

Exploring robbing patterns of *Bombus occidentalis* on *Ipomopsis aggregata* and *Linaria vulgaris*

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Introduction

Over 100 million years of evolution have produced a relationship between plants and pollinators where pollinators receive pollen and sometimes nectar and the plants receive pollination, or the transfer of pollen, and subsequent reproduction. This mutualistic relationship, however, is not always the case. Nectar robbers are birds or insects that pierce a hole into a flower, remove the nectar, and the flower often receives no reward in return. Floral larcenists are exploiters of the mutualistic relationship between plants and pollinators and can be broken down into two different types: nectar robbers and nectar thieves (Inouye 1983). Nectar robbers often bypass a plant's sexual organs by making a hole in the flower; whereas, nectar thieves enter the plant via a legitimate pollinator pathway but provide little or no pollination service to the plant. Nectar robbers have been documented on every continent except Antarctica and nectar robbing has been demonstrated in at least 214 plant species in 59 different families (Irwin and Maloof 2002). As widespread as nectar robbing may be, little is known about the robbing patterns from the larcenist's perspective.

Studies have explained the effects of nectar robbing on plant reproductive success extensively, and have found nectar robbing can have positive, negative, or neutral effects on plant reproduction. One direct negative effect is that robbing can cause damage to floral reproductive organs (Galen 1983, 1999). When robbers can also drive away legitimate pollinators, this is an indirect negative effect (Roubik 1982). Plant fitness can decrease in self-incompatible and pollen limited species when the amount of nectar decreases, causing pollinators to desert the plant after a brief visit or avoid the plant completely (Irwin and Brody 1998, 2000). For example, in *Ipomopsis aggregata*, robbing causes a 50% loss in both male and female reproductive success due to pollinator avoidance of robbed plants and flowers (Irwin and Brody 2000). Finally, sometimes robbers produce a direct positive effect by pollinating some or all of the flowers they rob (citations in Maloof and Inouye 2000).

Of at least five species of bumblebees at the Rocky Mountain Biological Laboratory (RMBL), *Bombus occidentalis* is the primary nectar robber (Pyke 1982). *Bombus occidentalis*, to our knowledge, robs at least eight different plant species around RMBL. With a mean tongue length of 7.1 mm (Irwin and Maloof 2002), it is unable to attain nectar from plants with long corollas, such as *Ipomopsis aggregata*, through legitimate pollinator methods.

Instead, *B. occidentalis* uses its toothed mandibles to chew a hole into the corolla of the flower, inserts its proboscis, and sucks out the nectar of the plant.

The objective of this project was to assess spatiotemporal variation in robbing levels and to study nectar robbing from the nectar robber's perspective. I asked two questions: 1. Do levels of nectar robbing vary temporally and spatially? By measuring the number of flowers in bloom, number of flowers robbed, and number of times each flower was robbed, I tested how the time of season related to the amount of nectar robbing occurring. I hypothesized that robbing levels would vary spatially and temporally, potentially due to competition for nectar resources. There may also be an increase in the number of bees that rob. In addition, I also compared robbing levels measured in my study to those from previous field seasons at RMBL.

2. Do nectar robbers learn where to make their nectar-robber hole on flowers? Little is known about whether the bees' robbing habits are learned or innate. I hypothesized that the distance between nectar-robber holes and calyx decreases over time, from which I will infer that *Bombus occidentalis* is learning where to rob these plants of nectar by trial and error or by communication among the bee colony. Alternatively, if there is no learned component of where to put the robber hole, I would expect that the distance between the hole and the nectary will not change systematically with the time of season.

Methods and Materials

Study System

Fieldwork was conducted in the Elk Mountains of Colorado on or near the RMBL from June until August, 2006 (RMBL; latitude 38°45'N, longitude 106°59'W, altitude 2,900 m).

