Breaking the trade-off: rainforest bats maximize bandwidth and repetition rate of echolocation calls as they approach prey

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Both mammals and birds experience a performance trade-off between producing vocalizations with high bandwidths and at high repetition rate. Echolocating bats drastically increase repetition rate from 2–20 calls s^{-1} up to about 170 calls s^{-1} prior to intercepting airborne prey in order to accurately track prey movement. In turn, bandwidth drops to about 10–30 kHz for the calls of this 'final buzz'. We have now discovered that Southeast Asian rainforest bats (in the vespertilionid subfamilies Kerivoulinae and Murininae) are able to maintain high call bandwidths at very high repetition rates throughout approach to prey. Five species of *Kerivoula* and *Phoniscus* produced call bandwidths of between 78 and 170 kHz at repetition rates of 140–200 calls s^{-1} and two of *Murina* at 80 calls s^{-1}. The 'typical' and distinct drop in call frequency was present in none of the seven species. This stands in striking contrast to our present view of echolocation during approach to prey in insectivorous bats, which was established largely based on European and American members of the same bat family, the Vespertilionidae. Buzz calls of *Kerivoula pellucida* had mean bandwidths of 170 kHz and attained maximum starting frequencies of 250 kHz which makes them the most broadband and most highly pitched tonal animal vocalization known to date. We suggest that the extreme vocal performance of the Kerivoulinae and Murininae evolved as an adaptation to echolocating and tracking arthropods in the dense rainforest understorey.

Keywords: echolocation; bandwidths; repetition rate; approach calls; buzz

1. INTRODUCTION

The late Donald Griffin, who unravelled the mystery of how bats orient in complete darkness, also came up with the first detailed description of the echolocation of flying insects by bats (Griffin *et al*. 1960). Call

sequences during approach to and capture of airborne prey have since been studied intensively, especially for the large group of vespertilionid bats (Kalko & Schnitzler 1989; Schumm *et al*. 1991; Surlykke *et al*. 1993; Kalko 1995; Britton & Jones 1999; Siemers & Schnitzler 2000; Siemers *et al*. 2001). The approach is characterized by a reduction in pulse duration and pulse interval; it typically terminates in a group of calls at minimum duration and minimum pulse interval, the so-called 'buzz'; the final part with minimum intervals, constant at values around 6 ms, is often referred to as 'buzz II'. The increase in call rate provides the bats with frequently updated echo-information on prey position and might also be used for post hoc evaluation of unsuccessful capture attempts (Melcon *et al*. 2007).

An abrupt drop in call frequency and bandwidth of the first harmonic typically marks the onset of buzz II (Kalko & Schnitzler 1989; Kalko 1995). It has been suggested that the cricothyroid muscles in the bats' vocal tract might be not fast enough to produce broadband signals at very high repetition rates (Griffin *et al*. 1960; Schumm *et al*. 1991). Also in songbirds, motor constraints on vocal production impose a trade-off between trill rate and frequency bandwidth (Podos 1997), although female preference exerts a selection pressure for the maximization of both song parameters (Draganoiu *et al*. 2002). Given that similar performance constraints seem to be imposed on both the mammalian larynx and the avian syrinx, the repetition rate–bandwidth trade-off appears as a rather general phenomenon in vertebrate vocalization. However, we have discovered here that Southeast Asian vespertilionid bats (from the sister taxa Kerivoulinae and Murininae; Hoofer & Van den Bussche 2003) that are adapted to foraging in dense rainforest interior (Kingston *et al*. 1999, 2006) are able to maintain high call bandwidths at very high repetition rates throughout approach to their prey.

2. MATERIAL AND METHODS

(a) *Capture and husbandry of bats*
Fieldwork was conducted from February to May 2009 in Malaysian lowland rainforest at Kuala Lompat Research Station, Krau Wildlife Reserve, Pahang, Peninsular Malaysia (3°43′ N, 102°10′ E) under licence of the Economic Planning Unit Malaysia (UPE: 40/200/19/2359) and the Director-General of the Department of Wildlife and National Parks. See Kingston *et al*. (1999) for details of the field site. Bats were caught in harp traps positioned across trails in the forest understorey. We identified species following Kingston *et al*. (2006). Animals were kept individually or in pairs for a number of days in round mesh cages (30 cm diameter, height 40 cm) under a natural light regime. Bats received food during the experiments and were handfed additional mealworms (larvae of *Tenebrio molitor*, Coleoptera) to sustain their capture weight if necessary. Bats received a vitamin supplement; water was available ad libitum. After completion of the experiments, all bats were released at the site of capture.

