Fall 2016

Trends in Nectar Concentration and Hummingbird Visitation: Investigating different variables in three flowers of the Ecuadorian Cloud Forest: Guzmania jaramilloi, Gasteranthus quitensis, and Besleria solanoides

Sophie Wolbert
SIT Study Abroad

Trends in Nectar Concentration and Hummingbird Visitation:

Investigating different variables in three flowers of the Ecuadorian Cloud Forest: *Guzmania jaramilloi*, *Gasteranthus quitensis*, and *Besleria solanoides*

Author: Wolbert, Sophie
Academic Director: Silva, Xavier Ph.D
Project Advisor: Beck, Holger
Scripps College
Organismal Biology
South America, Ecuador, Nanegal, Santa Lucía Cloud Forest Reserve
Submitted in partial fulfillment of the requirements for Ecuador: Comparative Ecology and Conservation, SIT Study Abroad, Fall 2016

(Image on previous page: Male Violet-tailed Sylph, camera trap footage by Sophie Wolbert)

Abstract
Many variables affect hummingbird visitation to flowers, including nectar concentration, time of day, corolla length and other aspects of flower morphology, and elevation. Nectar concentration was measured from flowers of three Ecuadorian subtropical montane species: *Guzmania jaramilloi*, *Gasteranthus quitensis*, and *Besleria solanoides*. Concentration values were compared across varying times of day, corolla lengths, and elevations to determine the effects of these variables on flower nectar concentration. Camera traps were also used to observe hummingbird visitation to the above three flower species. After running regression and ANOVA analyses, nectar concentration was found to be positively correlated with time of day, negatively correlated with corolla length, and negatively correlated with elevation. Additionally, both flower morphology and elevation were determined to affect the species of hummingbirds that visited each flower species.

ISP Topic Codes: Botany (613), Ecology (614), Plant Physiology (620)

Resumen
Muchas variables afectan la visitación de colibrís a los flores, incluyendo la concentración de néctar, la hora del día, el largo de la corola y otros aspectos de la morfología de flores, y la altura. La concentración de néctar fue medido de flores de tres especies subtropicales en las montañas de Ecuador: *Guzmania jaramilloi*, *Gasteranthus quitensis*, y *Besleria solanoides***.** Los valores de concentración fueron comparados entre diferentes horas del día, largos de corola, y alturas para determinar los efectos de estas variables en la concentración de néctar de los flores. Cámaras trampas fueron usados para observar la visitación de colibrís a las tres especies de flores. Después de ejecutar análisis de coeficiente de regresión y ANOVA, la concentración de néctar fue determinado a ser correlacionado positivamente con hora del día, correlacionado negativamente con largo de corola, y correlacionado negativamente con altura. También fue determinado que la morfología de los flores y la altura afectan las especies de colibrís que visitaron cada especie de flor.

Introduction

Many flower species produce nectar to attract pollinators, like bees and hummingbirds. In fact, nectar production in some flowers can represent up to 30% of the energy output of the flower (Pleasants & Chaplin, 1983). Flowers have certain adaptations and morphologies that attract the correct pollinator; for example, a flower with a long, curved corolla will tend to exclude bees and attract hummingbirds with long, curved bills (Maglianesi, Böhning-Gaese, & Schleuning, 2014). The concentration of the nectar can also be a determining factor for which type of pollinator will visit the flower. Hummingbirds, for example, have such a fast metabolic rate that sugar concentration in the nectar they feed upon is extremely important; they almost always prefer nectar that has a concentration that will maximize their energy intake rate (Tamm & Gass, 1986). Additionally, flowers that produce nectar for pollinators such as hummingbirds and bees are able to homeostatically regulate the content and quality of their nectar in order to continue attracting those pollinators to the flowers (Castellanos, Wilson, & Thomson, 2002). A study that surveyed flowers pollinated by bees and hummingbirds in Costa Rica and California determined that bee flowers have significantly higher average sugar concentrations than hummingbird flowers (Baker, 1975). There are a few potential explanations for this, including the higher levels of pollination when hummingbirds must visit multiple flowers, water as a necessary nutrient for the birds, and increased viscosity in higher concentrated nectar. Bolten and Feinsinger posit that hummingbirds will almost always prefer the higher concentrated nectar when given a choice between a higher and a lower concentration, but that hummingbird flowers secrete more dilute nectar to deter nectar-robbing bees (1978). The average nectar concentration found across a range of hummingbird-pollinated flowers in various habitats was between 20-25%, which is significantly lower than the average of bee flowers, but still enough to attract hummingbirds (Baker, 1975).

In the following investigation, nectar was taken from flowers of three species: *Guzmania jaramilloi* (Bromeliaceae), *Gasteranthus quitensis* (Gesneriaceae), and *Besleria solanoides* (Gesneriaceae). *G. jaramilloi*, the bromeliad, is an epiphyte found at various heights in several different tree species. The inflorescence is red and cone-like, but each individual flower emerges long and yellow, with the nectary at the base. *G. quitensis* is an abundant ground plant with small, red flowers. The flowers resemble shoes, with an opening on top and nectary in the heel part of the shoe. *B. solanoides*, also of the Gesneriaceae family, is a tree-like bush with many clusters of orange flowers. The flowers are small and tubular, with the nectary at the back of the flower.

Figure 1. From left: *G. jaramilloi, G. quitensis,* and *B. solanoides.*

This investigation seeks to answer the following questions: 1) How do time of day, corolla length, and elevation affect nectar concentration? 2) How do these same variables affect the feeding behavior of hummingbirds? 3) Which species of hummingbirds visit which plants (of the three plant species I studied)?

I predict a positive correlation with time of day and nectar concentration; that is to say, the nectar concentration in flowers should increase as the day goes by. Plants need sunlight to undergo photosynthesis, the process by which sugar is produced. Therefore, at night, when there is no light, plants do not produce sugar or nectar. This also makes sense because the hummingbirds that feed on the nectar of plants are not active at night. Valtueña, Ortega-Ollivencia, & Rodriguez-Riaño found that in a Fabaceae species, nectar volume is highest in the morning and decreases throughout the day but that nectar concentration is lowest in the morning and increases throughout the day (2007). I would expect to see a similar trend in the flowers I studied, because the plant would have more time in the sun as the day goes on to produce sugar. Additionally, a plant needs to continue attracting pollinators throughout the day, so it would not make sense for nectar concentration to decrease over the course of a day.

Corolla length has been shown to play a role in sugar production in flowers; in two separate studies, significant positive associations were found between sugar production and corolla length (Harder & Cruzan, 1990; Ornelas et al., 2007). While the differences in corolla length within species are not large, there is greater variation in corolla length between the three species in this study. I would therefore expect a similar trend to that in the aforementioned studies; nectar concentration should increase with increasing corolla length, within and between species.

As altitude increases, temperature tends to decrease and therefore viscosity tends to increase. Because of this, nectar at higher altitudes is more viscous than nectar at lower altitudes. Ornelas et al. found that sugar concentration in nectar decreases with altitude due to the fact that the more viscous the nectar, the slower hummingbirds can take it up. As a result, I would expect nectar concentration to decrease as elevation increases. However, in the small altitudinal range of my study, temperature tends to stay relatively constant across elevations. Therefore, temperature and viscosity should not have a significant effect on nectar concentration.

In terms of hummingbird visitation, I would expect to observe more total visits in the morning than in the afternoon. Additionally, different hummingbird species are present at different altitudes, so I predict that elevation will have an effect on which species visit the flowers. Maglianesi, Böhning-Gaese, and Schleuning found that flower morphology, including traits like corolla length, has a significant effect on which hummingbirds visit flowers (2014). It follows that *G. quitensis*, which has a slightly curved corolla, will attract more hermit hummingbirds, who have long and curved bills. I further predict that hummingbirds with shorter bills, like the Violet-tailed Sylph and the Booted Racket-tail, will record more visits to *B. solanoides*, which has a small tubular flower. *G. jaramilloi*, with a long tubular flower, should attract hummingbirds with longer bills, like the Brown Inca.

Methods

Study site

Data was collected in the Santa Lucía Cloud Forest Reserve (SLCFR), in the northwest section of the Pichincha Province in northern Ecuador. The SLCFR lies in the western Andean cloud forest that connects to the Chocó forest. In SLCFR, mornings are generally clear to cloudy, with more clouds setting in as the day goes on. In the rainy season (late October to late May), it tends to rain almost every afternoon and often rains overnight as well. The average annual temperature and average annual rainfall of a nearby town, Nanegalito, are 18.3˚C and 2071, respectively (Edson, 2015). The trails on which data was collected had an elevation range of 1649m-2145m.

