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Food selection and feeding patterns in nectarivorous bats: *Leptonycteris yerbabuenae* and *Glossophaga soricina*

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Sympatric species reduce competition for resources due to differences in one or more of their niche dimensions. Biotic interactions between pollinators and variations in the availability and quality of resources are important factors that determine food selection in bats. The nectarivorous species *Leptonycteris yerbabuenae* and *Glossophaga soricina* coexist temporarily in much of their distribution. These species have similar requirements but differ in the way they obtain food. Previous field studies have not reported competition for resources when evaluating the diet of these species; however, it remains unclear how competition is involved or whether this segregation is based on resource characteristics. We therefore analyzed nectar selection and feeding patterns in these two bat species under captive conditions. We conducted experiments in which we controlled resource type and its availability by offering the bats different artificial nectar solutions, while we removed interspecific interactions. These solutions differed in concentration and type of sugar, and some were similar to the nectar offered by chiropterophilic plant species. The bat species presented differences in food selection; *G. soricina* fed mainly on resources similar to *Ipomoea* and sucrose sugar. In contrast, *L. yerbabuenae* preferred those resources similar to the nectar of cacti. In addition, the timing of feeding for each solution also differed . These results may suggest low levels of competition between species under abundant resources and low density of individuals; however, such conditions are not always found in nature, and patterns may change with increased food scarcity and a high density of competitors.

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Abstract

Sympatric species reduce competition for resources due to differences in one or more of their niche dimensions. Biotic interactions between pollinators and variations in the availability and quality of resources are important factors that determine food selection in bats. The nectarivorous species *Leptonycteris yerbabuenae* and *Glossophaga soricina* coexist temporarily in much of their distribution. These species have similar requirements but differ in the way they obtain food. Previous field studies have not reported competition for resources when evaluating the diet of these species; however, it remains unclear how competition is involved or whether this segregation is based on resource characteristics. We therefore analyzed nectar selection and feeding patterns in these two bat species under captive conditions. We conducted experiments in which we controlled resource type and its availability by offering the bats different artificial nectar solutions, while we removed interspecific interactions. These solutions differed in concentration and type of sugar, and some were similar to the nectar offered by chiropterophilic plant species. The bat species presented differences in food selection; *G. soricina* fed mainly on resources similar to *Ipomoea* and sucrose sugar. In contrast, *L. yerbabuenae* preferred those resources similar to the nectar of cacti. In addition, the timing of feeding for each solution also differed. These results may suggest low levels of competition between species under abundant resources and low density of individuals; however, such conditions are not always found in nature, and patterns may change with increased food scarcity and a high density of competitors.

Introduction

Coexistence between sympatric species is possible because species use resources differently. This phenomenon is known as niche partitioning (Schoener 1974; Tavizon 1998; Griffin & Silliman 2011; Denzinger & Schnitzler 2013; Salinas-Ramos et al., 2015). Niche partitioning can occur in two main dimensions: feeding time and diet niche flexibility (Schoener 1974; Griffin & Silliman 2011). Nectarivorous species, such as birds and bats, have a high energetic cost of flying, making them highly dependent on food resources (Hainsworth & Wolf 1972; Winter & Helversen 1998; Winter et al.,1998). This dependence on food resources of nectar-feeding vertebrates may lead to the adoption of strategies to avoid competition among sympatric species (Tschapka 2004).

Nectar is a reward that varies in volume and composition between plant species. It is composed mainly of sugars such as glucose and fructose, as well as small amounts of sucrose and water. Additionally, it can contain proteins, amino acids, lipids, organic acids, and antioxidants (Baker 1977; Wolff 2006; Nicolson & Thornburg 2007; Rodríguez-Peña et al., 2016). These nutritional characteristics of nectar make it a highly valuable resource to pollinators over which they can compete. Other nectar properties, such as volume, concentration, and composition of sugars, can vary depending on the environmental conditions, plant species characteristics, and biotic interactions (Tschapka & Dressler 2002; Nicolson & Thornburg 2007). Specifically, plants pollinated by bats may produce a volume of nectar ranging from 100 µl to 20 ml in one night (Tschapka & Dressler 2002; Nicolson & Thornburg 2007), and nectar sugar concentration can vary significantly from 3% to 33% (Baker et al., 1998, Rodríguez-Peña et al., 2007). However, the most common sugar concentration ranges from 18% to

