

Do the differing pollen foraging strategies of *Megachile sp.* and *Bombus spp.* result in differing pollen removal and deposition rates in *Lupinus bakeri*?

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Abstract

Mutualistic interactions between flowering plants and bees are a pairwise interaction, but exist in a network of similar interactions (Bronstein, 2001). The outcomes of these interactions differ, for example, the costs and benefits for flowering plants caused by floral visitors (Bronstein, 2001). *Lupinus bakeri* is a pollen-rewarding plant species that controls the amount of pollen removed by each floral visitor (Gori 1989; Harder 1990a). The bee genera *Bombus spp.* and *Megachile sp.* remove pollen differently from *Lupinus bakeri*, perhaps affecting the reproductive success of the plant differently. We assessed whether pollen removal and deposition rates are different for each bee genus through a interview stick based observational study. More *Bombus spp.* visited *Lupinus bakeri* than *Megachile sp.*, but we did not detect a statistically significant difference in removal and deposition rates between bee taxa.

Introduction

Though species interactions are often treated as pairwise, interactions between any two species are usually part of a network of similar interactions experienced by each partner species (Bronstein, 2001). Flowering plants and their floral visitors provide a good example. The traits of species in mutualistic relationships can affect the outcomes of these interactions between organisms (Bronstein, 2001). The interactions among floral visitors, such as bees, and flowering plants can have variable outcomes (Bronstein, 2001). These outcomes have to do with visitations by bees to flowering plants. Visitations by bees to plants have costs associated with them and the benefit these plants receive from bees is pollen deposition. These costs and benefits from bees to flowering plants vary, thus their effectiveness as mutualists varies too.

These visitations occur because flowering plants offer a reward to the floral visitor (Bronstein, 2001). In many cases, pollen can act as a reward for floral visitors. Pollen is a particularly important for bees because it is their main source of protein, acting as a resource for reproductive females and growing larvae (Harder 1990a). As bees visit flowers for the pollen reward, they benefit flowering plants by depositing pollen from conspecific flowers on the stigmas of flowers, increasing the reproductive success of the plant. The costs of reproductive success vary, depending on the ratio of pollen that is removed and deposited on conspecific flowers (Bshary and Bronstein, 2004). For example, when there is a higher removal to deposition ratio this indicates a higher rate of pollen loss due to consumption by visitors (Harder, 1990b). Therefore, there is a higher ratio of cost to benefit for the plant (Harder, 1990b). Understanding how species balance the ratio of costs and benefits is then essential to better understanding mutualistic relationships (Bronstein, 2001). Being able to quantify the removed and deposited pollen may then act as an indicator for the quality of mutualistic relationship there is between species (Bshary and Bronstein, 2004).

The goal of this project was to observe the differing foraging strategies of bees on a particular plant species, and determine whether pollen removal and deposition differed between bee taxa. We compared pollen removal and deposition in the pollen rewarding species *Lupinus bakeri* by two genera of bees with differing foraging strategies. If there were a difference in the ratio of removal to deposition for the flowering plant, then this would have possible implications for lupine reproductive success. Some of these differences in pollen foraging could be a high removal and high deposition rate, a high removal and low deposition rate, a low removal and low deposition rate, or a low removal and high deposition

rate. These rates could then differ between the two genera of bees, implying that each genus of bee would have a different impact on the flowering plant.

Methods

Sites and Studied Organisms

In the Gunnison National Forest, CO I visited two sites: Deer Creek Trail (38°56'59.4"N, 106°59'06.1"W) and Quigley Creek (39°00'12.6"N, 107°01'42.5"W).

The bee genera that I observed in this study were *Bombus spp.*, and *Megachile sp.*, and both genera forage for pollen on lupine. Two samples of *Megachile sp.* were identified in the laboratory, but all *Bombus spp.* were identified in flight. I did not lethally sample any *Bombus*.

The plant species that was observed in this project was *Lupinus bakeri*. *Lupinus bakeri* is a pollen-rewarding plant species, and is pollinated by bees (Gori 1989; Harder 1990a). This species controls the amount of pollen removed by each visitor (Gori 1989; Harder 1990a). Like other papilionaceous legumes, the stamens and pistils are hidden under the keel petals of the flower, but unlike many other legumes the margins of the keel petals are fused, except at the keel-pore of the flower (Harder 1990a). Before anthesis, the stamens shed their pollen and the pollen is moved to the tip of the keel petals around the stigma (Harder 1990a).

