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WHAT ARE THE PLANT REPRODUCTIVE CONSEQUENCES OF LOSING A NECTAR ROBBER?

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Abstract—Pollinator declines worldwide are having strong negative consequences for plants. In many communities, antagonistic flower visitors, including nectar robbers, have likely declined in abundance as well. Given the negative effects that these visitors can sometimes inflict, might declines in their populations benefit plants? During the 1970s, the floral visitor community of the Colorado columbine, *Aquilegia caerulea* (Ranunculaceae), was documented near Gothic, Colorado. At that time, *Bombus occidentalis*, the Western Bumble bee, was one of its many pollinators, but more commonly acted as its only known nectar robber. *Bombus occidentalis* abundance has declined precipitously throughout the Western USA since the 1970s. In 2016, we documented the floral visitor community in sites near to those used in the original survey. We then experimentally quantified the effects of nectar robbing, allowing us to estimate the reproductive consequences of losing *B. occidentalis*. We also quantified the potential pollination services of muscid flies (Muscidae, Diptera). The floral visitor community was dramatically different in 2016 compared to the 1970s. *Bombus occidentalis,* a frequent *A. caerulea* visitor from 1969-1976, was infrequently observed visiting the plant, and nectar robbing was negligible. Our experiments suggested that a high level of nectar robbing would lead to significantly reduced fruit set, although not seeds per fruit. Fly visits to flowers were dramatically higher in 2016 compared to the 1970s. We show that, in the absence of bumble bee pollinators, muscid flies significantly reduced fruit set below the self-pollination rate. The negative effect of the increase in these flies likely outweighed any positive effects *A. caerulea* experienced from the absence of its nectar robber. Although the field observations were conducted in a single year, when they are interpreted in combination with our manipulative experiments, they suggest how *A. caerulea* may fare in a changing visitation landscape.

Keywords—*Bombus occidentalis*, bee declines, nectar robbing, floral larceny, *Aquilegia caerulea*, Muscidae

INTRODUCTION

Plant-pollinator interactions involve over 500,000 species worldwide. Animals provide pollination services to > 87.5% of all flowering species, creating a scaffold for biodiversity and ecosystem services (National Research Council 2007, Burkle & Alarcón 2011, Ollerton et al. 2011). In recent years, anthropogenic influences on the environment have expanded and accelerated, resulting in significant impacts on pollinator communities, including pollinator declines across all continents (Hegland et al. 2009: Burkle & Alarcón 2011; Ferreira et al. 2013; Newbold et al. 2015; Irwin et al. 2020).

Extensive work has been done to quantify how anthropogenically mediated pollinator declines affect plant reproductive success (Biesmeijer et al. 2006; Gallai et al. 2009; Potts et al. 2010). In contrast, minimal consideration has been given to the effects of anthropogenic change on the relationship

between plants and the many species that use flowers but that do not serve as effective pollinators. Strong negative effects of introduced floral antagonists have been measured in a few native plant species (e.g., Dohzono et al. 2008, Chalcoff et al. 2022); conversely, Mackin et al. (2021) have experimentally documented costs of native antagonists on an introduced plant species. Some antagonists are clearly shifting in abundance and phenology as climates warm, with effects postulated on the plants whose flowers they attack (Liu et al. 2011, Hamaan et al. 2021). Here, we investigate a previously unexplored potential consequence of anthropogenic impacts on pollination systems: what would be the consequences for a plant of losing some or all of its floral antagonists? In particular, would it experience elevated reproductive success as a consequence?

One of the most common exploitative interactions in pollination systems is nectar robbing, a form of floral larceny, whereby animals feed on nectar via holes made in flowers (Inouye 1980), often bypassing the anthers and stigmas (Irwin et al. 2010). Primary robbers make the holes through which they then feed; secondary robbers feed through robbing holes that are already present (Inouye 1980, Irwin et al. 2010). Common in almost all plants with tubular flowers or nectar spurs, nectar robbing can have significant consequences for reproductive success. In some well-studied systems, robbing is detrimental to both female and male reproductive function (Irwin et al. 2010). In other systems, female function alone is affected (Richman et al. 2018). Increased reproductive success resulting from nectar robbing has also been reported, although this appears to be less common than documented negative consequences (Higashi et al. 1988; Navarro 2000; Zhu et al. 2010).

From 1969 to 1976, Miller (1978, 1981) studied the pollination ecology of the Colorado Columbine, *Aquilegia caerulea* (Ranunculaceae), near Gothic, Colorado, USA. He noted that *Bombus occidentalis*, the Western Bumble bee, was one of its more common pollinators, but also a frequent primary nectar robber, with the number of bees acquiring nectar by biting through spurs sometimes outnumbering those foraging legitimately. Miller (1978) recorded a high rate of

nectar robbing in this species (43-64% of nectar spurs exhibiting robbing holes).

