

## RESEARCH ARTICLE

# Morphological specialization influences nectar extraction efficiency of sympatric nectar-feeding bats

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### SUMMARY

Mammals frequently use nectar as a supplementary food, while a predominantly nectarivorous lifestyle with morphological specializations for this feeding mode is rare within the class. However, Neotropical flower-visiting bats largely depend on nectar resources and show distinct adaptations to a nectar diet. Glossophagine bats form local guilds of 2–6 species that may differ distinctly in skull morphology. It is still unknown how and to what extent this morphological diversity influences the efficiency of nectar extraction and hence resource partitioning within the local bat guild. As foraging behaviour is a key factor for niche partitioning of co-existing species, we compared nectar extraction behaviour and efficiency at different flower depths among sympatric bat species with different degrees of morphological specialization (*Glossophaga soricina*, *Leptonycteris yerbabuena* and *Musonycteris harrisoni*). In flight cage experiments with artificial flowers, at deeper nectar levels all species showed a distinct decrease in the amount of nectar extracted per visit and an increase in the time spent hovering at the flower, indicating increased energetic cost when foraging on longer tubed flowers. The lowest nectar extraction efficiency ( $\text{g s}^{-1}$ ) was found in the small *G. soricina* and the highest in the largest species *L. yerbabuena*. However, when also considering the different energy requirements of the different-sized bat species, the morphologically most specialized *M. harrisoni* consistently showed the highest foraging efficiency. Our data suggest that the long rostrum and tongue of the extremely specialized *M. harrisoni* are probably not evolved for monopolization of co-evolved deep flowers but for allowing efficient access to the broadest range of the local chiropterophilous flower resources.

Key words: Glossophaginae, Phyllostomidae, foraging behaviour, daily energy expenditure.

Received 21 November 2011; Accepted 22 July 2012

### INTRODUCTION

Specializations on particular food resources are frequently correlated with morphological adaptations that aid in efficient foraging (Wainwright, 1996; Irschick and Losos, 1998; Vanhooydonck et al., 2000; Muchhala, 2006). In predominantly nectar-feeding animals, such specializations often include elongations of the feeding apparatus that aid in extracting nectar from floral tubes, such as long proboscises in butterflies, bumblebees and flies, or beaks of varying length and shape in hummingbirds (Wasserthal, 1993; Freeman, 1995; Wasserthal, 1996; Winter and von Helversen, 2003). Such morphological characteristics may facilitate access to floral resources, and the differences between co-existing species may contribute to resource partitioning within the local nectarivore guild. Many mammals (e.g. monkeys, opossums, procyonids, rodents) utilize nectar only as a supplementary food; therefore, morphological specializations to a nectar diet are rather rare within the class. A diet composed mainly of nectar is reported for the honey possum (*Tarsipes rostratus*) and also for some taxa of bats (Feldhamer et al., 2007). Distinct morphological specializations to a nectar diet are found within the Old World flying fox subfamily Macroglossinae (Pteropodidae) and especially in the Neotropical Glossophaginae (Phyllostomidae). Our objective was to determine whether and to what extent the different degrees of morphological specialization found among sympatric glossophagine species translate into differences in feeding efficiency.

The phyllostomid subfamily Glossophaginae (Chiroptera: Phyllostomidae) is a diverse group of ca. 40 species of bats that show distinct morphological, physiological and behavioural adaptations for feeding on floral nectar (Howell and Hodgkin, 1976; Gardner, 1977; Willig, 1983). Morphological adaptations include a long tongue with brush-like papillae, an elongated rostrum and reduced dentition (Freeman, 1995). These characteristics vary widely among the different species of glossophagines, from the generalist *Glossophaga* species to *Musonycteris harrisoni*, the glossophagine bat with the morphologically most specialized cranium (Tschapka et al., 2008). An important behavioural adaptation is the ability to extract nectar from flowers on the wing. Similar to the case in hummingbirds, hovering flight allows glossophagine bats to visit a larger number of flowers per time unit and therefore improves overall foraging efficiency (Tschapka and Dressler, 2002). Nectar-feeding bats, as well as hummingbirds, combine a high metabolic rate (Berger and Hart, 1974; Arends et al., 1995) with flight, an expensive mode of locomotion (Speakman and Thomas, 2003). These high energetic requirements are met by the consumption of large amounts of nectar. Consequently, glossophagine bats may consume up to 150% of their body mass in nectar per day (von Helversen and Winter, 2003). The daily energy expenditure (DEE) of a nectar-feeding bat determines the overall amount of energy an individual bat needs to obtain on a daily basis (von Helversen and Winter, 2003).

Foraging efficiency of glossophagine bats is highly selected for and may be influenced by the skull adaptations related to feeding and the characteristics of the nectar-feeding apparatus, mainly the tongue (Freeman, 1995; von Helversen and Winter, 2003), but also by body mass and wing morphology, which affect flight costs (Winter, 1998; Winter and von Helversen, 1998; Voigt and Winter, 1999; von Helversen and Winter, 2003). The ability to effectively use the local nectar resources depends crucially on the species' efficiency of nectar extraction from flowers with corollas of different shape (Nicolay and Winter, 2006).

