Trophic niche flexibility in *Glossophaga soricina*: how a nectar seeker sneaks an insect snack

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Summary

1. Omnivory enables animals to fill more than one trophic niche, providing access to a wider variety of food resources with potentially higher nutrient value, particularly when resources become scarce. Animals can achieve omnivory using different strategies, for example opportunistic foraging, or switching between multiple trophic niches.

2. The Neotropical bat *Glossophaga soricina* (Pallas, 1766) is a common and widespread species known for nectar feeding, but it also eats fruit and insects. Approaching stationary objects (flowers and fruits) or moving objects (insects) poses different sensory tasks and should require different echolocation behaviours. Here we tested the contrasting hypothesis that *G. soricina* can approach both stationary and moving objects using the same echolocation behaviour, thus feeding at different trophic levels by a single sensory mechanism.

3. Using DNA barcoding, we demonstrate that *G. soricina* eats beetles (Coleoptera), flies (Diptera) and noctuid moths with bat-detecting ears. Laboratory observations show that *G. soricina* actively hunts for prey so insect consumption does not appear to be opportunistic. After capture, individuals consumed prey while perched and manipulated them with jaw, thumb, wrist and wing movements, but food handling was longer and chewing rate slower than in obligate insectivores.

4. In contrast to most insectivorous bats, the echolocation calls of *G. soricina* are of high frequency and low intensity, and *G. soricina* did not produce feeding buzzes when approaching insects. An acoustic model of detection distances shows that its low-intensity calls fail to trigger the auditory neurons of eared moths, allowing *G. soricina* to overcome auditory prey defences.

5. Individuals achieved niche flexibility using a unique but generalist behavioural approach rather than employing two different specialist methods. Our findings provide a novel insight into the functional mechanisms of insect capture in *G. soricina* and highlight the importance of considering niche flexibility in classifying trophic links in ecological communities.

Key-words: acoustic modelling, bat, molecular scatology, predator–prey, stealth echolocation

Introduction

Morphological and sensory characteristics determine a species' ability to exploit available food resources, and increasing specialization may restrict access to increasingly smaller subsets. The spatial and temporal availability of food strongly influences individual species and the patterns of their interactions, for example, between competitors for resources and between predators and prey (reviewed in Martin & Pfennig 2009). In response to limited resources, selection can lead to generalist strategies and niche flexibility to exploit alternative resources (Grant & Grant 1987; Tebbich *et al.* 2004). Alternatively, resource limitation can drive the evolution of morphological or behavioural

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specialization when competition leads to disruptive selection for individuals who specialize on less common but under used resources (Martin & Pfennig 2009).

Omnivory is very common and frequently evolves from a more specialized ancestor and usually requires physiological and morphological adaptations (Diehl 2003). Omnivory can be driven by many factors, including: limitations of a qualitatively superior food, the necessity to diversify in order to achieve adequate nutrition, to avoid toxins, to follow changing resource distributions or to minimize foraging risks (Singer & Bernays 2003). While these factors may cause specific changes in resource use, many omnivores simply employ a generalist strategy by consuming anything they can capture. In that case, their diet is defined by the frequency with which they encounter particular food items (Rosenheim & Corbett 2003; Bastille-Rousseau *et al.* 2011). In this context, omnivory as a result of random encounters is likely to be common. In contrast, omnivory can also be a result of switching between two highly specialized behaviours or sensory modalities, particularly due to spatial–temporal variation in food resources (Singer & Bernays 2003). Bat assemblages may contain specialists and omnivores as well as species that specialize on a primary food type while retaining omnivorous capabilities. The latter strategy is particularly common among the New World Leaf-nosed bats (family Phyllostomidae), for which it has been argued that dietary flexibility is partly responsible for the taxonomic and trophic diversity within the family (Rex *et al.* 2010).

For frugivorous and nectarivorous bats, variation in fruiting time and nectar availability may limit resources. This is particularly true for bats thought to specialize on nectar and pollen, and many nectarivores exhibit species-specific responses to food restrictions. For example, when nectar availability is low in the Caribbean wet forest of Costa Rica, resident *Glossophaga commissarisi* may switch to a frugivorous diet while the sympatric *Hylonycteris underwoodi* exploits the remaining flowers, and *Lonchophylla robusta* and *Lichonycteris obscura* track resources between habitats (Tschapka 2004). More dramatically, in Mexico, *Anoura* and *Glossophaga*, particularly *G. soricina,* may be seasonal insectivores, switching between trophic levels (Howell 1974).

G. soricina (Phyllostomidae, subfamily Glossophaginae) is abundant from southern Mexico to northern South America (Alvarez *et al.* 1991) and is an efficient and well-studied nectarivore that also eats fruit and insects (Howell 1974; Alvarez *et al.* 1991; Herrera *et al.* 2001; Tschapka 2004). Accounts of the ecology of *G. soricina* have alternatively described this species as mostly frugivorous (Willig 1986), a seasonal insectivore (Howell 1974), an obligate nectarivore (Heithaus, Fleming & Opler 1975) and an omnivore (Henry & Stoner 2011), depending on when and where the populations were studied (Howell 1974). Some studies have also reported sex-based variation in the use of insects (Herrera *et al.* 2001). The published evidence suggests that *G. soricina* can flexibly switch between its trophic niches, but has not suggested a functional mechanism.

