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Trends in Nectar Production and Concentration of Hummingbird-Pollinated Flowers:

An investigation of three flowers of the Ecuadorian Cloud Forest: *Palicourea demissa*, *Mezobromelia capituligera*, and *Kohleria affinis*

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South America, Ecuador, Nanegal, Santa Lucía Cloud Forest Reserve

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Abstract

Nectar concentration and sugar production both impact and are impacted by pollinator activity through a complex system of coevolution. Additionally, a variety of morphological and environmental factors influence the nectar properties of animal-pollinated flowering plants. Nectar concentration and nectar production rate (NPR) of three hummingbird-pollinated plants, *Palicourea demissa*, *Mezobromelia capituligera*, and *Kohleria affinis*, were measured. These flowers occur and were studied in the southern section of the Chocó-Andean subtropical montane rainforest, in the Pichincha province of Ecuador. Flowers were bagged for 24 hours and sampled at approximate two-hour time intervals for 12 or 24 hours. Nectar concentration and sugar production (used interchangeably with NPR) were compared across varying times of the day, number of open flowers per inflorescence (umbel size), and corolla length. Nectar concentration is negatively correlated with time of day for *P. demissa* and *M. capituligera*, and positively correlated with time of day for *K. affinis*. Volume of nectar and sugar production are significantly negatively correlated with time of day for *P. demissa* and *M. capituligera*. *P. demissa* was found to have the highest cumulative sugar production after both 12 and 24 hours than the other two species. Cumulative sugar production after 12 hours is significantly positively correlated with umbel size in *P. demissa* and significantly negatively correlated with umbel size for *M. capituligera*. No significant correlation was found between cumulative 12-hour sugar production and corolla length for any of the three species. For *P. demissa*, time of day accounts for 20.2% of variation in sugar production, umbel size accounts for 44.9% of variation in cumulative 12-hour sugar production, and corolla length accounts for 0.12% of variation in cumulative 12-hour sugar production. For *M. capituligera* the effect sizes of the above variables are 23.5%, 28.1%, and 7.8% respectively, and for *K. affinis* are 0.0014%, 28.1%, and 7.8% respectively.

Resumen

La concentración del néctar y la producción de azúcar, ambos afectan y son impactados por la actividad de los polinizadores a través de un sistema complejo de la coevolución. Además, una variedad de factores morfológicos y ambientales influyen las propiedades del néctar de las plantas polinizadas por los animales. La concentración del néctar y la tasa de la producción del néctar (TPN) de tres plantas polinizadas por los colibríes, *Palicourea demissa*, *Mezobromelia capituligera*, y *Kohleria affinis*, fueron medidos. Estas flores fueron encontradas y estudiadas en la zona del sur de la selva subtropical montañosa Chocó-Andina, en la provincia de Pichincha en Ecuador. Las flores fueron colocadas en bolsas de tul por 24 horas y fueron examinadas en intervalos de 2 horas durante 12 o 24 horas. La concentración del néctar y la producción de azúcar (utilizado indistintamente con TPN) fueron comparados durante varias horas del día, por el número de flores abiertas por inflorescencia (tamaño de la umbela), y por la longitud de la corola. La concentración del néctar en *P. demissa* y *M. capituligera* es negativamente correlacionada durante la hora del día, y en *K. affinis* es positivamente correlacionada durante la hora del día. El volumen del néctar y la TPN en *P. demissa* y *M. capituligera* son negativamente correlacionados durante la hora del día. Se encontró que *P. demissa* tiene la mayor producción acumulada de azúcar después de 12 y 24 horas en comparación con las otras especies. La
producción de azúcar acumulada después de 12 horas es significativa y positivamente correlacionada con el tamaño de la umbela para *P. demissa* y significativa y negativamente correlacionada con el tamaño de la umbela para *M. capituligera*. No se encontró una correlación significativa entre la producción de azúcar acumulada de 12 horas y la longitud de la corola para ninguna de las 3 especies. Para *P. demissa*, la hora del día explica el 20,2% de la variación en la producción de azúcar, el tamaño de la umbela explica el 44,9% de la variación en la producción acumulada de azúcar después de 12 horas, y la longitud de la corola explica el 0,12% de la variación en la producción acumulada de azúcar después de 12 horas. Para *M. capituligera*, los tamaños del efecto de las mismas variables son 23,5%, 28,1%, y 7,8% respectivamente, y para *K. affinis* son 0,0014%, 28,1%, y 7,8% respectivamente.

