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FLORAL MORPHOLOGY AND CROSS-POLLINATION IN ERYTHRONIUM GRANDIFLORUM (LILIACEAE)[1]

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ABSTRACT

In bumblebee visits to flowers of *Erythronium grandiflorum* (Liliaceae), the ratio of self- to nonself- ("outcross") pollen grains deposited on the stigma is positively correlated with the degree of stylar exsertion beyond the anthers. Natural populations show substantial, continuous variation in stylar exsertion.

IN THEIR REVIEW of devices that promote outcrossing in angiosperms, Faegri and van der Pijl (1979, p. 27) suggest that "herkogamy, the spatial separation of anthers and stigma, is so much the rule as to be mostly taken for granted and passed by unnoticed like M. Jourdain's talking prose." An equally obvious corollary is the prediction that the ratio of outcross- to self-pollen grains deposited on the stigma of a flower (hereafter, "outcross ratio") should vary with the degree of separation of stigma and anthers. Work in this area has concentrated on the amount of stylar exsertion relative to the anthers. It should be possible to make inferences about relative outcross ratios of different plant populations by examining anther-stigma separations if the expected relationship holds. However, the literature suggests that the relationship between stylar exsertion and cross-pollination is more complex than would be expected, given its apparent mechanical inevitability.

First, there have been relatively few quantitative studies of intraspecific floral variation and outcrossing. A clear role of floral morphology has been shown in *Lycopersicon pimpinellifolium* (Solanaceae; Rick, Fobes and Holle, 1977; Rick, Holle and Thorpe, 1978) and in the distylous self-compatible *Amsinckia vernicosa* var. *furcata* (Boraginaceae; Ganders, 1975). Intrageneric comparisons that are consistent with an outcrossing role for floral morphology have been made by Ennos (1981) for *Ipomaea purpurea* and *I. hederacea* (Convolvulaceae) and by Vasek (1964, 1965) for *Clarkia exilis* and *C. unguiculata* (Onagraceae) (but see Vasek and Harding, 1976 for an account of inconsistencies within *Clarkia* species).

Second, some investigators have looked specifically for the expected relationship but have been unable to make definite statements about floral morphology. The work of Breese (1959) on *Nicotiana rustica* (Solanaceae) and of Schoen (1982a; see also Grant, 1954; Schoen, 1977, 1982b) on *Gilia achilleifolia* (Polemoniaceae) illustrate some of the practical difficulties in establishing what would seem to be an obvious effect. Breese selected lines for increased and decreased herkogamy, establishing that style-anther separation was highly heritable but that it was correlated with changes in dichogamy. Subsequent tests of outcrossing rate (via marker genes) found correlations between stylar exsertion, degree of dichogamy, and outcrossing; however, the variation in floral morphology did not explain a significant amount of variation in outcrossing beyond what was explained by the parallel variation in the timing of male and female functions. Schoen looked at interpopulational variation in outcrossing rate (via allozymes), and also found that dichogamy influenced outcrossing more than stylar exsertion did. Stylar exsertion was insignificantly rank-correlated with outcrossing in a sample of six populations, although the data showed a trend in the expected direction. Schoen (1982a, p. 357) concluded that "inferences about plant breeding systems based solely on floral morphological grounds may often be misleading."

It is quite possible that the expected relation

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between morphology and outcrossing does hold in both Breese's and Schoen's plants; despite Schoen's valid but conservative conclusion, certainly neither study constitutes a resounding *negation* of such a relationship. However, in Breese's study the unplanned response of dichogamy to selection for heterostathmy, and in Schoen's the necessarily small number of populations sampled, are complications that would make a relationship hard to establish. The importance of stylar exsertion should be most easily demonstrated in a system in which outcrossing can be simply measured on a per flower, rather than a per population, basis, and in which differences in timing of the sexual functions are nonexistent or can be controlled experimentally.

