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Frequency alternation and an offbeat rhythm indicate foraging behavior in the echolocating bat, *Saccopteryx bilineata*

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Abstract The greater sac-winged bat, *Saccopteryx bilineata* (Emballonuridae), uses two distinct echolocation call sequences: a 'monotonous' sequence, where bats emit ∼48 kHz calls at a relatively stable rate, and a frequency-alternating sequence, where bats emit calls at ∼45 kHz (low-note call) and ∼48 kHz (high-note call). The frequencies of these low–high-note pairs remain stable within sequences. In Panama, we recorded echolocation calls from *S. bilineata* with a multi-microphone array at two sites: one a known roosting site, the other a known foraging site. Our results indicate that this species (1) only produces monotonous sequences in non-foraging contexts and, at times, directly after emitting a feeding buzz and (2) produces frequency-alternating sequences when actively foraging. These latter sequences are also characterized by an unusual, offbeat emission rhythm. We found significant positive relationships between (1) call intensity and call duration and (2) call intensity and distance from clutter. However, these relationships were weaker than those reported for bats from other families. We speculate on how call frequency alternation and an offbeat emission rhythm might reflect a novel strategy for prey detection at the edge of complex habitat in this ancient family of bats.

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Abbreviations

dB SPL RMS	Decibel sound pressure level re. 20 μPa root mean square
PF	Peak frequency

Introduction

The greater sac-winged bat, *Saccopteryx bilineata*, belongs to the family of sheath-tailed bats (Emballonuridae), a pantropical group comprising approximately 90 extant species the origins of which date back to the Eocene (Teeling et al. 2005). Found from Mexico to Brazil and on the islands of Trinidad and Tobago (Yancey et al. 1998), *S. bilineata* weighs ∼10 g and forages in a variety of habitats (Bradbury and Vehrenkamp 1976; Kalko 1995; Jung et al. 2007) on wings well suited to edge habitats and semi-open spaces (Norberg and Rayner 1987). *S. bilineata* is insectivorous and takes a wide range of small to medium sized prey (Bradbury and Vehrenkamp 1976; Yancey et al. 1998). Unlike the more recently derived molossids and vespertilionids, most emballonurids, including *S. bilineata*, emphasize the second, rather than first, harmonic in their echolocation calls. We note that over some of its range, *S. bilineata* uses slightly lower call frequencies (e.g. Biscardi et al. 2004) than those we report below.

S. bilineata is unusual among echolocating bats in that it uses two distinct call sequences. One is a remarkable frequency-alternating sequence, where bats emit pairs of calls: a first call at ∼45 kHz followed by a second call at ∼48 kHz. Call intervals between pairs are roughly

1.5 times longer than intra-pair intervals (Pye 1980; Barclay 1983; Jung et al. 2007). Peak frequencies (maximum energy) of the first and the second call remain stable within a sequence. The second sequence type is monotonous, characterized by calls with frequencies ∼48 kHz emitted at a relatively steady rate (Pye 1980; Jung et al. 2007). Monotonous sequences are regarded as typical of many echolocating bats while they are in transit or prior to detecting prey (Simmons et al. 1979; Surlykke and Moss 2000). Greater sac-winged bats are more often observed emitting frequency-alternating sequences than they are monotonous sequences (Barclay 1983; Kalko 1995; O'Farrell and Miller 1997; Jung et al. 2007).

Several hypotheses have been proposed to explain the existence and predominance of the frequency-alternating echolocation call sequences, none of which is necessarily mutually exclusive (Jung et al. 2007). Here, we divide them into two classes: (1) sensory hypotheses with a functional explanation for alternating sequences pertaining to echolocation itself, and (2) social hypotheses, suggesting a secondary social function. We consider monotonous sequences under roughly these same classes.

With respect to sensory hypotheses, call frequency alternation may help bats avoid ambiguity in call-echo matching, because returning echoes are effectively 'marked' as echoes originating from either 45 or 48 kHz calls. For example, echoes returning from a 45 kHz call should be recognizable to a bat even if a 48 kHz has been emitted in the intervening period (Behr and von Helversen 2004; Jung et al. 2007). In contrast, bats using monotonous sequences are not expected to be able to reliably match echo to call if an audible echo returns after a subsequent call has already been emitted (Schnitzler and Kalko 2001). Another hypothesis, which has been proposed in only the most general of terms, is that *S. bilineata* produces frequency-alternating sequences when hunting for prey and monotonous sequences when in transit between roosts and foraging sites (Pye 1980). Other sensory hypotheses have been put forward [e.g. two closely spaced calls of different peak frequency (PF) increase effective bandwidth] but have since been refuted (see Barclay 1983; Jung et al. 2007 and references therein).

Possible social functions for alternating sequences include acoustic partitioning (Jung et al. 2007). However, adding a 45 kHz call for the purpose of partitioning acoustic channels seems unlikely. On the one hand, no sympatric emballonurid's echolocation calls in Central America typically produce calls with peak frequencies around 48 kHz (see Jung et al. 2007). On the other hand, a 45 kHz call is close to those used in the monotonous sequences of *Balantiopteryx plicata* (∼43 kHz) and *Centronycteris centralis* (∼41 kHz) (Jung et al. 2007). Furthermore, by adding, for example, 51 kHz calls to

sequences with 55 kHz calls, the acoustic channels used by congeneric *S. leptura* are brought closer to those frequencies used by *S. bilineata*. Typical interpretations predict sister species should instead select channels further apart from one another if acoustic partitioning was a driving force in their evolution (Kingston and Rossiter 2004).

