ANTHROPOGENIC CHANGE, EFFECTS ON BEE POPULATIONS,

AND CONSEQUENCES FOR A SUB-ALPINE PLANT COMMUNITY

By

TREVOR ANTHONY LEDBETTER

_____

A Thesis Submitted to The Honors College

In Partial Fulfillment of the Bachelor of Science Degree
With Honors In

Ecology & Evolutionary Biology

THE UNIVERSITY OF ARIZONA

M A Y   2 0 1 7

Approved by:

_____

Dr. Judith Bronstein
Department of Ecology & Evolutionary Biology

Abstract

Declines in pollinator populations due to anthropogenic influences have been well documented in recent years. However, the effects that these declines have had on floral visitors that utilize floral resources in exploitative manners have received little to no attention. Not all floral visitors are pollinators. Some rob nectar, which may impose fitness effects that range from negative to positive. Some plant-pollinator systems have seen dramatic declines not only in pollinators, but in nectar robbers, in recent years. What are the consequences to plants of these declines? I studied consequences of such a decline in *Aquilegia caerulea*, the Colorado Columbine.

During the 1970s, the floral visitor community and nectar robbing rate of *A. caerulea* were documented by Miller (1978). *Bombus occidentalis*, the Western Bumblebee, acted at that time as both the major floral visitor and an active nectar robber. In the subsequent 45 years, many studies have reported declines in *B. occidentalis*, earning it an IUCN Vulnerable Red List classification. Building from this information, I quantified fitness effects of nectar robbing as well as differences in the floral visitor community to *A. caerulea* compared to past studies. I determined the effects and consequences of these changes, particularly in relation to nectar robbing, on the floral community. I also quantified the presence and ability of flies (Diptera), whose abundance has recently been exceptionally high, to act as pollinators in the absence of effective pollinators (bumble bees).

I found that the floral visitor community has changed drastically since the studies of Miller (1978). *Bombus occidentalis* was nearly absent from the system in 2016, resulting in severely reduced nectar robbing rates. Nectar robbing was found to have no significant effect on fruit set, while Diptera floral visitors, which dominated the system in 2016, significantly reduced fruit set below the self-pollination rate for the flower in the absence of bumble bees. As a whole, the absence of a nectar robber for this system is insignificant as nectar robbing has no influence on the reproductive success of the plant, however, in the absence of all bumble bees, Diptera, now a primary floral visitor, does have a significant, negative influence on reproductive success.

Introduction

Pollination is the process by which pollen from a flower's anthers is transferred to the stigma(s) of the same or a different flower, resulting in fertilization of one or more ovules, giving rise to one or more seeds. In most systems, pollination is mediated by an animal vector, where an individual visits a floral opening, coming into contact with floral reproductive structures, in search of floral resources. This visitation results in the transfer of pollen to the individual in exchange for a nectar or pollen reward, an interaction that is widely defined as a mutualism, in which both partners mutually benefit from the interaction (Bronstein 2001, 2006).

The availability of these rewards, however, creates a system in which exploitation can readily occur on both sides of the interaction. Exploiters obtain a benefit without a reciprocal benefit offered in return (Bronstein 2001). Within plant-pollinator systems, exploitation is widespread and common (Bronstein and Ziv 1997, Bronstein 2001, Schaefer and Ruxton 2009).

One of the most common of these exploitative interactions is nectar robbing. This is a behavior performed by certain floral visitors in which individuals with the mouthparts capable of cutting or piercing floral tissue create a hole in a flower to access nectar, bypassing the floral opening and reproductive structures in the process (Inouye 1980, Bronstein 2001, Irwin et al. 2010). This behavior, common in almost all plants with tubular flowers or nectar spurs, can have consequences for reproductive success. Nectar robbing impois typically detrimental to both female and male reproductive function (Irwin 2010). Although there are instances of increased reproductive success resulting from nectar robber visitation, such systems are rare (Higashi et al. 1988, Navarro 2000, Zhu et al. 2010). Other systems show no measurable effects of nectar robbing on plant reproductive success (Morris 1996, Rojas-Nossa et al. 2016, Heiling et al. in preparation).

Globally, plant-pollinator interactions involve over 500,000 species, with animals providing pollination services to >87.5% of all flowering species, together creating the scaffolds for increased ecological biodiversity and ecosystem services (NRC 2007, Burkle & Alarcon 2011, Ollerton 2011). In recent years, much attention has been given to anthropogenic influences within ecosystems and to global climate change as these influences have expanded and accelerated, resulting in significant impacts on pollinator communities across the planet (Hegland et al. 2009, Newbold et al. 2015, Burkle & Alarcon 2011, Ferreire at al. 2013). These impacts have resulted in nearly ubiquitous pollinator declines across all continents, primarily driven by climate change, landscape alteration, agricultural intensification, non-native species introductions, and the spread of pathogens (Potts et al. 2010, Hooke et al. 2012, Gonzalez-Varo et al. 2013, Ferreira et al. 2013).

Much work has been done to quantify the effects of pollinator declines on pollination services (Biesmeijer et al. 2006, Gallai et al. 2009, Potts et al. 2010). However, little consideration has been given to the effects of declines on species that rely on floral rewards such as nectar robbers. In my thesis research, I sought to quantify the fitness effects of nectar robbing as well as changes in the floral visitor community to *Aquilegia caerulea*, the Colorado Columbine, and determine the effects and consequences of these changes, particularly in relation to nectar robbing, on the floral community.

