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THE EFFECT OF DELPHINIUM NELSONII POLLEN ON SEED SET IN IPOMOPSIS AGGREGATA, A COMPETITOR FOR HUMMINGBIRD POLLINATION[1]

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ABSTRACT

Sympatric plant species can compete for pollination services in several ways. For example, pollinators may move between species and deposit heterospecific pollen on stigmas, which in turn may reduce the efficacy of conspecific pollen. We explored this possibility by determining the effect of *Delphinium nelsonii* pollen on seed set in *Ipomopsis aggregata*. These montane herbs are pollinated by hummingbirds, experience heterospecific pollen deposition in nature, and suffer reduced seed set in each other's presence. We hand-pollinated flowers of *I. aggregata* with either pure conspecific pollen or a mixture of pollen of the two species. Resulting pollen loads on stigmas ranged from 0–865 *D. nelsonii* grains and from 10–336 *I. aggregata* grains; mean seed set per flower was 11.3. There was no detectable effect of *D. nelsonii* pollen load on *I. aggregata* seed set. It is possible that seed set reductions seen in previous studies of competition for pollination between these species were caused by pollen wastage, pollen layering on the pollinator, or the temporal sequence of pollen arrival at the stigma.

ANIMAL POLLINATORS, even some of those reputed to have strong flower constancy, frequently move between flowers of different species (Waser, 1983b, 1985). Such interspecific visits are likely to result in pollen of one species being deposited on floral parts of others. One or more species may suffer a reproductive loss thereby, because of pollen wastage and related mechanisms when pollen or pollinator visits are limited, or because heterospecific pollen on the stigma interferes with deposition, adhesion, germination, tube growth, or ovule fertilization of conspecific pollen (Crosby, 1966; Waser, 1978b, 1983a; Rathcke, 1983; Campbell, 1985; Campbell and Motten, 1985; see also Ganders, 1979).

Fitness losses related to interspecific visitation constitute a form of competition for pollination whose mechanism can be described as interspecific or improper pollen transfer (Waser, 1978a, b, 1983a; Rathcke, 1983). As Waser (1978b, 1983a), Rathcke (1983), and Campbell and Motten (1985) discuss, this form of competition and a form in which the mechanism is described as pollinator preference (without implying conscious choice; N. Waser, unpubl.) are expected to differ in their evolutionary effects on floral traits. Specifically, interspecific pollen transfer is likely to promote stable floral character divergence among sympatric species, whereas pollinator preference is likely to select for increased floral reward and advertisement by nonpreferred species. These differences make it especially interesting to determine how plants compete for pollination.

In this paper we explore one mechanism by which *Delphinium nelsonii* Greene (Ranunculaceae) could influence *Ipomopsis aggregata* (Pursh) V. Grant (Polemoniaceae). These herbaceous wildflowers co-occur in subalpine meadows in Colorado. They flower in sequence, but a brief period of flowering overlap exists during which hummingbirds cause interspecific pollen transfer. Waser (1978a) found that both species suffered reduced seed set during this natural flowering overlap and in artificial mixed-species plots exposed to hummingbird pollination. Furthermore, hand-pollinations that mimicked hummingbird visitation caused interspecific pollen transfer and seed set reductions in *I. aggregata* similar to those observed in nature and in experimental plots. These results suggest that interspecific pollen transfer is involved in the reproductive losses suffered by *I. aggregata* when it flowers with *D. nelsonii*. Here we discuss a hand-pollination experiment designed to explore whether seed set reductions can be ascribed to direct effects of *D. nelsonii* pollen deposited on *I. aggregata* stigmas.

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METHODS—In June 1983, we dug up 29 *I. aggregata* plants in bud stage from a pasture near Almont, Gunnison Co., Colorado (elevation 2,450 m). Plants were potted in native soil and moved 35 km to a greenhouse at the Rocky Mountain Biological Laboratory, Gothic, Colorado (RMBL, 2,900 m elevation). Ten plants were subsequently used as conspecific pollen donors and the rest as pollen recipients. We also potted 20 *D. nelsonii* plants from a meadow near the RMBL to serve as heterospecific pollen donors. Potting at different elevations was necessary to obtain plants of the two species that would flower simultaneously.

We removed the first 10 flowers that opened on each of the 19 recipient *I. aggregata* plants and counted ovules by examining squashed ovaries at 25×. Subsequent flowers were hand-pollinated as described below, except that ovules were again counted for up to 10 flowers per plant that developed after the pollination experiment was terminated. We also counted ovules of the first and last 10 flowers produced by 10 plants in the field in 1984.

