Pollen Tube Attrition in Erythronium grandiflorum

Author(s): Mitchell B. Cruzan

Source: *American Journal of Botany*, Apr., 1989, Vol. 76, No. 4 (Apr., 1989), pp. 562-570

Published by: Wiley

Stable URL: https://www.jstor.org/stable/2444351

Amer. J. Bot. 76(4): 562–570. 1989.

POLLEN TUBE ATTRITION IN ERYTHRONIUM GRANDIFLORUM[1]

MITCHELL B. CRUZAN

Department of Ecology and Evolution, State University of New York at Stony Brook,
Stony Brook, New York 11794, and
The Rocky Mountain Biological Laboratory, Crested Butte, Colorado 81224

ABSTRACT

Seed set after selfing in *E. grandiflorum* is often reduced relative to seed set after crossing; however, the compatibility patterns seen are not due to genes of major effect (i.e., S alleles). There is quantitative variation in the proportion of pollen tubes reaching the base of the style after both self- and cross-pollinations. Pollen tubes require between 24 and 72 hr to reach the ovary, but pollen tube growth ceases after 72 hr. When styles were removed from the ovaries 5 days after pollination, between 10 and 80% of the pollen tubes in the stigma had not reached the base of the style. The number of pollen tubes at the base of the style is a much better predictor of seed set than is the number of pollen tubes in the stigma. Pollen tube attrition is not affected by the age of the recipient flower or by the number of pollen donors contributing to the stigmatic pollen pool. The number of pollen tubes reaching the base of the style is dependent upon the source of the pollen and appears to be a decelerating function of the number of pollen tubes present in the stigma.

SEED PRODUCTION in angiosperms depends upon a sequence of steps, including pollen transfer to the stigma, pollen germination and pollen tube growth, ovule fertilization and seed development, and finally seed maturation and dispersal. Attrition during each step determines the upper limit on the number of participants (i.e., gametes or zygotes) in subsequent processes, and variation in the number of gametes or embryos successfully passing a particular stage contributes to variation in the reproductive success of the sporophytes involved in a particular mating. Although much attention has been given to pollen transfer (Jones and Little, 1983; Real, 1983) and more recently to patterns of postfertilization ovule and fruit abortion (Stephenson, 1981; Bertin, 1982; Lee and Bazzaz, 1982), less effort has been made to quantify variation in the fertilization success of pollen tubes after compatible crosses, especially in noncultivated species. The populations of *Erythronium grandiflorum* used in this study do not appear to contain compatibility genes of major effect as have been recorded for other plant species (i.e., S loci) (unpublished data). Plants often produce fruit and varying amounts of seed after selfing, but usually not as many seeds as can be obtained through crossing (Thomson and Cruzan, unpublished data). The quantitative variation in seed production seen after selfing also occurs for crosses between different individuals, and is largely due to variation in the degree of pollen tube attrition occurring in the style (see below). Quantitative variation in the fertilization success of pollen tubes after single-donor crosses has generally been described as partial incompatibility or pseudo-self-compatibility (de Nettancourt, 1977) and has usually been attributed to a breakdown of an S allele based self-incompatibility system (Ascher, 1984).

The work described here grew out of the observation that seed set after single-donor hand pollinations often was much less than seed set in open-pollinated flowers. It was also observed that this reduction in seed set was largely due to the attrition of pollen tubes during growth in the style. Some of the ways in which natural pollination can differ from hand pollinations are 1) the age of the flower at the time of pollination, 2) the number of pollen donors contributing to the stigmatic pollen pool, 3) the total amount of pollen received as indicated by the number of pollen tubes in the stigma, and 4) the number of pollinations received. In this paper I consider how the first three factors affect the growth of pollen tubes in the style; in addition, I determine the rate at which pollen tubes reach the ovary after single pollinations.

METHODS—*Erythronium grandiflorum* Pursh (Liliaceae) is a perennial herb arising from a buried corm. The two strap-shaped

---

[1] Received for publication 12 April 1988; revision accepted 10 October 1988.

Assistance in the field was provided by J. Brunet, C. Cruzan, L. Rigney, and B. and J. Thomson. J. Thomson, N. Waser, M. Price, and C. Cruzan made comments on the manuscript. This work was supported by NSF dissertation improvement grant BSR431-2986A, and BSR861-4207 to J. Thomson. This is contribution 627 from the Graduate Program in Ecology and Evolution, SUNY at Stony Brook.

leaves appear above ground in early spring shortly after snowmelt, and plants flower soon after the leaves appear. Inflorescences bear from one to several flowers, with one- and two-flowered plants being most common. Flowers are large (2 to 3 cm long), with six yellow tepals and six large anthers in two whorls of three anthers each. Anthers in both whorls are indehiscent at the time of anthesis, and anthers in the outer whorl usually dehisce on the same day. By the third day of anthesis, anthers in both whorls are typically fully dehisced and on the fifth day the tepals usually begin to senesce. The cleft in each of the three stigma lobes is continuous with a channel of the hollow, fluid-filled style. The three channels are open to each other to form a single lumen, Y-shaped in cross section. Although pollen tubes from different lobes tend to grow down the channels of the style separately, they intermingle at the base of the style, so that pollen tubes originating from any of the three lobes can fertilize ovules in any of the three locules (personal observation). Other species of *Erythronium* commonly reproduce vegetatively (Blodgett, 1900; Banks, 1980), however this mode of reproduction rarely occurs in *E. grandiflorum* (D. Inouye, personal communication).