The elongated rostrum and tongue and hovering flight of *G. soricina* suggest that it is well adapted to a nectarivorous lifestyle, although its morphological adaptations are less extreme than those of some its relatives [e.g. *Anoura fistulata* (Muchhala 2006) or *Musonycteris harrisoni* (Tellex & Ortega 1999)]. Moreover, its well-developed incisors and ectoloph molars (Howell 1974) are retained from insectivorous ancestors (Santana, Strait & Dumont 2011) and may increase the range of food it can consume. Glossophagine bats have been described as having one of the most specialized diets of all mammals (Kelm *et al.* 2008) and may have specific adaptations such as the ability to accommodate hyperglycaemia (Kelm *et al.* 2011). Nevertheless, nectar is a relatively poor source of nutrition, and nectarivores must supplement their diet. Finding and feeding on stationary food sources such as nectar-bearing flowers and fruit present similar sensory challenges, but detecting and capturing moving insects require entirely different strategies.

Insectivory has been documented in *G. soricina* (e.g. Howell 1974; Alvarez *et al.* 1991; Herrera *et al.* 2001; Tschapka 2004), but the echolocation behaviour involved in detecting and tracking insect prey has not been well described [though see a preliminary description provided by Howell (1974)]. Fruit- and nectar-feeding bats such as *Glossophaga* likely detect stationary fruit and flowers using multimodal information including scent, echolocation and spatial memory (e.g. Henry & Stoner (2011), Winter, von Merten & Kleindienst (2005), Thiele & Winter (2005) and Carter, Ratcliffe & Galef (2010)). At least 1000 plant species are bat-pollinated and many have evolved characteristic strong, multidirectional echo signatures that can guide foraging bats, such as *Glossophaga*, to nectar and/or advertise its presence (von Helversen & von Helversen 1999, 2003; Simon *et al.* 2011). Thus, the typical echolocation task for nectarivorous, and perhaps frugivorous bats, is to detect an object that has specifically evolved to be conspicuous.

In contrast to feeding on stationary resources, the detection, identification and tracking of small moving targets such as insects, often in cluttered environments, pose different demands on the sensory system. The echolocation behaviour of foraging insectivorous bats typically consists of three phases. During the search phase, echolocation calls are spaced at regular intervals until an insect is detected. During the approach phase, bats increase the rate of calling as they approach the target. The approach phase is followed by a feeding 'buzz' composed of extremely rapid calls often with two distinct subphases. In buzz 1, call rate can rise to 100 calls per s while in buzz 2, call rate can increase to $\geq$160 calls per s (Kalko & Schnitzler 1989; Schnitzler & Kalko 2001; Elemans *et al.* 2011).

To escape from predatory bats, tympanal ears evolved in seven orders of insects at least 19 times independently (Yager 1999). Noctuid moths are a classic, well-studied example. They possess ears with just two sensory cells and a two-staged evasive response consisting of directional flight away from distant bats and erratic flight in response to close bats (e.g. Roeder 1967; Fenton & Fullard 1979;

Conner & Corcoran 2011). Despite their ears, the European bat *Barbastella barbastellus* largely feeds on noctuid moths. It uses a unique form of 'stealth' echolocation by emitting echolocation calls of low intensity that do not trigger the bat-detecting ears of noctuid moths at distances where the bat can detect the moths (Goerlitz *et al.* 2010).

It is not clear how, or even if, *Glossophaga* uses echolocation to locate insects. Many plant-visiting phyllostomids, including *Glossophaga*, use low-intensity echolocation during foraging to limit the returning echoes from dense vegetation clutter (Brinklov, Kalko & Surlykke 2009). These species are sometimes called 'whispering bats' (Griffin 1958), though they are capable of producing high-intensity calls (Brinklov, Kalko & Surlykke 2009). This raises the possibility that *Glossophaga* either uses its low-intensity calls to detect both stationary and moving food items, or that it employs an alternative acoustic strategy (e.g. alternating between low- and high-intensity calls) for stationary vs. moving targets. The first possibility suggests flexibility in the application of a single strategy and would represent a unique foraging strategy among bats; the second would require a substantial switch in echo-acoustic behaviour [omnivory in the context of exploiting two different specializations (Singer & Bernays 2003)].

Here we tested the hypothesis that *G. soricina* uses a unique form of echolocation when hunting insects that differs from the strategy employed by 'typical' insectivorous bats and that individuals achieved niche flexibility using a unique generalist behaviour rather than by exploiting two different specialist methods. Here we define a 'typical' insectivore as one that uses high-intensity calls and produces a feeding buzz in the final phase of an attack and feeds only within its insectivorous niche. Given the relatively poor diet afforded by nectar alone and previous documentation of insect consumption, we predict that insects, including eared moths, are a consistent part of *G. soricina*'s diet and that its echolocation and attack behaviours enable it to prey on insects. Specifically, we predict that its hunting style may resemble the stealth-like echolocation to date reported only from *B. barbastellus* rather than the high-intensity echolocation of a typical insectivorous bat. We used field surveys and molecular analyses to determine the frequency and types of insects consumed by *G. soricina*. With a captive colony of bats, we measured the intensity of echolocation calls and documented the acoustic and behavioural techniques used by individuals to detect, capture and consume insect prey. Using neural measurements of hearing in eared noctuid moths and our measurements of echolocation, we modelled maximum detection distances for this predator–prey system.

Materials and methods

DIETARY ANALYSIS

We caught individual *Glossophaga soricina* using mist nets in the Area de Conservación de Guanacaste, Costa Rica, during a 7-week period from late May to early July 2009. We placed individual bats in cloth bags for *c*. 1 h and froze guano produced during that period. For molecular analysis, we soaked each guano sample in 95% ethanol until it could be easily fragmented using sterile forceps. We removed individual insect fragments following Clare *et al.* (2009, 2011) and stored them separately in 96-well plates containing ethanol. We evaporated the ethanol in a 56 °C incubator for 60 min and added 45 μL of lysis buffer and 5 μL of proteinase K to each fragment, and the samples were again incubated for 12–18 h. DNA was extracted using an automated glass fibre technique (Ivanova, deWaard & Hebert 2006). We amplified a 648-bp target region of the mitochondrial cytochrome oxidase *c* subunit 1 (COI) gene using the primers LepF1 ATTCAACCA ATCATAAAGATATTGG and LepR1 TAAACTTCTGGATGT CCAAAAAATCA following Hebert *et al.* (2004). We visualized PCR products on a 1·2% agarose gel and sequenced each product unidirectionally using LepF1 following Hebert *et al.* (2004).

