Fall 2017

Hummingbird Foraging Preference: The Hierarchical Impacts of Color, Position, and Concentration on Visitation Frequency

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Biology and Spanish

South America, Ecuador, Chocó Cloud Forest, Santa Lucia Reserve

Submitted in partial fulfillment for Ecuador Comparative Ecology and Conservation,

SIT Study Abroad, Fall 2017

Abstract

Hummingbirds are well adapted to efficiently locate high quality nectar sources, but relatively few studies have examined the mechanisms of that process under natural conditions in the field. This study investigates the visual signals that allow hummingbirds to do just that, as well as the external factors that limit their choices in foraging. More specifically, feeder color and nectar concentration were manipulated to determine the effect of those independent variables on visitation frequency. An initial experiment was conducted in order to explore color preference among the visiting species. Red, orange, and blue feeders were filled with nectar of equal concentrations and the feeders were rotated three times to ensure each color was observed in each position. Once preference was established, a second experiment was conducted in which the feeder of least preferred color, blue, was placed in the least preferred position and filled with a higher nectar concentration, while two red feeders remained at the initial concentration. Eight hours of observation were completed each day, with the following data collected for each foraging bout: feeder color and position, species and sex of visiting individual, time of arrival, number of visits, and behavioral notes. Territoriality played a major role in determining visitation to each feeder, with first a male violet-tailed sylph and then a brown Inca asserting their role as dominant territorialists that chased indiscriminately. It was discovered that, when nectar reward was equal for each feeder, position was the primary factor in determining visitation frequency. However, visitation to each position was influenced by feeder color, each position receiving the most visits when the red feeder was in place. During the second experiment, no clear preference for the higher concentration was shown by the end of the four days, but visitation to that feeder exhibited an upward trajectory and increased consistently each day, which suggests that preference would eventually be indicated. The findings of this investigation indicate the presence of a hierarchy of factors impacting feeder choice, pointing to nectar concentration with the strongest influence, followed by position and then color.

Los colibríes están bien adaptados para poder encontrar fuentes de azúcar en una manera muy eficiente, pero pocos estudios han investigado los mecanismos por los que funciona ese proceso bajo condiciones naturales fuera de un laboratorio. Esta investigación examina las señales visuales que permiten que los colibríes hagan exactamente eso, además los factores externos que limitan sus opciones en el forrajeo. El color de bebedero y la concentración del néctar fueron manipulados para averiguar cómo afectan esos variables independientes a la frecuencia visitación. Un experimento inicial se realizó con el propósito de explorar la preferencia del color entre los colibríes visitantes. Tres bebederos de rojo, naranjo, y azul se llenaron de néctar de concentraciones iguales y los bebederos fueron rotados tres veces para asegurar de que todos los colores se habían puesto en cada posición. Una vez que se estableció la preferencia, un experimento adicional se realizó en lo que el bebedero del color menos preferido fue colocado en la posición menos preferida, lleno de néctar de una concentración aumentada, mientras dos bebederos rojos se quedaron con la misma concentración que se usó al inicio. Ocho horas de observación se completaron cada día del estudio, con los siguientes datos colectados para cada episodio de forrajeo: el color y la posición del bebedero visitado, la especie y el sexo del individuo visitante, la hora de llegada, el número de visitas, y notas del comportamiento. Territorialidad hizo un papel importante en determinar la frecuencia de visitas a cada bebedero, con primero un violet-tailed sylph y después un brown Inca que afirmaron su papel como territorialistas dominantes. Se descubrió que, cuando la recompensa del néctar era igual para cada fuente, la posición era el factor principal en determinar la visitación. Sin embargo, las visitas a cada de las posiciones se vieron influenciadas por el color del bebedero, y cada posición recibió la mayor cantidad de visitas cuando el bebedero rojo estaba en su lugar. Durante el segundo experimento, no se demostró una preferencia clara para la concentración alta por el fin de eses cuatro días, pero el número de visitas a ese bebedero mostró una trayectoria ascendente y aumento de manera consistente cada día, lo que sugiere que eventualmente la preferencia sería indicada. Los resultados de esta investigación indican la presencia de una jerarquía de factores que afectan la elección de bebedero, apuntando a la concentración de néctar con la mayor influencia, seguida de la posición y luego del color.

ISP topic codes: Biology 609; Ecology 614; Forestry and Wildlife 608

Acknowledgments

I would like to thank my professors, Xavier Silva and Javier Robayo, for all of their enthusiasm, advice, and flexibility in helping this project come to life. I would also like to thank my advisor, Holger Beck for his help in the designing of this project and aid in the face of numerous technological crises. Thank you to Simon Lowen for his brainstorming abilities and for never allowing there to be a dull moment. I would also like to thank Ben Weinstein for his ideas and for tirelessly working to solve my technological issues. Endless thanks to Noé Morales for always having the answers. Thank you to Biche and Cati Vela for welcoming us into their home, keeping us alive and sane in la Delicia and especially for teaching us how to play cuarenta. Additional thanks to Eduardo Tapia, Edison Tapia, Edwin Urquia, and of course Freddy, for keeping Santa Lucia functioning, lively, and full of good food and music.

Introduction

With extremely high levels of metabolic activity, hummingbirds must be efficient in locating high quality nectar sources and must be able to quickly adjust to changes in their environment (Powers *et al*., 1994). Visual signaling plays an important role in that process, allowing hummingbirds to recognize flower morphology and create an association with certain nectar concentrations. Several studies have shown that many species of hummingbird associate primary and secondary floral attractants with nectar concentrations and tend to select for those traits associated with higher reward (Fenster *et al*., 2006; Maglianesi et., 2015). The ability to create such associations is an invaluable mechanism for maximizing energy intake. A widely-held belief is that hummingbirds are partial to red objects and flowers. The results of one study indicated that pollinator-mediated selection among plants resulted in directional selection that favored wide corolla tubes as well as intense red coloration (Campbell *et al.*, 1997).

However, Bene (1941) found that certain hummingbird species do not show preference for red over other colors, but rather that preference may be conditioned. In a separate study, hummingbirds showed a spontaneous preference for red over white flowers with equal rewards, but the preference shifted to white when those flowers were made the most rewarding food source (Melendez-Ackerman & Campbell, 1993), indicating that there was a previously learned association between red and a high reward. Going even further down the road of color-concentration association, Stiles (1976) and Wagner (1946) demonstrated that among several species in Mexico, hummingbird response to color changed seasonally along with the color of the most rewarding food flower. Conditioning for color preference could have interesting implications for a study that manipulates both feeder color and nectar concentration. At feeders, energetic parameters are somewhat equalized, as nectar is "unlimited" in a sense, meaning that the major factor affecting energetics is sugar concentration (Stiles, 1976). Relatively few studies have examined hummingbird flower color preference under natural conditions in which flower color preference varies within a species. The purpose of this study was to determine color preference among the visiting hummingbird species at Santa Lucia and to observe how they track the signal-reward pattern through an association of color with higher nectar concentration.

In terms of foraging habits, hummingbirds tend towards one of two behavior niche types: territorial and trap lining. There are six distinguishable community roles for hummingbirds for foraging on various flower types: high-reward trapliners, which visit but do not defend nectar-rich flowers with long corollas; territorialists, which defend clumps with high quantities of shorter flowers; low-reward trapliners, which forage on widely dispersed nectar-poor flowers; territory-parasites; and generalists, which shift among various resources (Feinsinger & Colwell, 1978). Several species observed on the feeders exhibited territorial behavior and aggression including violet-tailed sylph (*Aglaiocercus coelestis*), empress brilliant (*Heliodoxa imperatrix*), fawn-breasted brilliant (*Heliodoxa rubinoides*), and brown Inca (*Coeligena wilsoni*). All of those species have small bodies, short wings, and relatively short, thin beaks. Short wings have been demonstrated to have a correlation with faster wing beats, which are more energy-consuming (Feinsinger & Chaplin, 1975). However, while it is energetically costly, it is also beneficial as it allows for faster movement and change in direction, which is a powerful tool for aggressive territorial hummingbirds that are often expending energy chasing. To counteract that energy expenditure, territorial birds tend to perch unless they are feeding or interacting with other individuals (Feinsinger & Colwell, 1978). While larger hummingbirds with longer beaks, specifically high-reward trapliners, tend to be more specialized in flowers with longer corolla tubes (Maglianesi *et al.*, 2015), these smaller territorialists are able to take advantage of small flowers and any accessible resource because they do not need to counteract the energy spent by hovering. Therefore, it would be interesting to see if territorialists are less sensitive to changes in concentration.

During the investigation, three feeders of different colors (red, yellow-orange, and dark blue) were filled with nectar of the same concentration and rotated every three days to determine whether color or position of feeder impacted visitation frequency across species. Spatial location of feeders adds an additional variable to the experiment. Hummingbirds do use spatial information in foraging. On one spatial scale, they are able to learn which patches of nectar sources, such as flowers or feeders, are the most energetically profitable, and preferentially revisit those patches (Sutherland 1984). Therefore, in order to account for position and ensure that it does not have a greater impact than color, the colors were rotated so that each was observed in each position. Following the color experiment, the feeder of least preferred color was placed in the least preferred position with an increased nectar concentration while two other feeders (both red) remained at the initial concentration, all in order to see whether the hummingbirds would eventually select for the higher concentration feeder regardless of color or position. Should the hummingbirds demonstrate a clear shift in preference in favor of the high concentration feeder, that feeder would then be shifted to different positions in order to see how quickly the concentration could be tracked using colors. With regard to this part of the investigation, I expected that the hummingbirds would shift to the high concentration feeder faster each time it was moved, demonstrating a learning curve and a strengthening association between color and concentration. I hypothesized that all species would preferentially visit the red feeder regardless of position in the initial phase of the investigation and that more territorial interactions would be observed at that feeder. Additionally, I hypothesized that, once concentration had been changed in the least preferred feeder, the hummingbirds would shift their preference to that feeder and there would also be an increase in territorial behavior surrounding that feeder.