A wide variety of hummingbird species exist in the SLCFR, with the most common species being: the Tawny-bellied Hermit, the Violet-tailed Sylph, the Booted Racket-tail, and the Brown Inca (Beck, 2016).

Data collection took place for three weeks in November of 2016, Monday through Friday for nectar collection and every day of the week for camera trapping.

Nectar concentration

Before extracting the nectar from each flower, the corolla was measured using a Whitworth 0-150mm Digital Caliper. Nectar was then extracted from flowers using a VWR Ergonomic High-Performance 100-1000μL micropipette. In *G. quitensis*, the tip was inserted into the corolla and pushed back into the heel of the shoe-like flower, where the nectar was. In most cases, it was not necessary to remove the flower from the plant. In *G. jaramilloi*, one open flower was removed from the rest of the inflorescence and the petals were peeled back in order to insert the micropipette tip and remove nectar. In *B. solanoides*, one open flower was removed from the plant and the tip was inserted to collect nectar. In nearly all cases, the amount of nectar collected from each flower was very small, in the range of 2-10μL. The nectar was then pipetted onto a Vee Gee BX-50 Refractometer and the nectar concentration was measured. The refractometer was cleaned with boiled water between each measurement. The elevation of each plant was also recorded using a Garmin Etrex 10 GPS device. The time of day and the trail on which the plant was found were also recorded.

Data was not collected at times when it had very recently rained or when it was currently raining because of potential for rainwater to enter the flowers and dilute the nectar.

Camera traps

Plotwatcher Pro: Day 6 Outdoors cameras were used to monitor certain flowers of the above species for hummingbird activity. Pictures were taken once every second. Cameras were put out for 2-3 days at a time, and the results include data from my collection period (November 2016) and from an ongoing camera trap study that began in 2013. The elevation range of the cameras from the ongoing study is slightly broader (1371m-2178m), leading to a higher diversity of hummingbird species observed.

Data Analyses

Correlation and regression analyses, including ANOVA, were run for all concentration data.

Camera footage was run through MotionMeerkat, a type of camera trap analysis software, to pick out all frames with motion, and then the images were analyzed visually to identify each hummingbird species that visited the flowers. The term "visit" in this case refers to a hummingbird probing and/or feeding from one or more flowers. Some images were too blurry to make true identification possible, so these images were excluded. Species, sex (if known), elevation, and time of day were recorded for each image. The number of visits was then analyzed with respect to elevation and time of day for each plant species, and correlation and regression analyses were performed.

Results

Nectar Concentration Study

Nectar was collected from 376 samples in total: 87 from *G. jaramilloi*, 132 from *G. quitensis,* and 157 from *B. solanoides*. The nectar concentrations were lower than concentration averages of hummingbird flowers from the literature (see Baker, 1976), and *G. quitensis* had both the highest average nectar concentration and the widest range of concentrations of the three species. The nectar concentrations generally increased as the day went on, with the afternoon time block having the highest average nectar concentration for all three species. The elevation ranges of the three species were similar, with *G. quitensis* found at a higher abundance at lower elevations (Table 1).

Table 1. General results for *G. jaramilloi*, *G. quitensis*, and *B. solanoides*. Average nectar concentrations had standard deviations of 4.14% (*G. jaramilloi*), 7.84% (*G. quitensis*), and 4.25% (*B. solanoides*). The standard deviations for corolla length were 2.86mm (*G. jaramilloi*), 2.17mm (*G. quitensis*), and 1.58mm (*B. solanoides*).

	G. jaramilloi	*G. quitensis*	*B. solanoides*
Average nectar concentration (%)	13.3	18.15	10.28
Range of nectar concentrations (%)	5-19.5	1-27.5	2-18.5
Average corolla length (mm)	34.2	21.84	15.25
Range of corolla lengths (mm)	26-42	15.5-27	11.5-19.5
Average elevation (m)	1861.87	1828.33	1868.5
Range of elevations (m)	1696-2103	1646-1939	1693-2145
Average early morning (6:00-9:30 AM) concentration (%)	12.5	18.29	9.87
Average mid-morning (9:30-1:00 PM) concentration (%)	13.43	17.68	10.09
Average afternoon (1:00-4:00 PM) concentration (%)	13.47	19.92	11.71

Nectar concentration was compared across times of day for all three species (Figures 2-4). In *B. solanoides*, nectar concentration was found to be positively correlated with time of day (Figure 4, $p<0.05$). No significant correlation was found between nectar concentration and time of day in either *G. jaramilloi* or *G. quitensis*.

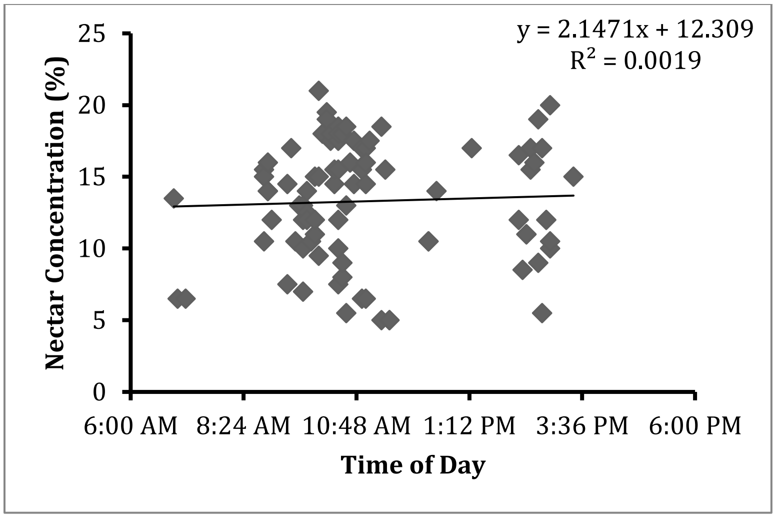

Figure 2. Nectar concentration across time of day for *G. jaramilloi*. No significant correlation was found ($p=0.67$, 95% confidence interval).

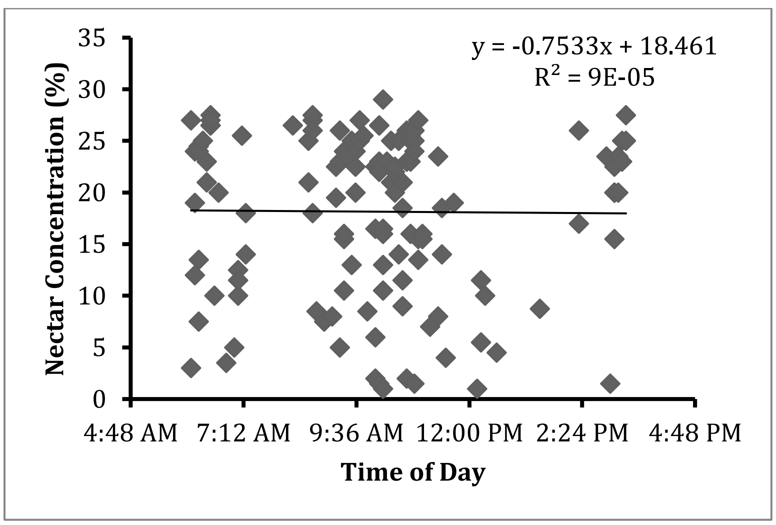

Figure 3. Nectar concentration across time of day in *G. quitensis*. No significant correlation was found ($p=0.69$, 95% confidence interval).

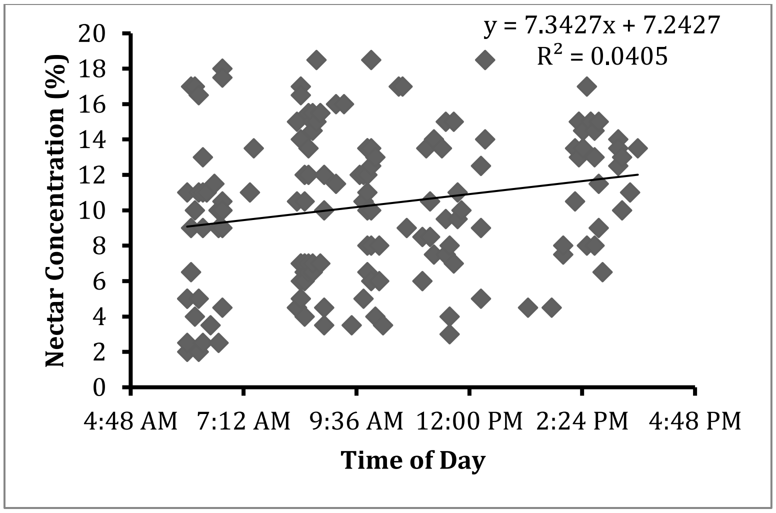

Figure 4. Nectar concentration across time of day in *B. solanoides*. A weak positive correlation was found, with a correlation coefficient of 0.20 (p=0.016, 95% confidence interval).

