

**Comparing *Bombus* pollinator efficacies in *Delphinium barbeyi*
(Ranunculaceae) reproductive success**

Amanda Cooke

**Mentors - Berry Brosi and Heather Briggs
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Abstract

Pollinator populations are decreasing globally constituting a major conservation concern. A decline in a particular pollinator species may cause plant population declines. Certain *Bombus* species may be more effective pollinators; i.e. they contribute more to female reproductive success of a flower species than others. This study determined the pollination efficacy of five *Bombus* species in *Delphinium barbeyi* reproductive success in a subalpine ecosystem. I determined the pollinator efficacy of each bee species by the amount of conspecific pollen (CP) and percentage of CP deposited on *D. barbeyi* stigmas and the resulting seed set of each flower visited. *Bombus nevadensis* was the most effective pollinator due to its high CP deposition and resulting seed set. *B. appositus* was the least effective pollinator because it deposited the least amount of CP and was related to the lowest seed set. *B. californicus*, *flavifrons*, and *kirbyellii* were of intermediate pollinator efficacy. These results suggest bee morphology such as body size and bee behavior such as floral fidelity may contribute to pollinator efficacy in this system. This study provides a model with which to determine bumblebee pollination efficacy in a wildflower population.

Introduction

Pollinator populations are decreasing in many locations constituting a major conservation concern whose causes and consequences require further research (Potts et. al. 2010). A decline in a particular pollinator species may have implications for the plant species they pollinate such as plant population declines (Potts et. al. 2010). One or a few pollinator species may be more effective pollinators; i.e. they contribute to female reproductive success of a flower species more than others (Ne'eman et. al. 2009). Previous research has not examined the pollinator efficacies of bee species within the same genus.

Some pollinators may provide more CP per visit than others due to differences in morphology or behavior such as floral fidelity or duration of visit (Ne'eman et. al. 2009). Bee morphology differences that can contribute to differences in pollinator efficacy include differences in body size and body hair quantity and quality such as length and density. Larger bees and those with thicker or longer hairs may be able to carry and deposit more pollen (CITE). Bumblebees with higher floral fidelity may bring more CP to a flower (Arceo-Gomez et. al. 2011). High ratios of CP:HP receipt lead to increased reproductive success of flowers (Arceo-Gomez & Ashman 2011).

Some research argues that more effective pollinators can reduce pollen limitation (Ne'eman et. al. 2009). Pollen limitation (PL) occurs when a flower does



Figure 1. *Bombus appositus* visiting *Delphinium barbeyi* at Cold Springs Ranch Site, Crested Butte, CO 2013.

not receive enough CP to fertilize all available ovules when resources are not limiting (Knight et. al. 2005). Pollen limitation may lead to a decrease in female reproductive success (Ashman et. al 2004) and thus a decline in population (Potts et. al 2010). Pollen limitation may occur when pollinator populations decline and the amount of CP deposited on a flower decreases (Ashman & Mitchell 2008). More effective pollinators may reduce PL and increase female reproductive success because they deposit more outcross CP on the stigma than they remove (Ne'eman et. al. 2009).

This study sought to determine the efficacy of five bumblebee species that visit *D. barbeyi* in a Colorado subalpine ecosystem. Conspecific pollen deposition, percentage of conspecific pollen of the total pollen deposited on female receptive stigmas and the resulting seed set of *D. barbeyi* provide an analysis of the pollinator efficacies of *Bombus appositus*, *californicus*, *flavifrons*, *kirbeyellis* and *nevadensis*.

Methodology

I examined the efficacy of five common bumblebee pollinators of *Delphinium barbeyi* (Subalpine larkspur), an herbaceous perennial wildflower that grows in moist meadows and forest clearings in much of the southwestern US (Elliot & Irwin 2009). *Bombus appositus* (Fig 1.) and *B. flavifrons* most commonly pollinate *D. barbeyi* along with *B. bifarius*, *B. frigidus*, *B. californicus*, *B. kirbeyellis*, *B. occidentalis*, and *B. nevadensis* (Inyoue 1976, Waser 1982, Williams 2001, Elliot et. al. 2009).

