

COSTS OF SELF-POLLINATION IN A SELF-INCOMPATIBLE PLANT, *POLEMONIUM VISCOSUM*¹

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ABSTRACT

Plants of *Polemonium viscosum* are strongly self-incompatible, yet have floral features that enforce spatial and temporal separation of pollen and stigma presentation. In this study, we address the widely held view that such barriers to selfing are maintained because they reduce interference by self pollen in performance of outcross grains. In the first year of the study we found that 24 hr precedence of self pollen on the stigma (24S), reduced outcross pollen germination by 32%, and seed set by 40% in flowers of *P. viscosum*. In the second year significant negative effects of self pollen on compatible pollen germination were seen when self pollen was applied four hours before (4S) or immediately prior (S×) to outcross pollen. Surprisingly, however, in the second year of study, seed set was not reduced with as much as 50% inhibition of compatible pollen germination. Results suggest that by interfering with normal functioning of outcross grains, incompatible self pollen can increase pollen limitation in *P. viscosum*. Reduction in seed production as a result of this phenomenon should select for displacement of pollen presentation from stigma receptivity.

SEPARATION of male and female functions in self-incompatible flowering plants is thought to improve the efficacy of pollen dispersal (e.g., Dulberger, 1981; Lloyd and Yates, 1982; Webb and Lloyd, 1986; Lloyd and Webb, 1986) and ovule fertilization (Bawa and Opler, 1975; Zapata and Arroyo, 1978; Wyatt, 1983; Lloyd and Webb, 1986; Webb and Lloyd, 1986). Such reasoning implies that in the absence of gender separation, either male or female function will suffer. Surprisingly few studies have attempted to assess whether interference between sexual functions exacts such costs. While it is clear that deposition of pollen on stigmas of incompatible flowers will decrease the number of grains available for effective dispersal, the results of studies testing for negative consequences of self-pollination on seed production by outcross pollen have not been convincing (see Shore and Barrett, 1984, for review). Only in one case has a large reduction in the number

of outcross pollen tubes resulted from the presence of self pollen on the stigma and there the effect was transient (Ockendon and Currah, 1977). In a variety of other systems self pollen had little or no demonstrable effect on germination, tube growth, or fertilization success of outcross pollen grains (Shore and Barrett, 1984). Here we experimentally test for potential negative effects of self pollen receipt at these three stages of sexual reproduction in the entomophilous alpine plant, *Polemonium viscosum* (Polemoniaceae).

Polemonium viscosum is an herbaceous perennial that is widely distributed above timberline in the Rocky Mountains of western North America. Plants depend on insect visitors for pollination, and in populations that we have studied seed set is strongly limited by pollinator activity (Galen, 1985). Individuals are self-incompatible with self-pollination reducing seed set by 80% relative to outcrossing (Galen and Kevan, 1980). Despite this physiological barrier to selfing, herkogamy, the spatial separation of sex organs, and dichogamy, the temporal separation of male from female function, are widespread in the species. Anthers are located about 2.6 mm below the stigma lobes and dehisce about one day before the clasped stigma lobes recurve and expose their receptive surfaces (Galen and Kevan, 1980). The objective of the present study was to obtain insights into the functional significance of dichogamy and herkogamy by investigating consequences of self pollen receipt for female reproductive success.

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TABLE 1. Analysis of variance in the number of pollen grains adhering on the stigma per pollination, pollen grains germinating, and seeds per flower after self pollen precedence (24S) vs. outcross pollination control (×) treatments. Randomized block ANOVAs were performed. Values for pollen germinating per stigma were square-root transformed before analysis to meet the assumptions of ANOVA

Dependent variable	Effect	df	Mean square	F	P<
Pollen adhering	Full model	15	45,952.4	1.52	ns
	Treatment	1	625.6	0.02	ns
	Plant	14	49,189.9		
	Error	14	29,944.3		
Pollen germinating	Full model	15	12.24	2.89	0.03
	Treatment	1	22.69	5.36	0.04
	Plant	14	11.69		
	Error	14	4.23		
Seeds per flower	Full model	15	29.81	2.07	0.04
	Treatment	1	66.15	4.60	0.04
	Plant	14	20.81		
	Error	44	14.39		