Nectar concentration was also compared across increasing corolla length for the three species (Figures 5-7). *B. solanoides* showed no significant correlation between corolla length and nectar concentration, but the two other species did have significant negative correlations. Nectar concentration decreased as corolla length increased in both *G. jaramilloi* and *G. quitensis* (Figure 5, $p<0.05$; Figure 6, $p<0.05$).

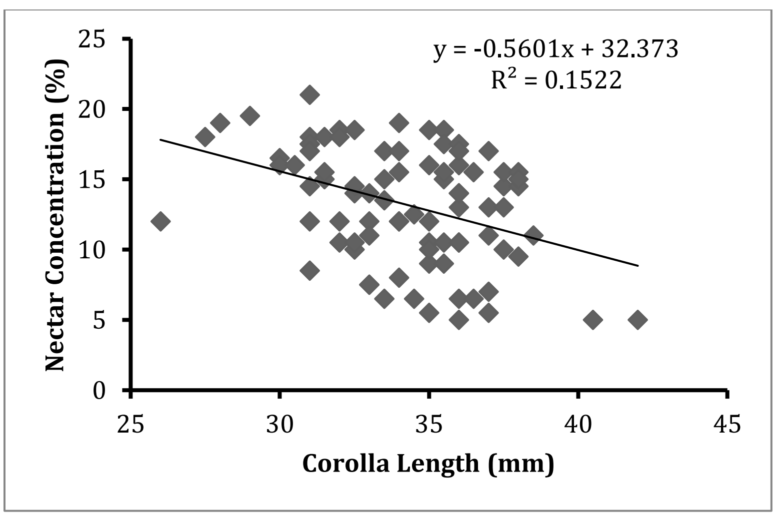

Figure 5. The effect of corolla length on nectar concentration in *G. jaramilloi*. A moderate negative correlation was found, with a correlation coefficient of -0.39 (p=0.00006, 95% confidence interval).

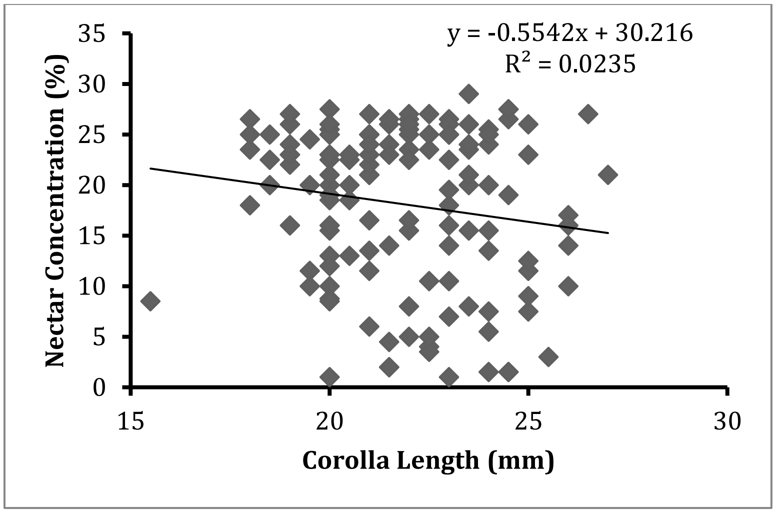

Figure 6. The effect of corolla length on nectar concentration in *G. quitensis*. A weak negative correlation was found, with a correlation coefficient of -0.15 (p=0.032, 95% confidence interval).

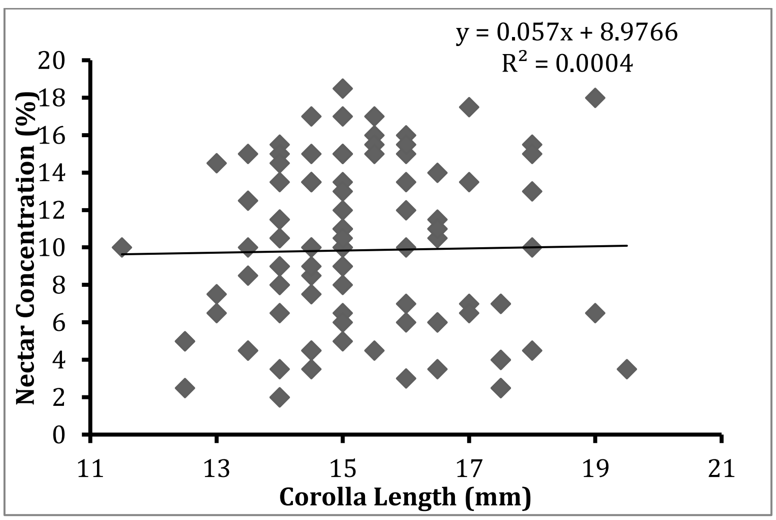

Figure 7. The effect of corolla length on nectar concentration in *B. solanoides*. No significant correlation was found (p=0.83, 95% confidence interval).

Finally, nectar concentration was compared across increasing elevations for the three study species (Figures 8-10). Concentration decreased with increasing elevation for both *G. quitensis* and *B. solanoides* (Figure 9, p=0.0065; Figure 10, p=0.02), but no significant correlation was found in *G. jaramilloi*.

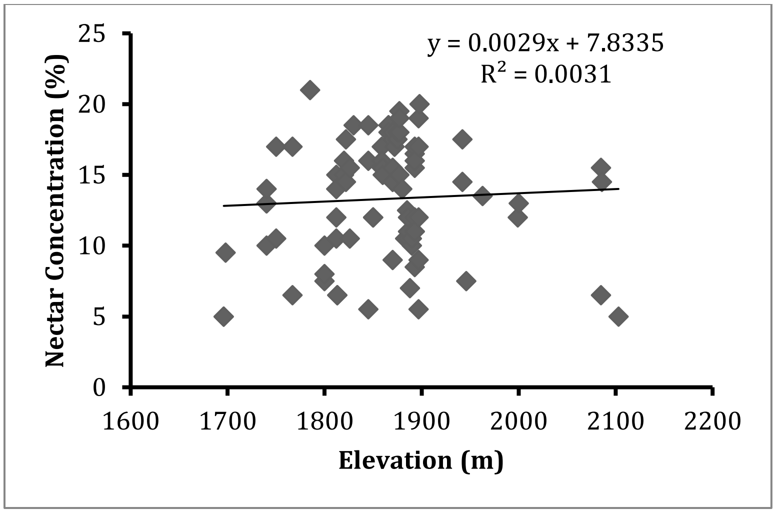

Figure 8. Nectar concentration across increasing elevation in *G. jaramilloi*. No significant correlation was found (p=0.96, 95% confidence interval).

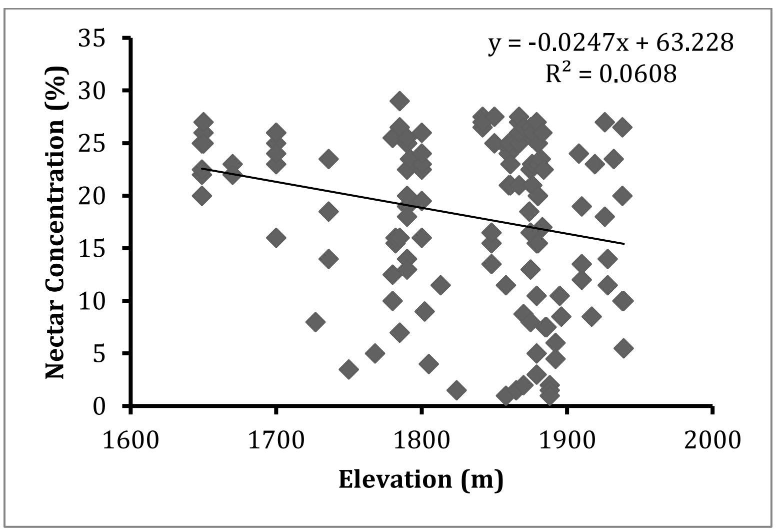

Figure 9. Nectar concentration across increasing elevation in *G. quitensis*. A weak negative correlation was found, with a correlation coefficient of -0.25 (p=0.0065, 95% confidence interval).

