

Effects of nectar robbing on pollen deposition and hummingbird-pollinator behavior in
Ipomopsis aggregata

Author: Paige Howell
Advanced Undergraduate Research
Mentor: Rebecca Irwin

Abstract

Nectar robbing can affect both male and female components of plant reproduction. To understand the mechanisms driving those plant-reproductive outcomes, I studied the effects of nectar robbing on pollen deposition and pollinator behavior in the hummingbird-pollinated host plant *Ipomopsis aggregata*. To determine the effects of robbing on pollen deposition, I conducted a field experiment in which I observed single hummingbird visits to emasculated robbed and unrobbed flowers. Once a flower was visited, I collected the stigma, stained it, and counted pollen. I hypothesized that less pollen would be found on robbed flowers than unrobbed flowers. Consistent with this hypothesis, the amount of pollen deposited on unrobbed flowers was twice as much as robbed flowers, indicating that the quality of visits to robbed flowers was lower than to unrobbed flowers. To determine the effects of nectar robbing on hummingbird behavior, I first investigated liquid as an honest signal. Previous studies had found that hummingbirds avoided robbed plants and flowers without nectar, and I focused on the honest signal that nectar provides. I predicted that liquid itself provided a cue that hummingbirds used to make decisions about which plants to visit. I observed hummingbird visits to flowers with either nectar, water or nothing and calculated the mean visitation rate. Contrary to my hypothesis, flowers with nectar had the highest visitation rate driven by the number of visits per day and the percentage of flowers probed. Then, I tested the hypothesis that hummingbirds hovering over flowers caused them to vibrate at different amplitudes and frequencies depending on the amount of nectar. I set up an experiment in the lab with a fan blowing over flowers with varying amounts of nectar and recorded them with a video camera. Flowers with no nectar had significantly higher vibration amplitudes than plants with any amount of nectar. Plants with no or low amounts of nectar vibrated at significantly lower frequencies than plants with higher amounts of nectar. Taken together, these results suggest that robbing may affect not only pollinator visit quantity but also quality, and that the cues associated with hummingbird-avoidance of robbed plants may be associated with a flowers' vibration frequency and amplitude.

Keywords: pollination, nectar robbing, *Ipomopsis aggregata*, *Bombus occidentalis*, hummingbird

Introduction

Flowering plants often rely on a mutualism with animals for pollination (Cushman and Beattie 1991). These mutualistic relationships are a sort of compromise between the plant and pollinator: the plant relies on pollination for sexual reproduction while the pollinator benefits from food rewards and other floral resources (Waser and Price 1983) and both the plant and the pollinator typically experience costs in the relationship (Bronstein 1994). With these mutualistic relationships is the potential for cheating. Other animals may be able to obtain the reward, such as nectar, produced by plants for the mutualists without performing pollination services. These animals are referred to as nectar robbers if they pierce flowers to extract nectar, instead of entering the flower as pollinators do. Nectar thieves enter the flower in a way similar to pollinators, but still transfer little or no pollen, often due to a morphological mismatch between the floral visitor and the flower (Inouye 1980).

Nectar robbing often occurs in hummingbird-pollinated plant systems (e.g., McDade and Kinsman 1980, Roubik et. al. 1985, Irwin and Brody 1998, Irwin 2000). In many of these hummingbird-pollinated systems, nectar is a limiting resource for hummingbirds given their high energy demands during flight and hovering (Carpenter, 1978). Hummingbirds may not only compete with other hummingbirds for nectar, but also with bees that act as nectar robbers. Nectar robbers may compete with hummingbirds via interference competition and exploitation competition. In the case of interference competition, a study of nectar robbing on the tropical shrub *Pavonia dasypetala*, *Trigona ferricauda* bees claim and rob the nectar from flowers of this plant. In nectar-robbed plants, reduced seed production was caused indirectly by the

deterrence of hummingbird pollinators by bee attacks (Roubik 1982). In a study of exploitation competition, nectar robbing by the bumblebee *Bombus occidentalis* has been shown to affect pollination indirectly by influencing the behavior of hummingbird pollinators. Hummingbirds tended to avoid heavily robbed plants and flowers, and hummingbird visitation rate was significantly higher to plants and flowers in lighter robbing treatments (Irwin and Brody 1998).

The purpose of this study was to examine the effects of nectar robbing from both the perspective of the plant and the perspective of the hummingbird pollinator. The study system I focused on was the hummingbird-pollinated host plant *Ipomopsis aggregata* that is nectar robbed by the bumble bee *Bombus occidentalis*. In this system, high levels of robbing reduce seeds sired and seeds produced due to hummingbird avoidance of nectar-robbed plants and flowers (Irwin and Brody 1998, 2000). First, from the plant perspective, I investigate how nectar robbing affects per-flower pollen deposition. Previous research has found that there is typically a 20% decline in hummingbird visitation to robbed plants, but a 50-80% decline in seed production and seeds sired (Irwin and Brody 1998, 2000). This suggests that the effects of robbing may not be solely driven by hummingbird visitation numbers but also other factors, such as how good hummingbirds are at depositing pollen onto the stigmas of robbed vs. unrobbed flowers. I hypothesized that hummingbirds would deposit fewer pollen grains on robbed compared to unrobbed flowers because robbed flowers have less nectar and so hummingbirds may spend less time per flower. Studies in previous systems have shown that pollinators that spend less time per flower deposit fewer pollen grains per visit (Thompson 1986, Harder 1990, Mitchell and Waser 1992).

Second, from the hummingbird's perspective, I explored the proximate cues that hummingbird pollinators use to avoid nectar-robbled plants and flowers. In a previous study, hummingbirds avoided plants and flowers without nectar, regardless of the presence or absence of spatial cues as well as the presence or absence of visual cues such as the nectar-robber hole. The only factor that had an effect on hummingbird foraging behavior was the absence of nectar (Irwin 2000). Hummingbirds most likely use the cue of nectar absence to avoid plants or flowers visited by any type of floral visitor. However, the more proximate signals that hummingbirds use to cue in on flowers with nectar and avoid those without nectar remain unknown. First, I tested the hypothesis that the liquid in flowers provides some cue or "honest signal" that a flower has nectar. Second, I tested the hypothesis that the vibration of flowers by hummingbird wing-beats as they hover in front of a flower provides a clue to the presence of nectar.

Materials and Methods

Study site and organisms

I conducted this study from June to August 2009 at the Rocky Mountain Biological Laboratory (RMBL), elevation 2800 m, in the Elk Mountains of Central Colorado, USA. *Ipomopsis aggregata* (Polemonicaeae) is a long-lived monocarpic perennial. It produces a basal rosette of leaves for 1-7 years; once it accumulates enough resources, it sends up a flowering stalk, flowers once, and dies. Plants typically produce a single inflorescence with numerous, red, trumpet shaped flowers (Waser and Price 1989). During its year of flowering, these plants produce flowers for 4-8 weeks and individual flowers that last for between 3 and 5 days. *Ipomopsis aggregata* flowers

have nectaries located at the base of the flowers, and nectar is produced at a rate of 1-5 μL of nectar per flower per day, with a concentration of 20-25% sucrose equivalents. The flowers of *I. aggregata* are hermaphroditic and protandrous (Pleasants 1983).