Erythronium grandiflorum (Liliaceae) is a good subject for such studies. Captive bumblebees, which are major pollinators in the wild, will pollinate cut flowers in normal fashion, and the existence of a strong pollen-color dimorphism (see Thomson and Plowright, 1980; Thomson, 1985) allows the outcross ratio of pollen deposition to be estimated quickly by direct grain counts. Unpublished work by Allison Snow shows that *E. grandiflorum* is largely self-compatible.

MATERIALS AND METHODS—We worked at the Rocky Mountain Biological Laboratory in Gothic, Colorado, during June 1983. We collected buds of the yellow-pollen and red-pollen color morphs and let the flowers open and the anthers dehisce indoors. We chose especially dark red-pollen flowers for our tests and covered their stigmas with gelatin capsules before any anthers had dehisced (Fig. 1A).

Two *Bombus occidentalis* queens did the pollinating. In a laboratory room, we induced them to visit several yellow-pollen flowers and then presented a freshly uncovered red-pollen flower (with all six anthers dehiscent). After the bee had completed a single visit to the red-pollen flower, we carefully severed the style from the ovary, avoiding applying any further pollen, and immediately counted the yellow (outcross) and red (self) grains on the papillose region of the stigma, at 50 ×. The morphs could be distinguished unambiguously. The bees were allowed to fly freely indoors and to feed from yellow-pollen flowers only between runs. We allowed sufficient time for them to groom away essentially all red grains, based on our prior experience (Thomson, 1985).

After the grains had been counted, we reunited the cut style to its stump and used a 6 × optical comparator to measure the distance ("stylar exsertion") from the distal tip of the stigma to the distal end of the longest anther, holding the stamens and pistil in juxtaposition to make the measurement. Anther-stigma separation could not be measured prior to removal because of the likelihood of contamination by self-pollen. We made the same measurement on a large, random collection of flowers from a natural population along Trail 401 near Gothic. We measured only flowers with 3 of the 6 anthers dehiscent, to standardize flower age.

RESULTS—Figure 1B shows a sample of flowers demonstrating variation in stylar exsertion. Figure 2 shows that stylar exsertion varies substantially and continuously in the Trail 401 population ($\bar{x}$ = 1.46, SD = 1.65, C.V. = 111%). Figure 3 shows that the fraction of outcross pollen applied in a single visit varies significantly with stylar exsertion ($r = 0.71$, 18 df, $P < 0.01$ for arcsine-transformed fractions of self-pollen). The fraction of self-pollen was independent of total pollen load, which ranged from 289 to 703 grains. Although the flowers used in our experiments do not span the full range of stylar exsertion found in natural populations, the outcross fraction differs very substantially in our sample. If natural conditions are similar to those in our experiments, we might expect a somewhat greater range of outcrossing fractions in nature.

DISCUSSION—*Mechanics*—Because stylar exsertion beyond the longest anther was the only floral character we measured, we cannot prove that stylar exsertion per se, rather than some correlated character, is the cause of the relationship with cross-pollination. Several studies (e.g., Grant, 1954; Rick et al., 1977;