(b) *Recording of echolocation calls*
Bats were flown singly in a large screen tent (Eureka, Breezeway; ground area 3.5 × 3.5 m, 2.5 m central height) during their natural activity period. They were trained for one to maximally four nights to catch mealworms from a nylon string (0.16 mm diameter). For recordings, the mealworms were hung 64 to 5 cm in front of a vertically positioned sheet of acoustic foam (147 cm high, 92 cm wide), in the centre of which a microphone was inserted from behind (see the electronic supplementary material, figure S1). We tried to induce the bats to approach prey and microphone on-axis by erecting mesh side walls and a roof around the foam screen.

Calls were recorded with custom-made equipment (condenser microphone ± 3 dB between 30 and 120 kHz, above -0.2 dB kHz^{-1}; A/D conversion with 480 kHz sampling rate, 16-bit depth; Department of Animal Physiology, University of Tübingen, Germany) and stored on a laptop computer. Video documentation of the bats' behaviour was recorded under infrared-illumination and synchronized with sound recordings via a video time code controller. Owing to technical problems, we used a different digital ultrasound recording system for one and a half weeks (D1000X Detector, Pettersson Elektronic AB, Sweden; sampling rate 500 kHz, 16-bit depth, ± 5 dB between 30 and 120 kHz, but sensitive up to 250 kHz).

We recorded echolocation during prey capture for 18 adult individuals from the following seven species; *Kerivoula intermedia* (Kein, one female, two males), *Kerivoula papillosa* (Kepa, four, one), *Kerivoula pellucida* (Kepe, one, one), *Murina cyclotis* (Mucy, three males), *Murina suilla* (Musu, one, two), *Phoniscus atrox* (Phat, one male) and *Phoniscus jagorii* (Phja, one male).

(c) *Sound analyses*

Calls were displayed as colour spectrograms (256 fast Fourier transform (FFT), Hann window; software SELENA, Department of Animal Physiology, University of Tübingen). Reading accuracy was improved by FFT overlap (96%) to 0.002 ms and by zero-padding to 0.47 kHz. For each downward sweep, we measured starting frequency and terminal frequency at 25 dB below maximum instantaneous call intensity using a half-automated custom routine (for details see Siemers & Kerth 2006). Occasional initial upward modulated elements (cf. figure 1b) were not included in quantitative call measurements. All measurements were taken on the first harmonic; higher harmonics were only rarely visible and much weaker than the first. Where available, we analysed two successful approach sequences with high recording quality per individual. Time of prey-interception was extracted from the video recordings. Statistical tests were calculated using SYSTAT 12.

3. RESULTS

All seven species used broadband, downward frequency modulated echolocation calls. For all species, pulse interval decreased, and thus repetition rate increased, throughout the approach to prey (figures 1a, 2 and table 1; see the electronic supplementary material, figures S2–S7). Approach sequences typically lasted for 0.5–1 s. Towards the end of the buzz, pulse intervals reached about constant values around 5–7 ms in the three *Kerivoula* species and the two *Phoniscus* species, whereas in the two *Murina* species it stayed at 12–13 ms (table 1 and figure 1b). By contrast, neither call bandwidth nor starting or terminal frequency decreased in any of the seven bat species. For *K. papillosa* and *M. cyclotis*, there was even a significant increase in call bandwidths from the beginning to the end of the approach sequence; *K. pellucida* showed a trend in the same direction (table 1 and figure 2; see the electronic supplementary material, figures S3 and S6). All species used high ultrasonic frequencies throughout, with mean starting frequencies of the FM sweep between 154 and 236 kHz and mean terminal frequencies between 66 and 96 kHz (table 1). Average call bandwidths for the different species were thus between 78 and, in the extreme case of *K. pellucida* buzz calls, 170 kHz. The first harmonic remained by far the strongest harmonic throughout the approach in all species (estimated at 80–100 dB sound pressure level at 1 m; second harmonic typically $\gg 15$ dB below).