I selected 8 sites (Fig. 2) and 10 *D. barbeyi* plants per site, controlling as best I could for abiotic conditions such as soil moisture and elevation. I placed a bag on one raceme on each plant of study before any stigmas on any flowers were receptive to pollination to prevent unrecorded pollination visits from occurring. After several flowers on the raceme became female, I removed the bag for one *Bombus* pollinator visit and replaced the bag for pollen tube development. The bumblebees were allowed to visit only three flowers on each raceme before being driven away. I marked the order of the flowers visited and the position of the flower on the raceme. I also recorded the positions of the flowers visited on the raceme and the number of flowering racemes on each plant at the time of the visit. I recorded five *Bombus* species as visitors to *D. barbeyi*, including *B. appositus*, *B. californicus*, *B. flavifrons*, *B. kirbeyellis*, and *B. nevadensis* (N=7 for all for the subsample analyzed for this writing).

I harvested stigmas after pollen tubes had developed and determined the amount of CP and CP:HP ratio deposited on the stigma after mounting them on microscope slides using a fuschin gel pollen stain (Kearns et. al 1993). After the fruits matured, I determined the seed set of each flower by harvesting and counting aborted and non-aborted seeds in each carpel of each flower visited.

Since both the number of pollen deposited and seed set are count (non-continuous) data, using such data would violate basic assumptions of ANOVA and other statistical tests for continuous data. Instead, I used generalized linear models ("GLMs") with Poisson errors, which are appropriate for count data, using R statistical program.

Results

A generalized linear model showed each *Bombus* species deposited different amounts of pollen on *D. barbeyi* stigmas ($P < 2e-16$ ***) except in the case of *B. californicus*, which did not differ significantly when compared to *B. appositus* ($P = 0.945$). The low P value indicates that *B. appositus* and *B. californicus* deposited similar amounts of pollen (Fig. 3). *B. nevadensis* deposited the most conspecific pollen ($\bar{x} = 81.4$) followed by *B. flavifrons* ($\bar{x} = 48.8$), *B. appositus* ($\bar{x} = 33.4$) and *B. californicus* ($\bar{x} = 33.6$), and *B. kirbeyellis* ($\bar{x} = 22.7$) (Fig. 3).