On the west coast of Mexico, several species of nectar-feeding bats with a wide range of morphological specialization co-exist; namely, the small generalist *Glossophaga soricina* Pallas 1766, the endemic *M. harrisoni* Schaldach and McLaughlin 1960, an only slightly larger resident bat with highly specialized cranial morphology (Tschapka et al., 2008; Sperr et al., 2011), and the large, migratory *Leptonycteris yerbabuena* Martinez and Villa-R. 1940. We compared nectar extraction from artificial flowers at different depths among these three bat species to test the hypothesis that the species-specific morphology should influence their respective foraging efficiency. We expected that because of its highly specialized cranial adaptations and relatively small size *M. harrisoni* would be the most efficient of the sympatric nectar-feeding bats in the area.

## MATERIALS AND METHODS

### Animals and flight cage

The study was conducted in Callejones, Colima, Mexico (18°48'52.3"N, 103°37'59.3"W). Bats were captured with mist nets set in front of flowers. Body mass of glossophagines was recorded with a Pesola spring balance ( $\pm 0.5$  g); snout length was measured with a ruler as the distance between the end of the eyes and the tip of the lower lip ( $\pm 0.5$  mm). For the analysis of morphological parameters, we used data from all the individuals that we caught during mist netting (*G. soricina*,  $N=733$ ; *L. yerbabuena*,  $N=347$ ; *M. harrisoni*,  $N=20$ ).

Experiments were conducted with three glossophagine species that occur together over the entire year in the study area (Sperr et al., 2011) and represent the entire morphological and size range of the subfamily (Fig. 1A–C, Fig. 2): *G. soricina* ( $N=5$ ), *L. yerbabuena* ( $N=5$ ) and *M. harrisoni* ( $N=10$ ). Experiments were performed in a flight cage, consisting of an aluminium frame (4×4×3 m) covered by shade cloth with 70% light permeability. Prior to an experiment, a bat was kept in the flight cage for acclimation and training with *ad libitum* access to an artificial feeder containing honey water. Depending on the individual bat, this training phase lasted 1–2 nights before the experiment was started. After the experiments, bats were released unharmed at the site of capture. We followed all IACUC protocols recommended by the American Society of Mammalogists (Gannon and Sikes, 2007).

### Nectar extraction experiment design

The experimental setup consisted of a feeder (glass test tube, inner diameter 9 mm) filled with honey water of 17% sugar concentration. Sugar concentration was measured using a hand refractometer (Krüss Co., Hamburg, Germany; range 0–30% mass/mass). The feeder was placed on an analytical balance (Mettler Labstyle 152, precision 1 mg; Fig. 1D), which allowed us to record the amount of nectar removed by a bat during a single visit. An infrared light beam at the entrance of the feeder registered bat visits to the LPT port of a personal computer. The time of each status change of the light beam (interrupted to uninterrupted and *vice versa*) was logged with a

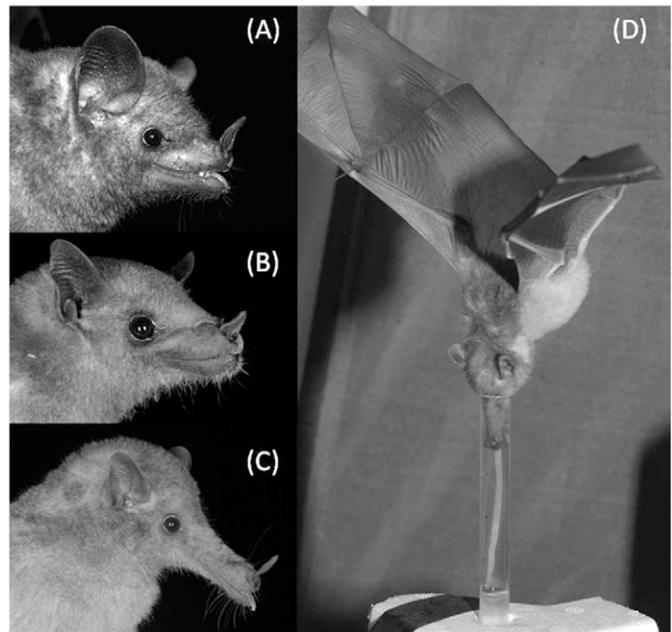


Fig. 1. Study species *Glossophaga soricina* (A), *Leptonycteris yerbabuena* (B) and *Musonycteris harrisoni* (C). (D) *Musonycteris harrisoni* hovering above the feeder. Note the long tongue reaching down to the fluid.

precision of 10 ms by a custom-written program (Turbo Pascal 5.0) into an ASCII file. Time differences between status changes were subsequently calculated using Excel 2007 and provided the duration of each hovering visit. We collected data on nectar removal and hovering duration at up to 7 different depths of nectar (from 1 to 7 cm), depending on the bat species. We recorded at least 10 visits for each individual bat for each depth, while manually maintaining the nectar level constant.

By combining the amount of nectar extracted (g) with the respective hovering duration (s) we obtained an index for the ratio between benefit and cost during a flower visit that in this paper we will call nectar extraction efficiency  $E$  ( $\text{g s}^{-1}$ ). However, as DEE of glossophagine bats increases with body mass (von Helversen and Winter, 2003), a given amount of nectar will contribute a different fraction to the daily requirements of different-sized species. The same amount of nectar covers relatively more of the daily energetic needs of a smaller compared with a larger species. For a biologically meaningful comparison of nectar extraction efficiency among the species, we therefore calculated standardized nectar extraction efficiency ( $E_s$ ) by dividing  $E$  by the specific DEE:

$$E_s = \frac{E_x}{DEE_x}, \quad (1)$$

where  $E_x$  is the efficiency of nectar extraction of species  $x$  and  $DEE_x$  is the daily energy expenditure of the same species. For calculating DEE ( $\text{kJ day}^{-1}$ ) we used the following equation (von Helversen and Winter, 2003):

$$DEE_x = 1555M_x^{0.755}, \quad (2)$$

where  $M_x$  is the body mass of species  $x$  in kg.