The amount of nectar robbing varies among plant species. *Bombus occidentalis* is known to rob at least eight different species at or around the RMBL. Four of the more extensively studied are *Delphinium nuttallianum* (Ranunculaceae), *Ipomopsis aggregata* (Polemoniaceae), *Corydalis caseana* (Fumariaceae), and *Linaria vulgaris* (Scrophulariaceae). The mean robbing level ± 1 SE (%) for *D. nuttallianum* is $56.50\% \pm 8.50\%$, *I. aggregata* $66.14\% \pm 12.14\%$, *C. caseana* $67.44\% \pm 3.49\%$, *L. vulgaris* $79.25\% \pm 15.34\%$ (Irwin and Maloof 2002). In these cases, nectar robbing was seen from the plant's perspective rather than the robbers' and focused primarily on plant reproduction.

Bombus occidentalis has an annual life cycle. In the spring, fertilized queen bees will build nests, lay eggs, and supply the eggs and themselves with nectar and pollen for food. When the non-reproductive larvae hatch, they will provide the colony with food. During this period the queen will forage infrequently and only when it is essential to do so. Non-worker male bees, or drones, as well as new queens will be produced at the end of the summer. When cold weather arrives, however, all of the bees will die except for the newly fertilized queen bees. The queen bees that survive the winter will then carry on the same cycle aforementioned.

Ipomopsis aggregata has a lengthy blooming season from early June to mid September. It is monocarpic and self-incompatible. Its red tubular flowers are primarily pollinated by hummingbirds (*S. platycercus* and *S. rufus*) (Waser 1978) and it experiences high amounts of nectar robbing (66.14 ± 12.14 SE %) from *Bombus occidentalis*. *Linaria vulgaris* is also self-incompatible. It is a yellow perennial and is zygomorphic. *Linaria*'s nectar spur is robbed.

Field Methods

1. Do levels of nectar robbing vary temporally and spatially?

I. aggregata was studied at five different sites on or near the Rocky Mountain Biological Laboratory (RMBL): Copper Creek, Orehouse, Wounded Knee, Gothic Campground, and East River (Table 1). *Linaria vulgaris* was studied at two different sites; Purple and McLeod (Table). Plots of 10 m by 10 m were set at each site. Plants were numbered haphazardly from 1-50. The number of plants in bloom and flowers in bloom were counted biweekly within the 10 m by 10 m area to provide an estimate of *I. aggregata* total floral abundance. Individual *I. aggregata* flowers bloom for approximately three days. To ensure that robber holes were not counted more than once, the plots were only visited twice per week. Plots of *L. vulgaris* were measured only twice a week as well. Each flower was examined to determine the number of flowers robbed, number of flowers open, and how many times each flower was robbed biweekly.

Table 1. Latitude and longitude points for the corners of each research plot.

	Copper Creek	Orehouse	East River
Latitude	1)38.961587408 2) 38.961577086 3) 38.961487725 4) 38.961504878	38.960127921 38.960200924 38.960155057 38.960082086	38.971187294 38.971270501 38.971239308 38.971153874
Longitude	1) -106.973006604 2) -106.973115883 3) -106.973095048 4) -106.972982055	-106.991008035 -106.991074555 -106.991159443 -106.991086658	-106.995804625 -106.995851497 -106.995950150 -106.995901486

	Wounded Knee	Gothic Campground
Latitude	1) 38.979752213 2) 38.979828521 3) 38.979793565 4) 38.979709184	38.981047536 38.981142729 38.981091489 38.981015635
Longitude	1)106.999906635 2)-106.999966345 3)-107.000057774 4)-107.000018336	-107.000535078 -107.000566254 -107.000680312 -107.000631957

Statistical Analysis

For each *Ipomopsis aggregata* at each census period, I calculated robbing level as: number of flowers robbed divided by number of flowers open. Robbing levels were non-normally distributed due to a large number of zeros in the dataset. As a result, I used nonparametric tests to assess whether robbing levels varied among sites and among weeks.

In addition, I also combined my robbing data collected in 2006 with robbing data on *I. aggregata* collected from 1995-2005 to visually compare robbing levels among years. From 1995- 2001, I used data published by Irwin and Maloof (2002). In 2002, data were collected from two sites: Orehouse with 30 plants and Vera Falls with 22 plants. Robbing was measured every four days throughout the blooming season. In 2003, there was no data. In 2004, data were collected from two sites: Orehouse with 30 plants and Richards with 30 plants. Robbing was measured once per week throughout the blooming season. In 2005, there was no data.

