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Pollen precedence and stigma closure: a mechanism of competition for pollination between *Delphinium nelsonii* and *Ipomopsis aggregata*

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Summary. Previous experiments showed that the sympatric herbs *Delphinium nelsonii* and *Ipomopsis aggregata* compete for hummingbird pollination and that deleterious effects of the former species on seed set of the latter involve interspecific pollen transfer. However, seed set was not reduced when pollen of both species was applied simultaneously to *I. aggregata* stigmas. Hence a competitive effect may require arrival of foreign pollen before conspecific pollen. To explore this possibility we subjected *I. aggregata* flowers to a "competition" treatment in which they received *D. nelsonii* pollen 6 h before *I. aggregata* pollen, or to a "control" in which they received only the conspecific pollen. Foreign pollen precedence decreased mean seed set by almost 50%, which is consistent with effects observed in previous experiments. Reduced seed set can be explained by the fact that foreign pollen often caused stigma lobes to close together within 1.5–6 h, reducing subsequent receptivity. Stigma closure was also elicited by conspecific pollen, but not by mechanical stimulation, and was influenced by size of the pollen load and identity of the plant being pollinated.

Key words: Pollination – Stigma closure – Competition – *Delphinium – Ipomopsis*

Competition for pollination refers to an interaction among sympatric flowering plants in which the use of common pollinators depresses reproduction. The interaction is more subtle than sometimes imagined: it may occur when pollinators themselves are plentiful, but move between flowers of different species (see Waser 1978a, b, 1983).

In the Rocky Mountains of Colorado, USA, the sympatric herbaceous wildflowers *Delphinium nelsonii* Greene (Ranunculaceae) and *Ipomopsis aggregata* (Pursh) V. Grant (Polemoniaceae) share pollination services of hummingbirds (broad-tailed, *Selasphorus platycercus* Swainson; and rufous, *S. rufus* Gmelin). Previous experiments showed that these species compete for pollination (see Waser 1978a). The evidence can be summarized as follows, with emphasis on *I. aggregata*. (1) The species bloom in sequence, but there is a short "flowering overlap" period during which each receives the other's pollen as a result of interspecific hummingbird flights. Seed set is lower during overlap than

at other times; in 1976 *I. aggregata* flowers suffered a 30% reduction. (2) Seed set also is lower in outdoor mixed-species arrays of potted plants visited by hummingbirds than in single-species arrays; in 1975 and 1976 *I. aggregata* flowers suffered reductions of about 35%. (3) Artificial interspecific pollinations that mimic hummingbirds reduce seed set of *I. aggregata* but not *D. nelsonii*. Reductions in three 1976 and 1977 experiments were about 25%.

In all the situations just described, fitness loss to *I. aggregata* was associated with pollinator flights or pollen transfer between species, suggesting that this underlies competition. However, Kohn and Waser (1985) found that *D. nelsonii* pollen had no reproductive effect when applied to *I. aggregata* stigmas at the same time as conspecific pollen. There are several possible explanations for this apparent discrepancy. First, interspecific pollinator flights may depress fecundity because they entail a loss of *I. aggregata* pollen that fails to adhere to the pollinator, is covered over by foreign pollen, or is deposited on *D. nelsonii* flowers (Kohn and Waser 1985; Campbell and Motten 1985). Second, interspecific flights will cause foreign pollen to arrive on stigmas before conspecific pollen in some cases. Pollen precedence may reduce subsequent adhesion, growth, or fertilization success of conspecific pollen (cf. Armbruster and Herzig 1984).

We used hand-pollination of *I. aggregata* to explore whether precedence of *D. nelsonii* pollen could explain seed set reductions. Here we show (1) that pollen precedence substantially depresses seed set; (2) that the deleterious effect is associated with closure of stigma lobes; and (3) that stigma closure is related to size of the foreign pollen load. We also discuss other treatments that elicit stigma closure.