### Statistics

Statistics were calculated using SPSS Statistics 17.0 and SigmaPlot 11.0. As the morphological data were not normally distributed we

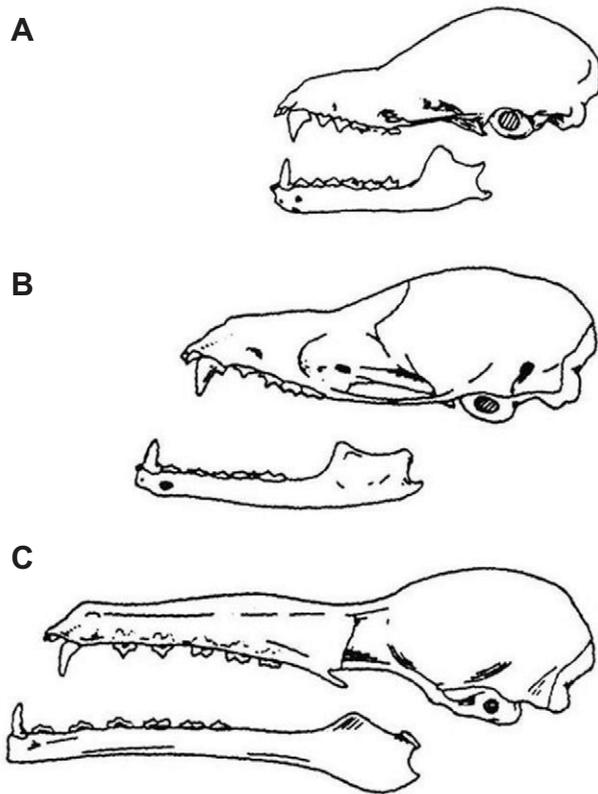


Fig. 2. Skulls and mandibles of the three study species: *G. soricina* (A), *L. yerbabuena* (B) and *M. harrisoni* (C). All skulls are drawn to the same scale (modified from Hall, 1981).

used a Kruskal–Wallis ANOVA to compare body mass, rostrum length and standardized rostrum length among the bat species. We used linear regressions to test for the effect of nectar level on the amount of nectar taken. We compared the time spent hovering and efficiency of nectar extraction among species using general linear mixed models (GLMMs; normal distribution, identity link function). Fixed effects were species, nectar level and species  $\times$  nectar level; individual bats were included as random effect. Dependent variables were hovering time, efficiency of nectar extraction or standardized efficiency of nectar extraction. Bonferroni corrections were used for *post hoc* pairwise comparisons.

## RESULTS

### Behaviour of bats during experiments

Before drinking at the feeder, bats often circled around it multiple times. Once they began visiting the feeder, bats would typically perform 2–3 visits within 1 or 2 min and then would return to their roosting place, resuming visitation after 10–15 min. Bats visited the

feeder throughout the night, but activity varied distinctly between individuals of all species. During the more than 1500 registered visits, all individuals of the three species used hovering flight for extracting the nectar from the artificial feeder. After initially contacting the test tube with their snout, bats lapped the nectar with their tongues while in hovering flight. With decreasing nectar levels, bats also inserted the rostrum into the tube (Fig. 1D). Bats with a longer rostrum were able to successfully extract nectar with a shallower insertion of the snout. Frequently, the animals closed their eyes while drinking.

### Morphological parameters of bats

Rostrum length differed significantly among the three species (Kruskal–Wallis ANOVA on ranks  $H=736.073$ ,  $d.f.=2$ ,  $P<0.001$ , *post hoc* test: all  $P<0.05$ ). *Musonycteris harrisoni* had the longest and *G. soricina* the shortest rostrum. We standardized the rostrum length to body length, in order to have an index of the degree of specialization per species. The three species have a significantly different standardized rostrum length (Kruskal–Wallis ANOVA on ranks,  $H=505.474$ ,  $d.f.=2$ ,  $P<0.001$ , *post hoc* test: all  $P<0.05$ ); after standardization *M. harrisoni* showed by far the greatest standardized rostrum length of the three species, while *G. soricina* and *L. yerbabuena* showed similar values. All three species also differed significantly in body mass (Kruskal–Wallis ANOVA on rank,  $H=728.518$ ,  $d.f.=2$ ,  $P<0.001$ , *post hoc* test: all  $P<0.05$ ; Table 1). The largest glossophagine bat was *L. yerbabuena* with more than twice the body mass of *G. soricina*, the smallest nectar-feeding bat in the area.