In the decades since Miller (1978) began his observations, *B. occidentalis* has been greatly affected by anthropogenic stressors (*e.g.,* Jacobson et al. 2018; Graves et al. 2020). Once one of the most common bumble bees across the American West, it has declined by 40% on average across its native range (Hatfield et al. 2015). Its range has shrunk by 93% in the past 21 years (Graves et al. 2020). Although *B. occidentalis* is still found in isolated areas, primarily in the Rocky Mountains (Rao & Stephen 2007; Evans et al. 2008) and remains a common bumble bee species in some years and sites near Gothic, Colorado, USA (Irwin et al. 2018), its abundance across much of its range is lower now than at any point in the past hundred years (Evans et al. 2008; Cameron et al. 2011; Hatfield et al. 2015). As a consequence, *B. occidentalis* has been moved into the International Union for Conservation of Nature's (IUCN) Vulnerable Red List Category (Hatfield et al. 2015).

We took advantage of extremely low visitation of *B. occidentalis* near Gothic, Colorado in 2016 to explore the consequences for *A. caerulea* of potentially losing this pollinating and nectar-robbing associate. We addressed the following questions. (1) How did the floral visitor community of *A. caerulea* in 2016 differ from the one Miller (1978) reported from 1969-1976? (2) Was the observed change in *A. caerulea*'s visitor community accompanied by a change in the rate of nectar robbing? (3) Does *A. caerulea* suffer reduced reproductive success from nectar robbing? If nectar robbing leads to reduced reproductive success in this species, then declines in robbing are potentially beneficial. In the course of this study, we noted extremely high densities of muscid flies (Muscidae spp., Diptera) on *A. caerulea*. We therefore also asked (4) Do flower-visiting Diptera, which were infrequent floral visitors at the study site in the 1970's but extremely common in 2016, transfer *A. caerulea* pollen, such that they might be able to compensate for a lack of visitation by other pollinators?

MATERIALS AND METHODS

STUDY SPECIES

Colorado blue columbine (*Aquilegia caerulea*, Ranunculaceae) is a self-compatible, perennial

herbaceous plant distributed in the USA throughout the Rocky Mountains, from southeastern Utah and northeastern Arizona to southern and central Colorado, as well as throughout New Mexico, occurring at elevations from 2100 – 3700 m (Miller 1978; Whittemore 1997; Brunet 2009). Miller (1978), working near the Rocky Mountain Biological Laboratory (RMBL) in Gothic, Colorado, noted that it bloomed approximately from 1 July – 1 August. Like many other species in the region (CaraDonna et al. 2014, CaraDonna and Inouye 2015), its flowering season has since advanced and now lasts longer, from approximately 20 June – 5 August (A. Iler, *unpubl. data*). The flowers are radially symmetrical, with 5 petals that alternate with 5 sepals. Petals are differentiated into an upper flattened lamina and an elongated spur, approximately 50 mm in length, with a nectary located at the base of each spur (Miller 1978, 1981). Nectar production, which averages 3.9 μL/fl/d, 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 5-12 unfused carpels, each capable of independently developing into a mature follicle (fruit), all of which is surrounded by approximately 50-130 stamens (Fig. 1 in Miller 1981; Brunet 1996, 2009). Plants produce new stalks each year, bearing one to >20 individual stalks, each with 1-15 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 of *A. caerulea* (Miller 1978, 1981; Brunet 2009). In the desert Southwest, it is likely adapted for pollination by long-tongued hawkmoths (Miller 1981). The plant has since expanded its distribution into areas, including montane Colorado, where it is more dependent on bumble bees due to the unpredictable presence of hawkmoths from year to year (Chase & Raven 1975; Miller 1981). Most bumble bee species can only forage for its pollen, as they are unable to reach the nectar at the bottom of its nectar spurs. This is equally true of the short-tongued *Bombus*

occidentalis. However, *B. occidentalis* can also feed as a primary nectar robber by using its toothed mandibles to cut into the spur, circumventing the floral opening and the reproductive structures (Fig. 3 in Miller 1978). This species has been documented to primary- as well as secondary-rob other long-tubed flowers in the area as well (Inouye 1983; Irwin & Maloof 2002, Burkle et al. 2007, Richman et al. 2017).

Miller (1978) noted 10 bumble bee species visiting *A. caerulea* at his sites (see below and Table 1), of which *B. occidentalis* was the most common and the only one he observed primary-robbing nectar. This species is easily recognized by its distinctive white pile on the apex of its abdomen (Rao & Stephen 2007). At one site (Emerald Lake), Miller also commonly observed floral visits by hummingbirds (*Selasphorus platycerus*, the broad-tailed hummingbird) and hawkmoths (*Hyles lineata*, the white-lined sphinx moth). In addition, he identified 23 species of Diptera making visits to flowers, including representatives of the families Anthomyiideae (9 species), Dolicopodidae (1), Muscidae (6), Rhagionidae (1), and Syrphidae (6). The most common genera were *Lasiops* (Muscidae) and *Hylemya* (Anthomyiidae).