21% between chiropterophilous plant species (Von Helversen & Reyer 1984; Baker et al., 1998; Rodríguez-Peña et al., 2016). Sugar content in the nectar of bat-pollinated plant species may be primarily composed of glucose, fructose, and small amounts of sucrose (Wolff 2006, Rodríguez-Peña et al., 2016). This constancy in nectar composition among chiropterophilous plant species may limit the dimensions into which niche partitioning may occur, as this may increase the likelihood of species preferences overlapping. Despite such variations in nectar properties, experimental studies have not reported differences in bat preference for a given sugar type when in equal concentrations; however, results from these studies suggest that nectarivorous bats prefer highly sugar-concentrated nectars (Rodríguez-Peña et al., 2007; De Santiago-Hernández 2013).

The coexistence of bats with similar food preferences may increase the likelihood of competition (Bloch et al., 2011). Modeling competition studies in nectarivorous bats suggest that body size alone cannot describe niche differentiation and coexistence (Bloch et al., 2011). Foraging behavior is an important component of niche differentiation and may promote coexistence between bat species that share food sources, have high energetic requirements, and have similar phenotypical adaptations (Novella-Fernandez et al., 2020). Thus, a specialized diet in a few specific food items or a generalized diet in most items may promote the coexistence of bats with similar energetic requirements. Specialist bats may have more adjustments in foraging behavior when sharing foraging sources than generalist bat species, which may use all available resources.

Materials & Methods

Study species

Leptonycteris yerbabuenae is one of the largest nectarivorous bats in America (Cole & Wilson 2006). This species can fly up to 100 km per night to reach feeding areas (Horner et al., 1998), and northern populations perform migrations during the winter, tracking flowering plants (Wilkinson & Fleming, 1996; Herrera-Montalvo, 1997; Rojas-Martinez et al., 1999; Cole & Wilson, 2006; Morales-Garza et al., 2007). In contrast, *G. soricina* is a smaller nectarivorous bat (Álvarez et al., 1991) and has much more reduced mobility than the other species, flying up to three km per night in search of feeding areas (Aguiar et al., 2014). Resident *G. soricina* populations occur throughout its distribution range (Fleming et al., 1993), probably due to low mobility (Aguiar et al., 2014). Although both species of bats use nectar as the principal food resource, fruit and insect consumption may occur when nectar is partially unavailable (Howell 1979; Rojas-Martínez et al., 2012). However, insect and fruit consumption is more common in *G. soricina* than in *L. yerbabuenae* (Gardner 1977, Estrada-Chávez et al., 2019).

Study site and bat sampling

Bats were collected in the "La Bonetera" area in Lázaro Cárdenas, southern Michoacán, Mexico (ca. 18°05'N, 102°25'W). This area is covered mainly by tropical deciduous forests and patches of semi-deciduous forests (Sandoval-Soto 2013). It has a marked dry season from November to June and an average annual temperature of 27 °C (Cristóbal-Pérez 2011). The area has reported six species of nectarivorous bats, with *L. yerbabuenae* and *G. soricina* as the most abundant species (Sandoval-Soto 2013; pers.

obs.). We used mist nets at ground level to capture 6 non-reproductive adult males each of *L. yerbabuenae* and *G. soricina* (Estrada-Chávez et al., 2019). At the end of the study all individuals were returned to their natural conditions. We followed Mexican laws for animal care, use, and handling (SEMARNAT permit no. SGPA/DGVS/03702/17) to YHD. Care and housing of bats, experimental design, and data analysis were prepared prior to initiating the study.

