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American Journal of Botany, Vol. 75, No. 12 (Dec., 1988), 1898-1903.

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EFFECT OF CROSSING DISTANCE AND MALE PARENT ON IN VIVO POLLEN TUBE GROWTH IN *CHAMAECRISTA FASCICULATA*¹

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ABSTRACT

Gametophytic competition among pollen grains has been proposed as an important mechanism of sexual selection in plants. The purpose of this paper is to examine the contribution of pollen source on in vivo pollen tube growth in *Chamaecrista fasciculata*. We addressed two questions: 1) Is pollen tube growth affected by the genetic relatedness between the pollen source and the pollen recipient? 2) Is there significant phenotypic variation among pollen donors for pollen tube growth? We compared pollen tube growth by measuring the number of pollen tubes which germinated, reached quarter length of style, and reached the ovary resulting from self- and outcross-pollinations. The outcross pollinations included three interplant distance classes: near (within genetic neighborhood, ca. 1 m), far (between far neighborhoods and within subpopulation, ca. 20 m), and distant (between neighborhoods and adjacent subpopulations, ca. 50–100 m). Our results show that pollen tube growth was not affected by genetic relatedness, by differences between self and outcross, nor by differences due to phenotypic variation among pollen donors. In contrast, maternal environment had a strong impact on pollen tube growth. These results suggest a lack of gametophytic competition and indicate little opportunity for sexual selection on pollen tube growth in *C. fasciculata*.

GAMETOPHYTIC COMPETITION among pollen grains has been proposed as an important mechanism of sexual selection in plants (Stephenson and Bertin, 1983). Three necessary conditions for sexual selection to operate on pollen tube growth are 1) phenotypic variation among male pollen donors for the selected trait (Crow, 1958; Arnold and Wade, 1984), 2) underlying genetic basis for variation of the trait (Haldane, 1954; Lande and Arnold, 1983), and 3) an absence of pollen limitation (Stephenson and Bertin, 1983). In vitro (Schemske and Fenster, 1983; Bookman, 1984; Mazer, 1987)

and in vivo studies (Hill and Lord, 1986; Marshall and Ellstrand, 1986) have found significant differences among donors in pollen tube growth. However, the significance of in vitro studies is limited because pollen performance is not measured in the maternal environment where all ecologically significant interactions for pollen tube growth occur (Charlesworth, Schemske, and Sork, 1987).

An additional source of variation in pollen tube growth among males is the effect of the interaction between the paternal and maternal genotypes. The genetic relatedness among parents may influence pollen performance. Some studies have shown that outcross pollen are more likely to fertilize than self-pollen (e.g., Bateman, 1956; Smith and Clarkson, 1956; Bowman, 1987). When comparing pollen tube growth among males, it is important to control for the genetic relatedness among parents. Because gene flow often is limited in plants (Ehrlich and Raven, 1969; Levin, 1981), populations may be subdivided so that interparent distance reflects the genetic similarity between parents (Wright, 1969; Schaal, 1974; Levin, 1984; Epperson and Clegg, 1986). Therefore, variation among pollen donors for pollen tube growth may not be due to the individual male genotype nor reflect opportunities for sexual selection, but may simply reflect male/female interactions.

In this paper we examine the contribution

¹ Received for publication 15 December 1987; revision accepted 31 March 1988.

CF was supported by funds from a NIH Genetics Training Grant, Sigma XI, the University of Chicago Hinds Fund, and NSF Grant BSR-8501229. VS was supported by NSF Grant RII-8503512 and the University of Missouri Weldon Springs Fund. We thank the Illinois Dept. of Conservation and the Illinois Nature Preserve Commission, J. Nyhoff and S. Villalobos of Gooselake Prairie State Park, and Sue Yamins, Betty McCarthy, and Wilbur Schroeder from the University of Chicago greenhouse. We also thank D. Schemske for advice and encouragement, P. Feinsinger for pollen tube staining techniques, and S. Weller for use of an epifluorescent microscope. The following individuals commented on earlier versions of this manuscript: D. Schemske, M. DeMauro, M. Dudash, D. Buckley, L. Mule, L. Dorn, and N. Pavlovic.