Methods

(1) Pollen recipients and donors

In June 1985 we potted 22 *I. aggregata* individuals in bud stage from a meadow near the Rocky Mountain Biological Laboratory (RMBL, 2,900 m elevation), and moved them into a greenhouse. Flowers were emasculated to prevent self-pollination. When they became pistillate, as indicated by reflexed stigma lobes (Kohn and Waser 1985), they received either a "competition" hand-pollination treatment in which *D. nelsonii* pollen was applied at 10 AM followed 6 h later, at 4 PM, by *I. aggregata* pollen; or a "control" in which only conspecific pollen was applied, also at 4 PM.

In the former treatment we attempted to apply conspecific pollen even when stigma lobes had closed together by 4 PM (see below), sometimes pushing appressed lobes apart slightly in the process. This was meant to simulate the rapid and forceful entry of a hummingbird into a flower. Pollinations were made daily from 3–7 July, and on a given day the 2 treatments were alternated among randomly-chosen flowers on each plant. We achieved approximately balanced replication of treatments on each recipient; in all we pollinated 224 flowers.

After 7 July we carried out further pollination treatments at 10 AM; these consisted of applying *I. aggregata* pollen or of mechanically stimulating stigmas with toothpicks carrying no pollen. The latter is a control for hand-pollination, since all treatments were made using toothpicks to collect fresh pollen from anthers of donor flowers and apply it promptly to stigmas.

We scored all flowers 6 h after initial pollination for closure of stigma lobes, and scored a subset 1.5 h after pollination. We also examined some flowers at senescence to see if stigmas remained closed.

Conspecific pollen donors for hand-pollinations were collected as needed within 10 m of the site of origin of recipients (see Waser and Price 1983). When possible we collected *D. nelsonii* inflorescences from the same meadow. Peak anther dehiscence occurs in late morning for *D. nelsonii* and mid-afternoon for *I. aggregata*, which conveniently allowed us to use freshly-dehisced pollen of each species in experimental treatments.

(2) Stigma pollen loads and seed set

Studies of pollen tube growth (N. Waser and M. Price unpublished work) indicate that *I. aggregata* pollen fertilizes ovules within 72 h. We waited this long to collect stigmas of hand-pollinated flowers, squashed them on a microscope slide with a drop of Alexander's (1980) stain, and scored germinated and ungerminated *I. aggregata* pollen. In the case of *D. nelsonii* we scored total pollen loads, since germinated grains were difficult to distinguish with this stain.

After collecting stigmas we allowed fruits from competition and control treatments to mature and counted seeds. We calculated dose-response relationships for each treatment between conspecific pollen load on stigmas and seed set, using the NLIN procedure of SAS (SAS Institute 1982). This procedure determines the least-squares fit to a specified model, in our case a negative exponential of the form $y = K$ $[1-\exp(-bx)]$ where y is seed set and x is conspecific pollen load (see Kohn and Waser 1985 for justification of this model).

We scored pollen loads on *I. aggregata* stigmas from natural situations of flowering overlap with *D. nelsonii*. Stigmas were scored with Alexander's stain as described above, or by staining with 0.05% aniline blue, counterstaining with 0.01% ethidium bromide (Hough et al. 1985), and viewing under an epifluorescence microscope.

Results

(1) Seed set reduction

Mean seed set of flowers receiving the competition treatment was almost 50% lower than that of control flowers (Table 1a). The absolute reduction averaged 4 seeds per flower. This was highly significant when individual seed set values were analyzed by factorial ANOVA (Table 1b) or when treatment means within each plant were compared (pairwise $t = 4.4$, df $= 21$, $P < 0.001$, 1-tailed). ANOVA showed no significant interaction between plant identity and treatment effect.

(2) Stigma pollen loads

The competition treatment had no effect on germination of *I. aggregata* pollen (Table 1), and best-fit dose-response relationships between seed set and conspecific pollen load were similar for the two treatments (Fig. 1; cf. Kohn and Waser 1985). Compared to controls, the competition treatment had a slightly lower asymptotic seed set (a smaller value of the variable K in the nonlinear regression; 9.9 vs 12.4) and a slightly lower initial "slope" (a larger value of the variable b; 0.008 vs 0.007). In each case, however, the mean for one treatment was contained within 95% confidence limits of the other (K: upper limit in competition $=$ 16.5, lower limit in control $= 7.9$; b: upper limit in competition $= 0.17$, lower limit in control $= 0.001$).

