

Quantifying the effects of pollen load and heterospecific pollen ratio on the species
Delphinium barbeyi (Ranunculaceae)

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Abstract

To date there are few studies that have carefully studied the impact that varying pollen quantity and proportion of heterospecific pollen has on reproductive rate of a flowering plants. My project aimed to assess the relationship between pollen quantity and composition on the subalpine larkspur, *Delphinium barbeyi*, by conducting hand-pollination treatments that varied in pollen composition (proportion of heterospecific pollen) and quantity. Across 6 different sites, a total of 96 racemes of *D. barbeyi* were subjected to different treatments consisting of *D. barbeyi* and *Helianthella quinquenervis* pollen in different proportions. A significant relationship was found between seed set and pollen quantity, when looking at a subsample of the data collected.

Introduction

With many species of pollinators declining across the globe, there has been a demand for additional studies on pollination (Gallai et al. 2009; Roubik 2001), especially in light of the fact that a large quantity (roughly 85% in Europe) of crops grown to sustain the human population are dependent on pollinators (Williams 1994). As pollinator numbers decrease, there is potential for heightened competition between flowering plants for the remaining pollinators (Waser & Fugate 1986). This increase in flower competition could result in more generalized behavior for the pollinators, with remaining pollinators visiting sympatric heterospecific flowering plants, and causing a decrease in reproductive success from the loss of conspecific pollen (henceforth CP) that could be deposited (Fishman & Wyatt 1999; Waser 1978). It has also been hypothesized that the increase in the amount of heterospecific pollen (henceforth HP) deposited on flowering plants could also contribute a decrease in plant reproductive success (Jakobsson et al. 2008; Kwak et al. 1991; Waser 1978; Arceo-Gómez & Ashman 2011). Different mechanisms are thought to drive the relationship between increases in HP deposition and the resulting loss of reproductive success. The most widely accepted mechanisms are: loss of receptive stigma surface area, HP may physically or chemically disrupt CP germination, and stigma closure. (Waser 1978; WAITES et al. 2004; Waser & Fugate 1986; Brown & Mitchell 2001; Galen et al. 2013). Few studies have carefully assessed the consequences of varying pollen quantity and pollen identity (CP and HP) deposited on stigmas (Morales & Traveset 2008). Furthermore, no experiments have been done to see if there is any interaction between pollen quantity and CP:HP ratio.

During this study, I assessed how stigmatic pollen composition (i.e percentage of conspecific pollen deposition) impacts seed set in *Delphinium barbeyi* and if these effects depend on the total amount of pollen deposited. I choose *Helianthella quinquenervis* as the HP donor because this species is often found co-flowering with *D. barbeyi*. As these two species are often found in the same area, it is a likely that pollen from *H. quinquenervis* would be transferred to *D. barbeyi* via a pollinator and I sought to assess the impact of this potential heterospecific pollen transfer.

I hypothesized that the HP's impact on the seed set is dependent on the overall quantity of stigmatic pollen load. At low levels of pollen quantity, I theorized that the resulting seed set will be low, irrespective of the ratio of pollen identity. The reverse should be seen for high levels of pollen quantity; seed production should be relatively high, independent of the ratio of pollen identity. However, at intermediate levels of pollen deposition, HP may have the greatest effect on the seed set created as there is not a surplus or deficit of CP.

Methodology

Focal Species

D. barbeyi (commonly referred to as the subalpine larkspur), is a perennial member of the buttercup family (Ranunculaceae) (Inouye et al. 2002). *D. barbeyi* tends to begin blooming at the end of June or early July (Williams et al. 2001).

H. quinquenervis (also known as the Aspen Sunflower), is a perennial member of the sunflower family (asteraceae). *H. quinquenervis* is commonly found in areas with rocky soil (Knapp & Smith 1988). Peak flowering for *H. quinquenervis* is from mid to late-July (Inouye et al. 2008).

Experimental Design

Site setup

I choose six different plots of *D. barbeyi* in areas around the Gothic Valley (Figure 1). Sites needed to be within a meadow-like area with at least 40 individual *D. barbeyi* plants. During site setup, 20 to 25 racemes of *D. barbeyi* from different individuals were bagged. I tied a red string just above the highest bloomed flower to mark and prevent any of the flowers that had bloomed from being used in the experiment. This ensured that the flowers used in the study were virgin, and did not have any pollen deposited by pollinators.

Two days after the initial bagging, I revisited the site and emasculated three flowers from each raceme to prevent self pollination. I used fine forceps to remove all anthers from the flowers as well as the guard petals. I only emasculated flowers if there was either no dehiscence or a low amount of anthers had been dehisced (one to three).