We edited sequences using Sequencher v.4.5 (Gene Codes, Ann Arbor MI, USA) and manually aligned the sequences in BioEdit v.7.0.9 (Ibis BioSciences, Carlsbad, CA, USA). We constructed a neighbour-joining tree of recovered sequences using the K2P parameter using MEGA v.4 (Tamura *et al.* 2007). Sequences are available from the authors on request.

We compared each sequence against the available reference sequences derived from arthropods from the same study location that were present in the Barcode of Life Data System (Ratnasingham & Hebert 2007). We assigned identifications following the 'strict' method outlined by Ross, Murugan & Li (2008), where sequences that matched a reference sequence (using both sequence similarity and phylogenetic considerations) without a close match to any other species were identified as deriving from that species. In the absence of an exact match, we recorded the percentage of the best match but consider these preliminary hypotheses of identification only (see Clare *et al.* (2011) and Bohmann *et al.* (2011) for a discussion of sequence identification).

For each prey species identified, we measured the prey surface area to determine the acoustic target strength of prey items, which was used in a model of echo-based detection distance (below). We measured surface areas from photographs of pinned preserved specimens (*n* = 1 per species) captured at the same site as the wild bats were caught. Since insect size varies between individuals, we used images with mean wing size in the image data base for Caterpillars of the Area de Conservación de Guanacaste (http://janzen.sas.upenn.edu/caterpillars/database.lasso). Surface area measurements were made using the software ImageJ (http://rsbweb.nih.gov/ij/index.html) after manually outlining the shape of the prey wings and body.

BEHAVIOURAL EXPERIMENTS

We conducted behavioural experiments with a captive colony of *G. soricina* held at the University of Bristol. This colony had been in captivity for many generations and fed an exclusively plant-based diet and were thus 'naive' to insects as prey. We conducted behavioural experiments in a flight room (1·8 × 2·4 × 2·8 m), which housed the entire colony (five males and 10 females) and was kept at a constant photoperiod of 12 h light starting at midnight followed by 12 h of dark starting at noon and was temperature and humidity controlled (24 °C, 70% relative humidity). The bats were kept in these conditions for years before the start of the behavioural trials so were habituated to the conditions. The bats were normally offered fresh nectar at the start of the dark photoperiod. The behaviour of the bats indicated that they anticipated the offering of new food when 'dark' commenced. On experimental days, rather than new food, we presented them with mealworms at the same location that they normally found nectar encouraging them to view these as 'food'.

We recorded insect attacks and captures for 1–1·5 h on nine consecutive days by hanging live mealworms on monofilament 10 cm under an ultrasonic microphone in front of an infrared camera. Echolocation behaviour was recorded with a calibrated microphone (CO-100K; Sanken, Tokyo, Japan) on a computer running Avisoft Recorder software (Avisoft Bioacoustics, Berlin, Germany), and flight behaviours were recorded using an infrared camera (Swann SecuraView SW244-SVM, Milton Keynes, UK) connected with a Sony MiniDV handycam digital video recorder (DCR-TRV9E, Sony, Tokyo, Japan). We synchronized recordings using a broad-spectrum sound recorded both on the camera and the microphone. During experiments, we watched attacks in real-time on a video screen and noted the time of each attack for later reference. We viewed recorded video files in IMovie 7.1.4 (Apple Inc., Cupertino, CA, USA) to classify attacks as 'flower like' (if the bat paused and hovered) or 'insect like' (without hovering) and analysed the audio files in SASLab Pro (Avisoft).

We compared the pulse interval (PI; end of one call to end of next call) of call sequences during insect attack between *G. soricina* and field recordings of the obligate insectivore *Myotis lucifugus* (family Vespertilionidae) using the last 11 calls of each approach (i.e. the 'buzz 2' phase of an attack by a typical insectivorous bat). We chose to compare *G. soricina* to the obligate insectivore *M. lucifugus* because they are similar in size, the foraging calls and feeding behaviour of *M. lucifugus* are exceptionally well-studied, and it employs the 'typical' insectivore tri-phasic echo-acoustic foraging strategy. We specifically chose to compare *G. soricina* with an insectivore from another family because we suspect that many other phyllostomids may use the same strategies as *G. soricina* making comparisons within the family a potential confounding variable. Recordings of *M. lucifugus* were made over many years of field surveys in Ontario (Canada) as full spectrum wav files using a single channel Avisoft UltraSoundGate 116 CM16/CMPA (Avisoft).