Care and housing of bats

All bats were transferred to the housing facilities in Morelia, Mexico, where they were marked with plastic collars, and their health status (weight) was constantly monitored. We kept bats inside cages (0.75 x 0.75 x 0.75 m), covered with shade cloth, in groups of three individuals. The place where the experiment was performed remained in darkness at night and with little illumination during the day. The temperature was maintained constant at around 26-27 °C, and relative humidity was around 50%; these conditions were similar to the capture area conditions and used in other experimental studies (Rodríguez-Peña et al., 2013). Bats were fed daily at 20:00 hrs. with a maintenance diet composed of milk powder, cereals, sugar, and fruit (mango or banana), complemented with vitamins and minerals (multivitamin tonic, "Cariño", Mexico) (Mirón et al., 2006).

Experimental design

We prepared different types of solutions (Table 1) based first on sugar concentration, for which we prepared solutions using separately the hexoses glucose and fructose and

the disaccharide sucrose in a concentration of 20% (weight/weight), where 20% of the solution weight corresponds to the sugar type and 80% to water weight. In addition, we also prepared sugar solutions combining the three sugar types aiming to emulate sugar content to the nectar of chiropterophilic plants that bats commonly use (Estrada-Chávez et al., 2019): *Ipomoea ampullacea* (Convolvulaceae) (24.42 ± 1.03 °Brix), *Ceiba aesculifolia* (Bombacaceae) (18.03 ± 0.93 °Brix) and *Acanthocereus occidentalis* (Cactaceae) (27.13 ± 1.44 °Brix) (Rodríguez-Peña et al., 2016). Combining these solutions provided a gradient of concentrations from low to higher sugar concentrations from which both species could choose.

To evaluate food selection, we selected six individuals per bat species. Each individual was exposed to one experimental night trial; therefore, we conducted 12 trials. Every night, we placed one individual in the experimental area, consisting of a larger cage (3.6 x 1.6 x 2.6 m) with two resource patches placed at a height of 1 m on opposite sides. Each patch had 14 feeders, two feeders for each experimental solution. Each feeder was placed randomly within the patch and filled with 15 ml of sugar solution. In addition, to control the evaporation effect on the volume and sugar concentration, we used two control feeders of a known volume for each sugar solution in each experimental session. These control feeders were covered with mesh to prevent bats from feeding on them. Later, to determine the volume consumed, we quantified the evaporated volume and subtracted the evaporated volume of each sugar solution from the solution volume remaining in the experimental feeders.

Bat behavior was recorded during the night using two-night vision video cameras, one for each food patch. Recording began at 20:00 hrs. and ended at 07:00 hrs. We

discarded the first recording hour for analysis since the bats took around an hour to feed after the experimental solutions were placed in the cage. The video recordings were reviewed in slow motion using the software VLC-media-player 2.2.6. To evaluate foraging behavior and activity patterns, we recorded each time the bats approached a feeder and drank from it. We also recorded the feeder they visited and the time each feeding event occurred. No unexpected events occurred during the experimental sessions. All data are available in Supplementary File 1.

Data analysis

We use generalized linear models, one by bat species, using the GLIMMIX procedure implemented in the SAS 2003 version 9.3 software. To determine whether the frequency of visits differs between sugar solutions, we used the frequency of visits as a response variable and the sugar solution as a fixed factor. To know whether activity patterns differ between hours, we obtained activity patterns per hour per type of solution (from 22:00 to 06:00 hrs.) using the frequency of visits per hour. We used the frequency of visits as the dependent variable and the time as the independent variable. For all analyses, we used a Poisson distribution with an associated log link function and the ILINK function to back-transform data to the original scale.

