Oecologia (1989) 81:120–123

Oecologia

Interspecific pollen transfer as a mechanism of competition: consequences of foreign pollen contamination for seed set in the alpine wildflower, *Polemonium viscosum*

Candace Galen and Teresa Gregory
Department of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403, USA

Summary. Earlier studies showed that under natural conditions foreign pollen receipt is correlated with loss of seed set in populations of *P. viscosum*. Here, we report on experimental pollinations using foreign pollen from two co-flowering species that share pollinators with *P. viscosum*. Experiments were designed to ascertain whether improper pollen transfer causes reduced seed set under controlled conditions and, if so, to determine the stage at which interference occurs. In flowers pre-treated by pollination with either *Mertensia* or *Castilleja* pollen, adherence of subsequently deposited conspecific pollen grains was unaffected. However, conspecific grains had significantly lower germination and ovule fertilization success in flowers receiving foreign pollen than in controls. These results suggest that pollinator infidelity in high alpine plant communities can reduce female reproductive success of *P. viscosum* by inhibiting the performance of conspecific pollen grains. Negative effects of *Mertensia* and *Castilleja* pollen were comparable despite large interspecific differences in pollen grain morphology. Accordingly, susceptibility to foreign pollen interference appears to be an intrinsic property of *P. viscosum* that is not dependent on the unique structural properties of pollen produced by competitive species.

Key words: Pollination – Competition – *Polemonium* – *Mertensia* – *Castilleja*

Competition for pollination among co-occurring species of flowering plants has received considerable attention from experimental (e.g., Waser 1978a; Thomson et al. 1981; Campbell 1985a; Feinsinger et al. 1988) and theoretical (Waser 1978b; Campbell 1985b) perspectives. For such competition to take place, sympatric species must share the services of a pollinator population whose visits are important to male or female components of reproductive success. In fact, pollinator sharing of this kind characterizes a diversity of tropical (e.g., Armbruster and Herzig 1984; Feinsinger et al. 1986) and temperate (e.g., Levin and Anderson 1970; Waser 1978a, 1983; Campbell 1985a; Rathcke 1983, 1988) plant associations. Waser (1978b) explored the potential consequences of competition for pollination on female components of reproduction through simulation models. He concluded that, when the availability of compatible pollen, receptive stigma surface area, or pollinator visits limit ovule fertilization, then pollinator sharing should negatively affect reproductive success and, in the extreme, lead to competitive exclusion. Campbell (1985b) extended simulation studies to consider male function and showed that deposition of pollen on heterospecific stigmas should exact costs with respect to both the number of pollen grains effectively dispersed and the total number of recipients reached by a given donor. These studies underscore the premise that competition for pollination, regardless of its mechanism, may be a widespread phenomenon having important ecological consequences.

The evolutionary outcomes of such competitive interactions, however, are strongly mechanism-dependent (Rathcke 1983). Where pollinators prefer flowers of a given species over those of a neighboring one, selection should favor increases in attractiveness, phenological displacement, or specialization on alternative vectors by the less visited taxon. Conversely, if competitive effects are mediated by improper pollen transfer (sensu Rathcke 1983), then divergence in morphological traits affecting the placement of pollen on a vector's body may allow for coexistence in the face of pollinator sharing. In other words, knowledge of the mechanism as well as the magnitude of competition for pollination is necessary in order to identify plausible ecological and evolutionary responses of competing species. Yet, to the best of our knowledge, both attributes have been considered in only a few systems (Waser 1978a; Waser and Fugate 1986; Campbell 1985a; Campbell and Motten 1985; Feinsinger et al. 1986). The main purpose of the present study was to test experimentally whether improper pollen transfer can account for an observed negative relationship between pollinator infidelity and seed set in natural populations of the alpine sky pilot, *Polemonium viscosum* (Galen and Newport 1988). A second objective was to ascertain whether differences in pollen morphology between two potential competitors of *P. viscosum* influence the magnitude of deleterious effects on female reproductive success.

Study system. Plants of *P. viscosum* are herbaceous perennials common in Rocky Mountain tundra vegetation of western North America (Davidson 1950). Floral traits vary geographically, ecotypically, and within populations of this species. At high elevations in alpine tundra, most plants have broadly-flared, sweet-scented flowers adapted to bumble bee-mediated pollination (Galen et al. 1987; Galen and

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Offprint requests to: C. Galen

Newport 1987). On Pennsylvania Mountain (Park Co., CO, 30°15′N, 106°07′W), where we have studied the importance of animal pollinators to reproduction in *P. viscosum* over several years, bumble bee visits account for more than 90% of the seeds set by individuals in high tundra populations (Galen 1983).