ECHOLOCATION CALL PARAMETERS

To measure call peak frequency, duration and source level of search phase echolocation calls, we trained seven free-flying *G. soricina* to approach a feeder next to a microphone and tracked their three-dimensional positions with video monitoring. We had each individual fly alone in a flight room (2·98 × 1·8 × 2·39 m) for up to 2·5 h, allowing it to feed on sugar water from the feeder at one narrow end of the room. Bats approached the feeder along the long axis of the room, starting from a roost positioned at the opposite end of the room. Two rows of echo reflectors (seven poles per row, each pole of 2 m height and separated by 30 cm from the next one) were placed along the long sides of the room at a distance of 20 cm to the walls. A calibrated microphone (CO-100K; Sanken) was mounted above the feeder to record the calls of the approaching bats. It was angled at 32° downwards with its membrane 3·2 cm above the feeder. We recorded the amplified (QuadMic; RME, Haimhausen, Germany) microphone signal at 500 kHz sampling frequency using a high-speed soundcard (USB-6251; National Instruments, Austin, TX, USA) and Avisoft Recorder software (Avisoft). Calls were band-pass-filtered (30–160 kHz) and analysed in SASLab Pro (Avisoft) to obtain peak frequency (frequency with the highest intensity, 512 FFT), call duration and peak-to-peak level. Using the peak-to-peak level and the bat position (see below), we calculated the peak-equivalent call source level (dB peSPL re. 20 µPa RMS, that is, the RMS level re. 20 µPa of a sinusoid with the same peak-to-peak amplitude as the call) for each call, referenced to 10 cm in front of the bats mouth, by correcting for geometrical and atmospheric attenuation (temperature 24 °C, 70% relative humidity) of sound from the bat to the microphone.

We tracked flight paths in three dimensions (Goerlitz, ter Hofstede & Holderied 2009) based on frame-synchronized interlaced videos recorded at 25 frames per s (50 half-frames per s) on a video recorder (XM-DVR; Datatoys, Brown Deer, WI, USA) at 720 × 576 pixel resolution, using two calibrated video cameras (WAT-525EX; WATEC, Tsuruoka, Japan) equipped with IR lenses (Cinegon 1·8/4·8 mm; Schneider Kreuznach, Bad Kreuznach, Germany) under continuous IR illumination (850 nm, Raymax 100; Raytec, Northumberland, UK). We mounted the cameras above and below the feeder and monitored the air space between the feeder and roost. The microphone signal (see above) was also recorded on both audio channels of the video recorder at 44·1 kHz sampling frequency, generating a clipped signal that was used for synchronization between video and high-speed sound recordings. We manually marked bat positions in the videos using custom-written software for MATLAB (The Mathworks Inc., Natick, MA, USA), which were then converted into three-dimensional positions, using VMS software (www.geomsoft.com, Shortis & Harvey 1998). The flight paths were interpolated with cubic smoothing splines to obtain bat positions for each emitted call.

MODEL OF MAXIMUM DETECTION DISTANCES

We used echolocation call intensity and prey surface area measurements (above) to model maximum detection distances of prey echoes by bats and of bat calls by prey (Goerlitz *et al.* 2010), using a bat hearing threshold of 20 dB SPL and tropical climate (25 °C, 90% relative humidity). Since most identified prey were noctuid moths (see Results below), we chose fixed hearing thresholds of 55 and 75 dB SPL for the moth's A1 and A2 cell, respectively, which corresponds to the lowest thresholds measured for any noctuid moth species (Fullard 1982, 1984, 1988; Goerlitz *et al.* 2010; ter Hofstede *et al.* 2011), thus biasing our model in favour of the moths. The lowest known threshold belongs to the temperate moth *Noctua pronuba* (ca. 850 mm² surface area, ter Hofstede *et al.* 2011).

Since the moths eaten by *G. soricina* had smaller surface areas (ranging from 272 to 772 mm², see Results) and thus presumably higher auditory thresholds than those of *Noctua pronuba* (Surlykke *et al.* 1999), using this low threshold biased our model even further in favour of the moths. Since thresholds for *N. pronuba* were recorded for audiogram stimuli of 20 ms duration, we corrected them for the shorter call duration by −1·85 dB (mean for 'small' and 'large' moth species in Tougaard 1998) per halving of duration.

Moth target strengths (TS; echo attenuation relative to impinging sound at a fixed distance) were calculated from the measured surface area as:

$$TS \text{ (in dB)} = 10*\log(\text{surface area (in mm}^2)) - 39,$$ derived from the regression line for 100 kHz in Surlykke *et al.* (1999) and were on average $-12\cdot5 \pm 1\cdot8$ dB (mean ± SEM) at 10 cm distance from the moth.

We calculated the average maximum detection distance for the bat using the means of source level, peak frequency and call duration of *G. soricina* and target strength of moths. To obtain error bars for the average maximum detection distances, we used the standard errors of source level, peak frequency, call duration and target strength to obtain the upper and lower boundaries of the SEM of the maximum detection distances. To calculate the upper boundary, we added the SEM to the mean of source level, call duration and target strength and subtracted the SEM from the mean frequency, thus obtaining call parameters resulting in a larger detection distance. Likewise, to calculate the lower boundary, we subtracted the SEM from the mean of source level, call duration and target strength and added the SEM to the mean of frequency, thus resulting in a shorter detection distance.

We modelled detection distances for both A1 and A2 auditory nerve cells of moths. These cells have different response thresh-

olds; the sensitive A1 cell is thought to cause directional flight away from distant bats, while close bats trigger the less sensitive A2 cell and are evaded by erratic escape flight (Roeder 1967).

Results

DIETARY ANALYSIS

We caught 112 *G. soricina* (73 females, 39 males) from which we obtained 38 faecal samples. Of these, 25 (66%) were solid and contained primarily insect material while the others were liquid indicating nectar consumption. Of the recovered insect DNA sequences, half showed similarity to Coleoptera and Diptera, but could not be conclusively identified from available references sequences. The remaining sequences were identified as eight species of Lepidoptera from the tympanate family Noctuidae (Fig. 1). Surface areas of identified moth species were *Parachabora abydas* (272 mm^2), *Coscaga picatalis* (304 mm^2), *Coxina hadenoides* (512 mm^2), *Mimophisma de-*

lunaris (772 mm^2), *Magusa orbifera* (396 mm^2) and *Spodoptera latifascia* (668 mm^2) (images for other species could not be obtained).