Thus precedence of foreign pollen did not significantly reduce fertilization success of a conspecific grain, which was on the order of 5% (20 grains per seed set) for about the first 200 grains, beyond which seed set reached a maximum (Fig. 1). On the other hand, precedence did reduce the total conspecific load delivered to a stigma, by an average of almost 60% relative to control values (Table 1a; $t = 9.4$, df $= 217$, $P \ll 0.001$, 1-tailed). As a result conspecific loads in the competition treatment more often fell within the ascending portion of the dose-response function (Fig. 1), and seed set was reduced.

Conspecific loads delivered in the competition treatment were similar to those found in previous experiments and during periods of natural flowering overlap with *D. nelsonii* (Table 2), whereas foreign loads delivered were 1.5 to 4 times larger.

(3) Stigma closure

There was a strong tendency for *D. nelsonii* pollen to cause the reflexed lobes of receptive *I. aggregata* stigmas to close together. This occurred in 62 of 117 flowers receiving foreign pollen at 10 AM (competition treatment), as opposed to only 9 of 95 flowers receiving no pollen (control treatment; $X^2 = 42.6$, df $= 1$, $P < 0.0001$). The response was scored 6 h after pollination but may occur more rapidly. In a sample of 10 flowers with closed stigmas at 1.5 h, there was no additional response by 6 h. Observation of a sample of flowers indicated that closure persisted for the remainder of the pistillate-stage (cf. Stephenson 1979). The response increased with size of the foreign pollen load; competition flowers whose stigmas closed had about twice the average load of those whose stigmas did not close (Table 1a; $t = 4.8$, df $= 91$, $P < 0.001$, 2-tailed). The proportion of flowers with closed stigmas varied significantly among plants ($G_H = 33.9$, df $= 21$, $P < 0.05$) and ranged between 0 and 1.0.

Closure was even more strongly elicited by *I. aggregata* pollen; it occurred in 27 of 30 flowers to which we applied conspecific pollen at 10 AM ($X^2 = 68.2$, df $= 1$, $P < 0.0001$ for comparison with flowers receiving no pollen, see above). This includes outcross and self pollen, which had the same

Table 1. A Hand-pollination results. Values are means ±1 standard error (sample size). Sample sizes vary because we did not obtain all measurements from all flowers. "Load" is stigma pollen load, "germination" is pollen germination on stigmas. For the competition treatment values are given separately for flowers with closed and open stigmas, in addition to overall values. **B** Two-way ANOVA with raw seed sets

A	Treatment	Seed set	*I. aggregata* load	Germination	*D. nelsonii* load
	Control	8.1±0.6 (111)	193.5±10.5 (108)	0.39±0.02 (109)	0
	Comp	4.1±0.5 (113)	79.4± 6.1 (111)	0.38±0.02 (110)	380.4±25.6 (111)
	closed	2.3±0.5 (50)	54.0± 6.4 (50)	—	504.9±42.5 (50)
	open	6.7±0.8 (44)	107.7±11.0 (44)	—	257.1±28.5 (43)

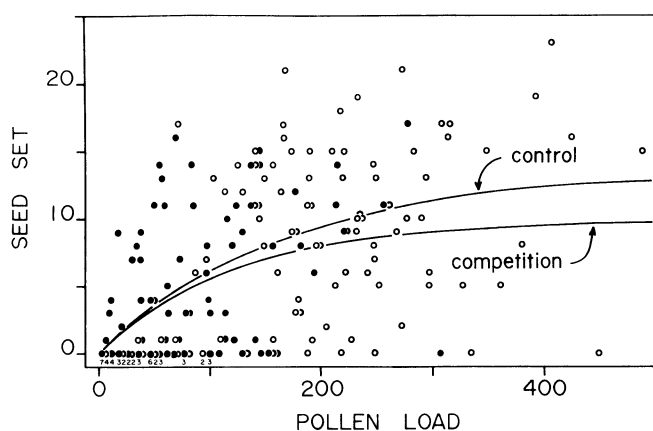| B	Source of variation	DF	MS	SS	F	P
	Treatment	1	644.73	644.73	23.06	<0.0001
	Plant	21	1552.50	73.97	2.64	0.0003
	Interaction	21	161.85	7.71	0.28	0.99
	Error	180	5032.83	27.96		