**Introduction**

The production of nectar in animal-pollinated flowers is integral to their survival and a fundamental element of their ecological role. The presence of nectar incentivizes pollinators to visit flowers, and trends in nectar production can influence pollinator behavior (Ornelas *et al*., 2007). Characteristics of animal-pollinated flowers such as nectar production, as well as color, shape, and odor, at once impact pollinator activity and are influenced evolutionarily by pollinator behavior; this relationship is part of a complex system of coevolution. Hummingbirds serve as particularly interesting subjects of study when examining this relationship, as they have extremely high energy needs. Given their small body sizes and behavior, which in large part consists of feeding on nectar mid-flight, hummingbirds have both a high energy demand and limited capacity to store food (Krüger *et al*., 1982). A 1986 study using large volume feeders found that rufous hummingbirds gave preference to sucrose concentrations that maximized their energy intake in a minimal amount of time (Tamm and Gass, 1986). However, other studies have found that the nectar produced by hummingbird-pollinated flowers is relatively dilute compared to that produced by insect-pollinated flowers (Baker, 1975; Pyke and Waser, 1981). Baker posits that this is true for three main reasons, the first being that cross-pollination increases when hummingbirds are compelled to visit more flowers, the second being that rapid ingestion of nectar with a high viscosity may pose difficulties to the hummingbirds, and the third being that the water present in more dilute nectar solutions may be a dietary necessity (Baker, 1975). The amount of energy per individual flower that is available to pollinators and that results in maximum cross-pollination is very much related to the energetic needs of the pollinator, and it is in this relationship that we can further our understanding of the complex process of coevolution taking place between plants and their pollinators (Heinrich and Raven, 1972).

A study by Castellanos *et al*. found that flowering plants subject to variation in pollinator visitation rates or weather conditions may have evolved mechanisms by which to regulate nectar production and characteristics, thus offering greater consistency in their nectar reward to pollinators (2002). In addition to pollinator needs and activity, nectar characteristics such as nectar production rate (NPR) are impacted by flower morphology and environmental factors (Pleasants, 1983). This study focuses primarily on nectar sugar concentration and NPR, which is typically measured by bagging flowers for 24 hours and measuring the amount of accumulated nectar (Pleasants, 1983). The NPR of a plant is as important to its pollinator syndrome as other characteristics such as color and odor, and largely informs – and, through coevolution, is informed by – pollinator species and activity (Pleasants, 1983). The nectar concentration and
NPR of a plant can be influenced by such morphological and environmental factors as umbel size, corolla length, period in flowering season, temperature, and time of day (see Pleasants and Chaplin, 1983; Lara and Ornelas, 2001; McDade and Weeks, 2004; Freeman and Head, 1990). The NPR of certain flowers is also impacted by flower age (Carpenter, 1976; Feinsinger, 1978; Pyke, 1978; Cruden et al., 1982) while in other flowers, age has little to no effect on NPR (Willson and Bertin, 1979; Cruden et al., 1982).

The following investigation seeks to understand how time of day, umbel size, and corolla length impact the nectar concentration and NPR of three hummingbird-pollinated flowering plants of the Ecuadorian Chocó cloud forest: *Palicourea demissa*, *Mezobromelia capituligera*, and *Kohleria affinis*. The purpose of this study is to advance understanding of plant-pollinator interactions by gaining insight into the energetic reward offered to hummingbirds (and other pollinators) by each of these three species. In order to study the NPR, flowers of each species were bagged for 24 hours and sampled at approximate two-hour time intervals during daylight hours.

*P. demissa* is a plant of the Rubiaceae family, producing one-day flowers and found in Ecuador, Colombia, and Venezuela (Govaerts, 2019). The flowers are pink, with the nectary found at the base. At the Santa Lucía Cloud Forest Reserve (SLCFR) observations have been made of visitation to *P. demissa* by the Booted Rackettail, Green-Crowned Woodnymph, Purple-bibbed Whitetip, and Violet-tailed sylph (Beck, 2018). *M. capituligera* is a bromeliad in the subfamily Tillandsioideae, also producing one-day flowers. Native to Ecuador, it is found in the coastal, Andean, and Amazonian regions, with an elevation range of 500–3500m (Grant, n.d.). *M. capituligera* is red, with thin white flowers extending out from each branch. The nectary is at the base of the flower. At SLCFR it is visited by the Brown Inca, the Collared Inca, the Fawn-breasted Brilliant, the Tawny-bellied Hermit, and the Violet-tailed Sylph (Beck, 2018). *K. affinis* is a flower of the Gesneriaceae family. It is purple in color, but the end of the corolla is light green with brown spots to attract non-hummingbird pollinators. Nectar is found at the base of the corolla. Across Ecuador’s cloud forest regions, *K. affinis* is visited by the Speckled hummingbird, the Violet-tailed Sylph, the Buff-winged starfrontlet, the Collared Inca, the Brown Inca, the Tyrian Metaltail, the White-booted Rackettail, and the Tawny-bellied Hermit (B. Weinstein, unpubl. data).

I hypothesize that nectar concentration and consequently NPR will be higher in the afternoon than in the morning. Past studies have shown diurnal patterns in nectar concentration and, for some species, in sugar production as well (Pleasants, 1983; Wolbert, 2016; McDade and Weeks)
I, 2004). I also predict that both nectar concentration and NPR will increase with increasing corolla length. Hainsworth and Wolf (1976) found through a series of choice experiments that hummingbirds selected nectar primarily based on sugar concentration, and that the preferred sugar concentrations were found in plants shown to be visited by hummingbirds, thus having longer corolla lengths. Finally, I hypothesize that nectar concentration and NPR per individual flower will decrease with increasing umbel size. In a study of *Asclepias quadrifolia*, Pleasants and Chaplin (1983) found umbel size and mg of sugar produced to be significantly negatively correlated, but only in umbels of above-average size.