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of pollen source on in vivo pollen tube growth variation in *Chamaecrista* (= *Cassia fasciculata*). In the study population, Fenster (1988) has found that genetic similarity between individuals, an index of genetic relatedness (Schwartz and Armitage, 1983; Wilkinson and McCracken, 1985), declines with distance. In order to control for genetic relatedness and to maximize the genetic variation among males, controlled pollinations were conducted among plants sampled across a range of interparent distances. We addressed two questions: 1) Is pollen tube growth affected by the genetic relatedness between the pollen donor source and the pollen recipient? 2) Is there significant phenotypic variation among pollen donors for pollen tube growth?

MATERIALS AND METHODS—*Chamaecrista fasciculata* Michx. (formerly known as *Cassia fasciculata*), is a self-compatible, highly outcrossing (Fenster, 1988), annual legume of old field, disturbed prairie and savanna. In northern Illinois *C. fasciculata* is exclusively bee pollinated (Lee and Bazzaz, 1982). Stigmatic pollen grain loads typically exceed ovule numbers, which range from 7–21 per fruit, by at least 2–3 times in open-pollinated, field-collected flowers suggesting that there is opportunity for gametophytic competition among pollen grains for access to ovules.

The study population is located at Gooselake Prairie Nature Preserve, Grundy Co., Illinois, a disturbed mesic prairie. The population is subdivided naturally into clumps of subpopulations 50–200 m apart. Each subpopulation contains 100 to >1,000 adult flowering individuals.

In early June 1985, juvenile plants were collected from each of 11 subpopulations at Gooselake Prairie. In the center of each subpopulation, a plant was selected randomly to be the maternal parent (totaling 11 females). Plants to be used as pollen donors were chosen at 3 distances from the female parent: near (ca. 1 m), far (15–20 m), and adjacent subpopulation (100 m). For each maternal parent, there were 2 pollen donors in each distance class. The first was chosen randomly and the second was chosen equidistant from the female but in the opposite direction to the first male plant. In this way, we attempted to maximize the genetic variation among males within a spatial scale by maximizing the scalar distance between donors. Males in the near class represent pollen donors within the genetic neighborhood (sensu Wright, 1943, 1946) of the maternal plant (Fenster, 1988), while far males were lo-

cated several neighborhoods from the maternal parent but within the same subpopulation.

Plants were grown in a greenhouse where they were watered as needed and fertilized weekly. Four types of hand-pollinations were conducted in a pollinator-free cage: single donor pollen loads representing self, near, far, and adjacent subpopulation pollen sources. During the early morning over 4 days, 2 flowers on each pollen recipient were self-pollinated. In addition, pollen recipients were crossed with single donor pollen loads from 2 different near donor plants, 2 different far donor plants, and 2 different distant donor plants. This procedure resulted in 8 flowers pollinated per female per day. Each male was represented by 4 pollinations on each female over the 4-day period. Pollen was prepared for hand-pollination by tapping the anthers of the donor flower over a petri dish. Pollen was transferred to the stigma with a wooden toothpick.

Pollen tube growth was interrupted at 10 hr following pollination by removing the pistils and placing them in FAA. (Ten hr was chosen because previous experiments showed that some but not all pollen tubes could reach the ovary in that time interval.) Prior to clearing, the FAA was evaporated to improve uptake of the stain. Pistils were cleared in 10 N NaOH for 48 hr, rinsed twice in water, and then transferred to a 0.1% solution of aniline blue in 33 mM K_3PO_4 for 24 hr (Martin, 1959). Pollen tubes were observed at 100 \times using a BG 12 excitor filter in conjunction with a 512 nm filter on a Zeiss Universal microscope, equipped with a mercury vapor lamp. Three measurements of pollen tube growth were taken: 1) number of pollen grains germinated on the stigma, 2) number of pollen tubes penetrating $\frac{1}{4}$ of the way down the style, and 3) number of pollen tubes penetrating into the ovary.