Statistical Analysis

For each *Linaria vulgaris* plant at each census period, I calculated robbing level as: number of flowers robbed divided by number of flowers open. Robbing levels were non-normally distributed due to a large number of zeros in the dataset. As a result, I used nonparametric tests to assess whatever robbing levels varied among sites and among weeks. Repeated measures ANOVA analysis was done to compare levels of nectar robbing among years and among sites.

In addition, I also combined my robbing data collected in 2006 to robbing data on *L. vulgaris* collected from 1998-2005 to visually compare robbing levels among years. Data from 1998- 2001 came from data published in Irwin and Maloof (2002). In 2004, Happy valley had 60 plants, Mt CB fitness trail had 60 plants and robbing was recorded 3 day/ week.

2. Do nectar robbers learn where to make their nectar robber hole on flowers?

Within the same plots, robber holes and distances were measured. Up to three robbed flowers per plant were selected haphazardly for examination. For *I. aggregata*, the distance from the base of the calyx to the flower notch was measured to the nearest 0.01 mm using digital calipers. Robber holes are measured from the base of the hole and the base of the flower. For *L. vulgaris* the total distance was measured from the nectar spur to the tip of the flower, and the holes were measured from the nectar hole until the nectar spur.

Nectar standing crop was measured to see if there was a correlation between the time of the season and the amount of nectar produced by *I. aggregata*. To obtain nectar standing crop, I used 5uL pipettes to draw the nectar from the plant and measured it using a digital caliper. Ten plants from each site, three flowers per plant, were haphazardly

chosen to measure nectar abundance once a week. A regression line comparing distance and weeks and nectar standing crop were done for statistical analysis.

Results

1. Do levels of nectar robbing vary temporally and spatially?

Ipomopsis aggregata. Over the past 8 years of study (1995- 2006), there has been tremendous variation among years (Figure 1). In 1995 there was 100% robbing at peak levels. From 1995 until 1999 robbing level remained high, ranging from 100% to 85%. From 2000 until my data, the levels of robbing have been declining. Peak robbing levels for 2006 were 2.25%, a dramatic decrease from 1995.

Variation in nectar robbing levels also occurred within a season among weeks (Figure 2 a,b). In weeks one, two, and three, the mean robbing level was 0. The highest level of mean nectar robbing was week 7 at 2.25% with weeks six and eight at .79% and .77% ($\chi^2 = 43.1552$, $df = 7$, $P < .0001$). Nectar robbing began very low and ended at a higher rate.

There was also significant variation in nectar robbing among sites ($\chi^2 = 13.1767$, $df = 4$, $P = .0104$). Copper Creek's mean robbing levels were the highest at .39% and Gothic Campground had the lowest robbing rate at .001% (Figure 3).

Linaria vulgaris peak robbing rates appear to have decreased from 1999 (100%) to 2006 (29.5%) but still remain higher than robbing to *I. aggregata* (Figure 4).

Between the two census periods, *L. vulgaris* robbing rates remained similar. Census week one had a level at 29.5% with week two at 27.9%; however, this difference is not significant ($\chi^2 = .0035$, $df = 1$, $P = .9530$).

The difference between *Linaria* sites was drastic (Figure 6). McLoed had mean robbing levels at .82%, whereas, Purple's levels were at 50% ($\chi^2 = 122.2788$, $df = 1$, $P = < .0001$).

2. Do nectar robbers learn where to make their nectar robber hole on flowers?

There is no relationship between the amount of nectar and the week ($DF = 1$, F Ratio for census week .0007, $P = .7767$; Site 1.9137 $P = .1102$). However, as the season progressed, the distance between robber holes and the calyx decreased. There is a significant effect of site on week by hole distance (p value .0007).