STUDY SITES

Miller (1978, 1981) studied *A. caerulea* in a meadow 5.6 km south of the RMBL, in a spruce and aspen stand at the RMBL, and in a rocky meadow near Emerald Lake, approximately 7.4 km north-northeast of the RMBL. The present study was conducted within a 10 km radius of these sites. The majority was conducted at a single site, 2.1 km north-northwest of the RMBL, approximately 50 m off-trail from the Mt. Avery trailhead (MA: 38° 58′ 33.94″N, 106° 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 h after sunrise, and flanked to the north and west by a permanent, spring-fed stream. The remainder of the data were gathered at two other sites in close proximity to the RMBL, 1.5 km to the north (Judd Falls, JF: 38° 58′ 16.67″N, 106° 59′ 46.64″W), and 2 km to the west in the lower Washington Gulch Valley (LWG: 38° 56′ N, 107° 01′ W). The floral communities of these sites, especially MA and JF, were similar. The plant community at LWG was

Table 1. Comparison of visitation rate to *A. caerulea* by dominant flower visitors in 2016 vs. 1969-1976. Visits/Hour refers to visitation rate observed over 12.9 hours in this study (2016); Visits/Hour (Miller 1978) refers to visitation rate observed over a total of 95 hours reported in that paper. Visitors are listed to the lowest taxonomic unit possible, identifiable on the wing.

Visitor	Visits/Hour	Visits/Hour (Miller 1978)
Bombus appositus	1.16	0.08
B. bifarius	0.53	0.13
B. flavifrons	4.81	0.25
B. occidentalis	0.07	1.68
Bombus spp.	0.00	0.99
Diptera	34.02	3.59
Megachilidae	0.30	0.07
Selasphorus platycercus	0.00	0.15
S. rufus	0.16	0.00
Sphingidae	0.00	0.63

somewhat more diverse but was similarly dominated by long-lived perennial herbs.

FLORAL VISITOR RATE & COMPOSITION

Observations of the floral visitor community of *A. caerulea* were made throughout the 2016 flowering season between 0900 h and 2030 h. From 27 June to 26 July 2016, approximately 13 h of observations were conducted, the large majority (94%) at MA and the rest at LWG; flowers at JF were all consumed by deer herbivores before observations could be conducted. Deer find *A. caerulea* highly palatable and have been documented to inflict extensive damage on it at this site (Waser et al. 2014). Bumble bees were observed during individual bouts as they visited *A. caerulea,* moved between *A. caerulea* individuals, and moved to and foraged at co-flowering species. A bout began when the individual was within view of the observer. The visitor species, sex (when possible), plant species visited, number of flowers visited, and lengths of time for each floral visit within a bout were recorded. A bout was considered concluded when the visitor left the observer's field of view. Bumble bees were identified to species on the wing based on distinctive pile markings.

In addition to, but separate from, bumble bee observations, we recorded the number and activity of flies (later identified as Muscidae, commonly known as houseflies; hereafter referred to as muscid flies) on randomly selected *A. caerulea* flowers. Depending on the density of flies present,

up to 4 flowers/plant were observed for up to 10 min. The number of flies that were actively interacting with the anthers and/or stigma of the flowers and the length of time that this occurred was also recorded. Unique individuals were not tracked. It was not possible to identify flies on the wing. Specimens were collected and preserved for later identification.

While it would have been preferable to align 2016 census methods with those of Miller (1978), this was unfortunately not possible. Miller (1978) states that he conducted floral visitor observations near Gothic from 0730-2000 h and 2000-2130 h during the summers of 1969, 1970, 1971, and 1976, but provided no other information, and no methods were described. He did note that several groups of smaller insects, including Diptera, were common on *A. caerulea* flowers, either on the anthers chewing pollen or in the spurs seeking nectar, but how these visitors were quantified was not explicitly stated. He provided a proportion of nectar-robbed spurs at each of his study sites, but presented no data on the number of flowers sampled or the year(s) in which these samples were collected.