APPROACH, ATTACK AND CONSUMPTION BEHAVIOUR

During the course of the behavioural experiment, we observed dozens of approaches, attacks or near attacks on mealworms by captive *G. soricina*. Of these, 23 met the criteria of having synchronized video and call recordings that could be quantified. Examination of video playback allowed us to classify 16 attacks by our criteria for 'insect like', while the remaining 7 showed a distinct hovering pause and were classified as 'flower like'. Mean and last PI (Fig. 2) of the final eleven calls in an attack were significantly longer in *G. soricina* (mean$_{PI}$ = 21·8 ms SD ± 16·4, last$_{PI}$ = 25·8 ms SD ± 24·9), than in *Myotis lucifugus* (mean$_{PI}$ = 6 ms SD ± 1·3, last$_{PI}$ = 5·6 ms SD ± 0·3) ($z = -13·411$, $P < 0·001$; $z = 1·67$, $P = 0·008$, respectively).
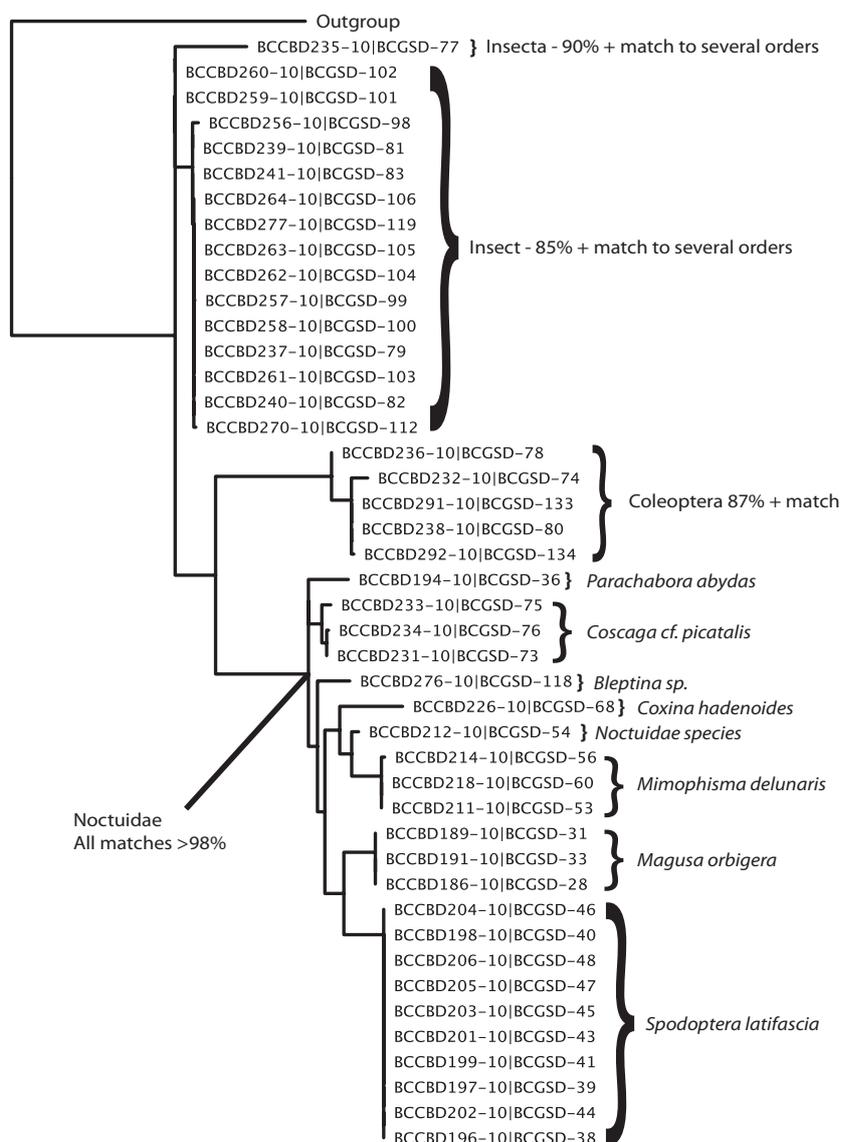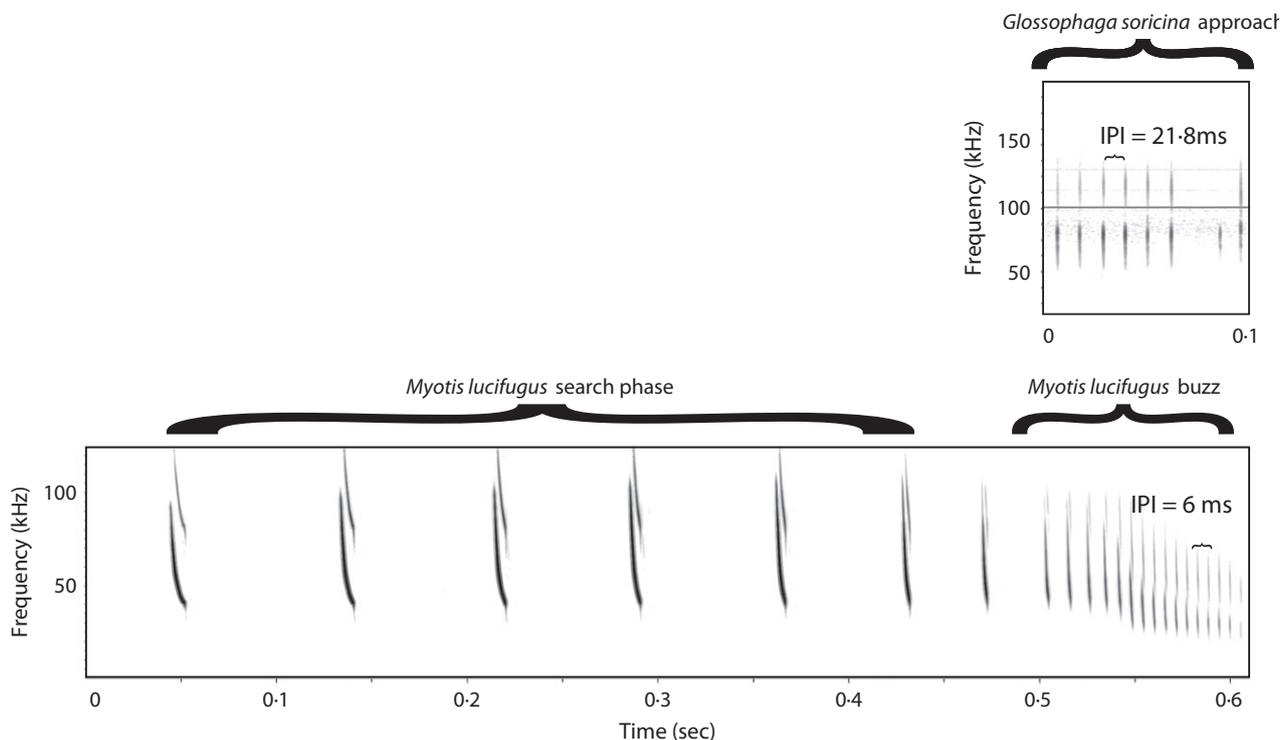