To analyze whether bat species differ in preferences for sugar solutions, we calculated the standardized specialization index Kullback-Leibler (*d'*) based on the Shannon diversity index that compares the distribution of the interactions of each bat species with each sugar solution (Kullback et al., 1951; Blüthgen et al., 2006). The *d'* values range from 0 to 1. Values close to 1 indicate maximum specialization. To discard whether

specialization *d′* index results from chance, we assessed a significance analysis comparing an interaction matrix between bat species and sugar solution with the interaction matrix obtained from a null model (Fischer & Lindenmayer 2002; Ulrich & Gotelli 2012 and 2013). To obtain the significance of the *d′* index, we use the r2dtable null model, which keeps the matrix sum and row/column sum constant (Dormann et al., 2008, R Core Team 2016). Significant P values ($P \leq 0.05$) indicate the probability that *d′* calculated from the null distribution will be higher than values obtained from empirical data. Calculations for specialization *d′* index were performed in the "bipartite" package ver. 2.18 implemented in R-software platform ver. 4.3.1.

Results

A total of 132 hours of video recordings were taken for each bat species. In the experiments, *G. soricina* individuals presented more feeding events during the night, with an average of 102 visits per night (confidence intervals = 41.57, 162.77, n=6). In contrast, *L. yerbabuenae* individuals presented an average of 65 visits per night (confidence intervals = 27.25, 103. 74, n=6).

Significant differences were found between frequencies of visits to each type of solution per bat species. *Leptonycteris yerbabuenae* visited the feeders with nectar similar to cacti (*A. occidentalis*) more frequently (x^2= 218.28, df= 6, p<0.0001). At the same time, *G. soricina* presented a higher number of visits to feeders with a solution similar to *Ipomoea ampullacea* nectar (x^2= 371.36, df= 6, p<0.0001). We also observed that each bat species recorded a low number of visits to the solution preferred by the other species (Fig. 1). For the rest of the solutions, the sucrose solution was visited mainly by

G. soricina and fructose, and glucose solutions were visited similarly by both bat species. The water and the *Ceiba aesculifolia* solutions presented the lowest number of visits by both bat species. The bat species not only fed from different solutions but also at different times; *L. yerbabuenae* presented a feeding peak at 03:00 hrs (x^2= 68.16, df= 11, p<0.0001) while *G. soricina* presented its highest number of feeding events around midnight (x^2= 98.46, df= 11, p<0.0001).

The frequency of visits to each treatment varied throughout the night. The bats apparently made some recognition visits to the feeders and then chose the resources they used for the rest of the night (Fig. 3). In the case of *L. yerbabuenae*, the consumption of water was restricted to the first hours of feeding only and was not consumed during the rest of the night. In contrast, *G. soricina* consumed water throughout the night, although the frequency of visits was low. Both species alternated nectar consumption between different solutions. However, it was observed that, from 23:00 hrs. onwards, *L. yerbabuenae* fed mainly on nectar similar to cacti. This pattern was maintained until 06:00 hrs. when it changed to favor the sucrose and *Ipomoea* solutions (Fig. 3). *Glossophaga soricina* also alternated solutions consumption among the different treatments before midnight, but after that time, it mainly consumed *Ipomoea* and sucrose (Fig. 3). Specialization *d'* index was higher for *L. yerbabuenae* than *G. soricina*. The null model analysis indicates that the empirical *d'* index significantly differs from the chance (p<0.0001) (Fig. 4).

Discussion

Our results support our initial hypothesis of segregation of both species in the type of food resource, the concentration of nectar used, and the time of food resource visit. We found differences in resource use between bat species along a time axis and in foraging behavior. *Glossophaga soricina* was more generalist than *L. yerbabuenae*, while the latter species was selective and chose the most energetic resources. One possible explanation is that resource characteristics such as nectar quality could drive the feeding pattern (Gonzalez-Terrazas et al., 2012). Concerning the activity patterns, we observed that bat species presented differences in their activity peaks. These activity peaks were similar to those observed previously for both species in the field (Chávez-Estrada et al., 2019). Our results also indicate that the duration of the feeding time per species was higher than that found previously under a high density of bats (Chávez-Estrada et al., 2019). Therefore, the ability of nectarivorous bats to select distinct available food resources may promote a successful strategy to allow the coexistence of bat species that use similar floral resources.