Discussion

Little is known about nectar robbers and their interactions with their environment. What studies have been done were only a few years and only a couple of plants (Roubik 1982; Arizmendi 1996; Goulson 1998; Traveset 1998). By examining the data set for both *L. vulgaris* and *I. aggregata* from years between 1995- 2006, we can get a better

understand about how nectar robbing varies temporally. The results show there is variation throughout time and throughout space.

There is variation between sites, weeks, and years in *Ipomopsis*. The variation in weeks is possibly due to more nectar robbers being born and thusly, more competition. When more nectar robbers begin to appear, the amount of flowering by *Ipomopsis* decreased (Figure 2b). This could possibly be an evolutionary link to have flowering earlier in the season to avoid nectar robbing and provide *Ipomopsis* with a potentially higher reproductive success. Decrease in nectar robbing over the years could be attributed to an increase in the nectar robbing in *Linaria*. *L. vulgaris* has a higher sugar content per flower than *I. aggregata* providing the nectar robber with a greater reward.

There is variation between sites and years in *Linaria*. This the decrease in robbing may be due to a reduced number of *Bombus occidentalis*, and thus a decrease in robbing. Also, a significant increase in blooming *L. vulgaris* might look like a decrease in robbing as the mean peak robbing is calculated by dividing # flowers in bloom by the amount of robbing. More flowers means a higher probability that that flower won't be robbed if the number of *Bombus occidentalis* stays consistent.

Nectar standing crop stayed consistent throughout the season. The change in robber hole distances cannot be attributed to the nectar standing crop of *Ipomopsis*. Nectar robbers, over time, will place their holes closer to the nectary because they may be learning that this is where they will receive the greatest reward. However, robbers do not place their holes closer to the nectary because of a decrease in nectar standing crop.

In the future, replication of these studies could provide more valuable information. To determine whether or not nectar robbing levels on *Ipomopsis* will remain extremely low or will they, for some reason, increase would be interesting. In addition, it would be interesting to continue to track *Linaria*'s nectar robbing. It would be interesting to look at how the significant amount of robbing affects other indigenous plants species as well as bee species at RMBL. It would be interesting to speculate how robbing rates vary among sites throughout the years.

Robbing levels vary over time and space. Selection of flowering and floral traits may depend on these differences in spatiotemporal variation. Nectar robbing, being as common as it is, has not been studied significantly making it very important to have more studied to better understand the significance and extent of the exploitation of plant- pollinator mutualisms by nectar robbers.

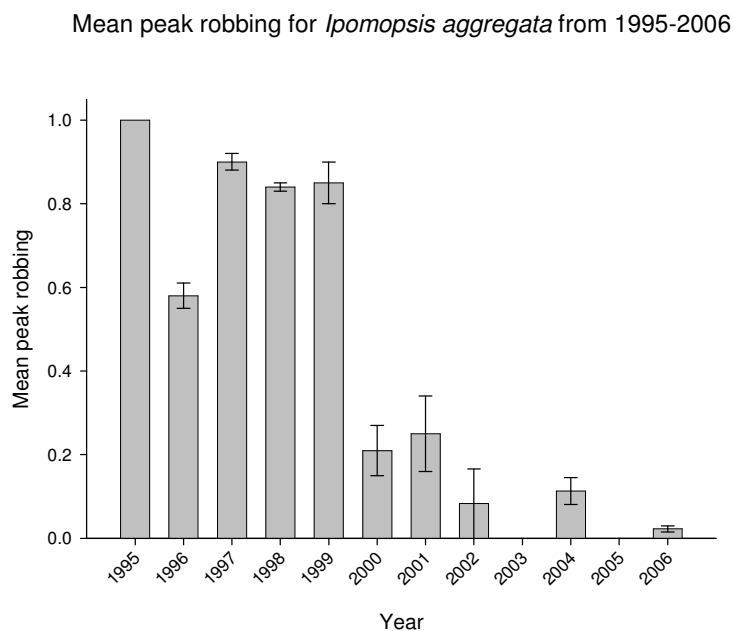
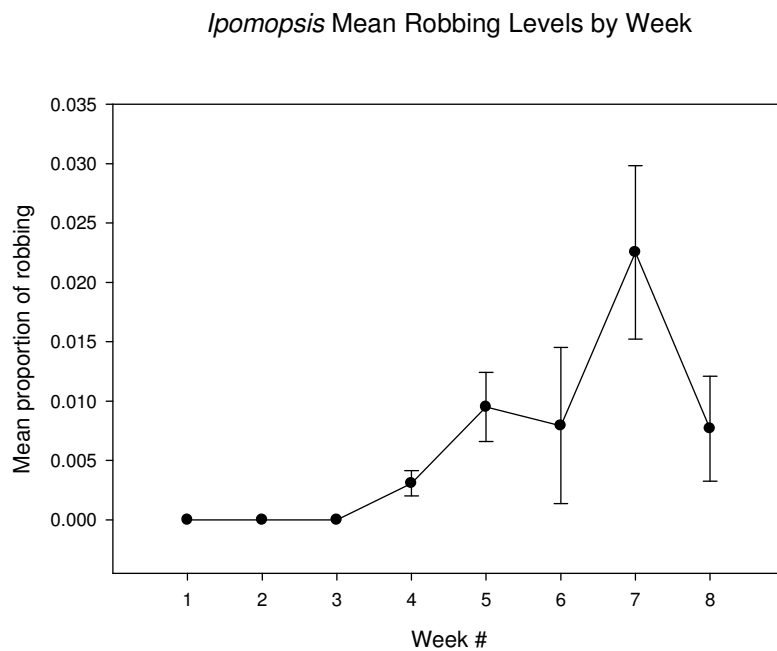
Figure 1. Mean peak robbing in *Ipomopsis aggregata* from 1995- 2006.Figure 2a. *Ipomopsis* mean robbing levels are increasing by week.