Whereas *S. bilineata's* unusual frequency-alternating sequences have garnered much attention from researchers in the context of foraging behavior, the function of the monotonous sequences have not, to our knowledge, been as thoroughly considered. As the monotonous sequences are typically associated with bats flying near to the roost (e.g. Pye 1980; Jung et al. 2007) their relative simplicity may reflect a greater reliance on spatial memory (Ratcliffe et al. 2005), reduced demand for prey-background resolution and/or serve an as yet unknown secondary social function when flying in familiar space in the presence of familiar individuals (Fenton et al. 2002).

In this study, we used a multi-microphone array to record flying *S. bilineata* at two locations, one close to a known roost where bats flew close to vegetation and the ground, and one nearby a known foraging patch where bats flew slightly higher and further away from background (E. K. V. Kalko, unpublished data). Our purpose was plain: to document the differences, if any, between the (1) echolocation call designs and (2) call emission patterns used at the two sites with respect to the following parameters: (1) call frequency, duration, and intensity and (2) call interval, pattern and flight speed. We then input call intensity data to a simple acoustic model and estimated the intensity of echoes returning to a bat based on the bat's estimated distance from (1) the array (as proxy for foremost background), (2) virtual insects in front of the array and outside of the call-echo-overlap zone (Schnitzler and Kalko 2001), (3) virtual background from which echoes would return as the call window closes and (4) virtual insects from which echoes would return at this same point in time (Holderied and von Helversen 2003). We discuss our results, paying particular attention to measured call intensities and those estimated for echoes, within the context of the hypotheses outlined above.

Materials and methods

Study site

Our study was conducted in March 2008 during one of the biannual dry seasons on Barro Colorado Island (BCI), Panama; a field station of the Smithsonian Tropical Research Institute (STRI) near the Panama Canal (9°11′N, 79°50′W). BCI is characterized by semi-deciduous, moist tropical lowland forest and home to over 70 species of

echolocating bats representing several families (Jung et al. 2007; Kalko et al. 2008). Recordings of free-flying *Saccopteryx bilineata* were made at two locations: one site close to a known day roost; the other, a volume of airspace commonly used for foraging by this and other insectivorous bat species (e.g. *Myotis nigricans* (Vespertilionidae), Siemers et al. 2001).

The known roost was the vertical exterior wall of a concrete building, a roosting choice common for this species (Fenton et al. 2001). It was surrounded by three edges of forest: within this area bats flew beneath the canopy (2–4 m from the ground) and were never more than 5 or 6 m from the wall or forest edge, which together formed a perimeter with sides of 10–12 m. Hereafter, we refer to this as the roost site.

The center of the known foraging airspace was approx 10 m in front of the balcony of an old dining hall and here bats foraged approx 2–5 m above the ground. Most of the site was without canopy cover and surrounded by far-away edge (>10 m), hence it was a more open space than the roost site. Hereafter, we refer to it as the foraging site.

To minimize the array's influence on echolocation call design, we embedded it within preexisting clutter. At the foraging site it was built directly onto the dining hall balcony (∼3 m above the ground), at the roost site it was set flush with forest edge (∼3 m above the ground).

Sound recordings

At each site, we began making sound recordings at sunset (approx 1900 h) and continued until bat activity had peaked and declined, usually around 2200 or 2300 h. Echolocation calls were recorded using a cross-shaped multi-microphone array covered with sound-absorbing cotton batting (Fig. 1). Seven ¼″ 40 BF G.R.A.S. microphones [grids off; frequency response flat (±1 dB) from 1 to 100 kHz] were positioned equidistant from one another along the long,

horizontal axis of the cross (x plane). Two Avisoft CM16 condenser microphones were positioned perpendicular to and at an equal distance from the horizontal axis, above and below the fourth G.R.A.S. microphone; together these three microphones comprised the shorter vertical axis of the cross (z plane). At the roost site, the seven microphones were set 50 cm apart, the two Avisoft microphones 75 cm above and below. At the foraging site, the microphones were set 70 cm apart horizontally, the two Avisoft microphones 70 cm above and below (Fig. 1). Avisoft microphones were used only for positioning the bats in three-dimensional space and were not used to estimate intensity.

In all instances, all microphones protruded approx 20 cm from the y plane of the cross on thin aluminum rods, well past the cotton batting. G.R.A.S. microphones were amplified 40 dB using 12 AA G.R.A.S. amplifiers (with custom 13.5 kHz high pass filters), and signals were sampled using a 12-channel Avisoft 1216 ultrasound gate (sampling rate: 500 kHz/channel) and stored onto a laptop (IBM X60). We calibrated the G.R.A.S. microphones using a Brüel and Kjær sound calibrator (type 4231) and measured temperature and relative humidity at the beginning and end of each recording session.