Recipient flowers were emasculated before anthers dehisced to avoid contaminating stigmas with self pollen, which is incompatible (Waser and Price, 1983). We pollinated almost daily, between 0800 and 1230 hours, from 27 June to 16 July. Pollen was collected from most male-phase flowers on donor plants and usually came from flowers that had opened on the day of collection. We mixed pollen from all donors and applied it with fine watercolor brushes, with each flower receiving either pure conspecific pollen or a mixture of conspecific and heterospecific pollen. We alternated pollination treatments (i.e., pure—mixed) to flowers on each recipient; if an odd number of flowers were receptive, first and last flowers received mixed loads. We used reflexed stigma lobes as an indicator of receptivity. Tests for the presence of esterase (1-ANS, Mattson et al., 1974; FDA, Heslop-Harrison and Heslop-Harrison, 1970) indicate that this is as accurate as possible, but there may have been some variation in receptivity that remained uncontrolled. Three days after flowers were pollinated, we removed and stained stigmas, squashed them gently, and counted pollen at 100×. Grains of the two species are different shapes and sizes (45–55 μm diam for *I. aggregata* vs. 20–25 μm for *D. nelsonii*). Subsequently, we removed undehisced fruits and counted seeds. We excluded from analyses fruits attacked by fly larvae (Zimmerman, 1979), but included undamaged flowers that failed to set fruit or seed.

Finally, we assessed natural conspecific and heterospecific pollen loads by examining 240 stigmas from five different *I. aggregata* patches near the RMBL. We also counted pollen loads on 46 *I. aggregata* stigmas from Waser's (1978a) previous hand-pollination experiment. These values were then compared to those obtained in the present experiment.

RESULTS—Mean ovule number was higher for the first 10 flowers on pollen recipients than for flowers measured after the end of pollinations (means = 28.1 vs. 21.8; paired $t = 11.8$, $df = 18$, $P < 0.001$, two-tailed). Thus, the maximum number of seeds a flower could set declined on average during the experiment. Mean ovule counts in the field in 1984 were 23.3 for early flowers and 19.7 for late flowers on the same plants (paired $t = 3.50$, d.f. = 9, $P < 0.01$, two-tailed).

Of 454 hand-pollinated stigmas from the 19 pollen recipients, 172 contained *I. aggregata* pollen alone and 282 contained pollen of both species. Pollen loads ranged from 0–865 *D. nelsonii* grains ($\bar{x} = 108.1$, SD = 141) and from 10–336 *I. aggregata* grains ($\bar{x} = 87.3$, SD = 51.1). Amounts of pollen of the two species on individual stigmas were positively correlated ($r = 0.26$, $df = 281$, $P < 0.001$). The mean seed set of hand-pollinated fruits was 11.3 (SD = 5.6, $N = 454$).

Stigma loads of *D. nelsonii* and *I. aggregata* pollen produced by our experiment fell within the ranges of 1–977 and 3–357 obtained for the two species, respectively, in Waser's (1978a) hand-pollinations. Field-collected stigmas from 1983 carried 0–309 heterospecific grains, a somewhat smaller range than in the experiment, and 0–478 conspecific grains ($\bar{x} = 119$, SD = 80.5, $N = 240$), some of which were certainly incompatible self grains. Similarly, R. Mitchell (pers. comm.) found 0–156 *D. nelsonii* grains and 36–372 *I. aggregata* grains on *I. aggregata* stigmas collected during natural flowering overlap of the species in 1984.

The dose-response relationship for seed set resulting from pure *I. aggregata* pollen (Fig. 1) should be fit reasonably well by a negative exponential function of the form $y = a\ [1 - \exp(-bx)]$, which is asymptotic to some maximum value of y (seed set) as x (pollen load) increases. The values in Fig. 1 are poorly fit by a linear model (Table 1). Log-transformation of pollen loads and an exponential function do explain slightly more of the variance (Table 1). Even the best-fitting model explains very little of the variance in seed set, however.

Multiple linear regressions with log-transformed pollen loads (Table 2) indicate that there is no detectable effect of *D. nelsonii* pollen on

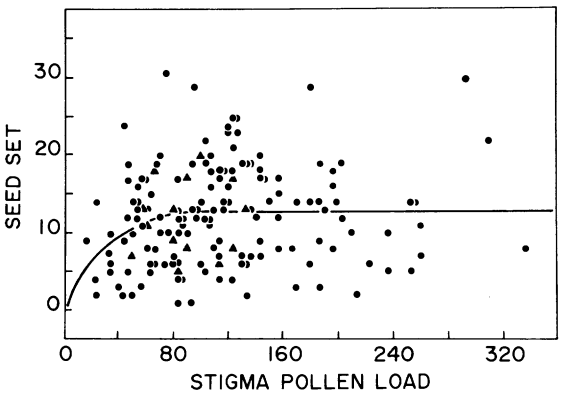

Fig. 1. Dose-response relationship between seed set and pollen load, from the 172 *I. aggregata* flowers receiving pure conspecific pollen. Closed circles indicate single observations, triangles indicate double observations of the same value. The best-fit negative exponential relationship is shown (see Table 1).