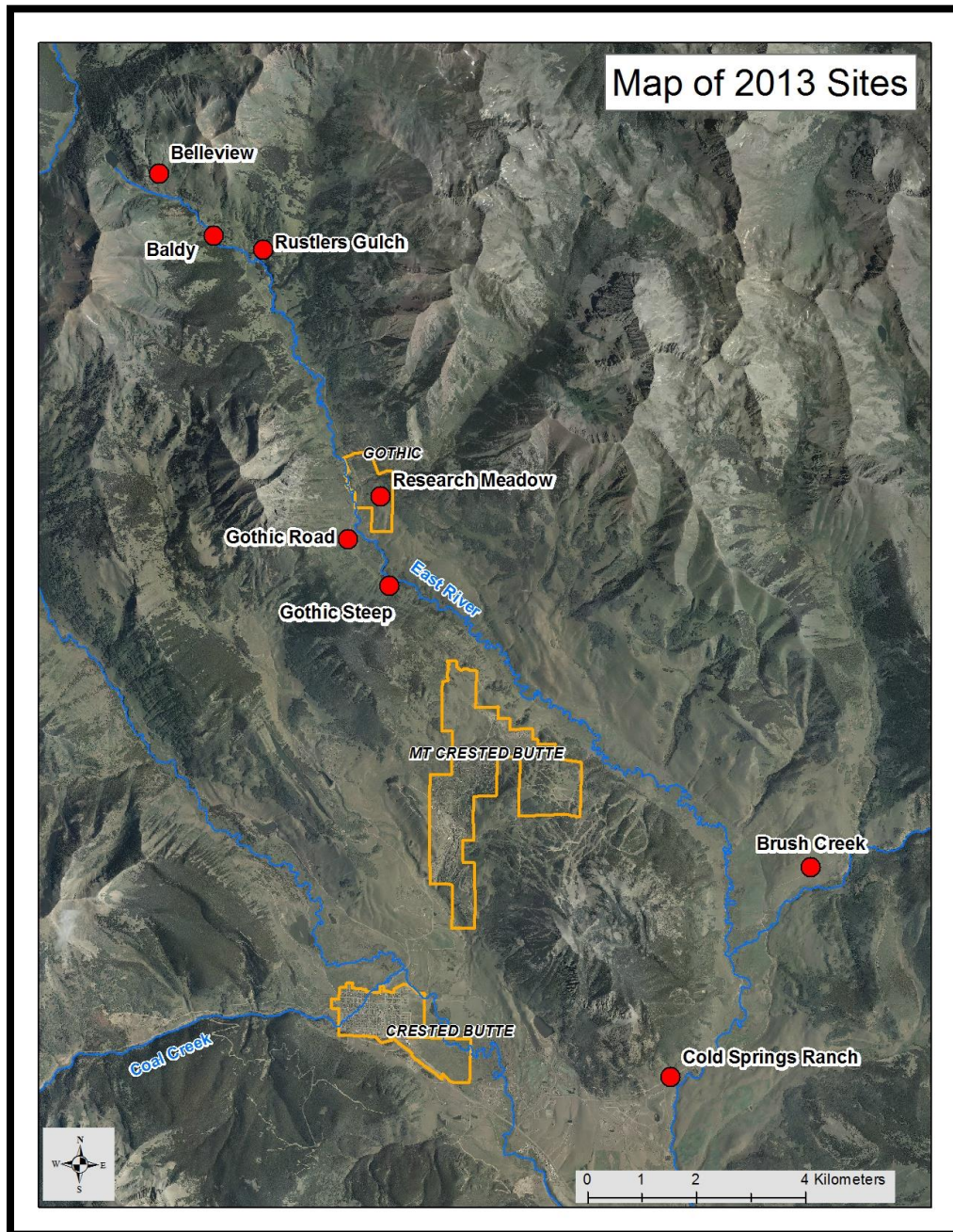


Figure 2. Map of research sites of the study in 2013. All sites are located in the East River and Brush Creek Valleys.

B. nevadensis deposited the highest percentage of conspecific pollen of the total amount of pollen deposited on *D. barbeyi* stigmas followed by *B. flavifrons* ($\bar{x}=89.2\%$), *B. californicus* ($\bar{x}=82.8\%$), *B. appositus* ($\bar{x}=77.2\%$), and *B. kirbeyellis* ($\bar{x}=76.6\%$) (Fig. 3). *B. californicus* deposited a higher percentage of conspecific pollen than *B. appositus* though they both deposited the same amount of conspecific pollen (Fig. 2 & 3). In other words, *B. appositus* deposited more heterospecific pollen (Fig. 3).

The bee species included in this study were related to different levels of seed set of *D. barbeyi* (Fig. 5, all $P < 2e-16^{***}$, except *B. flavifrons*, $P < 0.0352^*$). *B. nevadensis* was related to the highest seed set overall with a mean seed set of 13.1 followed by *B. californicus* ($\bar{x}=10.3$), *B. flavifrons* ($\bar{x}=7.3$), and *B. appositus* ($\bar{x}=6.1$) (Fig. 5). At the time of writing, I had not collected the *D. barbeyi* seed set related to *B. kirbeyellis* due to immature fruits.

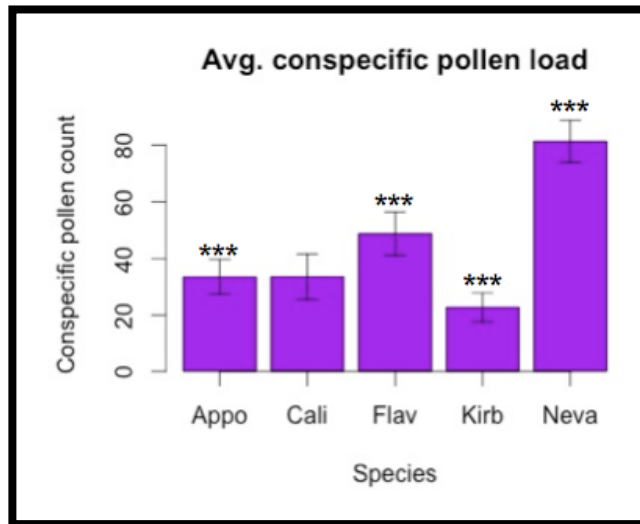


Figure 3. Average amount of conspecific pollen deposited by *Bombus* pollinator species onto *Delphinium barbeyi* stigmas. Significance codes: '***' = $P < 0.001$, '**' = $P < 0.01$, '*' = $P < 0.05$