### Amount of nectar extracted per visit

Species with a longer rostrum extracted nectar down to deeper levels than species with a shorter rostrum. *Glossophaga soricina* successfully extracted nectar to a maximum depth of only 3 cm and *L. yerbabuena* barely reached 5–6 cm, while *M. harrisoni* successfully extracted nectar up to a depth of 7 cm (Fig. 3). All species showed a significant negative relationship between nectar level and amount of nectar extracted (Table 2). At shallow nectar levels, bats always extracted more nectar per visit than when having to reach deeper into the feeder. *Leptonycteris yerbabuena* had a very high extraction capacity at levels of 1–3 cm (0.16–0.19 g) but it decreased dramatically towards deeper nectar levels. At a level of 4 cm, this species extracted only a third of the value at 1 cm. *Glossophaga soricina* extracted the smallest amount of nectar at all depths. In *M. harrisoni* the decrease between subsequent levels was less pronounced than in the other two species, resulting in a distinctly lower slope in the regression model. *Glossophaga soricina* and *L. yerbabuena* showed extremely similar slopes, corresponding to the similar standardized rostrum length (Fig. 3, Table 2).

### Hovering duration

In all species, hovering duration showed a tendency to increase with increasing nectar depth, particularly towards the species' maximum

Table 1. Morphological characteristics and DEE of the three nectar-feeding bat species

Species	Rostrum length (mm)	Standardized rostrum length (rostrum length/total body length)	Body mass (g)	DEE (kJ day <sup>-1</sup> )
<i>Musonycteris harrisoni</i>	17.4 $\pm$ 1.8	0.23 $\pm$ 0.02	11.4 $\pm$ 1.2	53.1
<i>Leptonycteris yerbabuena</i>	10.1 $\pm$ 0.7	0.14 $\pm$ 0.01	24.26 $\pm$ 3.1	93.8
<i>Glossophaga soricina</i>	7.3 $\pm$ 0.6	0.12 $\pm$ 0.01	10.75 $\pm$ 1.4	50.8

Length and mass data are means  $\pm$  s.e.m.

Daily energy expenditure (DEE) was calculated after von Helversen and Winter, 2003.

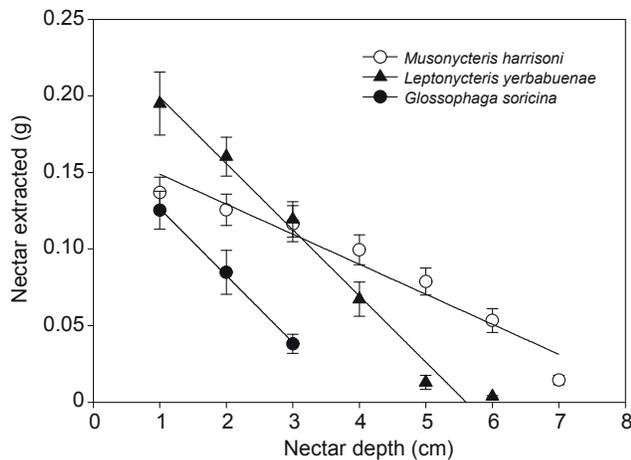


Fig. 3. Amount of nectar extracted (means  $\pm$  1 s.e.m.) per visit at different nectar depths, measured as the distance between the upper rim of the feeder and the nectar level. Lines are regression lines (see Table 2).

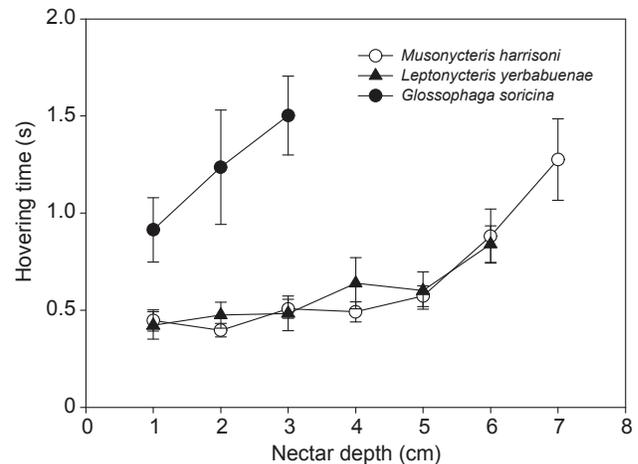


Fig. 4. Time spent in hovering flight for extracting nectar at different nectar depths (means  $\pm$  1 s.e.m.).

extraction depth (Fig. 4). We found a significant effect of species, nectar level and their interaction on the time each species spent hovering at the feeder (GLMM; species:  $F_{2,45.6}=3.294$ ,  $P=0.046$ ; depth:  $F_{1,81.3}=38.339$ ,  $P<0.0001$ ; species  $\times$  depth:  $F_{2,81.5}=5.711$ ,  $P=0.005$ ; AIC=47.608). The smallest species, *G. soricina*, consistently showed the longest visits, and hovered at all levels more than twice as long as *L. yerbabuena* and *M. harrisoni* (*post hoc* test: both  $P<0.0001$ ); however, there was a high degree of individual variation. *Musonycteris harrisoni* and *L. yerbabuena* invested similar hovering time at most nectar levels (*post hoc* test:  $P=1$ ; Fig. 4).