Fig. 1. Neighbour-joining tree of identified insects consumed by *Glossophaga soricina*.

Fig. 2. During an attack on an insect, the buzz phase call of a typical insectivore such as *Myotis lucifugus* consists of short duration calls at decreasing intervals until the capture. The pulse interval between elements in the approach phase of *Glossophaga soricina* is significantly longer.

Search call source level was 92.3 ± 3.4 dB peSPL re. 20 µPa RMS at 10 cm from the mouth, peak frequency was 90.2 ± 9.9 kHz and duration 1.3 ± 0.4 ms (mean $\pm$ SD of individual means, $n = 7$ individuals).

We were opportunistically able to observe the consumption of the mealworm (both visually, $N = 5$, and in video recordings, $N = 2$, when we went in to replace a mealworm (see Movie S1 in Supporting information)). These individuals roosted to consume prey rather than eating on the wing and extensively manipulated their prey using both thumbs and the membrane near the wrists (see Movie S1, Supporting information). Consumption time for a mealworm was calculated from the video recordings as a minimum of 157 s in the first individual (measured from when we first observed the bat chewing to consumption) and a minimum of 360 s in the second individual. The rate of chewing was observed to range from 2.5 to 3.5 mouth movements per s (measured for 15-s intervals between manipulations with the wings or digits).

ACOUSTIC MODEL

Based on echolocation call parameters of *G. soricina*, we calculated maximum detection distances of moth echoes by the bat and of bat calls by moths (Fig. 3). Maximum detection distances are a function of call source level and differ between bats and eared prey (Fig. 3a). This is because of differences in hearing thresholds between bats and moths and the one-way travel distance of sound from the bat to the moth for eliciting a prey response vs. the two-way sound travel distance of the returning echo for the bat to perceive the prey. Bats have larger detection distances than prey at low source levels (below parity, i.e. the point where both perceive the other at the same distance), whereas prey have larger detection distances at high source levels (above parity, Fig. 3a). Both *G. soricina* and the moth's A1 cell detect each other at about the same distance of 1.5 m (Fig. 3b). In contrast, the detection distance of bat calls by the A2 cell is much smaller at <0.5 m. It is thus unlikely that moths will start erratic escape flight before *G. soricina* has already detected the moth.

Discussion

Niche flexibility provides the opportunity for species to exploit alternative resources, particularly in times of resource limitation. We used a variety of approaches, including field surveys, captive behavioural experiments, acoustic analyses and genetic methods to assess niche flexibility in *G. soricina* and describe the behavioural and acoustic mechanisms of insect capture and the species of insects targeted. Our results suggest that *G. soricina* actively targets insects using a non-visual aerial hawking approach which resembles that of purely insectivorous bat species, except for its low-intensity calls and lack of a final buzz. This allows the bat to effect a stealth approach towards insects without eliciting an erratic escape flight by prey with auditory-based defences. This behaviour is similar to the
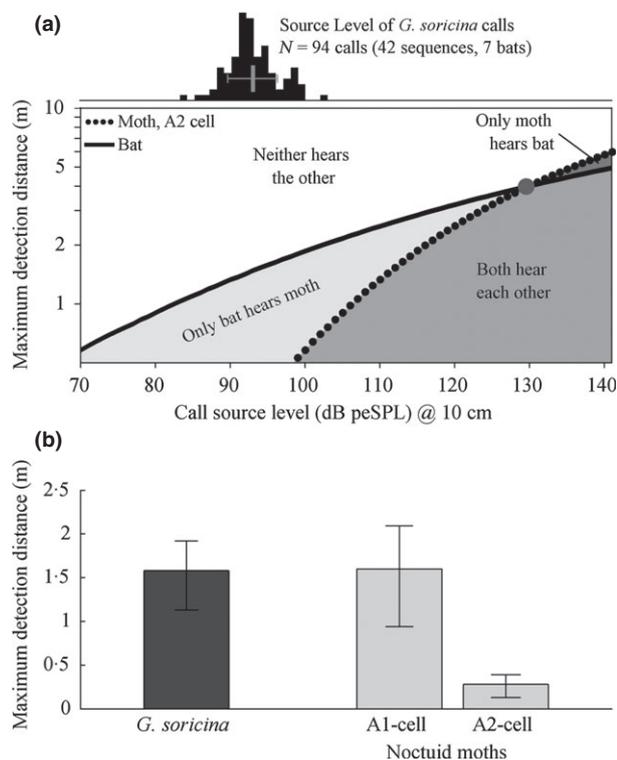
Fig. 3. Maximum detection distances of moths and bats. (a) Detection distances of bats (solid) and moths (dotted) depend on call source level. At the source level of *Glossophaga soricina* (histogram), bats detect moths before the A2 cell detects the bat and elicits an escape response. The large dot where the solid and dotted lines cross indicates the source level where both moth A2 cell and bat would detect each other at the same time (parity). (b) Mean (± SEM) maximum detection distances by *G. soricina* and the A1 and A2 cell of Noctuid moths.