MATERIALS AND METHODS—Experiments reported in this paper were conducted during the summers of 1987 and 1988 on Pennsylvania Mountain (Park Co., CO, 30°15'N, 106°07'W), at 3,500–3,640 m elevation. Vegetation in this area resembles that surveyed by Komorakova (1979). Detailed descriptions of the populations of *Polemonium viscosum* studied at this site are given in Galen (1983, 1985).

Tests for negative effects of self pollen precedence—In June 1987, fifteen individuals of *P. viscosum* located at 3,500 m were selected at random, surrounded by fine mesh before flowering to exclude flying insects, and protected with tanglefoot from floral damage by ants. Two flowers on each plant were randomly assigned to each of the following treatments: self pollen precedence (24S) and outcross control (×). Flowers in both groups were marked individually and then emasculated before their anthers dehisced to prevent accidental selfing. At the onset of stigma receptivity, 24S flowers were self-pollinated by brushing a dehiscing anther from an untreated flower in the same inflorescence across the receptive stigma lobes. Twenty-four hr later, 24S flowers were outcrossed with pollen taken from a donor located at least 3 m away. Control flowers were not pollinated on the first day of receptivity. Rather, we waited 24 hr, then cross-pollinated each, as above.

At the end of anthesis, stigmas were excised from one flower per treatment on each plant, mounted individually on glass slides, and stained with Alexander's solution for pollen germination counts (Alexander, 1969; Galen and Newport, 1988). Staining in this manner differentiates empty and presumably germinated grains from full (ungerminated) ones.

Since mounting procedures can dislodge pollen from the stigma surface, all grains under the coverslip were counted. Counts were made under the light microscope at 250×. We scored total pollen delivered and the number of germinated grains. Screening was removed from plants after all flowers had wilted. Six to eight weeks later, fruit capsules were harvested from the four treated flowers per plant and the seeds in each capsule were counted.

To correct for the fact that 24S flowers were pollinated twice and C flowers only pollinated once, values for total pollen delivered to stigmas of selfed flowers were divided by two before testing the null hypothesis of equal pollen adhesion. Counts of germinated pollen grains per stigma were square root transformed before statistical analysis to correct for heteroscedacity. All statistical analyses were performed using the Statistical Analysis System (SAS), General Linear Models (GLM) package (SAS Institute, 1982). Differences in average pollen adhesion and germination between 24S and C groups were tested for significance by randomized block ANOVA without replication (plant = random; treatment = fixed effect). We pooled flowers left intact with those from which stigmas were excised for analysis of treatment effects on seed set, since stigma removal did not reduce seed production ($F_{1,58} = 0.08$, $P \gg 0.05$). Differences in mean seed set between treatment groups were tested by randomized block ANOVA with replication. For seed set, the treatment effect was tested over the pooled error mean square, because the plant by treatment interaction was not significant ($F_{1,14} = 1.54$, $P \gg 0.05$; Zar, 1984).

Tests for effects of the time interval between self and outcross pollination on performance of

outcross grains—In 1988, another 15 individuals of *P. viscosum* located at 3,640 m elevation were randomly selected, enclosed as above to exclude insects, and subjected to experimental pollinations to determine how time delays between arrival of self vs. outcross pollen affect self pollen interference. One flower on each plant was assigned to each of the following five treatment groups in a randomized block design without replication: complete outcrossing (\times), complete selfing (S), 4-hr self pollen precedence (4S), selfing followed immediately by outcrossing ($S\times$), and outcrossing followed immediately by selfing ($\times S$). Pollination treatments were randomized with respect to the sequence in which flowers opened, and carried out as described above. Styles were removed from wilted flowers in all treatment groups and preserved in 3:1 (ethanol:acetic acid) for counts of pollen tubes penetrating the stigma lobes. Pollen tubes were observed with fluorescence microscopy after staining the styles with aniline blue (Ramming, Hinrichs, and Richardson, 1973). After flowers wilted, mesh enclosures were removed. Six to eight weeks later, ripened fruits were collected for seed counts. We analyzed treatment effects on pollen tube density and seed set using ANOVA. Differences between treatments were tested for significance at the 95% confidence level with Duncan's multiple comparisons test.