I conducted the experiments near the Rocky Mountain Biological Laboratory in Gothic, Colorado, during May and June of 1986 and 1987. In all cases where pollen was mixed prior to pollinations (see below), I collected fresh pollen from plants within the same population as the recipients by randomly selecting plants along a 50 to 100 m transect. I made pollen mixtures by combining an equal number of freshly dehisced anthers from each donor. Since outcrossing distance can have an effect on the degree of pollen tube attrition (Cruzan, unpublished data), these pollen mixtures typically contained pollen from several distance classes for each recipient. Using a thick piece of fishing line, I pollinated plants in situ by evenly coating stigmas with pollen. Pollen clings readily to nylon fishing line due to its static properties, making it an excellent tool for hand pollinations. With the exception of the flower age experiment (see below), I made all pollinations two to three days after anthesis. I protected stigmas from pollen deposition by pollinators by placing hollow dried grass straws over their gynoecium. Straws were slit at their base in order to obtain a snug fit on the ovary. Five days after pollination I removed the grass straws, excised the styles, and fixed them in 70% ethanol. Removal of styles at this stage has no effect on subsequent fruit and seed set (see below). Ovaries were either excised five days after pollination or were left until fruits were mature.

I made observations of pollen tubes using a Wild stereoscopic microscope at ×50. I carefully slit styles longitudinally, laid them flat, and stained them for 10 min with one drop of acidified 0.1% aniline blue (acidified with hydrochloric acid until the solution turned dark blue) and one drop of acetocarmine (40% acetic acid saturated with carmine). By using wet mounts of styles, I could manipulate pollen tubes with a fine insect pin to facilitate counting. I counted pollen tubes within 1.0 mm of the tips of the stigma lobes and within 1.0 mm of the cut end at the base of the style. I analyzed data either as raw counts of pollen tubes in the stigma (TS) and at the base (TB) of the style or as the fraction of pollen tubes not reaching the base of the style (i.e., [TS − TB/TS]), which I term "stylar attrition of pollen tubes." For those flowers that set fruit, I counted the number of seeds set (SD) and determined the "ovarian attrition of pollen tubes" as (TB − SD/TB). Ovarian attrition includes all sources of failure of pollen tubes in the ovary, including failure to fertilize an ovule and postfertilization abortion of ovules.

The above measures were entered as dependent variables in analyses of variance or covariance using the GLM procedure of SAS (SAS Institute, 1982). Fruit set patterns were analyzed using log-linear models with the CATMOD procedure of SAS (SAS Institute, 1982).

Pollen tube growth rate—I pollinated flowers and collected styles at 1, 2, 3, 4, or 5 days after pollination to determine the amount of time required for pollen tubes to reach the ovary. Using two-flowered plants as recipients in a balanced incomplete block design, I gave each plant a random pair of the above treatments. I pollinated each flower on the second day after anthesis with an equal mixture of pollen collected each day from a different set of five plants. When making pollinations, I recorded the time of pollination for each flower and excised and fixed the style after the assigned interval for pollen tube growth.

Flower age—In order to determine the effect of recipient flower age on pollen tube growth, I pollinated flowers either on the day of anthesis or three or five days after anthesis. I applied the above three treatments in pairs to two-flowered plants in a balanced incomplete block design. I collected pollen from five different plants and made equal mixtures of their pollen each day. Five days after pollinating flowers, I excised and fixed their styles.

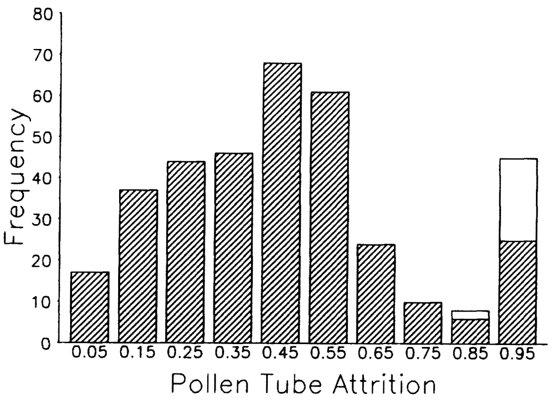

Fig. 1. Pollen tube attrition in individual styles of *Erythronium grandiflorum.* The data are pooled from the flower age experiment, the self/outcross experiment, and from both donor number experiments. The crosshatched portion of the bars represents cross-pollinations; the empty portion of the bars, self-pollinations.