NECTAR ROBBING RATE SURVEYS

Nectar robbing was quantified throughout the flowering season by recording robbing damage on *A. caerulea* flowers. Primary robbers typically cut a hole cut halfway up the spur approximately 2 cm from the tip (T. Ledbetter, *pers. obs.*). MA was surveyed during the peak of the flowering season,

on 7, 16, 21, and 27 July 2016. LWG was surveyed twice at the peak of the flowering season, on 16 and 21 July 2016. JF was surveyed only once, on 12 July, due to the high rate of deer herbivory. At each site on each census date, all nectar spurs on all plants (N = 10-18 plants) were surveyed for damage, with the exception of MA on 7 and 16 July, at which all spurs on a subset of haphazardly selected plants were examined (N = 20 plants). The number of spurs with more than one robbing hole were also recorded. The proportion of all surveyed spurs that were robbed was calculated following Miller (1978). This method quantifies primary nectar robbing (damage left by robbers that chew holes) only; secondary nectar robbing, in which a visitor feeds through a pre-existing hole (Inouye 1980), leaves no signature. Thus, it is possible that total robbing rates (i.e., primary plus secondary robbing) were higher than the rates reported here.

CONSEQUENCES OF EXPERIMENTAL NECTAR ROBBING

To determine whether nectar robbing affected fruit and seed set, we artificially robbed flowers and followed their subsequent fates. Near the onset of the flowering season, we haphazardly selected 30 plants at the Mount Avery (MA) study site. As flowers opened (27 June – 12 July 2016), we assigned plants to one of three treatments: *Control (No Robbing), Low Robbing,* or *High Robbing* (n = 10 plants per treatment). In the *Low Robbing* treatment, a single nectar spur per plant was artificially robbed, and in the *High Robbing* treatment all five spurs were robbed. Miller (1978) recorded a robbing rate of 43-64% of spurs robbed; the *Low Robbing* treatment used a robbing rate substantially lower, and the *High Robbing* treatment used a robbing rate substantially higher. For plants with 3-6 visible buds, we manipulated 1 flower/treatment/plant; for plants with > 6 visible buds, we manipulated 2 flowers/treatment/plant. Robbing damage was simulated by cutting into the nectar spur(s) of a flower, approximately 2 cm from the tip, using dissecting scissors, and withdrawing all available nectar using 5 μL microcapillary tubes (Drummond Scientific, Broomall, PA, USA). This technique does not damage the reproductive structures of a flower or nectary in other nectar-robbed plant species (e.g., Irwin et al. 1999; Richman et al. 2017). Nectar was withdrawn through the same hole each day until flowers withered or fell off. Nectar spurs of flowers

in the *Control* treatment were squeezed lightly to control for effects of handling but were otherwise undisturbed. To prevent herbivory by deer, all selected flowers were staked using a 16-gauge wire stake and lightly tied along the stalk to ensure stability while minimizing interference from the wire. 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 both robbed and unrobbed spurs. 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 (27 July – 15 Aug), they were collected and dried in a drying oven at 50°C for approx. 48 hr. Fruit set was determined by dividing the number of mature fruits by the number of carpels. A fruit was considered mature if it contained at least one mature seed. Fruits were later dissected, and the average number of seeds for each fruit was calculated by dividing total mature seed production by the number of fruits produced. Mature seeds were dark green or blue-green in color.

FLORAL VISITOR EXCLUSIONS

Muscid flies were exceedingly common on flowers in 2016. To determine if they were capable of pollinating *A. caerulea*, an exclusion experiment was conducted at MA. We haphazardly selected 50 flowers from which to fully exclude all floral visitors, and another 40 flowers from which to exclude all visitors except flies. For plants with at least two visible buds, we manipulated one flower/treatment/plant; for plants with four or more visible buds, we manipulated two flowers/treatment/plant. Flowers were staked as described above. All flowers were bagged using a lightweight mesh bridal veil 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 to prevent any unwanted visitation and were staked 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 kept in place until the flower wilted or abscised. Bags were removed and flowers exposed during the morning hours, when muscid flies were common but

bumble bees and other floral visitors were rare. Each exposed, open flower was observed daily for approximately 15 min to confirm that the only floral visitors were muscid flies before the bag was replaced. Flowers from which all floral visitors were excluded opened between 13-19 July. Flowers exposed only to muscid flies opened between 13-16 July.

As with the nectar robbing manipulations, the number of carpels were recorded once the flower wilted or abscised. At the end of the reproductive season when fruits had fully matured (15-16 Aug), fruits were collected, dried, and processed as described above.