#### Nectar extraction efficiency

The efficiency of nectar extraction of a glossophagine bat (g nectar  $s^{-1}$  hovering time) illustrates the nutritional gain obtained by the animal per energetic investment in hovering flight. In all species, efficiency decreased towards deeper nectar levels and showed significant interspecific differences (Fig. 5). Both species and nectar level, as well as their interaction, had a significant effect on nectar extraction efficiency (GLMM; species:  $F_{2,74.3}=28.419$ ,  $P<0.0001$ ; depth:  $F_{1,82.8}=154.517$ ,  $P<0.0001$ ; species  $\times$  depth:  $F_{2,83.2}=19.146$ ,  $P<0.0001$ ; AIC=-239.664). *Glossophaga soricina* showed by far the lowest efficiency and differed significantly (*post hoc* test: both  $P<0.0001$ ) from the two larger species, which showed a comparable decline in extraction efficiency with decreasing nectar level. At shallow nectar levels, the relatively large *L. yerbabuena* showed more efficient nectar extraction, while *M. harrisoni* was more efficient than *L. yerbabuena* at levels deeper than 4 cm (Fig. 5).

After correcting for bat size through standardization of nectar extraction by DEE of the respective species (Table 1), the respective positions on the graph of the two larger species shifted distinctly

(Fig. 6). Both species and nectar level, but not their interaction, had a significant effect on standardized nectar extraction efficiency (GLMM; species:  $F_{2,79.0}=18.874$ ,  $P<0.0001$ ; depth:  $F_{1,84.1}=131.655$ ,  $P<0.0001$ ; species  $\times$  depth:  $F_{2,84.5}=0.017$ ,  $P=0.983$ ; AIC=-1055.236). There were significant differences in standardized nectar extraction efficiency between all species (*post hoc* tests: all  $P\leq 0.003$ ). The morphologically most specialized and comparably small *M. harrisoni* consistently showed the highest values of all species, the large *L. yerbabuena* was intermediate and the smallest *G. soricina* was at all depths less efficient than the other two species (Fig. 6).

#### DISCUSSION

Our experiments demonstrate differences in nectar extraction abilities among closely related, sympatric nectar-feeding bat species that are correlated with specific morphological specializations. While all species showed a distinct decrease in nectar extraction capability towards deeper nectar levels, they differed in the amount of nectar extracted, in hovering time at artificial flowers and also in nectar extraction efficiency. These differences may have a distinct influence on niche partitioning of sympatric species.

#### Morphological parameters of bats

Tongue length is an indicator of the degree of specialization among nectar-feeding bats, and generally is correlated with rostrum length (Nicolay, 2001; Winter and von Helversen, 2003). Tongue length imposes limits to the depth of the flowers a nectar-feeding bat can successfully visit. It also determines how deep the head has to be inserted into a flower when feeding (Nicolay and Winter, 2006). For more specialized nectar-feeding bats, at shallow nectar levels there is no need to insert the entire snout, which may allow them to keep alert and probably diminishes their predation risk (Nicolay,

Table 2. Linear regressions for the amount of nectar extracted and nectar level

Species	$R^2$	Model	$F$	d.f.	d.f. error	$P$
<i>Musonycteris harrisoni</i>	0.8671	$y=-0.0191x+0.1658$	74.389	6	47.156	<0.0001
<i>Leptonycteris yerbabuena</i>	0.9691	$y=-0.0438x+0.2436$	56.665	5	17.189	<0.0001
<i>Glossophaga soricina</i>	0.8823	$y=-0.0436x+0.1699$	17.521	2	8	0.001

Linear regressions were performed separately for each bat and then averaged per species to obtain values for  $R^2$ , intercept and slope. GLMMs with amount of nectar extracted as dependent variable, nectar level as fixed factor and bat as random factor were used to calculate values for  $F$ , d.f., d.f. error and  $P$ .

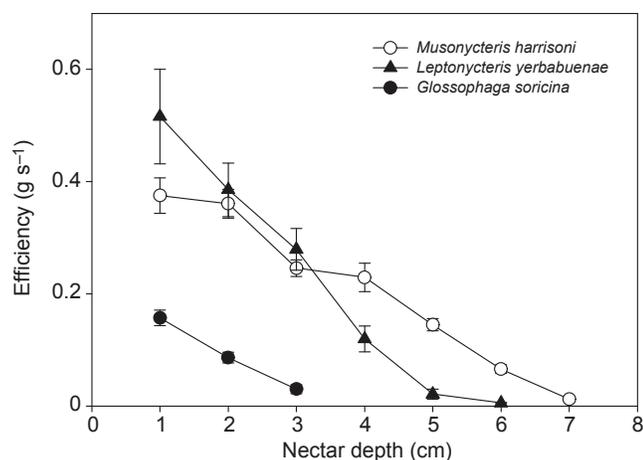


Fig. 5. Nectar extraction efficiency at different nectar depths (means  $\pm$  1 s.e.m.).

2001). In contrast, less specialized bats, such as *G. soricina*, need to insert the entire snout, often close their eyes even at shallow depths and spend more time in hovering flight in front of the flower. This combined with the fact that bats stop echolocating while drinking nectar from flowers could make them more vulnerable to predators.

#### Amount of nectar extracted per visit

All species of nectar-feeding bats showed the same basic pattern; a decrease in the amount of nectar extracted per visit with decreasing nectar levels. The same pattern was found in laboratory experiments combining nectar-feeding and frugivorous bats (Nicolay and Winter, 2006). However, that experimental setup included depths up to only 4 cm, which is clearly less than some of the flower depths available to the bats in our study area, e.g. 7–9 cm corolla depth in the cactus *Pachycereus pecten-aboriginum* (Tschapka et al., 2008).