TABLE 2. *Multiple regression of log-transformed pollen load on seed set in the 454 flowers receiving mixed conspecific and heterospecific pollen. The regression model is y = alogx + blogz + c, where x = number of* I. aggregata *pollen grains, y = seed set, z = number of* D. nelsonii *grains, and a and b = partial regression coefficients. The table includes tests of the null hypothesis that regression parameters equal zero*

I. REGRESSION PARAMETERS

Variable	df	Parameter estimate	SE	*t*	*P*
a	1	6.7	1.1	6.1	0.0001
b	1	−0.01	0.06	0.1	0.9
c	1	−1.2	2.0	0.6	0.5

II. ANOVA TABLE

Source of variation	df	SS	MS	*F*	*P*
Model (r^2 = 0.09)	2	1,311.2	655.6	22.9	0.0001
Error	451	12,890.5	28.6		

I. aggregata seed set in flowers receiving pollen of both species. However, heterospecific pollen need not have a log-linear effect on seed set. For example, it could be that deleterious effects occur only when the stigma load of conspecific pollen falls below some threshold number. To examine this possibility we used factorial ANOVA with the following class variables: *I. aggregata* pollen grains (<50, 50–100, >100); *D. nelsonii* pollen grains (0, 1–100, >100); identity of recipient plant; and time of pollination (first, second, or last one-third of pollinations to a plant). The results (Table 3) show that *I. aggregata* seed set was significantly influenced by all variables except *D. nelsonii* pollen grains and the interaction between *D. nelsonii* and *I. aggregata* pollen grains.

DISCUSSION—The decline in ovule numbers between early and late flowers on potted *I. aggregata* plants parallels that seen in nature. The change was greater than in nature, however, suggesting that confining plants to pots also played a role. ANOVA (Table 3) indicates a significant relationship between time of pollination and seed set in hand-pollinated plants; this was due to reduced seed set in later-developing flowers. Lower ovule numbers may account for seed set reduction, or both ovule and seed set declines may be due to a loss in vigor of potted plants over the course of the experiment, although we saw no evidence of this.

TABLE 1. *Summary of regressions of pollen load on seed set in the 172 flowers receiving pure conspecific pollen, with x = number of pollen grains and y = seed set*

Regression equation	Description	r^2
$y = 0.01x + 10.8$	linear	0.009
$y = 4.4(\log x) + 3.4$	log-linear	0.022
$y = 13.2[1 - \exp(-0.03x)]$	exponential	0.040

Ovule and seed counts indicate that flowers seldom matured all their ovules. Mean ovule number across plants was 28.1 at the beginning of the experiment, overall mean seed set was 11.3, and only occasionally did fruits produce more than 20 seeds. Seed sets of naturally pollinated plants around the RMBL are also in the range of 10–15 (Waser, 1978a; N. Waser and M. Price, unpubl.; A. Snow, unpubl.).

Very little of the seed set variation in our experiment was explained by conspecific pollen dosage (Fig. 1). This is not necessarily surprising: we know or can assume that individual flowers differed in multiple factors, other than pollen dosage, that determine seed set. These include ovule number, exact degree of stigma receptivity, viability of pollen received, and genetic congruity between pollen and pistil (Waser and Price, 1983). In addition, plants probably differed in overall vigor and thus in ability to allocate resources to seed development. Considerable variation in dose-response relationships has been observed previously with *I. aggregata* in the greenhouse and field (A. Snow, pers. comm.; D. Paton, pers. comm.; N. Waser, unpubl.) and in several other species as well (Snow, 1982; McDade and Davidar, 1984; D. Campbell, unpubl.; N. Waser, unpubl.). A notable exception is the tight dose-response relationship obtained by Silander and Primack (1978) with *Oenothera fruticosa*. Their plants were kept in a growth chamber under strictly controlled conditions, suggesting

TABLE 3. *Factorial ANOVA of* I. aggregata *seed sets*

Source of variation	df	SS	MS	*F*	*P*
Model ($r^2 = 0.41$)	28	5,840.0	208.6	10.6	0.0001
I. aggregata grains	2	756.1	378.1	19.2	0.0001
D. nelsonii grains	2	8.3	4.2	0.2	0.8
Plant identity	18	4,015.9	23.1	11.3	0.0001
Time of pollination	2	653.1	326.6	16.6	0.0001
D. nelsonii grains × *I. aggregata* grains	4	44.4	11.1	0.6	0.7
Error	425	8,361.0	19.3		

again that variation in environmental factors may contribute to seed set variation.