STATISTICAL ANALYSES

All analyses were performed using R version 4.0.2 (R Core Team 2020). Fruit set was calculated at the flower level by dividing the number of mature follicles (fruits) by the number of carpels. We used this response variable to test whether nectar robbing manipulations resulted in differential fruit set. Because of an abundance of zeroes in the dataset driven by low fruit production, we performed a zero-inflated generalized linear mixed effects model (binary error distribution) using the glmmTMB package (Brooks et al. 2017). To control for variation in phenology, flowering date was included as a random effect. We tested for overall treatment effects using a likelihood ratio test. We used estimated marginal means to make pairwise treatment comparisons (emmeans package, Lenth 2020). We performed a linear model to test whether robbing treatment affected the number of seeds per fruit. Flowering date was initially included as a random effect, but variance within flowering date was effectively zero, so we dropped it from the model. We tested for overall treatment effects using a likelihood ratio test. To test whether Diptera could act as effective pollinators, we performed a generalized linear mixed model (binomial error distribution; lme4 package, Bates et al. 2015) with fruit set, calculated as described above, as the response variable and exclusion treatment as the explanatory variable. Flowering date was included as random effect. We used a likelihood ratio test to assess overall treatment effects. We performed a second model testing the effect of treatment on seeds per fruit, using the

same techniques described above in the visitor exclusion fruit set analysis.

RESULTS

FLORAL VISITOR RATES & COMPOSITION

The floral visitor community of *A. caerulea* in 2016 differed notably from that recorded by Miller (1978) on unspecified dates but at nearby sites (Table 1). Visits of some insects were more frequent in 2016, one group dramatically so: visitation by flies in the family Muscidae was approximately 10x higher, from 3.59 visits/hr reported by Miller (1978) to 34.02 visits/hr in the present study. Collected and preserved individuals were identified to belong to at least four species (K. Kingsley, pers. comm.), which were unfortunately impossible to distinguish in the field. Visits by several bumble bees (*Bombus flavifrons*, *B. appositus*, and *B. bifarius*) and by bees from the family Megachilidae were also higher in 2016 than reported in the 1970's, with *B. flavifrons* increasing the most, from 0.25 visits/hr noted by Miller 1978 to 4.81 visits/hr in 2016.

All other major visitors recorded by Miller (1978) were observed visiting flowers less frequently or not at all in 2016 (Table 1). There was a particularly notable 98% decrease in observed visits by *Bombus occidentalis*: Miller (1978) recorded 1.68 *B. occidentalis* visits/hr (averaged across three sites), whereas in this study, we noted only 0.07 visits/hr (averaged across our three sites). Indeed, over 12.9 observation hours in 2016, we observed a single *B. occidentalis* individual foraging legitimately for pollen, a visit that lasted just 10 sec. No Sphingidae (hawkmoths) were observed, in comparison to 0.67 visits/hr reported by Miller (1978). Similarly, several bumble bee species (*Bombus centralis*, *B. frigidus*, *B. kirbyellus* (now *B. balteatus*), *B. mixtus*, *B. rufocinctus*, and *B. sylvicola*) were absent in 2016. Hummingbird visitation (*Selasphorus platycercus* and *S. rufus*) was approximately similar between the two studies (0.16 visits/hr in 2016 compared to 0.15 visits/hr reported by Miller).

NATURAL NECTAR ROBBING RATE SURVEYS

Natural nectar robbing was extremely rare in 2016. Miller (1978) reported a robbing rate ranging from 43-64% of all spurs; in contrast, in 2016 the robbing rate ranged from 0.6-2.3% of spurs (Table

Table 2. Comparison of nectar robbing rates across *A. caerula* flowers in 2016 (this study) vs. 1969-1976 (as reported by Miller 1978). Percentage represents spurs robbed across the total number of spurs examined, independent of individual plant identity.

Site	Spurs Robbed (%)	Spurs Examined	Study
S. Gothic Meadow	43.2	500	Miller 1978
Gothic (RMBL)	63.6	500	Miller 1978
Emerald Lake	50.6	500	Miller 1978
Lower Washington Gulch	2.3	394	This study
Judd Falls	1.2	654	This study
Avery	0.6	1836	This study

2). Across 12.9 h of observation in 2016, not a single bee was observed robbing *A. caerulea*. In comparison, Miller (1978) observed *B. occidentalis* robbing regularly, noting that at times, this behaviour was more common than pollen foraging.

CONSEQUENCES OF EXPERIMENTAL NECTAR ROBBING

Plants in the *High Robbing* treatment set significantly fewer fruits than plants in either the *No Robbing* or *Low Robbing* treatment (χ^2_2 = 12.55, *P* = 0.002, Fig. 1A). Although there was a treatment-level difference in fruit set, there was no statistically significant effect of treatment on seed set per fruit χ^2_2 = 1.28, *P* = 0.53, Fig. 1B).

FLORAL VISITOR EXCLUSIONS

Muscid flies did not serve as effective pollinators of *A. caerulea*. In fact, fly visits lowered fruit production by approximately 50% compared to no visits at all (χ^2_1 = 4.65, *P* = 0.03, Fig. 2A). Likewise, seed set per fruit was reduced 85% in plants visited by flies compared to plants that received no visits (χ^2_1 = 5.94, *P* = 0.01, Fig. 2B).

