

Does stigma-anther separation prevent  
sexual interference in dwarf bluebells  
(*Mertensia fusiformis*)?

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## **ABSTRACT**

Innumerable floral traits have been ascribed adaptive significance via a variety of mechanisms. One such trait is herkogamy, the spatial separation of the stigma and anthers. In self-incompatible plants, herkogamy is thought to reduce sexual interference, defined as any situation in which one sex function directly decreases the success of the other sex function. I investigated herkogamy in the dwarf bluebell (*Mertensia fusiformis*), and failed to find evidence for the presence of sexual interference or the predicted functional significance of herkogamy.

## **INTRODUCTION**

A wide array of the morphological, phenological, and biochemical traits exhibited by the flowers of angiosperms have been ascribed adaptive significance, whether via pollinator attraction, non-pollinator deterrence, outcross promotion, inbreeding avoidance, or any of a variety of other proposed mechanisms (Barrett 2002). One such trait is herkogamy, the spatial separation of the anthers and stigmatic surface in hermaphroditic flowers, which has traditionally been explained as an adaptive feature serving to prevent self-fertilization (Webb & Lloyd 1986). Although this explanation is potentially adequate

for self-compatible plants, herkogamy is also commonly found in self-incompatible plants, for which a spatial mechanism of self-fertilization avoidance seems unnecessary (Webb & Lloyd 1986). This apparent redundancy could be the result of the sequential evolution of herkogamy for self-fertilization avoidance, followed by the evolution of self-incompatibility either for further self-fertilization avoidance or for other reasons. However, the abundance of self-incompatible herkogamous species suggests that another explanation is required.

Webb & Lloyd (1986) proposed that herkogamy may serve to prevent sexual interference, defined as any situation in which one sex function directly decreases the success of the other sex function. This might result from anthers blocking cross-pollen import, self-pollen crowding the stigmatic surface and preventing cross-pollination, self-pollen triggering ovule degradation before cross-pollination occurs, or any of a number of other mechanisms (Barrett 2002). Although the concept of sexual interference has gained favor and is now commonly invoked, empirical tests demonstrating its existence or validating the efficacy of herkogamy for its prevention are limited (Barrett 2002; for example, see Broyles & Wyatt 1993 and Waser & Price 1991). Here, I looked for the existence of sexual interference and investigated the functional significance of herkogamy for female reproductive success in a self-incompatible angiosperm. In particular, I asked whether herkogamy contributes to sexual interference avoidance in dwarf bluebells

(*Mertensia fusiformis*), an angiosperm that exhibits considerable variation in the spatial arrangement of its stigmas and anthers.

## **METHODS**

*Experimental System.* The dwarf bluebell (*M. fusiformis*) is an early-flowering, self-incompatible, subalpine annual that exhibits substantial within-population variation in the presence, degree, and orientation of herkogamy. The location of the stigmatic surface ranges from approximately one half anther-length (about 5 mm) below the bottom of the anthers (i.e., reverse herkogamy) to approximately one half anther-length above the top of the anthers (approach herkogamy; Webb & Lloyd 1986). The variation between these two extremes is essentially continuous, with no evidence for the presence of discrete morphs, as would be expected if *M. fusiformis* were heterostylous. Additionally, within-plant variation in the presence, degree, and orientation of herkogamy is negligible, and variation in style length does not appear to be associated with any other morphological characters, including filament length. Flowers each have exactly four ovules, except in the case of rare developmental abnormalities. *M. fusiformis* is most commonly visited by bumble bee queens (primarily *Bombus bifarius*) and solitary bees (primarily *Osmia* spp.).

*Research Sites.* The investigation of sexual interference was performed near Virginia Basin (38°58'17"N, 106°59'04"W, 3211 m elevation). The investigations of stigma clogging and seed production were performed near Gothic (38°57'18" N 106°59'04"W, 2920 m elevation).

*Statistical Analyses.* All data analyses were performed in JMP 8 (version 8, SAS Institute Inc., Cary, North Carolina). All variables analyzed herein met normality assumptions without transformation, as indicated by normal quantile plots.

*Seed Maturation & Style Length.* Plants were classified as: short-styled if the stigma was located below the bottom of the anthers; intermediate-styled if the stigma was located above the bottom of the anthers but below the top of the anthers; and long-styled if the stigma was exerted beyond the top of the anthers. Short- and long-styled plants were considered herkogamous; intermediate-styled plants were considered aherkogamous. Ovules were scored as mature if seeds had reached full size and were hardening or had abscised and left scars indicative of maturation. Sufficient time was allowed to pass before ovules were scored such that all ovules capable of developing into mature seeds had done so; this maturation period typically lasted three weeks following flowering. Seed maturation was interpreted as indication of successful cross-pollen receipt, ovule fertilization, and embryo development.