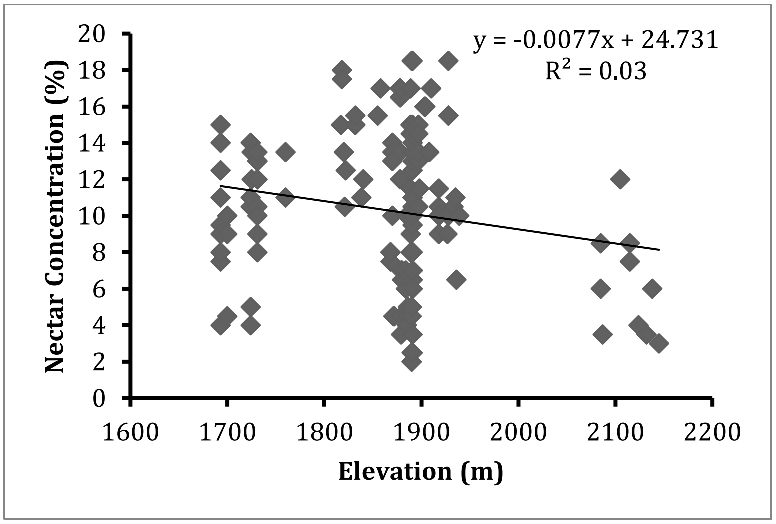

Figure 10. Nectar concentration across increasing elevation in *B. solanoides*. A weak negative correlation was found, with a correlation coefficient of -0.17 (p=0.02, 95% confidence interval).

Camera Trap Study

The footage from 48 cameras (34 from the ongoing study that began in 2013 and 14 just from November 2016) was analyzed and the results were compiled below. 177 total hummingbird visits to *G. jaramilloi* were documented, which was the most of the three plant species. Next was *B. solanoides*, with 68 total hummingbird visits, and last was *G. quitensis*, with 51 total visits.

Number of hummingbird visits was compared across half-hour intervals during the day in the three study species (Figures11-13). In *G. quitensis*, there was a significant decrease in number of visits as the day went on (Figure 12, p=0.032). *G. jaramilloi* and *B. solanoides* showed no significant correlation between number of visits and time of day.

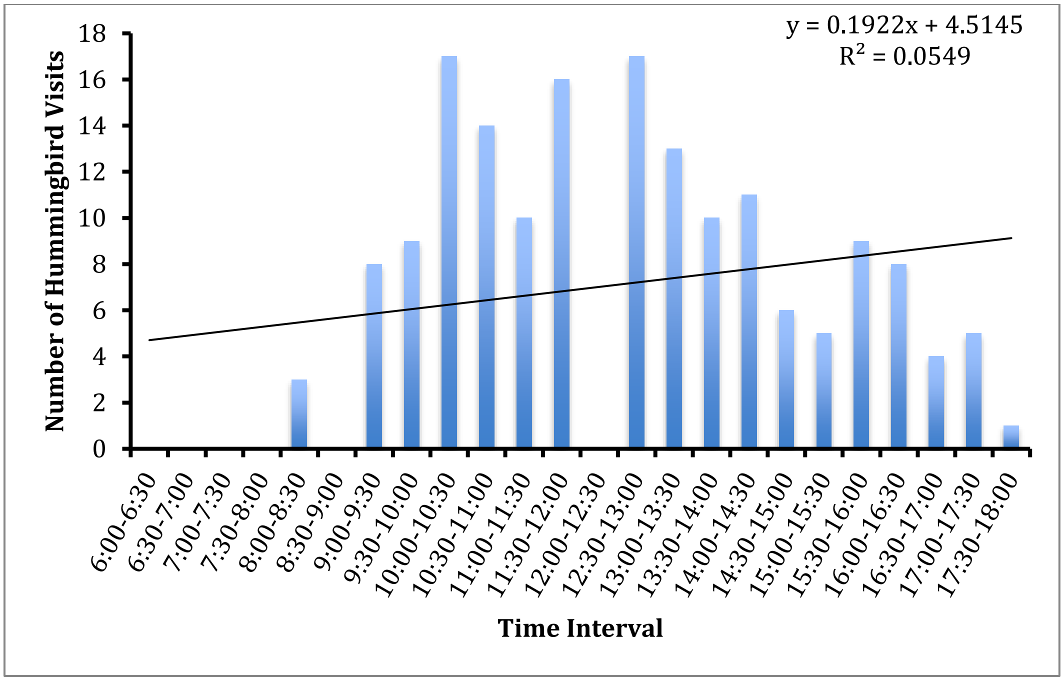

Figure 11. Number of hummingbird visits to *G. jaramilloi* per time interval. No significant correlation was found (p=0.46, 95% confidence interval).

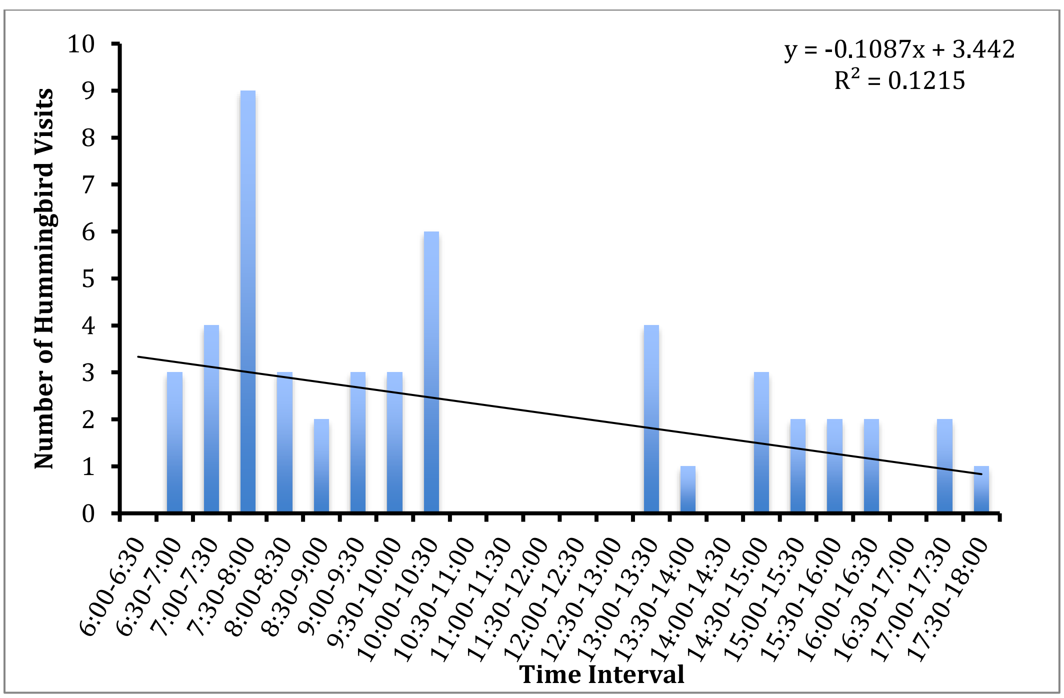

Figure 12. Number of hummingbird visits to *G. quitensis* per time interval. A weak negative correlation was found (p=0.032, 95% confidence interval).

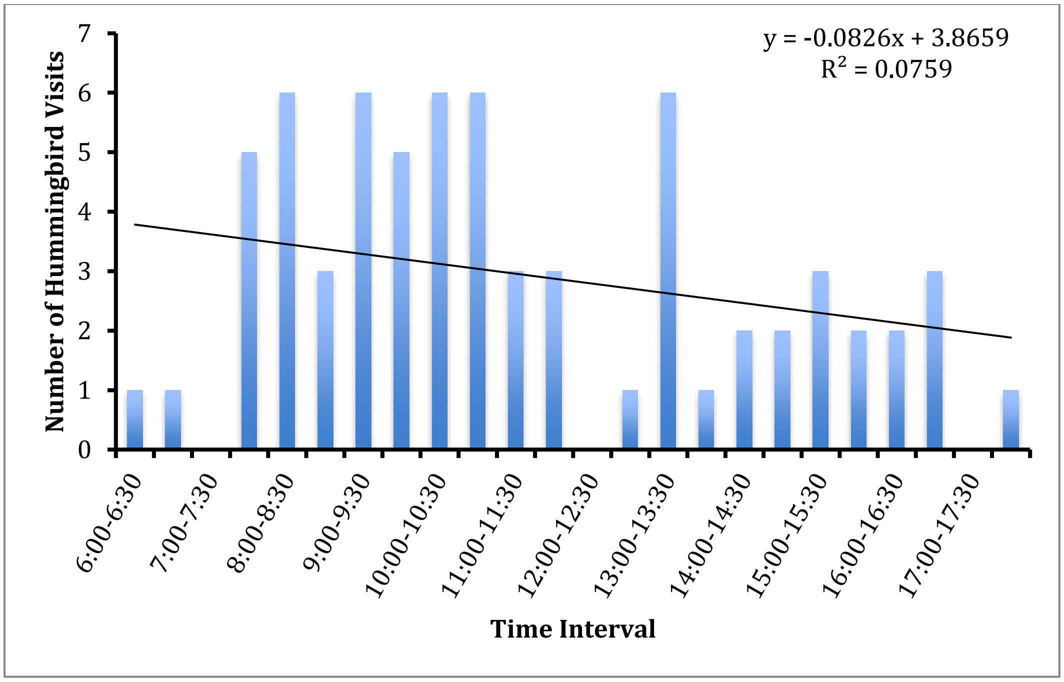

Figure 13. Number of hummingbird visits to *B. solanoides* per time interval. No significant correlation was found (p=0.09, 95% confidence interval).