Ipomopsis aggregata is the preferred host of broad-tailed (*Selasphorus platycercus*) and rufous hummingbirds (*Selasphorus rufus*) (Waser 1976, 1978). Broad-tailed hummingbirds arrive first to RMBL in the spring and rufous hummingbirds arrive later in mid-July (Waser 1976, Calder 1987, 1993). Both species spend about 10% of their day foraging for nectar to support their high metabolic rates. Hovering in flight costs hummingbirds about 2.0-3.1 mg sucrose per min, and so hummingbirds rely on nectar found in plants such as *I. aggregata* (Calder and Calder 1992).

In addition to attracting legitimate pollinators, the nectar of *I. aggregata* also attracts the bumble bee *Bombus occidentalis*. These bees are robbers and consume nectar without providing pollination services. To extract all of the available nectar, the bees use sharp, toothed mandibles to chew a hole through the corolla and insert their proboscis into the hole. This robbing does not damage the nectaries or reproductive parts of the flowers (Pleasants 1983, Irwin and Brody 1998). There is no evidence of aggressive behavior between these robbers and hummingbird pollinators that would directly affect pollination (Heinrich 1979, Pyke 1982, Pleasants 1983). Rather, the effect on plant pollination by bee nectar robbers has been shown to be indirect, as nectar robbing influences the behavior of hummingbirds by affecting their pollination behavior (Irwin and Brody 1998). In terms of male reproduction, plants subjected to high levels of robbing sired significantly fewer seeds than plants subjected to low levels of robbing. Plants were also less successful as females under high levels of robbing, as

indicated by a decrease in the percentage fruit set and seed set per fruit (Irwin and Brody 2000).

Experimental Procedures

Plant perspective: Does single-visit pollen deposition by hummingbirds vary between robbed and unrobbed flowers?

I potted 60 budding *I. aggregata* from a natural population growing behind Murray lab at the RMBL. I marked the locations of all potted plants in the field and returned the plants once my experiment was finished so that I minimized any effects on population-wide plant reproduction. I placed potted plants in peat pots, placed them in a WeatherPORT® (WeatherPORT Corp., Delta, Colorado, USA) for 4 to 5 days, and watered and fertilized them daily to allow recovery from the shock of transplant. When the plants developed elongated buds, I emasculated a series of buds on each plant by pulling out the anthers before they dehisced using fine-tip forceps.

Once the emasculated buds opened and were in female phase, I paired flowers, one robbed and one unrobbed, on each plant. To mimic nectar robbing, I used a pair of dissecting scissors to cut a hole approx. 1 mm in diameter in the side of the corollas and removed all available nectar with a 10 µl microcapillary tube. This method has been previously shown to accurately mimic nectar robbing by *B. occidentalis* on *I. aggregata* (Irwin and Brody 1998). Unrobbed flowers were handled to control for the robbing treatment. Robbed flowers were marked with a small red dot on their calyces using a permanent, felt-tipped marker to distinguish them from unrobbed flowers. Unrobbed flowers were marked with a black permanent, felt-tipped marker to control for any

effects of marking. I ensured that the paired robbed and unrobbed flowers on the plants were distinguishable visually as well by being on different sides of the plant or at different heights. If necessary, I pruned some flowers from the plants to distinguish the focal flowers of each treatment. At any given time, I maintained nine plants in the field, each with one robbed and one unrobbed flower.

Potted plants with paired robbed and unrobbed flowers were placed in the field behind Murray lab to observe hummingbird visitation. Plants were placed in the field for 3 hours immediately following robbing treatments so that additional production of nectar by the plant would not significantly affect the robbing treatment. Plants were placed in a 3x3 grid with 1 m in between plants. Around the grid, I placed up to six *Ipomopsis* that did not have emasculated flowers to act as pollen donors. In addition, naturally growing *Ipomopsis* were in bloom around the array that also acted as pollen donors.

I continually watched the plants during the 3 hour period of observation. Once a hummingbird visited one of the robbed or unrobbed flowers, I collected the stigma in a glassine envelope. I also noted the gender and species of hummingbird (broad-tailed or rufous) visiting each plant using a hand-held digital recorder. I washed my forceps in ethanol between stigma collections to avoid any potential pollen contamination of stigmas. I brought in the array once all of the treatment flowers were visited or after 3 hours had passed. Plants were observed in the morning, approximately from 0800-1100.

In the lab, I stained the stigmas in basic fuchsin dye, and I used a compound microscope to count the number of *Ipomopsis* (conspecific) pollen grains and pollen grains from other species (heterospecific) received by the stigmas. Heterospecific pollen

deposition on the stigmas was low (mean $\pm$ 1 SE: 6.56 ± 2.27 grains per stigma) and was not considered in subsequent statistical analyses. I repeated this study over a period of six days, with new flowers and sometimes new plants each day to have a total of 26 visits to robbed and 29 visits to unrobbed flowers. Although hummingbirds often avoid robbed flowers, they do make mistakes (Irwin 2000), so it was possible to observe visits to robbed flowers. Finally, I also collected stigmas from ten emasculated flowers that were not visited by hummingbirds to insure that there was no spurious pollen deposition on the virgin stigmas. These stigmas were also stained with basic fuchsin dye and I counted the number of *Ipomopsis* pollen grains.

Statistical analyses: The distribution for number of *Ipomopsis* pollen grains was right skewed; thus, I used a $\log(x) + 1$ transformation to improve normality. To determine if there was a significant difference between the mean number of pollen grains deposited in robbed vs. unrobbed flowers, I performed a t-test. I did not perform a paired t-test because both robbed and unrobbed flowers weren't always visited on each plant each day and doing a paired t-test would have meant losing visits to single flowers in some cases. I also tested for an effect of day on pollen deposition, and for an effect of plant on pollen deposition. In total, I used 28 different plants. Twenty-three of them I used two times, one was used three times and another was used four times. Even if I took the mean per plant for robbed and unrobbed flowers, I got a similar response, and so I treated flowers as independent replicates.

Pollinator perspective: What proximate cues do hummingbirds use to avoid robbed flowers?

Hypothesis One: Honest signal

To test the hypothesis of whether liquid in flowers is providing an “honest cue” to the hummingbirds, I used 60 plants set in a 6 x 10 plant array in the field behind Murray lab. There was 1 m between plants in the array. Treatments (described below) were assigned to positions within the array at random each day using a random number generator. I used three treatments performed at the whole-plant level: the addition of 2.5 μ L of water per flower, 2.5 μ L of nectar per flower, or the flowers were left empty. Before treatment application, nectar was removed from all flowers using 10 μ L microcapillary tubes inserted into the front of the flowers. Liquid was injected into the flowers using a 10 μ L glass syringe. For plants in the “nectar” treatment, I added 2.5 μ L of 25% sucrose solution to all open flowers. For plants in the “water” treatment, I added 2.5 μ L of water. For flowers that receive nothing, the same procedure was performed using the syringe, but nothing was injected into the flowers. On all plants, I recorded the number of flowers open.