*Sexual Interference.* To assess whether male function can interfere with female function and whether herkogamy can have any effect on this interference, I performed emasculations on open-pollinated plants and measured seed maturation. One hundred plants were identified nonrandomly at the Virginia Basin site, with efforts made to ensure that all style lengths were well represented. All experimental flowers were identified and treated following corolla opening but preceding anther dehiscence. Within a plant, flowers that were in this stage on the same day were paired, and one flower from each pair was randomly selected to be emasculated. If a plant had an odd number of flowers in this stage on a particular day, one flower was nonrandomly chosen to be excluded, and the remainder were paired and treated; if a plant had only one flower in this stage on a particular day, no flowers were treated. Between one and four pairs of experimental flowers were selected per plant. Plants were left uncovered and allowed visitation by natural pollinators. Seed maturation and style length were measured as described previously. Seed maturation values from all emasculated flowers and from all unmanipulated flowers were averaged within each plant. Only plants for which all experimental flowers could be located and scored at the time of seed counts were included in the analysis; some flowers were lost to grazing and frost damage.

*Stigma Clogging.* To assess whether self-pollen deposited on the stigma can reduce seed set, I performed hand-pollinations on plants isolated from pollinators and measured seed maturation. Forty plants were selected in the Gothic site and were equipped with pollinator exclusion bags prior to flowering. Following the onset of flowering, two open flowers with undehisced anthers were identified per plant, and both flowers were emasculated. One of these two flowers was randomly selected to receive geitonogamous self-pollen (i.e., pollen from another flower on the same plant) approximately 24 hours after emasculation. Both flowers received cross-pollen from two donor plants approximately 48 hours after emasculation. Donor plants were located at least 6 meters away from the experimental plant to which their pollen was applied. Pollinator exclusion bags were removed one week after experimental treatments. Seed maturation was measured as described previously.

*Seed Production.* To assess whether the presence or orientation of herkogamy is related to seed production, I measured seed maturation from whole, unmanipulated plants. Four transects were selected through patches of *M. fusiformis* at the Gothic site, and the first 20 to 30 plants within 25 centimeters of each transect were identified. Ovule counts were calculated by quadrupling the number of fully developed flowers. Seed maturation and style length were measured as described previously.

## RESULTS

*Sexual Interference.* Seed maturation did not differ significantly between emasculated and unmanipulated flowers (paired two-tailed t-test,  $t=1.35$ , 30 d.f.,  $p=0.18$ ). Emasculated flowers matured an average of 1.22 seeds, while unmanipulated flowers matured an average of 1.63 seeds (Fig. 1). Style length was not a significant predictor of the difference in seed number between the two treatments (ANOVA,  $F_{2,28}=0.82$ ,  $p=0.45$ ).

*Stigma Clogging.* Seed maturation did not differ significantly between flowers that received only cross-pollen and flowers that received both self-pollen and cross-pollen (paired two-tailed t-test,  $t=1.51$ , 26 d.f.,  $p=0.14$ ). Buds that received only cross-pollen matured an average of 2.96 seeds, while buds that received both self-pollen and cross-pollen matured an average of 2.33 seeds (Fig. 2).

*Seed Production.* Proportions of ovules that had developed or were developing into mature seeds met equal variance assumptions without transformation, as indicated by Bartlett's test ( $p=0.27$ ). Style length was not a significant predictor of proportion seed maturation (Fig. 3; ANOVA,  $F_{2,84}=0.55$ ,  $p=0.58$ ).

## DISCUSSION

Sexual interference theory predicts that male and female sex functions impinge upon each other's success, and that removal of one function should benefit the other. However, I found that removal of anthers had no significant effect on seed set, indicating that male function does not interfere with female function in this system. Sexual interference theory furthermore predicts that herkogamy should reduce the effects of any such interference, but herkogamous plants did not develop significantly higher proportions of their ovules into mature seeds than aherkogamous plants did. These results imply that the naturally-occurring variation in the presence, degree, and orientation of herkogamy exhibited by *M. fusiformis* lacks consequences for female fitness.

It is yet possible that female function interferes with male function in this system; this possibility was not investigated here. This category of interference might occur via mechanisms such as pollen discounting, in which the stigma blocks pollen export and reduces outcross siring success. It is furthermore possible that herkogamy is capable of reducing any such interference. However, the sheer quantity of pollen typically produced and the small size of the stigma and style relative to the anthers make it far more plausible that male function would interfere with female function than vice versa. Consequently, the results found here suggest that sexual interference is not present in *M. fusiformis*, and that

the naturally occurring variation in herkogamy lacks adaptive significance and is neutral with regard to selection. If this is indeed the case, it may explain why the variation in style length has been maintained and has not converged on an optimum.

