

Why doesn't nectar robbing decrease seed set in some bee-pollinated plants?

Student: Jisung Park  
Mentor : Rebecca Irwin, Jacob Hailing

Full Time Independent Research  
Summer 2015

## **Acknowledgement**

First, I give thanks to Binney Girdler, college senior thesis adviser, and Rebecca Irwin, chief mentor, for providing me a great opportunity to research nectar robbing bumble bees. I appreciate Jacob Heiling, direct mentor, and Emily Mooney, research coordinator, for guiding me how to design and write a senior thesis. I acknowledge Heather Ellison for helping me a collecting data and Shannon Sprott for guidance on spatial data. I thanks Lauren Morse and bee work crew members (Elinor Lichtenberg, Cody Phillips, Kelsey Brennan,Emily Graber, Rhiana j., Sean Griffin,Sarah Kelley Richman, Caity Winterbottom, Jenna Lea, Kevin Barry, Cherie Nelson,Lauren Howe-Kerr,Dan Papaj, Paul CaraDonna, and Amy Iler) for research technique help and support. Finally, I attribute Ian Billick and Jennifer Reithel (directors), and Rocky Mountain Biological Laboratory for great experiences.

## Abstract

Herbal impact on nectar robbing is species specific by pollinator species (Maloof and Inouye 2014). Unlike hummingbird pollinated plants, some bee-pollinated species does not suffer reproductive loss by nectar robbing (Maloof 2001, Mayer et al. 2014, Maloof and Inouye 2014). This observational study is investigating the probability that *Bombus.sp* forage either *Mertensia ciliata* or *Corydalis caseana* legitimately at least once before the flower is robbed. I also measured population-label robbing rate. I bagged the flower two days before, and on the days of experiment I waited in front of the uncovered flowers in order to voice- record Bumble bees' visit type (legitimate vs. robbing). Furthermore, I conducted transects on the flower patch, and every time I saw the bee I recorded their visit type. *Bombus sp.* (*B.flavifrons*, *B. mixtus*, *B. bifarius*, *B.appositus*) was very likely visit legitimately first before it robbed (94.12 %, n=32), than robbing first (5.88%, n=2). Populational robbing rate was 8.98% (n=167), 35.29% (n=34) for *C.caseana* and 6.77%(n=133) for *M. ciliata*. Though this is observational study, I speculate that *Bombus sp.* is able to recognize flowers with low nectar volume. Unlike hummingbird that avoid low nectar flowers(Irwin 2000), I am guessing bumble bee may rob the flower to extract last bit of nectar. Therefore, nectar robbing may be more of a "Plan B" then "Contract Breaking" to both *Bombus sp.* and studied plant species, for flowers may already receive pollination, and *Bombus sp.* could extract last bit of energy.

## Introduction

Plant pollinator mutualisms signify interdependent relationships between plants and pollinating animals. In a typical plant-pollinator mutualism, the plant provides nectar which the visitor uses as an energy source, and the pollinator provides reproductive opportunities for the

plant. This may be thought of as a contract that benefits each participant (Rodriguez 2009).

However, if the condition that background the mutualism is changing, this participant may “cheat” (Rodriguez 2009)

There are many ways that a pollinator could “cheat” (Inouye 1980). For instance, a bumble bee with a short tongue facing a flower with a long corolla could instead bite the corolla with the mandible to reach nectar (Inouye 1980). This is primary robbing (Inouye 1980). Secondary robbing happens when a forager visits a flower through an already formed robbing holes (Inouye 1980). Nectar robbing seems to fall in “contract breaking”, especially for pollinators who need to invest more on legitimate foraging (Inouye 1980).

Earlier studies have focused on negative impacts on nectar robbing. Darwin speculated that nectar robbing might negatively impact plant fitness (Irwin 2000). Heavily robbed (80%) *Ipomopsis aggregata* show significantly decreased fluorescent dye donation, pollen receipt and average seed set per fruit relative to unrobbed flowers (Irwin and Brody 1999). Broadtail and rufous hummingbirds (*Selasphorus platycercus* and *Selasphorus rufus*, respectively) distinguish between robbed and unrobbed *I. aggregata* flowers, and visit fewer robbed flowers, resulting in decreased fitness gains from robbed flowers (Irwin 2000). As an immediate effect, Nectar robbing reduces mean nectar volume.