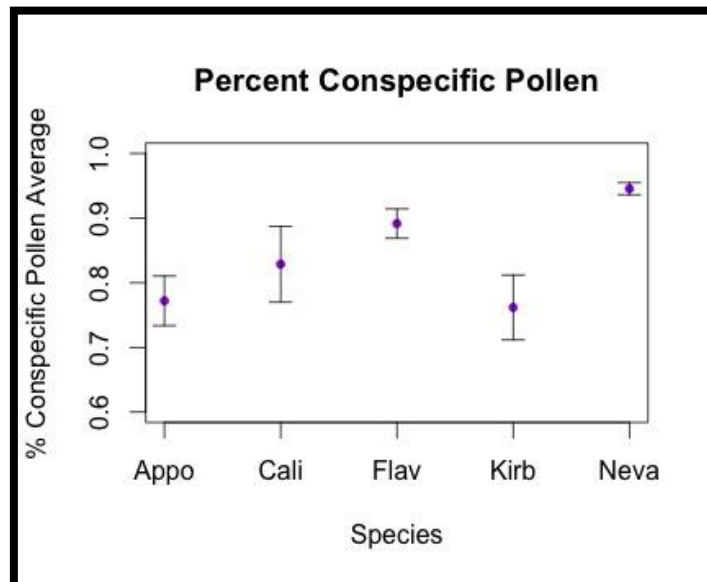


Figure 4. Average percent conspecific pollen of total pollen deposited by *Bombus* pollinator species onto *Delphinium barbeyi* stigmas.

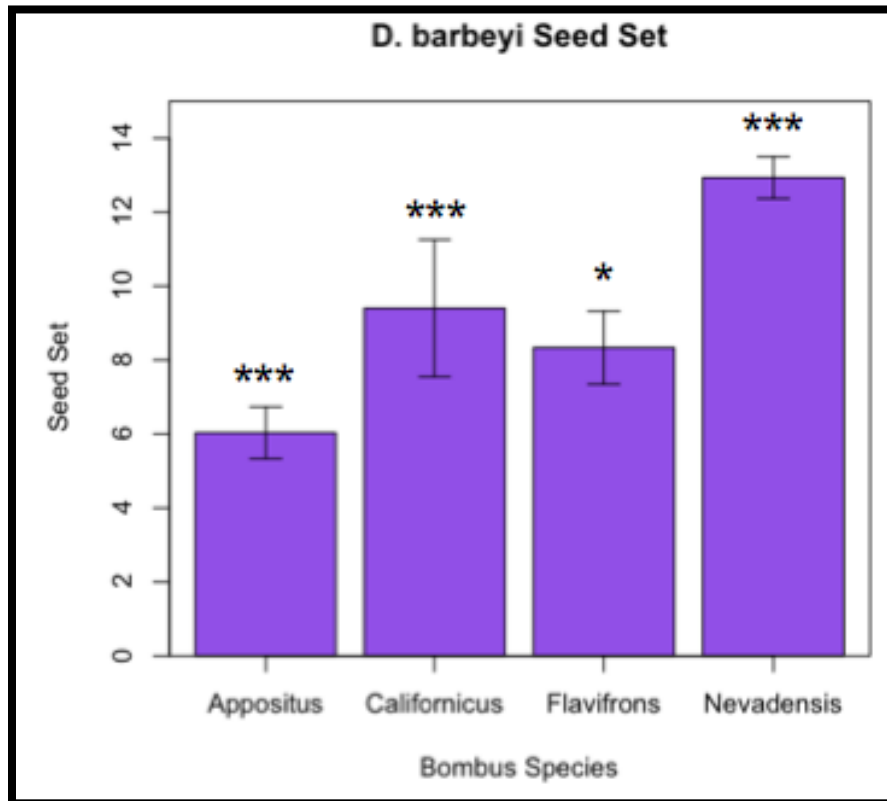


Figure 5. Average seed set of *Delphinium barbeyi* in relation to *Bombus* pollinator species. Significance codes: '***' = $P < 0.001$, '**' = $P < 0.01$, '*' = $P < 0.05$