Self/outcross pollinations—I made single-donor pollinations to 27 three-flowered plants using either self-pollen from the same flower or outcross pollen from a neighboring plant (within 2 m) to determine the average degree of pollen tube attrition for self- and cross-pollen. For each plant, I selfed one flower and crossed the other two, rotating the treatments among flower positions for different plants. Five days after pollinating, I collected styles and analyzed them to determine the degree of pollen tube attrition. I collected fruits when mature and counted the seeds from each fruit.

Donor number—I manipulated the number of donors contributing to the stigmatic pollen load to determine effects of pollen load diversity on pollen tube growth, and fruit and seed set. Using an equal amount of pollen from each donor plant (one anther per donor), I made mixtures of either one, three, or ten donors. I conducted this experiment in both 1986 and in 1987. In 1986, I used three-flowered plants and collected indehiscent anthers several days prior to pollinations. I stored anthers in air-tight vials at 2 C. On the day pollinations were made, I opened the vials containing anthers to allow anthers to dehisce. I applied all three treatments to each recipient plant with treatments rotated among flower positions. In 1987, I used two-flowered plants, and I collected the pollen used for pollinations each day from plants within the same local population as the recipients. I used the same three treatments in a balanced incomplete block design with pairs of treatments randomly assigned to the two flowers of each recipient plant. In both years, styles were collected five days after pollination and fruits were collected when mature. I counted and weighed seeds from each fruit as a group.

RESULTS—Pollen tubes in *Erythronium grandiflorum* were easily observed and counted using the methods described above. After staining, pollen tubes appeared dark blue against the light blue background of the wall of the stylar canal. Tubes adhered weakly to the style wall and to each other but were easily separated with a fine insect pin so that relatively precise counts could be made.

The number of pollen tubes reaching the base of the style was often substantially less than the number in the stigma (Table 1), and

TABLE 1. *Summaries of experiments conducted on* Erythronium grandiflorum *during the 1986 and 1987 field seasons. Sample sizes and means for the number of pollen tubes present in the stigma and at the base of the style and the percent pollen tube attrition are given for the styles analyzed. The number of fruits set, the mean number of seeds per fruit, the minimum number of unfertilized ovules (pollen tubes at the base of the style minus the total number of ovules), and the percent ovarian attrition (pollen tubes at the base of the style minus the number of seeds set, divided by the number of tubes at the base of the style) are given for those experiments in which fruits were collected. Sample sizes reported for pollen growth do not include crosses for which there were no pollen tubes present. The sample sizes for fruits give the total number of fruits set for each experiment. Numbers in parentheses are standard deviations*

Experiment	Pollen tubes			Stylar attrition	Fruits		Unfert. ovules	Ovarian attrition
	N	Stigma	Style		*N*	Seeds		
Flower age 1986	60	82.8 (39.8)	39.5 (19.2)	48.8 (17.3)	—	—	—	—
Self/outcross 1986	74	65.6 (47.6)	25.8 (25.8)	62.0 (30.1)	23	33.3 (19.0)	30.1 (22.2)	18.5 (36.3)
Donor no. 1986	43	54.2 (36.3)	31.1 (20.3)	40.7 (23.3)	31	28.9 (15.5)	31.2 (20.3)	22.4 (21.4)
Donor no. 1987	145	129.3 (49.1)	77.6 (30.8)	39.5 (20.4)	92	33.4 (14.8)	−14.9 (27.3)	56.9 (18.2)

TABLE 2. *The determination of seed set by the number of pollen tubes present in the stigma and at the base of the style in* Erythronium grandiflorum. *Results are given for each experiment in which fruits were collected. Values are the standardized partial regression coefficients (path coefficients) from a multiple regression analysis which included both parameters. Numbers in parentheses are the probabilities from t tests testing for a regression coefficient equal to zero*

Experiment	*N*	Stigma	Style
Self/outcross	23	0.045 (0.81)	0.648 (0.0025)
Donor no. 1986	31	0.233 (0.12)	0.699 (0.0001)
Donor no. 1987	92	0.076 (0.52)	0.365 (0.0028)

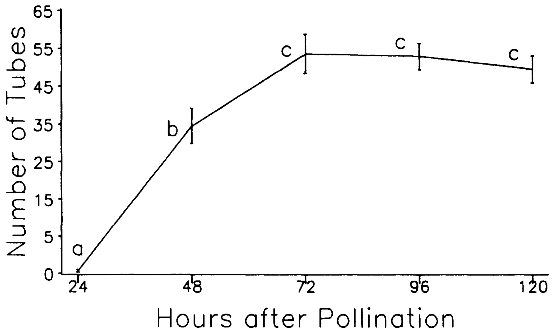

Fig. 2. The relationship between the growth interval and the number of pollen tubes reaching the base of the style in *Erythronium grandiflorum.* The vertical bars represent the standard error of the mean. Means associated with the same small case letter are not significantly different from each other.

there was considerable variation in the degree of pollen tube attrition in all of the experiments (Fig. 1). In two of the three experiments, many ovules typically remained unfertilized after single hand-pollinations, since there were more ovules present than there were pollen tubes at the base of the style (Table 1). In all three experiments where fruits were collected, the number of pollen tubes present at the base of the style was a much better predictor of seed set than was the number present in the stigma (Table 2). For these pollinations, seed set was virtually unaffected by the number of pollen tubes in the stigma.

