen production	1.2†	21.1**	0.0	3.7

† $P < 0.10$.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Pollen Competitive Ability

In flowers pollinated with two donors, each donor's tube growth in the style (when acting as the focal donor) was affected by the characters of both donors present (the focal donor and the nonfocal donor; Table 7). For the focal donor, stylar attrition increased when there were more tubes present in the stigma, and when the mean grain volume was greater (Table 7). The position of the donor flower also significantly affected the degree of stylar attrition, with pollen from second flowers (mean = 52.76%) having slightly less attrition than pollen from first flowers (mean = 53.29%) (Table 7). The focal donor's total pollen production had no effect on tube growth, but stylar attrition was greater when the nonfocal donor had greater pollen production (Table 7). Higher numbers of nonfocal donor tubes present in the stigma also tended to increase the focal donor's stylar attrition. In a path model examining among-donor variation in focal donor's tube growth, the only covariate having a strong effect was the number of focal donor tubes present in the stigma (Fig. 1).

Variation in ovarian processes was mostly among donors and was not affected by within-donor variation in pollen characters (Table 7, Fig. 1). When among-plant differences were held constant, none of the covariates measured had significant effects on donor performance and most of the variation was explained by differences among donors (Table 7). The path analysis for among-donor variation in donor performance indicates that average grain volume was strongly correlated with a donor's ability to sire seeds (Fig. 1). There was also a strong negative effect of the mean size of the nonfocal donor's pollen on the number of seeds produced that were fertilized by the focal donor. The nonfocal donor's total pollen production and number of tubes present had negative effects on the success of the focal donor, but these were not significant.

Synopsis of Results

Consistent effects for the donor and pollen characters measured were found for pollen tube growth in most experiments (Table 8). Pollen from second flowers produced longer tubes in vitro, did not differ in pollen tube growth rate in vivo, and had less pollen tube attrition than pollen from first flowers. Pollen production did not have any effect on any of the tube growth measurements, except for its influence on the stylar attrition of pollen from other donors growing in the same style. Mean pollen grain volume did not affect either tube length in vitro or growth

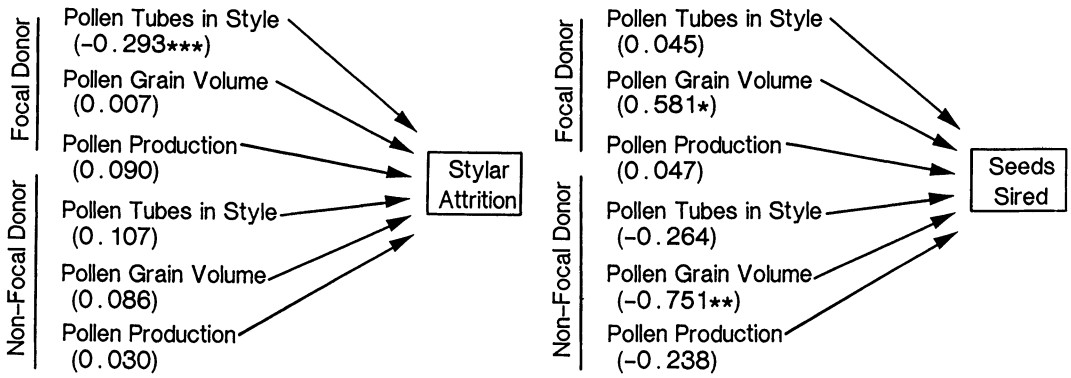

FIG. 1. Path models for the among-donor effects of pollen tube parameters and donor characters on the amount of stylar attrition and the number of seeds sired after pollinations with pairs of donors in *Erythronium grandiflorum*. Standardized partial regression coefficients (path coefficients) from multiple regression models that included parameters for a donor (focal donor) and for the other donor present (nonfocal donor) are given.

rate, but smaller pollen had less attrition in both experiments where attrition was examined. For ovarian attrition, neither donor flower position nor pollen production had any effects. Plants producing larger pollen, however, had greater seed siring ability than plants producing smaller pollen in both experiments where it was examined.