Once plants had been set up in the field, I recorded hummingbird visitation. I noted first visits to the experimental array as well as the gender and species of hummingbird, the number of times plants were visited in each treatment, and the number of flowers probed per plant (from which I calculated percent of flowers probed). The percent of flowers probed should reflect nectar rewards for the hummingbirds. Once the hummingbird(s) left the array, I refilled flowers according to their initial

treatments, re-randomized a subset of the plants and continued my observations. I conducted observations immediately after treating the flowers from 0900-1130 hours.

Statistical analysis: I determined a) the mean number of times plants were visited per day and b) the mean percentage of flowers probed per plant. For each plant, I calculated the mean visitation rate per day (mean percentage of flowers probed x mean number of times plants were visited). I also determined visitation rate by the first two visitors each day; the first visit, or first few visits, to the array is important because it is with naïve birds for that particular day who haven't probed any of the plants yet. To determine if there was a difference in the visitation rate for first visits per day and all visits across all days, I used the program EcoSim to perform a one-way analysis of variance (ANOVA) using a randomization procedure (Gotelli and Entsminger 2001). EcoSim compares the actual data to a null model that it simulates based on the actual data entered. The null model generates a number of pseudo-communities by randomizing the input matrix in a Monte Carlo simulation. The number of iterations generated in this case was 1000. EcoSim calculates tail probabilities by tabulating the number of times the observed statistic is greater than or less than that in the pseudo-communities. A p-value is derived to determine whether the actual data is significantly different from a null model. If I found a significant effect of treatment on visitation rate, I then broke visitation rate down into its components; number of plant visits and percent of flowers probed per visit, and performed ANOVAs on these two component measures to determine which was driving the effect on visitation rate. If I find an effect of treatment on number of plant visits, it suggests that hummingbirds can determine plant rewards prior to tasting; if I find effects on percent of flowers probed, it suggests that effects on

visitation rate are driven by hummingbird behavior once it has chosen to forage on a plant. This study was conducted in two years, 2008 and 2009, and I analyzed data from both years. There was an effect of year on visitation rate ($t_{628} = 3.31$, $p = 0.001$), so I analyzed each year separately.

Hypothesis Two: Vibration of flowers

When hummingbirds hover over flowers, their wings beat at approximately 28-200 beats per second (28-200 Hz). These wing beats may also cause flowers to vibrate. Depending on the amount of nectar, the flower may vibrate at a different frequency. If hummingbirds can cue in on this difference in vibration frequency or amplitude, it may provide a signal as to the presence or absence of nectar. To test this hypothesis, first, I set up plants in the WeatherPORT with 0, 2.5, 5, 10, and 15 μl of 25% sucrose solution (nectar) and had a fan blowing over the flowers to determine if I could detect the flowers vibrating. Behind each plant I placed a grid to measure maximum flower vibration amplitude. Using a Sony high-speed video camera (model HDR-HC9 camcorder with 60-240 fps speed) set on smooth slow motion recording speed, I filmed flower movement. I then transferred film clips to a computer using Picture Motion Browser software and edited clips using Windows Movie Maker. To determine maximum amplitude, I recorded how far away from its starting position on the grid the end of a flower petal moved. I then measured the distance on the actual grid. I took an average for each flower and repeated this procedure for five flowers on a plant. I then repeated this procedure for one plant in each treatment group for a total of five plants. The inverse of the amount of time it took to reach a maximum amplitude (multiplied by four

to obtain the period) was then used to calculate the frequency. Each video recording was a constant time-period of 48 seconds so that comparisons could be made across treatments.

Second, I recorded hummingbirds at plants placed in the field. The experimental set-up in the field was the same as used with the fans in the weatherport. This study was only conducted on calm days with no wind. I sat 1 m from the focal plants with the video camera and again recorded the flowers vibrating as hummingbirds visited. Visits were recorded; however, video software was not sophisticated enough to analyze the video clips.

Results

Plant perspective: Does single-visit pollen deposition by hummingbirds vary between robbed and unrobbed flowers?

I recorded 10 broad-tailed hummingbird bouts to 28 individual plants over 18 h of observations. The 10 foraging bouts represented the activity of at least three broad-tailed hummingbirds. Nectar robbing had a significant effect on single-visit pollen deposition by hummingbirds in *I. aggregata* ($t_{53} = -2.40$, $p = 0.012$). Stigmas from flowers that were unrobbed had almost 2x as much *I. aggregata* pollen than did robbed flowers (Figure 1). Stigmas from flowers that had not been visited by hummingbirds contained no *Ipomopsis* pollen and so there was no spurious pollen deposition on virgin stigmas. In addition, neither plant nor date had an effect on pollen deposition (plant effect: $F_{27, 54} = 1.26$, $p = 0.2$; date effect: $F_{4, 50} = 0.64$, $p = 0.63$).

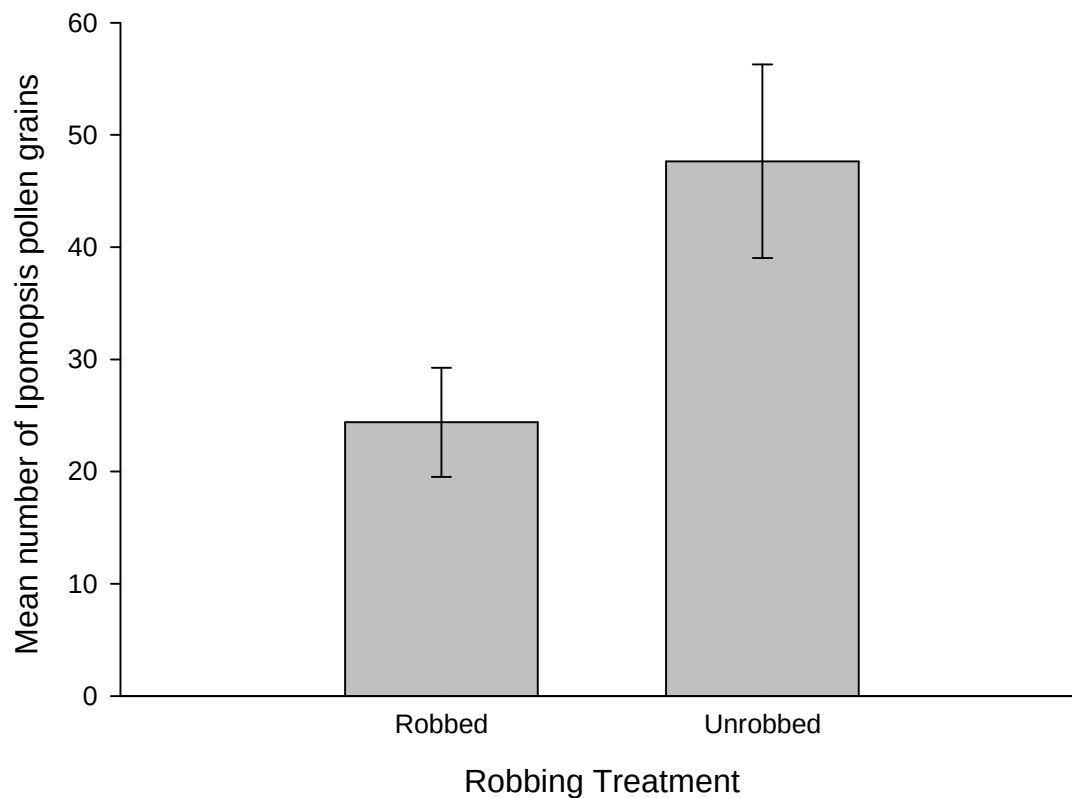


Figure 1. Mean number of *Ipomopsis aggregata* pollen grains (± 1 SE) deposited by hummingbirds in single visits to the stigmas of flowers that were robbed or unrobbed.