Plants of *P. viscosum* typically occur in association with other bumble bee-pollinated species including *Mertensia viridis, Castilleja occidentalis, Trifolium parryi* and *T. nanum, Silene acaulis*, and *Oxytropis sericea* (Macior 1974). All of these species co-flower with *P. viscosum* to some extent. However, we chose *M. viridis* and *C. occidentalis* for experiments reported in this study for two reasons. First, competition for pollinators is probable in this system. Flowers of both neighboring taxa are heavily visited by queen bumble bees of the species, *Bombus kirbyellus*. These are the most important bee pollinators of *P. viscosum* at our study site and elsewhere in Colorado, accounting for over 93% of bumble bee visitation (Macior 1974). On Pennsylvania Mountain, queens of *B. kirbyellus* forage indiscriminately within mixed species stands of *P. viscosum, M. viridis*, and *C. occidentalis*, switching among species on 47% of foraging trips (Galen and Lubinski, unpubl.). Observations of pollen loads received by flowers of *P. viscosum* in mixed species stands support the inference of extensive pollinator infidelity. On average, 23% of the grains deposited are of heterospecific origin and most loads contain some *Mertensia* pollen (Galen and Newport 1988).

Our second reason for focusing on *M. viridis* and *C. occidentalis* is that the two species diverge markedly in pollen morphology. Pollen grains of *Mertensia* are very small (about 10 μm in diameter) and bilaterally symmetrical. Those of *Castilleja* are larger (about 35 μm in diameter) and radially symmetrical. Consequently, comparing the effects of pollen transferred from these species on fertilization in *P. viscosum* could indicate whether the degree of interference varies with pollen morphology of the competitor. Pollen grains of both species are easily distinguished by shape (*Mertensia*) and pore arrangement (*Castilleja*) from those of *P. viscosum* (radially symmetrical and about 50 μm in diameter), facilitating observations on the adherence and germination of conspecific pollen in mixed stigmatic loads.

That pollinator infidelity may have adverse effects on seed set in *P. viscosum* is in line with the high degree of pollen limitation consistently observed in our study populations (Galen 1985; Galen et al. 1987). Moreover, a negative relationship between foreign pollen contamination and seed production characterizes naturally pollinated plants at this site (Galen and Newport 1988). However, because other uncontrolled factors may covary with heterospecific pollen receipt, these data do not definitively identify the mechanism or the potential magnitude of competitive losses to *P. viscosum* due to pollinator infidelity. To do so, an experimental approach is necessary.

Methods

Experimental design. Experiments described below were performed on the east-facing slope of Pennsylvania Mountain at an elevation of 3640 m during June–August, 1987. Nineteen plants of *Polemonium viscosum* were chosen at random and surrounded in bud by fine mesh screening to exclude animal visitors. Six flowers per plant were pollinated. Two of these were assigned to each of the following treatments in a randomized block design with replication: heterospecific pollination with *C. occidentalis* pollen (HC), heterospecific pollination with *M. viridis* pollen (HM), and control pollination (C). Plants of *P. viscosum* have from 8–15 flowers each. These open in a relatively synchronous manner over the course of 2–3 d. Accordingly, it was possible to assign flowers in the same stage of development to the three treatment groups. Flowers were uniquely marked according to treatment group with small dots of enamel paint applied to the persistent calyces. When all six flowers reached the pistillate (receptive) stage of anthesis, mesh screening was removed and heterospecific pollinations were performed. Heterospecific pollen was applied by brushing a full anther taken from a neighboring individual of the appropriate species across the receptive (unfurled) stigma lobes. In other studies we have used this pollination method for conspecific pollen transfer and in no case has handling the flowers per se, reduced subsequent seed production (e.g. Galen 1985). An average of 7300 ± 625 (s.d., $N = 5$) pollen grains of *M. viridis* and 481 ± 79 (s.d., $N = 5$) grains of *C. occidentalis* were deposited, respectively, on recipient stigmas of HM and HC flowers. Control flowers were marked but not otherwise manipulated. Plants were left unscreened for the remainder of the experiment. In this way, we could assess whether arrival of heterospecific pollen on the stigma interferes with subsequent fertilization of ovules under natural pollination regimes.