DISCUSSION

Pollen Resource Content

Pollen size varies both within (Bell, 1959; Bragg, 1969; Stanton et al., 1986) and among species (reviewed by Muller, 1979). Debate about the consequences of differences in pollen size between species began in the 1800s (Darwin, 1877), and continues today (refs. in Cruden and Lyon, 1985). Many of these accounts assume that differences in pollen size represent functional mating barriers between species and that pollen size has coevolved with style length. This interpretation presumes that pollen contains limiting resources for the growth of pollen tubes and is autonomous in its growth to the ovary. Physiological evidence conflicts with these hypotheses. As early as 1830 it was recognized that pollen grains must receive nutrition from the stylar tissue (Amici, 1830; cited in Cruden and Lyon, 1985). More recently, carbon labeling studies clearly demonstrate that microgametophytes are using external substrates for pollen tube construction (O'Kelley, 1955; Johri and Vasil, 1961; Campbell and Ascher, 1975; Vasil, 1974). Although pollen is known to contain storage materials (Todd and Bretherick, 1942; Baker and Baker, 1983), it is thought that these are used large-

TABLE 8. Summary of results from experiments for pollen source flower position, pollen grain production, and mean pollen grain volume effects on pollen tube growth and postfertilization siring ability in *Erythronium grandiflorum*. Results from the in vitro and in vivo experiments and the fertilization ability (single donor in situ) and the competitive ability (double donor in situ) experiments are given.

Experiment	Flower position	Pollen production	Pollen size
	A. Pollen Tube Growth		
In vitro tube growth	Second > first	Not tested	No effect
In vivo growth rate	No effect	Not tested	No effect
Single donor in situ	Not tested	Not tested	Smaller > larger
Double donor in situ	Second > first	No effect[1]	Smaller > larger
	B. Seed Siring Ability		
Single donor in situ	Not tested	Not tested	Larger > smaller
Double donor in situ	No effect	No effect	Larger > smaller

[1] No effect of a donor's pollen production on the attrition of its pollen was detected, but pollen from larger anthers tended to increase the attrition of pollen tubes from other donors growing in the same style.

ly for germination and early growth (Vasil, 1974; Cruden and Lyon, 1985); the majority of pollen tube growth is supported by the stylar tissue (reviewed in Vasil, 1974). There is still the possibility, however, that there is some limiting resource contained in pollen that is critical to a pollen tube's successfully reaching the ovary, and that larger pollen grains contain more of this vital substance.

Although it has been assumed that pollen grain volume is correlated with resource content, no data directly address this issue. Indirect support comes from observations of the distance that tubes grow either in vitro or in vivo after incompatible matings (Glover and Barrett, 1983; Anderson and Barrett, 1986); however, there is also evidence of this type to the contrary (Barnes and Cleveland, 1963). Pollen grown in media or in incompatible styles presumably ceases growth when internal limiting resources are exhausted (Johri and Vasil, 1961). In *E. grandiflorum,* mean grain volume was not related to pollen tube length in vitro. When mean grain size was held constant, pollen from second flowers tended to produce longer tubes. Anthers from the two flower positions dehisced at the same time, so this difference in tube length should not be due to a difference in pollen age. Together these results suggest that the resource content of pollen grains is not correlated with the measurement of grain volume used in this study. Pollen size in *E. grandiflorum* can vary substantially depending on its state of hydration (pers. observation). The volumes obtained from Coulter Counter measurements represent the fully hydrated state; this measure is not necessarily correlated with the minimal internal volume, which occurs in the dehydrated state. If pollen tube length attained in vitro is truly indicative of grain resource content, pollen grain volume as measured by the Coulter Counter does not correspond to grain resource content, and pollen from second flowers contains greater amounts of resources than pollen from first flowers.

Pollen Tube Growth in the Style

Within-plant differences in pollen appeared to have effects on its fertilization ability after pollinations to flowers, suggesting that plants may be able to influence the vigor of the pollen that they produce. Previous work indicates that pollen tube attrition in *E. grandiflorum* may be mediated by the recipient style (Cruzan, 1989), but evidence for some pollen-mediated control of pollen tube growth via interactions between growing tubes has also been found (Cruzan, 1990). Pollen vigor in this species probably represents the ability of pollen to circumvent compatibility barriers imposed by the style, and contributes to pollen-source-specific differences in pollen tube growth. Within-donor differences in pollen tube growth represent evidence that pollen donors can influence their fertilization success. Mean pollen size and the position of the donor flower both affected the amount of stylar attrition, perhaps because of associated characters that influenced pollen vigor. These effects were relatively weak and were not detected in all of the experiments, so the ability of plants to influence pollen vigor may be minimal. Among-donor differences in pollen vigor were greater than within-donor differences, but this may represent genetic rather than environmental influences on pollen vigor.