**Methods**

**Study Site**
Data were taken at the Santa Lucía Cloud Forest Reserve (SLCFR), located in the town of Nanegal in the Pichincha province of Ecuador. The forest in which the reserve is located is categorized as tropical montane rainforest, and connects to the Chocó forest. The lodge at which this investigation was based is situated at 1900m elevation. Data were collected Monday through Friday from April 17th, 2019 to May 1st, 2019. Given that Ecuador’s rainy season occurs from late October to late May, days at Santa Lucía were generally partly to fully cloudy in the morning, with rain starting around 1pm most afternoons. Climate data for Nanegal shows that the average annual temperature of the region is 18.3°C and that the average annual rainfall is 2071mm (Edson, 2015).

The three species chosen for investigation all have flowers that have been shown to be pollinated by hummingbirds. A large variety of hummingbird-pollinated plants are found at Santa Lucía, however their flowering seasons vary. *P. demissa*, *K. affinis*, and *M. capituligera* were identified at the beginning of the three-week study period as being enough in abundance to provide sufficient data for this investigation.

**Nectar Production**

![Figure 2. *K. affinis* covered with tulle bag to prevent visitation from hummingbirds or other pollinators.](image-url)
In order to study the production of nectar, individual flowers or entire inflorescences (depending on size) were bagged for 24 hours and sampled non-destructively approximately every two hours (Figure 2). The time of day during which the flowers were initially bagged varied, however sampling took place regularly for the following 12 to 24 hours, excluding during the night (6pm – 6am). Because P. demissa and M. capituligera both produce one-day flowers, sampling in most cases could only be done over a 12-hour period, as the flowers of each of these species tended to open and die during non-working hours. K. affinis, however, could be sampled for a period of 24 hours, as flowers live for several days.

Tulle fabric and rubber bands were used to bag each flower or inflorescence. Each flower sampled was tagged and assigned an individual ID, in order to keep track of individuals. To sample a flower, a 75mm CITOGLAS capillary tube was inserted into the nectar chamber of the flower to draw out the nectar. The volume of nectar inside the capillary tube was measured to two decimal places using a dialMax dial caliper. The length measurement was converted to volume using the ratio provided by the capillary tube instructions: 75mm is equal to 70µl. Once the volume measurement had been taken, the liquid inside the capillary tube was placed onto the surface of a handheld Bellingham and Stanley Eclipse refractometer, used to measure the Brix. One degree Brix is equal to one gram of sucrose in 100 grams of solution, and represents the concentration of the solution as percentage by mass (Bolten et al., 1979). Between samples, the refractometer was cleaned with boiled water. In addition to taking the nectar samples, the time was noted, the corolla length of each flower was measured, the number of open flowers and buds per inflorescence was counted, and the weather on a scale of one to five was recorded (1 = clear sky, 5 = raining). Finally, a GPS waypoint was created using the EasyTrails smartphone application.

Data Analysis
In order to calculate sugar production, the volume of nectar sampled was multiplied by the nectar concentration found (expressed in decimal form as percent mass). This calculation results in slight error because the conversion of volume to mass is not exact, however with small volumes the error is negligible (Bolten et. al., 1979).

Using Excel, correlation and regression analyses were run for data on concentration, volume, sugar production, corolla length, and umbel size. Data were organized according to the analyses to be performed; in order to draw comparisons between species and to better understand the effect of umbel size and corolla length on NPR, average cumulative volume and sugar production after 12 and 24 hours was calculated for each species (with the exception of M. capituligera, which did not have 24-hour data for either variable.)

Boxplots were created using the statistical coding program R in order to better visualize the difference in sugar production between morning and afternoon for each of the three species. Morning and afternoon sugar production were plotted as separate series.

Results
In sum, 228 nectar samples were taken from three species of hummingbird-pollinated flowers: 92 samples were taken from 21 individual flowers of P. demissa; 60 samples were taken from 23
individual flowers of *M. capituligera*; and 76 samples were taken from 15 individual flowers of *K. affinis*. Of the three species, *P. demissa* had both the highest average nectar concentration and the highest average nectar volume, at 12.5% and 7.5µl respectively (Table 1). The average daily sugar production of *P. demissa* and *M. capituligera* were comparable, however the range of sugar production in *P. demissa* was greater (Table 1). *K. affinis* had on average the lowest nectar concentration, the lowest nectar volume, and the lowest sugar production of the three species (Table 1). All three species were found at comparable elevations; *P. demissa* and *M. capituligera* had virtually no elevation range, while *K. affinis* had an elevation range of 111m (Table 1).