Patterns of resource utilization

Our experiments show significant differences in the number of feeding events, feeding preferences, and feeding time according to bat species and treatment. The specialization *d'* index suggests that individuals of *Leptonycteris yerbabuenae* were more selective in food resources than *Glossophaga soricina*. Differences in food preferences among nectarivorous species can be attributed to various factors, including physiological requirements, morphological specializations, nectar characteristics, and resource availability. (Freeman 1995; Nicolson & Thornburg 2007; Ayala-Berdon et al., 2011; Gonzalez-Terrazas et al., 2012).

241 *G. soricina* also recorded a higher number of feeding events in all treatments.
242 Explanations accounting for this pattern may include both bat species' intrinsic
243 characteristics and resource characteristics. Particularly, these results may be related to
244 differences in morphological characteristics between both species that enable nectar-
245 feeding habits in bats (Freeman 1995). Some studies have demonstrated that
246 morphological specialization, such as the length of the oral apparatus and the tongue, is
247 positively related to nectar extraction efficiency (Gonzalez-Terrazas et al., 2012). The
248 specialized species *L. yerbabuenae* is known to be more efficient at extracting nectar
249 than the less specialized nectarivorous *G. soricina* (Gonzalez-Terrazas et al., 2012).
250 Therefore, *G. soricina* must conduct more floral visits than *L. yerbabuenae* to extract the
251 same volume of nectar.
252 Floral visits may also depend on nectar concentration and nectar properties. Some
253 studies have shown that nectar properties, especially viscosity, can increase with nectar
254 concentration, making its consumption more difficult (Baker 1975; Kingsolver & Daniel
255 1983; Nicolson & Thornburg 2007). Thus, specialized species may prefer high-sugar
256 concentrated nectar because they could be more capable of consuming viscous nectar
257 than less specialized species. For a less specialized species, feeding on intermediate
258 concentrations may be more efficient than on more diluted or concentrated nectars
259 (Baker 1975; Kingsolver and Daniel 1983). Our results support this notion because the
260 less efficient nectarivore *G. soricina* preferred nectar with an intermediate sugar
261 concentration and an intermediate energetic level similar to the Ipomoeas. Nectar
262 properties such as energy content are important factors in determining bat species'
263 feeding times and events (Ayala-Berdon et al., 2011). In contrast, *L. yerbabuenae* fed

mainly on cacti solutions, our experiments' most concentrated and energetic resources. *Leptonycteris yerbabuenae* is one of the largest nectarivorous bat species, and feeding on the most concentrated solutions enables the bats to enhance their daily energy budget with less frequent feeding events (Helversen and Reyer 1984; Gonzales-Terrazas et al., 2012).

Other explanations for the feeder visiting patterns may be related to physiology. Nectar is composed mainly of sugar and water (Baker 1977), and then feeding behavior is also determined by the physiological capabilities of the bat to digest sugars and eliminate excess water (Ayala-Berdon et al., 2011). Pure water consumption is not expected in nectar-feeding bats, as it can be obtained from nectar; however, some studies have shown that nectarivorous species drink extra water when the nectar on which they feed exceeds 50% in concentration (Helversen & Reyer 1984). These results contrast with our results, in which we observed that *G. soricina* maintains a little water ingestion throughout the night, even with a low sugar concentration solution. Water consumption in nectarivorous species is a theme that remains largely unexplored, and further research is required.

Resource availability is another factor related to food selection in bats (Ayala-Berdon et al., 2009; Laurindo 2017). Previous studies in tropical dry forests have found that Bombacaceous plant species constitute one of the most important resources for nectarivorous bats (Stoner et al., 2003; Quesada et al., 2003). Since experimental bats were collected in a tropical dry forest, we expected they would feed mainly on these solution types. However, the sugar solution similar to *C. aesculifolia* nectar was one of the least visited resources in our experiments. One possible explanation is that bats use

Bombacaceous plant species because they have massive flowering that provides a higher volume of nectar than other plant species. For example, in a previous study, *Pseudobombax ellipticum* was the most commonly consumed plant species by *L. yerbabuenae* and *G. soricina* during its massive flowering (Chávez-Estrada et al., 2019). Nevertheless, our results indicate that both bat species do not prefer Bombacaceous nectar. The consumption of Bombacaceous nectar by the two bat species may be due to the large availability of flowers during mass flowering events rather than nectar quality.

