Pollen limitation, abortion rates, and the evolution of selfing in *Clarkia unguiculata*

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Abstract

Within the genus *Clarkia*, individual species have evolved floral traits that enable self-fertilization (selfing), as opposed to pollinator-dependent mating between genetically distinct individuals (outcrossing). Insufficient pollen deposition, which may reduce individual seed production, is hypothesized to have contributed to the evolution of selfing. If seed set is limited by pollen deposition (i.e., pollen limited), then natural selection may favor the evolution of traits associated with selfing. Our experiment confirmed that pollen limitation occurs in the annual self-compatible wildflower *Clarkia unguiculata*, a predominantly outcrossing species that often occurs in sympatry with its selfing sister species *Clarkia exilis.* In Spring 2010, we selected 600 plants at three different sites in the southern Sierra Nevada. We subjected two flowers per plant to one of the following experimental treatments: (1) naturally pollinated or (2) hand-pollinated with supplemental pollen from neighboring plants. We compared seed production between fruits derived from flowers that were pollinated naturally and those that were pollen supplemented. We detected pollen limitation in natural populations of *Clarkia unguiculata* early in the flowering season, and to a lesser degree in the late season. Advantages of receiving additional pollen may be nullified in the late season when plants allocate limited resources to a fixed number of seeds. As anthropogenic factors may influence the interactions and evolution of plants and pollinators in the Sierra Nevada in the future, it is important to note that a plant's ability to evolve selfing mechanisms may be influenced by resource availability.

Introduction

Numerous hypotheses exist on why certain plant species have evolved floral traits that enable self-fertilization (**selfing**) rather than relying on the use of pollinating vectors for mating between genetically distinct individuals (**outcrossing**). While selfing allows for reproduction when pollinators are absent, potential risks of selfing include weak, inbred offspring and reduced genetic variability within a population.

Our experiment tested the hypothesis that selfing has evolved as a response to **pollen limitation**, which is a reduction in seed production due to poor pollinator service.

Our study species, *Clarkia unguiculata*, is an outcrossing plant found in the Sierra Nevada Mountains that is also self-compatible. *Clarkia unguiculata* is an annual plant that flowers from mid-April to May, later in the season than its sister species *Clarkia exilis* (which is a selfer). Though both occur in sympatry, the outcrosser possesses a large floral display and exhibits high degrees of herkogamy (spatial separation between the male and female sex organs of the flower) and protandry (difference in timing between the emergence of male and female sex organs), whereas the opposite is true in the selfer.

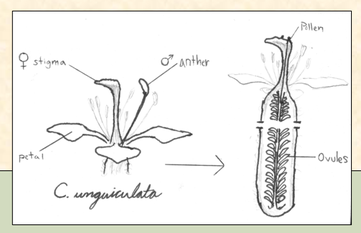

Clarkia unguiculata (outcrosser)


Clarkia exilis (selfer)

We tested for pollen limitation in *C. unguiculata* both during the onset of flowering (**early season**) and end of the flowering season (**late season**). We investigated whether pollen limitation early in the season may be the reason selfing has evolved in early flowering clarkia species and if harsher conditions in the late season may impede the development of floral traits associated with selfing.

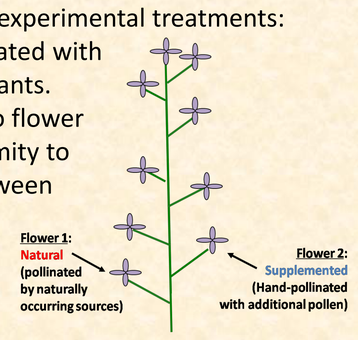
Floral Reproduction

When pollen comes into contact with a stigma (see figure below), it will germinate, releasing sperm to the ovules (analogous to the eggs of a human female). Each pollen grain has the ability to fertilize one ovule and a fertilized ovule becomes a seed. *Clarkia unguiculata* ovaries contain ~100 ovules on average. Under conditions where pollinators perform well, a higher seed set (number of full seeds / number of ovules prior to fertilization) is expected. Under favorable pollination conditions, the advantage of selfing would be nullified. However, if pollen limitation occurs we can expect to see a lower seed set than when pollen is widely available.

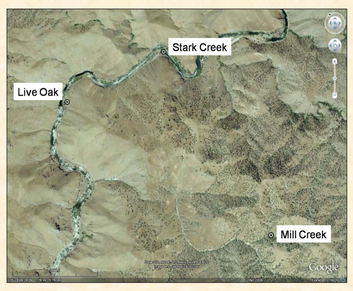


Methods

In order to quantify pollen limitation in *C. unguiculata* we subjected two flowers per plant to one of the following experimental treatments: (1) naturally pollinated or (2) hand-pollinated with supplemental pollen from neighboring plants. We alternated treatments with respect to flower position on the plant to control for proximity to light. We compared seed production between fruits derived from flowers that were pollinated naturally and those that were pollen supplemented.