Several pieces of evidence suggest that the role of nectar robbers as cheaters may oversimplify the relationship (Maloof and Inouye 2014). First, a study of *Corydalis caseana*, *Bombus occidentalis*, and *B. appositus* demonstrated no negative impact of nectar robbing on seed set and fruit set (Maloof 2001). On the contrary, bumble bees departing nectar robbed flowers flew significantly longer distance than the control group, increasing the pollen flow and decreasing geitonogamy (Maloof 2001). Similarly, when nectar volume was experimentally decreased by 40% in *Aconitum napellus* ssp. *lusitanicum* bees visited fewer flowers per inflorescence and spent less

time per flower, increasing the number of flowers visited per unit time (Mayer et al. 2014). This study also showed that bees leaving robbed flowers flew longer distances (Mayer et al. 2014).

Nectar robbing is species specific (Maloof and Inouye 2014). Unlike hummingbirds, many bee-pollinated species does not suffer reproductive losses by nectar robbing (Maloof and Inouye 2014). Like pollinators, plants species that have many flowers per inflorescence might benefit from nectar robbing via reduced geitonogamy (Maloof and Inouye 2014). Nectar robbers can pollinate flowers in some cases (Maloof and Inouye 2014). When a bee positions itself to rob a flower, the pollen could sometimes reach the bee's abdomen (Maloof and Inouye 2014). Finally, nectar robbing may have large evolutionary implications (Maloof and Inouye 2014). For example, having long corolla might attract not only legitimate pollinators but also nectar robber which could also pollinate. This is because long corolla prevents legitimate pollinator to drain all nectar, giving a chance to nectar robber. (Maloof and Inouye 2014)

Nectar robbing is also space specific. When primary robber decreased small volumes of nectar, next legitimate forager on the flower might not become a robber. (Maloof and Inouye 2014). For example, *Bombus occidentalis* leaves 20% of nectar after it robs nectar, resulting high standing crops (>80%) on *Corydalis caseana* (Maloof and Inouye 2014). If there are few alternative flower species that satisfy similar nutrition need, legitimate pollinators might not become a nectar robber (Maloof and Inouye 2014). This is because switching the flower has learning cost and bee is not willing to risk new flower species that have different nutrition contents. (Maloof and Inouye 2014).

Most of the nectar robbing study species that showed neutral and beneficial impacts on plant fitness focus on bee-pollinated plants (Maloof 2001, Mayer et al. 2014, Maloof and Inouye 2014). No study suggested that hummingbird species provide positive influences on plant reproductive success (Irwin 2000). If the immediate responses of nectar robbing, like damage in corolla and decreased nectar standing crop, is same for both pollinator species, why resulting plant fitness differ by species?

**The purpose of this study was to determine why nectar robbing does not negatively impact plant fitness in some species. I pose two questions: 1-What is the likelihood that *Bombus. sp.* would visit at least one legitimate visit before it is robbed? How frequently does *Bombus. sp* rob the flower?** In order to investigate, I set-up three to six stalks of *Mertensia ciliata* & *Corydalis caseana*, one species at a time, as a part of observation study. I measured *Bombus spp.* type, visit type, and visit duration. To investigate the second question, I conducted transects through the flower patch, observing whether *Bombus spp.* forage legitimately or not.

## Method

### Site information

Heather Ellison and I collected first visit probability data at subalpine meadow near Rocky Mountain Biological Laboratory

Table I. *C. caseana* and *M. ciliata* field site information.

| Site Name              | Focal plant       | Latitude | Longitude | Area   | Elevation (m) |
|------------------------|-------------------|----------|-----------|--------|---------------|
| Upper Washington Gulch | <i>C. caseana</i> | 38.95997 | -107.0338 | 1233.4 | 3149.8032     |
| West Kebler Pass       | <i>C. caseana</i> | 38.86496 | -107.1065 | 854.28 | 3070.2504     |
| Lower Washington Gulch | <i>M. ciliata</i> | 38.94049 | -107.023  | 1089.2 | 3024.835      |
| West Kebler Pass       | <i>M. ciliata</i> | 38.86497 | -107.1058 | 322.06 |               |
| Avery                  | <i>M. ciliata</i> | 38.97622 | -106.9961 | 306.98 |               |
| Yule                   | <i>M. ciliata</i> | 38.95838 | -106.9905 |        | 2895.6        |