We found no detectable effect of *D. nelsonii* pollen loads on *I. aggregata* seed set. A similar lack of relationship was observed by Campbell and Motten (1985) with a forest herb, and by S. Kinsman (1984, pers. comm.) with several tropical hummingbird-pollinated plants. Campbell and Motten applied heterospecific pollen to stigmas first, followed immediately by conspecific pollen. All these results contrast with those of Sukhada and Jayachandra (1980) and Thomson, Andews and Plowright (1981), the only other studies we know of that directly consider how heterospecific pollen on a stigma affects reproduction. Both of the latter studies reported strong deleterious effects of heterospecific pollen. However, both used heterospecific pollen known or thought to be allelopathic and recipient species unlikely to encounter this heterospecific pollen under undisturbed natural conditions. These features lead us and others (S. Kinsman, pers. comm.) to speculate that competition for pollination involving passive mechanical clogging of the stigma may be rare, especially if potential competitors have evolved in each other's presence. It seems reasonable that size of the receptive stigma surface would usually change rapidly in response to selection imposed by clogging, and that surfaces large enough to accept heterospecific loads without deleterious effect would result. The same might hold true for size of the stylar transmitting tissue, if heterospecific pollen germinates (see Martin, 1970) and its tubes interfere with conspecific ones. Many plant species might thereby be buffered not only against species they are normally sympatric with, but also against heterospecific pollen in general, unless allelopathic.

What may account for the approximately 30% seed set reductions seen in Waser's (1978a) competition experiments with *D. nelsonii* and *I. aggregata*? We can think of several plausible mechanisms other than passive stigma clogging or active interference by heterospecific pollen.

First, *I. aggregata* seed set reductions in the original experiments may have been caused by effective pollen limitation due to loss of conspecific pollen on *D. nelsonii* flower parts. Such a pollen wastage mechanism has been detailed for the forest herb *Stellaria pubera* (Campbell, 1985; Campbell and Motten, 1985). Other interactions might also reduce conspecific loads reaching *I. aggregata* stigmas and could also be classified as pollen wastage. For example, heterospecific pollen on a pollinator might reduce amounts of conspecific pollen picked up or might layer over conspecific pollen with only the top layer on the pollinator being deposited on a stigma.

Second, seed set reductions might have involved pollen layering in a different way than just described or may have involved the time course of pollen arrival at stigmas. Waser's (1978a) experiments involved potted plant arrays exposed to natural pollination and hand-pollinations that mimicked hummingbird visitation. In such cases it is possible that pollen from successive flowers is picked up, transferred, and deposited in layers (Lertzman, 1981; Price and Waser, 1982). If so, conspecific pollen often might not hydrate and germinate, or tubes might fail to reach the stigma surface, due to underlying layers of heterospecific pollen. Likewise, if each *I. aggregata* flower receives multiple visits (as several lines of evidence indicate; Waser, unpubl.), heterospecific pollen often will reach a stigma well before conspecific pollen. This might allow time for potentially deleterious structural changes—such as callose deposition (Dickinson and Lewis, 1973; Dumas and Knox, 1983)—to occur in stigma or stylar tissue. In our hand-pollinations, no such effects were possible, since we mixed conspecific and heterospecific pollens thoroughly and applied them to stigmas simultaneously.

Other studies have suggested that the mechanism of competition for pollination by which *D. nelsonii* influences *I. aggregata* involves interspecific pollen transfer, while the mechanism in the opposite direction involves pol-

linator preference (Waser, 1978a and unpubl.; R. Mitchell, unpubl.). Our experiment indicates that heterospecific pollen on stigmas is not responsible for the competitive effect on *I. aggregata*—at least when such pollen reaches stigmas well mixed with conspecific pollen. We can easily imagine, however, that seed set reductions in *I. aggregata* are caused by other effects of interspecific pollen transfer by hummingbirds. Further experiments are in progress to explore these possibilities.

ADDENDUM—A survey during the 1985 period of natural flowering overlap between the two species indicates again that mixed stigma loads are common. Of 63 *I. aggregata* stigmas examined, 29 (46%) contained *D. nelsonii* pollen. Loads of conspecific pollen ranged from 4–196 grains ($\bar{x}$ = 89.0, SD = 75.7), and those of heterospecific pollen from 1–431 grains ($\bar{x}$ = 61.2, SD = 109.3). We also discovered with fluorescence microscopy that *D. nelsonii* pollen tends to germinate on *I. aggregata* stigmas ($\bar{x}$ = 88% germination, SD = 12%, N = 20 stigmas) and that tubes often grow at least to the base of stigma lobes. This finding bolsters a speculation made in the Discussion section, that heterospecific pollen might have a detrimental effect if it precedes conspecific pollen on the stigma. There is now preliminary evidence (N. Waser and M. Fugate, unpubl.), in fact, that germinating *D. nelsonii* pollen induces stigma lobes to close together, thus reducing receptivity to later-arriving *I. aggregata* pollen.

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