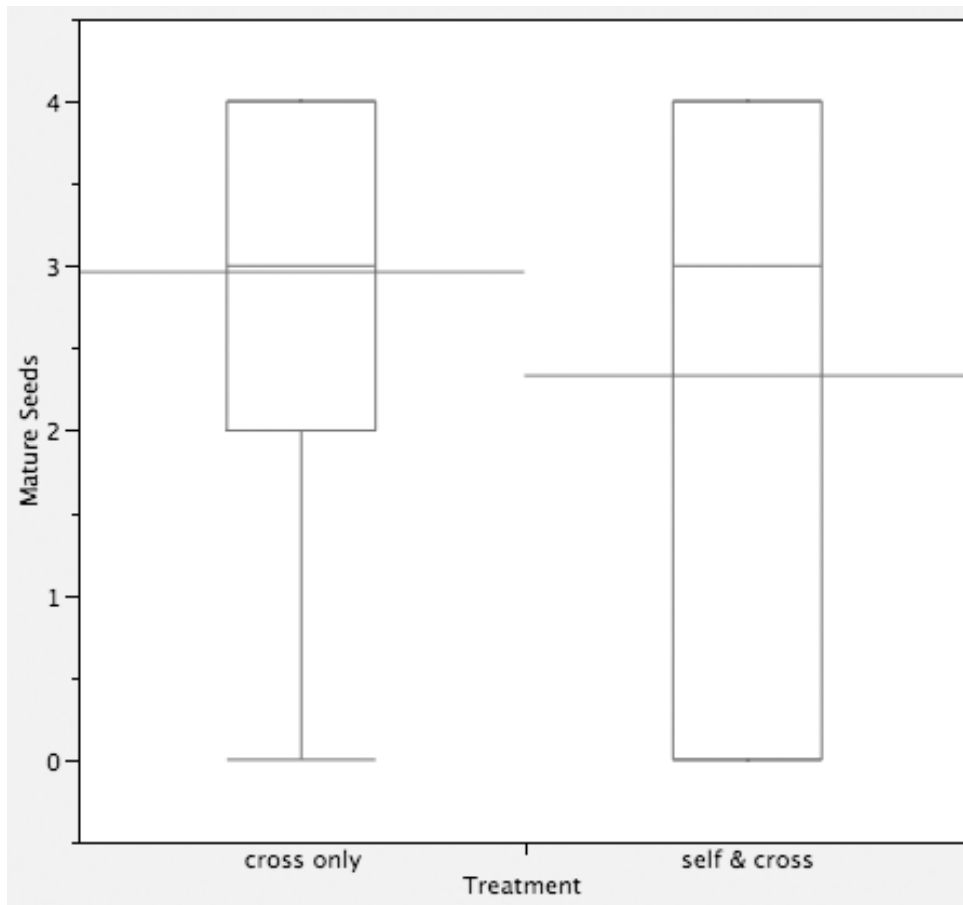
Although considerable time has passed since the original theoretical formulation of sexual interference in 1986, relatively few empirical demonstrations of its existence have been produced (Webb & Lloyd, Barrett 2002). Moreover, a considerable number of published studies have found little or no evidence for the existence of sexual interference (for example, see Barrett & Glover 1985, Kohn & Barrett 1992, and Shore & Barrett 1984). Rather than resulting from a dearth of investigations, this lack of empirical confirmation may bespeak the existence of a larger body of unpublished studies with negative results similar to those found here. Although the existence of sexual interference and the functional significance of herkogamy have been demonstrated in a limited number of systems (for example, see Bertin & Sullivan 1988, Broyles & Wyatt 1993, and Waser & Price 1991), sexual interference may be an inadequate explanation for the evolution and persistence of herkogamy in systems where sexual interference does not appear to be occurring. Consequently, invocation of sexual interference may warrant more caution than is currently employed, and alternative explanations should be given consideration. In light of the inadequacies discussed here, future theoretical and empirical work should consider

novel or overlooked explanations for the evolution and maintenance of spatially separated stigmas and anthers in hermaphroditic plants.