Feeding time patterns

Feeding activity patterns also differed between species. The peaks of maximum feeding observed in our experiments agree with results previously found in the field. However, the duration of the feeding time increased compared to those found under a high density of bats (Chávez-Estrada et al., 2019). *G. soricina* performed higher feeding events around midnight, earlier than the peak maximum feeding time of *L. yerbabuenae*. Field studies have suggested that foraging activity peaks may be related to a higher production of nectar during the night (Horner et al.,1998), and differences in foraging behavior may promote the coexistence of bats with similar requirements of resources (Chávez-Estrada et al., 2019); however, in our experiments, nectar was available for the bats throughout the night. One possible explanation for this is that activity and foraging behavior are related not only to food characteristics but also to intrinsic characteristics, such as the energetic requirements of the bat species (Fragaszy et al., 2004). Another factor that could impact foraging behavior is the field

experience of individual subjects. In the tropical dry forest, uncertainty in resource availability is common, so experimental bats may become accustomed to this and overlook the consistent availability of food resources in the experimental setup. Our results suggest that other nectarivorous animals, such as hummingbirds or insects, may follow similar strategies to nectarivorous bats, optimizing their food preference and modifying their behavior. Thus, experimental and field tests are needed to test these hypotheses.

Conclusions

Our results support our initial hypothesis, and we consider that *Leptonycteris yerbabuenae* and *Glossophaga soricina* may coexist due to food preferences and foraging behavior. However, many factors can influence foraging behavior under natural conditions, including biotic interactions, food quantity and quality, and physiological demands (Ayala-Berdon et al., 2009; Laurindo 2017). Since our experiments were carried out under conditions of high resource abundance and low density of individuals, we suggest future experiments in which natural conditions are considered.

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Table 1(on next page)

Composition of artificial nectar solutions provided to the bats during each experiment.

Three sugar solutions were elaborated to emulate the nectar of three chiropterophilous plant species. Three sugar solutions containing different sugar types and water as control. All nectar solutions are based on Rodríguez-Peña et al. (2016).

Solution	Plant family	Nectar concentration (°Brix)	% Glucose	% Fructose	% Sucrose	Energy content in 100 g of solution (kcal)
Ipomoea ampullacea	Convolvulaceae	24.42	40.93	46.58	14.49	95.18
Ceiba aesculifolia	Bombacaceae	16.85	47.18	49.82	2.99	65.33
Acanthocereus occidentalis	Cactaceae	27.13	17.06	28.97	53.98	105.39
Glucose	-	20	100	-	-	75.00
Fructose	-	20	-	100	-	80.00
Sucrose	-	20	-	-	100	77.4
Water	-	-	-	-	-	0.00

1

2

Figure 1

Average frequency of visits per treatment.

Frequency of visits per treatment. Significant differences were found for Leptonycteris yerbabuenae (χ2= 218.28, df= 6, p<0.0001) and Glossophaga soricina (χ2= 371.36, df= 6, p<0.0001).

