

The effects of primary and secondary nectar robbing of *Ipomopsis aggregata* on hummingbird
visitation

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Independent Research program and Research Training in Wildlife Biology

Summer 2015

Abstract:

Nectar robbers feed on the nectar obtained through holes that have been chewed into the flower. This behavior often leads to a reduction in effective pollination because nectar robbers avoid floral reproductive structures (Inouye 1980). Primary nectar robbers puncture the flower, creating an opening in the petal from which to remove nectar, while secondary nectar robbers use the holes created by primary robbers. Studies have shown that nectar robbing can negatively affect plant reproduction by deterring legitimate pollinators (Irwin et al. 2010). Furthermore, data have shown that secondary nectar robbing can result in increased reproduction costs beyond that caused by primary robbing alone (Richman et al. in prep). One reason this might be is that legitimate pollinators are further deterred as plants incur more nectar robbing. I have tested this hypothesis by collecting 30 *Ipomopsis aggregata* plants and subjecting them to varying levels of nectar robbing: none, primary robbing only, and secondary robbing. After applying treatments I arranged them in a field and observed hummingbird visitations, recording the sex and species of the bird as well as the specific plants visited and the number of flowers probed per plant. The data show that hummingbirds probed more flowers per plant of the control group than they did of either of the nectar robbed groups and they probed more flowers per plant of the primary group than they did of the secondary group. This suggests that hummingbirds will not stay at a plant as long if it has a smaller nectar reward.

Introduction:

Hummingbirds tend to visit plants with flowers that have wide tubular corollas (Fenster et al. 2004). They are able to extract the nectar from the floral opening, during which the pollen attaches to the bird's beak or feathers which will then be transferred to the next flower that it

drinks from, thus resulting in pollination. Studies have shown that nectar robbing, a type of floral larceny, can negatively affect plant reproduction by deterring these legitimate pollinators (Irwin et al. 2010).

Nectar robbing is a type of floral larceny that is very common in plants with flowers that have tubular corollas or long nectar spurs (Barrows 1980). Nectar robbers feed on the nectar obtained through holes that have been chewed into the corolla. This behavior often leads to a reduction in effective pollination because nectar robbers avoid floral reproductive structures (Inouye 1980). Primary nectar robbers puncture the flower, creating an opening in the petal from which to remove nectar, while secondary nectar robbers use the holes created by primary robbers. Nectar robbers remove nectar, potentially depriving legitimate pollinators of a reward. This might result in pollinator behavioral changes. For example, hummingbirds have been observed to not only bypass nectar robbed flowers, but to increase the distance between them and such flowers, likely in order to find alternative unrobbed sources of nectar (Irwin & Brody 1998). This results in decreased pollination of the nectar robbed plants and therefore decreased reproductive success (Irwin & Brody 1998). Compared with primary robbing, not nearly as much research has been done concerning secondary robbing (Irwin et al. 2010).

Data have shown that secondary nectar robbing can result in increased reproduction costs beyond that caused by primary robbing alone (Richman et al. in prep). However, the mechanism that drives this response is unknown. My experiment was designed to test the hypothesis that hummingbird pollinators are further deterred as plants incur more nectar robbing, i.e. secondary robbing.

Materials and Methods:

The study system:

This study was conducted at the Rocky Mountain Biological Laboratory town-site in Gothic, Colorado, USA, at an elevation of 2800 m (See Appendix A). The plant species studied was *Ipomopsis aggregata*, a native perennial montane herb that is mostly pollinated by Broad-tailed hummingbirds and Rufous hummingbirds, but also experiences nectar robbing by the bumblebee, *Bombus occidentalis* and secondary robbing by several *Bombus* species, most commonly *Bombus bifarius* (Irwin & Brody 1998).

Experimental design:

In order to test the hypothesis that hummingbird visitation decreases with increased nectar robbing, 50 newly bolted, single stalked *Ipomopsis* plants were collected from within the town site and transferred into individual flower pots and then moved to a weatherport by the research center at RMBL. The plants were treated for transplant shock using root stimulator for the first three days following collection. Extra plants were kept in case some plants did not survive transplant.

The *Ipomopsis* were haphazardly assigned to one of three treatments: control, primary, or secondary. (n = 10 plants per treatment) In the control group plants were not nectar robbed at all. In the primary group all flowers were artificially primary robbed once only. In the secondary group each flower was primary robbed once and subsequently nectar robbed every new treatment until the corolla abhisced.

Primary nectar robbing was simulated by perforating a small hole in the base of the floral corolla using dissecting scissors and then using capillary action to extract all of the available nectar using a 10 μ L microcapillary tube. Treatments were applied six days per week to all newly

opened flowers. New flowers were treated at the beginning of each experiment day, and the flowers on the secondary robbing treatment plants were re-robbed. All flowers were gently squeezed to control for manipulation of the flowers.