Sound analysis

Only sequences judged to be from a single *S. bilineata* (Fig. 1) and containing exclusively calls with call intervals of >25 ms and, thus, always without a terminal buzz were selected for analysis (signal to noise ratio >10 dB above background noise). Sound files containing buzz calls were inspected but only to determine whether approach calls preceding the buzz were frequency alternating or monotonous. Call intervals (i.e. time elapsed from the beginning of one call to the beginning of the next) of >25 ms should help prevent the inclusion of calls emitted after the bat has detected prey that it intends to intercept (i.e. late approach

Fig. 1 Typical flight-paths recorded at the foraging site from which calls that met criteria were selected. At both sites, all calls that met criteria were recorded as bats flew towards the microphone array. Oscillogram traces are superimposed onto the flight-paths. Horizontal microphones were 70 cm apart; Avisoft microphones were 70 cm above and 70 cm below
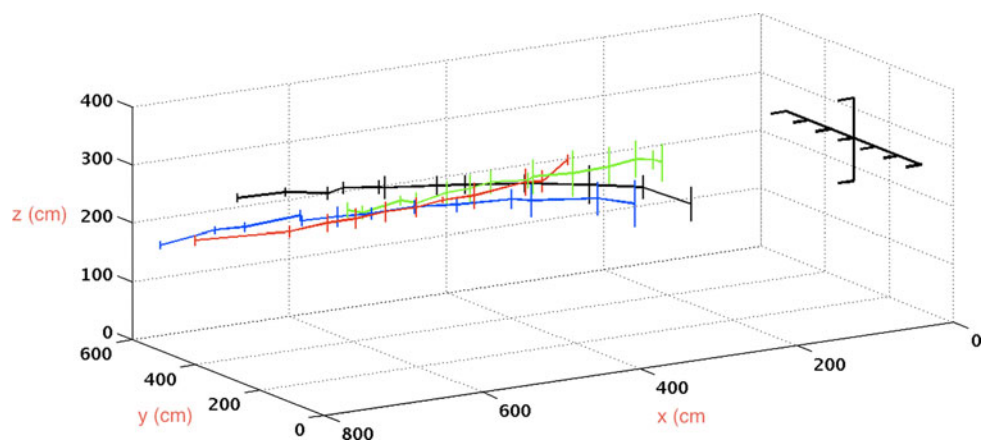
and buzz calls) (Griffin et al. 1960; Ratcliffe et al. 2011). All recordings were screened using either Audition 1.5 (Adobe) or BatSound v. 4.1 (Pettersson Elektronik). Sequences typically contained 10 or more calls; the majority of these calls, however, did not meet criteria for intensity measurement (see below).

Within sequences, individual bats were positioned at each detected call emission by triangulation based on time of arrival differences for each call at the nine microphones. Individual calls were then filtered using a third octave band-pass filter centered at 46 kHz and the received RMS pressure of the filtered signals was calculated. Source-levels were calculated by compensating the calculated RMS pressures for spherical spreading loss [20 $\log_{10}$(distance (m)/0.1 m)], atmospheric attenuation (ANSI 1978), and angle of incidence on the microphones (Brüel and Kjær 1982; for details see Brinkløv et al. 2010).

Only calls recorded when the bat was flying roughly towards the array and for which the most intense recording was made at one of the three central horizontal microphones were selected for intensity estimation. Call duration, PF and call interval of these calls (and those for calls emitted directly before and after them) were measured using BatSound on the microphone channel that recorded the most intense call (after compensation). Hereafter, we refer to this microphone as the 'on-axis microphone'. Call duration and call interval (the time elapsed from the beginning of that call to the start of the next call) were measured on the oscillogram (cross-checking for overlapping echoes on the spectrogram); call PF was measured from power spectra [automatic fast Fourier transform function (FFT), Hann window].

Modeling echo intensity at a bat's ears

We calculated the estimated intensity of echoes from clutter and potential prey returning to the bats' ears from (1) the location of the on-axis microphone and (2) the distance corresponding to the end of the call's interval, such that echoes return right before emission of the next call (the 'call window' following Holderied and von Helversen 2003). To estimate the echo from prey we input our data into a simple form of the sonar equation:

$$\text{Echo intensity} = \text{emitted call intensity} - \text{transmission loss} + \text{target strength}$$

Transmission loss includes spherical spreading of sound (6 dB per doubling of distance) and atmospheric attenuation of call frequencies at recorded temperature and relative humidity (ANSI 1978; Surlykke and Kalko

2008). For a point target (e.g. an insect), spherical spreading loss of an echo returning to the bat amounts to 40 $\log_{10}$(distance (m)/0.1 m). For clutter, the situation is different. Theoretically, spreading loss for an echo from an infinite wall is 20 $\log_{10}$(distance (m)/0.1 m). Based on measurements of reflection from vegetation (Jensen 2000), we estimated that the echo from a clutter background decreases slightly more than from a solid wall, i.e. spreading loss amounts to 24 $\log_{10}$(distance (m)/0.1 m). To the resultant value, we added frequency and condition dependent atmospheric attenuation (as above).