RESULTS—Negative effects of self pollen precedence—The presence of self pollen on the stigma had no apparent effect on the deposition of subsequently applied outcross pollen ($F_{1,14} = 0.02$, $P \gg 0.05$; Table 1). We obtained comparable estimates for the number of pollen grains delivered per pollination in 24S and \times treatment groups (Fig. 1). However, significantly fewer pollen grains germinated in flowers that had received self pollen than in controls ($F_{1,14} = 5.36$, $P < 0.04$; Table 1). Self pollen precedence suppressed pollen germination by 32% on average (Fig. 1). Interference of self pollen during germination of outcross grains was associated with a significant reduction in seed set ($F_{1,44} = 4.60$, $P < 0.04$; Table 1). Flowers selfed 24 hours before outcrossing set an average of 40% fewer seeds than those outcrossed only (Fig. 1). In other words, self pollen interference exacted a significant cost in seed production of 24S flowers.

Effects of time interval between self and outcross pollinations on performance of outcross grains—In 1988, we found significant variation in the mean number of pollen tubes per stigma among groups receiving different schedules of self and outcross pollen ($F_{4,41} = 29.44$,

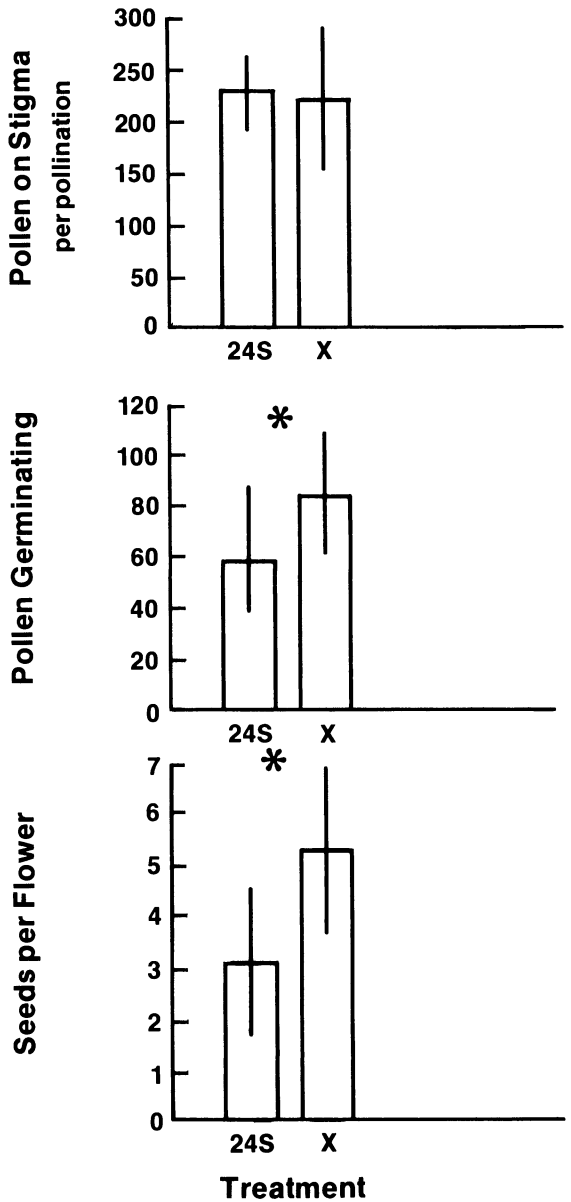


Fig. 1. From top to bottom: pollen deposited on the stigma per hand-pollination, pollen grains germinating, and seed set per flower after pure outcross pollination (\times) and 24 hr self pollen precedence (24S). Bar height indicates mean value; brackets show 95% confidence interval. Asterisks (*) denote significant differences between treatments at the 95% confidence level.