stealth approach mechanisms known only from *Barbastella barbastellus* in Europe. It allows *G. soricina* to exploit two trophic niches without switching foraging mechanisms; a tactic that has not previously been documented in bats.

Insects composed a large portion of the diet of *G. soricina* in Costa Rica (66% of the faecal pellets sampled contained insects). Furthermore, 43% of faecal samples (*n* = 75) collected from *G. soricina* in Belize contained insect fragments (E.L. Clare, unpublished data) while the rest contained only nectar or pollen. However, only a third of captured individuals produced a faecal pellet. It is possible that all other individuals had consumed only nectar (and thus did not readily produce a pellet). In this case, insectivory would be practiced by a minimum of 22% of the population; however, we suspect this to be an underestimate. First, many bats do not produce a faecal pellet in the short period they are held in captivity (regardless of their feeding niche), thus failure to produce solid guano pellets with insect fragments does not mean they did not consume insects (holding time was limited by permitting requirements). Secondly, in some cases, we were able to tell when a bat had consumed nectar as they produced a faecal pellet, but the consistency was different and no insect fragments were visible, thus lack of a faecal pellet cannot be

used as evidence for nectarivory rather than insectivory, it is best classed as unknown. These findings are in agreement with Rex *et al.* (2010) who assigned *Glossophaga* from several localities at a high trophic level based on nitrogen isotopes.

Only half of the insects were identified to species level, as the reference data base for the area is comprised almost exclusively of Lepidoptera. All of the identifiable prey were noctuids. The rest were likely Coleoptera and Diptera, in which a sense of hearing is not as widespread as in noctuids and thus can be caught without specialized echolocation. Species of insect identified in the diet of *G. soricina* were not associated with bat-pollinated flowers (Daniel Janzen, personal communication), supporting our conclusion that *G. soricina* actively hunts insects rather than opportunistically consuming prey present on flowers while the bat is feeding on nectar. The relative proportions of prey in the diet cannot be assessed using molecular techniques [see Clare *et al.* (2011), Bohmann *et al.* (2011) and supplemental material for a discussion of quantification in molecular diet analysis from Bohmann *et al.* (2011)]. However, the data do show that *G. soricina* consumes noctuid moths, although it is certainly not a noctuid specialist.

The consumption of insects by nectarivorous vertebrates is well known (e.g. hummingbirds, sunbirds (Kelm *et al.* 2011)) and likely provides much needed dietary supplements. Kelm *et al.* (2008) indicated the importance of supplementing nectar diet to replace missing protein and fibre. Ganzhorn *et al.* (2009) tested the protein content of fruits in the neotropics and determined that they contained sufficient protein to support primates without additional supplementation, whereas fruits in Madagascar were relatively protein deficient and unlikely to provide adequate nutritional supplementation. Ganzhorn *et al.* (2009) proposed that this was a significant driving factor in the success and radiation of frugivores in Central and South America. If the inability of nectar to provide sufficient nutrition underlies omnivory in chiropteran nectarivores such as *Glossophaga*, it would be of interest to explore the potential physiological adaptations of species such as *Anoura fistulata* (Muchhala 2006) or *Musonycteris harrisoni* (Tellex & Ortega 1999), which exhibit extreme morphological adaptations for nectarivory. The physiological adaptations of the gastrointestinal system of *G. soricina* to process insects are also of interest. Kelm *et al.* (2008) reported that *G. commissarisi* has a restricted ability to process fruit (compared to nectar) and implicated metabolic, physiological and behavioural limitations. It remains interesting then that they can consume insects (and presumably extract nutrition). The mechanisms of this process represent an excellent avenue for research.

In our opportunistic observations, captive *G. soricina* always roosted before eating mealworms. Our estimates of the time required to consume a mealworm (>360 s in at least one case) was much longer than that estimated for a similarly sized typical obligate insectivore *Myotis lucifugus* [*c.* 66 s (Fraser & Fenton 2007)], perhaps due to their

much slower chewing rate (2·5–3·5 mouth movements per s vs. *c.* 6 mouth movements per s in *M. lucifugus* (Fraser & Fenton 2007)). As observed for fruit consumption (Dumont 1999; Santana & Dumont 2009), *G. soricina* primarily chewed with its back molars. Bite force in *G. soricina* (and other nectarivorous phyllostomids) is lower than predicted by body size, and its teeth have reduced shearing crests relative to insectivorous phyllostomids (Dumont *et al.* 2011; Santana, Strait & Dumont 2011). These features may be associated with its longer food-handling times and slower chewing rates. *G. soricina* manipulated prey with the thumb and wing membrane, pressing items into the wing (see Movie S1, Supporting information) in a way that is reminiscent of how some insectivores use the tail membrane as a bowl to hold prey fragments while roosting to consume prey (e.g. *Myotis septentrionalis*, ter Hofstede, Ratcliffe & Fullard 2008). We also observed theft behaviour between colony members.