During Spring of 2010, we performed this treatment at three different sites in the southern Sierra Nevada Mountains near Lake Isabella, California. At each site 200 *C. unguiculata* plants were chosen, 100 early in the season, and 100 late in the season. Approximately four weeks after pollination, fruits were glued to prevent seed dispersal and allowed to mature until we returned to harvest them. Thereafter I dissected them in a lab and quantified seed set.



*Note: The number of ovules prior to fertilization was calculating by adding the number of mature seeds to the number of unfertilized ovules and aborted seeds

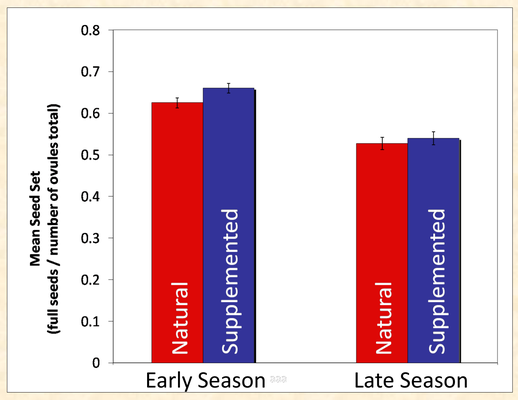
Analysis of Variance (ANOVA) was used to detect differences between the naturally pollinated fruits and supplemented fruits early and late in the season.

We also used ANOVA to detect differences in abortion rates between early and late flowering fruits. A plant may allocate its resources to a fixed number of seeds regardless of the number of ovules fertilized do to resource limitations. Hence, some seeds may be aborted and can be identified as such under a microscope. The abortion rate is calculated by dividing the number of aborted seeds by the total number of ovules prior to fertilization. We analyzed data from both early and late in the season in order to assess the possible influence of resource limitation on the abortion rates.

Results

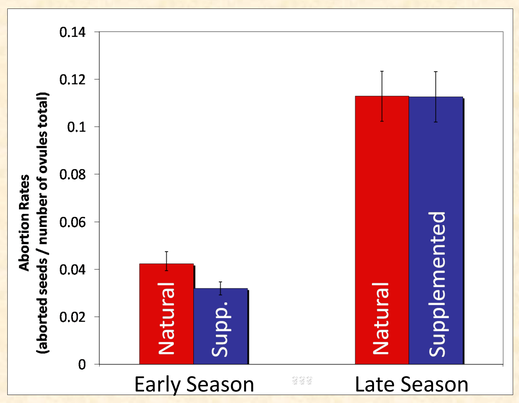
Pollen limitation occurs in *Clarkia unguiculata*

We detected pollen limitation in *C. unguiculata* (pollination treatment effect p=0.0473) in both early and late in the flowering season. Pollen limitation occurred at a lesser degree during the late season. The seed set was significantly higher in the supplemented fruits than the naturally pollinated. An analysis of the effect of site by ANOVA showed geographic location had no effect on the data set and the data was consistent throughout our sites.



Abortion rates are influenced by timing

Our analysis of the data showed the abortion rates to be significantly higher in the late season than the early season (p<0.0001). Abortion rates were nearly tripled in the late season for both treatment groups.



Discussion

After sampling at our locations and performing statistical analyses of the data, we confirmed that pollen limitation does occur in *Clarkia unguiculata.* While some of our samples fell victim to herbivory and/or fungal pathogens, 706 of the fruits were successfully dissected and analyzed at the Mazer Lab, located at University of California, Santa Barbara.

While pollen limitation was detected early and late in the flowering season, the degree to which it occurred was more pronounced in the early season than the late season. High abortion rates in the late season may have masked the degree to which pollen limitation occurred, since a plant may allocate its resources to a fixed number of seeds regardless of the amount of pollen it receives. This is consistent with the dryer and hotter conditions present in the late season.

The data is consistent with the fact that selfers have evolved to flower in the early flowering season and outcrossers in the later. Because pollen limitation occurs early in the season, the mechanism of selfing is advantageous. However in the late season the advantage of developing selfing mechanisms may be nullified if high abortion rates cause the plants yield a fixed number of seeds.

Habitat fragmentation and encroachment are known to disrupt plant-pollinator interactions, inevitably causing poor pollinator service to occur. While selfing may evolve as a mechanism to cope with these perturbations, the potential emergence of a drier climate in the Sierra Nevada may increase abortion rates and interfere with the ability of plant species to adapt to environmental change.

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Lake Isabella, CA

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