Nectar-feeding bats have extremely high energetic requirements (Winter and von Helversen, 2003), so they need to consume a large amount of nectar every night, which is obtained during hundreds of flower visits. A small glossophagine drinks more than its body mass in nectar per night (von Helversen and Reyer, 1984; Winter and von Helversen, 1998). Bats feeding on deeper flowers need more visits to obtain a given amount of nectar, which results in higher energetic investment for foraging and therefore reduces net energy gain. Species able to extract more nectar at greater depths can reduce the number of visits needed to satisfy their daily energy requirements.

Although all species showed a negative correlation between the amount of nectar extracted and nectar depth, we found interesting differences between the species. While the regression slope was almost the same for *Glossophaga* and *Leptonycteris*, it was only half as high in the more specialized *Musonycteris*, so the relative reduction in the amount of nectar extracted was much less pronounced for *M. harrisoni* than for *L. yerbabuena* or *G. soricina*. Between 1 and 4 cm, the amount of nectar extracted by *M. harrisoni* per unit time dropped by only 26%, while that of *L. yerbabuena* diminished by 65%. This reduced yield at greater depths translates directly into foraging efforts: while *L. yerbabuena* may obtain a given amount of nectar during 1000 visits to flowers of 1 cm depth, it would need to visit 2857 flowers to consume the same amount of nectar when only visiting flowers of 4 cm depth. In contrast, in the same situation *M. harrisoni* would need to visit only 1351 flowers of 4 cm depth. Clearly, while both species must visit a higher number

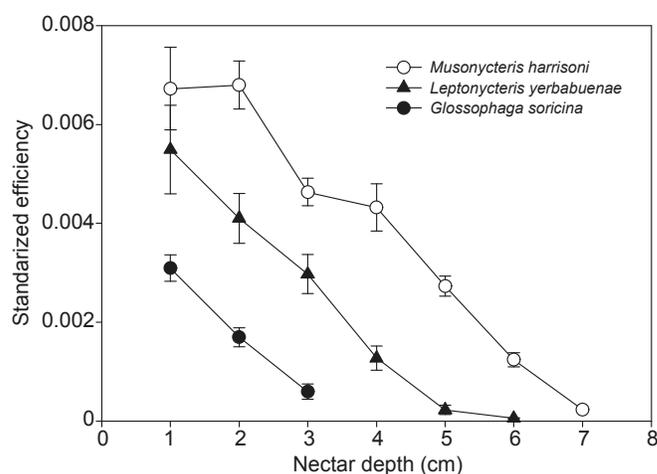


Fig. 6. Nectar extraction efficiency standardized by daily energy expenditure (DEE) at different nectar depths (means  $\pm$  1 s.e.m.).

of flowers with deeper nectar levels, the nectar extraction capability of *M. harrisoni* is less sensitive to increasing flower depths than that of *L. yerbabuena*.

#### Hovering time per visit

The time spent in expensive hovering flight at the flower represents a significant cost of the daily foraging budget for a nectarivore (Gass and Roberts, 1992). All nectar-feeding bat species in our experiments showed longer hovering times when extracting nectar from deeper flowers, probably due to compensating attempts to keep nectar intake per visit at a desired value. Foraging at deeper flowers therefore not only yielded less nectar but also required higher energetic investment than foraging at shorter flowers. While the increase in hovering duration with increasing flower depth was similar in *L. yerbabuena* and *M. harrisoni*, the former has nearly twice the body mass of the latter. As power input necessary for hovering flight increases with body mass (Voigt and Winter, 1999), the energetic cost for exploitation of deeper flowers is higher for larger species. Although hovering time was similar in the two species, the increase in flight cost with deeper flowers was more pronounced for *L. yerbabuena* than for the smaller *M. harrisoni*.

Increased hovering duration at deeper nectar levels near dawn has been shown in the field for nectar-feeding bats visiting the bromeliad *Werahia gladioliflora* in Costa Rica (Tschapka and von Helversen, 2007). Similar to the present experiments, these bats hovered longer at flowers with deeper nectar levels, probably in an attempt to extract nectar from almost empty flowers; thus, our experiments indeed reflect the natural behaviour of glossophagine bats. In flight cage pollination experiments, Muchhala and Thomson also found an increase in visit duration when bats were forced to visit longer flowers (Muchhala and Thomson, 2009).

#### Nectar extraction efficiency

Through a ratio of nutritional benefits (amount of nectar extracted) to energetic investment (hovering duration) we obtained a measure for the efficiency of nectar extraction. In all species the benefits diminished consistently with deeper flowers, while at the same time energetic investment increased, so overall feeding efficiency decreased noticeably towards deeper nectar levels. We found clear differences among the species, with *G. soricina* showing distinctly lower feeding efficiency than the other two species.

These species-specific differences became even more pronounced when considering the, biologically more relevant, standardized efficiency. An important variable in the energetic budget of nectar-feeding bats is body mass. The three species in our experiments differ distinctly in body mass and consequently also in DEE. Nectar-feeding bats keep their DEE relatively constant, even under different foraging conditions. von Helversen and Winter conducted laboratory experiments with *G. soricina* and manipulated the concentration of nectar (von Helversen and Winter, 2003). In these experiments the number of visits per night varied from 300 on 'high energy reward' nights to 2000 during 'low energy reward' nights. However, despite the large differences in the number of visits, the DEE remained largely the same. Analogous to these behavioural responses to changes in nectar concentration, a bat will have to visit more flowers when feeding at greater depth with diminished efficiency. Our method for standardization of nectar extraction efficiency by the species-specific DEE therefore not only considered the absolute nectar gain but also included the different energy requirements of each species, and allowed a direct comparison between the three sympatric nectar-feeding bats.