Fig. 1. Dose-response relationship between seed set and conspecific pollen load for flowers receiving competition (filled circles) and control (open circles) hand-pollinations. Best fit nonlinear regressions are $y = 12.4(1-\exp(-0.007x))$, $r^2 = 0.25$ for control flowers, and $y = 9.9(1-\exp(-0.008x))$, $r^2 = 0.22$ for competition flowers, where y is seed set and x is pollen load
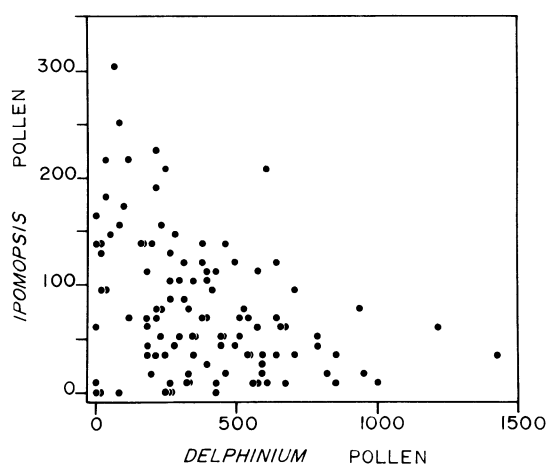

Fig. 2. Relationship between conspecific and foreign pollen loads on 110 *I. aggregata* stigmas receiving the competition treatment. The linear regression of *I. aggregata* load on *D. nelsonii* load is $y = -0.088x + 112.7$; $r^2 = 0.14$, df = 1, 109; F = 17.3; $P < 0.001$

Table 2. Ranges of pollen loads on *I. aggregata* stigmas in field-collected flowers subjected to overlap with *D. nelsonii* (F) and greenhouse flowers hand-pollinated with pollen of both species (G). Field collections include some flowers that did not experience interspecific pollen transfer

Sample	*D. nelsonii*	*I. aggregata*	Source
F 1983	0–309	0–478	Kohn and Waser (1985)
F 1985	0–431	0–306	This study
G 1977	1–977	3–357	Waser (1978a)
G 1983	1–865	10–253	J. Kohn (unpublished work)
G 1985	3–1425	0–307	This study

effect (closure in 12 of 14 flowers receiving outcross vs 15 of 16 receiving self). On the other hand, mechanical stimulation by itself did not elicit closure (cf. Guttenberg and Reiff 1958; Stephenson 1979). Of 13 stigmas brushed with toothpicks carrying no pollen, all remained open.

Stigma closure following application of foreign pollen

lowered subsequent receipt of conspecific pollen. Flowers in the competition treatment whose stigmas closed had conspecific loads reduced by 50% relative to competition flowers whose stigmas did not close (Table 1a; t = 4.2, df = 92, $P < 0.001$, 1-tailed) and by 70% relative to control flowers (Table 1a, t = 11.3, df = 156, $P < 0.001$, 1-tailed). There was a negative relationship overall between conspecific and foreign pollen loads on stigmas in the competition treatment (Fig. 2; contra Kohn and Waser 1985, Feinsinger et al. 1986).

Finally, the effect of stigma closure on conspecific pollen loads was large enough to explain seed set reduction. Mean seed set in competition flowers whose stigmas closed was reduced by 70% relative to controls (Table 1a; t = 7.2, df = 159, $P < 0.001$, 1-tailed), whereas in competition flowers whose stigmas did not close it was reduced by only 15% (Table 1a; t = 1.4, df = 153, $P > 0.05$, 1-tailed). Despite the relationship of closure and seed set, significant heterogeneity among plants in closure probability did not translate into a significant treatment by plant interaction in the ANOVA of seed sets (Table 1b).