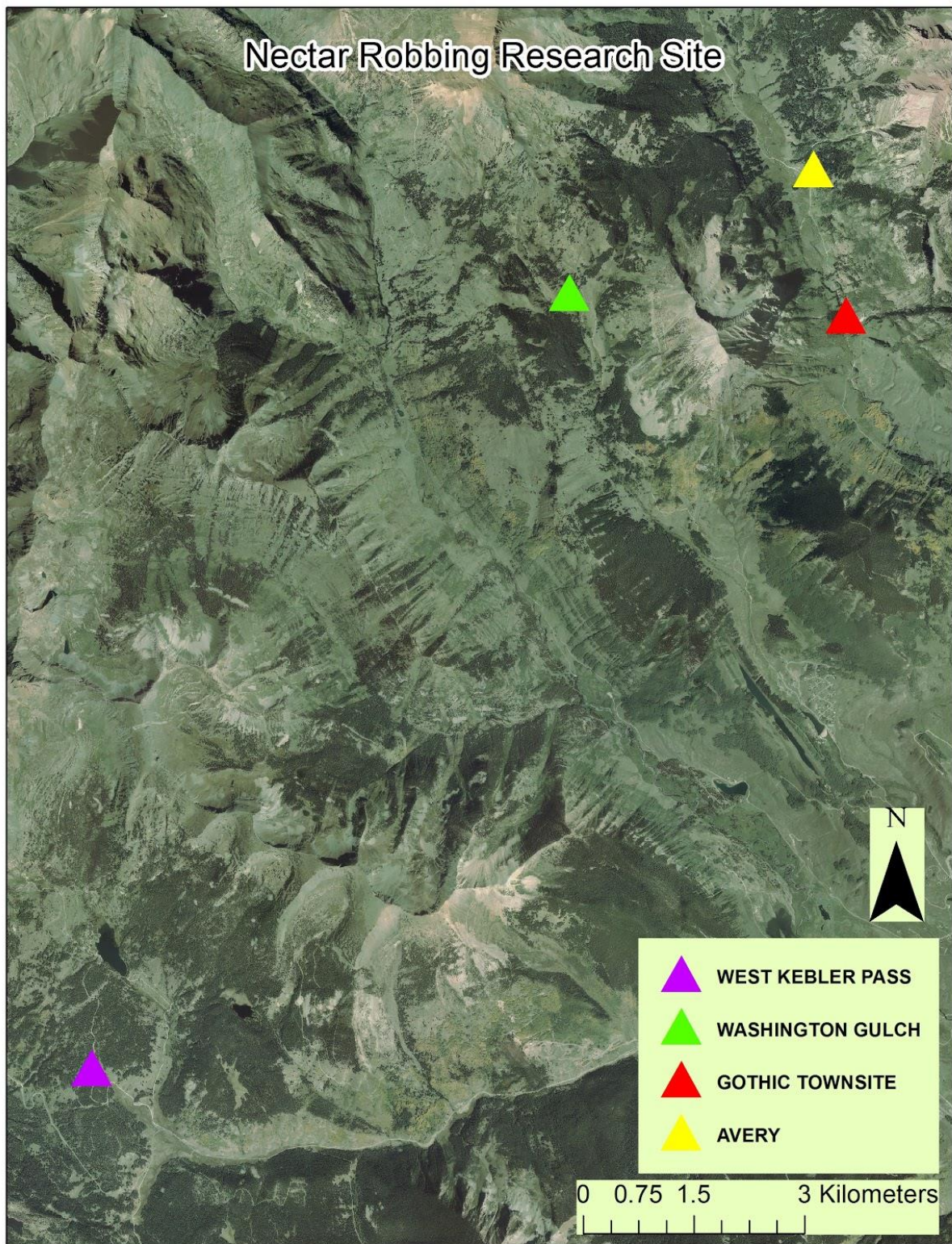


Figure 1. Field sites spatial location in Crested Butte, CO, USA

## Species Information

*Corydalis caseana* (*C. caseana*) is tall, reaching 1-2m. It is perennial herb, and researchers usually find it at subalpine meadows, where there are fresh streams (Maloof 2001). Researchers usually observe large monospecific patches. Unfortunately, *C.caseana* is not a common species because its distribution is scarce and apart from one patch to another (Maloof 2001). Though plant in earlier age starts with a single stem, a number of stems increases (2-25) as plants matures (7 years)(Maloof 2001). The top of those stems consist a terminal racemose inflorescence with 70 flowers and several side inflorescence with 70 flowers (Maloof 2001). Since the flower patches are so close from one another, it is very difficult to know which stems belong to which individual plants. Therefore, most bee behavioral studies focused on individual inflorescences (Maloof 2001). Because of the flower's long nectar spur, reaching approximately 12-16mm, long-tongued visitor like *Bombus appositus* is able to forage legitimately (Maloof 2001). Some short-tongued bumble bees are not able to reach the nectary, and therefore often robs by putting a hole in the petals (Maloof 2001). The nectar has approximately 35% sugar. If no bee is visiting, the plant produce nectar by 1mL per day (Maloof 2001). After flower blooms, it last for four days. Although *C. caseana* is able to self-pollinate, it require insect mediated pollination (Maloof 2001). Self-pollinated flowers give fewer fruits, fewer seeds, and tinier seeds than open or outcross pollinated flowers (Maloof 2001). This means inbreeding is less fit than outcrossing (Maloof 2001). *B. oppositus* was the main player of pollination, but smaller be like *B. mixtus* often visited the plant.

*Mertensia ciliata* (*M. ciliata*), also called as the alpine bluebell, is the perennial herb that live in wet subalpine meadows in Rocky mountain (Gerber 1985). When flowers bloom in the highest rate, a typical patch of the flower have 100-10000 flowers (Gerber 1985). *M.ciliata* have