The last call group (i.e. the buzz) of the *Kerivoula* and *Phoniscus* species contained on average between 23 and 38 calls (max. 61 calls in a *K. papillosa*; figure 1a). It is interesting to note that *Murina* hovered much more frequently than the other five species.

4. DISCUSSION

The fact that *Kerivoula* and *Phoniscus* produced call bandwidths of between 78 and 170 kHz with the first harmonic at repetition rates of up to 200 calls s^{-1} stands in striking contrast to our present view of echolocation during approach to prey in insectivorous bats. Interestingly, the 'classical' pattern was established largely based on temperate species of the same bat family that our seven species belong to—vespertilionids. These likewise emit their buzz calls at maximum repetition rates of 170–200 calls s^{-1}, but typically only reach bandwidths of 10–30 kHz for the first harmonic (Kalko & Schnitzler 1989; Schumm *et al.* 1991; Kalko 1995; Surlykke & Moss 2000; Siemers *et al.* 2001). Some bats increase overall bandwidths by shifting energy into higher harmonics (Surlykke *et al.* 1993; Siemers & Schnitzler 2000). The fact that the Kerivoulinae maintain large bandwidths at high repetition rates with only one harmonic might partly be linked to the longer duration of their buzz calls (1.3–1.9 ms versus 0.2–0.8 ms for other species; references as above). This, however, results in even shorter inter-pulse-intervals and higher duty cycles in the Kerivoulinae than in other vespertilionids.

The mean bandwidth of 170 kHz we measured for *K. pellucida* buzz calls is the largest bandwidth known for any bat call and, in a more general perspective, for any tonal animal vocalization. The mean starting frequency of these calls was 236 kHz and maximal values reached 250 kHz (cf. figure 1b). These again will be the highest values for a fundamental frequency ($=$ first harmonic) for any known animal vocalization, including all bat echolocation calls. The ability to separate prey from vegetation background is enhanced by broadband calls with high starting frequencies (Siemers & Schnitzler 2004), and we therefore suggest that the extreme vocal performance of the Kerivoulinae and Murininae evolved as an adaptation to echolocating and tracking arthropods in the dense rainforest understorey. How they overcame the trade-off between repetition rate and bandwidths is unclear as yet. Potentially, a specialization in their vocal apparatus allows these bats to maximize both parameters at the same time. It is interesting to note that the *Murina* attained the same good performance in catching tethered prey close to background as *Kerivoula* and *Phoniscus* with similar broadband calls, but lower final call rate. The European *M. nattereri* sustains bandwidths of more than 100 kHz at about the same low call rate during the initial part of the buzz (buzz I), but then drops bandwidths as repetition rate increases with the onset of buzz II (Siemers & Schnitzler 2000). The species under study use high bandwidths and high call rate already during search (Kingston *et al.* 1999), and approach sequences in our experiments lasted as long or longer than typical approaches in other vespertilionid bats (e.g. Kalko 1995). Thus these bats can produce broadband calls with short intervals over extended periods of time.