Pollinator perspective: What proximate cues do hummingbirds use to avoid robbed flowers?

Hypothesis 1: Honest Signal

I recorded 34 broad-tailed hummingbird bouts to 69 individual plants over 20 h of observations. The 34 foraging bouts represented the activity of at least three male and one female broad-tailed hummingbirds. The presence of 25% sucrose solution (nectar) had a significant effect on visitation rate by hummingbirds in 2008 and 2009 ($p = 0.002$, 0.015 respectively). In 2008, the mean visitation rate to plants with nectar was 1.64

times greater than plants with just water and 1.37 times greater than plants with nothing (Figure 2). In 2009, the mean visitation rate to plants with nectar was 1.43 times greater than plants with just water and 2.18 times greater than plants with nothing (Figure 3). In 2008 there was no effect of position on visitation rate ($F_{47, 228} = 1.3$, $p = 0.11$). Data for 2009 was organized by plant number (due to randomization of plants halfway through observation period) and there was no effect of plant number on visitation rate ($F_{76, 277} = 1.04$, $p = 0.40$)

Looking at the components of visitation rate separately, I found that the mean number of visits per day and the mean percentage of flowers probed were not the driving factors for the trend in visitation rate in 2008 ($p = 0.206$, 0.164 respectively). However, in 2009, both number of visits and mean percentage of flowers probed were significant driving factors of the trend in visitation rate when considered separately ($p = 0.012$, 0.027 respectively). The mean number of visits per day was 1.2 times greater in plants with nectar than with just water and 1.78 times greater than plants with nothing (Figure 4). The mean percentage of flowers probed was 1.19 times greater in plants with nectar than just water and 1.66 times greater than plants with nothing (Figure 5).

Looking at the first two visits per day in both 2008 and 2009, a similar trend in visitation rate was observed with plants containing the 25% sucrose solution (nectar) receiving a higher visitation rate than plants with either water or nothing ($p = 0.048$). The mean visitation rate to plants with nectar was 2.13 times greater than plants with just water and 1.35 times greater than plants with nothing (Figure 6). While the mean number of visits per day was not a significant driving factor of visitation rate ($p = 1.00$), mean percentage of flowers probed did significantly contribute to the trend in visitation

rate for naïve visits ($p = 0.0405$). The mean percentage of flowers probed to plants with nectar was 2.13 times greater than plants water and 1.35 times greater than plants with nothing (Figure 7). There was no effect of year ($t_{23} = -.358$, $p = 0.7238$).

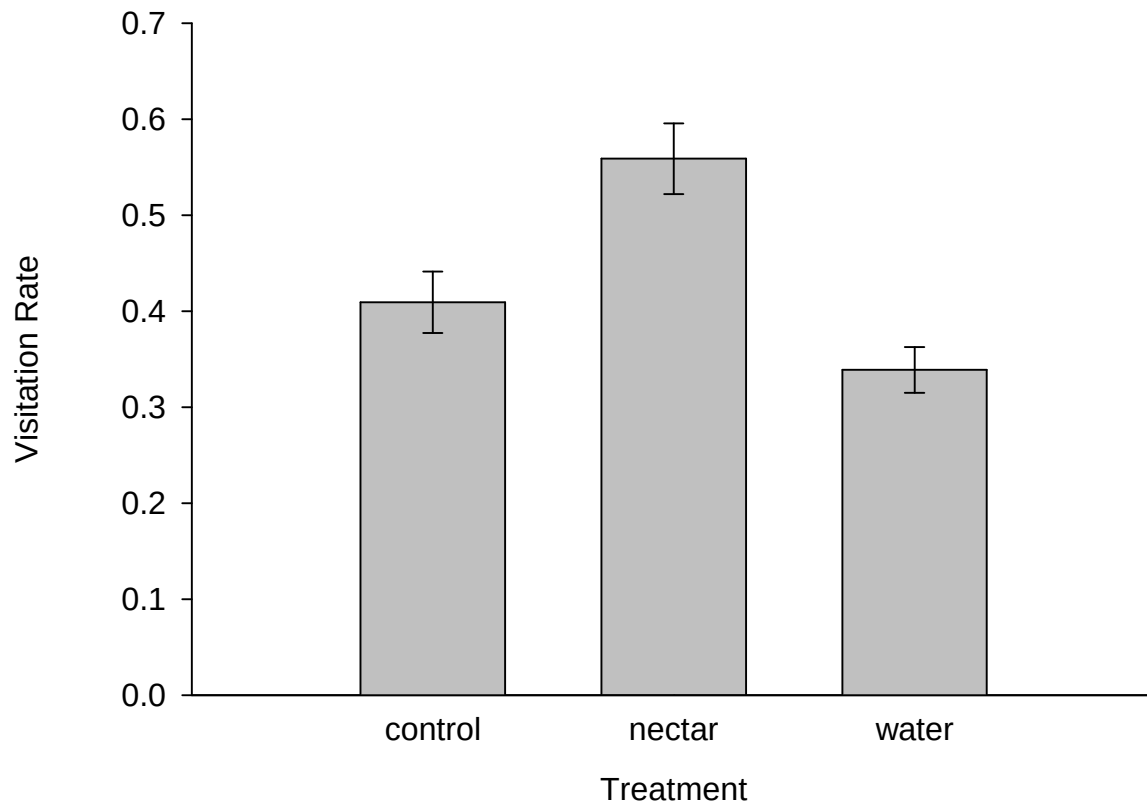


Figure 2. Hummingbird visitation rate (mean percentage flowers probed $\times$ visits per day, ± 1 SE) to flowers treated with nothing (control), 2.5 μ l of 25% sucrose solution (nectar) or 2.5 μ l of water (water) in 2008.