**Table 1.** General results

<table>
<thead>
<tr>
<th></th>
<th><em>P. demissa</em></th>
<th><em>M. capituligera</em></th>
<th><em>K. affinis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Average nectar concentration (% by mass) ± S.D.</td>
<td>12.5 ± 3.2</td>
<td>12.2 ± 4.1</td>
<td>10.8 ± 4.8</td>
</tr>
<tr>
<td>Range of nectar concentrations (% by mass)</td>
<td>1.2 – 18</td>
<td>0.1 – 16.8</td>
<td>1.9 – 16.6</td>
</tr>
<tr>
<td>Average nectar volume (µl) ± S.D.</td>
<td>7.58 ± 14.67</td>
<td>6.86 ± 8.38</td>
<td>6.17 ± 9.03</td>
</tr>
<tr>
<td>Range of nectar volume (µl)</td>
<td>0 – 63.56</td>
<td>0 – 39.81</td>
<td>0 – 38.8</td>
</tr>
<tr>
<td>Average sugar production (mg) ± S.D.</td>
<td>1.0 ± 2.1</td>
<td>1.0 ± 1.3</td>
<td>0.65 ± 1.1</td>
</tr>
<tr>
<td>Range of sugar production (mg)</td>
<td>0 – 10.6</td>
<td>0 – 6.6</td>
<td>0 – 6.9</td>
</tr>
<tr>
<td>Elevation range (m)</td>
<td>1910</td>
<td>1906 – 1908</td>
<td>1844 – 1955</td>
</tr>
</tbody>
</table>

**Figure 3.1.** Moderate negative correlation between nectar concentration of *P. demissa* and time of day. Correlation coefficient *r* is equal to –0.50 (*p = 0.000049, 95% confidence interval).
Figure 3.2. Moderate negative correlation between nectar concentration of *M. capituligera* and time of day. Correlation coefficient $r$ is equal to $-0.61$ ($p = 0.0000040$, 95% confidence interval).

Figure 3.3. Weak positive correlation between nectar concentration of *K. affinis* and time of day. Correlation coefficient $r$ is equal to $0.27$ ($p = 0.04$, 95% confidence interval).
Both *P. demissa* and *M. capituligera* show moderate negative correlations between nectar concentration and time of day (Figure 3.1, p<0.05; Figure 3.2, p<0.05). Contrastingly, *K. affinis* shows a weak positive correlation between nectar concentration and time of day (Figure 3.3, p<0.05).

![Graph](image)

**Figure 4.1.** Moderate negative correlations between a) volume of nectar produced by *P. demissa* and time of day, and b) sugar production of *P. demissa* and time of day. Correlation coefficient *r* is equal to –0.44 for volume of nectar produced (p = 0.000021, 95% confidence interval). Correlation coefficient *r* for sugar production is equal to –0.45 (p = 0.0000089, 95% confidence interval).
Figure 4.2. Moderate negative correlations between a) volume of nectar produced by *M. capituligera* and time of day and b) sugar production of *M. capituligera* and time of day. Correlation coefficient $r$ is equal to $-0.46$ for volume of nectar produced ($p = 0.00023$, 95% confidence interval). Correlation coefficient $r$ is equal to $-0.48$ for sugar production ($p = 0.000088$, 95% confidence interval).

Figure 4.3. Nectar volume and sugar production of *K. affinis* across time of day. No significant correlation was found for either variable ($p$ (volume) = 0.062, $p$ (sugar production) = 0.61, 95% confidence interval).
For *P. demissa*, a moderate negative correlation was found between a) nectar of volume produced and time of day, and b) sugar production and time of day (Figure 4.1, p (volume)<0.05, p (sugar production)<0.05). Similarly, a moderate negative correlation was found between each variable and time of day in *M. capituligera* (Figure 4.2, p (volume)<0.05, p (sugar production)<0.05). No significant correlation was observed between either variable and time of day in *K. affinis* (Figure 4.3, p (volume)>0.05, p (sugar production)>0.05).

Average cumulative volume and average cumulative sugar production after 12 and 24 hours from initial sampling were calculated (Table 2). The assumption in this analysis was that initial sampling took place at 6:00AM; in actuality, initial sampling generally took place between 6:00AM and 7:00AM. Samples from individual flowers were used in the calculation of average cumulative volume and sugar production. Because *M. capituligera* has one-day flowers and new flowers opened outside of working hours, there is no data on 24-hour nectar volume or sugar production. *P. demissa* had the highest average cumulative nectar volume and sugar production of the three species after both 12 and 24 hours (Table 2). While *M. capituligera* had the lowest cumulative volume after 12 hours, *K. affinis* had the lowest cumulative sugar production after 12 hours (Table 2).

**Table 2.** Average cumulative volume (µl) and sugar production (mg) per individual flower after 12 hours and 24 hours, beginning at 6:00AM.

<table>
<thead>
<tr>
<th>Time Elapsed</th>
<th>Species</th>
<th>12-HR</th>
<th>24-HR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>P. demissa</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume ± S.D.</td>
<td>29.20 ± 21.86</td>
<td>89.14 ± 0</td>
<td></td>
</tr>
<tr>
<td>Sugar Production ± S.D.</td>
<td>4.00 ± 3.05</td>
<td>14.07 ± 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>M. capituligera</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume ± S.D.</td>
<td>19.49 ± 6.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sugar Production ± S.D.</td>
<td>2.94 ± 2.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>K. affinis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume ± S.D.</td>
<td>22.96 ± 16.91</td>
<td>27.10 ± 23.28</td>
<td></td>
</tr>
<tr>
<td>Sugar Production ± S.D.</td>
<td>2.33 ± 2.54</td>
<td>3.65 ± 3.74</td>
<td></td>
</tr>
</tbody>
</table>
Figure 5.1. Sugar production of *P. demissa* in the morning (6:00AM to 12:00PM) and in the afternoon (12:00PM to 4:00PM). The morning mean and median sugar production are 1.8 and 0.4 respectively, and the afternoon mean and median sugar production are 0.5 and 0.01 respectively (95% confidence interval).