In addition to the lack of bandwidth reduction, a second 'typical' feature of vespertilionid buzzes was absent in the Malaysian bats: there was no drop in
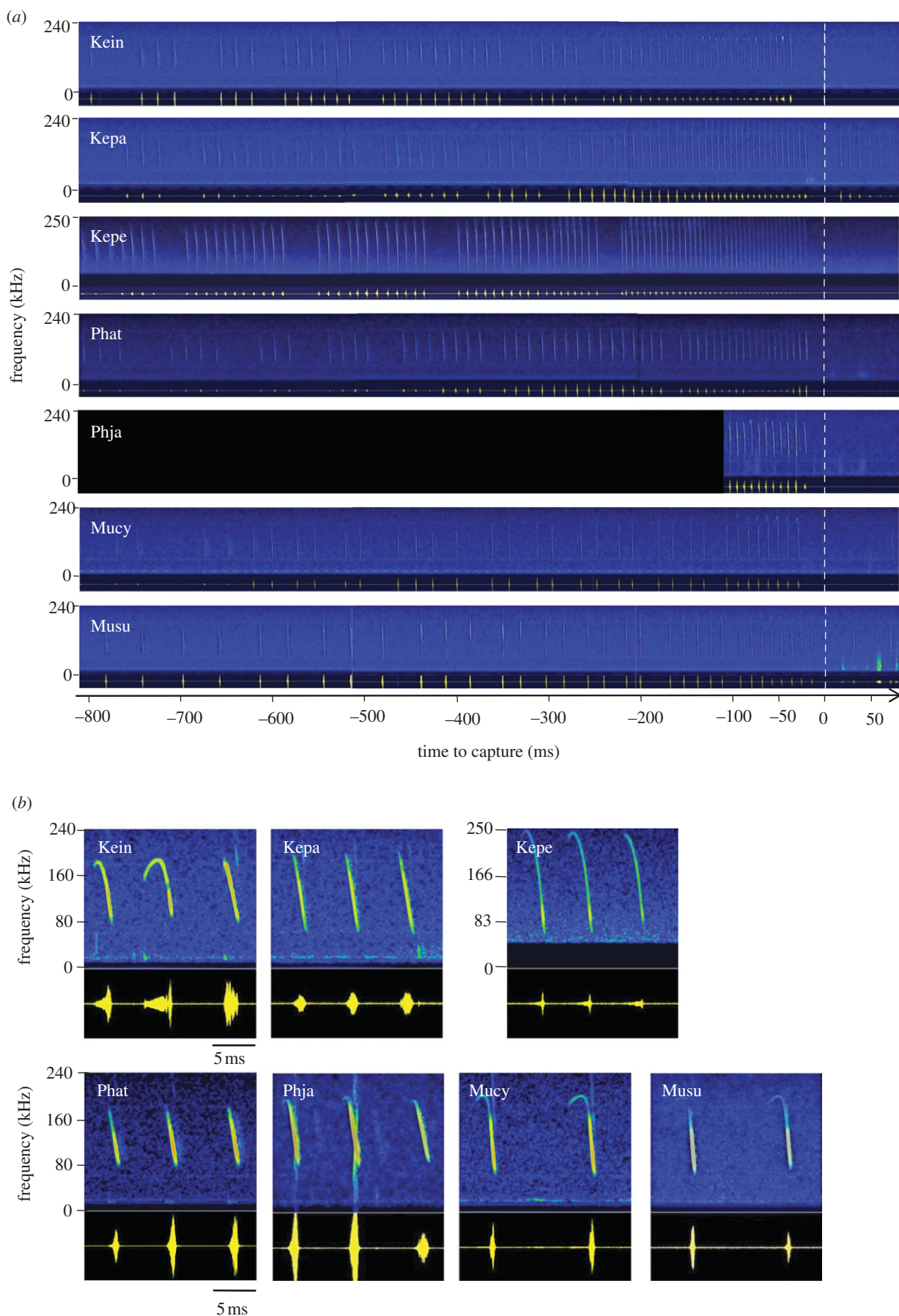
Figure 1. (*a*) Typical approach sequences with successful capture of prey (time zero; dashed lines) are shown in sonagram representation with oscillogram below. (*b*) Enlargement of the last 20 ms of the buzz for the above call sequences. Kein, *Kerivoula intermedia*; Kepa, *Kerivoula papillosa*; Kepe, *Kerivoula pellucida* (high-pass filtered to remove insect