The effects of mean grain size, flower position, and pollen production found probably represent characters that are correlated with some physiological characters that directly affect the fertilization ability of pollen. Because the primary characters affecting pollen vigor were not measured, it is not known what the cost of producing more vigorous pollen to the donor is, but we can assess the potential effects of the characters measured. Previous work indicates that second flowers of *E. grandiflorum* tend to have greater relative investment in male function than first flowers through higher pollen/ovule ratios and a reduced probability of setting a fruit (Thomson, 1989; Cruzan, unpubl. data), so increasing the amount of resources sequestered in pollen of second flowers might represent another trend in this direction. Producing smaller pollen grains also appeared to increase the fertilization ability of pollen, but there may be an associated cost in the expected longevity of smaller pollen. Lily pollen tends to be very susceptible to drying (Stanley and Linskens, 1974), and under field conditions, *Erythro-*

nium pollen loses viability within a few hours after anther dehiscence (Thomson, Cruzan, and Rigney, unpubl. data). Since smaller pollen grains have a higher surface-to-volume ratio, they would be expected to have reduced longevity, but this hypothesis remains to be tested. Although it would appear that pollen donors can influence the vigor of the pollen they produce, the within-donor differences in fertilization ability of pollen detected were relatively small, and the advantages might be offset by associated costs to the donor.

Seed Siring Ability

There were relatively large differences among donors in their siring success after both single and double pollinations; plants that produced large pollen also sired more seeds. The differences in siring success observed probably represent differences in postfertilization ovule abortion rather than selective fertilization of ovules. Selective inhibition of pollen tubes in the ovary could explain differences in the number of seeds sired; however, there was nearly a one to one correspondence between the number of tubes at the base of the style and the total number of ovules fertilized (seeds plus apparently fertilized and aborted ovules; unpubl. data). The difference between the number of tubes at the style base and the number of ovules fertilized was unrelated to the mean size of a donor's pollen and to the difference in size between the pollen of the two donors present (unpubl. data). Apparently, differences in the number of seeds sired by different donors were due to postfertilization processes rather than an inability to fertilize ovules.

Pollen parents may be able to manipulate the probability of an ovule's maturation by contributing extra nuclear material to the embryo or endosperm (Willson and Burley, 1983), which may explain the differences in postfertilization siring ability seen in *E. grandiflorum.* Transmission of pollen plastids to embryos is known by biparental cytoplasmic inheritance in some groups (Birky, 1976), but this has not been observed in closely related members of the Liliaceae. It is possible, however, that pollen tubes contribute plastids and other cytoplasmic inclusions (e.g., starch granules or lipid droplets) to the endosperm tissue. An example of pollen tubes contributing cytoplasm to the endosperm has been observed in *Plumbago,* where the contents of the pollen tube are deposited in the egg sack (Russell, 1980). The observed differences in postfertilization siring ability that corresponded to differences in mean grain size may be due to among-plant differences in the amount of cytoplasmic material contained in their pollen. This material might not contribute to tube growth, and may be reserved for deposition into the embryo sack. If so, within-plant variation in mean grain size would be expected to affect ovule maturation; however, within-plant differences in mean grain size may not be associated with the amount of cytoplasmic material present in pollen. On the other hand, pollen grains are relatively small compared to the female gametophyte ($<1\%$ by volume; pers. observation), so paternal contributions would be expected to be small. The possibility of an effect of cytoplasmic inclusions on ovule maturation cannot be eliminated without further experimentation, but it seems unlikely that paternal contributions to embryo growth could be responsible for the patterns of ovule abortion found.