When a bee visits the lupine flower, it pushes down the surrounding wing petals with its metathoracic legs and this causes the stigma to push through the keel petals and the pollen, offering the bee only a limited quantity of pollen each time the stigma is forced through the keel petals (Gori 1989; Harder 1990a). This mechanism is thought to control how much pollen is removed per visit (Harder 1990a; Harder 1990b). From the perspective of the plant, the controlled pollen removal may be optimized to the per-visit level of pollen removal

(Harder 1990a; Harder 1990b). Some visitors avoid this mechanism though (Heiling, *pers. comm*). Specifically, *Megachile sp.* act differently from *Bombus spp.* when visiting lupines. Whereas *Bombus spp.* take only the pollen that is presented by the flower from the keel-pore (Harder 1990a; Harder 1990b), some *Megachile sp.* split the keel open and take pollen directly from the anthers, which may result in higher pollen removal rates than the *Bombus spp.* foraging strategy does.

Interview Stick Method

In this study, I used the “interview stick” approach, as described in Thompson 1981, to collect data on pollen removal and deposition for the bee visitors. I collected inflorescences of *Lupinus bakeri* (one stalk per individual) taking about six stalks per-day.

The day before harvesting *L. bakeri* stalks, I removed all of the open flowers on the stalks that I was going to use the next day and I covered the stalks with a bridal veil bag. By removing all of the open flowers on a stalk and covering it with a veil bag I insured that when the flowers on the stalk opened they would be virgins. Thus, the flowers would not have had pollen removed or deposited onto them. I marked each sampled plant with either a numbered brown flag or with a piece of numbered green tape so that I would not accidentally resample a stalk.

On the following day, I cut stalks and stored them in water. I also also bagged new stalks during that time for the next day of observations. At the same site, either Deer Creek Trail or Quigley Creek, I attached the stalk to the interview stick. With the stalk attached to the interview stick, I presented the inflorescence to a bee foraging on *L. bakeri* and allowed it to

visit one flower. If it accepted the flower, I used a voice recorder to record the visitor species, the amount of time it spent on that flower, and the number identification of the plant. Once it finished on the flower, I shooed the bee gently off the flower and then gently removed the flower by using forceps and plucking the flower from the pedicel. Then with the same flower, I gently pushed down on the keel to uncover the stigma (Harder, 1990). Then, using clean forceps, I removed the stigma from the flower and placed it in an Eppendorf tube and then placed the flower in a separate Eppendorf tube. I repeated this same process of removing and storing the stigma and flower, but for the virgin flower that was adjacent to the removed flower. I immediately labeled the used tubes and stored them in a cooler on ice. I discontinued using a stalk after it was visited and I disposed all of the used stalks back at the laboratory.

Taking samples of visited flowers and visited stigmas provided me with an estimate of the mean quantities of pollen removed and deposited by both genera of bees. The total pollen load I found on a visited flower gave me the amount of pollen being removed by the floral visitor. The amount of pollen I found on a visited stigma then provided me with the amount of pollen being deposited by the bee. Then with these numbers, I drew comparisons between the two genera of bees based on their levels of removal and deposition on *L. bakeri*.

Counting Pollen Grains

In the lab, I mounted each stigma on a labeled glass slide with fuchsin gel (Kearns and Inouye, 1993). I counted the pollen grains on the stigmas using a compound microscope at a magnification of 40x, recording the con- and heterotrophic grains separately.

I sub-sampled the total pollen load in each flower in order to estimate the total pollen load. To sub-sample, I added 1.5mL of 70% ethanol to an Eppendorf tube with the flower samples. The samples were sonicated for approximately 10 minutes and then vortexed for approximately 15 seconds, or until the pollen was in suspension. Using a micropipette, I removed one 4uL drop of the pollen and added it to a slide. I counted the pollen grains on the slide from the flower sub-samples using a compound microscope at a magnification of 40x.

Statistical Analysis

I used R (R Core Team (2015)) for all data analysis and Microsoft Excel to calculate values and construct figures. I calculated values in Excel by first calculating the estimated single visit pollen load on an individual flower. The estimated single visit pollen load was calculated with the formula:

$$\frac{\text{sub - sample of single visit pollen load}}{4\mu\text{l}} = \frac{\text{estimate of single visit pollen load}}{1500\mu\text{l}} \quad (\text{Formula 1})$$

The sub-sample of single visit pollen was the counted pollen grains from the 4μl of sub-sample for each individual flower.(Figure 1).