$P < 0.0001$; Table 2). Significantly fewer tubes penetrated the stigma lobes after selfing than following either pure outcrossing or pollination with mixtures of self and outcross pollen (Fig. 2). Selfing followed by outcrossing reduced tube numbers by more than 50% relative to outcross controls, whether precedence was immediate ($S\times$) or delayed (4S) ($P < 0.05$ in both cases;

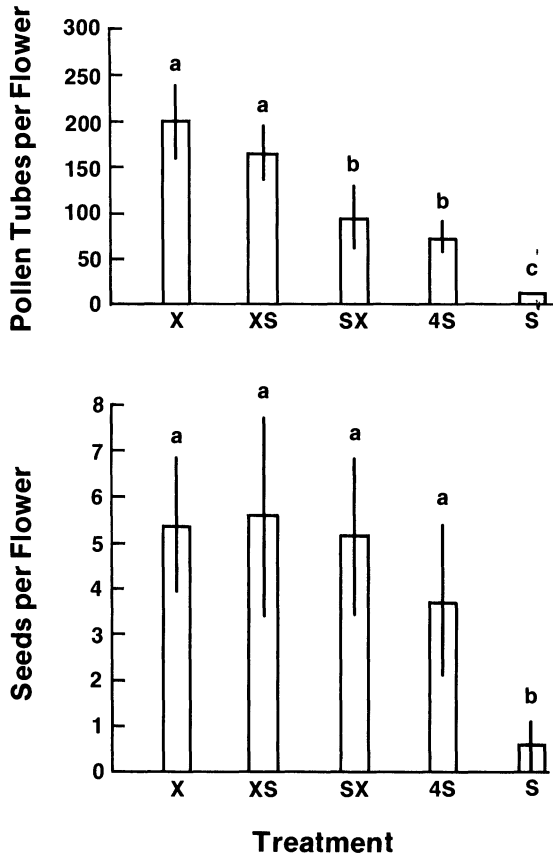


Fig. 2. Pollen tubes penetrating the stigma (top) and seed set per flower (bottom) after pure outcross pollination (\times), outcrossing followed immediately by selfing (\times S), self pollination followed immediately by outcrossing (S \times), 4 hr self pollen precedence (4S), and pure self pollination (S). Bar height indicates mean value; brackets show 95% confidence interval. Means of groups denoted by unique superscripts differ significantly at 95% confidence level according to Duncan's multiple comparisons test.

Fig. 2). Pure outcrossing (\times) and outcrossing followed immediately by selfing (\times S) achieved the highest tube densities.

In contrast to effects on pollen tube number, differences in seed set per flower among treatments in 1988 were less pronounced. Although pollination treatment explained a significant amount of the variation in mean seed set ($F_{4,51} = 7.73$, $P < 0.0001$), pure selfing was the only treatment to reduce seed set significantly (Fig. 2). Pure selfing reduced seed set by 85–90% compared to other pollination regimes. Equivalent seed set followed mixed vs. pure cross pollination, suggesting that when outcross pollen deposition equals or exceeds the levels applied in our 1988 experimental regimes, then self pollen precedence will not reduce female reproductive success in *P. viscosum*.