Figure 2b. Mean seasonal variation in nectar robbing levels over 8 weeks. The number of flowers bloom peaks around week 3, whereas robbing peaks around week 7.

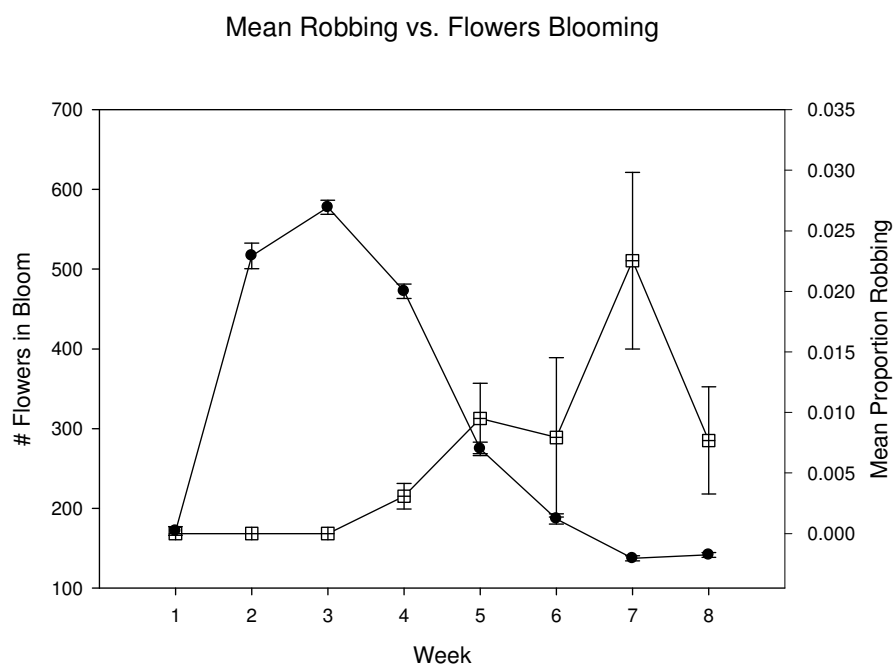


Figure 3. *Ipomopsis* robbing differs for every site.