Number of hummingbird visits was also compared across elevations for the three plant species (Figures 14-16). While the trendlines are slightly negative for all three species, none showed significant correlation between number of visits and elevation.

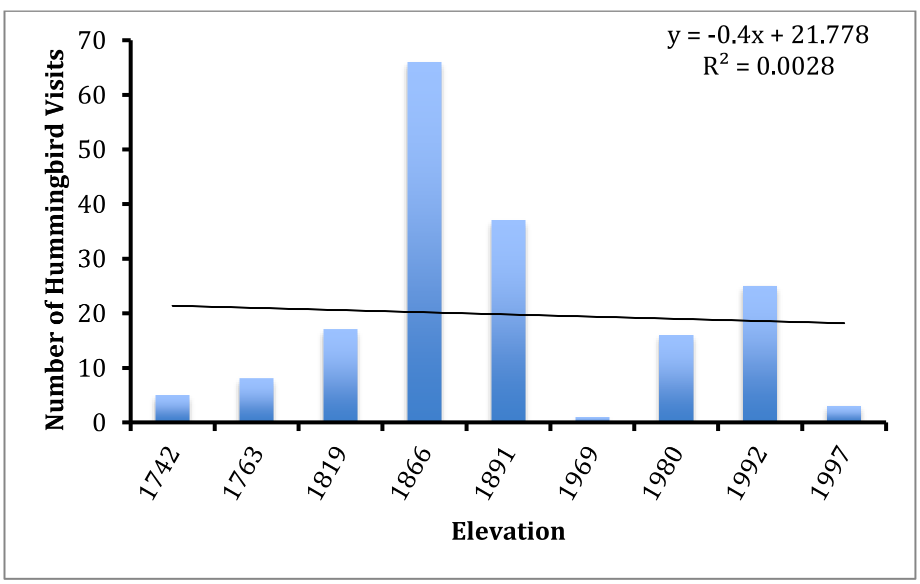

Figure 14. Number of hummingbird visits to *G. jaramilloi* at increasing elevations. No significant correlation was found (p=0.62, 95% confidence interval).

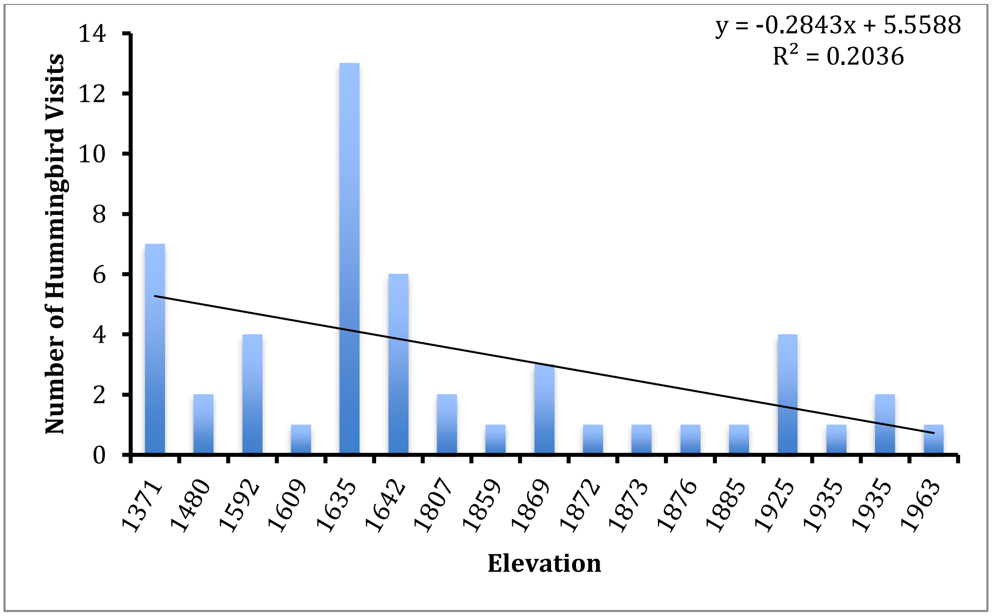

Figure 15. Number of hummingbird visits to *G. quitensis* at increasing elevations. No significant correlation was found (p=0.1, 95% confidence interval).

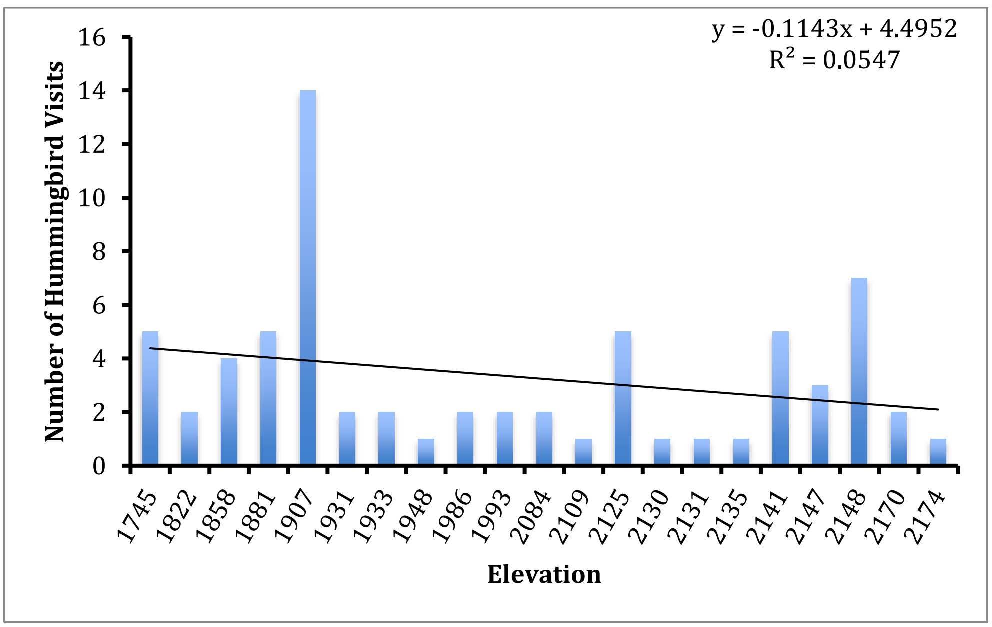

Figure 16. Number of hummingbird visits to *B. solanoides* at increasing elevations. No significant correlation was found (p=0.34, 95% confidence interval).

The elevations at which each hummingbird species appeared were compiled (Table 2). Some species (Empress Brilliant, Gorgeted Sunangel, Green-crowned Woodnymph, Purple-bibbed Whitetip, Wedge-billed Hummingbird, and White-whiskered Hermit) were only seen by one camera at a certain elevation, while the other more common species had larger elevation ranges.

Table 2. The eleven hummingbird species observed by cameras and the range of elevations in which they were observed.

Hummingbird Species	Elevation Range
Booted Racket-tail	1745-2170
Brown Inca	1763-2141
Empress Brilliant	1891
Gorgeted Sunangel	2174
Green-crowned Woodnymph	1635
Purple-bibbed Whitetip	1642
Stripe-throated Hermit	1371-1642
Tawny-bellied Hermit	1742-2148
Violet-tailed Sylph	1742-2148
Wedge-billed Hummingbird	1866
White-whiskered Hermit	1371

The Violet-tailed Sylph was the most commonly observed species at *G. jaramilloi* and *B. solanoides*, and the second most commonly observed species at *G. quitensis* (Figures 17-19). At *G. quitensis*, the Stripe-throated Hermit was the most common visitor. *G. quitensis* recorded 7 different species of hummingbirds, the highest number of species of the three plants (Figure 18).