DISCUSSION

The floral visitor community of the Colorado columbine, *Aquilegia caerulea*, near Gothic, Colorado was dramatically different in 2016 compared to the 1970's (Miller 1978, 1981). We stress again that our data were collected in a single year in a visitor community well documented to be highly dynamic interannually (e.g., Burkle & Irwin 2009; Ogilvie et al. 2017). Nevertheless, the degree of difference between this study and earlier studies is worth noting. Diversity and visitation rate of bumble bees and hawkmoths were lower, whereas

visitation by muscid flies was far higher. (Hummingbirds were rare visitors in both periods.) It is important to note that the number of
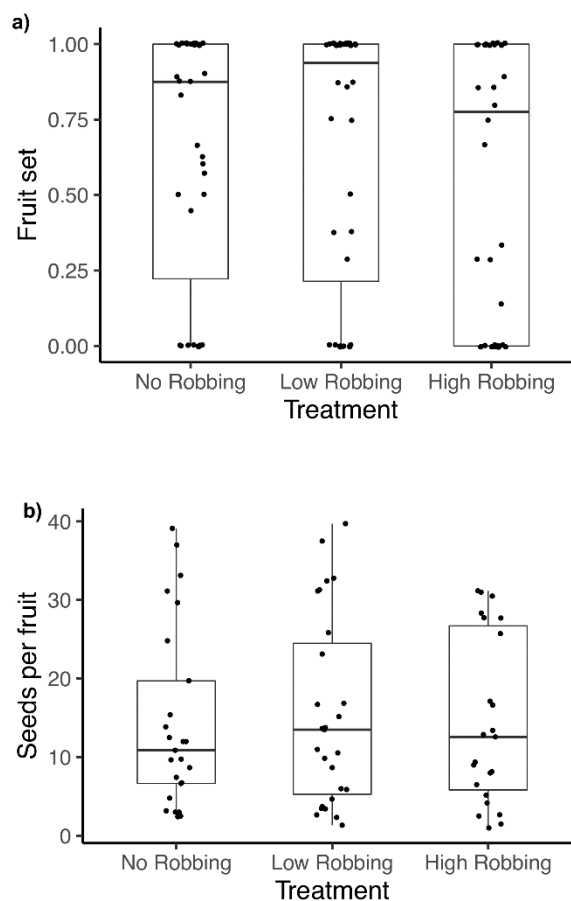

Figure 1. Results of the nectar robbing manipulation study on (A) percent fruit set and (B) seeds per fruit. Boxplots represent median, interquartile range, minimum and maximum data values (percent fruit set). Each point represents an individual flower; points are offset for visualization purposes. Flowers in the "Low Robbing" treatment had one spur/flower robbed; flowers in the "High Robbing" treatment had all spurs/flower robbed.
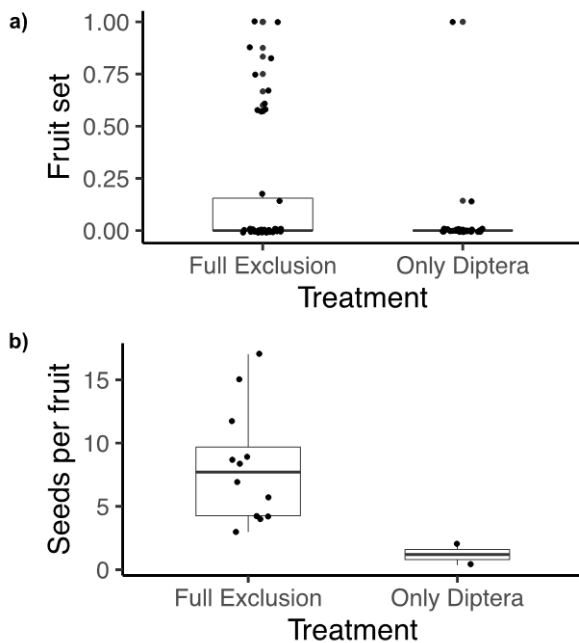
a)

b)

Figure 2. Results of the pollinator exclusion study on (A) percent fruit set and (B) seeds per fruit. Boxplots represent median, interquartile range, minimum and maximum data values (percent fruit set). Each point represents an individual flower; points are offset for visualization purposes. Flowers in the "Full Exclusion" treatment were enclosed in mesh bags from before the flower opened and until it closed. Flowers in the "Only Diptera" treatment had mesh bags removed when only Dipteran flower visitors were present.

visitor observation hours differed between Miller (1978) and this study. Miller (1978) collected 95 hours of observations over several years, whereas this study reports on 12.9 observation hours over a single year. Yet, we observed a much higher number of visits by Diptera. This, in conjunction with the overall declines in nearly every other visitor category in 2016, produced a markedly different visitor community, from one once dominated by bumble bees, hawkmoths, and solitary bees (53% of visitors) to one that was dominated by muscid flies (83% of visitors).