The morphologically most specialized, small *M. harrisoni* showed higher extraction efficiency than the other two species at all depths. For example, the efficiency of *M. harrisoni* at a flower depth of 4 cm was similar to that of *L. yerbabuena* at 2 cm, and its efficiency at 5 cm was the same as that of *G. soricina* at 1 cm. Because of its comparably low DEE, *M. harrisoni* can cover its daily energy budget with the same or even a fewer number of visits to flowers of 4 cm than *L. yerbabuena* feeding only at flowers of 2 cm depth.

Regular drinking from flowers deeper than 3 cm appears to be not very profitable for two of the three nectar-feeding bat species. *Musonycteris harrisoni*, however, may find a 'feeding refuge' in flowers of 4 cm and more, because it is the only species in the area that can extract nectar efficiently at great depths. A direct transfer of the size relationship from our study with standardized artificial feeders to natural flowers is not easy as it depends not just on flower depth but also on flower width, microstructure of the flower, location of nectar production, nectar viscosity, properties of the flower surface and probably also other factors. Nevertheless, the order of species-specific extraction efficiency established in our experiments should remain largely the same. Preliminary data from the two other, morphologically less specialized species of nectar-feeding bat found only in low numbers at the study site (the seasonal *Anoura geoffroyi* and the rare *Choeronycteris mexicana*) support our experimental results and show that *M. harrisoni* is the most efficient nectar feeder in the local guild (T.P.G.-T., personal observation), so our original hypothesis is confirmed. In similar experiments Nicolay and Winter found that *L. yerbabuena* always showed the highest nectar extraction efficiency, which was even greater than that of the morphologically more specialized *C. mexicana* (Nicolay and Winter, 2006). The authors suggested that the efficiency of nectar extraction in glossophagines may not necessarily always be associated with the morphological specialization of the cranium. It is worth mentioning that in their study the diameter of the test tubes used was 19 and 26 mm, which allowed the bats to insert not only the snout but also the head deep into the feeder and that the deepest nectar level tested was only 4 cm. Also, in their study they worked with bats that had lived over several years in captivity and were used to visiting an artificial feeder. In contrast, we used only naive bats that had spent no more than 2 nights in a flight cage and were accustomed to extracting nectar from a variety of flowers with different shapes in the field. Both factors might have influenced the performance and motivation of the animals. Therefore, a direct

comparison with our study is not easy. *Choeronycteris mexicana* may reach its tongue more than 65 mm into 9 mm test tubes (Winter and von Helversen, 2003) and thus has a tongue of similar length to that of *M. harrisoni*. It is feasible that in addition to the obvious cranial adaptations there are further, more subtle parameters, such as the ultra structure of the tongue (e.g. the amount and shape of filiform papillae) that may also contribute to differences in species-specific feeding efficiency.

#### Resource use and nectar extraction efficiency

Based on our results on standardized feeding efficiency, we can suggest which sort of floral resources are profitable for each species. If energy is limited and the cost-benefit ratio of foraging is critical, a pollinator should select which flowers to visit, and should avoid flowers where foraging costs are equal to or greater than the potential energy gain (von Helversen and Winter, 2003). Co-existing species of glossophagines can potentially choose from the same set of resources, among plants that may offer different cost-benefit ratios. This may result in a non-random use of nectar resources by different bat species and consequently in trophic structuring within the guild based on the energetic properties of the resources (Tschapka, 2004).

The most abundant nectar-feeding bat in the area is *G. soricina* (Gonzalez-Terrazas, 2008; Sperr et al., 2011), which has the lowest nectar extraction efficiency. In order to maintain a positive energy balance, *G. soricina* should exploit plants offering plenty of flowers and with easily accessible nectar. Field data from our study site showed that the most important natural floral resource of *G. soricina* during the dry season was the Capparaceae *Cleome spinosa*, a plant that presents nectar rather openly and occurs patchily and in high density, often along rivers and streams (Gonzalez-Terrazas, 2008; Sperr et al., 2011). *Glossophaga* spp. have the capability to change from a diet that consists mainly of nectar to one of insects and/or fruits, depending on the availability of resources (Petit, 1997; Herrera et al., 2001; Tschapka, 2004), which allows these small species to survive on alternative food types when nectar resources are scarce or when nectar extraction may be energetically too expensive.

*Leptonycteris yerbabuena* is the largest glossophagine bat in the area, and needs a higher amount of nectar to meet its daily energy budget. However, large species can fly faster and over larger distances than smaller species. An extended flight range allows foraging over a wide area, and exploitation of locally rich resources that are not depleted by the resident communities of smaller bats (von Helversen and Winter, 2003). Flowers of *C. spinosa* are also an important nectar source for *L. yerbabuena* in the area, which is consistent with our results, due to the plant providing easy nectar access and high density of flowers. Additionally, *L. yerbabuena* exploits the deep flowers of columnar cacti such as *P. pecten-aboriginum* with a flower length of 7–9 cm, which is possible as the external diameter of the flower is about 6 cm, allowing bats to insert the entire head into the flower (Molina-Freaner et al., 2004). *Pachycereus pecten-aboriginum* flowers produce large amounts of nectar (Molina-Freaner et al., 2004; Valiente-Banuet et al., 2004) that are out of reach for the smallest and most abundant *G. soricina*, so the cactus flowers are probably very profitable for *L. yerbabuena*. Over a large portion of its range, *L. yerbabuena* migrates in response to flower availability and leaves an area when nectar availability gets too low (Cockrum, 1991; Fleming et al., 1993; Fleming and Nassar, 2002). In the study area there is an influx of females during the dry season; however, the species is present in large numbers year round (Sperr et al., 2011).