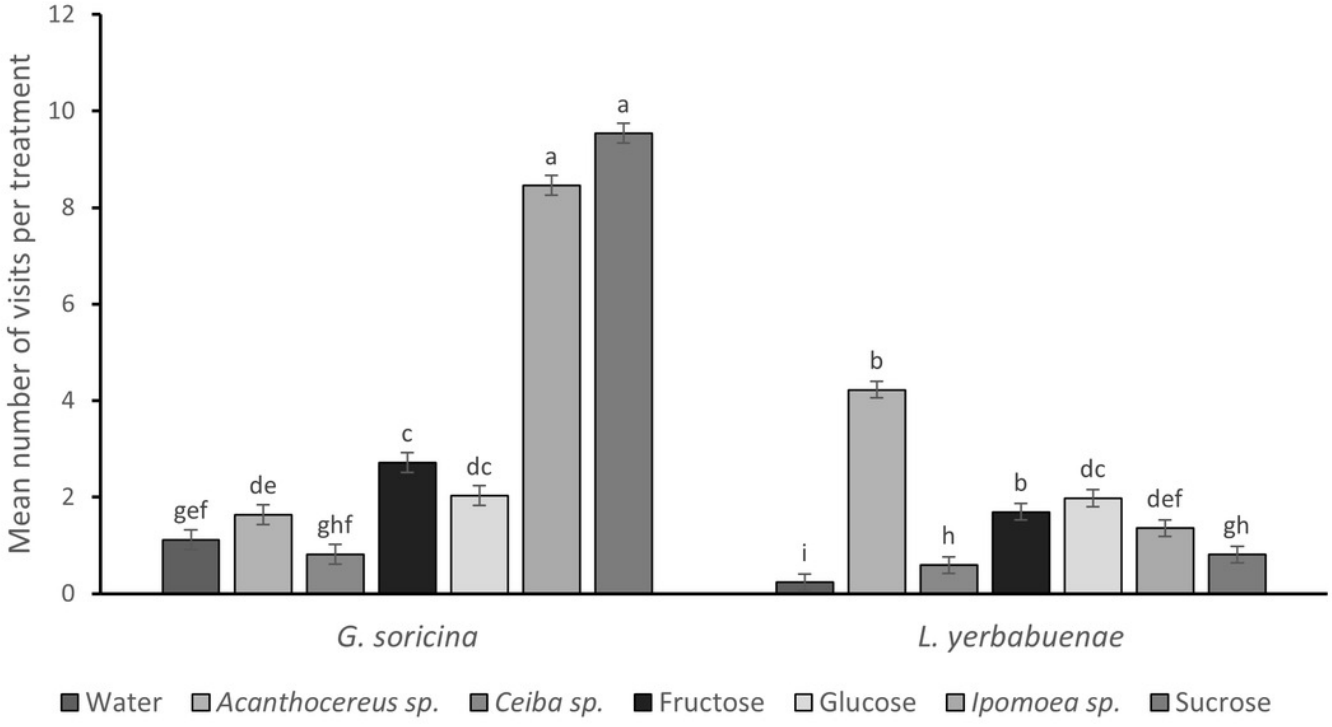

Figure 2

Average frequency of bat visits per hour.

Bat species fed from different solutions at different times. Leptonycteris yerbabuenae (χ2= 68.16, df= 11, p<0.0001), Glossophaga soricina (χ2= 98.46, df= 11, p<0.0001).

