

Assessing pollinators' contribution to the reproductive success of *Mertensia ciliata*

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Abstract

Shifting flowering phenologies as a result of climate change have the potential to disrupt plant-pollinator interactions through changes in temporal overlap of flowering and pollinator activity, decreasing plant fitness. Understanding current plant-pollinator interactions is key to predicting how plant fitness might be affected by such changes. The impact of temporal mismatch of flowering and pollinator activity on plant fitness should depend on how much each [pollinator](#) species contributes to a plant's reproductive success. Using [seed](#) set to determine effectiveness, this study investigates the differences in the pollinator effectiveness of visitors of *Mertensia ciliata* (Boraginaceae). We conducted single pollinator visit observations and obtained average seed set per flower for each type of *M. ciliata* visitor. Bumble bees and muscid flies were the two primary visitors to *M. ciliata*. We found that bumble bees had a higher visitation rate than muscid flies and that bumble bee single visits resulted in a higher seed set per flower than muscid fly single visits. We concluded that bumble bees were more effective pollinators for *M. ciliata* than muscid flies. To continue elucidating plant-pollinator interactions, future research should incorporate visitation rate with pollinator effectiveness to determine pollinator importance and consider how pollinator effectiveness and importance might be impacted by climate change.

Introduction

Global climate change has been shown to impact the flowering phenology, or timing of floral events, of many different plants ([e.g.](#), Sparks *et al.* 2000, Miller-Rushing 2008). Early snowmelt and warming temperatures advanced flowering time in several species of alpine wildflowers (Inouye 2008; Dunne *et al.* 2003). Measures of phenology such as first flowering, peak floral abundance, and last flowering can all be impacted differently by changing climate (CaraDonna *et al.* 2013). Changes in flowering phenology have the potential to impact many aspects related to plant fitness and reproduction including plant-plant interactions, herbivory, disease avoidance, and plant-pollinator interactions (Elzinga 2007).

Changes in flowering phenology could impact plant-pollinator interactions by causing decoupling, in which flowers and their pollinators emerge at different times, or as a result of temporal variation in pollinator effectiveness, or how much a pollinator contributes to plant reproductive success (Forrest 2015; Rafferty & Ives 2012). Current research suggests that advancing flowering phenology does not hinder adequate pollination since co-occurring pollinators are similarly affected by climatic variables and are emerging earlier than in the past (Forrest 2015). However, there is some evidence of temporal mismatch between plants and their pollinators. Rafferty and Ives (2012) found that earlier flowering time resulted in reduced seed set as compared to later flowering time due to differences in both the composition of available pollinators and the effectiveness of those pollinators over time. For animal-pollinated plants, this

suggests that changing flowering phenology as a result of climate change could impact reproductive success and potentially population persistence.

Mertensia ciliata (Boraginaceae) is a perennial subalpine wildflower native to the western United States, with the highest density located in Colorado (USDA 2016; Pelton 1961). While *M. ciliata* can reproduce vegetatively, it relies on insect pollination to maintain genetic variation in the population (Pelton 1961; Gallagher pers. comm.). Over a thirty-three-year period, *M. ciliata* average peak flower abundance has declined and date of first flowering has moved 3.3 days earlier per decade, with both trends best explained by early snowmelt (Miller-Rushing & Inouye 2009). Interestingly, the species remains abundant at higher altitudes around RMBL, but is disappearing at lower altitudes ($\leq 2,900$ m) (Miller-Rushing & Inouye 2009). Bumble bee species including *Bombus flavifrons*, *B. frigidus*, and *B. balteatus* are the most frequent visitor to *M. ciliata*, although solitary bees and syrphid and muscid flies have also been observed as pollinators (Pelton 1961; Gallagher pers. comm. 2016). Recent research on *M. ciliata* showed that although pollinator visitation rate declined over the course of the season, seed set did not (Gallagher pers. comm. 2016). The diversity of pollinator species also decreased from early to late in the season, with bumblebees making up about 70% of pollinators early in the season and increasing to 100% of pollinators by the end. These findings suggest that changes in the pollinator community paired with differences in pollinator effectiveness between bumble bees and other pollinators could explain the constant seed set as visitation rate decreases.