The average size of the pollen produced by plants appears to be correlated with genetically based differences in the competitive ability of the zygotes sired by a plant. Plants producing larger grains not only had lower rates of abortion for the zygotes they sired, but also appeared to induce greater amounts of postfertilization abortion for the zygotes sired by the other donor present. Since little more than nuclei are transferred from microgametophytes to the ovules in fertilization, variation among developing seeds sired by different donors probably reflects genetic differences among donors rather than differences in the paternal environment (Mazer, 1988). Although the differences between donors in their siring ability are probably not directly due to differences in their grain volume, the average size of the pollen grains produced by a plant appears to be a fairly good indicator of its postfertilization siring ability. If the size that pollen grains attain before maturity is dependent on their metabolic vigor then among-plant differences in mean grain vol-

ume may reflect differences in metabolic vigor.

Theoretical considerations of maternal investment and returns in offspring fitness predict that maternal parents should have complete control over the relative success and allocation of resources to their offspring (Smith and Fretwell, 1974). Observations of paternal influence on seed weight (in *Aralia hispida,* Thomson, pers. comm.; *Campsis radicans,* Bertin, 1982; *Raphanus sativus,* Marshall and Ellstrand, 1986; *R. raphanistrum,* Mazer, 1987; *Phlox drummondii,* Schlichting and Devlin, 1989) contradict this prediction and suggest that there may not be strict maternal control over zygote success. Physiological models of plant organ development also view fruits and ovules as competitors for a limited resource pool rather than being completely controlled by the maternal tissue; the success of an individual organ is related to its ability to maintain concentration gradients of assimilates and growth substances between itself and the maternal plant tissue influenced by other nearby organs (Gifford and Evans, 1981). In *E. grandiflorum,* ovule maturation patterns were more consistent with the paternal control hypothesis. When there was a large discrepancy between the mean sizes of the pollen produced by the two donors in a pair, there tended to be an increase in the number of apparently fertilized and aborted ovules (slope = -0.20, $P = 0.0299$ for the partial regression coefficient from a model holding the number of fertilizations and the total number of ovules constant). Presumably, these abortions represent less vigorous zygotes that were outcompeted by more vigorous ones. In addition, total seed production from fruits was negatively correlated with the sum of the mean grain sizes of the two donors (slope = -0.13, $P = 0.0602$ for the partial regression coefficient from a model as above). Apparently, when two pollen donors that produce vigorous zygotes sired ovules in the same fruit, competition among ovules resulted in increased rates of abortion for ovules fertilized by both donors, and reduced the overall fecundity of the maternal parent. Such a pattern would not have been predicted if the maternal parent had control over postfertilization ovule abortion.

The genotype of the paternal parent apparently affected postfertilization ovule abortion, but it may be the zygote's genotype that determines its ultimate success. Ovule growth and competition with other ovules may be viewed as the first round of selection on newly formed zygotes (Nakamura, 1986; Wiens et al., 1987). If success in the ovary is a result of metabolic vigor and growth rate, then pollen donors that had increased siring ability would also sire more vigorous offspring. This implies that pre- and postdispersal success may be positively correlated. If this were the case, selection would be predicted to minimize differences in siring ability. In an earlier experiment, ovarian processes did not contribute significantly to seedling vigor (unpubl. data), but pollen size differences among donors were not assessed in that experiment. It remains to be determined whether the differences in competitive ability observed in the ovary will also be expressed after seed germination.

Conclusion

Pollen donors sometimes differed in their pollen tube growth ability in *Erythronium grandiflorum,* but only a small portion of the differences in pollen vigor was associated with mean pollen grain size or total pollen production. Earlier experiments indicated that tube growth may be mediated by the style, possibly because of a multifactorial incompatibility system. Since there were within- and among-donor differences in fertilization ability, it is apparent that donors did have some influence on their fertilization success, but producing more vigorous pollen may cost the donor in terms of resources sequestered in pollen and pollen longevity. Although there can be among-donor differences in pollen vigor, within-donor differences in pollen vigor were not great, suggesting that plants may have limited control of this character.

Donor performance in the ovary, on the other hand, was more strongly influenced by differences among donors. Donors differed in their postfertilization siring ability, which was correlated with the mean pollen size, so that plants producing larger grains also sired more seeds. The differences observed among donors in their siring success

were probably genetically based, since the opportunity for paternal contributions to offspring is limited. When more than one donor sired seeds in the same ovary, the amount of ovule abortion depended on the mean pollen grain size of each donor, and the difference in size between the donors. The results are consistent with the hypothesis that zygotes compete with each other for maternal resources, and that resource allocation to ovules is not strictly controlled by the maternal parent.

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