Methods

Description of Study Site

The study was conducted in the Santa Lucia Cloud Forest Reserve, located at N00°07.090' W078°36.712' at an elevation of 1910 meters, approximately 300 meters from the main Santa Lucia lodge near the town of Nanegal in the Pichincha Province of Ecuador (Appendix, Figure A). The study was conducted from the 13th of November to the 1st of December. The climate at Santa Lucia was tropical and generally cloudy and there was minimal temperature variation. When the investigation began, it had not rained in the reserve for approximately ten days, which meant a decline in floral populations in the forest surrounding the lodge. It did not rain in the first week, during which no official observations were recorded but the feeders were left out in order to let the hummingbirds become accustomed to their presence. In the third week, during which the concentration experiment was conducted, it rained throughout three afternoon observation periods and for one morning observation period. Most mornings had very little cloud cover and sunny skies. Average annual temperature in the surrounding area is approximately 20.2 C and average annual rainfall is 2086 mm (Climate-data.org). The observation site was located in the orchid garden along the self-guided trail. The orchid garden consisted entirely of secondary forest and is home to many different orchid species, bromeliads, and other epiphytes. The most common species of flowering plants on which hummingbirds were observed in the observation site were Guzmania amplectens, Besleria solenoides, Columnea mastozoni, Columnea picta, and Gasteranthus cittensus. The reserve itself consists largely of reforested pasture that was previously used for grazing livestock, while 80% of the reserve remains primary tropical montane forest.

Feeder Setup

During the initial phase of the project, which sought to determine a color preference among the different species that visited, three hummingbird feeders were painted red, orange, and blue (Appendix, Figure B). The feeders were hung using a 25-meter rope tied in a triangular configuration using two trees and a fence post located in the orchid garden along the self-guided trail in the Santa Lucia Reserve, as depicted in Appendix Figure C. Feeders were secured to rope tied between the trees and post, placed approximately 50 centimeters from the Plotwatcher cameras that were secured to those fixtures. Feeders were at least 3 meters from one another, far enough that one territorial bird would not be able to protect all three feeders at once. Position A was 3.15 meters from position B and 3.34 meters from position C, while position B and C were 3.11 meters apart. Due to the slope of the hill, the feeders were fixed at different heights from the ground. One camera was trained on each feeder, and duct tape was used to block the nectar access ports that were opposite the camera to ensure that the visiting hummingbirds would always be in view of the camera. The feeders were filled with nectar of 25% sugar concentration and left out in the orchid garden for five days before data collection began to ensure that enough hummingbirds were visiting to obtain sufficient results for statistical analysis.

Color Preference Experiment

All three feeders were filled with the same nectar of 25% sugar concentration. Thirty-six hours of observation were performed in the first week of data collection. Due to logistical conflicts, feeders were only observed for six hours on Monday (9:00-13:00; 16:00-18:00) and Friday (6:00-8:00; 9:00-13:00), while eight hours of observations were done each day Tuesday through Thursday (6:00-8:00; 9:00-13:00; 16:00-18:00). Hummingbirds are active throughout the day due to their almost constant need to feed to counteract their extremely high levels of metabolic activity (Diamond *et al.*, 1986), meaning that observation could take place any time of day, with peak activity 6:00-8:00 and 16:00-18:00. The Plotwatcher cameras were set to record activity between 6:00 and 18:30 in order to collect more data when the observer was not present. Camera data was later uploaded and processed using the program MotionMeerkat created by Ben Weinstein. During each observation period, the following data were collected for each hummingbird foraging bout (which was defined as the entire time and all activities of one individual from first sighting at a feeder until the time they leave that feeder): feeder position, feeder color, sex (if possible) and species of the visiting hummingbird, time of arrival, number of visits (one visit defined as the time during which a hummingbird is perched on or actively drinking from the feeder), and behavioral notes that focused largely on territoriality (territorial behaviors included (1) perching and chirping (2) ramming (3) chasing (4) sticking tongue out (5) other displays, for example the puffing of feathers or hovering displays (Pitelka, 1942; Camfield, 2006). Visits were the primary focus of the study rather than bouts, because the goal was to determine the number of times that each hummingbird made the choice to revisit and drink from each feeder. Weather conditions were also recorded at the beginning of each observation period. After three days, the feeders were rotated, so that eventually each color was placed in each position. Three days was enough time for the hummingbirds to become accustomed to the new setup (Stiles 1976). Three rotations were completed during this part of the project.

Concentration/Signal-Reward Experiment

In order to determine whether the hummingbirds could track the signal-reward pattern and associate nectar concentration with a certain color, the blue feeder (least preferred) was placed in the B position (least preferred), both of which were determined during the first week of the experiment. The blue feeder was filled with 40% nectar while two red feeders were filled with the same 25% nectar and were placed in positions A and C. In doing so, it was probable that should the hummingbirds show preference for the feeder of higher concentration (Blue / Position B), it was not due to preference of position or color. The concentration in the two red feeders was not dropped below 25%, which one might speculate would result in a faster shift to the high concentration feeder, in order to ensure that the switch was made due to preference for a higher concentration rather than being driven from the lower concentration because they are not able to gain enough energy from the nectar. The feeders were observed during the same hours (6:00-8:00; 9:00-13:00; 16:00-18:00) each day, including the exceptions of Monday and Friday. The same data was collected for each foraging bout observed as in the previous color experiment.

Data Analysis

In order to analyze the general visitation data, total visits and percentages were calculated for the red, orange, and blue feeders in each position (A, B, and C). Three pie charts were used to represent the distribution of visits to each feeder during each of the three rotations. A two-way analysis of variance with replication with the independent variables color and position was performed to determine the effect of color and position on total visitation frequency. Because one day of data was lost due to a leak in the orange feeder, the averages for the other three days in that rotation were used to fill the missing data points for the ANOVA. Where the ANOVA results were significant, it was followed up by post-hoc two-sample t-tests that took into account the Bonferroni correction. The total number of visits by species was depicted using a bar graph with error bars. Then, number of visits and proportion of visits to each color and position across all three rotations was calculated by species. Then, two-way analysis of variance was repeated for the sums for each of the seven observed species to determine if there was significant difference for the factors of color and position. If significance was found, post-hoc t-tests with the Bonferroni correction were conducted between groups of each of the independent variables (color and position). Territorial behavior was analyzed by calculating the proportion of total aggressors (those that chased other individuals) by species and creating pie charts. This was done both for the color experiment (week 1) and the concentration experiment (week 2). The proportion of chases was also calculated for each color and position to look for any increased territorial behavior that may explain low visitation. The shift in preference during the concentration experiment was represented by creating a bar graph of the total visits to each feeder per day. The same was done for the number of chases.

Results

General visitation data - Color Experiment

Looking at the overall distribution of visits during the first week of observation (Appendix, Table A), during which the color preference experiment was conducted, the red feeder received 37.7% of the total visits, orange received 28.6%, and the blue feeder received 33.7% ($n = 6455$). The feeder at position A received 26.6% of the total visits ($n = 6455$), the feeder at B 13.2%, and the feeder at C received 60.2%. As seen in the pie charts in figures 1-3, which depict the proportion of visits to each feeder during the three position rotations, position C was the most preferred position across species during that part of the investigation. When the red feeder was present in the C position, it comprised 71.8% of the total visits during that rotation. In rotations 2 and 3, when blue and orange were present in that position, they made up 55.7% and 54.8% respectively. The same followed for position A which appeared to consistently fall second to position C in terms of preference. When the red feeder was placed in position A (rotation 3, Figure 3), 33.8% of all visits were made at that feeder, while 19.1% and 27.0% of visits were made to position A when the feeders were blue and orange, respectively (Figures 1 and 2). During all three position rotations, position B proved to be the least preferred position. The proportion of total hummingbird visits to that chose position B was highest with the presence of the red feeder (17.3%), followed by the blue (9.8%), then the orange (9.2%). These figures make it clear that, regardless of feeder color, the preference shown for feeders in certain positions is extremely consistent.

A two-way analysis of variance was conducted to compare the main effects of both color and position on visitation frequency at each feeder. All effects were statistically significant on the 0.05 level. The main effect for position yielded an F ratio of $F(2, 4) = 32.02$, $p < 0.001$, indicating a significant difference between position A (sum = 1716, avg. = 572), B (sum = 851, avg. = 283.7), and C (sum = 3888, avg. = 1296). Post-hoc two-sample t-tests using the Bonferroni correction showed that visitation to position C was significantly higher than to positions A ($p < 0.0167$) and B ($p < 0.001$), and also that position A received significantly more visits than position B ($p < 0.0167$). The main effect for color yielded an F ratio of $F(2, 4) = 4.25$, $p < 0.001$, indicating that the effect for color on visitation frequency was significant as well. Post-hoc two-sample t-tests using the Bonferroni correction showed that the total number of visits to red was significantly greater than that to orange ($p < 0.0167$). The interaction effect was also significant ($F(4, 27) = 9.36$, $p < 0.001$), indicating that there was an interaction between the two independent variables, color and position. In other words, color of feeder has an impact on the effect of position on visitation frequency. Looking at the plot of mean number of visits to each feeder throughout the three color rotations, it is clear that color has an effect on position and vice versa, however that effect is not necessarily consistent across combinations. At position C, the presence of the color red increased the mean number of visits. Orange was lowest in all positions except for

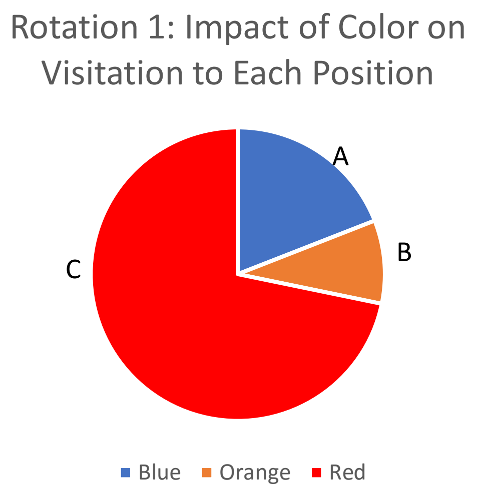

Figure 1. Total visits during rotation 1 of color experiment indicate significant preference for position C. All feeders contained the same nectar concentration of 25%. The blue feeder, located at position A, received 363 total visits making up 19.1% of the total visits during that rotation. The orange, located at position B, received 175 total visits and accounted for 9.2% of total visits. The red feeder, present at position C, received 1367 visits, making up 71.8% of total visits during that rotation. Visitation to C was significantly higher than that to position A ($p < 0.0167$) and position B ($p < 0.001$).