blue and tubular flowers, ranging from 12-15mm (Gerber 1985). It grows from cymes forming a cluster, and flower blooms basipetally (Gerber 1985). Pollen is attached to the anthers when the flower starts to bloom, but insect eliminate it within 1-2day (Gerber 1985). Stigma is active regardless of anther detachment until 4 to 5 days when flower wilt (Gerber 1985). The flower produces up to four seed (Gerber 1985). *M.ciliata* start flowering from early July to August (Gerber 1985). Single stalks maintain flowering for 3-5 week. Many bees visited the flowers, including *B. bifarius*, *B. flavifrons*, *B. mixtus*. *B.mixtus* is originally a rare species, but this year we observed increased abundance.

### Bee observation

#### Set up

I prepared three to six stalks of either *Mertensia ciliata* or *Corydalis caseana*. Each observation study consisted single species. I bagged the live flower that is close to each other two days before observation. When there were no stalks with only unopened flowers, I trimmed open flower to ensure no bee visitation. The cluster of bagged stalks was for a single round, so all the stalks observed in a day is from particular field sites. On the day of observation, I unleashed the bags, and randomly marked a sequential number on a leaf of each bagged stalk. For every round of trials, I counted the number of flowers of the numbered stalks. I did not resample the same plant, so I moved to next location after bee visited at least one of the flower. I also record the data when the bee visited more than one numbered stalks, or when the bee revisited either same numbered stalk or the same flower on the numbered stalks. I monitored stalks until bee finished visiting the plants. While the bee was visiting the flower, I voice recorded the duration of visits, total number of open flower, within stalk flower sequence, visitor species, visit type, trial and stalk number for identification and recording time that bee is away.

## Behavioral data

I analyzed voice recordings to determine flower visit type (whether they are for pollen, nectar, both, primary rob, or secondary rob). Ultimately I determined the robbing rate of the *Bombus sp.* community. Heather Ellison and I collected total 470 bee visit. Among those, 35 datasets signifying the first visit of each round is used for analysis.

## Data Analysis

I used chi-square test in order to determine likelihood that *Bombus sp.* would visit legitimately at least once before it is robbed. I defined the dataset to be the first visit of each trial, to ensure *Bombus. sp* did not visit the flower before. I used  $\alpha=0.05$  as my significance cutoff. I used JMP version 12.0.1 (SAS Institute Inc., Cary, NC, 1989-2007) and Excel version 6.1.7601 (Microsoft Corporation, USA) for statistical analysis and figure formulation.