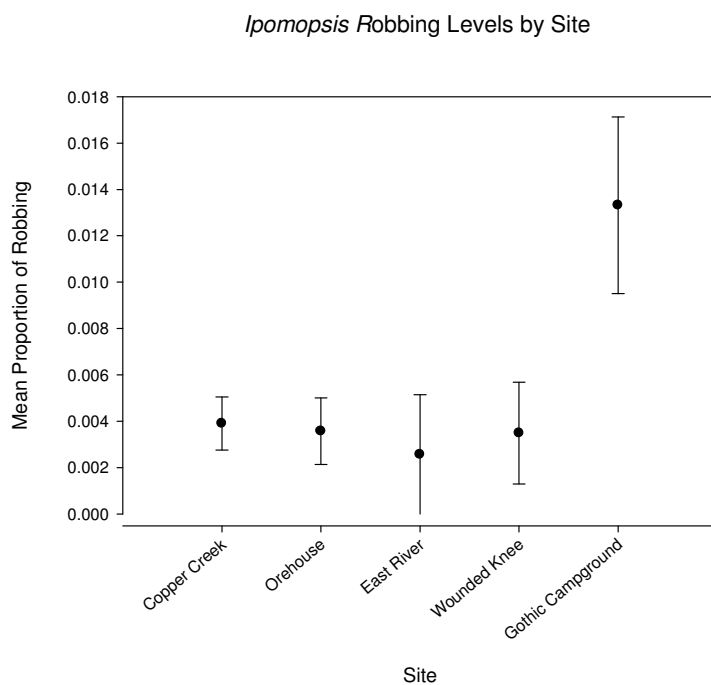


Figure 4. Mean peak robbing in *Linaria vulgaris* from 1998- 2006 showing a decrease in robbing but still a significant amount of robbing is occurring.

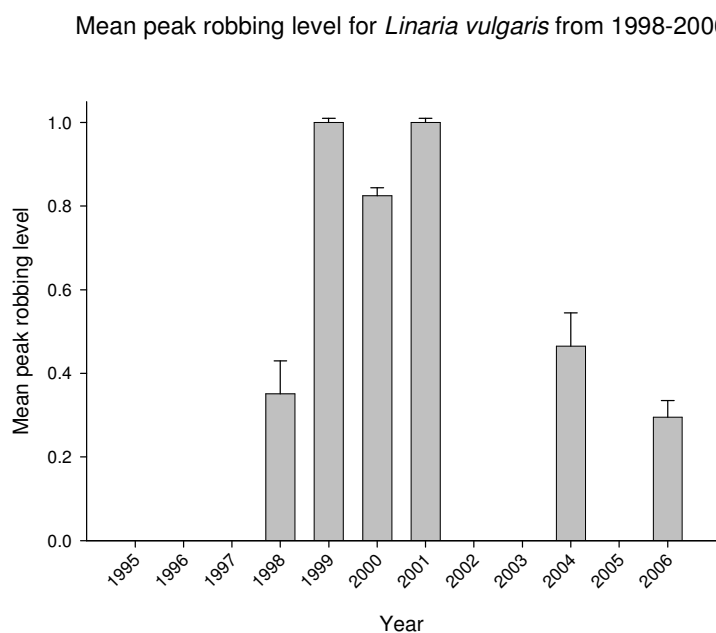


Figure 5. There is no significant difference in robbing on *Linaria* by week.