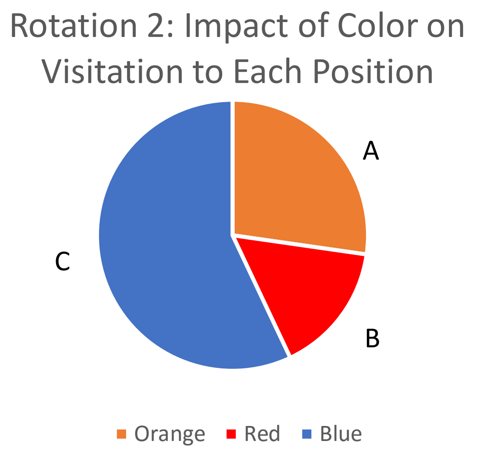

Figure 2. Total visits during rotation 2 of color experiment indicate significant preference for position C. All feeders contained the same nectar concentration of 25%. The orange feeder, located at position A, received 797 total visits making up 27.0% of the total visits during that rotation. The red, located at position B, received 512 total visits and accounted for 17.3% of total visits. The blue feeder, present at position C, received 1648 visits, making up 55.7% of total visits during that rotation. Visitation to C was significantly higher than that to position A ($p < 0.0167$) and position B ($p < 0.001$).

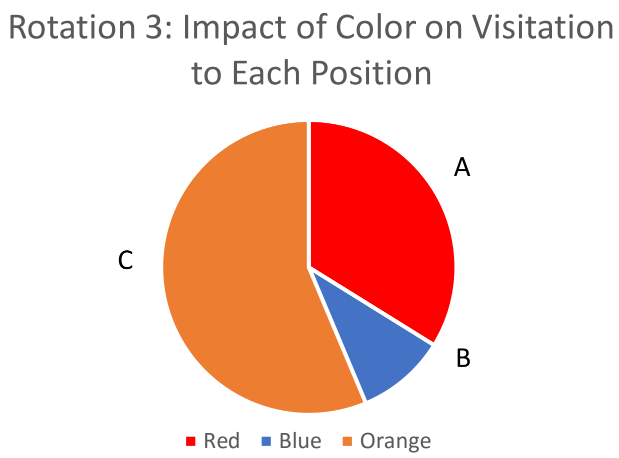

Figure 3. Total visits during rotation 3 of color experiment indicate significant preference for position C. All feeders contained the same nectar concentration of 25%. The red feeder, located at position A, received 556 total visits making up 34.9% of the total visits during that rotation. The blue, located at position B, received 164 total visits and accounted for 10.8% of total visits. The orange feeder, present at position C, received 873 visits, making up 54.8% of total visits during that rotation. Visitation to C was significantly higher than that to position A ($p < 0.0167$) and position B ($p < 0.001$).

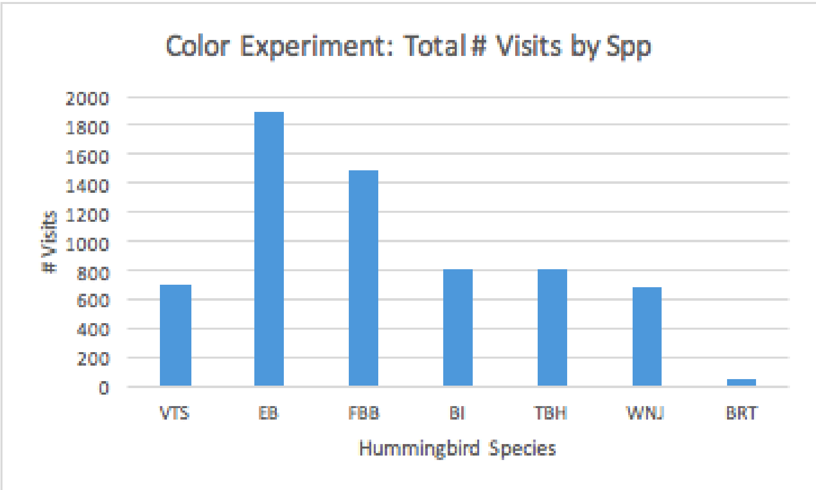

Figure 4. Number of visits by species during all rotations of color experiment. Male violet tailed sylph (VTS) visited a total of 707 times. Female empress brilliant (EB), who visited the most, made 1891 visits. Female fawn-breasted brilliant (FBB) visited 1489 times. Brown Inca (BI) visited 812 times, tawny-bellied hermit (TBH) 810, male white-necked Jacobin (WNJ) 692, and lastly female booted racket tail (BRT) 49 times throughout the experiment.

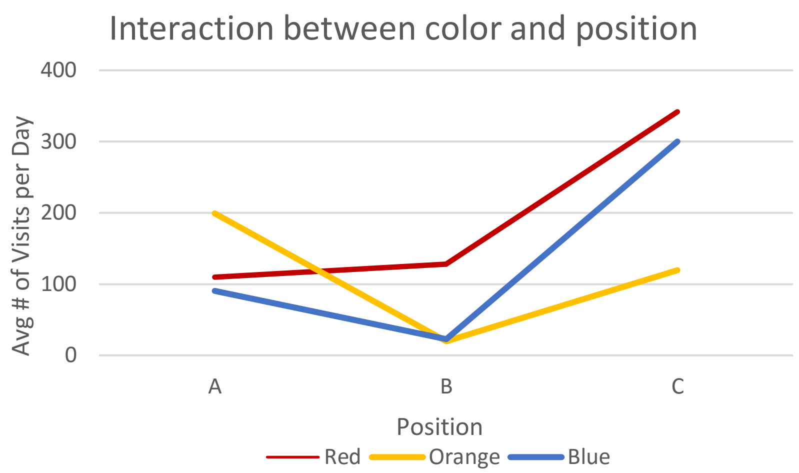

Figure 5. Plot of mean number of visits per day to each feeder by color and position represents the interaction effect of the two independent variables. The mean number of visits to position A for red, orange, and blue, respectively were 110, 199, and 91. Mean number of visits to position B for red, orange, and blue, respectively were 128, 20 and 23. The mean number of visits to position C for red, orange, and blue were 342, 119, and 300, respectively. It is important to note that the total number of visits changed each day.

Territorial Behavior - Color Experiment

Of the aggressive or territorial interactions observed at the feeders during the first week of data collection, 58.5% of the total chases took place at the feeder in position C (n = 282). Only 23.4% and 18.1% of chases took place at position A and B, respectively. Looking at distribution of chases across the different feeder colors, there was little favor shown to one color. 41.1% of the chases were from the red feeder, 26.2% from the orange, and 32.6% from the blue. One male violet-tailed sylph, identified with by his short tail and white spot on his shoulder, was responsible for 46.1% of the total chases, most of which were from position A, followed closely by empress brilliant (24.1%), which mainly chased individuals from position A. One female empress brilliant with a white spot where her wing met her chest was identified to be the dominant individual at position C. While she could not be identified during all of the aggressive interactions, she was identified as the aggressor in the majority of chases at position C (71.1%, n = 52).

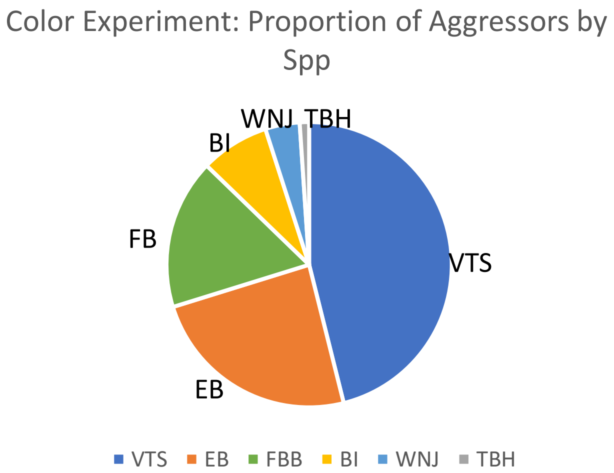

Figure 6. Proportion of total chases by aggressor during the color experiment indicates that violet-tailed sylph initiated the majority of aggressive interactions. The male violet-tailed sylph (VTS) chased 130 intruders (46.1% of total, n = 282). Female empress brilliant (EB) chased 68 individuals

(24.1% of total). Female fawn-breasted brilliant (FBB) chased 48 individuals (17.0%). Brown Inca chased 22 (7.8%). White-necked Jacobin chased 11 (3.9%), tawny-bellied hermit chased 3 (1.1%), and booted racket tail were the aggressors in no chases.

General Visitation Data - Concentration Experiment

During the concentration experiment, two red feeders were placed at positions A and B, both filled with nectar of 25% sugar concentration. A blue feeder was placed at position B, filled with 40% sugar concentration. The feeders were left in that position for the rest of the allocated research time, which ended up being four days. Due to logistical challenges, only six hours of observation were done on days 1 and 4, while eight hours of observation were done on days 2 and 3. Looking at the overall distribution of visits during that second week, feeder at A received 35.3% of the total visits, the feeder at B 23.0%, and the feeder at C received 41.3% (n = 2419). As seen in figure 7, on day 1 the majority of visits (61.5%, n = 576) were to the red feeder at position C. However, the number of visits to position C decreased over the four days, and on the final day of the concentration experiment 32.7% (n = 612) of the total visits that day were to position C. The other red feeder at position A increased from 22.9% (n = 576) to 37.9% (n = 612) of the total visits from day 1 to day 4. By the end of the four days, it appeared that the feeders at positions A, B, and C were receiving very similar numbers of visits. Two sample t-tests accepted the null hypothesis and indicated that there was no significant difference in visitation to the different positions on the last day of the experiment. In examining the progression of number of visits to the blue feeder with high nectar concentration as represented in figure 7, visitation to the blue feeder was on an upward trend throughout those four days. While the R^2 value for the blue trendline is 0.936, indicating that the data points fit the progression line in an extremely precise manner, the R^2 value for the progression of visitation at position A is much lower (0.199).