We recorded visitation rates of the Western Bumble bee, *Bombus occidentalis*, 98% lower than those documented over several years by Miller (1978) and Brunet (2009). A decline of *B. occidentalis* in association with *A. caerulea* at our study area was first reported about 25 years after Miller's studies. Brunet (2009) conducted a series of pollinator observations 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, northern Arizona, northern Colorado, and central Colorado near the RMBL (Emerald Lake, Flat). The Emerald Lake population is near to where Miller completed his work and in close proximity to MA, one of our own study sites. Brunet (2009) reported that prior to 2001, *B. occidentalis* was the most common bumble bee visitor to *A. caerulea* at this and all of her other sites; after 2001, however, *B. occidentalis* was not observed and *B. flavifrons* became the dominant bumble bee visitor. This change coincides with *B. occidentalis'* marked decline throughout the western USA (Evans et al. 2008; Cameron et al. 2011; Hatfield et al. 2015; Graves et al. 2020), although we stress that our visitation data to a single plant species in a single year cannot be used to infer *B. occidentalis* abundance.

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 2016), again raising caution about how much can be learned from a single flowering season. However, the consistent presence of *B. occidentalis* as *A. caerulea*'s primary floral visitor across all years and sites studied by Miller, as well as in Brunet's studies up until 2001, indicate that *B. occidentalis* was once a common and important floral visitor. We recognize the limitations of a single year of data (Stuble et al. 2021). For example, Kearns et al. (2017) documented high year to year variability of *B. occidentalis* from 2012 to 2014 at lower elevation sites in Colorado approximately 400 km from our study sites. Our own studies of nectar robbing near the RMBL also indicate high interannual variability in nectar robbing levels (Irwin & Maloof 2002) and the identities of primary nectar robbers, in some years (such as 2016) with *B. mixtus* as the dominant primary nectar robber (Lichtenberg et al. 2020), which we never observed robbing *A. caerulea* (and see below), and in other years with *B. occidentalis* as the primary nectar robber. Other studies of bumble bees around the RMBL have continued to note *B. occidentalis* as a member of the bee community with no consistent declines per se (e.g., Pyke et al. 2012), and our own observations suggest high interannual variability in this bee species at the RMBL (Irwin, *unpubl data*).

With regard to muscid fly visitation, 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 the RMBL. However, the visitation rates she reports generally align with those of Miller (1978), ranging from around 2.0 visits/hr to less than 0.25 visits/hr. These frequencies contrast markedly to the extremely high rate of 34.02 visits/hr recorded in this study. It is possible either that fly visitation to *A. caerulea* is increasing, or that 2016 was an unusual year. Given the high spatial and temporal variability noted by Brunet (2009), further studies would be needed to identify trends.

Although hawkmoths are among *A. caerulea*'s most effective pollinators (Miller 1978, 1981; Brunet 2009), we were unable to obtain evidence that they were present at our study site and visiting *A. caerulea* in 2016. Although the pollinator observations we report here were conducted in daylight hours, we did make two attempts to record nocturnal visitors, including hawkmoths. We observed flowers from 1800 – 2030 at MA on 27 June, 16 July, 25 July. In addition, we set up a blacklight to attract moths at LWG on one night in early July from 2000-2200 h. No hawkmoths were seen using either technique. Nor were they observed in daylight hours, although Miller (1978), whose observations were strictly diurnal, did record them (Table 1). This absence of hawkmoths is not necessarily indicative of a decline. Hawkmoths, particularly the known *A. caerulea* pollinator *Hyles lineata*, are highly variable in abundance in this region, fluctuating strongly from year to year and even week to week within a given season (Miller 1978; Brunet 2009; D. 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).

Consistent with the fact that the single known *A. caerulea* nectar robber, *B. occidentalis*, was almost never observed in 2016, nectar robbing rates in this study were dramatically lower than those reported by Miller (1978). The few robbing holes we noted in floral spurs were likely the result of rare and unobserved *B. occidentalis* visits; we did observe one *B. occidentalis* legitimate visit, indicating that they were not entirely absent. Another common bumble bee species, *B. mixtus,* is also capable of primary robbing (R.E. Irwin *pers. obs.* and see Morris 1996). However, in cage trials, *B. mixtus*

caught at sites where *A. caerulea* were abundant failed to forage for either pollen or nectar on it (data not shown). This remained true even when artificial holes were made in spurs to encourage secondary nectar robbing.