Pollen tube growth rate—The fastest growing pollen tubes required approximately 24 hr to reach the ovary for most pollinations (Fig. 2). The number of pollen tubes reaching the base of the style increased rapidly between 24 and 72 hr but few or no additional pollen tubes grew to the ovary after 72 hr (Fig. 2). The pollen tube growth interval had a significant effect on the number of pollen tubes at the base of the style, but the position of the recipient flower and the growth interval by flower position interaction did not (Table 3).

Flower age—The age of recipient flowers had a significant effect on the number of pollen tubes in the stigma but had no apparent effect on the number of pollen tubes at the base of the style or on the degree of pollen tube attrition (Table 4). Fewer pollen tubes were present in flowers pollinated on the first day after anthesis (mean = 79.0, SD = 29.3) than were present in flowers (86.3, SD = 33.4). The significant flower age by flower position interaction for the number of pollen tubes present in stigmas (Table 4) was due to second flowers having more pollen tubes in their stigmas than first flowers on the first day after anthesis, but fewer pollen tubes in their stigmas for third and fifth day pollinations. The mean pollen tube attrition in this experiment taken across treatments was 48.8% (SD = 17.3, $N = 58$). The number of pollen tubes at the base of the style tended to increase slightly with flower age (linear regression slope = 10.3), but this increase was not significant ($F = 2.51$, $P > 0.15$ for a test that the slope is equal to zero).

Self/outcross pollinations—Cross-pollinations typically resulted in lower levels of pollen tube attrition than did self-pollinations (Fig. 1). Although the numbers of pollen tubes present in the stigma were similar for both cross types ($F = 0.13$, $P > 0.71$, df = 1,65), pollen tube attrition was much greater for self- (94.3%, SD = 10.6%, $N = 22$) than for cross-pollinations (48.3%, SD = 24.6%, $N = 52$) ($F = 104.1$, $P < 0.0001$). Fewer pollen tubes were present at the base of the styles of self-pollinated (6.63,

TABLE 3. *The effect of time on the number of pollen tubes reaching the base of the style in* Erythronium grandiflorum. *The identity of the recipient plant was entered as a random variable; flower position and the amount of time pollen tubes were allowed to grow were fixed variables, and the number of tubes present in the stigma of each style was entered into the model as a covariate. Significance values are based upon the type III sums of squares from the GLM procedure (SAS Institute, 1982)*

Source	df	*F* value	*P*
Flower position	1	0.41	0.5273
Growth interval	4	17.38	0.0001
Interval • position	4	0.81	0.5286
No. of tubes	1	1.37	0.2491

TABLE 4. *The effect of flower age on the number of pollen tubes present in the stigma, the number of pollen tubes reaching the base of the style, and the degree of pollen tube attrition in* Erythronium grandiflorum. *The identity of the recipient plant and the pollination date were entered as random factors and flower age and flower position were fixed factors. The number of pollen tubes present in the stigma is the response variable in the first ANOVA and is treated as a covariate in the second two ANOVAs. Probabilities are based upon the type III sums of squares for GLM procedure (SAS Institute, 1982)*

		Tubes in stigma		Base of style		Attrition	
Source	df	*F*	*P*	*F*	*P*	*F*	*P*
Flower position	1	2.09	0.1699	0.02	0.9031	0.70	0.4203
Flower age	2	4.33	0.0343	2.52	0.1188	2.06	0.1699
Age • position	2	5.50	0.0173	0.76	0.4876	1.02	0.3885
Tubes in stigma	1	—	—	0.44	0.5198	4.60	0.0531

SD = 15.2) than cross-pollinated flowers (33.9, SD = 25.2) ($F = 26.32$, $P < 0.0001$). Only 2 of the 22 selfed flowers set fruit, whereas 21 of the 52 crossed flowers produced fruits.

Donor number—The number of donors contributing to the pollen pool had little effect on pollen tube growth, but some effect on seed set and seed weight. In both years there was no significant effect of donor number on the number of pollen tubes present in the stigma, at the base of the style, or on the degree of pollen tube attrition (Table 5). In 1986, there were effects of pollen donor number on the average number of seeds per fruit and average seed weight, but these effects were not detected in 1987 (Table 5). There were strong linear and quadratic effects of the number of pollen tubes present in the stigma on the number reaching the base of the style in 1986 and on the degree of pollen tube attrition in the style in both years (Table 5). The number of pollen tubes reaching