I calculated the mean virgin pollen load and virgin stigma counts, including their standard errors, for the *Lupinus bakeri*. I found this by calculating the estimated virgin pollen load with the formula:

$$\frac{\text{sub - sample of virgin pollen load}}{4\mu\text{l}} = \frac{\text{estimate of virgin pollen load}}{1500\mu\text{l}} \quad (\text{Formula 2})$$

The sub-sample of virgin pollen was the counted pollen grains from the 4μl of sub-sample for each individual flower.

The computer program R was used to construct linear models of the data. The pollen removal model and the pollen deposition model included the estimated pollen removed or deposited as a response, respectively, and visitor type as a predictor. I included time spent per flower as a covariate in the initial models, but removed it from the final models because it did not have a significant effect on the response variables.

Results

Virgin Lupinus bakeri

To estimate the amount of pollen there is present in *Lupinus bakeri* flowers before a bee visits, I counted pollen grains from virgin stigmas and sub-samples of pollen loads. Virgin flowers bore a mean of 17500 ± 2350 pollen grains, and 25.9 ± 4.48 pollen grains on virgin stigmas before pollen deposition.

Visited Flowers

The amount of pollen removed and deposited on *Lupinus bakeri* by *Megachile sp.* and *Bombus spp.* varied widely. There was no significant difference between the mean stigma pollen count, or pollen deposited on the stigma, for *Bombus spp.* and *Megachile sp.* The results for the

single test were $P=0.898$. Pollen removal did not differ between *Bombus spp.* and *Megachile sp.* The results for the single test were $P=0.5442$. The visitation rates differed with *Megachile sp.* having 9 visits and *Bombus spp.* having 18 visits to *Lupinus bakeri* (Table 1).

Discussion

The effectiveness of a floral visitor depends on the proportional amount of pollen they remove and deposit on each visit. The cost is the pollen that is consumed by the bee and the benefit to the plant is the amount of pollen they deposit on the flower in proportion to the amount they remove. As mentioned in the introduction, there are several possible rates of removal and deposition, which could be different between genera of bees. For example, if one of the bee genera had had a high removal and deposition rate then there would be a high cost and high benefit to the flower, but depending on the amount, the high removal and deposition could balance each other out in their impacts. If I had seen patterns of removal and deposition in comparison between the two bee genera, it would be mediated by the relative abundance of each visitor.

This project was looking at possible differences in deposition and removal rates between two genera of bees, *Bombus spp.* and *Megachile sp.* on the flowering plant, *Lupinus bakeri*. The pollen rewarding *L. bakeri* has a mechanism for pollination that offers only a certain amount of pollen on the stigma of the flower to a foraging bee (Gori 1989; Harder 1990a). *Bombus spp.* uses this mechanism to remove pollen from the stigma (Harder 1990a; Harder 1990b) while *Megachile sp.* circumvents this mechanism and takes pollen directly from the anthers (Heiling, pers. comm). These bees were thought to differ in their rate of removal and deposition due to

their different uses of the mechanism in the flower, possibly impacting the reproductive success of the flowering plant in different manners.

The results indicated that there was no significant difference between the removal and deposition rates for *Megachile sp.* and *Bombus spp.* However, the study appeared to lack sufficient power to detect any effect. There is a possible pattern in the visitation rates because there were a larger number of visitations from *Bombus spp.* than from *Megachile sp.* to *L. bakeri* (Table 1). The pattern in the data showed a higher rate of *Bombus spp.* visits to *L. bakeri*, but future studies would need to be conducted to further investigate this pattern. To improve the study in the future, I would go out earlier in the season in order to collect more data points throughout the season. With an increase in data, there would have likely been more significant results which could have had implications about the effects these two bees have on the reproductive success of *L. bakeri*.