## Robbing Rate

### Arrays

I utilized field site with abundant *Mertensia ciliata* & *Corydalis caseana*. Some sites (Washington gulch, Yule, and Avery) had only one abundant species, so I conducted transects of single species type. Another site (West Kebler Pass) had mixed patches both species, so I labeled the flower each observation and conducted mixed species transect. I made a particular trail per site that maximizes inspection of the various patch area. I considered completion of the trail to be one transaction. While I walked the trail, I monitored if the bumble bees foraging either *Mertensia ciliate* & *Corydalis caseana*. Every time I found the *Bombus sp.*, I voice recorded species type, visit type (legitimate, robbing), pollen load, cast (queen, worker) flower species, and the time of day. Typically I proceed 3-6 transaction in a day. I was aiming to find how often

does robbing happens. I kept the same path per site for consistency. I collected total 170 *Bombus* sp. visitation.

### Analysis

I determined robbing rate with following equation: (number of robbed foraging)/(total foraging)\*100. I used Jmp version 12.0.1 (SAS Institute Inc., Cary, NC, 1989-2007) and Excel version 6.1.7601 (Microsoft Corporation, USA) for statistical analysis and figure formulation.

### Result

Probability that a flower received at least one legitimate visit before it is robbed (94.12 %, n=32) was significantly higher (Chi square=14.47, d.f.=32;  $p<0.0001$ ; figure 2) than probability that flower is robbed first (5.88%, n=2). Overall robbing rate of *M.ciliata* and *C.caseana* in a population level was 8.98% (n=167), 35.29% for *C. caseana* (n=34) and 6.77% for *M.ciliata*(n=133)

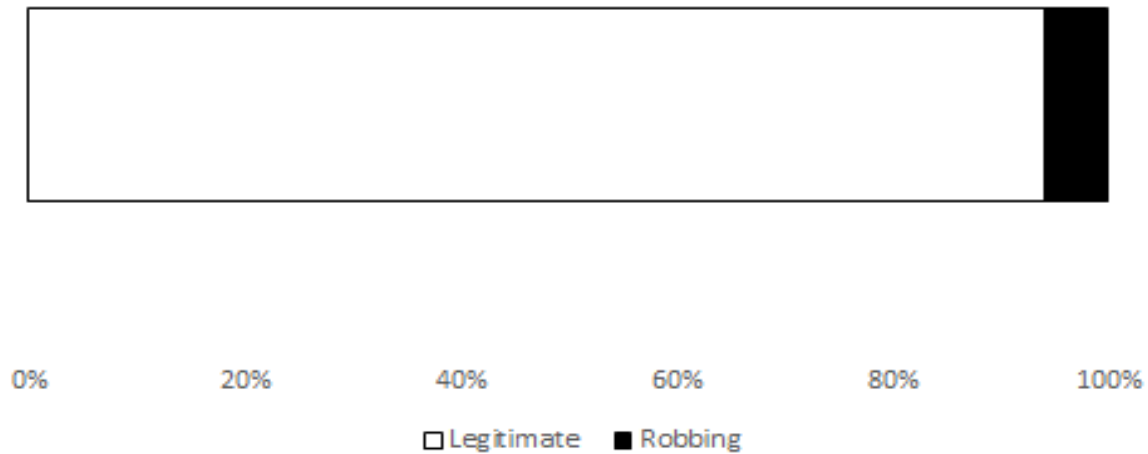


Figure 2. The probability that the bumblebee forage legitimately at least once before it robs either *M.ciliata* or *C.caseana* compared to the probability the bee would rob the flower first (Number of Legitimate Visit or Robbing visit/Total Visit\*100)

## Discussion

This observational study investigated why the nectar robbing on bee-pollinated flowers does not negatively impact plant reproductive success. The hypothesis is supported. The probability (94.12%) that the bumble bee would visit legitimately prior to robbing is significantly higher than the probability that robbing (5.88%) happens first (Chi square=14.47, d.f.=32;  $p < 0.0001$ ; figure 2). Therefore, bumble bees would pollinate legitimately first, so the damage occurred on later rob does not necessarily harm plant reproduction of the already pollinated plants. I speculate that bumble bees would choose to rob after other bees drained the nectar legitimately, though this might be a good topic for research for it is not yet proven. Moreover, since this is an observational study, I cannot imply that this correlation is not caused by other factors.

The actual robbing rate in the location I studied (8.98%) is higher than the first visit probability of robbing (5.88%). Even though the study shows higher robbing rate, first visit probability study shows that a flower is very likely to be legitimately visited before it is robbed. Therefore, it could be speculated that the higher populational robbing incidence occur after bee visited flower legitimately at least once. Due to the learning cost and distinct nutritional needs (Maloof and Inouye, 2014), bumble bees were less likely switch flower species, which benefit plant species because it maximizes pollination probability.