DISCUSSION—Explanations for the separation of male from female function in obligately outcrossing plants have often referred to potential costs of self-pollination to pollen dispersal and receipt (e.g., Dulberger, 1982; Lloyd and Yates, 1982; Webb, 1981; Webb and Lloyd, 1986). While the deposition of pollen on incompatible stigmas by definition reduces the number of grains available for effective matings, negative effects of self pollination on female function have been largely a matter of conjecture (for a review, see Shore and Barrett, 1984). In this study, we tested for such costs directly through experiment. Our results show that in self-incompatible *P. viscosum*, self pollen deposition prior to outcrossing reduces compatible pollen germination and hence intensifies pollen limitation of seed set. In the extreme, this phenomenon can lead to significant reproductive costs.

Thomson (1989) showed that in *Erythronium*, pollen germination proceeds from grains in contact with the stigma surface to more peripheral clumps of pollen. Essentially, as the inner grains germinate, they “melt” into the stigma surface and allow stigmatic fluids to reach outer grains. In *Polemonium viscosum*, as in other members of the Polemoniaceae (Zimmerman, personal communication; Pittman and Levin, 1986), most self pollen grains fail to germinate when deposited on stigmas. Thus, there is a correspondence between the stage of self pollen failure and that of self pollen interference with the normal functioning of outcross grains. If, as Thomson suggests, germination of outer pollen layers depends on that of grains closer to the stigma surface, then in *P. viscosum* incompatible self pollen may form an unhydrated layer that limits the access of later arriving compatible grains to a suitable medium for hydration and germination. Self pollen does not appear to affect pollen deposition, here or elsewhere (Ockendon and Currah, 1977; Shore and Barrett, 1984).

Under experimental conditions in 1987, early arrival of self pollen had a strong negative effect on the ability of outcross pollen grains to sire seeds in flowers of *P. viscosum*. Twenty-four hr self pollen precedence reduced seed set by 40% on average relative to that of outcross controls. However, in 1988 self pollen precedence had little effect on seed set despite comparably large decreases in compatible pollen germination. Possibly, the decreased time interval between self and outcross pollinations (24 hr in 1987 vs. 4 hr in 1988) could account for the failure to find a significant reduction in seed set in 1988. If so, however, one would expect to see a similar discrepancy between years in the effects of selfing on pollen ger-

TABLE 2. Analysis of variance in the number of pollen tubes penetrating the stigma lobes and seeds set per flower after different schedules of self and outcross pollen deposition. Randomized block ANOVAs were performed

Dependent variable	Effect	df	Mean square	F	P<
Pollen tubes per flower	Full model	18	18,958.33	7.78	0.0001
	Treatment	4	71,714.25	29.44	0.0001
	Plant	14	2,351.50		
	Error	41	2,436.16		
Seeds per flower	Full model	18	23.72	3.11	0.0005
	Treatment	4	58.94	7.73	0.0001
	Plant	14	11.82		
	Error	51	7.63		

mination. Alternatively, comparing pollen germination of outcrossed controls between years indicates that much more outcross pollen germinated on stigmas of all treated flowers in 1988 than in 1987 (200 vs. 85 grains germinating per stigma, respectively). These data suggest that either climatic conditions were more favorable for pollen germination or larger loads of outcross pollen were deposited during the second year of the study. Either factor could explain how, with as much as a 50% decrease in pollen germination on stigmas of $S \times$ flowers, seed set was not pollen tube-limited.

Two conditions will dictate whether stigma clogging enforces reproductive costs in nature: the ratio of self to outcross pollen that flowers receive and the timing of self vs. outcross pollen delivery. Earlier studies on Pennsylvania Mountain have shown that in spite of protandry and herkogamy the ratio of self to outcross pollen receipt in natural populations of *P. viscosum* approaches or exceeds that obtained in our experiments. Flowers of *P. viscosum* obtain an average of 43 outcross grains each and over five times as much self pollen during their lifetimes (Galen and Newport, 1988; Galen, unpublished data). Whether self pollen arrives before outcross pollen under natural conditions is more difficult to assess. Findings reported in this study support the view that in *P. viscosum*, plants having floral features which prevent the early arrival of self pollen will enjoy higher fecundity than individuals lacking such barriers. If so, features separating pollen dispersal from receptivity should be maintained.

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