TABLE 5. *The effect of the number of pollen donors contributing to the stigmatic pollen pool, position of the recipient flower, and the linear and quadratic contributions of the number of pollen grains on the stigma on pollen tube numbers, attrition, seed set, and seed weight in* Erythronium grandiflorum. *The identity of the recipient plant and pollination date were treated as random effects in the model. F values from type III or type IV sums of squares from the GLM proedure of SAS and probabilities for each test (in parentheses) are given. The number of pollen tubes in the stigma is treated as a dependent variable in the first column and as linear and quadratic covariates for the rest of the models. Each table contains the results from a separate year*

		Pollen tubes		Tube attrition			
	df	Stigma	Base	Style	Ovary	Seeds	Seed wt.
1986 Experiment source							
Flower position	2	1.33 (0.33)	1.02 (0.44)	1.05 (0.43)	3.71 (0.34)	2.20 (0.43)	1,693.80 (0.02)
No. of donors	2	0.28 (0.76)	1.34 (0.36)	1.84 (0.27)	4.19 (0.33)	159.11 (0.06)	2,285.01 (0.02)
Donors • position	4	0.37 (0.83)	0.06 (0.99)	1.73 (0.31)	1.27 (0.53)	10.17 (0.22)	379.09 (0.04)
Tubes in stigma (linear)	1	—	15.02 (0.02)	6.01 (0.07)	0.57 (0.59)	132.28 (0.06)	471.60 (0.03)
Tubes in stigma (quadratic)	1	—	9.42 (0.04)	8.74 (0.04)	0.41 (0.64)	41.65 (0.10)	70.55 (0.08)
1987 Experiment source							
Flower position	1	0.79 (0.38)	3.48 (0.07)	3.22 (0.08)	0.50 (0.49)	1.70 (0.21)	0.41 (0.53)
No. of donors	2	0.01 (0.99)	0.70 (0.50)	0.41 (0.66)	1.11 (0.35)	1.45 (0.26)	0.02 (0.98)
Donors • position	2	1.48 (0.24)	0.53 (0.59)	0.54 (0.59)	0.99 (0.39)	1.99 (0.18)	0.27 (0.77)
Tubes in stigma (linear)	1	—	0.00 (0.98)	19.00 (0.0001)	0.00 (0.96)	1.00 (0.33)	0.86 (0.37)
Tubes in stigma (quadratic)	1	—	1.47 (0.23)	7.78 (0.0079)	0.08 (0.79)	0.71 (0.41)	0.61 (0.45)

the base of the style increased nearly proportionally with the number of pollen tubes in the stigma at very low numbers, but decelerated as the number of pollen tubes in the stigma increased (Fig. 3).

The probability of fruit set for these pollinations was affected by the position of the recipient flower and the number of pollen tubes at the base of the style (Table 6). The number of pollen donors used had no significant effect on fruit set in either year (Table 6).

Table 6. *The effect of recipient flower position, number of pollen donors, number of pollen tubes at the base of the style, and the degree of pollen tube attrition on fruit set in* Erythronium grandiflorum. *Fruit set was the dependent variable in a log-linear model in which flower position and the number of pollen donors were entered as categorical variables and the number of pollen tubes at the base of the style and pollen tube attrition were entered as continuous variables. Chi square values are given for both years in which the experiment was conducted. Numbers in parentheses are probabilities*

	1986		1987	
Parameter	df	χ^2	df	χ^2
Intercept	1	0.32 (0.52)	1	0.04 (0.83)
Flower position	2	5.00 (0.08)	1	9.43 (0.0021)
Donor no.	2	2.61 (0.27)	2	1.93 (0.38)
Tubes at base	1	3.90 (0.05)	1	3.02 (0.08)
Tube attrition	1	2.88 (0.09)	1	0.05 (0.83)

Discussion—Pollen tube attrition in *Erythronium grandiflorum* is clearly an important factor affecting the reproductive success of individual plants acting both as pollen donors and recipients. The number of pollen tubes reaching the base of the style increased rapidly between 24 and 72 hr after pollination and few or no pollen tubes grew to the ovary after 72 hr. The number of pollen tubes present at the base of the style five days after pollination determined between 13 and 50% of the variation in seed set, whereas the effect of the number of pollen tubes present in the stigma was negligible. The reason for pollen tube failure during growth in the style in *E. grandiflorum* remains obscure, and the major sources of variation that determine the degree of pollen tube attrition were not revealed by the experiments conducted. The results do, however, provide some information about the nature of this phenomenon.

Both genetic and environmental factors may contribute to the degree of pollen tube attrition that occurs for a particular cross. It seems reasonable to examine environmental factors that affect incompatibility, since the physiological mechanism responsible for pollen tube attrition in *E. grandiflorum* may be the same that is responsible for incompatibility responses in other species, especially partial- or pseudoincompatability. Environmental factors that have been reported to effect incompatibility responses include ambient temperature and flower age (de Nettancourt, 1977). In *Lilium* self-compatibility responses have been reported to break down with recipient flower age (Ascher and Pelloquin, 1966). Pollen tube attrition in *Erythronium grandiflorum* did not change appreciably across the normal lifetime of flowers (Table 4). The number of pollen tubes present in the stigma was reduced for younger flowers, probably because of lower rates of pollen germination. Thomson (personal communication) reported slower germination rates for pollen on young stigmas in *E. grandiflorum* and speculated that this effect is probably due to the lower amounts of stigmatic exudate present. Ambient temperatures did not vary to any great extent during the experiments reported here, so this factor is also probably not important in determining the variation in pollen tube attrition observed.