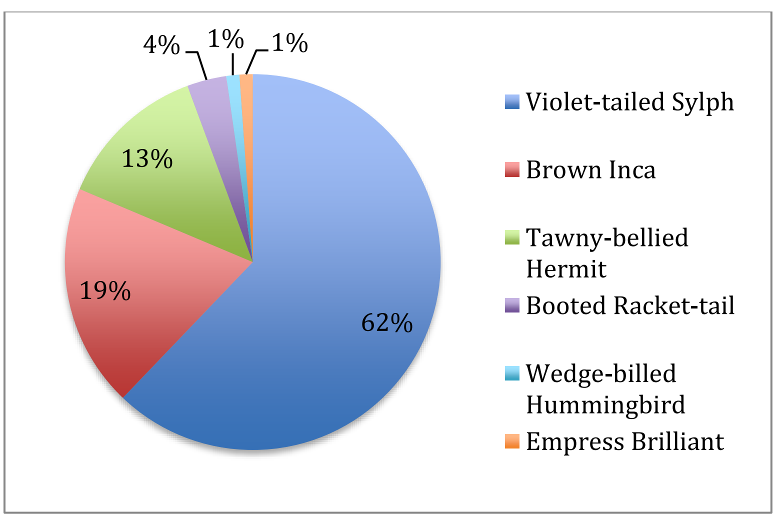

Figure 17. Percentage of hummingbird visits to *G. jaramilloi* by species.

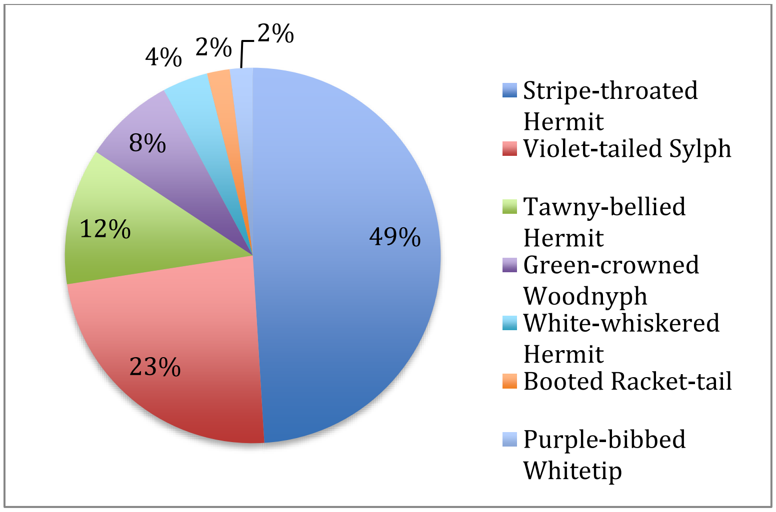

Figure 18. Percentage of hummingbird visits to *G. quitensis* by species.

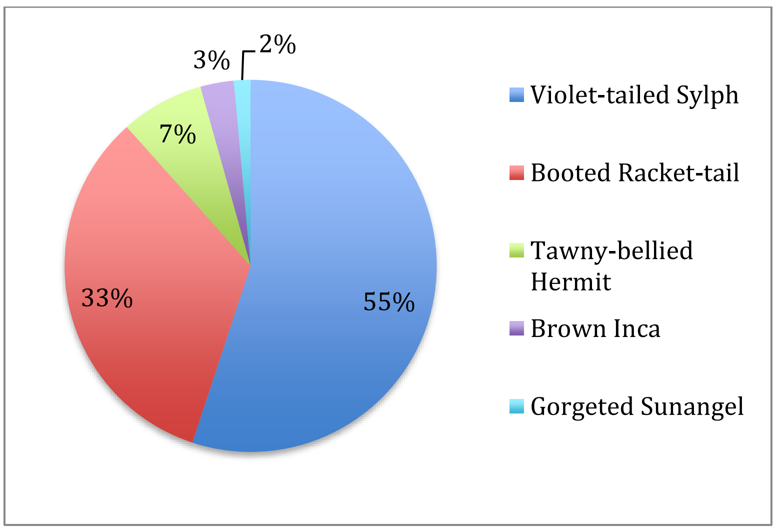

Figure 19. Percentage of hummingbird visits to *B. solanoides* by species.

The total number of visits by each hummingbird species were compiled, along with the number of visits to each flower species (Table 3). The Violet-tailed sylph recorded the most total visits and the most visits to both *G. jaramilloi* and *B. solanoides*. The Stripe-throated Hermit recorded the most visits to *G. quitensis*.

Table 3. Number of visits to each flower species by each species of hummingbird.

	G. jaramilloi	*G. quitensis*	*B. solanoides*	Total visits
Booted Racket-tail	6	1	23	30
Brown Inca	34	0	2	36
Empress Brilliant	2	0	0	2
Gorgeted Sunangel	0	0	1	1
Green-crowned Woodnymph	0	4	0	4
Purple-bibbed Whitetip	0	1	0	1
Stripe-throated Hermit	0	25	0	25
Tawny-bellied Hermit	23	6	5	34
Violet-tailed Sylph	110	12	38	160
Wedge-billed Hummingbird	2	0	0	2
White-whiskered Hermit	2	0	0	2

Discussion

Nectar Concentration Study

Average nectar concentrations found in this investigation (13.3%, *G. jaramilloi*; 18.15%, *G. quitensis*; 10.28%, *B. solanoides*, Table 1) were lower than averages found in hummingbird flowers in previous studies (21%, Percival, 1974; 20-24%, Baker, 1976). This could be due in large part to the fact that the three study species are adapted to a high-altitude, tropical, cloud forest climate in which it rains almost every day. As I will explain more in depth later in this paper, nectar can be diluted by precipitation and humidity (Park, 1929; Tadey & Aizen, 2001). The average concentrations found in this study align more with the averages found in a similar study also conducted in the SLCFR, in which three different but similarly adapted hummingbird flowers were used (6-15%, DeRycke, 2016). Additionally, holding with the findings of Bolten & Feinsinger, it would make sense for hummingbird flowers in this area, an area that has many more nectar-seeking insects than hummingbirds, to keep nectar concentrations at a level too low to interest nectar-robbing insects (1978).

The range of nectar concentrations found in this study is quite large (almost 30% for *G. quitensis*, Table 1). Some of the very low concentrations (1-4%) could have been measured on days after heavy rainfall, where precipitation could have entered flowers and diluted nectar. *G. quitensis* flowers have openings on the top, making it easy for rainwater or dew to enter the flower. *G. jaramilloi*, on the other hand, has a very long corolla and the flowers are often pointing down, so it follows that the lowest concentration measured for *G. jaramilloi* was 5% (Table 1). Furthermore, Krömer et al. found that bromeliads generally exhibit much lower intra-specific variability in nectar concentration, accounting for *G. jaramilloi* having the smallest range in concentrations of the three species (2008).

Average concentrations for *G. jaramilloi* and *B. solanoides* increased over the three time blocks and were highest in the afternoon (1:00-4:00 PM). *G. quitensis* also had the highest concentration in the afternoon, but its mid-morning concentration was lower than its early morning concentration (Table 1). Additionally, nectar concentration was found to be weakly positively correlated with time of day in *B. solanoides* (Figure 4). Both these findings are consistent with the prediction that time of day and nectar concentration would be positively correlated. Pleasants determined that in *Ipomopsis aggregata*, a hummingbird flower from the Polemoniaceae family, sugar concentration in nectar was highest in the afternoon and lowest in the early morning, which further supports the predictions and the findings of this study (1983). Although *B. solanoides* was found to have a positive correlation between nectar concentration and time of day, neither *G. jaramilloi* nor *G. quitensis* were found to have correlations for this variable (Figures 2-4). For *G. jaramilloi*, this could be due to the lack of data from the early morning. *G. jaramilloi*, as was discovered during the data collection period, seems to close its flowers at night and does not seem to reopen them until at the earliest 8:30 AM (personal observation). For obvious reasons, this made early morning data collection nearly impossible. Perhaps with more data from the early morning, a clearer trend might emerge. A similar, yet slightly opposite, problem occurred with *G. quitensis*; nectar volume seemed to decrease so much in the afternoon that it was difficult to find flowers after 2:00 PM that contained any nectar at all. This is consistent with the findings of Valtueña, Ortega-Olivencia, & Rodriguez-Riaño, that nectar secretion decreases as the day goes on (2007). Again, with a more consistent spread of data across the hours of the day, the data may have shown a stronger correlation.