Performance of conspecific pollen. We scored conspecific pollen performance in treated flowers at two stages: number of pollen grains adhering to the stigma surface and number of grains germinating. We then addressed whether pollen failure at either stage has negative consequences for fecundity by counting the ovules fertilized (seed set) per flower in each treatment. To observe pollen adherence and germination, the stigma was removed from one wilted flower per plant in each treatment group, placed on a microscope slide, and stained with Alexander's solution (Alexander 1969; Galen and Newport 1988). Staining in this manner distinguishes emptied (germinated) pollen grains from full (ungerminated) ones. Pollen loads were scored under a light microscope at 250 × magnification. The style of the remaining flower in each group was left intact to control for potential effects of stigma removal on seed production. About six weeks after flowering, mature fruits were harvested from all treated flowers, dissected, and scored for seed set. Analysis of variance (ANOVA) was used to test for differences among treatments in pollen adherence, germination, and ovule fertilization. Counts for pollen grains germinating per stigma were square root transformed prior to the analysis to correct deviations from normality. Because stigma removal had no effect on subsequent seed production ($F_{1,72} = 0.29$, $P > 0.05$), we used seed counts from all treated flowers for analysis of treatment effects on ovule fertilization.

For analysis of treatment effects on pollen adherence and germination, a randomized block ANOVA without replication was used. Differences in conspecific pollen performance within flowers receiving foreign pollen versus those of the control group were tested for significance at the 95% confidence level using Tukey's method of multiple comparisons. To test the significance of differences in seed set among treatment groups we used a two-way ANOVA (treatment = fixed, plant = random; Zar (1984) Model III).

For treatment, the F statistic was calculated as the ratio of the treatment mean square over the pooled error mean square, because the interaction effect was not significant ($F_{1,36}=1.07$, $P \gg 0.05$; Zar 1984).

Results

Neither *Mertensia* or *Castilleja* pollen interfered with the delivery and adherence of conspecific pollen grains on stigmas of *P. viscosum* (Table 1). On average, conspecific stigmatic pollen loads in the control group contained 642 ± 189 (95% C.I.) grains, compared to load sizes of 434 ± 121 grains and 486 ± 144 grains in HM and HC treatments, respectively. Pollen delivery was highly variable within groups (e.g., CV = 61% for control flowers), diminishing the likelihood of detecting significant treatment effects.

Table 1. Analysis of variance to test for effects of foreign pollen interference on adherence, germination, and ovule fertilization by conspecific pollen in flowers of *Polemonium viscosum*

Dependent variable	Effect	df	SS	MS	F	$P<$
Pollen delivered	Treatment	2	445189	222595	2.69	ns
	Plant	18	2581660	143426	1.73	ns
	Error	36	2978233	83729		
Pollen germinated[a]	Treatment	2	166.73	83.35	3.34	0.05
	Plant	18	838.36	46.58	1.87	ns
	Error	37	923.26	24.95		
Ovules fertilized	Treatment	2	120.27	60.14	3.14	0.05
	Plant	18	1446.25	80.33	4.20	0.0001
	Error	91	1742.3	19.15		

[a] Square root-transformed prior to ANOVA

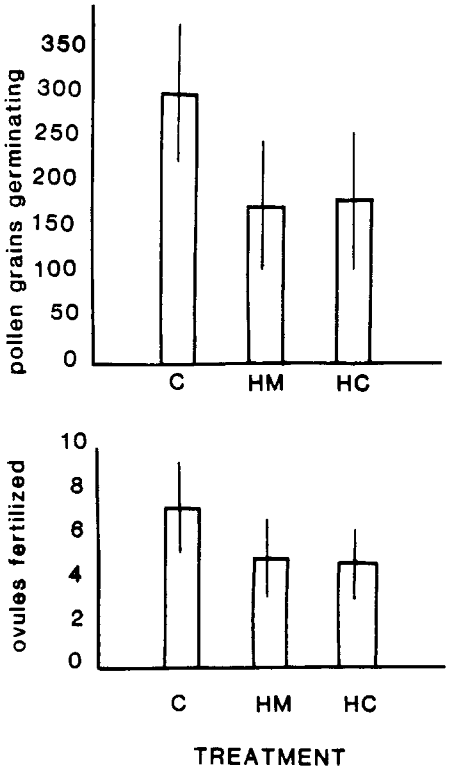

Fig. 1. Effects of interference due to *Mertensia* (HM) and *Castilleja* (HC) pollen precedence on germination (top) and ovule fertilization (bottom) of conspecific pollen in flowers of *Polemonium viscosum*. Bars show treatment means and brackets denote 95% confidence intervals. Both treatments differ significantly (Tukey's test, $P<0.05$) from the control group (C) in pollen germination and ovule fertilization

In contrast, both foreign pollen sources significantly suppressed the germination of conspecific grains (Fig. 1). Pollination treatment accounted for 43% of the variation in the number of pollen grains germinating ($F_{2,37}=3.34$, $P<0.05$; Table 1). *Mertensia* and *Castilleja* had comparable effects on the performance of *P. viscosum* pollen, reducing germination by 38% and 35%, respectively, relative to that in control flowers ($P<0.05$ in both cases; Fig. 1).