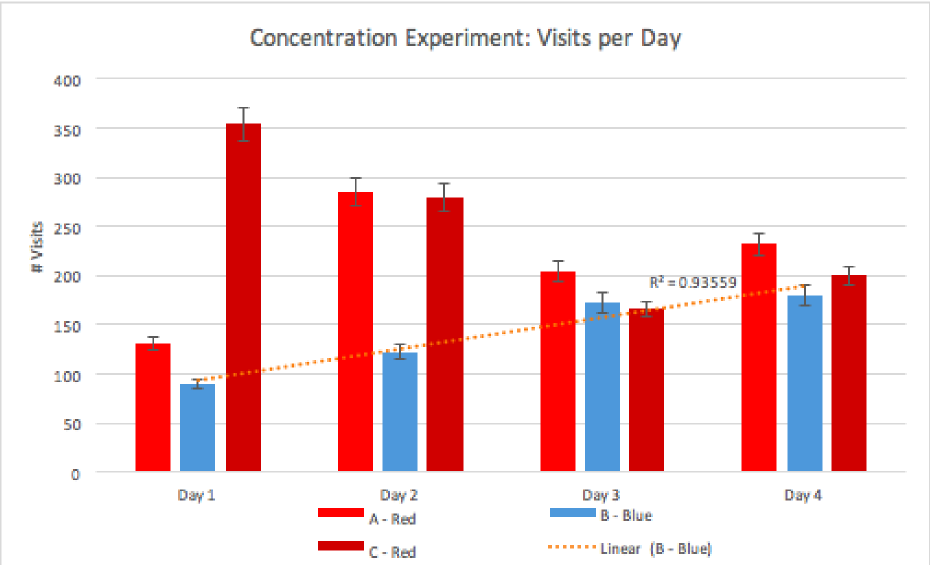

Figure 7. Visits to each feeder per day indicates an upward trend in visitation to the blue feeder of high concentration (40%) at position B. The trendline shown on the graph represents the increase in number of visits to the blue feeder (R^2 = 0.93559). Visitation to position A also increased in the absence of the dominant violet-tailed sylph. Total visits and visits by each species per day during the concentration experiment are represented in Table B in the appendix.

Territorial Behavior - Concentration Experiment

Of the total overtly aggressive interactions observed during this part of the investigation, 21.6% of the chases took place at the red feeder in position A. 34.4% of the chases were observed surrounding the red feeder at position C. Contrasting with the low number of visits to blue in comparison to other feeders, 44% of chases (n = 126) were observed at the blue feeder in position B. As shown in figure 8, after the first day in which concentration was manipulated, more chases were initiated at the blue feeder than any other feeder. Overall, the total number of chases went up each day, excluding day 4, during which number of chases dropped from 51 on day 3 (544 visits) to 16 chases on day 4 (612 visits).

Figure 9, which represents the proportion of aggressors by species, reveals that brown Inca was responsible for the majority of chases. Only 5 species were aggressors, compared to 6 species during week 1. Interestingly, while the violet-tailed sylph was the aggressor in most territorial interactions during the first week of observation, it was not involved in a single chase during the second week, though it made 18 visits in 2 foraging bouts to the blue feeder with higher sugar concentration on day 2. In the sylph's absence, one brown Inca became the dominant individual over the feeders. While only 23% of the total visits during week 2 of data collection were to the blue feeder (high concentration) at position B, 44% of the total chases during that period were initiated at that same feeder. 96.3% of those chases involved the brown Inca as the aggressor. Strangely, the brown Inca only visited the blue feeder at position B 7 times during the second week, making up 1.03% of the visits (n = 674) made by that species during that period.

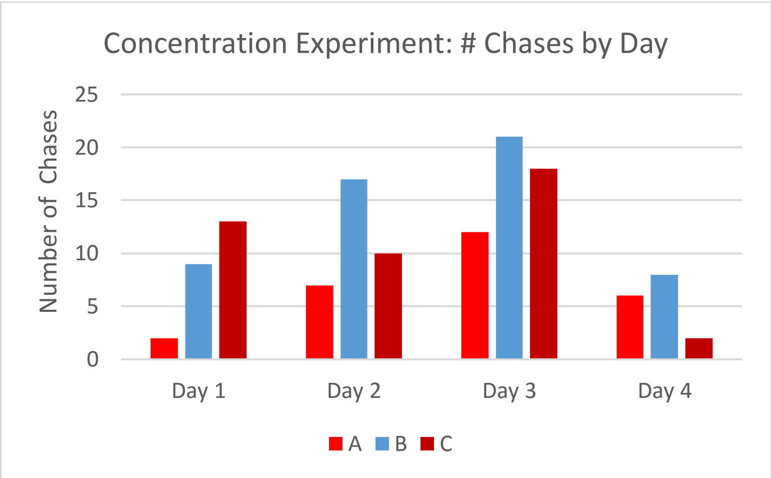

Figure 8. Number of chases during concentration experiment show the highest number of chases from position B, the high concentration feeder. 27 took place at position A (red), making 21.6% of the total chases (n = 126). 55 chases, 96% of which involved the Brown Inca as the aggressor, took place at position B, making 44.0% of the total chases. 43 chases were initiated at position C, making 34.4% of the total chases during that period.

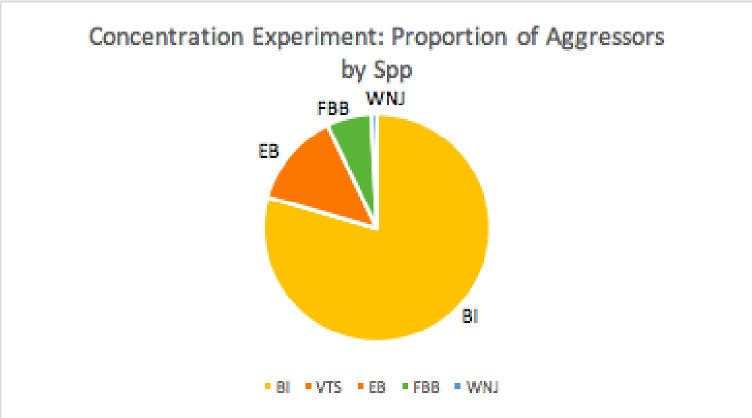

Figure 9. Proportion of total chases by aggressor during the concentration experiment indicates that Brown Inca initiated the majority of aggressive interactions. The brown Inca (BI) chased 100 intruders (79.4% of total, n = 126). Female empress brilliant (EB) chased 17 individuals (13.5% of total). Female fawn-breasted brilliant (FBB) chased 8 individuals (6.35%). Brown Inca chased 22 (7.8%). White-necked Jacobin chased 1 (0.79%) out of the total chases.

Breakdown by Species

<u>Violet-tailed sylph</u> (*Aglaiocercus coelestis*)

Examining visitation frequency by species, the violet-tailed sylph did not show preference for one color over another, but 76.5% of the sylph's visits were to position A. Two way analysis of variance indicated that there was a significant main effect for position (F (2, 4) = 7.09, $p = 0.0485 < 0.05$). Post hoc two-sample t-tests using the Bonferroni correction indicated that visitation to position A was significantly greater than that to position C ($p = 0.0128 < 0.0167$). The visits by violet-tailed sylph made up 11.0% of the total hummingbird visits during the color experiment (week 1). Only male violet-tailed sylphs were observed, with the exception of three visits by a female sylph in which she came to the feeder patch but was almost immediately chased away.

In terms of behavior, the violet-tailed sylph was observed to be the most aggressive species, recorded as the aggressor in 46.1% of the total chases in the first week before concentration was altered. One dominant male sylph, identified as having a tail shorter than most and a white spot on his shoulder, tended to protect the feeder in position A, regardless of the color of the feeder. The sylph was the aggressor in 52 of 66 (79%) chases from position C. The dominant sylph was often seen perched above the feeder in position A on a low branch or would perch for several minutes at a time resting on the rope just above the feeder at position A. Violet-tailed sylph were observed chasing away 130 individuals in the 36 hours done in the first week of observation, 52 of those chases taking place at position A. This species was the only to somewhat consistently ram other individuals, meaning that it flies full speed into the intruder, with an estimated 15% of violet-tailed sylph territorial interactions involving ramming. On several occasions, the sylph attempted to chase off an empress brilliant female that refused to leave, in which case he dove beneath her as she perched and flew directly up at her to stab her in the stomach with his beak. During each episode, that action was repeated five or six times until she would leave. There were also numerous occasions during which the violet-tailed sylph would attempt to chase extremely persistent intruders that would ignore its efforts. In those cases, the sylph often perched alongside the feeder, seemingly resting, for one or two minutes before once again beginning to drive the intruder away.

Empress brilliant (*Heliodoxa imperatrix*)

Empress brilliants also showed strong preference for position C, as indicated by the two way analysis of variance (F (2, 4) = 19.72, $p = 0.0085 < 0.05$). 78.5% of total visits made by empress brilliants during the color preference experiment were made to the feeder in position C regardless of color. Post hoc two-sample t-tests using the Bonferroni correction showed that visitation to position C was significantly greater than both that to position A ($p = 0.0059 < 0.0167$) and position B ($p = 0.0027 < 0.0167$). The total visits by all species was comprised of 29.3% visits by empress brilliant. Only female empress brilliants were observed on the feeders. Empress brilliant exhibited the second most frequent aggressive territorial behavior, initiating 24.1% of the total chases in the color preference experiment (week 1). Their preference for visiting position C was reflected in the fact that empress brilliants were the aggressors in 32% of chases from feeders at position C during the three position rotations.

In examining territoriality as expressed in the empress brilliant, this species chased away 68 intruders in the 36 hours done in the first week of observation, 52 of those chases taking place at position C. While at least five or six females of this species were observed visiting the feeders, one dominant empress brilliant, identified by a very small white patch on her chest and a white spot where her wing met her chest. There were instances in which an empress brilliant was observed chasing an intruder but did not perch and thus could not be identified. However, the dominant empress brilliant was identified and recorded as the aggressor in the majority of the chases by empress brilliant at position C (71.1%, n = 52). The empress brilliants were often observed glancing around, perching and chirping, preening, resting, puffing their feathers, and sticking their tongue out. On average, the empress brilliant chirped 15 times during each perch, though some perches involved very short visits and no chirping. They were also observed foraging in the orchid garden on Columnea picta and several flowering bromeliad species, Heliconia burleana and Heliconia impudica, all of which have bright red attractants.

Fawn-breasted brilliant (*Heliodoxa rubinoides*)

The two-way analysis of variance for the effect of position and color on visitation by fawn-breasted brilliant to the different feeders showed no statistically significant preference for either position ($p = 0.078 > 0.05$) or color ($p = 0.615 > 0.05$), though 68.7% of visits made by the species were to position C.