Contrary to prediction, nectar concentration was found to be negatively correlated with corolla length in both *G. jaramilloi* and *G. quitensis* (Figures 5-6). No correlation was found in *B. solanoides* (Figure 7). This could be partially due to nectar dilution from precipitation, or it could be the product of having too small of a sample size to account for individual variation. It was determined that the water in nectar evaporates more quickly from flowers with shorter corollas, leading to higher concentrations of sugar in the remaining nectar (Plowright, 1987). Additionally, Montgomerie posits that in order to maximize their energy intake rate, hummingbirds choose flowers with shorter corollas and higher nectar concentration (1984). This could mean that selective pressure is acting on these hummingbird flowers to have shorter corollas, accounting for the negative correlations reported in this study.

The prediction that nectar concentration would decrease with increasing elevation was supported by the data collected for two of the three study species: *G. quitensis* and *B. solanoides* (Figures 9-10). *G. jaramilloi*, however, showed no significant correlation between the two variables (Figure 8). This could be due to the negligible temperature differences among the different altitudes in the SLCFR, or to the fact that the majority of data collected for *G. jaramilloi* was collected in the middle of the altitudinal range. Had more data been collected at low and high elevations, a correlation might have been present.

Camera Trap Study

In accordance with the prediction that hummingbird visits would decrease as the day went on, *G. quitensis* showed a negative correlation between hummingbird visitation and time of day (Figure 12). Neither *G. jaramilloi* nor *B. solanoides* had correlations between the two variables, however, which, for *B. solanoides*, could be due to the fact that much fewer visits were recorded from the camera trap footage (Figures 11 & 13). *Meliphaga virescens*, a species of nectar-feeding bird, was found to drink nectar at a decreasing rate as the day went on (Collins & Morellini, 1979). Hummingbirds, which similarly feed on nectar throughout the day, should exhibit the same trend of visiting flowers more early in the day.

While elevation did not have a significant effect on hummingbird visitation rates, the prediction that different species would be observed at different elevations held true. For example, six of the eleven hummingbird species observed by camera were only seen at one elevation each (Table 2). Empress Brilliants (*Heliodoxa imperatrix*), while commonly observed at the hummingbird feeders in Santa Lucía, are rarely seen in the forest (Beck, 2016). The images from the camera traps only showed the Empress Brilliant feeding from *G. jaramilloi* at 1891m (Table 2). Empress Brilliants are large hummingbirds with medium-length, slightly curved bills, so it is not surprising that they feed on *G. jaramilloi*, a flower with a long, tubular corolla. The Gorgeted Sunangel (*Heliangelus strophianus*) was only seen visiting *B. solanoides* at an elevation of 2174m, one of the highest elevations of all data in this study (Table 2). Gorgeted Sunangels have a small range that includes tropical montane forests, and they are generally found at higher altitudes (McMullan, 2016). The Green-crowned Woodnymph (*Thalurania colombica*), a bird that is fairly common in the forest, was only captured by one camera feeding at *G. quitensis* at an elevation of 1635m (Table 2). This makes sense given that the observed elevation range in the SLCFR for the Green-crowned Woodnymph is 1300-1800m (Beck, 2016), and that its short, slightly curved beak makes it a perfect candidate to feed on *G. quitensis*. While also common at the Santa Lucía feeders, the Purple-bibbed Whitetip (*Urosticte benjamini*) was only seen by one camera at an elevation of 1642m, feeding on *G. quitensis* (Table 2). McMullan describes it as a sedentary, non-aggressive species that is found at locally lower altitudes, which fits with the camera trap observation (2016). The Wedge-billed Hummingbird (*Schistes geoffroyi*), a species with a broad altitudinal range, was observed visiting *G. jaramilloi* by a single camera at 1866m (Table 2). This species is a known parasitic flower-piercer; it uses its sharp, short bill to pierce flowers and rob nectar without pollination (McMullan, 2016). Given that such a short bill would not be long enough to reach nectar of *G. jaramilloi* flowers, it makes sense that the bird pierced the flower instead. The White-whiskered Hermit (*Phaethornis yaruqui*), a lower elevation hummingbird, was only seen by a camera at a low elevation of 1371m, feeding on *G. quitensis* (Table 2). With an elevation range of <1600m, the White-whiskered Hermit is not found at the Santa Lucía feeders (Beck, 2016; McMullan, 2016).

The other five species that were observed by the camera traps were: the Booted Racket-tail (*Ocreatus underwoodii*), the Brown Inca (*Coeligena wilsoni*), the Stripe-throated Hermit (*Phaethornis striigularis*), the Tawny-bellied Hermit (*Phaethornis syrmatophorus*), and the Violet-tailed Sylph (*Aglaiocercus coelestis*). These species were more abundant on the camera traps, especially the Violet-tailed Sylph (160 total visits, Table 3). The Booted Racket-tail, described by Beck as abundant in the forest (2016), was most commonly observed at *B. solanoides* (Table 3). Booted Racket-tails are very small birds with short beaks, so it makes sense for them to have visited most *B. solanoides*, a small flower with a short, tubular corolla. It makes less sense, however, for them to visit *G. jaramilloi*, which has long, tubular flowers. It is possible that the Booted Racket-tail pierces these flowers instead of feeding normally at them, since their bills are too short to reach the nectar inside the flowers. The Brown Inca, on the other hand, was seen almost exclusively at *G. jaramilloi* (Table 3). Its long, straight bill makes it a perfect candidate to feed at *G. jaramilloi*, and it was the second most common visitor at the flower (Figure 17). The most common visitor at *G. quitensis* from the camera trap data was a lower-elevation bird, the Stripe-throated Hermit (Figure 18). The Stripe-throated Hermit is a small hermit with a long, curved bill, which fits the morphology of *G. quitensis* flowers. It has an elevation range of 1300-1700m (Beck, 2016), which almost perfectly matches the range found with the cameras (1371-1642m, Table 2). A similar species, the Tawny-bellied Hermit, is a more generalist, traplining feeder, so it makes sense that the cameras observed it at all three flower species (McMullan, 2016) (Table 3). It was most commonly seen at *G. jaramilloi*, most likely because of its very long, curved bill that almost perfectly fits the corolla of *G. jaramilloi*. The Violet-tailed Sylph, also a generalist species, was the most common species observed at both *G. jaramilloi* and *B. solanoides*, and was second most common at *G. quitensis* (Table 3). Violet-tailed sylphs have relatively short, straight bills, and are known to pierce corollas of some flowers, so it seems reasonable that it pierced the corollas of *G. jaramilloi* to extract nectar (McMullan, 2016). The predictions that flower morphology would influence hummingbird visitation mostly held true; hermit hummingbirds were the most common visitors to *G. quitensis*, short-billed hummingbirds like the Violet-tailed Sylph and the Booted Racket-tail were most common at *B. solanoides*, and *G. jaramilloi* attracted the longer-billed Brown Inca.

Potential Sources of Error

Given the short duration (less than 3 weeks of data collection) of this study, there was a large potential for error. An issue that almost certainly affected the results was the small sample size. It is difficult to account for individual variation when the sample size is so small, especially in plants and flowers in which there exist a lot of individual variation. In addition to individual variation, age of the flowers (which was not tested) could have had an effect on nectar concentration. Valtueña, Ortega-Olivencia, & Rodriguez-Riaño found that older flowers in a Fabaceae species have smaller volumes of nectar and therefore higher nectar concentrations, which could have been a factor in my species as well (2007). Precipitation (and often, lack thereof) could have affected the results in two ways: precipitation and humidity have been shown to dilute nectar; lack of precipitation could lead to higher-than-normal nectar concentrations. During the first week of data collection, it rained heavily almost every night, but the second two weeks experienced almost no rain. The inconsistency and aseasonality of the precipitation trends during data collection could have affected nectar concentrations throughout the study. Another possible cause for dilution of nectar is that for the first few days of data collection, some flowers were bagged overnight in an attempt to prevent nectar robbers from accessing the flowers. However, I noticed that condensation tended to build up inside the bags when they were left on flowers for hours at a time, so bagging of flowers was stopped. It is possible that some data points from these bagged flowers have more dilute nectar than they otherwise would have. A final potential source of error for the nectar concentration study is that flowers were not closely inspected for holes before nectar was collected. Nectar in flowers with holes in the corolla has been shown to evaporate much faster; 6-10% of the water in nectar can evaporate through robber holes, leading to more highly concentrated nectar (Pleasants, 1983). As for the camera trap study, it is possible that the motion-detecting software did not detect all hummingbird movement and that some visits were missed. Additionally, it is possible that a few of the hummingbirds were misidentified or mislabeled due to human error.