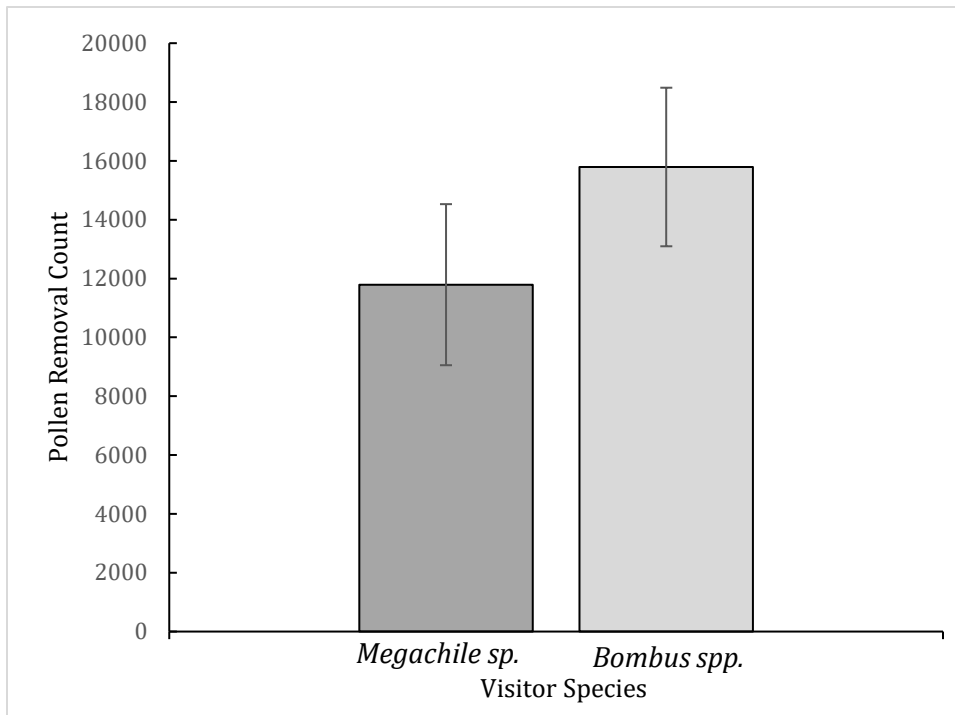


Figure 1 The amount of pollen that was found and counted in a 4 μ l sub-sample of a 1.5mL ETOH sample with a visited flower. These values indicate the amount of pollen that was removed by the bee genera. There was not a significant difference between *Megachile sp.* and *Bombus spp* removal. Error bars (± 1 SE).

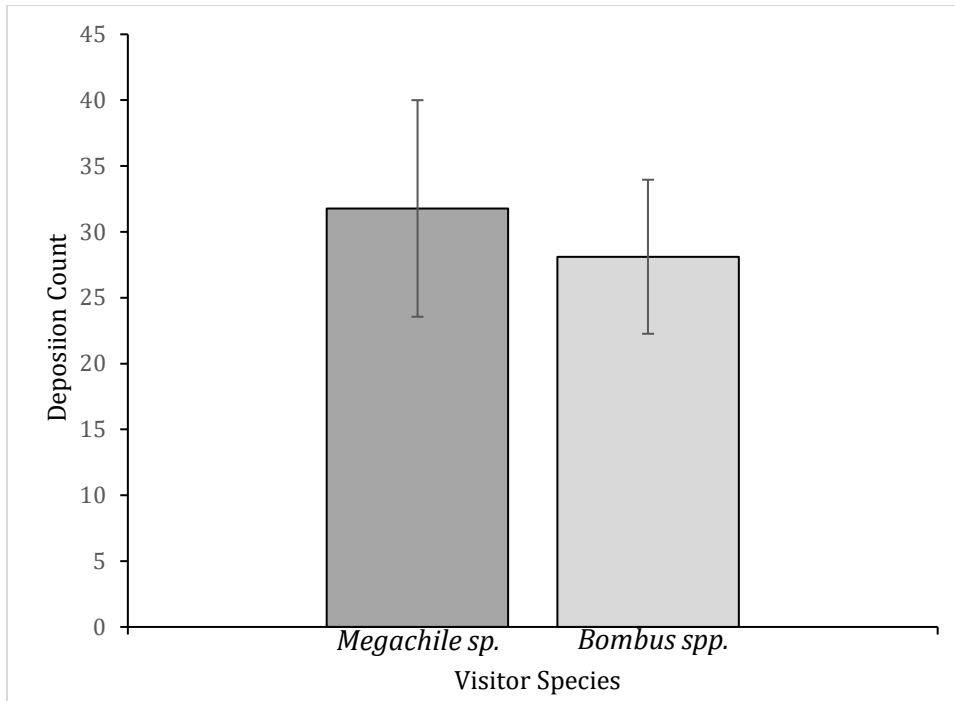


Figure 2 The amount of pollen that was found and counted on visited stigmas of both visitor genera. These values indicate the amount of pollen that was deposited by the bee genera. There was not a significant difference between the mean values for *Megachile sp.* and *Bombus spp.* Error bars ($\pm 1SE$).

Table 1 The number of times each species of bee visited a flower on a stalk of *Lupinus bakeri*. Through the interview stick method, bees were allowed to visit one flower on a stalk and the species was recorded.

Visitor species	Number of visits
<i>Megachile sp.</i>	9
<i>Bombus appositus</i>	1
<i>Bombus bifarius</i>	15
<i>Bombus centralis</i>	2



Figure 3 The sites at which the interview stick method was carried out.

Citations

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