In terms of behavior, fawn-breasted brilliant, of which only females were observed at the feeders, were aggressors in 17.0% of the chases during the first week of observation. 40 out of 48 interactions in which fawn-breasted brilliant chased away other hummingbirds during the first week of observation took place at position C. Fawn-breasted brilliant were also observed glancing around, perching and chirping (though much less frequently than empress brilliant), sticking their tongue out, puffing their feathers, and resting. During the concentration experiment of the second week of data collection, fawn-breasted brilliants were aggressors much fewer of the total chases, only 8 out of 126 chases. Fawn-breasted brilliant was the species that was most responsive to concentration change during the second week. Across the four days of observation, visitation to the blue feeder was as follows: day 1- 33; day 2- 76; day 3- 54; and day 4- 90 (Appendix, Table B).

Brown Inca (*Coeligena wilsoni*)

Brown Inca showed strong preference for position C, which made up 73.5% of total visits made by the species. The two way analysis of variance for the effect of position and color on visitation by brown Inca pointed to significant main effect for position, yielding an F ratio of $F(2, 4) = 25.1$, $p = 0.0055 < 0.05$. Post-hoc two-sample t-tests that took into account the Bonferroni correction signaled that visitation by brown Inca to position C (sum = 567) was significantly greater than visitation to position A ($p = 0.0123 < 0.0167$, sum = 147) and therefore to position B, which received even fewer visits by brown Inca during the color experiment in the first week of observation (sum = 68).

In terms of foraging behavior, during the color experiment in the first week of data collection, brown Inca were among the more subordinate species and rarely perched at feeders, but rather hovered and visited an average of 3 times per foraging bout. While not at the feeders, brown Inca were often seen foraging on Guzmania amplectens (Appendix, Figure D) located in the orchid garden. While brown Inca did occasionally chase, they were only responsible for 22 of the 282 total chases during week 1. Additionally, they very rarely attempted to chase birds that appeared to be more dominant such as the empress brilliant. Instead, during the first week, brown Inca largely chased away tawny-bellied hermit, white-necked Jacobin, and fawn-breasted brilliant.

During the second week of observation, beginning with the concentration experiment, one brown Inca of unidentified sex asserted itself as the dominant individual over all of the feeders. It was identified as one individual because it always returned to the same low hanging branch to perch after each chase and after many of its foraging bouts. This individual was the aggressor in 79.4% of all chases during the second week (n = 126). The dominant brown Inca was often seen perched on its favorite branch, typically vocalizing with long bursts of quick chirps that would last up to two minutes. While during week 1 brown Inca almost never perched at or above the feeder it was visiting, during week 2 that became a common behavior. While only 23% of the total visits during week 2 were made to the blue feeder (high concentration) at position B, 44% of the total chases (n = 126) during that period were initiated at that same feeder. 96.3% of those chases involved the brown Inca as the aggressor. Interestingly, the brown Inca only visited blue/position B 7 times during the second week, making up 1.03% of the visits it made during that period.

<u>Tawny-bellied hermit</u> (*Phaethornis syrmatophorus*)

The two-way analysis of variance for the effect of color and position on visitation by tawny-bellied hermit yielded no significant main effect for neither position ($p = 0.277 > 0.05$) nor color ($p = 0.378 > 0.05$). The most visits by the tawny-bellied hermit were to the orange feeder when in position C (16.7% of all visits by that species), with 46% of visits to orange when sorted by color and 49% of visits to position C when sorted by position.

With respect to foraging behavior, the tawny-bellied hermit rarely perched during foraging bouts. While it did sometimes perch on the rope above the feeder it was visiting, though rarely, it never perched on the feeder itself while drinking. When they did perch, the hermits wagged their tails up and down, seemingly for balance. They often hovered between visits directly by or above the feeder, drinking from the feeder an estimated 7 times on average during each foraging bout, sometimes up to 17 times in one bout. Tawny-bellied hermits were rarely recorded as the aggressors in any interactions with other hummingbirds, though on several occasions it appeared that they unintentionally scared away other individuals and would themselves also fly away before returning to drink a few seconds later. At least two tawny-bellied hermits were present on the feeders, as on one occasion two were seen at the same time at the feeder in position C.

White-necked Jacobin (*Florisuga mellivora*)

White-necked Jacobin were shown to have no preference for position or color. Two-way analysis of variance yielded no main effect for position ($p = 0.383 > 0.05$) or color ($p = 0.465 > 0.05$). While it may not be statistically significant so as to say that white-necked Jacobins showed preference, 54.5% of its visits were made to the red feeder throughout all position rotations (n = 692).

The white-necked Jacobin was not observed to be a particularly aggressive species, of which only males were observed at the feeders. In week 1, white-necked Jacobins chased 11 times, only from the feeder at position C. 5 of those interactions involved chasing members of the same species. It also chased brown Inca, fawn-breasted brilliant, and on one occasion a female empress brilliant. Then, during the second week of observation, a white-necked Jacobin was observed to be the aggressor in only one interaction, chasing a booted racket tail from the red feeder at position A.

Booted racket tail (*Creates underwood*)

The booted racket tail made the least visits out of all the species observed on the feeders, with a total of 49 visits during the first week. That species made up 0.76% of the total visits across feeders. They did not begin visiting until the end of the first week. The booted racket tail females demonstrated preference towards red (65.3% of visits) and position A (63.3% of visits) during week 1. The two-way analysis of variance conducted to look for the effect of color and position on number of visits by booted racket tail produced p-values that indicated significant main effect for color ($p < 0.05$) and not for position ($p > 0.05$). During the concentration experiment, booted racket tails made a total of 88 visits, 79 (89.8%) of which were to the red feeder at position A. Two-way analysis of variance indicated significant difference ($F(2, 9) = 32.65$, $p < 0.001$). Post-hoc tests showed that there were more overall visits by booted racket tail to the red feeder at position A than either of the other feeders ($p < 0.016$). The only visits made to the feeders at positions B and C were on day 4. Booted racket tail visits made up 3.64% of all visits during the concentration experiment.

In terms of behavior, the female booted racket tails seen on the feeders were the most subordinate visiting species. They were never observed to be the aggressor in a chase. During the color experiment, they were chased four times, only from the red feeder. During the concentration experiment, the booted racket tail was chased away three times, all from the red feeder at position A. They were chased by a brown Inca, a white-necked Jacobin, and a fawn-breasted brilliant. Due to their extremely small body size, the booted racket tails were able to perch on the feeders themselves, meaning that they tended to sit for several seconds and drink several times without leaving the feeder. When did they did perch near the feeder, it was usually on a small branch or leaf rather than on the rope just above the feeder.

Discussion

Color Preference

While there was preference for the color red during the first experiment, as demonstrated by the fact that the proportion of visits to each position was highest when the red feeder was in place, color was not the factor with the most influence on visitation frequency. Somewhat inconsistent color preference for the color red was demonstrated by the total number of visits across species. It is clearly represented in figures 1-3 that visitation is not severely but is somewhat impacted by color. However, serving as a secondary factor, color influenced the effect of position on visitation frequency.

Examination of visitation to position A, B, or C will yield the conclusion that visits were higher to each position when red was present. Position C, the most popular received 71.8% of visits during that rotation when red, while it received 55.7% and 54.8% when blue and orange were present. The same can be said for visitation at the least preferred position B: the red feeder received 17.3% of the total visits during that rotation, and the blue and orange feeders both received 10.3% and 9.2% of total visits during their respective rotations. Once a food source has been located, color may then act as an important discriminator stimulus, but there is no evidence from this or other studies that hummingbirds have an innate or strongly developed preference for one color, including red. Broken down by species, only female booted racket tail and male white-necked Jacobin showed significant preference for red, 54.5% and 65.3% of their total visits made to red, respectively. One explanation for this phenomenon is that booted racket tail and white-necked Jacobin, both subordinate species that appeared to pose few threats to the territory of the dominant individuals, may have been able to fly under the radar, so to speak, of those dominant individuals. In that way, their low rank in the dominance hierarchy may have granted them more freedom to visit feeder as they pleased

Despite persistence of the belief that hummingbirds show an innate preference for the color red, experimental evidence from numerous studies reject that belief and prove that color preference is learned. While in the current study, there was slight initial preference demonstrated for red, it cannot be concluded that it was the result of innate preference, but rather that it was the result of a learned association by the hummingbirds between the color red and high-reward nectar sources in their surrounding environment. It should also be noted that, throughout the year, red hummingbird feeders are set up directly outside the Santa Lucia lodge, providing a seemingly unlimited supply of nectar for the hummingbirds that learn to visit. Therefore, while there was not an overwhelming abundance of red flowers in the forest during the investigation, hummingbirds that recognized the feeders as food sources likely had a previously learned association between high nectar reward and red coloration. During a season in which flower populations are much more abundant, one might expect the presentation of color preference in favor of the most abundant flower color when the investigation is conducted.

Position Preference

Position appears to have greater impact on visitation than does the color of feeder. Looking at the results from total visits during the color experiment, a clear preference for position C is shown. Regardless of the color of feeder present, position C received the most visits during each of the three color rotations. Therefore, while one could point out that position also has an impact on the effect of color on visitation frequency, it seems apt to conclude that position is the primary factor in determining visitation frequency. When broken down by species, the visitation results paint a slightly different picture. Violet-tailed sylph was partial to position A, demonstrating a statistically significant preference ($p < 0.0167$). Position B was closest to the self-guided trail and also the lowest to the ground due to the slope of the hill. Position A was furthest in the direction from which the violet-tailed sylph tended to enter the feeder patch, which may suggest that his potentially pre-existing territory included only that feeder. Position C was furthest from the self-guided trail and had a large epiphyte in the tree above it, the leaves of which may have provided more cover for visiting hummingbirds. Position C's location further from the trail on which tourists sometimes walked past may have provided more appealing escape routes in the event of an attack by an aggressive hummingbird or predator. While the differences between positions did not seem significant to the observer, perhaps those differences appear much more dramatic in the energetic calculations of the hummingbirds as they assess each feeder.

One study on the impact of color and position of nectar sources on the feeding patterns of hummingbirds found similar results, suggesting a hierarchy of factors affecting hummingbird foraging choice. This study, performed under natural conditions in the field, demonstrated that the use of equally profitable feeders in arrays is more strongly influenced by position than by color (Miller *et al.*, 1984). Stiles found that, in laboratory experiments exploring color preference of hummingbirds, the birds would often visit the feeder in the position of the previous day's preferred solution for the first hour or two before visiting the other feeders and perhaps changing their preference (1976). Those findings suggest that there is increased latency in response to change in position under natural conditions, likely due to factors such as territoriality as observed in the current investigation. The same study on the impact of color and position on foraging habits came to the conclusion that hummingbirds use appropriate color cues, which depend on the time and location of foraging, in the initial search for floral nectar and, after finding a profitable source, then use spatial cues for subsequent visits (Miller *et al.*, 1984). While those results are very interesting and suggest further implications for this study, the presence of significant territorial behavior surrounding the feeders means that such conclusions cannot be assumed from the results of this study; since each hummingbird was not isolated and thus was under pressure from uncontrollable variables like aggression from other individuals, there is no way to be certain whether position is truly what attracted them to the feeder or whether they were simply driven from the other positions.