Discussion

The results of this study suggest *B. nevadensis* may be the most effective pollinator of *D. barbeyi* because the species deposits the highest conspecific pollen load and percentage of CP and is related to the highest seed set when compared to the other *Bombus* species studied. *B. nevadensis* may have deposited the most conspecific pollen due to several factors. *B. nevadensis* has the largest body size out of the bee species studied (on average 17mm for a worker), which may have contributed to its ability to deposit a high level of pollen and result in a higher seed set (Fig. 3, 4, & 5). *B. nevadensis* is also known to have high fidelity to *D. barbeyi* which explains the high CP percentage results (Brosi & Briggs unpublished data). *B. nevadensis* may be reducing pollen limitation in *D. barbeyi* reproduction more than the other bee species studied.

The *B. flavifrons*, *B. californicus* and *B. appositus* results do not follow as logical trends as *B. nevadensis*. While *B. flavifrons* deposited more CP total and %CP than *B. californicus*, it was related to a lower seed set than *B. californicus*. An abundance of aborted seeds of study plants occurred at sites (Research Meadow, Fig. 1) where I collected *B. flavifrons* data may be causing this unexpected result. The high abortion rates might be leading to a depression in the *B. flavifrons* seed set results. A similar depression may be occurring with *B. appositus* seed set data because the harvested fruits came from similar sites as *B. flavifrons*. The low seed sets related to *B. appositus* challenges expectations of high seed set considering the

higher *B. appositus* floral fidelity to *D. barbeyi* than other *Bombus* species. *B. appositus* may be leading to pollen limitation in *D. barbeyi* as the low seed set results suggest.

While *B. californicus* deposited the same amount of conspecific pollen as *B. appositus*, it deposited a higher percentage of CP. The higher %CP deposited by *B. californicus* may explain the higher seed set related to *B. californicus*. Though *B. flavifrons* is smaller in size than *B. appositus*, *B. flavifrons* may have deposited more CP than *B. appositus* because of its long body hairs that may allow higher pollen carriage despite *B. flavifrons* generalist foraging behavior and small body size (9-12mm in length) (Brosi & Briggs unpublished data).

These data suggest bee behavior and morphology play a major role in pollinator efficacy. Previous research has supported this hypothesis (Neinhuis 2009, Young et. al 2007). For example, bees that forage for pollen are less efficient at depositing pollen on stigmas ("high removal-low deposition") than bees that forage for nectar ("low removal – high deposition") (Young et. al. 2007). Examining the bees species of this study for proboscis length and corbicula (pollen basket) pollen loads in addition to foraging behavior may elucidate important information regarding the underlying mechanism causing differences in pollen deposition. Perhaps *B. appositus* is a "high removal-low deposition" pollinator while *B. nevadensis* is a "low-removal-high deposition" pollinator, which may explain the differences in CP deposition and seed sets between the two bee species. Grooming behavior may also influence pollinator efficacy (Young et. al 2007). When bees groom pollen from their faces to their corbiculae they reduce the likelihood of depositing pollen on the stigmas of plants they visit (Young et. al. 2007). *B. nevadensis* may also deposit more outcross pollen, a factor not looked at in this study that would increase seed set, because it may visit more flowers from different plants versus the same plant.

The CP deposition and seed set results suggest that *D. barbeyi* and *B. nevadensis* may be better adapted to each other's morphologies and *D. barbeyi* to *B. nevadensis* foraging behaviors than those other bees. The *D. barbeyi* flower may be better suited morphologically to *B. nevadensis* body size and shape reflecting what other researchers have suggested as one of the most critical aspects of pollinator effectiveness (Neinhuis et. al. 2009).

Conclusions

This study serves as a model in determining pollinator efficacy in bumblebee pollinated plant reproductive success as measured by total conspecific pollen and %CP stigma deposition and the resulting seed set. As pollinator populations decline and their conservation becomes a greater priority, information regarding their efficacy becomes crucial when considering their impact on flowering plants. Further research may consider examining the mechanisms underlying these differences in pollen deposition and seed set such as the differences in bee morphology and behavior. In addition, information regarding visitation rates and floral fidelity to *D. barbeyi* of these bee species should be analyzed in conjunction with the CP, %CP, and seed set data included here to provide a more comprehensive description of each bee species' pollination efficacy.

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