Pollen tube attrition in *Erythronium grandiflorum* is not strictly a characteristic of pollen donors or pollen recipients, but is strongly increased after self-pollinations and can vary for individual donors when crossed with different recipients (Cruzan, unpublished data). In this way it resembles a multilocus incompatibility system such as the "heterosis model" proposed by Mulcahy and Mulcahy (1983). However, patterns of pollen tube attrition do not strictly follow the predictions of a multilocus incompatibility model, as can be seen when single-donor crosses are compared to multiple donor crosses. Increasing the number of pollen donors contributing to a stigmatic pollen load would present a more representative sample of the compatibility types present in a population to pollen recipients. Although the mean response of recipients in terms of the degree of pollen tube attrition would not be expected to change from single to multiple donor crosses, the variance among responses should decrease when a more representative sample is presented to individual recipients. The variance in the degree of pollen tube attrition did not differ significantly among the single-donor (mean = 32.6%, s^2 = 826.2) and ten donor (mean = 45.6%, s^2 = 511.4) treatments in 1986

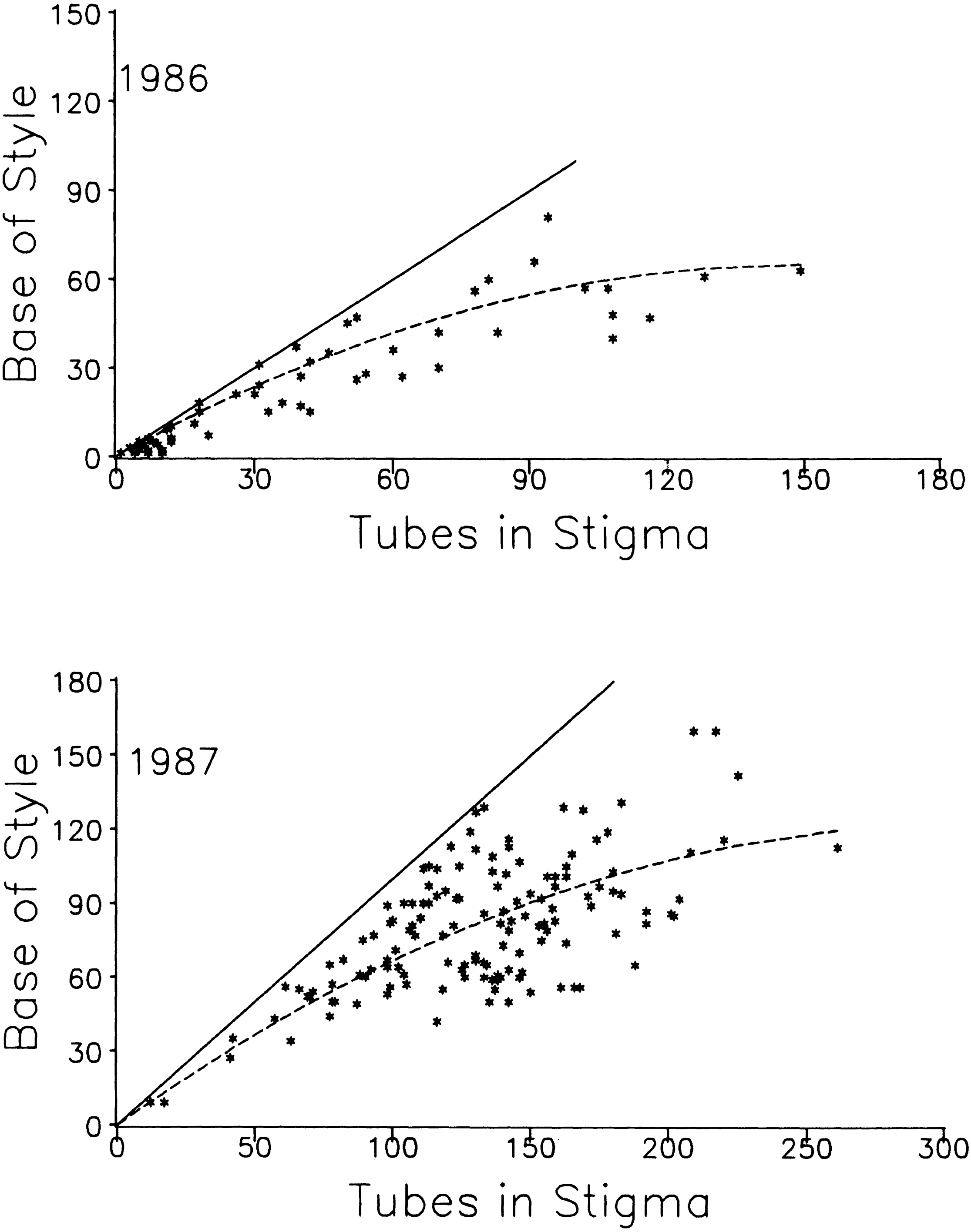

Fig. 3. The number of pollen tubes reaching the base of the style as a function of the number of pollen tubes in the stigma for *Erythronium grandiflorum*. Separate plots are given for 1986 and 1987. The solid lines represent the relationships expected if equal numbers of pollen tubes were present in both the stigma and at the base of the style. The dashed lines are the best fit lines from polynomial regressions with intercepts of zero; ($TB = [0.874]TS + [-0.0029]TS^2$) for 1986 and ($TB = [0.797]TS + [-0.0013]TS^2$) for 1987.

($F = 1.62$, $P > 0.25$ with 9/22 df). In 1987 the variance was greater for the ten donor treatment (mean = 41.3%, $s^2 = 522.8$) than it was for the single-donor treatment (mean = 39.0%, $s^2 = 305.9$) ($F = 1.71$, $P < 0.05$ with 46/46 df). If pollen tube attrition were strictly due to many incompatibility loci, each having a small effect, then the variance in the degree of pollen tube attrition should have decreased when more donors were present. Although genetic factors may be important in determining the degree of pollen tube attrition, simple genetic complementarity does not completely explain the patterns of pollen tube attrition found.

A decrease in the variance of the compatibility response when more pollen donors are present would also be seen if there were mentor effects (de Nettancourt, 1977; Visser and Marcucci, 1987) between pollen from different donors. Mentor effects occur in mixtures of pollen from different sources when pollen of one incompatibility type mediates the incompatibility response of pollen of a different incompatibility type. Mentor effects would tend to decrease the variance among fertilization abilities of individual pollen tubes when more donors were present to an even greater extent since the overall compatibility response should follow the average compatibility of all the pollen tubes present.