Plants were placed in a field for three hours each day in a 5 by 6 array one meter apart from each other and all hummingbird visits were recorded using a voice recorder. The species and sex of each hummingbird that enters the experimental grid of plants was recorded. For each hummingbird foraging bout I recorded which plant was visited and the number of flowers visited per plant. The plants were returned to the weatherport after each three hour observation period in order for them to replenish their nectar volume over the following 24 hours. No natural nectar robbing occurred during the observation periods.

Two ANOVA tests were performed using R studio to analyze the data. The first compared the proportion of flowers probed to the three treatment types and the second compared the number of times plants were visited per day to the three treatment types.

Results:

The proportion of flowers probed per plant was analyzed per treatment using an ANOVA test (Figure 1). The data show that the control group had the highest average proportion of flowers probed (0.63) with a standard error of 0.05 followed by the primary group (0.58) with a standard error of 0.04. The secondary group had the lowest proportion of flowers probed (0.54) with a standard error of 0.04. The proportion of flowers probed did not differ among the treatments ($F = 0.0975$, $P = 0.381$).

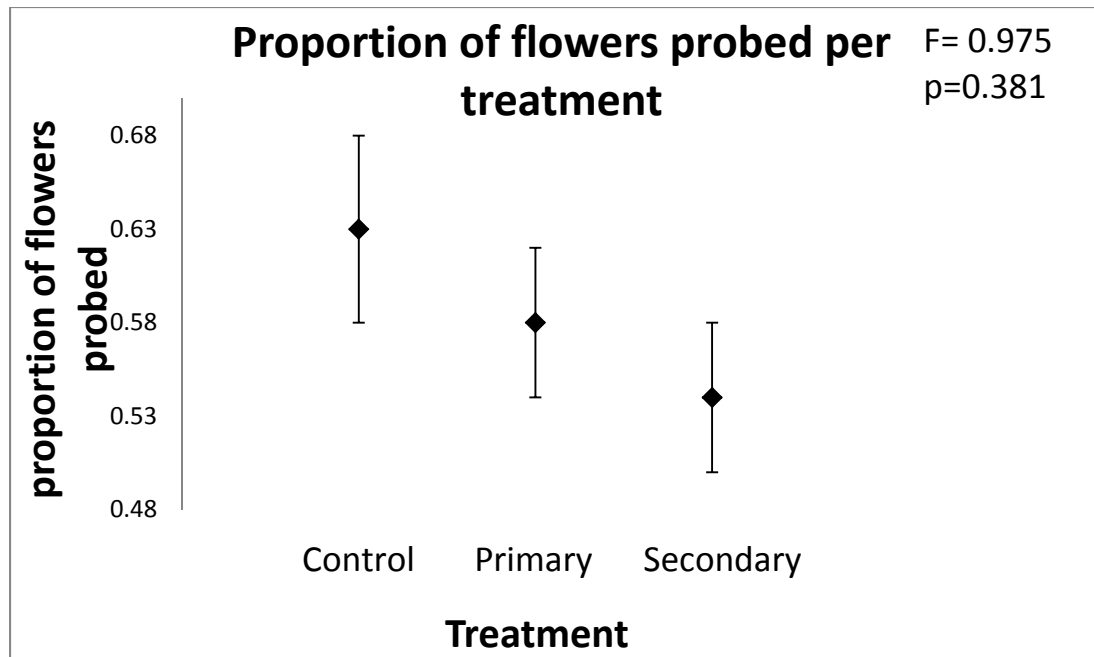


Figure 1: The average proportion of flowers probed per treatment: control, primary, and secondary. The dots represent mean value of proportion of flowers probed per treatment and the lines represent the standard errors. The graph shows that the control group had the highest proportion of flowers probed out of the three treatments and the secondary group had the lowest.

A second ANOVA test was used to analyze the number of times plants were visited per day per treatment (Figure 2). The average number of times plants were visited per day was highest in the control group (0.32) with a standard error of 0.04 followed by the secondary group (0.31) with a standard error of 0.05. The primary group had the lowest number of visits per day (0.26) with a standard error of 0.05. The number of plants visited per day did not differ among

the treatments ($F = 0.262$, $P = 0.77$).