Since we embedded our microphone array into the existing clutter at each site, we used the distance from the bat to the on-axis microphone as an estimate of the distance of the bat to clutter. Distance for an insect just outside of the target-clutter echo overlap zone was estimated as that from the bat to the on-axis microphone minus a distance equal to 0.5 × call duration × 348.8 m/s (thereby removing the target-background overlap zone; Schnitzler and Kalko 2001). For virtual echoes returning from either clutter or insects just before the close of the call window, distance was calculated as half the total distance the call could have traveled over the duration of the call window (i.e. the time from one call to the beginning of the next). For alternating sequences, but only if calls are 'marked' by frequency, this window would extend from the time of the 45 kHz call in one pair to the emission of the 45 kHz call in the next pair.

We used target strengths of −0 dB (reference distance 10 cm) to estimate the reflectivity of the buildings and vegetation into which our array was embedded (Jensen 2000) and −20 dB (reference distance 10 cm) to estimate the target strengths returning from a small flying insect (Surlykke et al. 1999). To judge when echoes were detectable by the bats, we assumed an echo detection threshold of 0–20 dB (Hoffmann et al. 2007; Jung et al. 2007; Moss and Schnitzler 1995).

Flight speed calculation

We estimated the average flight speed (m/s) between successive calls based on the calculated distance flown between calls (from positioning data) divided by the measured call intervals (from oscillograms).

Statistical analyses

All statistical analyses were conducted using JMP v. 8.0 (SAS Institute, Cary, NC, USA). All tests were two-tailed and for each α was set at 0.05.

Results

Call intensity correlations

A total of 181 *S. bilineata* calls from the two recording sites taken from 60 sequences met our criteria (Table 1). Sixty-one of these calls had a PF of approx 45 kHz (mean $\pm$ SE = 45.4 $\pm$ 0.1), the remaining 134 had a PF of approx 48 kHz (47.7 $\pm$ 0.1). Call intensity was positively related to the bat's distance from the on-axis microphone ($F_{1,179}$ = 22.78, r^2 = 0.11, $P < 0.0001$; Fig. 2a) and also to call duration ($F_{1,179}$ = 27.79, r^2 = 0.13, $P < 0.0001$; Fig. 2b). Call intensity was not significantly related to call interval ($F_{1,179}$ = 0.16, $r^2 < 0.001$, $P = 0.69$; Fig. 2c). Each night that we made recordings, there were at least 20 flying bats in the vicinity of the roost. At the foraging site there were at least five bats over the course of a night, probably more.

Site-specificity in monotonous versus frequency-alternating call sequences

At the roost site, 63% of *S. bilineata* echolocation calls that met criteria were from monotonous (48 kHz) sequences (Fig. 3a; Table 1), and 21 of 37 sequences considered consisted only of monotonous calls. At the foraging site, all calls that met criteria were components of frequency-alternating sequences (Fig. 3b; Table 1). The distribution of sequence categories differed significantly between the two sites (Fisher exact test, $P < 0.001$).

At both sites, for those files containing identifiable post-detection calls, search and approach phase calls were always frequency alternating (based on inspection of 25 attack sequences from the roost site and 39 from the foraging site) and, as such, the distribution of terminal buzz

calls differed significantly between sequence categories (Fisher exact test, $P < 0.001$). However, bats sometimes emitted a short series of exclusively 48 kHz calls immediately after producing a terminal buzz (Fig. 3d). This pattern was observed in 28 of 64 attack sequences inspected (8 of 25 at the roost site, 20 of 39 at the foraging site). In these instances, the bat returned to using a frequency-alternating pattern after emitting two to six 48 kHz calls. Never, at either site, did we observe a bat to proceed from monotonous calls into an attack sequence; however, bats transitioning from an alternating sequence to a monotonous sequence of calls and vice versa were sometimes recorded (Fig. 3c).

Relationships between call parameters, site, and distance to array

Frequency-alternating sequences were recorded at distances from the microphone arrays that did not differ significantly between sites (Table 1). Monotonous sequences (roost site only) were recorded closer to the array than alternating sequences at either site (ANOVA, $F_{4,176}$ = 9.1, $P < 0.0001$; TUKEY HSD post hoc tests; Table 1).

In frequency alternating and monotonous sequences, call intervals for 48 kHz calls were approx 80–90 ms, an interval duration which did not differ significantly between recording sites (Table 1). In alternating sequences, call intervals for 45 kHz calls were approx 60 ms and did not differ in duration between sites (Table 1). The 45 kHz call intervals were significantly shorter than the 48 kHz call intervals at both sites and from both alternating and monotonous sequences (ANOVA, $F_{4,176}$ = 7.83, $P < 0.0001$; TUKEY HSD; Table 1).