noise); Mucy, *Murina cyclotis*; Musu, *Murina suilla*; Phat, *Phoniscus atrox*; Phja, *Phoniscus jagorii*. (beginning of sequence not shown because overloaded). Note the differently scaled frequency axis for *Kepe*.
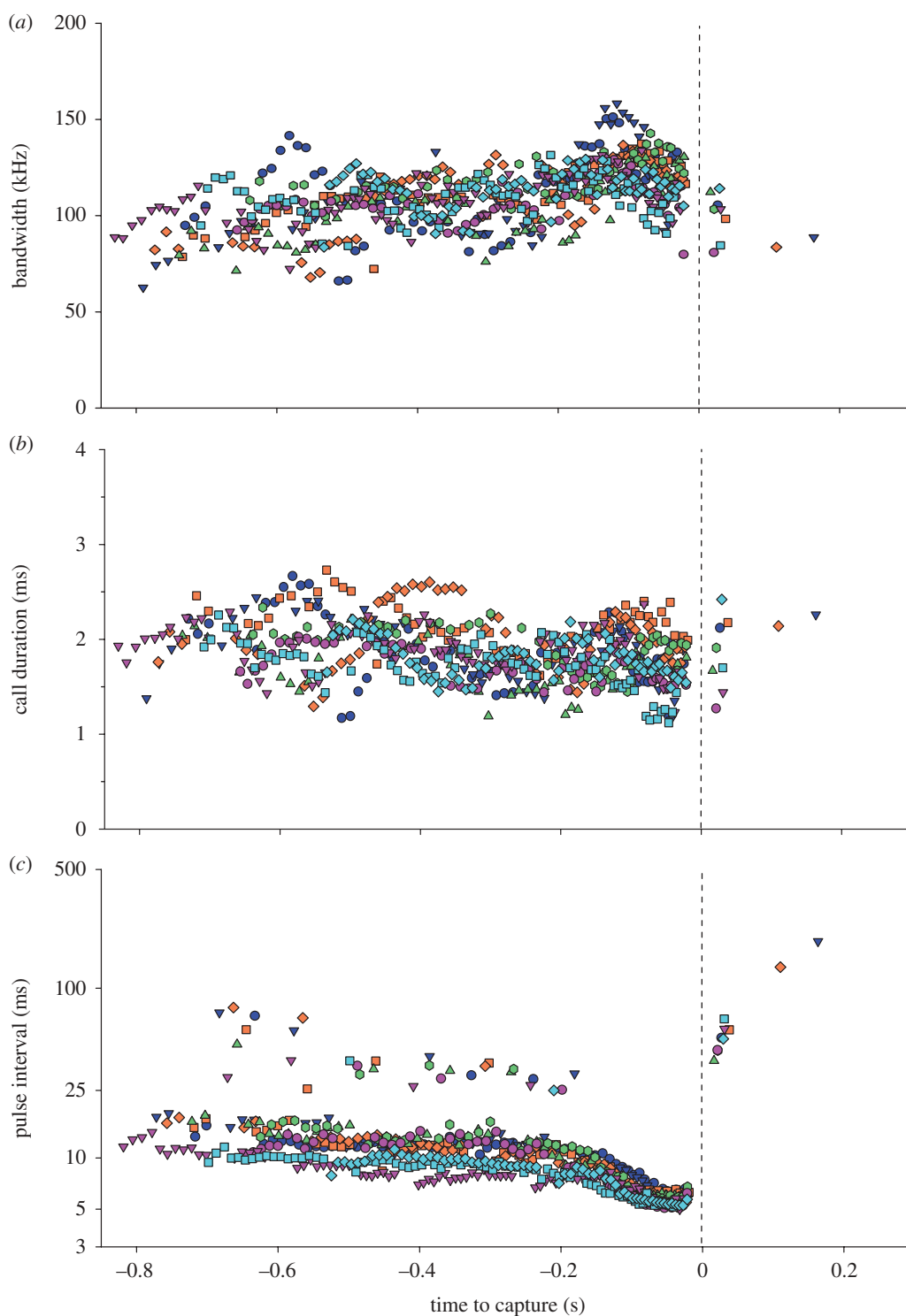
Figure 2. (*a*) Bandwidth, (*b*) call duration and (*c*) pulse interval for 10 approach sequences from five *Kerivoula papillosa* individuals. Each sequence is depicted by a different symbol; one colour per individual. Time zero (dashed line) marks prey capture. Note that the *y*-axis in (*c*) has a logarithmic scaling.