Treatment Preparation

I choose three different proportions of pollen mixtures for this study. The proportions of the three were 5 to 30, 14 to 36, and 20 to 26 (*D. barbeyi* anthers to *H. quinquenervis* anthers). These proportions were meant to approximate $\frac{1}{4}$ percent, $\frac{1}{2}$ percent, and $\frac{3}{4}$ percent CP mixtures, respectively.

I created the treatments from a mixture of the anthers of both species. I collected the anthers in fields close to the established sites. *D. barbeyi* anthers were collected before dehiscence and left within a microcentrifuge tube in natural light for a day or two until dehiscence. I used *H. quinquenervis* in the mixtures directly after removal. Anthers were placed within microcentrifuge tube in the correct proportion for the mixture being made. Alcohol was added and then the anthers were masticated using forceps. The mixture was then shaken using a vortex and the anther material was removed. The remaining mixture was then placed in the centrifuge for a few minutes. Afterwards, the mixture was left open and placed under a light to allow the remaining alcohol to evaporate.

Treatment application

Three to four days after the site was emasculated, I performed hand-pollination treatments on the 12 – 16 racemes that showed receptive flowers. I applied the treatment to two of the three emasculated flowers on each raceme. Each raceme was assigned a treatment at random. There was a total of 12

possible different treatments (Table 1). Treatments varied in both composition (different mixtures) and quantity of pollen deposited. Amount of pollen deposited was controlled by using different applicators and applying the same treatment a certain number of times. Either a toothpick or an insect pin was used to apply the pollen treatment depending on the total amount of pollen deposition needed. There were four different methods of applying the treatments: one dab with the insect pin (applying roughly 100 grains), two dabs with the insect pin (200 grains), three dabs with the insect pin (300 grains), and one dab with the toothpick (600 grains).

Stigma and Fruit collection

After the treatment application, I allowed enough time for the pollen tubes to grow and then collected the stigmas approximately 3 days after hand-pollination. Stigmas were collected out in the field to prevent any loss in pollen grains that could occur during transportation. I clipped the stigmas with fine forceps and placed them on a slide with fuchsin jelly. The carpels were marked before stigma collection to later pair the stigma with its corresponding fruit (and seed set). Fruits were scheduled to be collected four days after hand-pollination, but were allowed to mature if the fruits were still growing.

Data Analysis

I collected the fruits and counted the total number of viable and nonviable seed and those totals were recorded for each stigma. Stigma slides were used to find the approximate number of pollen grains of each species that were applied during hand-pollination. I used a generalized linear mixed effects model (GLMM) with binomial errors (OLOFSSON & SHAMS 2007) to analyze the effect of pollen quantity and HP:CP ratio on total seed set in *D. barbeyi* as well as the effect of interaction between pollen quantity and HP:CP ratio on seed set in *D. barbeyi*.

Results

Examining the GLMM allows us to see a significant effect for both total stigmatic pollen quantity (GLMM, $P = 0.00893$) and for the interaction of both stigmatic pollen quantity and composition (GLMM, $P = 0.01151$). Heterospecific pollen percentage, by itself, did not have a significant effect on the resulting seed set (GLMM, $P=0.335$). A visual representation of the predictions of this model was created (Figure 2).

Discussion

Within the range of the data analyzed, I am able to say that seed set is affected by pollen quantity and the interaction between both pollen quantity and composition, but pollen composition does not seem to have a significant effect on the resulting pollen quantity independently.

At this time, only subsample of this data has been analyzed. Out of the total of 433 stigmas used in this experiment, only 63 stigmas were able to be processed at this time (Table 2). As each stigma used led to the production of a fruit (whether aborted or not), the same proportion of fruits were analyzed at this time. The data generated from these stigmas and seeds were used in a GLMM. However, only data points with pollen counts below 100 grains were used as some of the outliers were orders of magnitude higher, and we have not seen a natural stigma with that much pollen.

However, when looking at mounted stigmas, I was unable to achieve both the precise amounts of pollen to deposit and the correct proportions of pollen on the hand-pollinated stigmas. Treatments tended to deposit low quantities of pollen that had high proportions of CP pollen (Figure 2, Table 3).

Stigmas that received high levels of pollen depositions did consistently produce a higher seed set rather than lower levels of pollen deposition. This does fit my expectations, as it can be assumed that more pollen grains deposited on the stigma would provide a higher chance that a pollen tube growth would be successful and lead to the germination of all ovules. However, these results are difficult to relate back to natural setting as the pollen quality might be much different in hand-pollination application when compared to natural pollination (Ashman et al. 2004).