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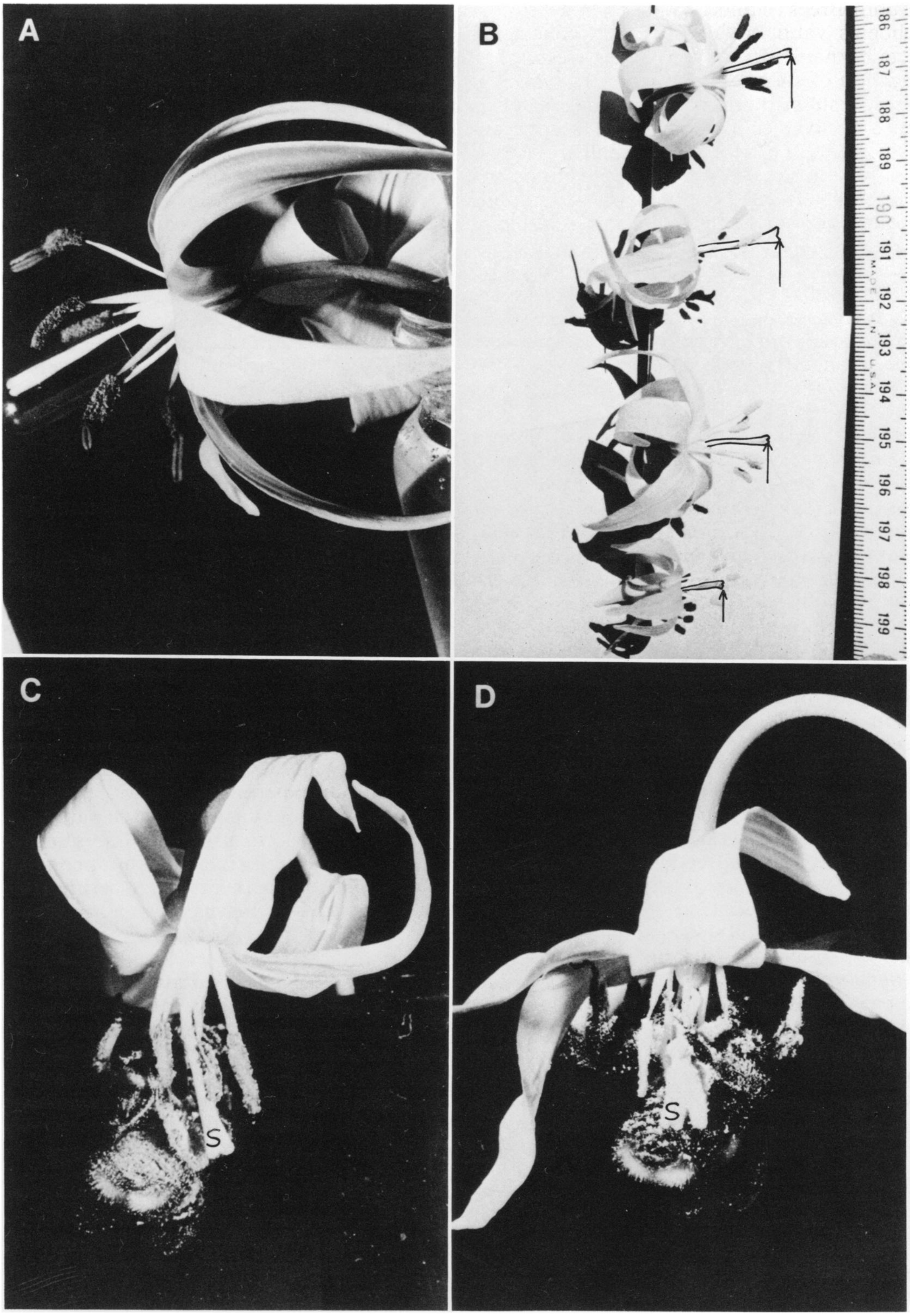
Fig. 1. **A.** Half of a gelatin capsule is used to protect the stigma of a red-pollen *Erythronium grandiflorum* flower until the experimental visit. Note the partial dehiscence of the second whorl of anthers. **B.** A sample of flowers showing variation in the degree of stylar exsertion. Arrows point to the stigmas. Units on the rule are cm. Note the contrast between the leftmost red-pollen flower and the other yellow-pollen flowers. Style outlines are darkened to improve visibility. **C.** A *Bombus occidentalis* queen visiting a red-pollen flower with a highly exserted style. **D.** A *Bombus occidentalis* queen visiting a yellow-pollen flower with a slightly exserted style. The transfer of self grains is more likely than in a flower such as the one shown in C.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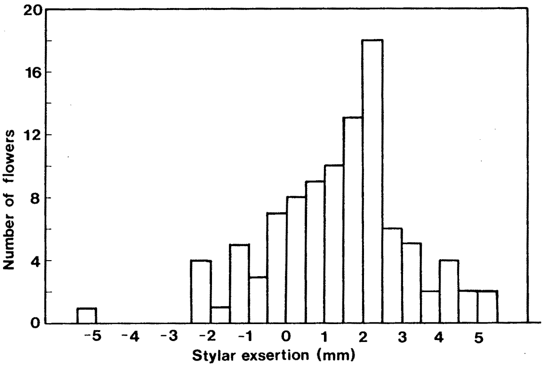

Fig. 2. The frequency distribution of stylar exertion (i.e., the distance by which the distal tip of the stigmatic surface protrudes beyond the longest dehiscent anther) for a random sample of *Erythronium grandiflorum* flowers measured near Gothic, Colorado. All flowers were at the same developmental stage (see text).