Conclusions

The variables predicted to have an influence on nectar concentration in the three study species were determined to have the following effects: nectar concentration was found to be positively correlated to time of day in *B. solanoides*, negatively correlated to corolla length in *G. jaramilloi* and *G. quitensis*, and negatively correlated to elevation in *G. quitensis* and *B. solanoides*. Hummingbird visitation was higher in the morning and decreased as the day went on, as predicted. Flower morphology had an effect on which hummingbird species visited which flowers, as did elevation.

In order to more fully understand the effects of these variables on nectar concentration, it would be helpful to repeat this study with a larger sample size. Additionally, this study has now been performed with two different sets of three Ecuadorian Cloud Forest plant species (this study; DeRycke, 2016), but it could be interesting to repeat the study with even more plant species to gain a more general survey of variables affecting nectar concentration and hummingbird visitation in this region. Another variable that could be included in similar future studies is temperature. Furthermore, as precipitation was potentially an unstudied variable in this study, a study that focuses on precipitation and dilution of nectar would be helpful for future research.

Acknowledgements

I would like to thank my advisor, Holger Beck, for all his help before, during, and after the research process. I am also very grateful to the staff of the Santa Lucía lodge and office, including Eduardo Tapia, Edison Tapia, Noé Morales, Vicente Molina, Juan Molina, Andrés Molina, Marcela Toapanta, Edwin Urquia, Patricio De La Torre, and Graciela Santos, for keeping us very comfortable in our time at Santa Lucía. I would also like to thank Xavier Silva, Javier Robayo, Diana Serrano, and Leonore Cavalleros for their continued support throughout the ISP process. Finally, I'd like to thank Briana Grether for her friendship and moral support on rainy days.

Works Cited

Baker, H. G. (1975). Sugar concentrations in Nectars from Hummingbird flowers. *Biotropica*, *7*(1), 37. doi:10.2307/2989798

Beck, H. (2016). Hummingbirds of Santa Lucía.

Bolten, A. B., & Feinsinger, P. (1978). Why do Hummingbird flowers Secrete dilute nectar? *Biotropica*, *10*(4), 307. doi:10.2307/2387684

Castellanos, M. C., Wilson, P., & Thomson, J. D. (2002). Dynamic nectar replenishment in flowers of Penstemon (Scrophulariaceae). *American Journal of Botany*, *89*(1), 111–118. doi:10.3732/ajb.89.1.111

Collins, B. G., & Morellini, P. C. (1979). The influence of nectar concentration and time of day upon energy intake and expenditure by the singing Honeyeater, Meliphaga virescens. *Physiological Zoology*, *52*(2), 165–175. doi:10.2307/30152561

DeRycke, E. (2016). *Impacts of Coevolution: Variables affecting nectar concentration, and how these variables influence hummingbird visitation*.

Edson, G. (2015, August 9). Nanegalito. Retrieved December 2, 2016, from Climate Data, http://en.climate-data.org/location/180088/

Harder, L. D., & Cruzan, M. B. (1990). An evaluation of the physiological and evolutionary influences of Inflorescence size and flower depth on nectar production. *Functional Ecology*, *4*(4), 559. doi:10.2307/2389323

Krömer, T., Kessler, M., Lohaus, G., & Schmidt-Lebuhn, A. N. (2008). Nectar sugar composition and concentration in relation to pollination syndromes in Bromeliaceae. *Plant Biology*, *10*(4), 502–511. doi:10.1111/j.1438-8677.2008.00058.x

Maglianesi, M. A., Böhning-Gaese, K., & Schleuning, M. (2014). Different foraging preferences of hummingbirds on artificial and natural flowers reveal mechanisms structuring plant-pollinator interactions. *Journal of Animal Ecology*, *84*(3), 655–664. doi:10.1111/1365-2656.12319

McMullan, M. (2016). *Field Guide to the Hummingbirds* (1st ed.). Colombia: Ratty Ediciones.

Montgomerie, R. D. (1984). Nectar extraction by hummingbirds: Response to different floral characters. *Oecologia*, *63*(2), 229–236. doi:10.1007/bf00379882

Ornelas, J. F., Ordano, M., De-Nova, A. J., Quintero, M. E., & Garland, T. (2007). Phylogenetic analysis of interspecific variation in nectar of hummingbird-visited plants. *Journal of Evolutionary Biology*, *20*(5), 1904–1917. doi:10.1111/j.1420-9101.2007.01374.x

Park, O. W. (1929). The influence of humidity upon sugar concentration in the nectar of various plants. *Journal of Economic Entomology*, *22*(3), 534–544. doi:10.1093/jee/22.3.534

Percival, M. (1974). Floral ecology of coastal scrub in southeast Jamaica. *Biotropica*, *6*(2), 104–129. doi:10.2307/2989824

Pleasants, J. M. (1983). Nectar production patterns in Ipomopsis aggregata (Polemoniaceae). *American Journal of Botany*, *70*(10), 1468. doi:10.2307/2443345

Pleasants, J. M., & Chaplin, S. J. (1983). Nectar production rates of Asclepias quadrifolia: Causes and consequences of individual variation. *Oecologia*, *59*(2-3), 232–238. doi:10.1007/bf00378842

Plowright, R. C. (1987). Corolla depth and nectar concentration: An experimental study. *Botany*, *65*(5), 1011–1013. doi:10.1139/b87-139

Tadey, M., & Aizen, M. A. (2001). Why do flowers of a hummingbird-pollinated mistletoe face down? *Functional Ecology*, *15*(6), 782–790. doi:10.1046/j.0269-8463.2001.00580.x

Tamm, S., & Gass, C. L. (1986). Energy intake rates and nectar concentration preferences by hummingbirds. *Oecologia*, *70*(1), 20–23. doi:10.1007/bf00377107

Valtueña, F. J., Ortega- OlivenciaAna, & Rodríguez- RiañoTomás (2007). Nectar production in Anagyris foetida (Fabaceae): Two types of concentration in flowers with hanging Droplet. *International Journal of Plant Sciences*, *168*(5), 627–638. doi:10.1086/513482

References

A, A., Marcelo. (2003, November 1). Down-facing flowers, hummingbirds and rain. Retrieved December 3, 2016, from Taxon, http://www.ingentaconnect.com.ccl.idm.oclc.org/content/iapt/tax/2003/00000052/00000004/art00003

Feinsinger, P., & Chaplin, S. B. (1975). On the relationship between wing disc loading and foraging strategy in Hummingbirds. *The American Naturalist*, *109*(966), 217–224. doi:10.1086/282988

Gill, F. B. (1988). Trapline foraging by Hermit Hummingbirds: Competition for an Undefended, renewable resource. *Ecology*, *69*(6), 1933–1942. doi:10.2307/1941170

Henderson, J., Hurly, T. A., Bateson, M., & Healy, S. D. (2006). Timing in free-living Rufous Hummingbirds, Selasphorus rufus. *Current Biology*, *16*(5), 512–515. doi:10.1016/j.cub.2006.01.054

Hixon, M. A., Carpenter, L. F., & Paton, D. C. (1983). Territory area, flower density, and time budgeting in Hummingbirds: An experimental and theoretical analysis. *The American Naturalist*, *122*(3), 366–391. doi:10.2307/2461022

Irwin, R. E. (2000). Hummingbird avoidance of nectar-robbed plants: Spatial location or visual cues. *Oikos*, *91*(3), 499–506. doi:10.1034/j.1600-0706.2000.910311.x

Lara, C., & Ornelas, J. (2001). Preferential nectar robbing of flowers with long corollas: Experimental studies of two hummingbird species visiting three plant species. *Oecologia*, *128*(2), 263–273. doi:10.1007/s004420100640

Reed Haisworth, F., & Wolf, L. L. (1976). Nectar characteristics and food selection by hummingbirds. *Oecologia*, *25*(2), 101–113. doi:10.1007/bf00368847

Snow, B. K., & Snow, D. W. (1972). Feeding niches of Hummingbirds in a Trinidad Valley. *Journal of Animal Ecology*, *41*(2), 471–485. doi:10.2307/3481

Snow, D. W., & Snow, B. K. (1986). Feeding ecology of hummingbirds in the Serra do Mar, southeastern Brazil. *El Hornero*, *12*(4), 286–296.