Figure 5.2. Sugar production of *M. capituligera* in the morning (6:00AM to 12:00PM) and in the afternoon (12:00PM to 5:00PM). The morning mean and median sugar production are 1.4 and 0.8 respectively, and the afternoon mean and median sugar production are 0.3 and 0.06 respectively (95% confidence interval).
Figure 5.3. Sugar production of *K. affinis* in the morning (6:00AM to 12:00PM) and in the afternoon (12:00PM to 6:00PM). The morning mean and median sugar production are 0.8 and 0.3 respectively, and the afternoon mean and median sugar production are 0.5 and 0.3 respectively (95% confidence interval).

Sugar production in the morning (6:00AM to noon) and in the afternoon (noon to latest 6:00PM) was plotted for each flower species. *P. demissa, M. capituligera,* and *K. affinis* showed similar patterns with average sugar production being higher in the morning (1.8 mg, 0.4 mg, and 0.8 mg for each respective species) than in the afternoon (0.5 mg, 0.3 mg, and 0.5 mg respectively) (Figures 5.1–5.3).

The effect of umbel size and corolla length on average cumulative sugar production after 12 hours was analyzed (Table 3). For *P. demissa*, a moderate to strong positive correlation was found between umbel size and daily sugar production, with a correlation coefficient of 0.67 (p = 0.0022, 95% confidence interval). No significant correlation was found between corolla length and daily sugar production (p = 0.89, 95% confidence interval) (Table 3). For *M. capituligera*, a moderate negative correlation was found between umbel size and daily sugar production, with a correlation coefficient of −0.53 (p = 0.02, 95% confidence interval). No significant correlation was found between corolla length and daily sugar production (p = 0.26, 95% confidence interval), however the correlation coefficient of 0.28 indicates a weak positive relationship between the variables (Table 3). For *K. affinis*, no significant correlation was found between umbel size and daily sugar production (p = 0.53, 95% confidence interval), however with a correlation coefficient of −0.32 there is a weak negative relationship between the two variables (Table 3). Likewise, there is no significant correlation between corolla length and daily sugar production (p = 0.46, 95% confidence interval) but the correlation coefficient of 0.28 indicates a weak positive relationship between the variables (Table 3).
Table 3. Effect of umbel size and corolla length on average cumulative 12-hr sugar production (6:00AM – 6:00PM).

<table>
<thead>
<tr>
<th>P. demissa</th>
<th>M. capituligera</th>
<th>K. affinis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average umbel size ± S.D.</td>
<td>5 ± 4</td>
<td>9 ± 6</td>
</tr>
<tr>
<td>Range</td>
<td>1 – 9</td>
<td>2 – 14</td>
</tr>
<tr>
<td>r</td>
<td>0.67</td>
<td>–0.53</td>
</tr>
<tr>
<td>p</td>
<td>0.0022</td>
<td>0.02</td>
</tr>
<tr>
<td>Average corolla length ± S.D.</td>
<td>20.45 ± 1.16</td>
<td>40.38 ± 2.97</td>
</tr>
<tr>
<td>Range</td>
<td>17.2 – 23.2</td>
<td>33.21 – 46.94</td>
</tr>
<tr>
<td>r</td>
<td>–0.035</td>
<td>0.28</td>
</tr>
<tr>
<td>p</td>
<td>0.89</td>
<td>0.26</td>
</tr>
</tbody>
</table>

The effect sizes of each of the three independent variables (time of day, umbel size, and corolla length) were calculated. For P. demissa, time of day accounted for 20.2% of the variation in sugar production (Figure 4.1). In looking at cumulative sugar production after 12 hours, umbel size accounted for 44.9% of variation, and corolla length accounted for 0.12% of the variation (Table 3). For M. capituligera, 23.5% of the variation was accounted for by time of day (Figure 4.2). Umbel size was responsible for 28.1% of the variation and corolla length was responsible for 7.8% of the variation in cumulative sugar production after 12 hours (Table 3). For K. affinis, time of day accounted for 0.0014% of observed variation in sugar production (Figure 4.3). Umbel size accounted for 28.1% of variation and corolla length accounted for 7.8% of variation in cumulative sugar production between 6:00AM and 6:00PM (Table 3).

Discussion

Nectar Concentration

The average nectar concentrations of P. demissa, M. capituligera, and K. affinis are 12.5%, 12.2%, and 10.8% respectively, concentrations which are almost shockingly low compared to studies of other hummingbird-pollinated plants (Table 1). Pleasants (1983) found that the concentration of Ipomopsis aggregata dipped below 15% only during the early morning, and during an overcast period. Baker (1975) found that for a variety of hummingbird-pollinated flowers found in Costa Rica and California, the mean nectar concentration ranged from 20-24%. A study by Pyke and Waser (1981) describes the nectar of 202 plant species which are known or assumed to be pollinated by hummingbirds, providing a mean nectar concentration of 25.4% sucrose-equivalent sugars. Theoretical predictions of nectar sugar concentrations typically fall between 22 and 26% (McDade and Weeks I, 2004).