At either site, 45 and 48 kHz calls from alternating sequences did not differ from one another in duration

Table 1 *Saccopteryx bilineata* call intensity, duration, interval and distance from microphone at emission for site, sequence, and call frequency classifications (N_{total} = 181)

| Habitat, sequence, frequency	Source level (dB) [median (quartiles)]	Duration (ms) (mean $\pm$ SE)	Interval (ms) (mean $\pm$ SE)	Distance (m) (mean $\pm$ SE)	N
Roost					
Monotonous					
48 kHz	113.5 (111, 116.1)	6.43 $\pm$ 0.16	93 $\pm$ 4.4	4.25 $\pm$ 0.22	62
Alternating					
45 kHz	112.6 (110.5, 115.2)	5.51 $\pm$ 0.27	57.8 $\pm$ 7.2	6.16 $\pm$ 0.35	23
48 kHz	112.5 (110.6, 113.9)	4.72 $\pm$ 0.36	80.3 $\pm$ 9.6	6.20 $\pm$ 0.47	13
Foraging site					
Alternating					
45 kHz	117.7 (114.5, 119.1)	8.26 $\pm$ 0.22	60.9 $\pm$ 5.9	5.52 $\pm$ 0.29	34
48 kHz	115.5 (113.8, 117.1)	8.22 $\pm$ 0.18	88.0 $\pm$ 4.9	5.71 $\pm$ 0.24	49
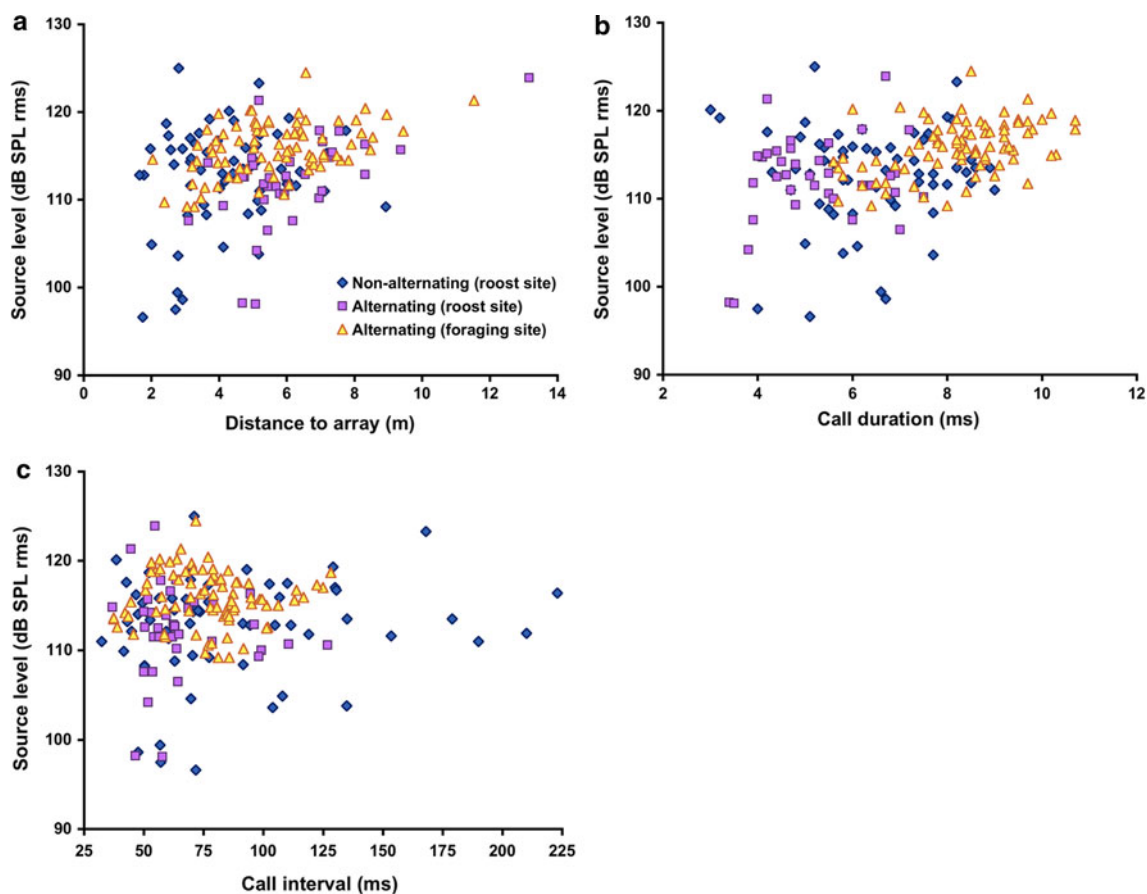
Fig. 2 Source levels (dB SPL RMS) emitted by *S. bilineata* in three contexts as a function of **a** distance to on-axis microphone, **b** call duration and **c** call interval

(Table 1). However, calls of both frequencies from alternating sequences recorded at the foraging site were significantly longer than those from alternating sequences recorded at the roost site (Table 1). Last, 48 kHz calls from monotonous sequences at the roost were significantly longer than calls from alternating sequences recorded there, but significantly shorter than calls from alternating sequences recorded at the foraging site (ANOVA, $F_{4,176} = 40.07$, $P < 0.0001$; TUKEY HSD; Table 1).

At the foraging site, the intensity of 45 and 48 kHz calls did not differ significantly (Table 1). However, 45 kHz calls from the foraging site were significantly more intense than calls from all call/sequence combinations recorded at the roost site, while 48 kHz calls recorded at the foraging site were not (ANOVA, $F_{4,176} = 7.37$, $P < 0.0001$; TUKEY HSD; Table 1).