From 1969 to 1976, Russel Miller characterized the pollination ecology of *A. caerulea*, near the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado. He conducted detailed observations on the floral visitor community and characterized morphological features of the plant and flowers (Miller 1978). *Aquilegia caerulea* has five long nectar spurs and likely adapted to visitation by hawkmoths in the desert Southwest (Miller 1981). It has since expanded its distribution, becoming more dependent on pollination by pollen foraging bumble bees (Chase & Raven 1975, Miller 1981). Due to the length of the flower's nectar spurs, bees visiting *A. caerulea* must either forage for pollen, or nectar rob the flower to access nectar resources.

Miller (1978) recorded a high rate of nectar robbing in *A. caerulea,* from 43% up to 64% of nectar spurs. He also noted that *Bombus occidentalis*, the Western Bumblebee, was one of its primary floral visitors, but that *B. occidentalis* was also a frequent nectar robber, and that at times, the number of bees biting spurs outnumbered those foraging for pollen (Miller 1978).

In the 48 years since Miller began his observations, *B. occidentalis* has been greatly affected by anthropogenic influences. It has declined by 40.32% on average across its native range (Hatfield et al. 2015). Several studies have noted that *B. occidentalis* has declined in both range and persistence, and that its relative abundance is lower now than at any point in the past hundred years (Evans et al. 2008, Cameron et al. 2011, Hatfield et al. 2015).

Aquilegia caerulea provides an ideal system to ask the following questions:

1.) Has the floral visitor community of *A. caerulea* changed in the 45 years since it was originally characterized? I predict that the abundance and general visitor composition to *A. caerulea* will be significantly different from that of the 1970s;

2.) If there has been a change in the abundance or diversity of the visitor community to *A. caerulea*, how has this impacted natural nectar robbing rates? I predict that if abundance and diversity have decreased, then nectar robbing rates should also decrease;

3.) Is there a fitness consequence to nectar robbing in *A. caerulea* that could lead to a change in its reproductive success when the nectar robber is absent? I predict that if there is a negative fitness effect to nectar robbing, then a change in the visitor community that negatively affects *B. occidentalis* should be beneficial from the plant perspective; alternatively, if there is no net fitness effect to nectar robbing, then any change in the nectar robbing community should have no measurable effect on the reproductive success of *A. caerulea.*

Additionally, if all bee species are in decline, and/or overall floral visitor compositions are changing, a previously discounted set of visitors, Diptera, could become more influential in the reproductive success of *A. caerulea* in the absence of primary pollinators. Hence, I also asked: 4.) Can Diptera serve as effective pollinators? I predict that Diptera will have no significant effect on the reproductive success of *A. caerulea*.

Methods & Materials

Study Species

Aquilegia caerulea (Ranunculaceae), the Colorado Columbine, is a self-compatible, perennial herbaceous plant, distributed throughout the Rocky Mountains from southeastern Utah and northeastern Arizona to southern and central Colorado and throughout New Mexico, occurring at elevations from 2,100-3,700m (Miller 1978, Whittemore 1997, Brunet 2009). Miller (1978), working near RMBL, noted that *A. caerulea* bloomed approximately from July 1 – August 1. It appears that the flowering season has since advanced and lasts longer, from approximately June 20 – August 5 (Amy Iler unpubl. Data, pers. obs.). The flowers are radially symmetrical with five petals that alternate with five sepals. Petals are differentiated into an upper flattened lamina and an elongated spur, approximately 50mm in length, with a nectary located at the base of each spur (Miller 1978, 1981). Nectar production, which averages 3.9 μL/flower/day, begins well before the anthers mature and even before the flower opens, with unopened buds often containing substantial amounts of nectar (Miller 1978). The female reproductive organ consists of five to twelve unfused carpels, each capable of independently developing into a mature follicle, all of which is surrounded by approximately 50-130 stamens (Fig. 1 in Miller

1981, Brunet 1996, 2006). Plants produce new stalks each year, bearing one to >20 individual stalks, each bearing one to 15 flowers. Flowers open sequentially, beginning with distal flowers (Brunet 1996, 2009). Flowers are strongly protandrous with anthers dehiscing over 2-3 days, followed by stigma receptivity beginning 1-2 days later, which favors functional maleness between early and late flowers, reflected in poor late-season seed set (Brunet 1996).

Bumble bees and hawkmoths are the most effective pollinators (Miller 1978, 1981, Brunet 2009). Most bumblebee species can only forage for pollen on *A. caerulea* as they are unable to reach nectar at the bottom of the nectar spurs. *Bombus occidentalis*, however, can also rob the flower's nectar spurs by using its toothed mandibles to cut into the spur, circumventing the floral opening and the reproductive structures (Fig. 3 in Miller 1978).

Bombus occidentalis, is present throughout western North America, occurring throughout the western United States and southwestern Canada (Rao & Stephen 2007, Evans et al. 2008). Up to the mid-1990s, *B. occidentalis* was one of the most common bumble beesacross the West and since 1998, *B. occidentalis* has declined most dramatically throughout the coastal West, though is still found in isolated areas, primarily in the Rocky Mountains (Rao & Stephen 2007, Evans et al. 2008). Due to these declines, *B. occidentalis* was recently moved into the International Union for Conservation of Nature's (IUCN) Vulnerable Red List Category (Hatfield et al. 2015). *Bombus occidentalis* is easily recognized by its distinctive white pile on the apex of its abdomen (Roa & Stephen 2007).

Study Sites

Miller conducted his research in a meadow 5.6 km south of the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, in a spruce and aspen stand at RMBL, and in a rocky meadow near Emerald Lake, approximately 7.4 km north-northeast of RMBL. My study was conducted within a 10 km radius of Miller's. The majority conducted at a single site, 2.1 km north-northwest of RMBL, approximately 50m off-trail from the Mt. Avery trailhead (Avery Site – 38º 58' 33.94"N, 106º 59' 45.69"W). The site was an open field of mixed floral resources characterized by long-lived perennial herbs, flanked to the east by a large ridge, limiting direct sunlight exposure to approximately 3.5 hours after sunrise, and flanked to the north and west by a permanent, spring-fed stream. Supplementary data were gathered at two other sites in close proximity to RMBL, 1.5 km to the north (Judd Falls Site – 38º 58' 16.67"N, 106º 59' 46.64"W), and 2 km to the west in the lower Washington Gulch Valley (LWG Site – 38º 56' N, 107º 01' W). Floral communities of these sites, especially the Avery and Judd Falls sites, were similar. LWG contained a slightly more varied community, dominated by long-lived perennial herbs.