Although other studies have found nectar concentrations that are higher than those identified in this study, the nectar concentrations of hummingbird-pollinated flowers are typically lower than
those of flowers pollinated by insects (Baker, 1975). Baker (1975) found that in some cases, flowers pollinated by bees could have nectar concentrations double that of hummingbird-pollinated flowers. In many studies it has been posited that the nectar produced by hummingbird-pollinated flowers is higher in sucrose as a result of the high energy demands of hummingbirds. A number of studies attempt to explain why this is not the case. Heinrich and Raven (1972) write that for a plant to be reproductively successful, it must experience sufficient levels of cross-pollination. In order to achieve this the pollinator must be somehow incentivized to visit multiple flowers and flowers of distinct plants. This can be achieved by lowering the energetic reward of nectar consumption (Heinrich and Raven, 1972). Baker (1975) cites this explanation and provides two others: digestive difficulties may arise for hummingbirds when consuming overly-viscous nectar, and the presence of water in nectar may be a dietary necessity for the birds.

Another possible explanation for the relatively low nectar concentrations is the extreme humidity and precipitation of the subtropical montane rainforest in this region and during the time of year in which the investigation took place. It is possible for nectar to be more dilute as a result of humidity and precipitation (Park, 1929; Aizen, 2003). Aizen (2003) found that down-facing flowers are associated with hummingbirds and other pollinators which remain fairly active during rain events. Park (1929) found that nectar concentration is negatively correlated with relative humidity. Thus, it is highly possible that the lower nectar concentrations found in this study can be attributed to these two factors.

Interestingly, *K. affinis* has the lowest nectar concentration of the three species, and it is the only species of the three which is not solely pollinated by hummingbirds (Table 1). In this investigation, only the sucrose content of the nectar was measured and analyzed. In plants pollinated by hummingbirds, moths, and long-tongued-bees, sucrose is the dominant component of nectar; in plants pollinated by passerines, short-tongued bees, and Neotropical bats, hexose is the primary component (Valtueña et. al., 2007). *K. affinis*, being also insect-pollinated, may have other, potentially more dominant, nectar components that were not taken into account in this study.

This investigation found a significant negative correlation between nectar concentration and time of day in both *P. demissa* and *M. capituligera*, and a significant positive correlation between nectar concentration and time of day for *K. affinis* (Figures 3.1–3.3). I hypothesized that the nectar concentration of all three species would be higher in the morning than in the afternoon; both *P. demissa* and *M. capituligera* confirmed this hypothesis. *K. affinis* demonstrated the opposite trend. A study by Valtueña et. al. (2007) found that the nectar concentration of *Anagyris foetida* of the Fabaceae family gradually rises from morning to night. It has been hypothesized that evaporation occurring in the afternoon may account for increasing nectar concentration over the course of the day (McDade and Weeks I, 2004). During the study period, mornings were warmer and sunnier than afternoons, which were consistently cloudy, rainy, and cold. Perhaps mornings had higher rates of evaporation than afternoons, resulting in an inverse relationship between concentration and time of day for *P. demissa* and *M. capituligera*. However, it is also possible that evaporation is less relevant in humid montane rainforests, especially in flowers with long, tubular corollas (McDade and Weeks I, 2004).
Two previous studies on nectar concentration of hummingbird-pollinated flowers have taken place at SLCFR. DeRycke (2016) carried out a study of *Centropogon solanifolius*, *K. affinis*, and *Fuchsia macrostigma*. Average nectar concentrations were 14.83%, 6.37%, and 11.45% respectively (DeRycke, 2016). Both *C. solanifolius* and *K. affinis* demonstrated a negative correlation between nectar concentration and time of day (DeRycke, 2016). It is interesting that DeRycke’s results regarding *K. affinis* differed from my own, despite the fact that both studies were carried out during the same time of year. One factor which differentiates these studies is methodology; DeRycke sampled flowers destructively, cutting the flowers just above the base of the corolla and placing the opened flower directly onto the surface of the refractometer (DeRycke, 2016). This study, on the other hand, used capillary tubes to extract nectar from inside the flower. It is possible that alternate methodologies may yield differing results.

A second study done at SLCFR, carried out by Wolbert (2016) found that nectar concentration was significantly positively correlated with time of day for *Besleria solanoides*. No significant correlation was found between nectar concentration and time of day for *Guzmania jaramilloi* or *Gastheranthus quitensis*. Like DeRycke (2016), Wolbert (2016) sampled flowers destructively. The study was also carried out during the month of November, which in this region is considerably less rainy than the months of April and May. Despite the absence of the same level of rain and humidity present during this investigation, Wolbert (2016) also found relatively low nectar concentrations. Taking into account DeRycke’s study as well, the more dilute nectar found in these cloud forest species might be attributable to other environmental or morphological factors.