Target and prey echo intensity at the bat's ears

Figure 4 illustrates that the estimated intensities of echoes returning from clutter or an insect at the distances from the bat to the on-axis microphone (i.e. actual distance for

clutter or, for an insect, this distance minus the target-background overlap zone) would have been at or above a bat's hearing threshold, as would have been echoes reflected by clutter at the far periphery of the effective call window. Conversely, echoes returning from an insect at this periphery would not have been heard by the bat.

Flight speed

Flight speed did not differ between the time elapsed between 45 and 48 kHz calls versus the time elapsed between 48 and 45 kHz calls in alternating sequences at either site (2 two-tailed *t* tests, $P > 0.5$ for both). Thus, for each site flight speeds between 45 and 48 kHz and 48 and 45 kHz calls were pooled. Flight speed did not differ significantly between sites nor between alternating and monotonous sequences [flight speed (mean ± SE): roost site (monotonous): 6.3 ± 0.5 m/s; roost site (alternating): 6.7 ± 0.7 m/s; foraging site (alternating): 7.3 ± 0.4 m/s; ANOVA, $F_{2,125} = 1.04$, $P = 0.36$). Flight speeds correspond well with those previously reported for this species (Morrison 1980).

Fig. 3 a Frequency-alternating sequence, **b** monotonous sequence, and **c** a transitional call sequence from monotonous to frequency-alternating calls. Terminal buzzes were always preceded by frequency-alternating echolocation calls (**d**)
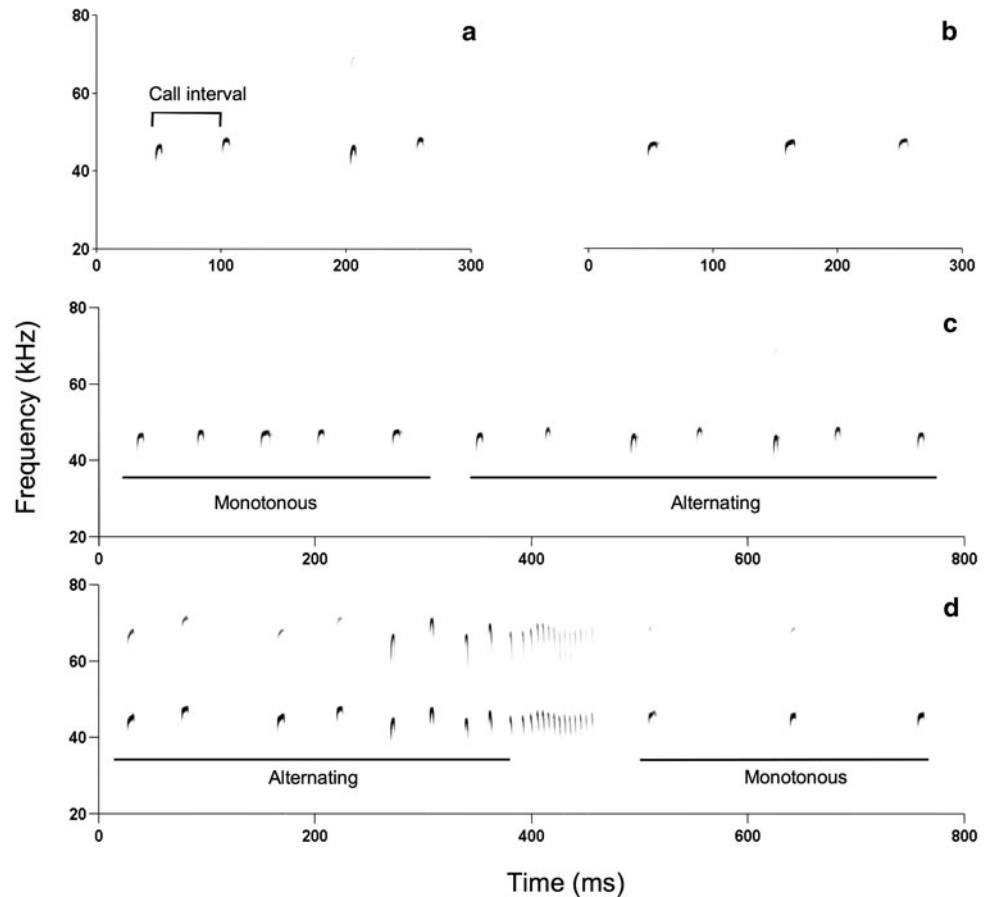
Discussion

We found that the greater sac-winged bat, *Saccopteryx bilineata*, produced only frequency-alternating call sequences (45/48 kHz pairs) at the foraging site and mostly monotonous sequences (48 kHz) near the roost. Calls from monotonous sequences were recorded from bats at distances significantly closer to our microphone arrays than calls from alternating sequences. Calls from monotonous sequences were, however, significantly longer than those recorded from alternating sequences at the roost. Our results show that call intensity was significantly correlated to both call duration and distance from the microphone. However, neither relationship was as tight or as profound as those observed in bats from more recently derived families (Brinkløv et al. 2009 and references therein). We stress here that the statistically significant mean intensity differences between sites and call designs are small (±2 dB, Table 1) and do not reveal the biologically meaningful relationship between call intensity and habitat as has recently been discovered for the trawling leaf-nosed bat, *Macrophyllum macrophyllum* (Phyllostomidae) (Brinkløv et al. 2010).