Floral Visitor Abundance & Composition

In order to determine if the floral visitor community of *A. caerulea* was different from that noted by Miller in the 1970s, floral visitor observations were made throughout the season at varying times of day. Observations were recorded with a digital voice recorder. From June 27th to July

26th, approximately 30 hours of observations were conducted at the Avery and LWG sites. I observed bumble bees during individual bouts, both on and between *A. caerulea* and among co-flowering species. Upon entering the view of the observer, a bout began. The visitor species, sex (when possible), plant species, number of flowers visited, and lengths of time for each visit within a bout were recorded. The bout concluded when the visitor left my field of view. The number of flies present on one to four floral reproductive structures per plant were also recorded, with the change in number of flies actively combing the reproductive structures during the observation period noted.

Natural Nectar Robbing Rate Surveys

In order to determine the rate of natural nectar robbing and whether it has changed since the 1970s, primary nectar robbing rates were quantified throughout the flowering season by recording damage caused by floral visitors that cut into the tissue of nectar spurs. Avery was surveyed approximately once weekly throughout the peak of the flowering season on July 7, 16, 21, and 27. LWG was surveyed twice at the peak of the flowering season on July 16 and 21. Judd Falls was surveyed only once on July 12, due to high rates of deer herbivory throughout the remainder of the flowering season. During surveys, plants were haphazardly selected and all nectar spurs on all flowers of selected plants were examined for robbing damage. Any spurs with more than two robbing holes were also recorded. The proportion of perforated spurs out of total spurs surveyed was calculated in the same manner as Miller (1978).

Artificial Nectar Robbing Manipulations

I haphazardly selected 30 plants near the onset of the flowering season. As flowers opened (June 27 to July 12), I applied the following treatments to flowers on each plant: *Control, High Robbing, and Low Robbing* . For plants with 3-6 visible buds, I manipulated 1 flower/treatment/plant. For plants with >7 visible buds, I manipulated 2 flowers/treatment/plant. Flowers in the *High Robbing* and *Low Robbing* treatments were experimentally robbed by cutting into the nectar spur(s) of a flower, approximately 1cm from the base, using dissecting scissors and withdrawing all available nectar using microcapillary tubes (Drummond Scientific, Broomall, PA, USA). This method of artificially robbing a nectar spur does not damage the reproductive structures of the flower, nor the nectary (Richman et al. 2017). Robbed spurs were re-robbed each day until flowers withered or fell off. Flowers in the *Control* treatment were squeezed lightly to control for effects due to squeezing required to remove nectar in the *High Robbing* and *Low Robbing* treatments, but were otherwise undisturbed. Due to high levels of herbivory by mule deer, all selected flowers were staked using a 16-gauge wire from flag markers and lightly tied along the stalk to ensure stability. Chicken-wire cages were also placed around each plant to further deter herbivory. Flowers were not covered during the experiment, allowing all visitors access to the floral opening as well as to the artificial nectar robbing holes. The total number of carpels within each flower was recorded once the petals and sepals fell away from the reproductive structures.

Once fruits had fully matured (July 27 to August 15), they were collected and dried in a drying oven at 50ºC. Fruits were later dissected, and the total number of mature follicles and viable seeds recorded. A follicle was considered mature if it contained at least one apparently mature seed. Mature seeds of *A. caerulea* appear dark green or blue-green in color. Fruit set was determined by dividing the number of mature follicles by the number of carpels.

Floral Visitor Exclusion Manipulations

In order to determine if flies are capable of providing pollination services in the absence of bumble bees, two exclusion manipulations were performed at the Avery site. I haphazardly selected 50 flowers from which to fully exclude all floral visitors, and another 40 flowers from which to exclude all visitors, except Diptera. Flowers were staked as described above. All flowers were bagged using a lightweight mesh bag large enough to accommodate each flower, with the stakes providing support to the bag. The bags were lightly tied at the base of the flower, around the stalk to prevent any unwanted visitation and were tied to prevent the bag from touching the flower. Flowers from which all floral visitors were excluded were bagged prior to opening and the bags were not removed until the floral structure had fallen way. Flowers that were exposed to Diptera were observed to ensure only Diptera visitations occurred, at times in which bumble bees and other visitors were scarce but Diptera were common, typically throughout the morning hours. Flowers from which all floral visitors were excluded opened between July 13 to July 19, while flowers from which just bees were excluded (Diptera allowed to visit) opened between July 13 to July 16.

As with the nectar robbing manipulations, the number of carpels were recorded once the floral structure had fallen away. At the end of the reproductive season when fruits had fully matured (August 15 to August 16), fruits were collected, dried, and processed as described above.

Statistical Analyses

I calculated fruit set at the flower level, quantifying the ovule to carpel ratio. I used this response variable to test whether artificial nectar robbing manipulations resulted in differential fruit using one-way ANOVA, with treatment as the explanatory variable. To test whether Diptera could act as effective pollinators, I calculated fruit set as described above, and performed a one-way ANOVA, using treatment as the explanatory variable. All analyses were performed using R version 3.3.1 (*R Core Team 2016).