**Nectar Volume**

The average nectar volumes of *P. demissa*, *M. capituligera*, and *K. affinis* are 7.58 µl, 6.86 µl, and 6.17 µl respectively (Table 1). *P. demissa* showed the greatest range in nectar volume collected, from 0 µl minimum to 63.56 µl maximum (Table 1). For both *P. demissa* and *M. capituligera* a moderate negative correlation was found between volume of nectar produced and time of day, and for *K. affinis* no significant correlation was found (Figures 4.1–4.3).

Pleasants and Chaplin (1983) found in a study of *Asclepias quadrifolia* that hourly nectar production was highest in the morning (7:00AM – 1:00PM), decreasing as the day went on. Valtueña et al. (2007) found that for *A. foetida*, while concentration increases from morning to evening as mentioned previously, the volume of nectar secreted is highest in the morning. In order to explain this phenomenon, the authors discuss the various factors that may impact the volume of nectar secreted by a flowering plant. Flowers that are first visited by a pollinator during the first half of antithesis accumulate more nectar than flowers first visited by a pollinator in the second half of antithesis. The cumulative volume of nectar secreted by a flower of this species is less in flowers that are visited once each day than it is in flowers that are visited three times in a day (Valtueña et al., 2007). This can be explained in part by the ability of some flowers to reabsorb unused nectar, a property held only by plants with contact between nectariferous tissue and secreted nectar. This property may also provide an explanation for plants that produce excess nectar after repeated visits in comparison to other flowers visited less frequently (Valtueña et al., 2007). These findings support the earlier work of Castellanos et al. (2002), which found that removal of nectar brings about replenishment; when nectar was
removed each hour for six hours from *Penstemon barbatus*, the cumulative volume was twice that of when nectar was only drawn at the start and end of the six-hour period.

During my study I did not analyze the total cumulative volume relative to the number of samples taken from each flower, which poses a limitation to this investigation. Flowers were not sampled equal numbers of times, nor at exactly equal intervals. Thus, it is impossible to know whether any of the three species investigated here were in fact stimulated by more frequent nectar sampling.

Cumulative volume after 12 and 24 hours was analyzed for *P. demissa* and *K. affinis*. It was not possible to study 24-hour cumulative production of *M. capituligera* given the fact that it is a one-day flower that opened and died during non-working hours. *P. demissa* had the highest cumulative volume after both 12 and 24 hours of being bagged, while *K. affinis* had the lowest cumulative volume (Table 2). Between 12 and 24 hours, the cumulative nectar volume and sugar production of *P. demissa* increased by 305% and 351%, respectively, while for *K. affinis* the magnitude of increase was lesser, at 118% and 157% respectively (Table 2). Because nectar was not sampled between 6:00PM and 6:00AM, it is not possible to know exactly how the rate of nectar secretion changes overnight. If it is true that nectar production slows during the night in *P. demissa*, it is puzzling that the volume produced should have increased by greater than threefold between 12 and 24 hours (which corresponds to 6:00PM to 6:00AM). Important to note here is that like *M. capituligera*, *P. demissa* also has one-day flowers which typically opened and died during non-working hours. By chance, one individual flower was successfully bagged and sampled for 24 hours, and that data is the only data represented from 12 to 24 hours for *P. demissa*. This individual was the only flower open of its inflorescence, and as will be discussed further, the minimal umbel size may have influenced the seemingly disproportionate increase in nectar produced overnight.

**Nectar Production Rate**

The NPR of each species corresponds closely to the volume of nectar produced (Figures 4.1–4.3). For *P. demissa* and *M. capituligera*, sugar production is moderately and negatively correlated to time of day, as is volume (Figures 4.1, 4.2). For *P. demissa*, time of day accounts for 20.2% of the observed variation in sugar production (Figure 4.1). For *M. capituligera*, 23.5% of the observed variation in sugar production is accounted for by variation in time of day (Figure 4.2). Time of day accounts for a negligible amount of variation in the sugar production of *K. affinis* (Figure 4.3).

Following the same trend as volume, cumulative sugar production after 12 and 24 hours is highest for *P. demissa* and lowest for *K. affinis* (Table 2). Additionally, morning and afternoon NPRs were compared. The mean morning sugar production of *P. demissa* is 3.6 times greater than the mean afternoon production (Figure 5.1). The morning NPR of *M. capituligera* is 4.7 times greater than the afternoon production, and for *K. affinis* the morning sugar production is 1.6 times greater than the afternoon production.

Several factors might account for the temporal trends observed in nectar production. McDade and Weeks (2004, 1) observed that among 12 species of Neotropical hummingbird-pollinated plants, time of day accounted for between 10 and 50 percent of the observed variation in nectar
production. Additionally, the study found diurnal patterns in both concentration and cumulative volume in flowers that had been covered for 24 hours. The authors point to evaporation as a potential cause of temporal variation in the flowers studied, but emphasize the high levels of variability within and between species that made more difficult the detection of clear patterns (McDade and Weeks I, 2004).