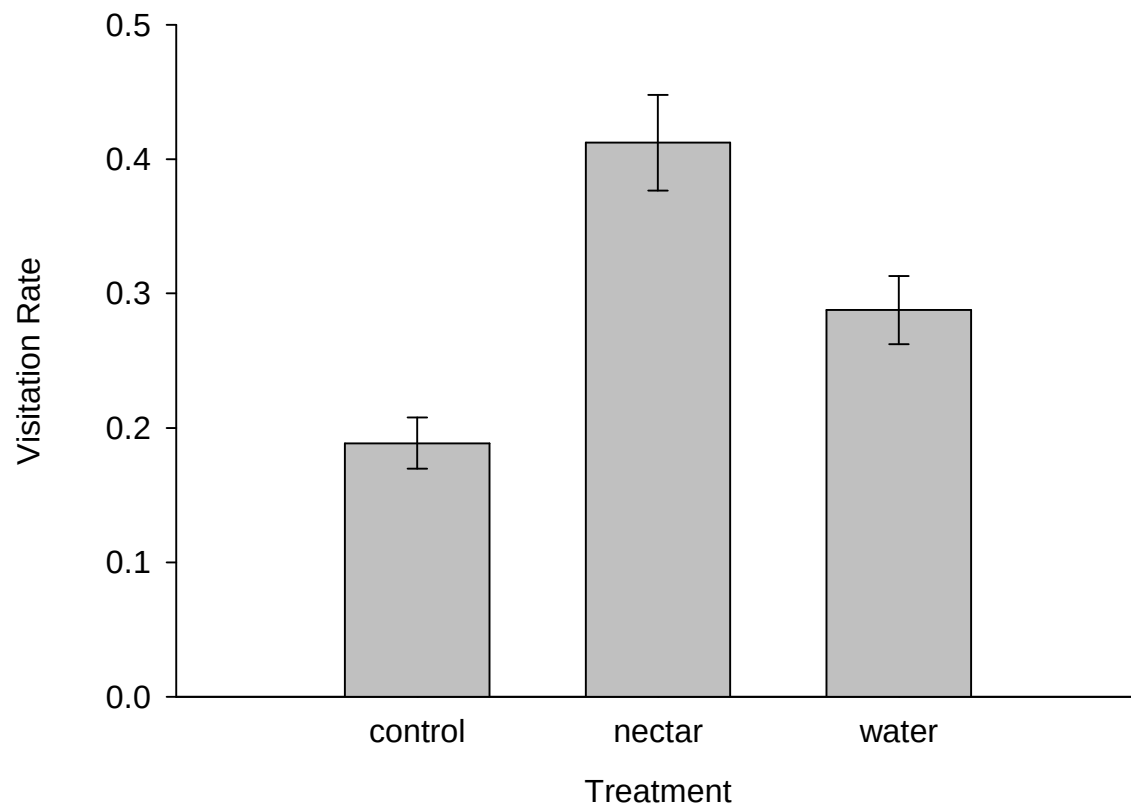


Figure 3. Hummingbird visitation rate (mean percentage flowers probed x visits per day, ± 1 SE) to flowers treated with nothing (control), 2.5 μ l of 25% sucrose solution (nectar) or 2.5 μ l of water (water) in 2009.

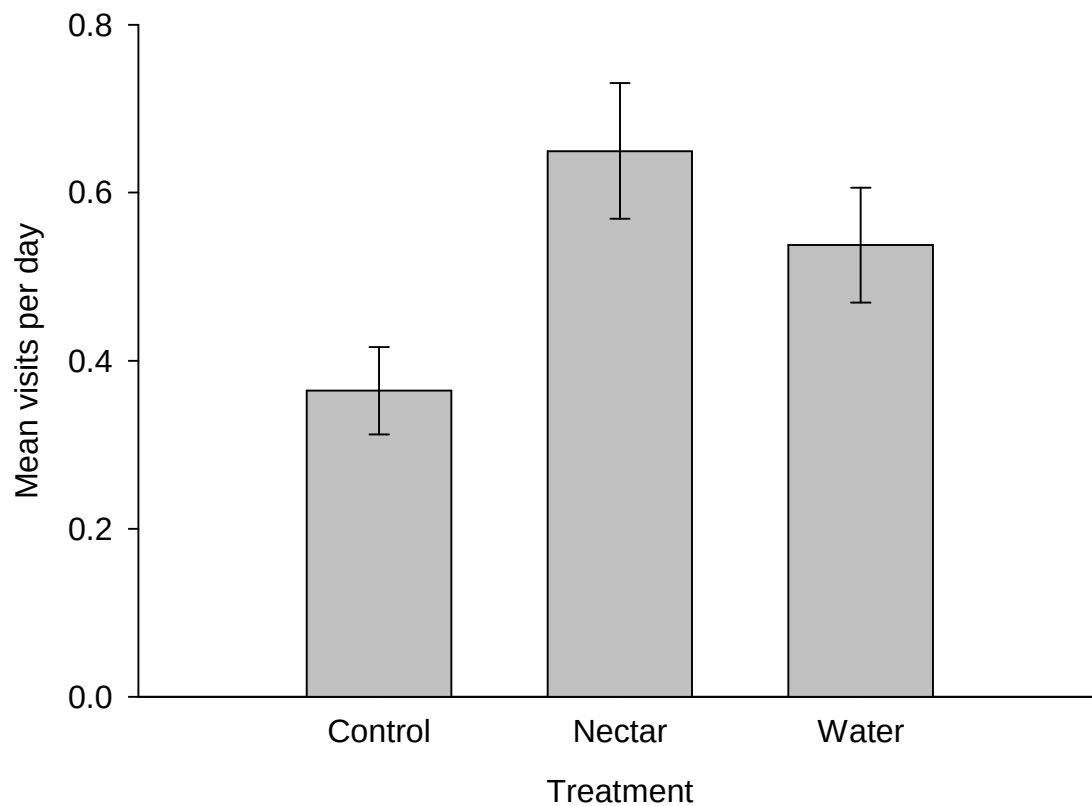


Figure 4. Mean number of hummingbird visits per day (± 1 SE) to flowers treated with nothing (control), 2.5 μ l of 25% sucrose solution (nectar) or 2.5 μ l of water (water) in 2009.