Results

Floral Visitor Abundance & Composition

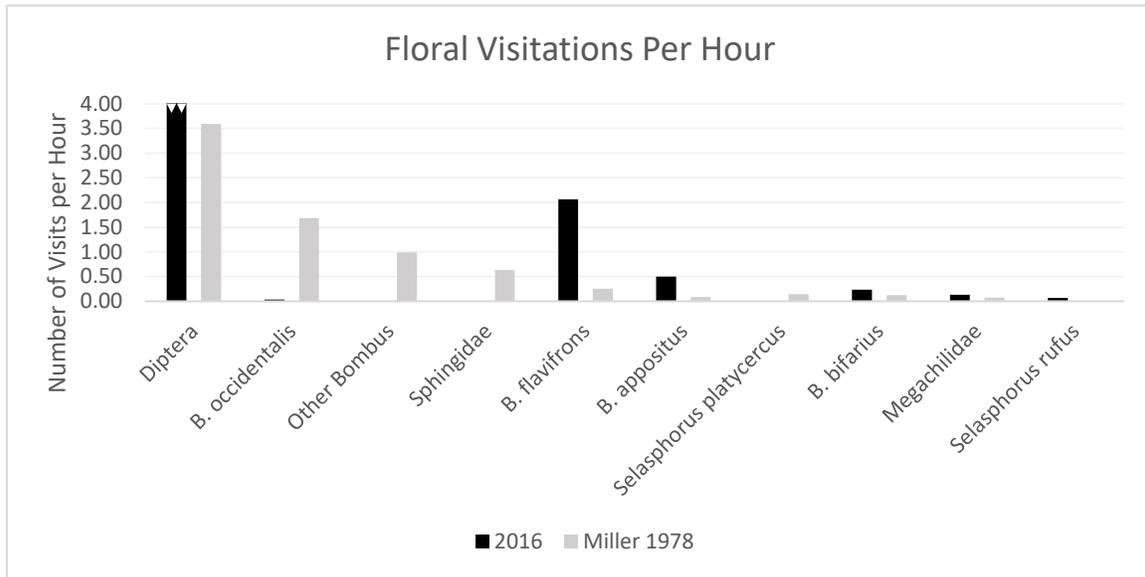*Is the floral visitor community of A. caerulea from when it was originally characterized 45 years ago?*

Figure 1: Total abundance of different floral visitors to A. caerulea as seen during the 2016 flowering season and as adapted from Miller 1978, standardized to number of visitations per hour of observeation. Approximately 30 hours of observations were conducted in 2016 and 95 hours were conducted across 1969, 1970, 1971, and 1976.

The floral visitor community of *A. caerulea* has clearly changed in both abundance and diversity between Miller (1978) and this study. One of the most dramatic changes since 1978 has been the drop in abundance of *B. occidentalis* by 98% from 1.68 visits/hr in Miller (1978) to 0.03 visits/hr 2016 (Figure 1). The 2016 observation was of a single individual foraging for pollen and whose visit lasted just 10 seconds. However, Diptera visits rose by 408%, from 3.59/hr in Miller (1978) to 14.63/hr in 2016. Visits by *B. flavifrons, B. appositus, B. bifarius*, and Megachilidae all rose as well compared to 1978, with *B. flavifrons* increasing the most, from 0.25 visits/hr in Miller 1978 to 2.07 visits/hr in 2016.

All other major visitors decreased in abundance and diversity from 1978 to 2016. No Sphingidae (hawkmoths) were observed, in comparison to 0.67/visits hour reported by Miller. Other bumble bee species, (*Bombus centralis, B. frigidus, B. kirbyellus, B. mixtus, B. rufocinctus,* and *B. sylvicola*) similarly were absent in 2016. Hummingbird visitation (*Selasphorus platycercus* and *S. rufus*) declined overall, from 0.15 visits/hr in Miller (1978) to 0.07 visits/hr in 2016.

Natural Nectar Robbing Rate Surveys

If there has been a change in the abundance or diversity of the visitor community to A. caerulea, how has this impacted natural nectar robbing rates?
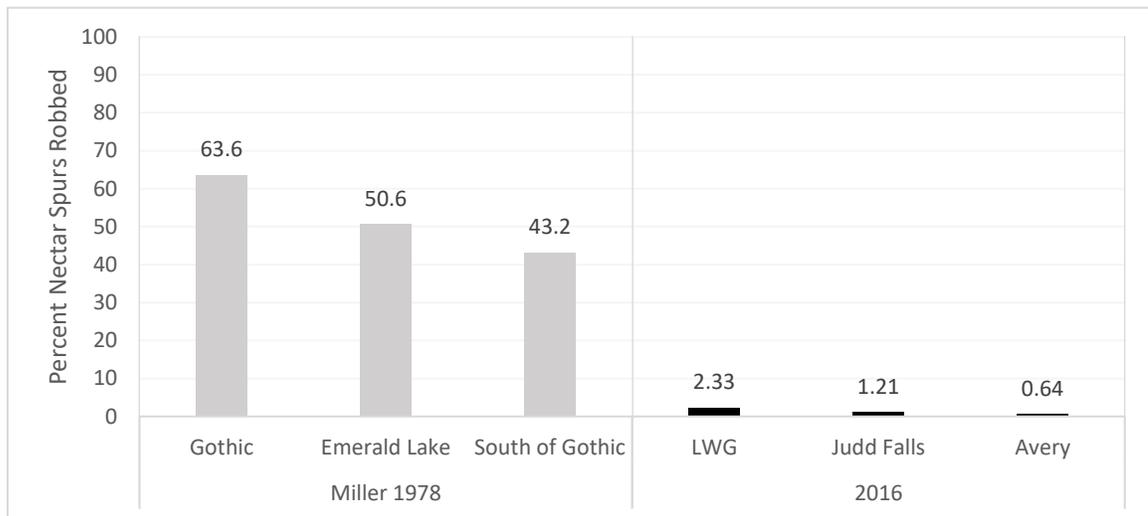

Figure 2: Percent robbing levels across sites during the 2016 flowering season and as redrawn from data reported in Miller 1978. Except for the 2016 Lower Washington Gulch (LWG) site, all sites in both studies occurred within the East River Valley near RMBL.