In our data analyses, we used only pistils with at least 1 germinated pollen grain on the stigma, excluding approximately 25% of the pistils. We adopted this criterion because we could not be sure that the lack of germination was not due to unreceptive stigmas or immature pollen grains. This decision did not bias our analyses: a multiple contingency table analysis (using PROC CATMODE, SAS, 1985) of data we kept vs. those we excluded showed no significant differences among distance classes ($G = 0.11$, $df = 3$, $P > 0.05$) nor among females ($G = 17.72$, $df = 10$, $P > 0.05$). Data analyses for ANOVAs were based on raw data which had been log transformed using the method of Wright (1968, p. 232) to reduce heteroscedasticity of the variances.

To examine the effect of interparent distance

TABLE 1. *Mixed model ANOVA results for three measures of pollen tube growth in C. fasciculata with the main effects of female (random) and interparent distance (fixed): self, near (within genetic neighborhood), far (between far neighborhoods and within subpopulation), and distant (between neighborhoods and adjacent subpopulations)*

Source	No. pollen grains germinated			No. pollen tubes reaching ¼ style			No. pollen tubes reaching ovary		
	df	MS	F	df	MS	F	df	MS	F
Female	10	1.746	3.6***	10	0.245	3.3***	10	0.548	2.8**
Distance	3	0.712	1.2	3	0.029	0.4	3	0.116	0.7
Female × distance	30	0.608	1.2	30	0.079	1.1	30	0.178	0.9
Error	189	0.491		178	0.073		189	0.199	

** $P < 0.01$; *** $P < 0.001$.

(cross) on pollen tube growth, a 2-way mixed model ANOVA was performed with cross (model I) and female (model II) as the main effects. To examine the effect of male on pollen tube growth, a nested model II ANOVA was performed with male nested within female. For this analysis, we used only females that were represented by pollinations at all 4 distance classes. By examining the contribution of pollen donor source using males from 3 spatial classes of neighborhoods [near, far (both within same subpopulation), and distant (adjacent subpopulation)], we probably increased the genetic variation for pollen tube growth far beyond what would occur on stigmas in natural populations and should have increased the likelihood of detecting a male effect on pollen tube growth. If the effect of cross is not significant in the first ANOVA, then the use of males from near, far, and distant neighborhoods should not confound the role of genetic relatedness with variation among pollen donors.

All analyses were conducted with the SAS statistical package (SAS, 1985). The GLM procedure was used for the ANOVAs, and the calculations of variance components were based on the SAS type IV sums of squares.

RESULTS—Using interparent distance as an index of genetic relatedness, there was no significant difference among pollen from self, near, far, and adjacent subpopulation distance classes for any of the three measures of pollen tube growth—mean number of pollen grains germinated, mean number of pollen tubes reach-

ing ¼ length of the style, and mean number of pollen tubes reaching the ovary (Table 1; Fig. 1A–C). As intended, the mean number of pollen tubes observed at quarter length of style and at the ovary was less than the maximum of 21 that could possibly fertilize all the ovules (Fig. 1B, C). However, number of germinated pollen grains counted on stigmas (range = 0–53) often exceeded the number of ovules.

The analysis of pollen tube growth among six individual males nested within eight different females revealed that there were no differences among males for any of the three measures of pollen tube growth (Table 2).

Both ANOVA analyses showed a strong female effect on pollen tube growth (Tables 1, 2). The variation among females for pollen germination and pollen tube growth (Fig. 2A–C) was much greater than that observed for the four distance classes (Fig. 1A–C). In the analysis of the male effect (Table 2), the maternal effect accounted for 14.2% of the variation in number of pollen grains germinated while the male effect accounted for only 1.5%. For number reaching a quarter length of the style, the maternal effect accounted for 15% and male effect 2.6%. For number reaching the ovary, the maternal effect accounted for 6% and the male effect for 1%.