Our colony had been in captivity for many generations and was housed in a closed room behind a double-door system where exposure to insects was unlikely. Their immediate and consistent response to the introduction of insect prey suggests that insectivory and associated behaviours may be innate in *G. soricina*, though additional experimentation is required to fully test that hypothesis. Yet, capture attempts increased over time, which might suggest that some learning (either through repeated attempts or social learning) was involved that increased the willingness to exploit a novel food source.

Stealth echolocation, that is, low-intensity echolocation calls not readily detected by eared prey, was described as a novel adaptation giving the vespertilionid bat *Barbastella barbastellus* in Europe an advantage for the capture of eared moths (Goerlitz *et al.* 2010). Similarly, we predicted that the high-frequency, low-intensity calls of *G. soricina* measured here (and common within the family Phyllostomidae) would not be detected by the A2 cell of eared prey, whose activity is correlated with the initiation of erratic escape flight behaviours. As such *G. soricina*, and potentially other low-intensity phyllostomids, appears to exploit this advantage to capture prey. We conducted our call-level measurements in a flight room, and many bats reduce the intensity of their calls in confinement. For example, the closely related *Macrophyllum macrophyllum* reduced its call intensity by 5–10 dB when it was in a flight cage (Brinklov, Kalko & Surlykke 2010). This suggests that our measurements of intensity may underestimate the call intensity of *G. soricina* in the wild. To compensate for this, our model is deliberately biased in favour of the moth by choosing very sensitive hearing thresholds of 55 and 75 dB SPL for the A1 and A2 cell. Therefore, our model is a deliberately conservative approach, which likely underestimates the actual echo-acoustic advantage that *G. soricina* has over its eared prey. Even given these biases, the model predicts that more than a 30-dB intensity increase would be required in wild bats to trigger a response in the noctuid A2 cell at the same distance as the moth is detected

by the bat (parity Fig. 3). Given this, slightly higher source levels expected in wild bats would not significantly change the outcome of our model or our conclusions about *G. soricina's* ability to sneak up on eared prey.

We compared the approach phase calls of *G. soricina* (family Phyllostomidae) to those of *Myotis lucifugus* (family Vespertilionidae) because the noted dietary flexibility of phyllostomids (e.g. Santana, Strait & Dumont 2011) suggests that many of these species may also employ undocumented atypical acoustic strategies, thus making comparisons within the family questionable. While in our comparison we did not observe a typical feeding buzz in *G. soricina* approaches, this is not an obligate trait among insectivores (e.g. Fenton & Bell 1979) and would not preclude capture success in the wild. Our observations suggest that the regular approach characteristics of *G. soricina* using call groups are efficient for localizing moving prey and that similar strategies for both nectarivory and insectivory may be used. Rather than being a disadvantage, the low-amplitude echolocation used by *G. soricina* (and many phyllostomids) may provide a distinct advantage over the acoustic defences of insects. While *G. soricina* did not employ the feeding buzz, they did use similar echolocation for localizing insects as that reported for approaching stationary nectar-feeding stations. The pulse interval measured here during insect attack (mean = 21·8 ms) is similar to the pulse interval recorded for *G. soricina* approaching a feeder [10–50 ms < 0·5 m from a feeder (Drapeau 2012)]. Pulse interval decreased linearly with the distance to the feeder and with increasing clutter [e.g. mean of 26·6–29·5 ms (Drapeau 2012)], but did not reach the pulse interval characteristic of a typical insectivorous buzz (Fig. 2). *Glossophaga soricina* thus achieved niche flexibility using a unique generalist behavioural approach rather than exploiting two different specialist methods.

Of the many hypotheses advanced to explain omnivory (e.g. Singer & Bernays 2003), omnivory in *Glossophaga* is likely driven by a combination of the nutritional requirements to augment a nectar diet with nutrients from insect prey (Kelm *et al.* 2008) and the requirement to compensate for resource limitations (Howell 1974; Tschapka 2004). As nectarivory is a derived trait (Wetterer, Rocman & Simmons 2000), this omnivorous behaviour follows the trend proposed by Diehl (2003) of evolving from a more specialized (in this case insectivorous) ancestor. The echo-acoustic mechanism to achieve omnivory, however, is thus far unique, suggesting an independently evolved behavioural technique.

Our observations suggest a new avenue for research. Most frugivorous and nectarivorous phyllostomids are known to eat some insects (Rex *et al.* 2010), though no mechanisms to detect and capture insects have been suggested. The strategies identified here in *G. soricina* may, in fact, be widespread within the family. We predict that the characteristic low-intensity echolocation known from many phyllostomids provides a consistent advantage over insect prey; thus, other phyllostomids may also exploit this

generalist strategy to achieve trophic niche flexibility. It will be particularly interesting to determine whether other frugivorous or nectarivorous phyllostomids use a feeding buzz in pursuit of insect prey.

In conclusion, low-intensity echolocation combined with highly manoeuvrable flight (Sharon Swartz, personal communication) makes the nectarivorous *G. soricina* surprisingly well suited for insectivory, confers greater niche flexibility than previously understood and may actually make them particularly well adapted to exploit eared insect prey. *Glossophaga soricina* is best classified as a highly flexible omnivore that employs novel acoustic strategies for resource exploitation across trophic levels. Our analysis of *G. soricina* provides a model for exploring insectivory among other nectar bats.

Acknowledgements

Data accessibility

Data deposited in the Dryad repository: doi:10.5061/dryad.n7j27 (Clare *et al.* 2014).

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Supporting Information

Additional Supporting information may be found in the online version of this article:

Movie S1. A movie depicting insectivory by captive *Glossophaga soricina*.