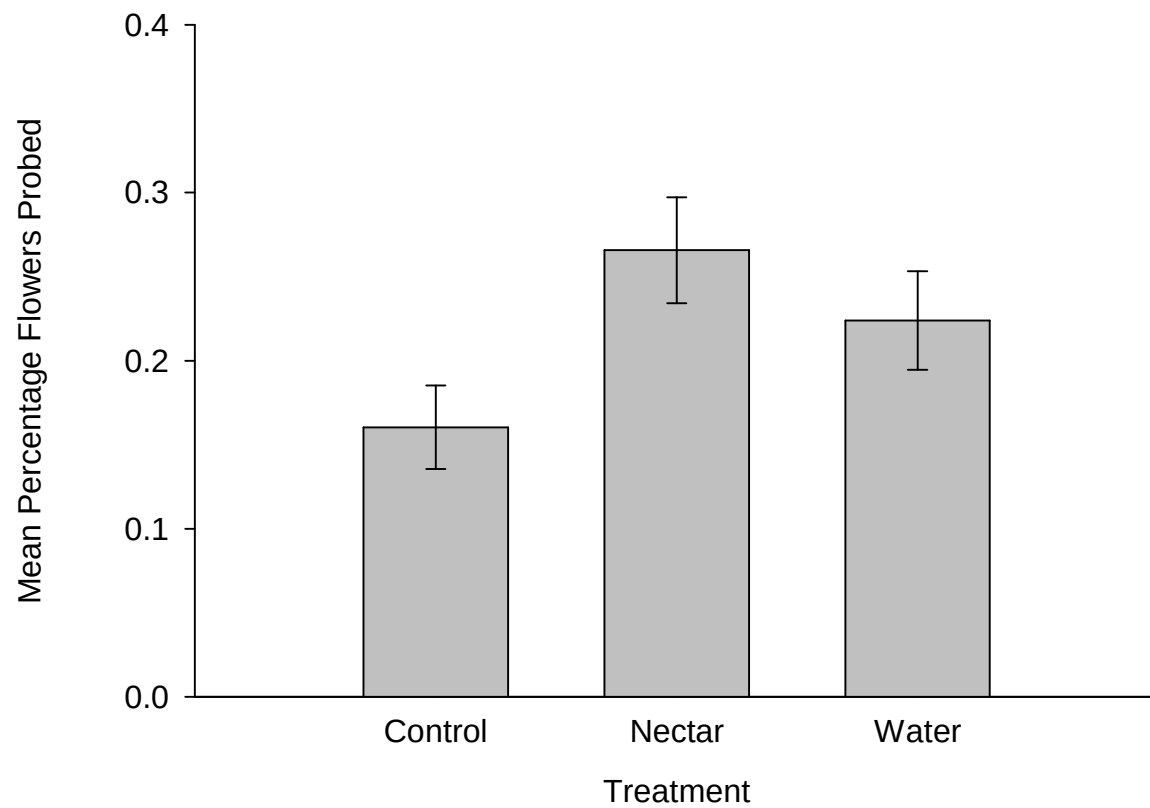


Figure 5. Mean percentage of flowers probed (± 1 SE) for flowers treated with nothing (control), 2.5 μ l of 25% sucrose solution (nectar) or 2.5 μ l of water (water) in 2009.

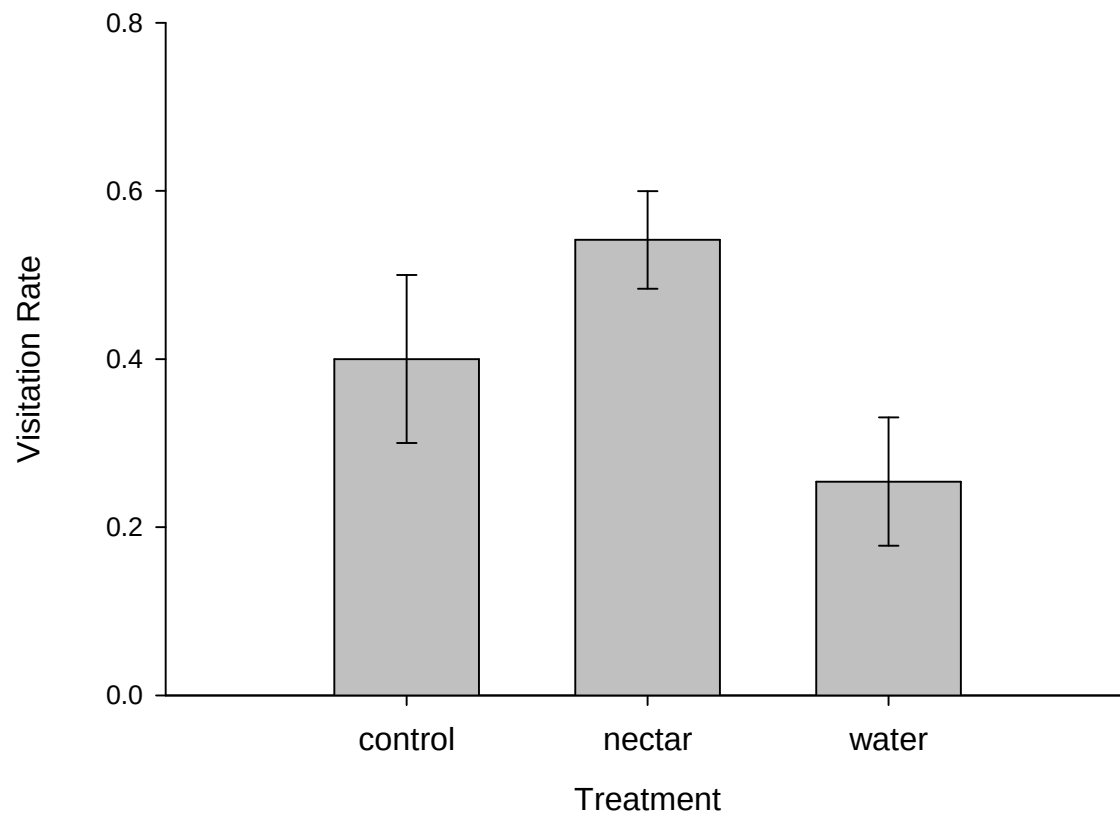


Figure 6. Hummingbird visitation rate (mean percentage flowers probed x visits per day, ± 1 SE) to flowers treated with nothing (control), 2.5 μ l of 25% sucrose solution (nectar) or 2.5 μ l of water (water). Data represents first two visits each day from 2008 and 2009.