DISCUSSION—Our results suggest that interparent distance, an estimate of genetic similarity, does not influence pollen tube growth. In species with evidence of inbreeding depression, it might be advantageous for a plant to distinguish between self and outcross pollen.

TABLE 2. *Nested ANOVA results for three measures of pollen tube growth in C. fasciculata for male nested within female (excluding self pollen)*

Source	No. pollen grains germinated			No. pollen tubes reaching ¼ style			No. pollen tubes reaching ovary		
	df	MS	F	df	MS	F	df	MS	F
Female	7	3.200	3.6**	7	0.087	3.6**	7	0.203	2.1
Male (female)	40	0.879	1.1	40	0.024	1.1	40	0.099	1.2
Error	90	0.837		82	0.022		90	0.096	

** $P < 0.01$.

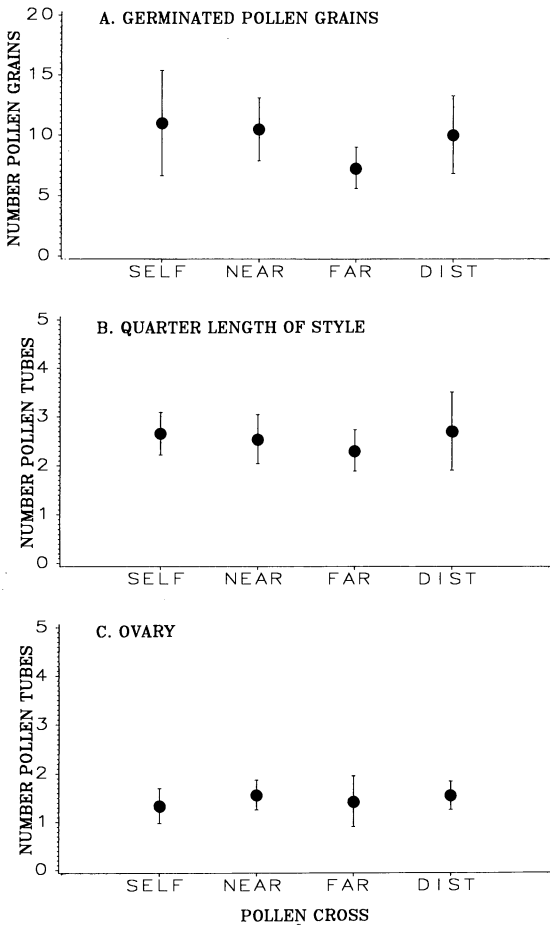


Fig. 1. Mean number (± 2 SE) of germinated pollen grains (A), pollen tubes which reached $\frac{1}{4}$ length of style (B), and pollen tubes reaching the ovary (C) for four classes of pollen crosses based on interparent distance: self, near (1 m), far (20 m) and distant neighborhood (100 m). Means are less than the number of ovules because the experiment was terminated before all pollen tubes reached the ovary.