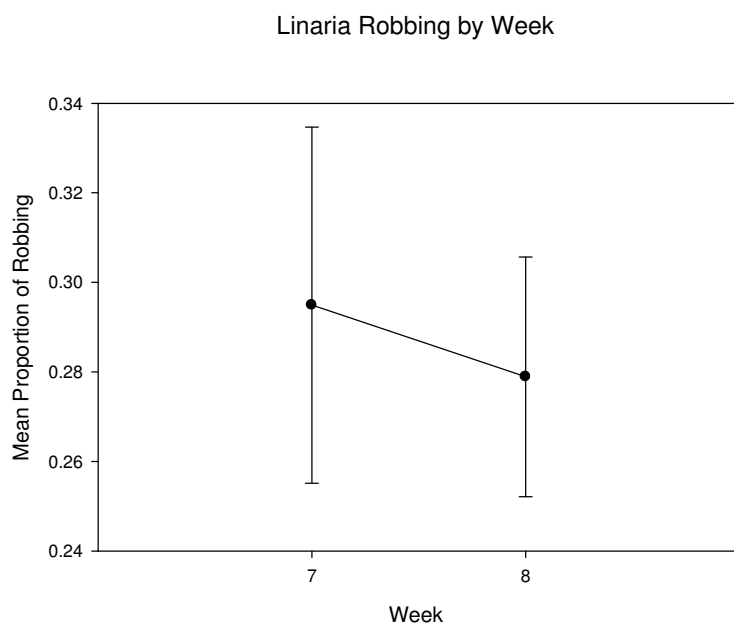
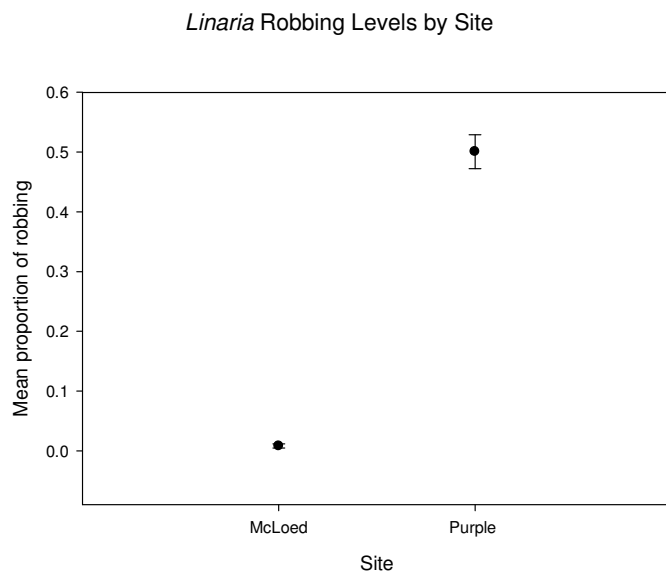


Figure 6. There is a significant difference between robbing in *Linaria* sites.



References

- Arizmendi MC, Dominguez CA, Dirzo R (1996) The role of an avian nectar robber and of hummingbird pollinators in the reproduction of two plants species. *Funct Ecol* 10: 119-127
- Galen C (1983) The effect of nectar-thieving ants on seedset in floral scent morphs of *Polemonium viscosum*. *Oikos* 85:245-249
- Galen C (1999) Flower and enemies: predation by nectar-thieving in relation to variation in floral form of an alpine wildflower, *Polemonium viscosum*. *Oikos* 85:426-434
- Goulson D, Stout JC, Hawson SA, Allen JA (1998) Floral display size in comfrey, *Symphyrum officinale* L. (Boraginaceae): relationships with visitation by three bumblebee species and subsequent seed set. *Oecologia* 113: 502- 508.
- Inouye DW (1983) The ecology of nectar robbing. In: Bentley B, Elias T (eds) *The biology of nectarines*. Columbia University Press, New York, pp 153-173.
- Irwin RE, Brody AK (1998) Nectar robbing in *Ipomopsis aggregata*: effects on pollinator behavior and plant fitness. *Oecologia* 116:519-527.
- Irwin RE, Brody AK (2000) Consequences of nectar robbing for realized male function in a hummingbird-pollinated plant. *Ecology* 81: 2637-2643.
- Irwin RE, Maloof J (2002) Variation in nectar robbing over time, space, and species. *Oecologia* 133:525-533
- Maloof J, Inouye DW (2000) Are nectar robbers cheaters or mutualists? *Ecology* 81:2651-2661.
- Pyke GH (1982) Local geographic distributions of bumblebees near Crested Butte, Colorado: competition and community structure. *Ecology* 63: 555-573.
- Roubik DW (1982) The ecological impact of nectar-robbing bees and pollinating hummingbirds on a tropical shrub. *Ecology* 63:354-360
- Traveset A, Willson MF, Sabag C (1998) effect of nectar-robbing birds on fruit set of *Fuchsia magellanica* in Tierra del Feugo: a disrupted mutualism. *Funct Ecol* 12: 459-464.