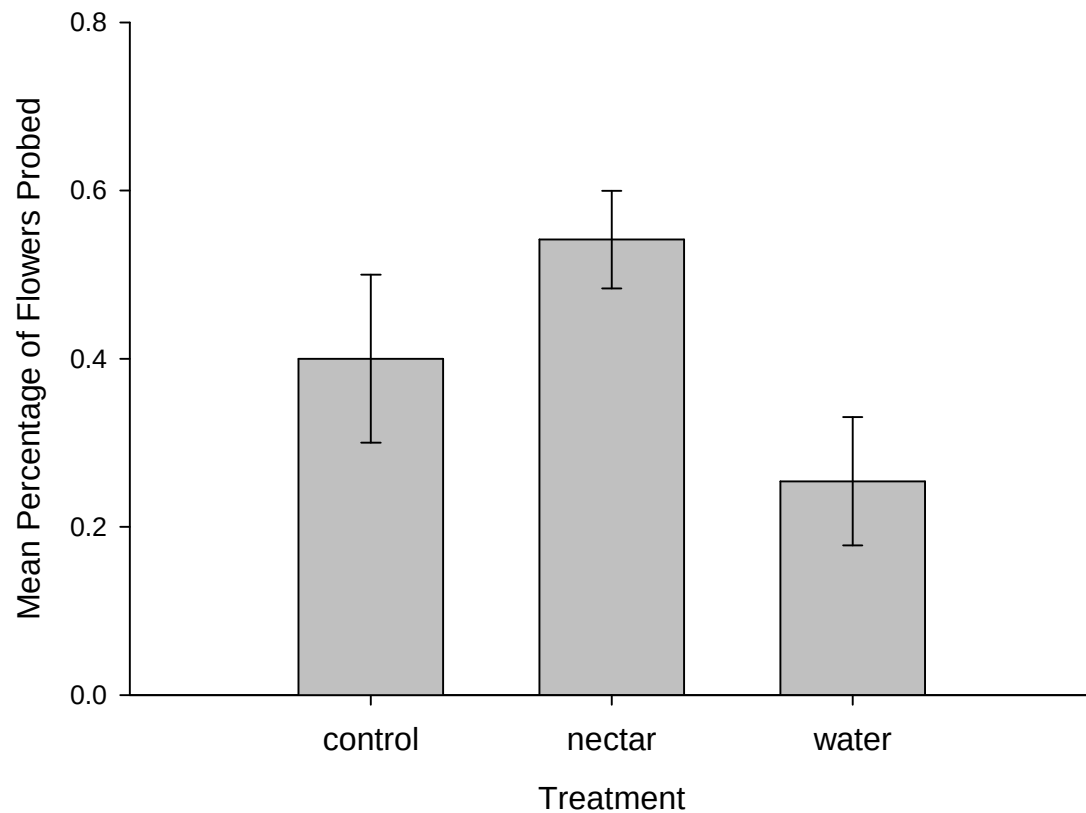


Figure 7. Mean percentage of flowers probed (± 1 SE) for flowers treated with nothing (control), 2.5 μ l of 25% sucrose solution (nectar) or 2.5 μ l of water (water). Data is from first two visits each day in 2008 and 2009.

Hypothesis 2: Vibrations

I recorded flowers with different amounts of nectar while they were subjected to a fan blowing air towards them. The mean maximum amplitude was higher in control flowers than in flowers with any amount of nectar. The amplitude of control flowers was twice as large as flowers with any other amount of nectar (Figure 8). The frequency was lower in control plants and plants with 2.5 μ l or 5 μ l of nectar when compared to plants with 10 μ l or 15 μ l of nectar (Figure 9).

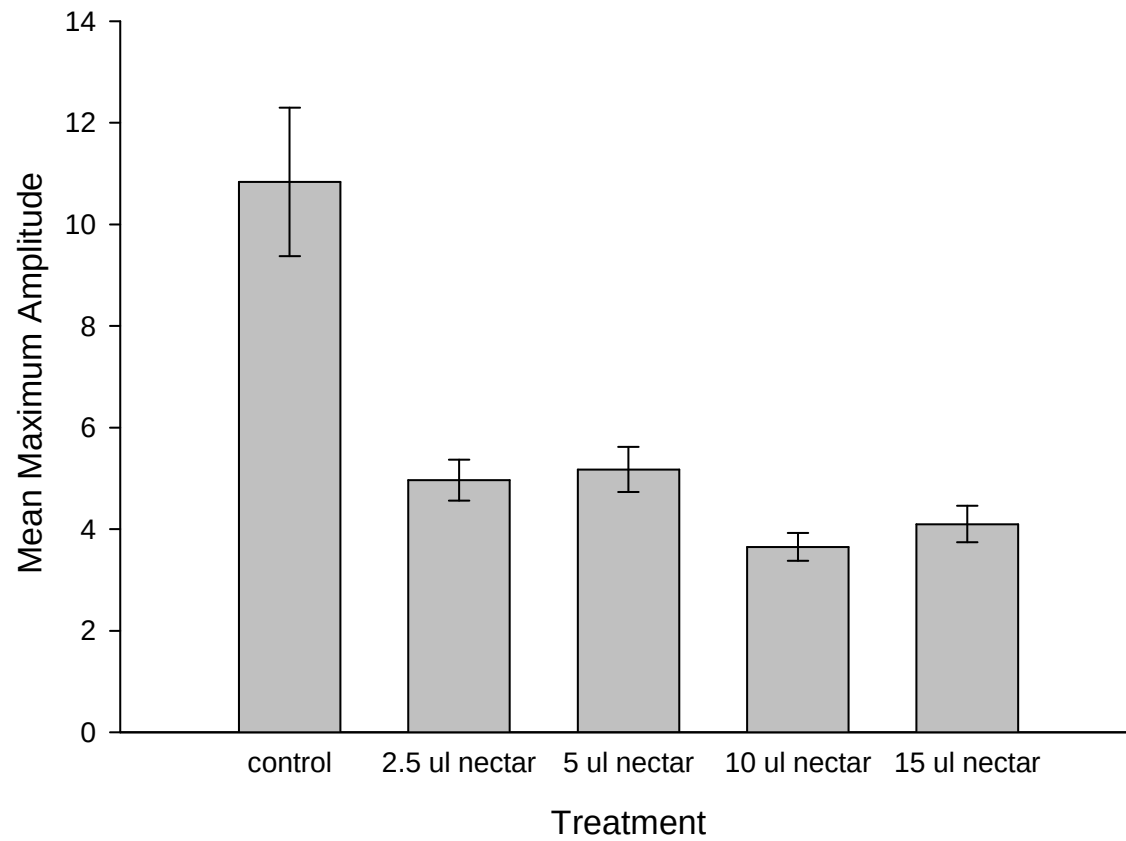


Figure 8. Mean maximum of amplitude (mm) of vibrating flowers with 15 μ l, 10 μ l, 5 μ l, 2.5 μ l or no (control) nectar (± 1 SE).

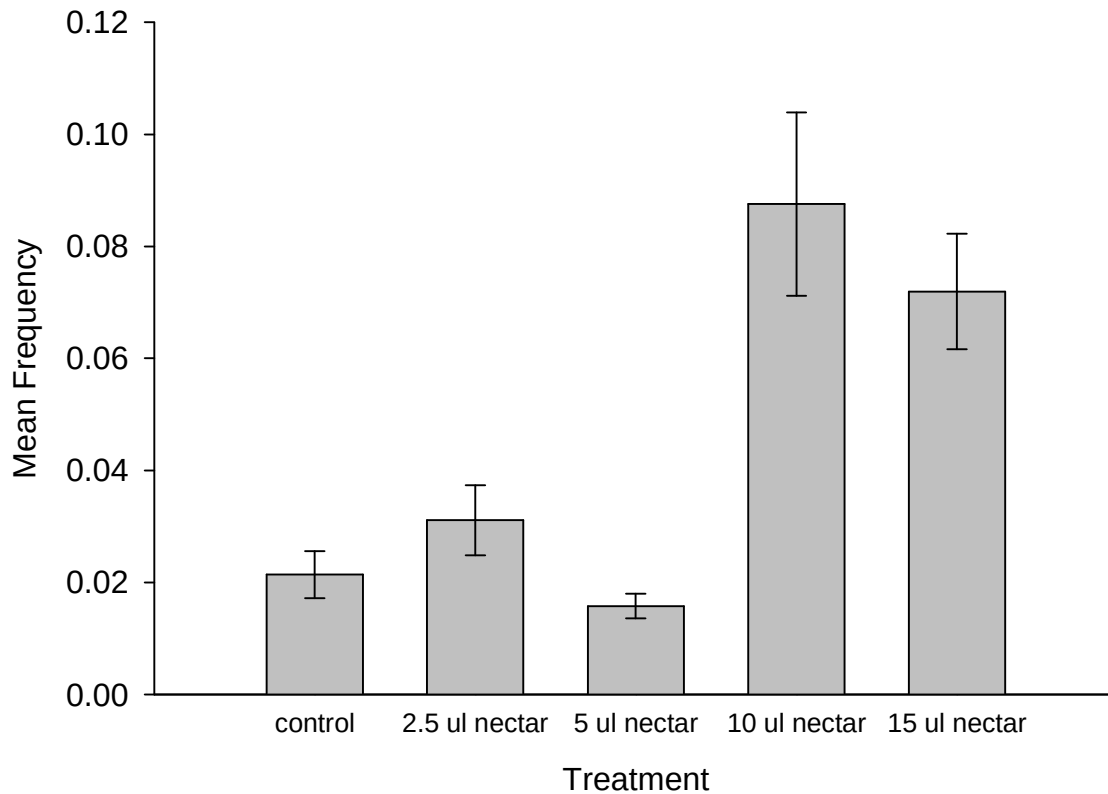


Figure 8. Mean frequency (1/s) of vibrating flowers with 15 μ l, 10 μ l, 5 μ l, 2.5 μ l or no (control) nectar (± 1 SE).