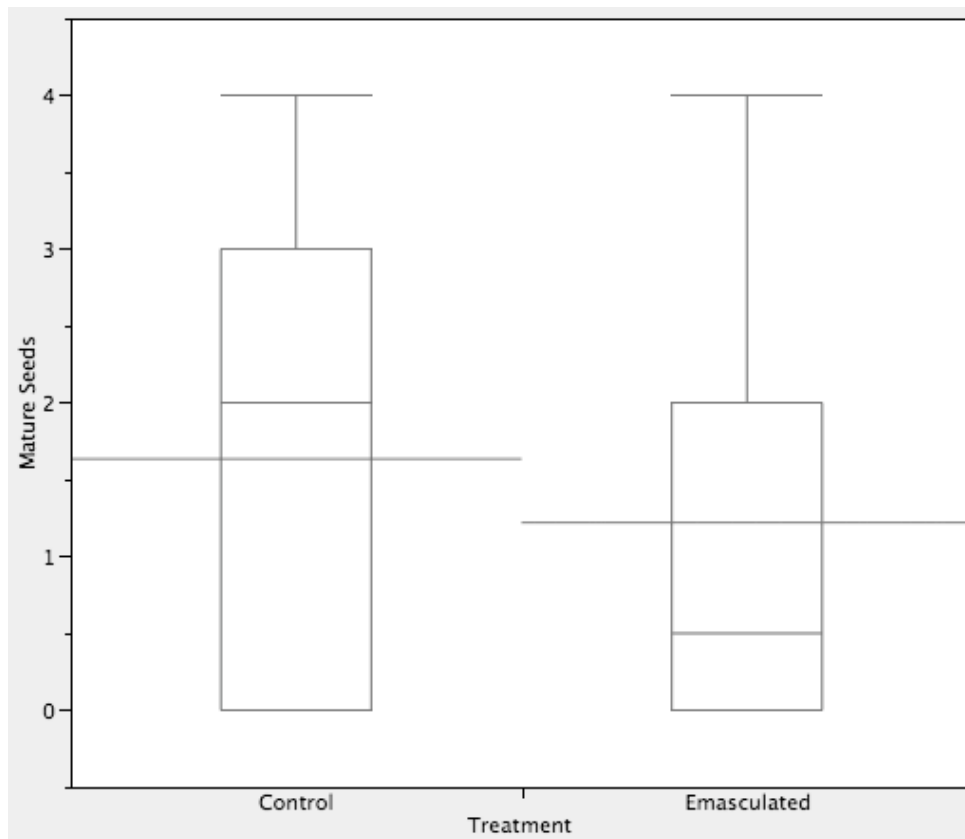
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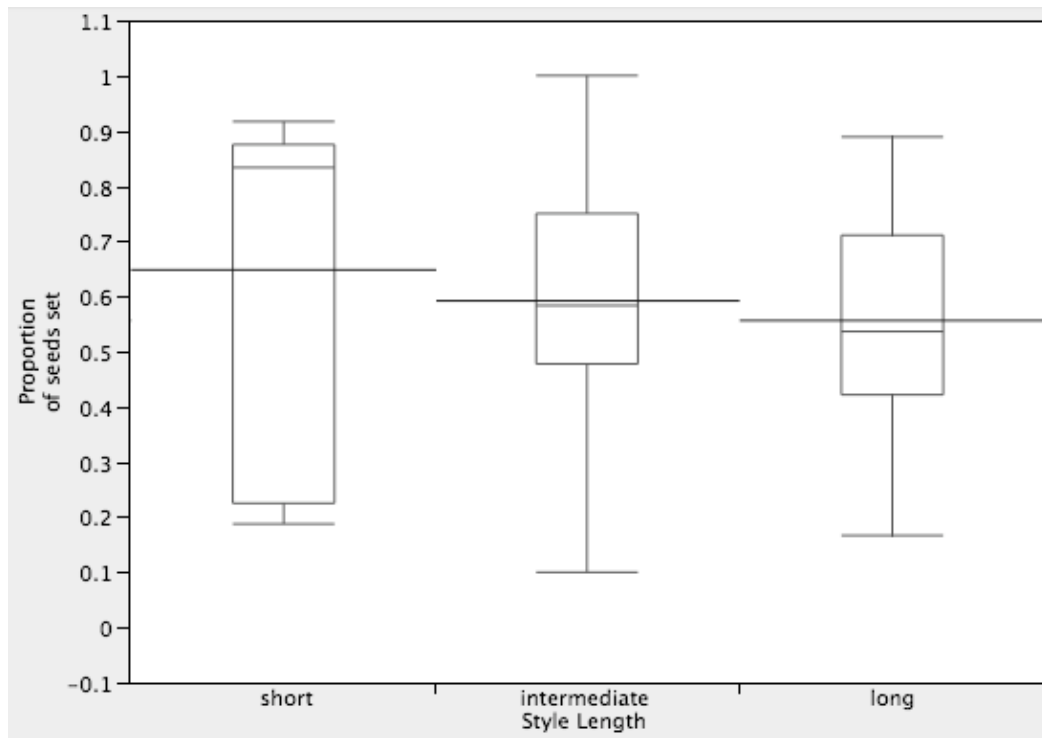
## FIGURES



**Figure 1.** Number of mature seeds produced by unmanipulated and emasculated flowers. Box-and-whisker plots indicate quantiles; extended lines indicate means.



**Figure 2.** Number of mature seeds produced by flowers hand-pollinated either with only cross-pollen or with self-pollen followed by cross-pollen. Box-and-whisker plots indicate quantiles; extended lines indicate means.



**Figure 3.** Proportion of seeds set by plants of different style lengths. Box-and-whisker plots indicate quantiles; extended lines indicate means.