Discussion

By promoting stigma closure and reducing conspecific pollen deposition, precedence of *D. nelsonii* pollen had a striking deleterious effect on fecundity of *I. aggregata* flowers. Seed set reduction occurred in 19 of 22 experimental plants and averaged almost 50%. This suggests that competition for pollination can act as a strong force of directional selection maintaining *I. aggregata* traits, such as later flowering, that reduce interaction with *D. nelsonii* (Waser 1978a, b, 1983).

This does not imply strong competition at present, however (see Connell 1980). Even assuming that loss in some flowers is not counterbalanced by gain in others (i.e., there is overall pollen limitation; Hainsworth et al. 1985), only a fraction of *I. aggregata* flowers open early enough in the season to experience competition. We can estimate whole-plant fecundity loss as follows. Overlap with *D. nelsonii* flowers on a local scale (that of pollinator flight distances) occurs during the first quarter of the *I. aggregata* flowering season (which lasts 30–40 days; Waser and Real 1979). Flowering is fairly synchronous across plants and approximately normally distributed through time. Thus at most the first quarter of a normal distribution of flower production on a plant, or 15% of all flowers, is exposed to foreign pollen. Assuming that 50% of these flowers actually receive *D. nelsonii* pollen (Kohn and Waser 1985), that the foreign pollen arrives first half the time, and that it causes a 50% seed set reduction when it arrives first (our experimental results), the present-day loss to an average plant represents <2% of total fecundity.

We have assumed that hand-pollination results are representative of nature; this is supported by the following observations. First, conspecific pollen loads were similar to those found during natural flowering overlap (Table 2). Foreign loads were somewhat larger, but the range that elicited closure in hand-pollinations (3–1425 grains) substantially overlapped loads in nature. Second, our results depended on precedence of foreign pollen of at least 1.5 h, the minimum estimate for stigma closure. We have not recorded visitation history of *I. aggregata* flowers, but dye transfer experiments (N. Waser and M. Price unpublished work) suggest an average 12 h interval between visits. Thus stigma closure is likely before a second visit occurs. Finally, hand-pollinations caused seed set reductions resembling those during natural flowering overlap with *D. nelsonii* (see Introduction), which suggests our experiment was realistic.

If stigma closure reduces subsequent receptivity, why should it occur, especially after receipt of a foreign pollen? A differential response may be difficult for plants to achieve if the stimulus for closure is pollen germination in general (Linskens 1976; Sedgley and Blesing 1982). Pollen of *D. nelsonii* often germinates on *I. aggregata* stigmas (Kohn and Waser 1985; compare Martin 1970). Furthermore, natural selection might favor closure once a certain stigma load is reached, if on average this increases adhesion and germination of conspecific pollen (see Linskens 1976).

The *D. nelsonii* – *I. aggregata* system remains one of few in which competition for pollination and its underlying mechanisms have been investigated experimentally (Waser 1983; Rathcke 1983; Armbruster and Herzig 1984; Campbell 1985b; Campbell and Motten 1985; Ford and Kay unpublished work). Our results suggest that competitive mechanisms are more complex than was imagined from early work with the system (Waser 1978a, b). In general, interspecific pollen transfer can cause competition because it decreases the chance of pollen reaching a conspecific stigma, or because foreign pollen delivered to stigmas reduces receptivity to conspecific pollen. Among conceivable deleterious effects of foreign pollen are clogging and actively disrupting the stigma surface, blocking the stylar transmitting tissue, and eliciting flower abscission or stigma closure. The taxonomic extent of "slow" stigma closure such as we have described appears to be unknown, since the available literature (Linskens 1976, SL Buchmann pers comm) concentrates on immediate responses to mechanical stimulation ("thigmotropism"). While closure seems sufficient to explain competitive effects in *I. aggregata*, it may be that other mechanisms also play a role (e.g., Campbell 1985a). We are exploring this possibility and examining competition in the reverse direction (R. Mitchell, unpublished work), i.e., the mechanism by which *D. nelsonii* suffers in the presence of *I. aggregata*.

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