The reduced floral community, including known nectar robbers, was accompanied by a lower nectar robbing rate. Miller (1978) reported the robbing rate in *A. caerulea* to range from 43% (216 of 500 nectar spurs surveyed robbed) in a meadow south of Gothic (RMBL) to 64% (318 of 500) in Gothic, to 51% (252 of 500) at Emerald Lake (Figure 2). In 2016, the robbing rate was dramatically lower. It ranged from 0.64% (11 of 1836) at Avery to 1.21% (10 of 654) at Judd Falls to 2.33% (8 of 394) at Lower Washington Gulch (LWG). No bees were observed robbing *A. caerulea* in 2016. In comparison, Miller (1978) noted that at times, spur-biting by *B. occidentalis* could be more commonly seen than pollen foraging in *A. caerulea* populations.

Artificial Nectar Robbing Manipulations

Is there a fitness consequence to nectar robbing in A. caerulea that could alter the reproductive success of the plant in the absence of a nectar robber?
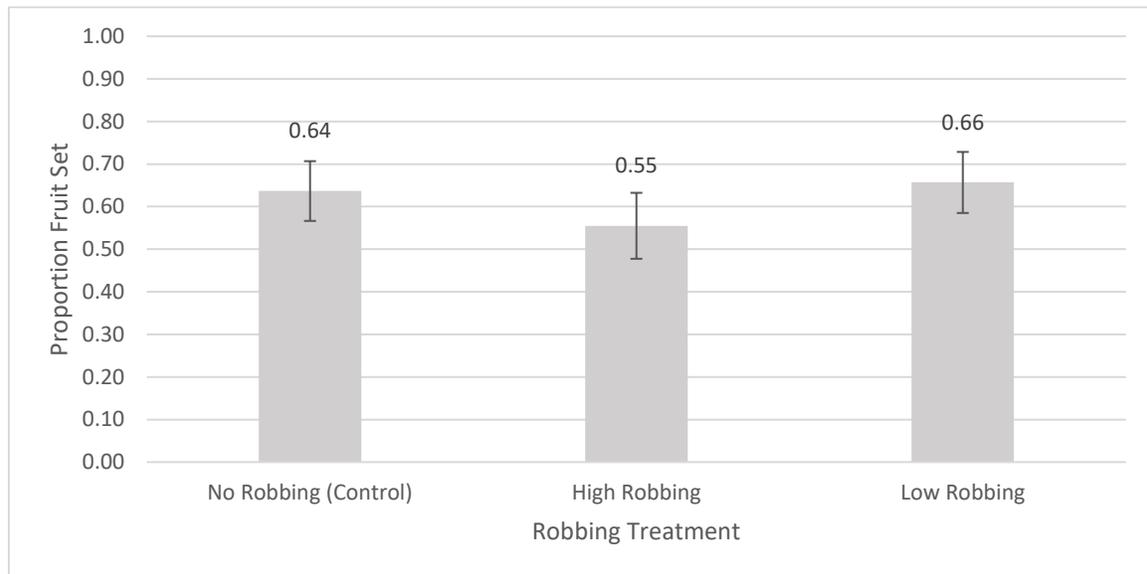

Figure 3: The effect of robbing treatments on fruit set across two artificial robbing treatment levels and a control (n=35). There was no significant difference in fruit set across treatments or compared to the control; ANOVA: F=0.538, P=0.585. Vertical bars represent ±1 S.D.

The manipulative experiments revealed no significant reproductive costs or benefits of nectar-robbing. Fifty-five percent of flowers within the high nectar robbing treatment set fruit, compared to 66% within the low nectar robbing treatment (Figure 3). Fruit set did not differ significantly between the high and low nectar robbing manipulations, nor between either treatment and the control (ANOVA: F=0.538, P=0.585).
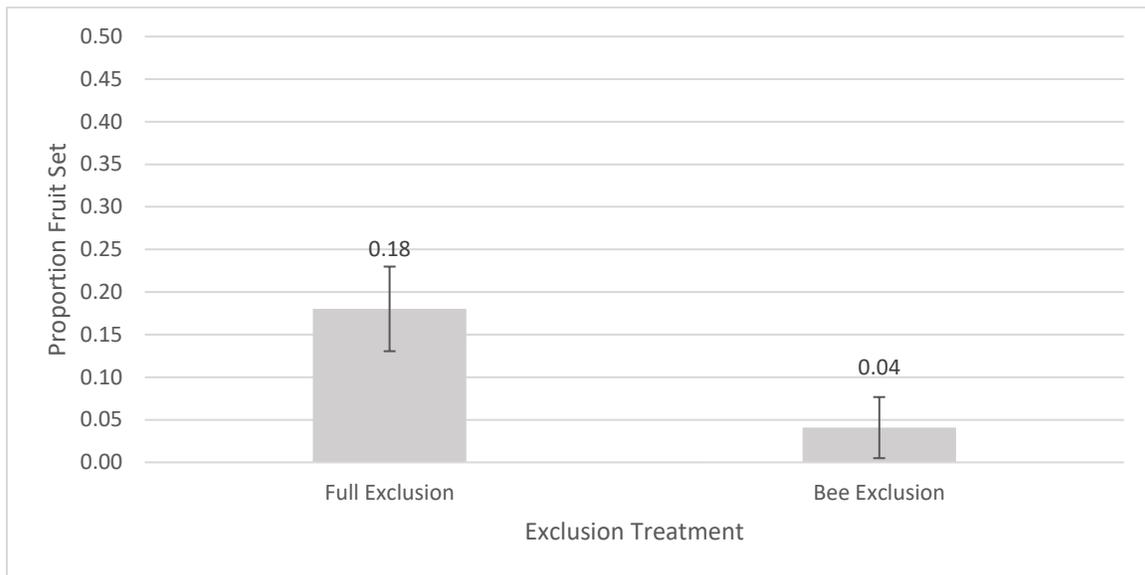
Can Diptera, serve as effective pollinators?