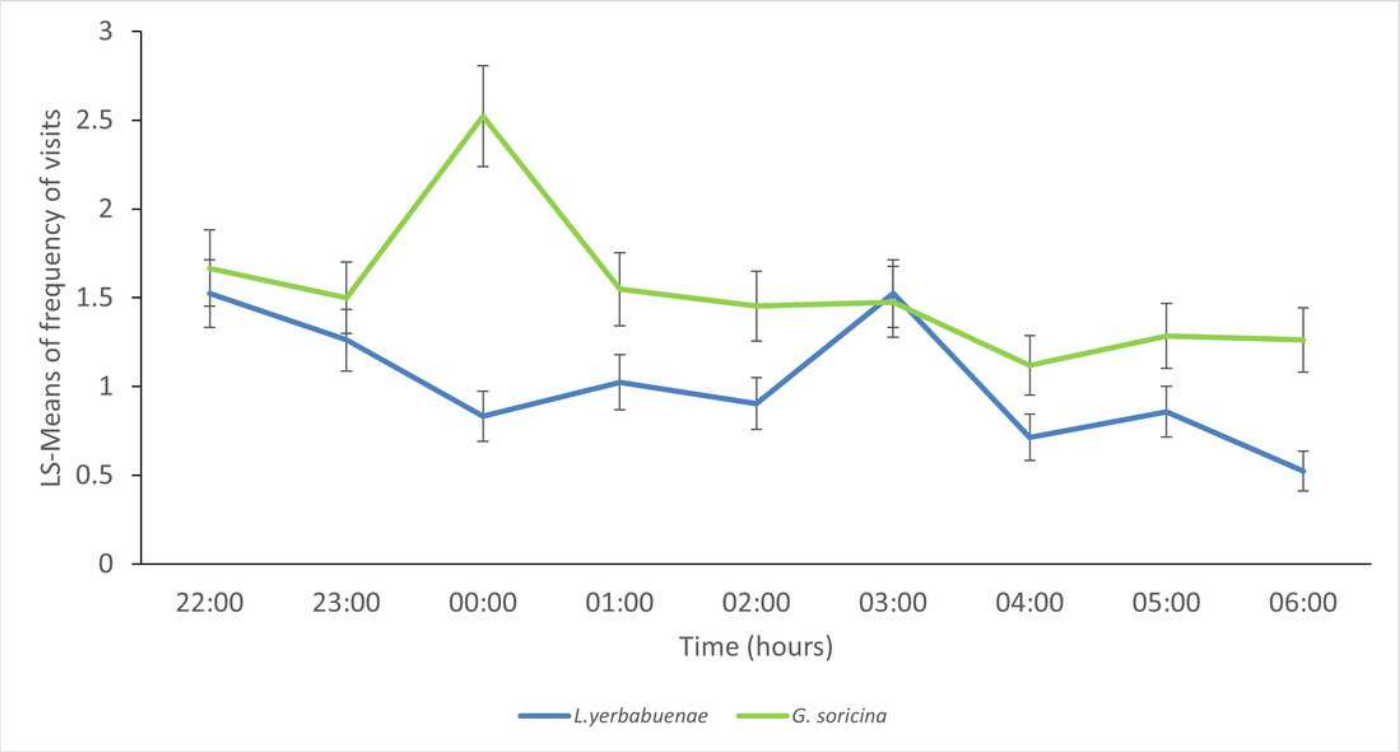

Figure 3

Alluvial plot showing the frequency of visits to each solution.

Foraging activity of Leptonycteris yerbabuenae (Ly) and Glossophaga soricina (Gs) to different sugar solutions at different times.

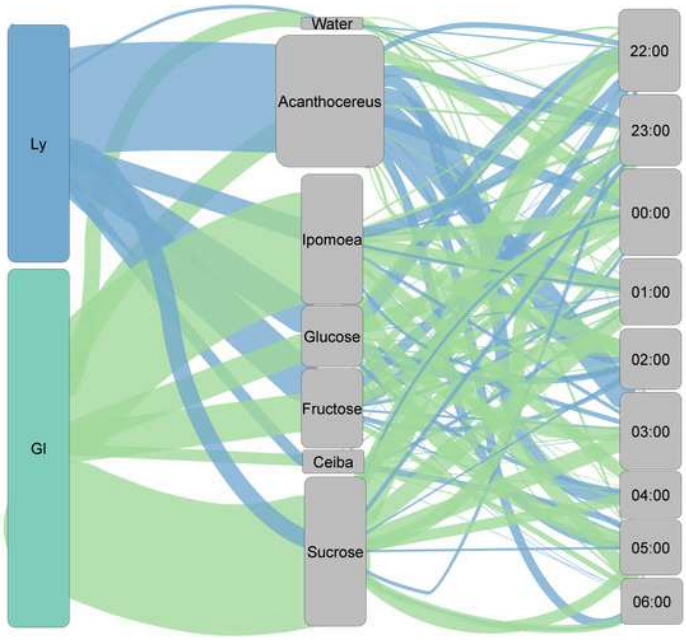

Figure 4

Specialization *d'* index for Leptonycteris yerbabuenae and Glossophaga soricina.

d' specialization index for each bat species based on visitation frequency to nectar feeders. Bars represent standard error obtained from a null model analysis.