In order to understand how changing flowering phenology might impact plant-pollinator interactions, we must first understand those interactions as they currently exist. This study investigates the importance of different pollinators to *M. ciliata* reproduction using the approach suggested by Ne'eman et al. (2010). According to this approach, contribution to plant reproductive success can be measured as the seed set produced by a single visit for each type of pollinator. Based on pollinator visitation rate, pollinator diversity, and seed set data collected last summer, we hypothesized that bumble bees will have increased pollinator effectiveness for *M. ciliata* relative to other visitors. We predicted that single visits by bumble bees will result in greater seed set per flower relative to other floral visitors. This study builds on previous research by investigating how the various pollinators differ in their contribution to seed set.

Methods

Study site

This research was conducted from June 27 to August 11, 2016 using a natural population of *M. ciliata* located north of the Rocky Mountain Biological Laboratory (latitude: 38°57'29" N, longitude: 106°59'06" W, altitude: 2,900 m a.s.l.). The first three weeks of observation were done at Rustler's Gulch and the subsequent two weeks of observation were done at Schofield Pass. Rustler's Gulch (2,992 m a.s.l) is a subalpine wet meadow in the Gunnison National Forest. Plants were located in two large, densely populated patches and two small, sparsely populated patches. Schofield Pass (3,263 m a.s.l) was used later in the season since *M. ciliata* flowers later there. Plants at Schofield pass were located in a mixed conifer forest in one densely populated patch and five small, sparsely populated patches. Plants at both sites received a mixture of shade and sun.

Pollinator effectiveness

We measured pollinator contribution to plant reproductive success by counting the seed set resulting from single pollinator visits. Flowers were enclosed in fine mesh jewelry bags prior to the corollas opening to ensure virgin stigmas. Two of the three plants were opened to pollination and the flowers were observed until at least one flower was visited by a single pollinator. Visitors were only counted as pollinators if they crawled inside of the corolla. The plants were then rebagged to prevent further pollinator visits. Pollinator identity, number of flowers available, number of flowers visited, visit length, observation duration, and nectar robbing behavior were recorded. For each 'single visit' ramet, two additional ramet with open flowers were bagged prior to pollinator observations to serve as controls. The first control remained bagged throughout the experiment to serve as control for self-pollination. If this group produced few or no seeds it would indicate that *M. ciliata* are unlikely to self-fertilize. The second control group was made up of flowers that were bagged prior to opening, were made available to pollination, but not visited, and then rebagged to serve as a control for observer error and self-pollination.

The visited flowers were marked with permanent marker for stigma collection, corolla measurements, and seed set collection. We harvested the stigmas of each visited flower, as well as one randomly selected flower from each open, un-pollinated control plant within a week of

pollination events. The stigmas were then stained with fuchsin gel (Kearns and Inouye 1993) and the slides were stored for the future counting of pollen grains to assess how the amount of pollen deposited varies by pollinator species. We measured corolla width at the opening of the tube and corolla length from the base of the calyx to a randomly chosen corolla lobe. Three corolla length and width measurements were taken from visited plants and averaged. When possible, the corollas of all visited flowers were measured, but oftentimes these had already fallen off at the time of measurement. Since trial studies had previously determined that less than 10% of corolla trait variance is within plants (Gallagher, unpublished data), we used the measurements of randomly selected flowers to characterize the entire plant. We counted the number of nutlets produced per flower (measured as described by Forrest and Thompson 2010) and collected and weighed the seeds as an indicator of seed quality and pollen quality. Seed set per flower for each plant was calculated as number of mature nutlets / number of flowers that made seed. The primary visitors of our *M. ciliata* plants were bumble bees and muscid flies (n=43, n=44). *Osmia* were also observed pollinating the plants (n=8), but for the purposes of this study we will focus only on the two primary visitors.

Statistical Methods

We analyzed the effect of pollinator identity and location on seed set using a linear model, which provided a better fit than other model distributions, as judged by a lower value for Akaike Information Criterion (AIC), with pollinator identity, including open and bagged controls as well as means for each type of pollinator, and site as predictors. Both site and its interaction with pollinator identity were found to be insignificant and were removed from the model for the sake of simplicity. To determine if there was a significant difference between the seed set of insect-pollinated plants and control plants and between bumble bee-visited plants and muscid fly-visited plants, we used generalized linear hypothesis test function in the multcomp package in R to set a priori contrasts (Hothorn et al. 2008). To provide a measure of how much a single visit by one type of pollinator contributes to the reproductive success of the plant relative to plants that, presumably, are not pollen limited, we used seed set of experimental plants, as well as seed set of open pollinated plants from adjacent phenology transects, to calculate pollinator effectiveness using an equation proposed by Spears (1983).

$$PE_i = \frac{(P_i - Z)}{(U - Z)}$$

P_i = mean number of seeds set / flower by a plant population receiving a single visit from species i

Z = mean number of seeds set / flower by a population receiving no visitation.