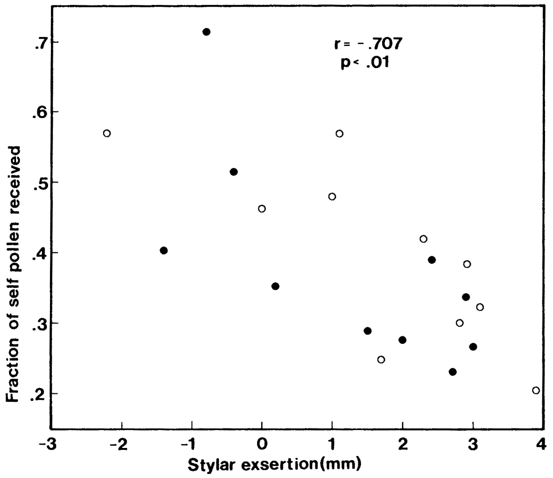

Fig. 3. The fraction of self pollen deposited on stigmas of *Erythronium grandiflorum* during single visits by *Bombus occidentalis* queens, as a function of stylar exsertion. The two symbol types represent data from two different bees.

Nothmann, Rylski and Spigelman, 1983) have found positive correlations between stylar exsertion and flower size, and size is known to affect pollination as well. However, observation of the bees leads us to believe that the correlation of stylar exsertion and cross-pollination does reflect a true causal relationship. In landing on these nodding flowers, the bees usually grasped the pistil and stamens with all six or the four hindmost legs while they pushed their heads upward toward the nectaries at the tepal bases (Fig. 1C, D). In seeking footholds, they often pulled anthers into contact with the style, depositing numerous self-grains on the stigmas of less-exserted styles. Also, in flowers with pronounced stylar exsertion, the bees often contacted the protruding stigma with their hind legs or abdomen before those body parts had received self-pollen from the anthers. When the stigma was at or below the anther level, in contrast, the outcross pollen on the bees was often overlain with self-pollen before any stigmatic contact was made.

Genetics—It should be obvious that our terms "outcrossing" vs. "selfing" actually refer to pollen brought to a stigma from other flowers vs. pollen put on the stigma of its own flower. While the latter is indubitably selfing, the former may represent geitonogamous selfing within inflorescences or between inflorescences within clones. Also, if there are differences between outcross and self-pollen with regard to germination, tube growth, fertilization of ovules, or selective abortion of resulting zygotes, the true outcrossing fraction may deviate from our estimates. Combining pollen marker analyses with allozyme analyses of progeny should be good method for studying these sources of discrepancy. What our mechanical analysis still indicates, however, is that when flowers of different stylar exsertion occur together in similar circumstances—as they do in these populations—the more exserted flowers will on average receive more outcross pollen. Although our argument has considered the effects of variable herkogamy on cross-pollination, it is by no means clear that *Erythronium* has evolved this variation under natural selection acting on outcrossing rate.

Animals—Bumblebees are not the only visitors of *Erythronium grandiflorum.* Hummingbirds and solitary bees are also common, and although the birds seldom pollinate the flowers, the solitary bees often do. We have not investigated the outcross ratios of pollen deposited by the small bees, and the relationship with stylar exsertion may be less clear than it is for *Bombus.* Casual observation suggests that greater exsertion will still increase outcrossing, but that the solitaries will generally provide more self-pollination due to their tendency to land on the anthers before touching the stigma.

CONCLUSIONS—A mechanically comprehensible relationship between variation in floral morphology and the ratio of self- to non-self-pollination by bumblebees is easy to demonstrate in *Erythronium grandiflorum* due to a pollen-color dimorphism. Future work should assess the reliability of floral morphology as an indicator of breeding system under completely natural conditions.

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