Another factor which affects the degree of pollen tube attrition is the apparent upper limit on the number of pollen tubes allowed to grow to the ovary. The number of pollen tubes successfully passing to the base of the style was not a linear function of the number of pollen tubes present in the stigma, but decelerated so it appeared to asymptote near 90 to 100 pollen tubes (Fig. 3). The exact shape of this curve for individual crosses may be dependent upon the particular combination of pollen donor and recipient involved in a cross. Towards the upper limit of pollen tubes there may be physical or physiological limits to greater numbers of pollen tubes growing to the ovary. One possibility is that the constriction of the lumen at the base of the style does not allow large numbers of pollen tubes to pass. Similar cases have been reported for other species where a large decrease in the size of the transmitting tissue occurred at the top of the style (Modlibowska, 1942; Cruzan, 1986). However, the ends of pollen tubes of *E. grandiflorum* after five days of growth are typically evenly distributed along the length of the style rather than accumulated at any particular point (personal observation). It may be more likely that the pollen tube growth substances supplied by the stylar tissue are limited or that individual pollen tubes are inhibited from further growth.

Although I usually applied a sufficient amount of pollen to fertilize all of the ovules present, many ovules were left unfertilized because of pollen tube attrition (Table 1). This occurred for all experiments except the 1987 donor number experiment, where stigma load sizes were very large and less pollen tube attrition occurred (Table 1). It is possible that those tubes not growing to the ovary were inherently incapable of doing so, but this is not a completely adequate explanation since the degree of attrition is often not consistent for individual donors across recipients (Cruzan, unpublished data). Since plants should be under strong selection to produce highly vigorous pollen which would fertilize as many ovules as possible (Haldane, 1966; Janzen, 1977), it is more likely that pollen tubes were inhibited from growing to the ovary due to interactions with the stylar tissue. Stylar tissue or style exudates have been implicated in modifying pollen tube growth in several instances (Pfahler, 1967; Sari Gorla, Bellintani, and Ottaviano, 1976; Malti and Shivanna, 1985; Mulcahy and Mulcahy, 1986). It would seem reasonable to presume that inhibition of certain pollen tubes in favor of others would confer some advantage to pollen recipients, possibly by modifying offspring vigor (Mulcahy, Mulcahy, and Ottaviano, 1975; McKenna and Mulcahy, 1983). There is no evidence, however, whether those pollen tubes that are inhibited from further growth would actually produce inviable or low quality offspring.