When *S. bilineata* emits calls of alternating frequency, the call interval follows an offbeat rhythm such that calls are emitted in pairs where the 45 kHz call precedes the 48 kHz call by approx 60 ms. The first call (always 45 kHz) of the next pair is emitted approx 90 ms later (see also Barclay 1983; Jung et al. 2007; Fig. 3a). Inserting 45 kHz calls should be energetically expensive because, assuming a steady flight speed from call to call (as our flight speed calculations indicate, see also Morrison 1980), the rhythm of alternating sequences renders one call to one wing-beat synchronization impossible and accordingly the energetic savings such synchronization should confer (Speakman and Racey 1991). During monotonous call sequences, *S. bilineata* is, at times, expected to match call emission to wing-beat cycle (Schnitzler and Kalko 2001; Jung et al. 2007) and should thereby realize these savings in some instances. We therefore assume frequency-alternating sequences are, overall, a more expensive means of orientation than monotonous ones, and that neither serves primarily a social function. Below, we consider what some of the potential advantages of this uncommon behavior might be.
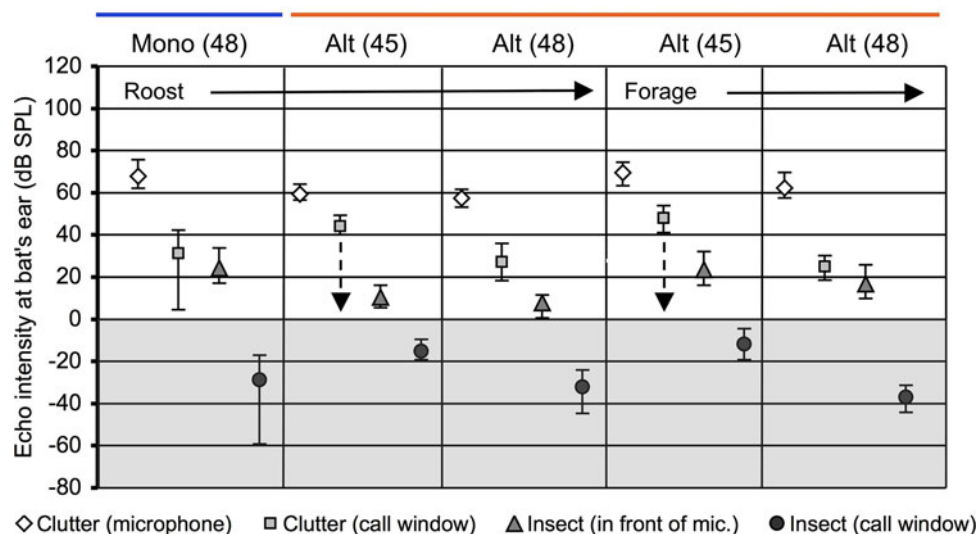
Fig. 4 Estimated intensity of echoes at the bats' ears after returning from actual clutter (*open diamonds*) at the distance of the on-axis microphone, *light gray squares* virtual clutter at distance corresponding to half of call interval (i.e. echoes returning at the end of call window), *gray triangles* virtual insect just outside target-clutter overlap zone (microphone distance = clutter distance, overlap calculated using actual call duration (Schnitzler and Kalko 2001), and *dark gray circles* virtual insect at distance corresponding to half of call interval (i.e. same distance as for *light gray squares*). Shapes' position indicates median dB value, bars the third and first quartiles.

Note, for bats under optimal acoustic conditions threshold for best frequencies (i.e. call PF) are approx 0 dB (Moss and Schnitzler 1995), and the detection threshold for bats in flight likely lies somewhere between 0 and 20 dB on the y axis (see text for details). Echoes returning at intensities below 0 dB (*shaded gray*) are expected to be inaudible to the bats under all conditions. *Dashed lines* with arrows indicate the extended range for unambiguously 'marked' echoes from the first call (i.e. 45 kHz calls, low note) in the pairs of alternating sequences (see text and Jung et al. 2007 for details)

Pye's (1980) sensory hypothesis revisited and revised

Holderied and von Helversen (2003) demonstrated that several species of vespertilionid bat flying in open space match the intensity of their calls to call interval such that still audible echoes should return just as the call-to-call window closes. Behr and von Helversen (2004) proposed that *S. bilineata* might extend the range of each successive call still further by marking calls with different frequencies, and thereby reduce potential ambiguity in matching echoes to their call. Jung et al. (2007) tested this prediction using estimated call intensities (≤130 dB SPL) and found that should echoes return from an object falcon-sized or larger just before the bat emitted its next call those echoes would be detectable. However, the bats observed never flew far enough from background to realize the full distance that the measured call windows would, theoretically, have allowed (Jung et al. 2007). They concluded that their results refuted the 'extended call window' hypothesis for prey detection, but that such an extension might have some benefit for detecting larger objects (e.g. birds of prey and vegetation) should the bat find itself flying in open space. Based on our own model, we too argue frequency alternation's primary function is not extending the range of prey detection in open space. Figure 4 illustrates that echoes for insects returning at the close of the call window would be

inaudible to the bat while clutter echoes, presumably, would be audible.