Discussion

My study demonstrates the nectar robbing has an effect on both pollen deposition and hummingbird behavior. *Ipomopsis aggregata* flowers that were unrobbed received almost twice as much pollen as flowers that had been robbed. The presence or absence of nectar affected which flowers hummingbirds chose to visit. In both 2008 and 2009, hummingbirds preferred to visit flowers with nectar over flowers with water or nothing. When considering all visits in 2009, the driving factor for this trend in visitation rate was the mean visits per day as well as the mean percentage of flowers probed. In

2008, neither factor significantly contributed to the trend in visitation rate when considered separately. Looking at just the first two visits each day for both 2008 and 2009, nectar was also the cue hummingbirds used to select flower. The driving factor for this trend was the mean percentage of flowers probed. Because mean percentage of flowers probed was the driving factor, it indicates that nectar flowers had higher visitation rates because hummingbirds tried more flowers once they made the decision to visit a plant. Looking more closely at nectar as a proximate cue, the amplitude of vibrating flowers that were empty was twice as large as flowers containing any amount of nectar. The frequency of the same vibrating flowers was between 3 and 7 times higher in flowers with 10 μ l or 15 μ l than flowers with 5 μ l, 2.5 μ l or no nectar.

From the plant perspective, nectar robbing affects single visit pollen deposition. Previous studies have found that there is typically a 20% decline in hummingbird visitation to robbed plants, but a 50-80% decline in seed production and seeds sired (Irwin and Brody 1998, 2000). My data suggest that not only is it the quantity of visits, but also the quality of the visits that affects female plant fitness. Perhaps hummingbirds probe flowers less deeply when nectar is absent. This may contribute to the length of time hummingbirds spend at a flower and consequently to the quality of visits to flowers. Studies in previous systems have shown that pollinators that spend less time per flower deposit few pollen grains (Thompson 1986, Harder 1990, Mitchell and Waser 1992). This could be the case in this study as well in that hummingbirds may spend less time at flowers when they have been robbed due to no nectar rewards.

From the pollinator perspective, my study suggests that the absence of nectar is the signal that hummingbirds use to cue in on flowers that have been robbed rather than

just the presence of liquid itself. This was contrary to my original hypothesis that liquid would provide an “honest signal” that hummingbirds cue in on. In previous studies of other systems, honest signals may be used by plants to attract legitimate pollinators. Several plant species on Mauritius have colored nectar which functions as an honest signal that allows pollinators to determine the presence and size of a reward prior to visitation (Hansen *et. al.*, 2006). In another study of frugivorous birds, fruit color was used by birds as an honest signal of dietary antioxidant rewards (Schaefer *et al.*, 2007). In my study, visitation rates to plants with nectar were higher than to plants with water or nothing. This trend was driven by both the number of visits as well as the percentage of flowers probed. The percentage of flowers probed indicates that once hummingbirds have decided to visit a plant with nectar, they stay longer at that plant than at plants without nectar. The number of visits is interesting because it demonstrates that hummingbirds are able to determine which plants will have a nectar reward without having to sample the plant first. This does not however reveal what it is about nectar that the hummingbirds are cueing in on.

When hummingbirds hover over a plant, the beating of their wings causes the flowers themselves to vibrate (personal observation). The presence, and amount, of nectar in a flower causes the flower to vibrate at a different frequency and amplitude. Amplitude was highest in flowers without any nectar compared to plants with any amount of nectar. This makes sense because the more mass an object has, the more inertia it possesses and the more it resists a change in its state of motion. Objects with less mass (i.e., flowers with less nectar) should move farther than objects with more mass when subjected to the same force because they have less mass to move. Flowers

without nectar had a lower frequency than flowers with nectar. Interestingly, flowers with little nectar (2.5 and 5 μ l) also had much lower amplitudes than flowers with high amounts of nectar (10 or 15 μ l). This makes sense because as the mass is increased, frequency increases as well. In a study of body mass and wing-beat frequency, insects with higher body mass also beat their wings at a higher frequency (). Perhaps when there is only a small amount of nectar, hummingbirds are able to detect the movement of a flower from a starting center point thus cueing in on the amplitude. When there is a larger amount of nectar, the hummingbirds may be using the amplitude as well as the frequency. Further observations using high-speed video recording will provide insight into these predictions.

Taken together, the results of my two studies on nectar robbing, one from the plant's perspective and one from the hummingbird-pollinator's perspective, help to reveal the mechanisms by which exploitation affects plant performance and animal behavior in a hummingbird-pollinated system. In the future, it may be interesting to investigate these mechanisms further. Although the quality of a pollinator visit affects the amount of pollen donated, it might help to elucidate what about the visit is actually affecting its quality and consequently pollen deposition. It may be the amount of time hummingbirds spend at a flower or it could be something else. Perhaps hummingbirds are able to cue in on flowers or whole plants with nectar and so they visit these plants or flowers first. Then when they visit a robbed flower (perhaps on the same foraging bout, but on an exploratory visit) they have already donated most of the pollen to the unrobbed flowers and so there is less to be deposited on the stigmas of robbed flowers.

Because the vibration experiment was only performed in the lab with a fan, it is necessary to perform future experiments in the field in which hummingbirds are recorded at flowers with varying amounts of nectar. If differences in amplitude and frequency can be observed in the field, then this may be the proximate cue that hummingbirds are using to detect the presence of nectar. However, if the differences in vibration cannot be replicated in the field, then the proximate cue may be something different. Perhaps there is a chemical cue associated with nectar (or sugar water) that hummingbirds are able to detect.

While this study helps us to better understand this system we can perform similar experiments in other locations or with other organisms to gain further general insights into the mechanisms affecting plants in nectar-robbed systems. Hummingbirds may use similar cues when pollinating other plants subjected to robbing or they may use different cues altogether. Other pollinators may also use the same or different cues to determine nectar robbing. With different organisms, it would be interesting to know whether pollen deposition is affected in a similar way by nectar robbing. If it is, then we may want to figure out the mechanism, whether it is the time spent at a plant or something else. More information on the mechanisms behind pollen deposition and pollinator decision making will help us to obtain a more comprehensive view of plant-pollinator systems in the face of exploitation.

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