Pleasants (1983) found the NPR of *I. aggregata* to be relatively constant during the day and reduced during the night. The coefficient of variation for nectar production within a single plant was 23.5%, indicating that total variation in NPR is impacted more by external factors. The study did not find significant differences between average nectar concentrations of different age classes, however Pleasants and Chaplin (1983) found that the nectar production of *A. quadrifolia* decreased with increasing flower age. My study did not take into account the ages of the flowers studies, and thus it is not possible to discuss possible variability brought about by age differences. The stage in the flowering season may account for some variation in nectar sugar concentration; for *I. aggregata* there is an observed decline in NPR as the flowering season declines (Pleasants, 1983). In sum, it appears that there still exist several prominent hypotheses attempting to explain temporal variation in nectar concentration and production.

Finally, this investigation sought to understand how umbel size and corolla length effect the nectar sugar production of the three species studied. A significant positive correlation was identified for *P. demissa* and a significant negative correlation was identified for *M. capituligera* (Table 3). Pleasants and Chaplin (1983) studied the impact of umbel size and root weight on individual variation in nectar production rates of *A. quadrifolia*. 57% of the variance in nectar production of individual flowers was explained by the two variables, with 33% of the total variance explained by umbel size. A significant negative correlation was found between flowers per umbel and sugar production, but only for umbels that were classified as being relatively large (Pleasants and Chaplin, 1983). This negative correlation is observed due to physiological properties of inflorescences. The amount of carbohydrate that can be allocated to the flowers in an umbel is limited by the single peduncle from which the umbel grows, thus a greater number of flowers results in limited nectar production per individual flower (Pleasants and Chaplin, 1983). An additional explanation posed by the authors is that larger umbels can attract the same rate of visitation by pollinators while keeping the cost of nectar production lower (Pleasants and Chaplin, 1983).

No significant correlations were found between corolla length and cumulative sugar production (Table 3). However, both *M. capituligera* and *K. affinis* have correlation coefficients of 0.28, indicating a weak positive relationship between the two variables. Wolbert (2016) found a weak negative correlation between corolla length and nectar concentration in *G. quitensis*, and DeRycke (2016) found a moderate negative correlation between the two variables for *F. macrostigma*. Field surveys of nine different species of flowers showed that the relationship between corolla length and nectar concentration was also influenced by environmental conditions, and that nectar evaporates more quickly from flowers with lesser corolla depths (Plowright, 1987). The results from that study suggest that the relatively dilute nectars produced by hummingbird-pollinated flowers are related to the evolution of long, tubular corollas (Plowright, 1987).
Sources of Error

*K. affinis* proved difficult to study for a variety of reasons. During the three-week study period, the plant was reaching the end of its flowering season and thus was in decline each day. Additionally, the flowers seemed especially prone to flower-piercing by certain hummingbirds, and consumption perhaps by insects. Another limitation of this study was the inability to study components of nectar apart from sucrose. It is possible that the other components of nectar, which include glucose and fructose, might demonstrate trends other than those seen in the sucrose concentration. Additionally, flowers were not sampled for nectar during the night and very early morning. In order to better understand how the production of nectar changes over a 24-hour period, it is important to have data from the night hours.

Attempting to look at cumulative nectar production at exact time intervals was not possible in this study, because nectar could not be sampled at exact time intervals. To avoid this problem, cumulative nectar volume and production was only analyzed for 12 and 24-hour time intervals. The rain posed a problem during afternoon work; in order to read the nectar concentration using the refractometer, nectar had to remain undiluted. Additionally, it is possible that afternoon rain and humidity diluted the nectar inside the flowers prior to my sampling. A rainshield could have helped avoid this problem, however that piece of equipment was not available to me.

There were a variety of other factors not taken into account in this report that could have helped elucidate the variation in nectar production for these three species. Such factors include flower age, elevation, and period in flowering season, among others.

Conclusions

The concentration and production of nectar in hummingbird-pollinated flowers influence and are influenced by an ongoing system of coevolution between plants and their pollinators. This study sought to understand how time of day, umbel size, and corolla length impact the nectar concentration and NPR of *Palicourea demissa*, *Mezobromelia capituligera*, and *Kohleria affinis*, which are found and were studied in the southern section of the Chocó-Andean subtropical montane rainforest. To study production flowers were bagged for 24 hours and sampled at approximate two-hour time intervals for 12 or 24 hours. The investigation found that nectar concentration is negatively correlated with time of day for *P. demissa* and *M. capituligera*, and positively correlated with time of day for *K. affinis*. Volume of nectar and sugar production are significantly negatively correlated with time of day for *P. demissa* and *M. capituligera*. *P. demissa* was found to have the highest cumulative sugar production after both 12 and 24 hours than the other two species. Cumulative sugar production after 12 hours is significantly positively correlated with umbel size in *P. demissa* and significantly negatively correlated with umbel size for *M. capituligera*. No significant correlation was found between cumulative 12-hour sugar production and corolla length for any of the three species.

This study has furthered prior work at SLCFR (DeRycke, 2016; Wolbert, 2016) by taking into account nectar production, rather than solely nectar concentration, in relation to several environmental and morphological factors. To advance the findings presented by this study it would be useful to a) increase the sample sizes of all three species, b) carry out the same study at a different point in the flowering season of these species and/or during different times of the
year, and c) to include more variables (such as flower age, elevation, etc.). A method by which to limit dilution by rainwater, such as a rainsheet, would also be useful.

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