The exceedingly low nectar robbing rates we observed might have been expected to benefit *A. caerulea* relative to years and sites where robbing was more common. Costs of primary robbing and, to a lesser extent, secondary robbing have been documented in detail for some plant species, including ones robbed by *B. occidentalis* at or near our study sites (Irwin & Brody 1998, Irwin & Maloof 2002, Burkle et al. 2007). Negative effects can be attributed, for instance, to pollinator avoidance of robbed flowers, direct damage to ovules, and reduced pollen quantity or quality (Irwin et al. 2010; Richman et al. 2018). In our experimental manipulations of nectar robbing, we found evidence that *A. caerulea* fruit set was significantly lower when subjected to high rates of robbing, suggesting a potential benefit to the absence of this robber. However, seed set per fruit was unchanged regardless of robbing rate. Fruit set typically provides an index of visitation rates by pollinators for species that are self-incompatible, whereas seed set per fruit typically provides an index of both the quality of pollen delivered or the ability of the plant to utilize the delivered pollen to produce seeds (e.g., Waser & Price 1989). Although *A. caerulea* is self-compatible, it is highly protandrous, and so pollinators are typically required to carry pollen from one flower to another (within or among plants) (Brunet 1996). Thus, nectar robbing may have altered pollinator behaviour in a way that reduced pollen delivery and subsequent fruit set. However, *A. caerulea*'s most effective pollinators, bumble bees, are unable to access the nectar legitimately and visit the flowers to obtain pollen instead. While this should be examined directly, it seems unlikely that pollen-foragers would alter their behaviour in response to *A. caerulea* robbing damage or nectar removal. Indeed, they seem unlikely to encounter it, given that they feed well away from the nectar spur. In contrast to bumble bees, hawkmoths do visit *A. caerulea* for nectar. In a site or year with high hawkmoth visitation, nectar robbers might indeed deter visitation, although to our knowledge, it is not known whether hawkmoths avoid flowers with robbing damage. The mechanism driving

lower fruit set in robbed vs. unrobbed flowers is curious and warrants further investigation of both pollinator visitation to robbed and unrobbed flowers and pollen receipt to those stigmas.

Miller (1978) reported the presence of groups of smaller insects, including a variety of Diptera, on *A. caerulea* flowers. He noted that they were likely responsible for some level of pollination, but were probably relatively unimportant as they did not approach bumble bees and hawkmoths in size, foraging speed, or efficiency in pollen transfer (Miller 1978). However, in the face of reduced bee visitation, the very high abundance of muscid flies, at least in 2016, raises the question of whether these abundant visitors have the potential to benefit *A. caerulea*. Muscid flies are ubiquitous flower visitors in habitats worldwide, often found in exceptionally high numbers on sweet-smelling species (Willmer 2011). While not widely known as major pollinators (but see Orford et al. 2015), they are extremely important in certain habitats, notably the High Arctic (Tiusanen et al. 2016) where other floral visitors are rare.

This study soundly rejects the possibility that muscid flies are significant pollinator of *A. caerulea*. In the absence of bee visitors, Diptera had a negative, not positive, impact upon plant reproductive success. In fact, they reduced fruit set and seed production to nearly zero, significantly lower than in the entire absence of visitors. We suggest that this is because muscids remove pollen that would otherwise contribute to self-pollination. These flies typically sit on the reproductive structures of an *A. caerulea* flower, combing the anthers and stigma, gathering pollen for consumption, and rarely moving between flowers (T. Ledbetter, *pers. obs*). They also meticulously comb their bodies and legs, removing pollen from their bodies. These behaviours should reduce pollen transfer when an individual does move between flowers. Thus, in *A. caerulea*, muscid flies essentially act as pollen thieves, floral visitors that collect pollen in such a way that pollination is impeded without associated floral tissue damage (Inouye 1980).

While knowledge is rapidly accumulating on consequences for plants in a world with fewer pollinator mutualists, how they will fare with associated reductions in antagonistic floral visitors is less well known. Addressing this question empirically requires consideration of a plant's performance with and without such exploiters, ideally conducted in a system in which selective loss of these visitors or their behaviours is biologically meaningful. Here, we have described studies of the Colorado blue columbine, *Aquilegia caerulea*, whose floral visitors were well-documented in the 1970's and 1990's but that has more recently experienced a dramatic change in the visitor community. Nectar robbing, inflicted solely by a bumble bee known to be in steep decline in the western United States, was virtually absent on *A. caerula* in the year of this study. Loss of nectar robbers could benefit the plant: experimentally robbed plants produced fewer fruits, but similar numbers of seeds per fruit, which should result in reduced total plant reproduction if fruit set and seeds per fruit are multiplicative in affecting plant reproduction. Concurrent changes in the floral visitor community are likely outweighing the effects of robber loss in this system. This work argues for a community-level approach to disrupted mutualisms that take into account non-mutualistic associates as well.

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