Miller *et al.* (1984) also found that in laboratory experiments, although hummingbirds learn quickly to return to a single profitable feeder in a linear array of unprofitable ones if marked by a consistent color cue, their learning is strongly influenced by position in the absence of color cues. Such a conclusion leads to the concept that the spatial memory of hummingbirds may be limited in resolution and that they may use visible landmarks to improve their performance. That is consistent with Miller's hypothesis that landmarks facilitate a foraging individual's limited ability to learn and remember profitable locations in their environment.

Unlike the majority of studies that examined the influence of color and position on hummingbird foraging habits, most of which took place in a controlled laboratory environment, this investigation was done under natural conditions with hummingbirds that could not be isolated. It is evident that territoriality played a role in determining position preference, and perhaps in deterring visitation that may have indicated color preference, seeing as visitation increased considerably to the red feeder in position A in the absence of the dominant male violet-tailed sylph during the second week of data collection. Between day 1 and day 2 of the concentration experiment, the proportion of total visits for each day to position A increased from 22.9% (n = 576) to 41.5% (n = 687). After the first day, the number of visits to position A was always greater (though not significantly so) than that to either position B or C. The largest notable factor that changed between the first and second week was the disappearance of the territorial sylph, and that is very likely what explains such a shift in position preference, seeing as both the feeder at position A and C had the same color and nectar concentration.

Concentration Preference

While there was not a great enough difference in visitation to the feeders over the four days to conclude that there was certainly a preference for the blue feeder in position B with higher (40%) concentration, it appears that preference was in the process of shifting from the red feeders to the blue. On the final day of observation (day 4 of concentration experiment), each feeder received a relatively equal number of visits (A/Red = 232; B/Blue = 180; C/Red = 200). Compared to day 1, during which 61.5% of the visits (n = 576) were made to position C, that is a substantial shift. It is also important to take into account the number of chases were higher at position B throughout the four days, even though the number of visits remained lower than hypothesized. That finding suggests that, since the number of visits per day to the blue feeder increased consistently, that increase might continue until a clear preference was shown for the high concentration feeder. However, such an assumption does ignore the limiting impact that territoriality had on visitation frequency to the blue feeder. Stiles (1976) found that hummingbird taste preferences quickly override color preferences. That study focused not on concentration, but on type of sugar, using sucrose and glucose to determine a preference for one or the other. It was determined that all of the hummingbirds in the experiment eventually chose the preferred sugar regardless of the color of feeder, 80% of those choices occurring on the first day (Stiles, 1976). No hummingbird required more than 6 trials to make the requisite 3 consecutive choices for the preferred sugar type (Stiles, 1976), which in comparing to the results of this study in which a clear preference was not shown over four days, might suggest that sugar type has a greater influence on feeder preference than does concentration of sugar. However, it would be necessary to conduct the concentration study within a controlled laboratory environment, as was said taste experiment.

Energetics and optimal foraging theory come into play in analyzing the results of the concentration experiment. With territoriality being a clear factor in altering visitation frequencies at the feeders, the visiting hummingbirds faced a risk of being chased away. Chasing not only prevents the gain of energy from feeding, but also causes the intruder to spend more energy to avoid being charged or rammed. Therefore, the hummingbirds had to weigh the reward gained by visiting one of the feeders, especially those that were more protected by a dominant territorial individual like the violet-tailed sylph and the brown Inca. Experimental investigations of color preference or color discrimination among hummingbirds have demonstrated that individuals are able to quickly make associations and learn the correct choices of colors for nectar rewards in arrays of artificial feeders (e.g. Miller & Miller, 1971; Goldsmith & Goldsmith, 1979). However, throwing territorialists like the violet-tailed sylph and brown Inca into the mix make the balance of energy much more complicated. It makes sense that, during the initial days in which the concentration was at 40% in the blue feeder, there were fewer visits. Perhaps the majority did not realize the change in concentration at first and did not consider the risk of visiting that feeder worth a nectar concentration equal to that of the other feeders.

Out of 101 foraging bouts in which hummingbirds approached the blue feeder to visit during the concentration experiment, they were chased from the feeder 55 times. 18 of those chases prevented the individual from making any visits to the feeder, and during the majority of foraging bouts during which a hummingbird was chased away, it appeared that they were planning to visit at least once more but were deterred by the aggressor. Therefore, the assumption can be made that in the absence of a territorial individual, more visits would have been made to the high concentration feeder.

The latency of increased visitation to the high concentration blue feeder at position B provides further confirmation of the result that the combination of the blue feeder and position B is the least preferred among the rotations. Because there was no phase in which all feeders were identical in terms of color with the high concentration in position B, it is not certain whether hesitation to visit was due to distaste for the color or the position, though it was likely the latter. However, as the least preference was shown for the color blue and position B during the first part of the study, it can be concluded that the increase in visitation to that feeder is solely based on preference for the higher concentration. It should also be noted that, during the three weeks in which the feeders were set up in the orchid garden, the number of visiting hummingbirds on the feeders directly next to the Santa Lucia lodge dropped substantially, to the point where only two or three individuals would be seen in an hour. The Santa Lucia feeders are usually filled with nectar of 15% sugar concentration, while the feeders for this investigation were filled with 25% and 40% for the duration of the experiment. Therefore, it is entirely likely that the higher nectar concentration drew the birds at the lodge to the orchid garden, some 300 meters away.

The impact of concentration, position, and color preference among hummingbirds on foraging habit has interesting implications for plant evolutionary response. Given that position, or the spatial relationship between feeders, strongly influenced the probability of an individual returning to a feeder, hummingbirds learn and remember the location of profitable food sources and return to them preferentially, as long as the reward remains sufficient. Spatial relationships among flowers within inflorescences influence how well hummingbirds avoid revisiting empty flowers (Hainsworth et al., 1983). The results of this current study support that concept, additionally presenting the possibility that relevant cues such as color are important for spatial learning at the relatively specific level of choices among feeders.

Territorial Behaviors

The indiscriminate overt aggression displayed largely by one male violet-tailed sylph, one brown Inca, and one female empress brilliant around the feeders is consistent with the findings of several studies that observed behaviors of males of territorial species (Pitelka, 1942; Powers & McKee, 1994). The majority of studies thus far on territoriality in hummingbirds have focused on males of territorial species, but some have observed female territoriality during breeding seasons around nesting sites (Pitelka, 1942). Territoriality in hummingbirds can aggressively reduce the occurrence of other species at their food source, in this case the feeders (McNally & Timewell, 2005). The presence of territorial behavior surrounding the feeders is interesting, in that it suggests that there could potentially be a decline in naturally-occurring food availability, as territorial behavior is only adaptive when the benefits of exclusive use of an area or other resource exceed the cost of defense (Powers *et al*., 1994). Low food availability would be consistent with the qualitative observation that floral populations in the orchid garden and the surrounding forest were in decline due to seasonality combined with little to no rainfall for approximately ten days prior to the initiation of the study. A 300 meter transect was completed before data collection began with the initial intent of comparing color preference to the abundance of flower color in the surrounding environment, but the numbers were so low that a clear connection could not have been made. 2000 *Besleria solenoides* flowers were counted in 300 meters, which is extremely low compared to when they are in season according to Holger Beck, the main advisor on the project. However, the finding that there were low numbers of flowers during the research period pertains to this concept of increased aggression with low food availability.

In connection to low resource availability, several studies have shown that as energy availability decreases, the tactics used for defense change dramatically and energetically inexpensive tactics are used and the owner spends less time defending the territory (Ewald *et al.*, 1978; Powers *et al.,* 1994). It seems that there may be a complex balance pertaining to territorial defense, which pushes towards more aggressive defensive behavior while high quality resources are abundant, but also pulls in the direction of conservative territorial behavior when resources are low, while still allowing the territorial individual to fight for its food source. In other words, the territorial individual must find a balance, largely weighed by energetic gain and cost, between "weakening" its defensive tactics without totally giving up possession of the food source it seeks to defend. At a certain point, of course, the energy gained from the food source is less than or equal to the energy spent even on minimal territorial behavior, and they must move on.

On several occasions, the dominant individuals were observed perching for several minutes alongside their preferred feeder without visiting. Individuals of other species, such as fawn-breasted brilliant and tawny-bellied hermit, would sometimes attempt to do the same, but were in most cases chased away after at most one minute of perching. As individuals other than the dominant violet-tailed sylph, brown Inca, and empress brilliant were not identified on the individual level, it is unclear whether one individual of the other species visited more frequently than the other of its same species or whether the distribution of visits was relatively even across the more subordinate species. Previous studies have found that these long perches are used for two purposes, which include crop emptying, where the hummingbird conserves energy and digests the nectar it recently consumed, and guarding its territory by through occupation (Feinsinger & Chaplin, 1975; Diamond *et al.*, 1986).

Energetic Costs of Territoriality

One thing that is especially interesting to examine is the comparison between the energy spent by territorial birds protecting their territory and the energetic benefit they receive by feeding at the food source they protect. There was one male violet-tailed sylph that protected the feeder at position A, regardless of color, throughout the first week of observation. The sylph chased away 130 total individuals during the three rotations of the color experiment, and violet-tailed sylphs were observed feeding at the feeders 450 times during the periods in which the observer was present. Camera trap data was disregarded for this part of the territoriality analysis, as chases could not be consistently observed using the cameras and could only be compared with the number of visits made during the period in which the observer was present. 52 (40%) of the chases and 319 (71%) of the visits by the male violet-tailed sylph were observed at position A. It was expected before the investigation began that a greater proportion of chases from the dominant individual's preferred feeder, representing energy spent protecting its food source, to be closer to that of the number of visits to that feeder, representing the energy gained from the food source. Interestingly, the sylph chased 34 individuals away from the feeder placed in position B, but those 34 chases only occurred when the feeder in that position was red (rotation 1 of color experiment). Therefore, while the sylph did not show statistically significant preference for red out of the overall visits, it seemed more set on protecting feeders when red was present, suggesting an association with higher reward. It is possible that the sylph associated the color red with a higher food reward, perhaps based on the flowers in its surrounding environment, and assumed that visiting that least preferred position (B) would be worth the energetic benefit of feeding from a red, higher-reward nectar source. However, an alternative possible explanation is that the hummingbirds began visiting the feeders somewhat evenly, but over time other visiting species showed lower preference for position B, and therefore the sylph had no reason to chase intruders from the feeder in that position.