U = mean number of seeds set / flower by a population receiving unrestrained visitation.

All analyses were done using RStudio version 0.99.02. For models including interaction terms we calculated type III sums of squares using the *car* package in R (Fox and Weisberg 2011) and with a contrast setting that sets the row sum to zero. For models without interaction terms, we report type II analyses.

Timeline

Date	Activity
June 27- July 29	Pollinator observations, stigma collection, corolla trait measurements
August 1-4	Seed set collection and data analysis
August 4	Summer research symposium
August 5-15	Writing
August 15	Submit final paper

Results

Pollinator identity was a significant predictor of seed set per flower, and bumble bee single visits resulted in **higher** seed set per flower than muscid fly visits, bagged plants, and unbagged, unpollinated plants (ANOVA, $F_{3, 207} = 7.19$, $P < 0.0005$, Figure 2). Bumble visits produced 0.6 ± 0.16 (Mean $\pm$ SE) more seeds per flower than muscid fly visits (Table 1). Control plants also produced seed, contrary to expectations, but *a priori* contrasts showed that the seed set per flower was significantly higher for insect pollinated plants than for control plants ($T = -3.15$, $P = 0.002$). *A priori* contrasts also showed that there was a significant difference in seed set per flower between bumble bees and muscid flies ($T = 2.83$, $P = 0.005$). In addition to seed set, a direct measure of pollinator effectiveness was employed (Spears 1983). By this measure, bumble bees were 107% more effective than flies (Table 1).

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Discussion

This study suggests that bumble bees are more effective pollinators for *M. ciliata* than muscid flies. Single visits by bumble bees resulted in higher mean seed set than was produced by single visits by muscid flies. Pollinator effectiveness calculated from Spears' measure was also higher for bumble bees than muscid flies.

Our results are consistent with previous research showing differences in the pollinator effectiveness of bees and flies (Inouye et al. 2015). For two species of alpine wildflowers, bees transferred three times as much germinating pollen to *O. glandulosa* and ten times as much germinating pollen to *W. albomarginata* than *Allograpta* flies did (Bischoff et al. 2013). Bees were more effective pollinators than muscid flies for *Linum lewisii* in the Rocky Mountains (Kearns and Inouye 1994). However, both of these studies incorporated pollinator visitation rate in addition to pollinator effectiveness to assess overall pollinator performance. Bischoff et al. found that including pollinator visitation rates showed flies to be even less important to the plants' reproduction than was indicated by the pollinator effectiveness alone. Kearns and Inouye, on the other hand, found that higher visitation rates for flies than for bees made flies more important pollinators of *L. lewisii* than indicated by pollinator effectiveness alone. These studies show that while pollinator effectiveness is a good place to start, it hardly gives the whole picture of pollinator importance to plant reproductive success. Future studies on *M. ciliata* pollination should address pollinator visitation rates in order to adequately assess pollinator importance.

Differences in pollinator effectiveness that we observed may be attributed to morphological and behavioral differences between bumble bees and flies that make bumble bees better able to transfer pollen. Both queen and worker bumble bee visitors are larger than muscid flies, hoverflies, and osmia, allowing them to deposit more pollen on the stigma per visit (Willmer and Finlayson 2014, Inouye et al. 2015). Some bumble bees like *Bombus flavifrons*, which was a frequent visitor of our plants, use buzz pollination, in which they vibrate at a particular frequency that causes the anthers to release their pollen (Buchmann 1985). This likely allows them to gather more pollen from a single flower than flies can. Bumble bees are also known to use scent marks to avoid visiting flowers that have already been visited and depleted by themselves or another pollinator, which increases their foraging efficiency (Goulson 1999). Additionally, bumble bees can also target the most rewarding flowers by assessing the pollen content and age of flowers (Goulson 1999). Discriminating based on age and pollen content

means that bumble bees are more likely to carry pollen from flowers and to deposit pollen on receptive stigmas than flies, which are not known to discriminate.