Figure 4: The effect of exclusion treatments on fruit set. There was a significant difference between the two treatments. ANOVA: F=4.185, P=0.0446. Vertical bars represent ±1 S.D.

Diptera did not serve as effective pollinators. Rather, their visits lowered reproductive success in the absence of bee visits. Eighteen percent of flowers within the full exclusion manipulation set fruit, whereas within the bee exclusion manipulation, in which only Diptera species were allowed to visit, fruit set was significantly lower, falling to 4% (Figure 4); ANOVA: F=4.185, P=0.0446).

Discussion

Floral Visitor Abundance & Composition

The floral visitor community of *A. caerulea* near Gothic, Colorado is drastically different now as compared to that reported by Miller (1978). Diversity and abundance of bumble bees, hawkmoths, and hummingbirds are all lower, while Diptera have increased greatly. For *B. occidentalis,* the noted change of 98% is of special interest in the context of its range-wide decline.

Brunet (2009) conducted a series of pollinator observation in six *A. caerulea* populations over 1-4 years (distributed across 2000-2005), measuring visits/flower/hr, totaling 438 hours. These

sites were located in southeastern Utah (Cedar Breaks, Archery), northern Arizona (Original), northern Colorado (Falls Creek), and central Colorado near RMBL (Emerald Lake, Flat). The Emerald Lake population is near to where Miller completed his work, in close proximity to the Avery site in this study. In this work, Brunet spoke to a similar trend of *B. occidentalis* decline, reporting that prior to 2001, *B. occidentalis* was the most common bumble bee visitor to *A. caerulea* populations in central Colorado and all other populations observed. However, she reports that after 2001, *B. flavifrons* became the dominant bumble bee visitor, with *B. occidentalis* not being observed after 2001. Again, this decline in central Colorado coincides with *B. occidentalis'* decline throughout the western United States (Evans et al. 2008, Cameron et al. 2011, Hatfield et al. 2015).

It is likely that a portion of the drastic change in *B. occidentalis* abundance reported here is due in part to spatial and temporal variation. However, it is likely that a majority of this change can be attributed to the documented range-wide decline of *B. occidentalis*.

It is important to note that the number of hours of floral visitation observations was different between Miller 1978 and this study. Miller collected 95 hours of observations over several years, while I collected 30 hours over a single year. Yet, I still observed a much higher number of visits by Diptera, representing nearly a third the total observation time. This, in conjunction with the overall declines in nearly every other visitor category, has resulted in a marked shift in the visitor community, from one dominated by bumble bees, hawkmoths, solitary bees, etc. (53% of visitors) to one dominated by Diptera (83% of visitors).

Both nectar robbing and floral visitation vary in space and time in *A. caerulea,* as in most plant-pollinator systems (Brunet 2009, Cuevas & Rosas-Guerrero 2015). However, the consistent presence of *B. occidentalis* across all four study years as the primary floral visitor at all three sites in Miller 1978 and in Brunet (1996) and (2009) up to 2001 indicate that *B. occidentalis* was indeed common and an important floral visitor to *A. caerulea* up to its decline in the late 1990s and early 2000s, whereas Brunet (2009) noted its absence thereafter, in line with my observations of its continued absence.

The 14.63 visits/hr I found in 2016 drastically higher than either Brunet or Miller. Brunet (2009) noted that the presence and abundance of Diptera was not predictable in any of her study populations for any geographical area, including central Colorado near RMBL. However, her observations more closely align with those of Miller (1978) for Diptera, ranging from around 2.0 visits/hr to less than 0.25 visits/hr, than with my own. It is possible that 2016 was an exceptional year for Diptera, with several senior scientists at RMBL noting that 2016 was the most abundant year for them in recent memory.

It is also important to note that the apparent change in Sphingidae visitation is consistent with previous observations that hawkmoths, particularly *Hyles lineata*, are highly variable, fluctuating sharply from year to year and even week to week within a given season (Miller 1978, Brunet 2009, Diane Campbell, *pers. comm.*). *Hyles lineata* breeds in the semiarid and desert regions of the Southwest and their presence in montane and subalpine habitats of central Colorado is transitory (Miller 1981). *Bombus occidentalis* and other bumble bee species, however, breed and live near to where they are observed. Therefore, changes in their abundance are more likely to reflect direct local in climate and vegetation.

Nectar Robbing Decline

Unsurprisingly, since *B. occidentalis* was nearly absent, nectar robbing rates across all three sites studied were dramatically lower than those reported by Miller (1978). With no other nectar robber in the system observed during this study, the low level of nectar robbing observed is likely due to rare and unobserved *B. occidentalis* visits. It is possible that *B. mixtus* could also be performing a very low level of primary robbing, as it has recently discovered to be capable of primary nectar robbing (R.E. Irwin pers. obs.). However, in caged trials, *B. mixtus* caught on flowers co-occurring with *A. caerulea* failed to forage for either pollen or nectar on *A. caerulea* (data not shown). This remained true even when artificial holes were made in spurs to encourage secondary nectar robbing. It is possible that the nectar spur of *A. caerulea* is too thick to permit robbing by *B. mixtus*, which is much smaller than *B. occidentalis*. This suggests that *B. mixtus* may be naïve to the nectar resources available in *A. caerulea*.