During the concentration experiment, when the violet-tailed sylph had disappeared for unknown reasons, the brown Inca was crowned the dominant territorial individual in the feeder patch. Overall, the brown Inca chased away 100 individuals over the four days, making it the aggressor in 79.4% of chases during that period, and made 674 collective visits to all of the feeders. While only 23% of the total visits during week 2 were made to the blue feeder (high concentration), 44% (52) of the total chases during that period were initiated at that feeder. The brown Inca was the aggressor in 96.3% of those chases from blue. In an extremely unexpected outcome, the brown Inca only visited that blue feeder 7 times during the entire four days, making up 1.03% of its total visits. Looking at those findings from the perspective of optimal foraging theory, it does not make much sense for the brown Inca to expend such a vast amount of energy to protect a food resource from which it gains very little energy. It is possible that the brown Inca indiscriminately protected the feeders within the patch simply because they were within his territory. Rather than thinking of each feeder as a separate territory in this context, it is likely that the brown Inca considered the entire feeder patch itself to be part of its larger territory in the surrounding forest of the orchid garden. Brown Inca was observed frequenting *Guzmania amplectens* (Appendix, Figure D) located nearby the feeders, and on two separate occasions chased a female booted racket tail and a female empress brilliant from that same flower. However, even if that accounts for the high number of chases, it is still difficult to explain why the brown Inca would not visit a higher-reward nectar source unless the concentration was so high that the species cannot efficiently metabolize it. While it would make sense for the brown Inca to spend more energy defending the feeders that it actually visited, it is likely that it was forced to chase more from blue simply because the number of individuals attempting to visit the blue feeder increased throughout the experiment.

Interestingly, a greater number of territorial interactions would be expected when the food resource is limited, which was not the case during this investigation. When using feeders, nectar is "unlimited" in a sense, and therefore one would expect the tensions of competition to be eased. There were several occasions during which the dominant individual perched alongside an intruder and allowed it to visit the feeder, sometimes for several minutes without chasing it away. That likely would not have occurred had the food source been limited. With territorialists such as the violet-tailed sylph and brown Inca, which were observed to be the dominant species during their respective periods, it would then make sense to follow with the assumption that in conditions in which the nectar was limited in the feeders, perhaps refilled less frequently, the number of aggressive interactions would increase.

Camfield found that more energetically expensive behaviors were used to defend nectar sources with higher rewards (2006). It would be interesting to see if, were the concentration experiment continued and the blue feeder left with a higher concentration for a longer duration, the defensive tactics used by the dominant individuals would be more energetically expensive. Alternatively, as 25% sugar concentration is already relatively high (the Santa Lucia feeders are normally filled with 15% nectar), dropping the concentration significantly may result in reduced chasing and ramming and instead more inconspicuous territorial behavior such as perching above the feeder and chirping. Camfield found that at feeders with higher concentrations, chases were longest and were more often supplemented by chirps and hovering displays than at feeders with lower concentrations (Camfield, 2006). It is unclear whether the duration of chases increased with reward because the intruders were more persistent, as seen in this investigation with the tawny-bellied hermit and white Jacobin, or because the dominant individuals were more aggressive in their defense of high-reward nectar sources. In any case, those results support the idea that intensity of defensive tactics reflect resource quality (Camfield, 2006; Grether, 2016). In the same study, it was discovered that the number of intruding hummingbirds was positively correlated to territory quality, largely determined by nectar concentration (Camfield, 2006). Camfield's results are consistent with those found during the concentration experiment, as the number of visits to the blue feeder (high concentration) increased consistently over the four days even though the most chases consistently took place at that same feeder. While quality of nectar source clearly plays a role in territoriality, the question still remains whether a reduced quantity of nectar at the same concentration might cause an increase in territorial behavior. As discussed earlier, feeders supply essentially unlimited access to nectar. Using smaller feeders or simply filling identical feeders with less nectar might heighten competition as well.

There appeared to be a learning curve among the individuals of the subordinate species, which tended to be chased away by the majority of other species. Several of the species such as tawny-bellied hermit, booted racket tail, and white-necked Jacobin showed up relatively late to the feeders. The first tawny-bellied hermit arrived on day 5 of the color experiment, while the booted racket tail arrived on day 6. Initially, the new arrivals went directly to the red feeder, located at position B, and made several attempts to visit. They were immediately chased away by the violet-tailed sylph, and this repeated three or four times before they began visiting the feeder at position C. This relates to optimal foraging theory, which predicts that an individual will maximize their foraging efficiency by balancing the energy spent to locate and access a food source and the energy gained by feeding from that source (Pyke, 1978). According to the marginal value theorem, a hummingbird will forage in a patch until the rate of energy gain is less than or equal to the potential energy gain from other patches within that habitat (Pyke, 1978). That theorem pertains to the experiments on color preference as well as concentration preference. Hummingbirds are especially sensitive to energetic costs, as their near-constant movement and high metabolism require that they spend at least 20% of their waking hours feeding, which for some species means feeding for several minutes 14-18 times per hour, with rest periods spent "crop-emptying," or digesting (Diamond *et al.*, 1986). Defending oneself from an attacker not only distracts a hummingbird from its feeding, making the foraging time longer than necessary, but it also requires more energy to change direction or flee to avoid being rammed into. After being chased, in order to risk another aggressive interaction while revisiting the feeder, they must exercise even more caution which may also distract from nectar extraction. This could also apply to the feeder patch as a whole. While chases in the surrounding environment outside of the feeders were not recorded, a qualitative estimate would suggest that fewer chases occurred away from the feeders. Therefore, a hummingbird, especially of an individual of a species more prone to being chased, may view the feeder patch as a food source of which the benefit of feeding does not outweigh the increased risk of being chased. As such, territoriality likely had a major impact on visitation frequencies to the various feeders.

Dominance Hierarchy

As has been previously alluded to, a dominance hierarchy took form around the three feeders. At the top of the pyramid sat the male violet-tailed sylph. He indiscriminately chased away individuals from all species. The one dominant female empress brilliant with the white spot on her wing chased away individuals of all species except for violet-tailed sylphs. Empress brilliant is the largest of the visiting species in terms of body size, though the sylph's extremely long tail likely adds an element of visual intimidation. She seemed to be especially preoccupied with chasing away members of her own species as well as fawn-breasted brilliant, which is just below empress brilliant in the hierarchy of aggressive behavior. While the dominant empress brilliant was the aggressor in most of the chases surrounding position C during week 1, other empress brilliants were also involved in aggressive interactions. However, those more subordinate empress brilliants tended to be more lenient when it came to subordinate species, sometimes perching above the feeder to passively watch another individual feed before going to visit themselves. The fawn-breasted brilliant initially appeared to be superior to the brown Inca in terms of aggression. They not only chased members of their own species and those below in the hierarchy, including white-necked Jacobin, tawny-bellied hermit, and booted racket tail, but also chased away empress brilliants several times, though in many of those cases the empress brilliant came back to retaliate and ram the fawn-breasted brilliant. During the first week, in the presence of the violet-tailed sylph, the brown Inca remained relatively passive and chased very few times, chasing individuals of many species but also being chased itself by fawn-breasted, white-necked Jacobin, and other brown Inca. However, in the absence of the violet-tailed sylph during the second week of data collection, the brown Inca seized possession of the now open territory and began indiscriminately chasing all species other than the sylph, which only made two foraging bouts to the feeders during that period. MacNally & Timewell (2005) demonstrate the presence of a dominance hierarchy among heterospecific nectarivores that describes larger species defending the best flowering areas, and smaller or subordinate species occupy lower quality nectar sources. The lack of aggression of the booted racket tail fits beautifully into that model of dominance as its size is significantly smaller than that of all other visiting species. Similar findings were found in a separate SIT Independent Study Project performed at Santa Lucia in the fall of 2016, indicating that booted racket tails were the subordinate species while violet-tailed sylphs displayed the most aggressive territorial behavior (Grether, 2016). Many of the chasing interactions were intraspecific, which could be explained by a lower risk or better use of energy than defending from heterospecifics (Noble, 2010).

Interestingly, there were a few species to which the sylph reacted less aggressively, and on a few occasions, did not react at all as they feed at its feeder in its presence. Among these species were the tawny-bellied hermit and white-necked Jacobin, which proved to be surprisingly persistent hummingbirds for their place in the dominance hierarchy. On four occasions, the violet-tailed sylph was witnessed attempting to chase a tawny-bellied hermit from the feeder at position A, but the hermit would fly a few feet away, hover, and then return immediately to the feeder. After two or three repeats of this, the sylph would give up, remain perched and simply watch the hermit visit. It is possible that, since those generally subordinate species were so low in the hierarchy that the violet-tailed sylph simply felt no real threat by their presence. Perhaps one of the risks of allowing an intruder to visit its preferred feeder, its territory, is not so much that the nectar will be depleted, but rather a risk of losing that territory to another individual. Perhaps chasing, as much as it is a means for protecting a food source, is also an effective method for asserting one's dominance over a territory.

Limitations of the Study

While having an observer present was necessary for recording and quantifying all territorial behaviors, an integral part of the study, human presence very well may have altered the behavior of the visiting hummingbirds. The observation perch was located approximately 4 meters from the feeder patch and the self-guided trail on which tourists would occasionally walk past, was located about 2 meters from position C (Appendix, Figure C). Reflecting on the methods of the study, it perhaps would have been better to have an initial phase of the concentration experiment in which only red feeders were set up, with the same configuration of 25% in position A, 40% in position B, and 25% in position C. In that case, it could be confirmed whether or not the number of visits did not increase significantly in the four days due to color or due to the influence of territoriality or some other undetected factor. As the study was conducted under natural conditions in the field, there are several variables that could not be controlled that ended up limiting the success of the study. As discussed earlier, territorial behavior by several individuals influenced the visitation frequencies that might otherwise have indicated a color preference among some of the species. For example, had the violet-tailed sylph not chased "intruders" from position A regardless of the color of the feeder, it is possible that some species would have followed the red feeder regardless of position, but were deterred by the sylph's aggression.