However, flies have some advantages over bees. Although flies generally have a lower visitation rate than bees, they may be active in a wider range of conditions than bees, increasing their significance as pollinators (Inouye et al. 2015). Flies generally have a longer handling time than bumble bees, but it is possible that they spend time in corollas for warmth or to rest rather than foraging for pollen (Inouye et al. 2015). While bees transfer a higher number of mean pollen grains than flies, they transfer more heterospecific pollen, which does not contribute to a plant's reproductive success. Additionally, it has been observed that pollen deposition by bees declined more steeply for subsequent floral visits than did fly pollen deposition (Campbell 1985, Inouye et al. 2015). However, Pellmyr and Thompson (1996) found that [for bumble bees the seed set](#) remained constant for the first three flowers visited on a plant, suggesting that decreasing pollen deposition does not impact pollinator effectiveness for the first few flowers visited. In our study, bumble bees visited significantly more flowers in a single visit than flies ($F_{1,81} = 17.44$, $P < 0.001$). It is possible that decreasing pollen deposition impacted seed set per flower on bee-pollinated plants that had more than three flowers visited. It is also likely that at least some of the pollen transferred to the later visited flowers on the plant was self-pollen and did not contribute to the plant's reproductive success. [While *Mertensia ciliata* is self-compatible, plants are known to exhibit inbreeding depression in the form of lower seed set and seed mass \(Geber 1985\), therefore pollinators that deposit large quantities of self-pollen may be less effective at producing viable seeds.](#)

Contrary to our expectations both bagged and open, un-pollinated flowers made seed. The difference between seed set of open and bagged plants was marginally significant ($T = -1.84$, $P = 0.068$), suggesting that we transferred self-pollen to the stigmas in the process of opening and closing the bags of the open plants and indicating that *M. ciliata* is unlikely to self-pollinate under natural conditions. If seed set of bagged and open plants is primarily the result of self-pollination, we expect that the seeds collected from those flowers will be smaller and lighter than seeds produced by insect-pollinated flowers. Additionally, some of the bags may have been too loose around the stem, and we observed muscid flies and small beetles inside several bags, so seed set may have resulted from flies or other small insects that pollinated flowers while they were still bagged. Seed set on open, un-pollinated plants [may also](#) have resulted from pollination

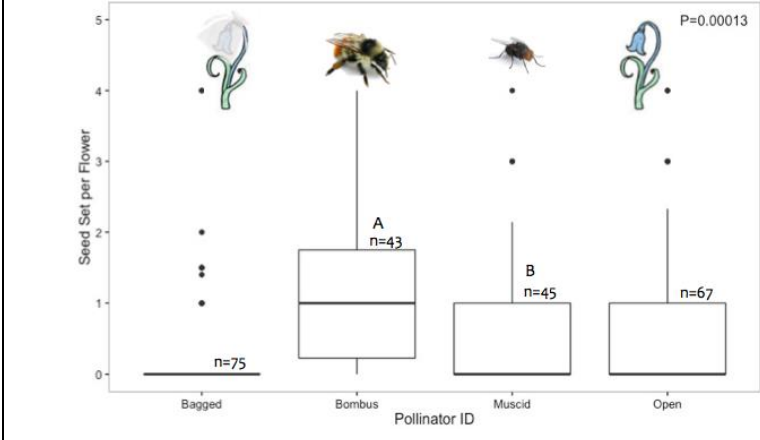
events that we failed to notice. Both osmia and muscid flies are quiet and small enough to fit inside a corolla without being easily noticed, so it is likely that some open plants were pollinated by insect visitors without our knowledge.

Climate change has already lead to reduced synchrony between bumble bee activity and community flowering timing and decreased bumble bee abundance in the Rocky Mountains (Pyke et al. 2016). This study found that for *M. ciliata*, bumble bees are more effective pollinators than flies. From these results, we postulate that declining bumble bee abundance and reduced temporal synchrony will hinder *M. ciliata* pollination and reproductive success, but more research into these potential effects is warranted.

Tables and Figures.

Table 1. Differences in pollinator effectiveness

	Seed set (Mean ±SE)	Spears' measure
Bombus sp.	1.20 ± 0.16	0.65
Muscid flies	0.58 ± 0.16	0.21



Seed Set per Flower

Pollinator ID

Bagged n=75

Bombus n=43

Muscid n=45

Open n=67

P=0.00013

A

B

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