Fitness Consequences of the Decline in Nectar Robbing

The change in abundance of *B. occidentalis* and associated drop in nectar robbing rates might have been expected to benefit *A. caerulea,* as nectar robbing often results in negative fitness effects (Irwin 2010). However, *A. caerulea* apparently experiences no significant effect on reproductive fitness from nectar robbing, even at the highest levels possible. This is perhaps not surprising as the primary, effective visitors to this flower, bumble bees, are visiting *A. caerulea* for its pollen, rather than its nectar, Consequently, we hypothesize that nectar robbing should have little to no bearing on the success, and therefore on the behavior, of pollen foragers. However, it is possible that in a year with high hawkmoth presence, or in populations of *A. caerulea* where nectar robbers and hawkmoths are both abundant, it may be possible to see some fitness reduction due to competition between nectar collecting species. Extreme rarity of hawkmoth visits to the region the year of the study prevented me from testing this hypothesis, however, it remains an intriguing open question.

Are flies alternative pollinators for A. caerulea?

Miller (1978) reported the presence of groups of smaller insects, including Diptera, on *A. caerulea* flowers. He noted that they were likely responsible for some level of pollination, but were probably relatively unimportant as they do not approach bumble bees and hawkmoths in size, foraging speed, or efficiency in pollen transfer. However, with the drastic shift toward a Diptera dominated visitor community, it seemed reasonable to conjecture that they contribute to overall plant fitness more now than in the past.

The results allow me to reject that hypothesis. In the absence of pollinators, Diptera had a negative, not positive impact upon plant reproductive success. In fact, they reduce fruit set to nearly zero, significantly lower than in the entire absence of visitors. We propose that this is because they remove pollen that would otherwise contribute to self-pollination. Diptera individuals typically sit on the reproductive structures of a flower, combing the anthers and

stigma, gathering pollen to eat, rarely moving between flowers. They also meticulously comb their bodies and legs, removing pollen from their bodies. This should reduce pollination when an individual does move between flowers.

Thus, in *A. caerulea*, Diptera essentially act as pollen thieves, floral visitors that collect pollen in such a way that pollination is impeded without floral tissue damage (Inouye 1980). It is probable that in this system, they function as "ugly" pollinators: they contribute to high pollen removal rates, but fail to deliver pollen to other flowers (Thomson and Thomson 1992).

Conclusions

It is clear that the floral visitor community of *A. caerulea* in the area surrounding Gothic, Colorado is dramatically different now that in was in the 1970's, from one dominated by bumblebees and hawkmoths, to one dominated by Diptera. Likewise, the primary robber within this system, and the primary floral visitor to this plant, has declined to almost zero in this region, resulting in markedly lower nectar robbing rates. However, nectar robbing appears to have no significant effect on fruit set for this plant, indicating that from the plant's perspective, losing its primary floral visitor and nectar robber, *B. occidentalis*, may be having no effect.

In spite of this, the complete decline of primary pollinators, largely bumble bees, would be detrimental for this plant. Diptera, which are highly abundant, provide no pollination service. Instead, they remove large amounts of pollen from flowers and do not transfer it, lowering fruit set to below that which the flowers could achieve through self-pollination. Under a worst-case scenario of complete removal of pollinator services to the system, Diptera could speed up the contraction of *A. caerulea* distribution into ranges where alternative, effective pollinators are present. These results should be applied to all future studies in which Diptera represent a large portion of floral visitors within a system. Their influences are clearly larger than many previous studies, including that of Miller (1978), have suggested.

What happens when a system loses a nectar robber that reduces fruit set, and how does this contribute to the larger picture of pollinator declines? Future work should explore this question in systems in which (unlike *A. caerulea*) it is known that nectar robbing imposes negative reproductive fitness effects. As plant-pollinator interactions experience impacts from anthropogenic effects, these influences could become more significant. Biesmejijer et al. (2006) proposed that in order to demonstrate a decline in pollinator services, one must demonstrate 1) declines in pollinator density, and/or 2) reduction in species diversity or shifts in visitor composition, and 3) declines in reproductive success or abundance of the plant species. At the moment, it does not appear that *A. caerulea* has seen a significant decline in pollinator services. However, we have demonstrated a decline in pollinator density (bumble bees), a reduction in floral visitor species diversity, and a shift in vistor composition, to a community now dominated by Diptera. Future work should investigate whether there has been a significant decline in the reproductive success or range of *A. caerulea*, particularly in areas closer in proximity to anthropogenic influences.

Acknowledgements

References

Biesmeijer, J. C., Roberts, S. P., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers, A. P., Potts, S. G., Kleukers, R., Thomas, C. D., Settele, J., & Kunin, W.E. (2006). Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science*, 313(5785), 351-354.

Bronstein, J. L. (2001). The exploitation of mutualisms. *Ecology Letters*, 4(3), 277-287.

Bronstein, J. L., & Ziv, Y. (1997). Costs of two non-mutualistic species in a yucca/yucca moth mutualism. *Oecologia*, 112(3), 379-385.

Bronstein, J. L., Alarcón, R., & Geber, M. (2006). The evolution of plant–insect mutualisms. *New Phytologist*, 172(3), 412-428.

Brunet, J. (1996). Male reproductive success and variation in fruit and seed set in *Aquilegia caerulea* (Ranunculaceae). *Ecology*, 77(8), 2458-2471.

Brunet, J. (2009). Pollinators of the Rocky Mountain columbine: temporal variation, functional groups and associations with floral traits. *Annals of Botany*, 103, 1567-1578.

Burkle, L. A., & Alarcón, R. (2011). The future of plant–pollinator diversity: understanding interaction networks across time, space, and global change. *American Journal of Botany*, 98(3), 528-538.