Out of the 63 fruits collected, a total of 41 fruits had a full seed set (i.e. – all ovules were germinated). This result is opposite of what was anticipated, as we believed that achieving our spread CP pollen ratios and stigmatic pollen deposition would result in varying proportions of developed ovules within each of the fruits. Though, it is interesting as some hand-pollinations experiments, including close relative *D. nelsoni*, have seen lower seed set through hand-pollination treatment when compared to natural pollination means (Bierzychudek 1981). Bierzychudek relates these decreases in seed set to detrimental effect caused by the treatment itself.

With only the subsample of data currently analyzed, it is difficult to make meaningful conclusion, even with the significant results received. While analyses will continue to take place as data continues to be processed, I believe it would be desirable to formulate methods of complementing this data set. It may be valuable to repeat this experiment with different focal species, as to avoid making *D. barbeyi* and *H. quinquenervis* be a model for all plant species interactions. Additionally, looking at the possible effects that could occur by using an invasive species as the HP pollen donor, as they has been an increasing trend in occurrence of invasive plant incursions (Brown & Mitchell 2001). As they are a wide range of species interactions, replications should be done in a manner that attempts to address all possible combinations.

Table 1 – One of the twelve possible treatments was given to two flowers on each raceme. IP stands for insect pin; TP stands for toothpick.

1 dab IP	2 dabs IP	3 dabs IP	1 dab TP
1 dab of 1/4 IP	2 dabs of 1/4 IP	3 dabs of 1/4 IP	1 dab of 1/4 TP
1 dab of 1/2 IP	2 dabs of 1/2 IP	3 dabs of 1/2 IP	1 dab of 1/2 TP
1 dab of 3/4 IP	2 dabs of 3/4 IP	3 dabs of 3/4 IP	1 dab of 3/4 TP

Table 2 - Site data from all the sites used in the analysis. Several variables are display to allow for the comparison of different sites.

Site	# of Stigmas	Total Pollen Grains Deposited	Average HP Percentage	Total Number of Viable Seeds Produced
Gothic Road Steep	11	4942	0.02029454	97
Gothic Townsite	31	545	0.151373192	320
Research Meadow	21	512	0.152489376	274

Table 3 – Data on the average CP found on the stigma slides created. This is a rough estimate of what the treatments actually deposited on the stigma.

Treatment	Average CP %
1/4	0.921002301
1/2	0.841528538
3/4	0.8761376



Figure 1 – Map of Gothic valley sites where hand-pollinations occurred.

Effects of Total Pollen Quantity and Proportion Heterospecific Pollen on Seed Set