call frequency. This drop of the first harmonic had been discussed both as the result of vocal limitation (Griffin *et al.* 1960; Schumm *et al.* 1991) and as an adaptive trait to achieve higher modulation rates with the second harmonic that would then cover the frequency range that is crucial for echo perception (Surlykke *et al.* 1993). Clearly, these rainforest bats do not need to drop call frequency for successful prey capture.

The case of the vespertilionids *Kerivoula*, *Phoniscus* and *Murina* highlights the great diversity of echolocation systems and their manifold ecological adaptations—providing surprises even within the putatively well-studied groups of bats. It will be interesting to look for corresponding adaptations in African members of the Kerivoulinae and in the phylogenetically distant, but ecologically similar neotropical rainforest bats.

Table 1. Call parameters for the initial and the terminal five calls of the approach sequences for all seven bat species (species means ± s.d.). (Note that only the terminal calls are buzz calls. *p*-values are reported from paired *t*-tests on sequence means where sample sizes allowed.)

	starting frequency (kHz)	terminal frequency (kHz)	bandwidth (kHz)	best-frequency (kHz)	call duration (ms)	pulse interval (ms)	sample size
Kerivoula intermedia							
initial	178.5 ± 7.3	93.6 ± 7.9	84.9 ± 8.2	144.9 ± 25.8	1.5 ± 0.3	18.3 ± 4.1	
terminal	173.2 ± 17.6	95.6 ± 7.7	77.7 ± 17.1	126.8 ± 7.6	1.5 ± 0.2	7.0 ± 0.6	
p-value	n.s.	n.s.	n.s.	n.s.	n.s.	*p* = 0.002	5 sequences/ 3 individuals
Kerivoula papillosa							
initial	172.6 ± 11.1	72.4 ± 3.4	100.2 ± 11.2	112.8 ± 16.0	2.0 ± 0.2	16.4 ± 5.7	
terminal	184.5 ± 6.1	69.4 ± 6.7	115.1 ± 10.2	109.6 ± 16.8	1.7 ± 0.2	5.8 ± 0.4	
p-value	*p* = 0.024	*p* = 0.071	*p* = 0.021	n.s.	*p* = 0.011	*p* = 0.000	10 seq/5 ind
Kerivoula pellucida							
initial	213.7 ± 21.8	74.4 ± 14.5	139.4 ± 10.5	135.2 ± 28.2	2.4 ± 0.3	17.2 ± 7.0	
terminal	235.6 ± 2.6	65.6 ± 3.8	170.0 ± 6.3	108.0 ± 26.0	1.9 ± 0.1	5.0 ± 0.3	
p-value	n.s.	n.s.	*p* = 0.085	*p* = 0.095	*p* = 0.054	*p* = 0.095	3 seq/2 ind
Phoniscus atrox							
initial	160.6 ± 12.6	76.9 ± 3.7	83.7 ± 9.0	103.7 ± 17.9	1.5 ± 0.1	17.3 ± 0.4	
terminal	166.4 ± 0.3	83.6 ± 0.8	82.9 ± 1.2	103.1 ± 6.1	1.3 ± 0.1	6.8 ± 0.0	2 seq/1 ind
p-value	sample size too low for testing						
Phoniscus jagorii							
initial	192.9 ± 6.9	90.2 ± 3.3	102.7 ± 10.2	124.1 ± 24.0	2.3 ± 0.4	18.1 ± 0.5	
terminal	191.4 ± 0.9	92.7 ± 3.4	98.7 ± 3.6	129.9 ± 8.2	1.5 ± 0.0	6.7 ± 0.2	2 seq/1 ind
p-value	sample size too low for testing						
Murina cyclotis							
initial	158.3 ± 7.1	69.1 ± 4.1	89.2 ± 4.4	95.1 ± 5.3	1.3 ± 0.1	29.2 ± 3.6	
terminal	171.4 ± 12.1	71.0 ± 6.2	100.3 ± 7.6	102.6 ± 2.4	1.0 ± 0.2	12.4 ± 1.8	
p-value	*p* = 0.034	n.s.	*p* = 0.038	*p* = 0.065	*p* = 0.014	*p* = 0.001	5 seq/3 ind
Murina suilla							
initial	153.9 ± 10.6	70.9 ± 5.5	83.0 ± 10.0	99.1 ± 11.5	1.3 ± 0.2	28.6 ± 5.1	
terminal	161.5 ± 12.4	74.0 ± 3.7	87.4 ± 12.5	109.1 ± 7.3	0.9 ± 0.1	13.1 ± 2.2	
p-value	*p* = 0.082	n.s.	n.s.	n.s.	*p* = 0.012	*p* = 0.003	5 seq/3 ind

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