Pollen tube attrition is an important phenomenon in determining the reproductive success of *Erythronium grandiflorum* plants, although the advantage that pollen recipients receive by limiting the number of ovules fertilized remains obscure. Quantitative variation in pollen tube attrition after compatible crosses has also been found in *Petunia* (Ascher, 1984; Cruzan, unpublished data), and *Delphinium nelsonii* (Waser et al., 1987). Patterns of variation in the reproductive success of plants has long been of interest to ecologists and evolutionary biologists (Willson and Burley, 1983; Stephenson, 1981; Bertin, 1982). In many of these studies, variation in seed production has been assumed to be caused by postfertilization seed abortion, although the exact causes are usually not determined (Bertin, 1982; Lee and Bazzaz, 1982). Simply measuring the number of seeds produced for crosses is not an adequate approach to this kind of problem, since seed set is the culmination of a series of processes

and patterns that occur at a particular stage may be obscured by variation at other stages. Better interpretations of the patterns we observe for seed production can be made with a more complete understanding of the patterns occurring at each stage in the reproductive process (Lyons et al., in press). More detailed studies of the reproductive patterns in plants on a population level are needed in order to assess the validity of several evolutionary hypotheses that have been suggested (Willson and Burley, 1983; Stephenson and Bertin, 1983).

LITERATURE CITED

ASCHER, P. D. 1984. Self-incompatibility. *In* K. C. Sink [ed.], Petunia, 92–109, Springer-Verlag, New York.

———, AND S. J. PELLOQUIN. 1966. Effect of floral aging on the growth of compatible and incompatible pollen tubes in *Lilium Longiflorum.* Amer. J. Bot. 53: 99–102.

BANKS, J. A. 1980. The reproductive biology of *Erythronium propullans* Gray and sympatric populations of *E. albidum* Nutt. (Liliaceae). Bull. Torrey Bot. Club 107: 181–188.

BERTIN, R. I. 1982. Paternity and fruit production in trumpet creeper (*Campsis radicans*). Amer. Naturalist 119: 694–709.

BLODGETT, F. H. 1900. Vegetative reproduction and multiplication in *Erythronium.* Bull. Torrey Bot. Club 27: 305–315.

CRUZAN, M. B. 1986. Pollen tube distributions in *Nicotiana glauca*: evidence for density dependent growth. Amer. J. Bot. 73: 902–907.

DE NETTANCOURT, D. 1977. Incompability in angiosperms. Springer-Verlag, New York.

HALDANE, J. B. S. 1966. The causes of evolution. Cornell University Press, Ithaca, NY.

JANZEN, D. H. 1977. A note on optimal mate selection in plants. Amer. Naturalist 111: 365–371.

JONES, C. E., AND R. J. LITTLE. 1983. Handbook of experimental pollination biology. Van Nostrand-Reinhold, New York.

LEE, T. D., AND F. A. BAZZAZ. 1982. Regulation of fruit and seed production in an annual legume, *Cassia fasciculata.* Ecology 63: 1363–1373.

LYONS, E. E., N. M. WASER, M. V. PRICE, J. ANTONOVICS, AND A. F. MOTTEN. In press. Sources of variation in plant reproductive success and implications for concepts of sexual selection. Amer. Naturalist.

MALTI, AND K. R. SHIVANNA. 1985. The role of the pistil in screening compatible pollen. Theor. Appl. Genet. 70: 684–686.

MCKENNA, M., AND D. L. MULCAHY. 1983. Ecological aspects of gametophytic competition in *Dianthus chinensis. In* D. L. Mulcahy and E. Ottaviano [eds.], Pollen: biology and implications for plant breeding, 419–424. Elsevier Science, New York.

MODLIBOWSKA, I. 1942. Bimodality of crowded pollen tubes in *Primula obconica.* J. Heredity 35: 187–190.

MULCAHY, D. L., AND G. B. MULCAHY. 1983. Gametophytic self-incompatibility reexamined. Science 220: 1247–1251.

———, ———, AND E. OTTAVIANO. 1975. Sporophytic expression of gametophytic competition in *Petunia hybrida. In* D. L. Mulcahy [ed.], Gamete competition in plants and animals, 227–232. Elsevier Science, New York.

MULCAHY, G. B., AND D. L. MULCAHY. 1986. More evidence on the preponderant influence of the pistil on pollen tube growth. *In* M. Cresti and D. Dallai [eds.], Biology of reproduction and cell motility in plants and animals, 139–144. University of Sienna Press, Sienna.

PFAHLER, P. L. 1967. Fertilization ability of maize pollen grains. II. Pollen genotype, female sporophyte, and pollen storage interactions. Genetics 37: 513–521.

SARI GORLA, M., R. BELLINTANI, AND E. OTTAVIANO. 1976. Competitive ability of maize pollen. Interaction between genotypes of pollen and stylar tissue. Maydica 21: 77–80.

SAS INSTITUTE. 1982. SAS user's guide: statistics. SAS Institute, Cary, NC.

STEPHENSON, A. G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. Annual Rev. Ecol. Syst. 12: 253–279.

———, AND R. I. BERTIN. 1983. Male competition, female choice, and sexual selection in plants. *In* L. A. Real [ed.], Pollination biology, 110–149. Academic Press, New York.

VISSER, T., AND C. M. MARCUCCI. 1987. The performance of double or mixed pollinations with compatible and self-incompatible or incongruous pollen of pear and apple. Euphytica 35: 1011–1016.

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

WILLSON, M. F., AND N. BURLEY. 1983. Mate choice in plants: tactics, mechanisms and consequences. Princeton University Press, Princeton.