Our behavioral data demonstrate that *S. bilineata* uses exclusively alternating sequences before commencing an attack at both sites and we propose instead that low-note, high-note pairs are a novel adaptation for short-range prey detection in complex habitat (Pye 1980). By reducing the call interval typical of monotonous sequences, the observed 45/48 kHz pairs will increase the rate of information update at the bat's ears (Moss et al. 2006). Additionally, the use of these two frequencies—although of little impact on overall bandwidth—should allow the bat to distinguish the 'marked' echoes returning from successive calls from a complex background (Hiryu et al. 2010). In other words, and as Fig. 4 illustrates, echoes of both call designs returning from background vegetation at the distance of the embedded microphones and beyond—through open spaces in the vegetation and past even the distance set by the call window—may be detectable to these bats.

Further, while echoes from insect prey at the extreme of the theoretical call window will not be detectable, echoes returning from insects in front of the target-background overlap zone should be (Fig. 4). Furthermore, because the alternating calls emitted at the roost site have a shorter duration than the monotonous ones, the overlap zone will also be smaller/shorter for alternating calls than

monotonous ones. We therefore suggest that *S. bilineata* switches from monotonous call sequences to using shorter calls (to reduce the probability of call-echo overlap) of alternating frequency (to reduce ambiguity), and produces them in pairs with a reduced call interval (to increase the rate of information update). All told, these differences should improve depth of field when hunting insect prey near complex habitat. In addition, we argue that the bat does so, in part, by using 'marked' echoes that return at still audible levels beyond the call-to-call window. Corroborating this explanation, the sympatric emballonurid, *Diclidurus albus*, uses monotonous sequences when foraging high in simple, open spaces and switches to frequency alternation only when it drops closer to the canopy in search of insects there (Jung et al. 2007). While our interpretations differ, Denzinger et al. (2001) have suggested that the vespertilionid, *Barbastella barbastellus* also alternates call type to improve foraging efficiency in edge and gap.

Plausible secondary social functions for each sequence type

Call design and emission pattern in both sequence types make clear each sequence type first serves echolocation. Changes in echolocation call duration and PF to avoid mistaking the calls and echoes of other bats as one's own might be considered 'social' since it is the presence of conspecifics that can influence call design in these ways (Ratcliffe et al. 2004). However, in neither alternating nor monotonous call sequences did we note changes in call duration or PF when more than one bat was present (see also Jung et al. 2007). Based on a stricter definition (e.g. species-recognition), our results do not strongly support a secondary social function for either sequence category. Although absence of evidence is not evidence of absence, we note that *S. bilineata* possess a diverse social call repertoire (Davidson and Wilkinson 2004; Knörnschild et al. 2006; Voigt et al. 2008) comprised of calls readily distinguishable from those used for echolocation (Behr and von Helversen 2004; Behr et al. 2009; Davidson and Wilkinson 2004; Voigt et al. 2008).

Further, the use of an alternating pattern to facilitate species-recognition would be energetically expensive, particularly as bats may be capable of the same based on individual, group, and species-specific signatures in monotonous sequences (Dechmann et al. 2009; Masters et al. 1995; Schuchmann and Siemers 2010; Voigt-Heucke et al. 2010). Lastly, no emballonurid species sympatric with *S. bilineata* produces 48 kHz calls (Jung et al. 2007). Expensive or not, adding an offbeat 45 kHz call to denote species identity seems unnecessary. However, we cannot rule out the possibility that frequency alternation may also

function as a warning to conspecifics and, perhaps, other insectivorous bat species exploiting a similar ecological niche not to trespass upon a patch. Observed aggressive interactions, including aerial chases, and anecdotal evidence that *S. bilineata*, unlike a number of other bat species, appears not to be attracted to the playbacks of conspecific call sequences suggest, at the very least, that frequency alternation is not a means of rallying group members to a rich foraging site (E.K.V. Kalko, unpublished data and observations).

Monotonous sequences, conversely, are linked to proximity to the roost (Behr and von Helversen 2004; Jung et al. 2007; Pye 1980; this study) and their simplicity may reflect greater reliance on spatial memory and the lower demands of orientation relative to insect capture. We further propose that if an individual's motivational state impacts what echolocation call sequence it uses, that same motivational state will influence what, if any, social calls are made. During territorial displays at the roost, when bats are not hunting, males use recognized social calls associated with aggressive behavior (Behr et al. 2006, 2009). These calls often appear bracketed by monotonous, rather than alternating, echolocation call sequences (Behr et al. 2006; this study). In our own recordings and based on a review of the literature, social calls rarely occur within the context of alternating sequences (e.g. Behr and von Helversen 2004; Behr et al. 2006). Should motivational state determine what sequence is used, we suggest that the presence of monotonous calls after a feeding buzz might indicate a bat that has successfully captured an insect and is now simply orienting and, for the moment, not motivated by food. Conversely, alternating calls suggest a bat that missed its target and is still hungry for the kill.

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