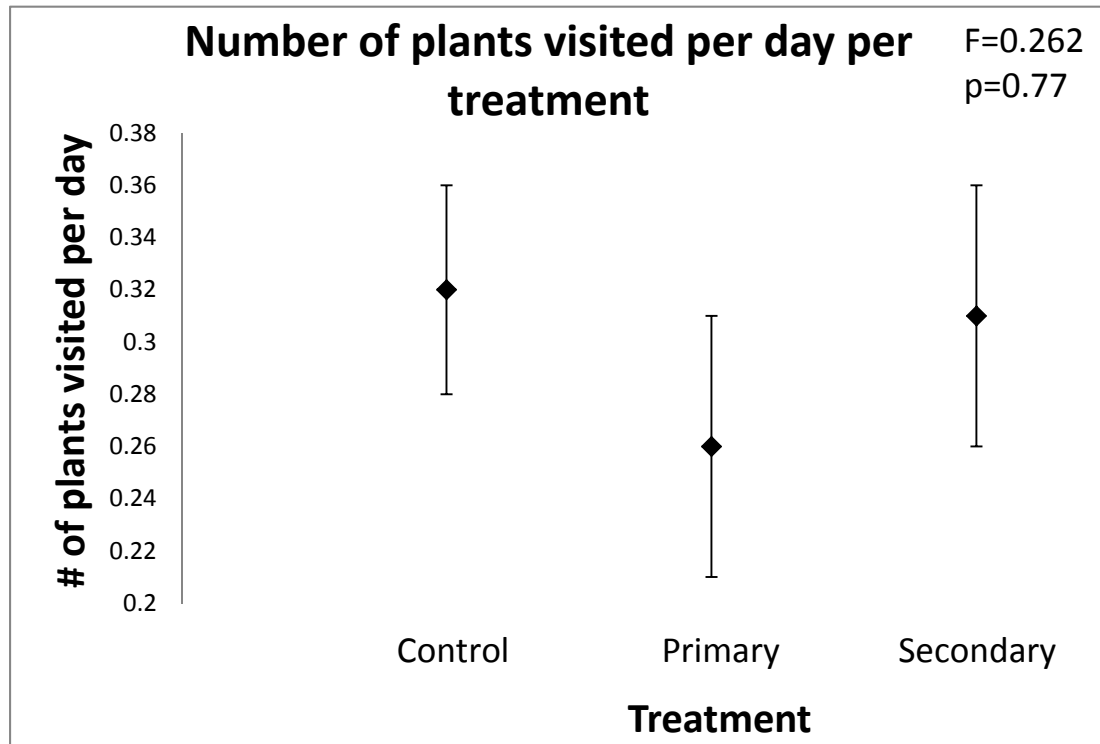


Figure 2: The average number of times plants were visited per treatment. The dots represent the average number of times plants were visited per day per treatment and the lines represent the standard errors. The graph shows that the control group had the highest number of hummingbird visits per day and the primary group had the lowest.

Discussion:

All animals must forage for food to meet their energetic needs; however, the process of doing so is energetically costly. Hummingbirds especially have a very high metabolism and therefore need to balance time spent searching for food with time spent feeding. According to the marginal value theorem, foragers use information at hand to determine the value of a particular patch of resources and make decisions on patch departure based on the assessed value of the patch (Charnov 1974). An optimally foraging organism should leave a patch of resources when

the rate of payoff falls short of the average rate of payoff of the entire area (Charnov 1974). A previous study testing the marginal value theorem in regards to hummingbirds found that the decision to leave was a factor of the number of flowers available on an inflorescence, the number of flowers visited, and the amount of nectar obtained at the last flower (Pyke 1978). If the marginal value theory is accurate then you would expect hummingbirds to spend less time probing plants with lower nectar rewards.

The data show that hummingbirds probed more flowers per plant of the control group than they did of either of the nectar robbed groups and they probed more flowers per plant of the primary group than they did of the secondary group. This suggests that hummingbirds will not stay at a plant as long if it has a smaller nectar reward. This is consistent with my hypothesis. Furthermore, a study has found that pollination success increases significantly with number of probes which means that plants with higher nectar production will benefit if pollinators respond by increasing probe numbers (Mitchell and Waser 1992). This is consistent with the results of my experiment that show a trend between the number of flowers probed and the level of nectar robbing,

Although the data show a trend hinting at a correlation between amount of nectar robbing and the proportion of flowers probed by hummingbirds, the p-value shows that the data are not significant. One reason this could be is because the number of hummingbird visits observed was too small. A larger sample size might also yield different results; a power analysis reveals that a sample size of 95 plants per treatment would be needed for the data to show any significant trends. The data also show no trend between the number of plants visited per day and the level of nectar robbing. This could be because there are other factors besides nectar volume contributing to a hummingbird's decision to visit a plant, such as plant height, number of flowers, flower size,

etc. Furthermore, future studies looking into the effects of primary and secondary nectar robbing on non-bird visitation might offer more insight into the complex ecological role that nectar robbers play.

Appendix A:

My 5 by 6 experimental array located at the Rocky Mountain Biological Laboratory town-site



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