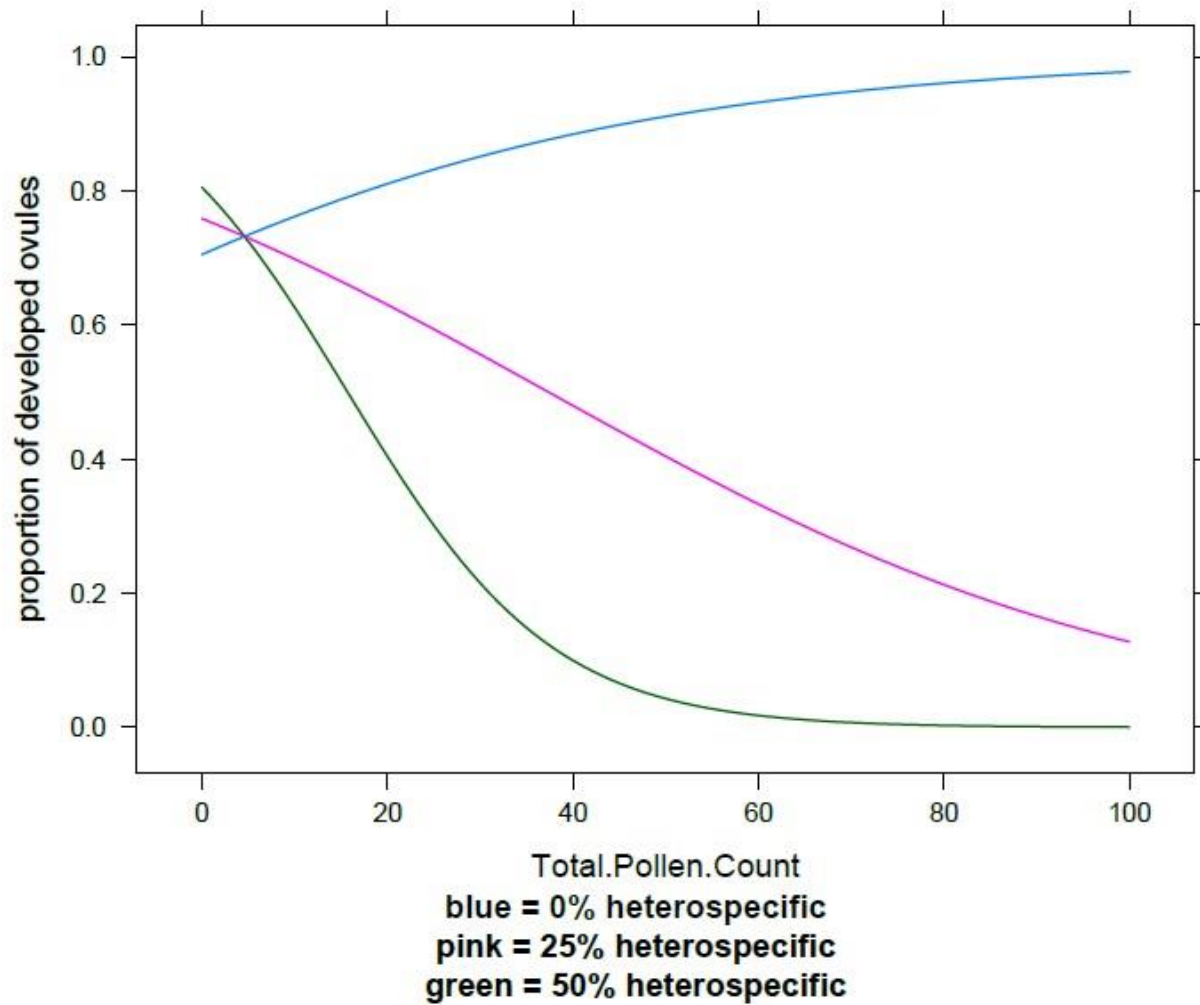


Figure 2 – Representation of the effect total pollen count on the proportion of developed ovules, separated into three different levels of heterospecific pollen percentage.

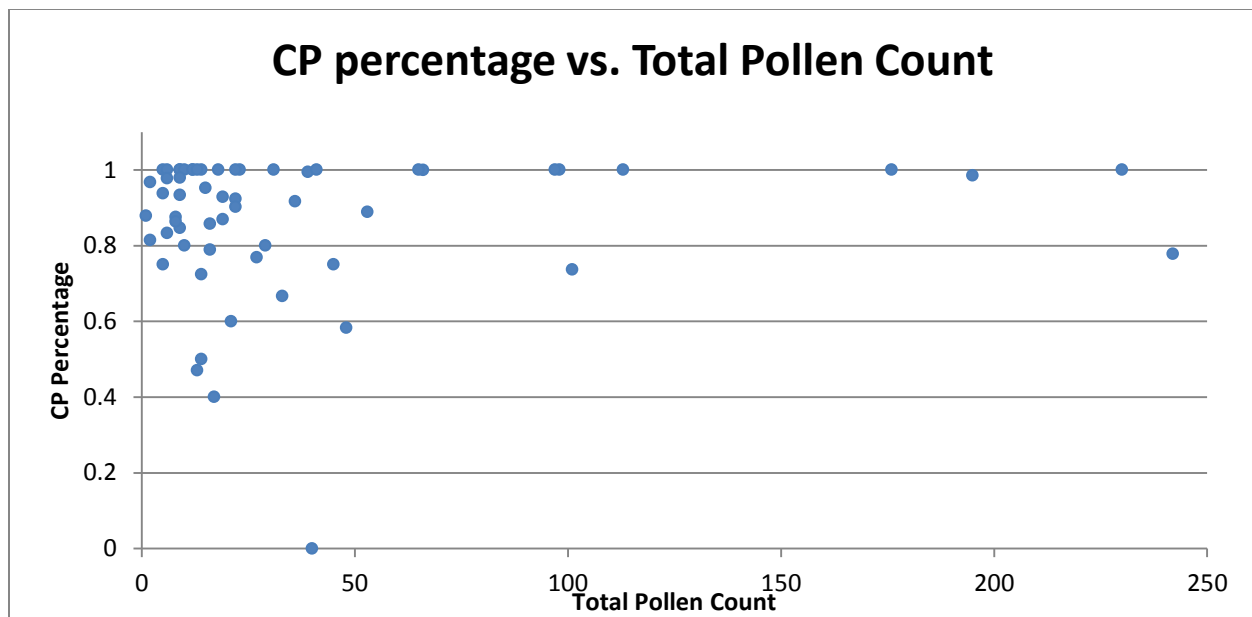


Figure 3 – Representation of what the treatments were able to place on the stigma. Treatments had low pollen quantity with a high proportion of CP.

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