Conclusion

The results of the investigation point to a hierarchy of factors that affect foraging habits of hummingbirds. During the initial experiment exploring color preference, position of feeder was the primary factor affecting visitation frequency, likely due to the presence of several dominant territorial individuals of violet-tailed sylph, empress brilliant, and brown Inca. Position C proved to be collectively the most popular feeder, while position A was dominated by a male violet-tailed sylph that chased indiscriminately and seemed to greatly reduce visitation to that feeder by other species. Although it is difficult to come to concrete conclusions regarding hummingbird color preference, due to the disproportionate impact of territoriality on visitation frequency at separate feeders, there appeared to be a preference for red coloration across species. Feeder color influenced the effect that position had on the visitation frequency to each feeder. During all three rotations of the color experiment, visitation was highest at each position in the presence of the red feeder. When foraging, hummingbirds use color in the initial search for nectar sources, and once a profitable source is found, use spatial cues for subsequent visits (Miller *et a*l., 1984).

With respect to higher nectar concentration, there was no clear preference for the high concentration feeder at position B by the end of the four-day experiment, but visitation frequency was on a consistently upward trajectory throughout. The number of aggressive territorial interactions was highest at that feeder, limiting attempts to visit and deterring other individuals from further visits. Energetics play a central role in hummingbird foraging, as their extremely high metabolic activity greatly limits their margin for error in foraging choice. Therefore, the fact that visitation to the high concentration feeder continued to increase despite the high number of chases indicates that the reward was great enough to risk being chased. The hummingbirds followed a loose dominance hierarchy, in which violet-tailed sylphs were superior. In the absence of the dominant sylph, a brown Inca became the main aggressor during the concentration experiment. Given that position strongly influenced the probability of an individual returning to a feeder, hummingbirds learn and remember the location of profitable food sources and return to them preferentially, as long as the reward remains adequate. That limited spatial memory is supplemented by visual signaling cues, such as color, which facilitate attraction of pollinators to flowers (Miller *et al*., 1984). Such tools for efficiently locating high quality nectar sources are an important evolutionary adaptation and may carry implications for hummingbird-pollinated plant response via co-evolution. This study leaves many questions to be answered, and a great deal more is left to be learned about the foraging preferences and behavior of these species as well as the implications for hummingbird foraging choice as a whole.

References

Bene, F. (1941). Experiments on the Color Preference of Black-Chinned Hummingbirds. *The Condor*, 43: 237-323.

Camfield, A. F. (2006). Resource value affects territorial defense by Broad-tailed and Rufous Hummingbirds. J. Field Ornithology, 77(2): 120-125

Campbell, D. R., Waser, N. M., Melendez-Ackerman, E. J. (1997). Analyzing Pollinator-Mediated Selection in a Plant Hybrid Zone: Hummingbird Visitation Patterns on Three Spatial Scales. *The American Naturalist*, 149(2): 295-315.

Climate: Nanegal. Retrieved December 2, 2017, from http://en.climate-data.org/location/180089

Diamond, J. M., Karasov, W. H., Phan, D., Carpenter, F. L. (1986). Digestive physiology is a determinant of foraging bout frequency in hummingbirds. *Nature*, 320 (6057): 62-63. doi:10.1038/320062a0

Ewald, P. W., Carpenter, F. L. (1978). Territorial responses to energy manipulations in the Anna hummingbird. *Oecologia*, 31(3): 277-292.

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.

Feinsinger, P., Colwell, R. K. (1978). Community Organisation Among Neotropical Nectar-Feeding Birds. *Integrative and Comparative Biology*, 4: 779-795.

Fenster, C. B., Cheely, G., Dudash, M. R., Reynolds, R. J. (2006). Nectar Reward and Advertisement in Hummingbird-Pollinated *Silene virginica* (Caryophyllaceae). *American Journal of Botany*, 93 (12).

Goldsmith, T. H. & Goldsmith, K. M. (1981). Discrimination of colors by the black-chinned hummingbird, *Archilochus alexandri*. *Journal of Comparative Physiology*, 130 (3): 209-220. https://link.springer.com/article/10.1007/BF00614607

Grether, B. (2016). Feeding Behaviors and Female Territoriality in Two Species of Tropical Hummingbird. *Independent Study Project (ISP) Collection*. 2473. http://digitalcollections.sit.edu/isp_collection/2473

Hainsworth, F. R., Mercier, T., Wolf, L. L. (1983). Floral arrangements and hummingbird feeding. *Oecologia*, 58(2): 225-229.

Maglianesi, M. A., Bohning-Gaese, K., Schleuning, M. (2015). Different foraging preferences of hummingbirds on artificial and natural flowers reveal mechanisms structuring plant-pollinator interactions. *Journal of Animal Ecology*, 84 (3): 655-664.

McNally, R. M., Timewell, C. A. R. (2005). Resource Availability Controls Bird-Assemblage Composition Through Interspecific Aggression. *American Ornithological Society*, 122 (4): 1097-1111. https://doi.org/10.1642/0004-8038(2005)122[1097:RACBCT]2.0.CO;2

Melendez-Ackerman, E., Campbell D. R. (1993). Adaptive Significance of Flower Color and Inter-Trait Correlations in an *Ipomopsis* Hybrid Zone. *Evolution*, 52 (5): 1293-1303.

Noble, E. (2010). To Defend, Or Not To Defend: That is the Question: The Effect of Resource Quality and Abundance on the Behaviour of a Hummingbird Guild in the Cloud Forests of Ecuador. *University of Leeds*, 1-50.

Pitelka, F. A. (1942). Territoriality and Related Problems in North American Hummingbirds. *The Condor*, 44(5): 189-204.

Powers, D. R., McKee, T. (1994). The Effect of Food Availability on Time and Energy Expenditures of Territorial and Non-Territorial Hummingbirds. *The Condor*, 96(4): 1064-1075.

Pyke, G. H. (1978). Optimal Foraging in Hummingbirds: Testing the Marginal Value Theorem, *Integrative and Comparative Biology*, 18 (4): 739–752, https://doi.org/10.1093/icb/18.4.739

Stiles, G. (1976). Taste Preferences, Color Preferences, and Flower Choice in Hummingbirds. *The Condor*, 78 (1): 10-26.

Sutherland, G. D. (1984). Spatial memory processes in hummingbirds: the influence of energetics and spatial distribution of food resources on foraging behavior. M. Sc. thesis, University of British Columbia, Vancouver, B. C.

Sutherland, G. D., Gass, C. L. (1995). Learning and remembering of spatial patterns by hummingbirds. *Animal Behaviour*, 50 (5): 1273-1286, https://doi.org/10.1016/0003-3472(95)80043-3

Wagner, H. O. (1946). Food and Feeding Habits of Mexican Hummingbirds. *The Wilson Bulletin*, 58 (2): 69-93

Appendix

Figure A. Google Earth image of Santa Lucia's location in Ecuador.

Figure B. Image of observation site. Orange feeder in position A, blue feeder in position B, and red feeder in position C. Photo taken from observation perch.

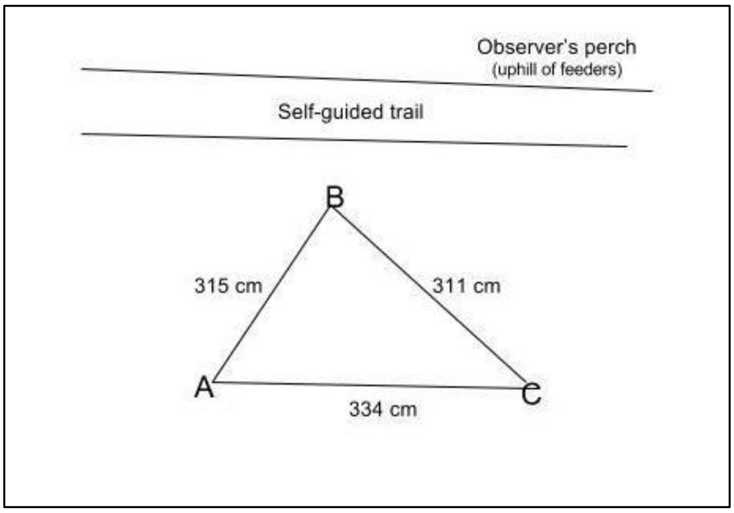

Figure C. Sketch of the observation site. Position A was 3.15 meters from position B and 3.34 meters from position C, while position B and C were 3.11 meters apart. Due to the slope of the hill, the feeders were fixed at different heights from the ground

Figure D. Image of *Guzmania amplectens* with visiting Brown Inca located alongside feeders.

Table A. Total visits for each combination of position and color during the color experiment.

	A	**B**	**C**	**Total for Color**
Red	556	512	1367	2435
Orange	797	175	873	1845
Blue	363	164	1648	2175
Total for Position	1716	851	3888	**Total overall visits:** 6455

Table B. Distribution of visitation to the three different feeders each day of the concentration experiment sorted by species. Day 1 of the concentration experiment corresponds to Day 14 of overall data collection. The feeders at positions A and C were both red and both were filled with nectar of 25% sugar concentration. The feeder at position B was blue and was filled with nectar with a concentration of 40%.

Species	**Days Within Concentration Experiment**	**A**	**B**	**C**
Violet-tailed sylph	1	0	0	0
	2	0	18	0
	3	0	0	0
	4	0	0	0
	Total	0	18	0
Empress Brilliant	1	35	24	155
	2	177	18	137
	3	46	66	84
	4	54	121	53
	Total	258	126	376
Brown Inca	1	27	7	74
	2	118	0	117
	3	77	0	115
	4	109	0	30
	Total	331	7	336
Fawn-breasted brilliant	1	44	33	139
	2	81	76	122
	3	32	54	45
	4	26	90	32
	Total	183	253	338
Tawny-bellied hermit	1	4	37	38
	2	0	33	76
	3	14	38	54

	4	1	6	33
	Total	19	114	201
White-necked Jacobin	1	0	0	0
	2	25	0	16
	3	7	0	12
	4	36	0	5
	Total	68	0	33
Booted racket tail	1	16	0	0
	2	14	0	0
	3	26	0	0
	4	23	5	4
	Total	79	5	4
	Total across species	992	1149	2629