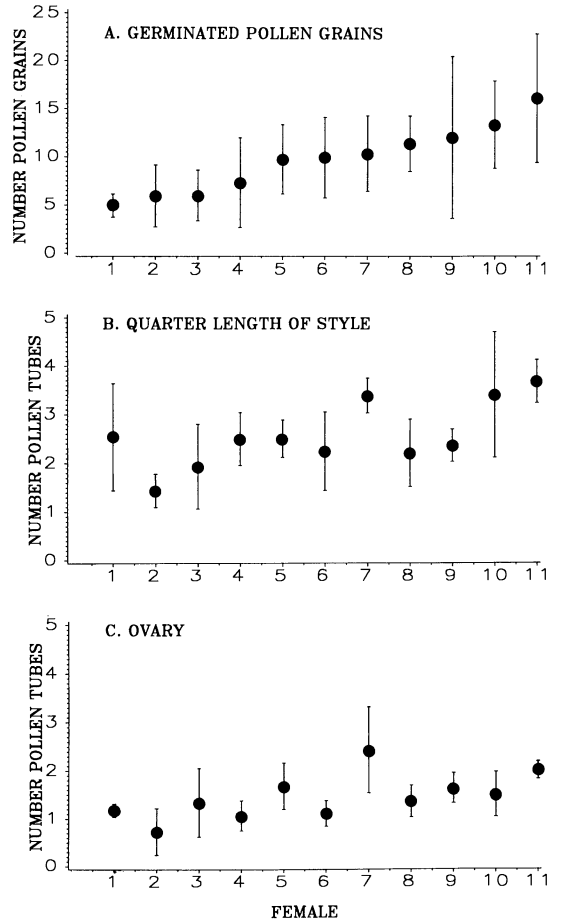


Fig. 2. Mean number (± 2 SE) of germinated pollen grains (A), pollen tubes which reached $\frac{1}{4}$ length of style (B), and pollen tubes reaching the ovary (C) for 11 *C. fasciculata* pollen recipient plants ranked from lowest to highest mean number of germinated pollen grains.

As discussed previously, some species do distinguish between self and outcross pollen (e.g., Bateman, 1956; Smith and Clarkson, 1956; Bowman, 1987). In separate studies on *C. fasciculata*, both of us (Fenster, 1988; Sork and Schemske, in preparation) have found a significant positive effect of interparent distance on progeny fitness and strong inbreeding depression (greater than twofold) following selfing. However, this experiment demonstrated that outcrossed pollen do not grow faster than self pollen even though this outcome might improve progeny fitness. A similar result was found by Hessing (1986) who studied *Geranium caespitosum*, an herbaceous perennial with known inbreeding depression.

The observation that there were no significant differences in pollen tube growth among

individual males indicates little opportunity for sexual selection on this trait. Because pollen donor sources were sampled from a number of genetic neighborhoods, we should have introduced sufficient genetic variation in pollen tube growth to detect phenotypic variation among males for this trait. In contrast to our findings, Hill and Lord (1986) found a significant difference of in vivo pollen tube growth between two pollen donors of *Raphanus raphanistrum*. Marshall and Ellstrand (1986) also observed a significant male effect of in vivo pollen tube growth among three males in a wild population of the self-incompatible *Raphanus sativus* but we do not know to what extent genetic similarity contributed to the variation among males. In both studies, it is difficult to assess the importance of variation in pollen tube growth for sexual selection since so few males were examined.

Most studies which examine the effects of pollen competition study the fitness consequences of different levels of pollen density from a single pollen source (e.g., Mulcahy and Mulcahy, 1975; Lee and Hartgerinck, 1986; McKenna, 1986) rather than compare the differences in pollen tube growth among pollen sources as we have. Lee and Hartgerinck (1986), using a pollen dilution approach to study pollen competition in *C. fasciculata*, found that progeny resulting from small pollen loads from a single donor had lower fitness than those resulting from high pollen loads when the progeny were grown under competitive conditions; however, these results could not be replicated (T. Lee, personal communication). The above studies show that increased gametophytic competition under heavy pollen loads from a single pollen source sometimes results in higher progeny fitness. Such findings address issues of gametophytic selection but are not comparable to studies which make comparisons between pollen sources.

Although we did not find that pollen source affected pollen tube growth, we did find that the maternal environment is a significant factor. Because pollen tube growth is a trait influenced by both male and female effects, its potential for evolution through sexual selection will be constrained by the amount of variation which can be attributed to the male effect (see also Mazer, 1987). In the case of *C. fasciculata* the environment where the pollen become deposited is much more important than the source of the pollen.

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