Seed set per flower was also reduced by foreign pollen interference (Fig. 1). Differences among treatment groups explained a modest, but significant, 4% of the variation in seed production ($F_{2,91}=3.14$, $P<0.05$; Table 1). Plant-to-plant differences were more important in this regard, accounting for 44% of the variance. Control flowers set 7.2 ± 2.1 (95% C.I.) seeds each, on average. Pollination by either *Mertensia* or *Castilleja* led to about a 30% reduction in seed set yielding 5.1 ± 1.6 seeds per flower and 4.9 ± 1.6 seeds per flower, respectively ($P<0.05$ in both cases; Fig. 1). Decreases in seed set parallel negative effects of foreign pollen on conspecific pollen germination and probably result from failure at that stage.

Discussion

Experiments reported in this study demonstrate that prior arrival of heterospecific pollen has the potential to reduce the amount of conspecific pollen germinating and the fertilization success of conspecific pollen grains in flowers of *P. viscosum*. Foreign pollen from *Mertensia* and *Castilleja* had comparable deleterious effects in spite of marked differences in shape, size and the numbers of grains applied. These findings indicate that susceptibility to foreign pollen interference is more likely to reflect the floral biology of the recipient species than structural features unique to the pollen of competitors. Waser and Fugate (1986) reached a similar conclusion concerning foreign pollen interference in *Delphinium nelsonii*. They found an inherent stigma closure response to foreign or conspecific pollen receipt that reduced the further arrival of conspecific grains.

Competitive effects of foreign pollen in *P. viscosum* do not significantly reduce pollen arrival but, instead, appear to reduce seed set by suppressing pollen germination. In other systems such findings have been indicative of chemical (allelopathic) inhibition (e.g., Sukada and Jayachandra 1980; Thomson et al. 1981). It is plausible that chemicals leaching onto the stigma surface from *Mertensia* or *Castilleja* pollen could interfere with the recognition of compatible grains. Alternatively, foreign pollen could physically block germination in outer clumps of conspecific grains, by impeding contact with stigma surface secretions. Thomson (1989) showed that in *Erythronium*, increasing distance of adhering pollen from the stigma surface delayed germination in purely conspecific loads. Evidently, outer clumps failed to germinate until inner grains were hydrated and began germination. *Mertensia* and *Castilleja* pollen grains never germinated on stigmas of treated flowers in our experiment. These ungerminated grains may form a barrier that prevents hydration of conspecific pollen by stigmatic fluids.

Amounts of foreign grains received by flowers in this study were 1–2 orders of magnitude greater than amounts arriving in nature (Galen and Newport 1988). Therefore, it is unlikely that the impact of competition from *Mertensia* or *Castilleja* under natural conditions is as large as that resulting from our experimental treatments. However, earlier observations support the view that pollinator infidelity in high alpine plant communities does reduce seed production in *P. viscosum* via improper pollen transfer (Galen and Newport 1988). The present study shows that correlations of pollen load impurity with lowered seed production have a causal basis and illuminates the stages at which competitive effects are mediated. In principle, selection to reduce competition for pollination could act on a myriad of floral traits in *P. viscosum* including flowering time, anther and stigma configuration, or floral attractants. However, because more than one neighboring species has potentially deleterious competitive effects in this system, selection for character divergence may be relatively ineffective.

Acknowledgements. We thank Hudson De Yoe and Barbara Steiner for help with laboratory work. Tia Lynn Ashman, Laura Galloway and John T. Rotenberry improved the manuscript with their helpful and insightful criticisms. Field facilities were provided by the University of Colorado at Colorado Springs. Computer access to statistical packages was made available at no cost by Bowling Green State University. This study was supported by NSF grant BSR-8604726 to C. Galen. T. Gregory's participation was funded through the NSF Research Experiences for Undergraduates program.

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Submitted February 14, 1989 / Accepted May 14, 1989