In the same area where we conducted this study, Tschapka and colleagues performed an extensive study on the food plants used by *M. harrisoni* (Tschapka et al., 2008). They found that *M. harrisoni* visits at least 14 plant species during the annual cycle and that most of the important food plants have short corollas and present the nectar rather openly. The only long-tubed flowers used by *M. harrisoni* were those of the columnar cactus *P. pecten-aboriginum*; however, these are also commonly visited by *L. yerbabuena* (Valiente-Banuet et al., 2004; Gonzalez-Terrazas, 2008; Sperr et al., 2011). Our experiments add to the previous, more descriptive results: *M. harrisoni* has the ability to extract nectar from a large flower depth range more efficiently than all other nectar-feeding bat species in the area and may exploit shallow flowers, but it may also more efficiently access deeper or largely emptied flowers that are beyond the reach of *G. soricina* or *L. yerbabuena*.

In contrast to the other glossophagines, its elongated rostrum probably impedes feeding on non-liquid, harder resources, such as fruit or insects, when nectar resources are scarce (Petit, 1997; Herrera et al., 2001; Tschapka, 2004). The study area (and indeed the entire species range of *M. harrisoni*) shows a marked seasonality and the highest abundance of chiropterophilous flowers occurs during the dry season. We suggest that *M. harrisoni* is able to stay year round in the area because of its great capability to efficiently extract nectar from a wide range of flowers. The small body size of *M. harrisoni* keeps energetic needs low, while at the same time the extremely elongated feeding apparatus permits profitable nectar extraction even at flowers with less accessible nectar rewards (von Helversen and Winter, 2003) and allows *M. harrisoni* to persist with the few nectar resources in the area during the rainy season (Sperr et al., 2011).

### Evolutionary implications

Foraging efficiency plays a major role in shaping patterns of resource use (Wainwright, 1996; Irschick and Losos, 1998) and should be a major evolutionary factor within bat–flower interactions (von Helversen and Winter, 2003). In our experiments bats had only a single artificial flower at their disposal, and therefore were forced to visit it even at very deep nectar levels that are difficult to feed on. However, in their natural habitat bats may choose among a range of flowers of different length. As nectar-feeding bats experience significantly diminished extraction efficiency at long-tubed flowers, they should prefer the shorter-tubed flowers within a plant population, while long flowers should be avoided and selected against. This effect is probably even more pronounced when bats can choose not only among individual flowers within one species but also between flowers of different species that show greater differences from each other. Energetic considerations should therefore render a tight co-evolution between species of long-tongued bats and long-tubed flowers unlikely. An interesting exception might be represented by the amazing glossophagine *Anoura fistulata* from the Northern Andes that has, as a result of unique morphological adaptations, the longest tongue of all Glossophagine bats (Muchhala, 2006) and is the only visitor of long-tubed *Centropogon nigricans* (Campanulaceae) flowers. Perhaps speciation and co-evolution happened in this case in a very specific situation, e.g. under isolation within a small mountain range in the Andes with very few chiropterophilous plants to choose from.

In conclusion, because of the distinct energetic restrictions it is likely that the long rostrum and tongue of *M. harrisoni* serve mainly to allow access to nectar from the broadest range of flowers available to bats in an area, but not for monopolization of a tightly co-evolved flower with a particularly deep calyx. The only long-tubed flowers in the study area are the flowers of columnar cacti, while most other

bat-pollinated flowers are short tubed and provide much easier nectar access (Sperr et al., 2004). In order to further understand factors influencing flower preferences and the dynamics within nectar-feeding bat guilds, it would be interesting to complement our data on feeding efficiency from simplified flowers (test tubes) with experiments using real flowers that differ not only in depth but also in shape, internal nectar distribution and nectar quality (volume, sugar concentration).

### ACKNOWLEDGEMENTS

Special thanks go to all the people who made our research in Callejones possible and enjoyable (Nena, Doña Eva, Don Javier, Doña Lilia, the Chapkitas and many more). To all the adventurous people that helped during field work, especially to the ever-present Tonatiuh Ruiz, muchas gracias. The Camioneta del Diablo deserves a special mention for (more or less) keeping up during the entire field work. Osiris Gaona provided technical and logistical support. Scientific collecting permits were provided to R.A.M. by the Secretaría del Medio Ambiente y Recursos Naturales (FAUT-0001). This is a contribution of the EcoHealth Alliance and of BIOCONCIENCIA, A.C. The manuscript was finalized while R.A.M. was on sabbatical at the Arizona-Sonora Desert Museum.

### FUNDING

This work was supported by Consejo Nacional de Ciencia y Tecnología [SEMARNAT-2002-01-C01-00357 to R.A.M.], Disney Worldwide Conservation Fund [to R.A.M.] and a grant from Bat Conservation International [to T.P.G.-T.].

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