The species specificity of nectar robbing applies here. Irwin (2000) suggested that hummingbird nectar robbing does not give positive impacts on plant reproductive success. I would speculate that the behavioral pattern of hummingbird is relatively different from that of bumble bees; this may means that the bumble bees' tendency to visit the flower legitimately prior to robbing may not be universal foraging pattern to hummingbird. For example, hummingbird might choose to avoid flower with low nectar volume (Irwin 2000), while the bumble bees might rob the flower with low nectar volume. I speculate the possibility that bee could also distinguish nectar volume on its own way, given that bee is likely to visit untouched flower legitimately first. Moreover, nectar robbing do pollinate the flower (Maloof and Inouye, 2014) but in a lower rate. I am guessing that flower may have a long corolla and more nectar not only because flower benefit from pollination by robbers (Maloof and Inouye, 2014), but also because it maximize chance of legitimate pollination with higher fertility probability.

I also noticed that the populational robbing rate is variable to time and location. Maloof and Inouye (2014) reported that the nectar robbing rate of *M. ciliata* is around 50%, much higher than my observation (6.77%). further research required on the variation of robbing rates. Though I was not able to establish causational relationship, My study supported Maloof and Inouye

(2014) who inspected that the Bumble bee's willingness to switch legitimate foraging behavior into robbing might be linked to the flower's fewer leftover nectar volume.

I am speculating that flowers with larger nectar volume might have higher rates of pollination than flowers with little nectar volume. Though my study was unable to determine whether nectar robbing itself give negative fitness, I am guessing nectar robbing on natural setting would be not trivial to plant's overall fitness because the flower receive multiple rounds of visit, which include legitimate one. The study did not investigate that how overall indifferent plant fitness effect correlates into changes of flying distances and geitonogamy, this could be a further topic for research.

One interesting observation is that most of nectar robbing that I observed is primary robbing. Maybe because I visited the plant while flowers are "fresh". Likewise, while I saw *B. appositus* pollinating *C.caseana* legitimately most of the time, I frequently saw smaller *B. fawcettii* and *B. mixtus* robbing the same plant species. My observation is consistent with Maloof (2001). I am speculating that the reason why *C.caseana* robbing rate was different with *M. ciliata* is because the visitor species composition per species is different.

Considering the bee's likelihood to visit first before robbing, nectar robbing is not a "contract breaking", rather it is "Plan B". This is because the plants are pollinated in lower rates or already pollinated fully before, and bees are able to extract last bit of nectar from already low volume nectar flower. However, in order to fully establish the relationship, similar study as experiments is necessary as further research topics.

## Reference

- Gerber, M. A. 1985. The Relationship of Plant Size to Self-Pollination in *Mertensia Ciliata*. *Ecology* 66:762-772
- Inouye, D. W. 1980. The Terminology of Floral Larceny. *Ecology* 61:1251–1253.
- Irwin, R. E. 2000. Hummingbird avoidance of nectar-robbed plants : spatial location or visual cues. *OIKOS* 91:499–506.
- Irwin, R. E., and A. K. Brody. 1999. Nectar-robbing bumble bees reduces the fitness of *Ipomopsis aggregata* (*Polemoniaceae*). *Ecology* 80:1703–1712.
- Maloof, J. 2001. The effects of a bumble bee nectar robber on plant reproductive success and pollinator behavior. *American Journal of Botany* 88:1960–1965.
- Maloof, J. E., and D. W. Inouye. 2014. Are Nectar Robbers Cheaters or Mutualists ? *Ecology* 81:2651–2661.
- Mayer, C. C. Dehon, A. L. Gauthier, O Naveau, C. Rigo, and A. L. Jacquemart. 2014. Nectar robbing improves male reproductive success of the endangered *Aconitum napellus* ssp. *Lusitanicum*. *Evol Ecol* :669–685.
- Rodriguez, H. D. E., and M. Josefina. 2009. Conditional outcomes in mutualistic interactions.

Free from grammatical and spelling errors

Active voice used

Writing concise and clear

Narrative thread present (a clear story emerges)

Discussion

Avoids explicitly repeating results

Presented as a narrative, with well-constructed paragraphs, that proceeds in logical order

Addresses whether hypothesis(es) is/are supported

Figure

Non-data ink minimized

Descriptive figure, table title