Cameron, S. A., Lozier, J. D., Strange, J. P., Koch, J. B., Cordes, N., Solter, L. F., & Griswold, T. L. (2011). Patterns of widespread decline in North American bumble bees. *Proceedings of the National Academy of Sciences*, 108(2), 662-667.

Chase, V. C., & Raven, P. H. (1975). Evolutionary and ecological relationships between *Aquilegia formosa* and *A. pubescens* (Ranunculaceae), two perennial plants. *Evolution*, 29(3), 474-486.

Cuevas, E., & Rosas-Guerrero, V. (2016). Spatio-temporal variation of nectar robbing in *Salvia gesneriflora* and its effects on nectar production and legitimate visitors. *Plant Biology*, 18(1), 9-14.

Evans, E., Thorp, R., Jepsen, S., & Black, S. H. (2008). Status review of three formerly common species of bumble bee in the subgenus *Bombus*. *The Xerces Society*, 1-63.

Ferreira, P. A., Boscolo, D., & Viana, B. F. (2013). What do we know about the effects of landscape changes on plant–pollinator interaction networks? *Ecological Indicators*, 31, 35-40.

Gallai, N., Salles, J. M., Settele, J., & Vaissière, B. E. (2009). Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics*, 68(3), 810-821.

González-Varo, J. P., Biesmeijer, J. C., Bommarco, R., Potts, S. G., Schweiger, O., Smith, H. G., Steffan-Dewenter, I., Szentgyorgyi, H., Woyciechowski, M., & Vilà, M. (2013). Combined effects of global change pressures on animal-mediated pollination. *Trends in Ecology & Evolution*, 28(9), 524-530.

Hatfield, R., Jepsen, S., Thorp, R., Richardson, L., Colla, S. & Foltz Jordan, S. (2015). *Bombus occidentalis*. *The IUCN Red List of Threatened Species 2015*.

Hegland, S. J., Nielsen, A., Lázaro, A., Bjerknes, A. L., & Totland, Ø. (2009). How does climate warming affect plant-pollinator interactions? *Ecology Letters*, 12(2), 184-195.

Higashi, S., O'Hara, M., Arai, H., & Matsuo, K. (1988). Robber-like pollinators: overwintered queen bumblebees foraging on *Corydalis ambigua*. *Ecological Entomology*, 13(4), 411-418.

Hooke, R. L., Martín-Duque, J. F., & Pedraza, J. (2012). Land transformation by humans: a review. *GSA Today*, 22(12), 4-10.

Inouye, D. W. (1980). The terminology of floral larceny. *Ecology*, 61(5), 1251-1253.

Irwin, R. E., & Maloof, J. E. (2002). Variation in nectar robbing over time, space, and species. *Oecologia*, 133(4), 525-533.

Irwin, R. E., Bronstein, J. L., Manson, J. S., & Richardson, L. (2010). Nectar robbing: ecological and evolutionary perspectives. *Annual Review of Ecology, Evolution, and Systematics*, 41, 271-292.

Irwin, R. E., Howell, P., & Galen, C. (2015). Quantifying direct vs. indirect effects of nectar robbers on male and female components of plant fitness. *Journal of Ecology*, 103(6), 1487-1497.

Miller, R. B. (1978). The pollination ecology of *Aquilegia elegantula* and *A. caerulea* (Ranunculaceae) in Colorado. *American Journal of Botany*, 65(4), 406-414.

Miller, R. B. (1981). Hawkmoths and the geographic patterns of floral variation in *Aquilegia caerulea*. *Evolution*, 35(4), 763-774.

Morris, W. F. (1996). Mutualism denied? nectar-robbing bumble bees do not reduce female or male success of bluebells. *Ecology*, 77(5), 1451-1462.

Navarro, L. (2000). Pollination ecology of *Anthyllis vulneraria* subsp. vulgaris (Fabaceae): nectar robbers as pollinators. *American Journal of Botany*, 87(7), 980-985.

Newbold, T., Hudson, L. N., Hill, S. L., Contu, S., Lysenko, I., Senior, R. A., ... & Day, J. (2015). Global effects of land use on local terrestrial biodiversity. *Nature*, 520(7545), 45-50.

Ollerton, J., Winfree, R., & Tarrant, S. (2011). How many flowering plants are pollinated by animals? *Oikos*, 120(3), 321-326.

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution*, 25(6), 345-353.

Rao, S., & Stephen, W. P. (2007). *Bombus* (Bombus) *occidentalis* (Hymenoptera: Apiformes): in decline or recovery. *Pan-Pacific Entomologist*, 83(4), 360-362.

Richman, S. K., Irwin, R. E., Nelson, C. J., & Bronstein, J. L. (2017). Facilitated exploitation of pollination mutualisms: fitness consequences for plants. *Journal of Ecology*, 105(1), 188-196.

Rojas-Nossa, S. V., Sánchez, J. M., & Navarro, L. (2015). Effects of nectar robbing on male and female reproductive success of a pollinator-dependent plant. *Annals of Botany*, 117, 291-297.

Schaefer, H. M., & Ruxton, G. D. (2009). Deception in plants: mimicry or perceptual exploitation?. *Trends in Ecology & Evolution*, 24(12), 676-685.

Thomson, J.D., & Thomson, B.A. (1992). Pollen presentation and viability schedules in animal-pollinated plants: consequences for reproductive success. In Ecology and Evolution of Plant Reproduction (ed. R. Wyatt), pp. 1–25.

Zhu, X. F., Wan, J. P., & Li, Q. J. (2010). Nectar robbers pollinate flowers with sexual organs hidden within corollas in distylous *Primula secundiflora* (Primulaceae). *Biology Letters*, online.