

ALTERNATION OF ECHOLOCATION CALLS IN 5 SPECIES OF AERIAL-FEEDING INSECTIVOROUS BATS FROM MALAYSIA

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We describe design of echolocation signals in 5 species of aerial-hawking insectivorous bats from Malaysia. These bats forage in open spaces above the forest or in large clearings and belong to 2 families: Molossidae (*Chaerephon johorensis*, *Mops mops*, *Cheiromeles torquatus*) and Vespertilionidae (*Hesperoptenus blanfordi*, *Pipistrellus stenopterus*). As is typical for aerial-hawking bats, all 5 species produced narrowband calls of long duration (6–21 ms) and low peak frequency (16–44 kHz). However, sequences recorded from bats flying at high altitude (>10 m) were characterized by an alternation between calls that differed in frequency of maximum energy (peak frequency), switching between high- and low-frequency calls. In some species, the types of calls also differed in duration and sound pressure level. We consider possible implications of the alternation of types of calls for the detection of prey in open spaces.

Key words: Chiroptera, echolocation, Malaysia, Molossidae, Vespertilionidae

Most aerial-feeding insectivorous bats use echolocation to detect, locate, and classify prey (Schnitzler and Kalko 1998). Design of echolocation signals depends to a large extent on foraging habitat (Neuweiler 1990). For bats that forage in open spaces above forests, or in large clearings, the key selection pressure on design of signals is long-range (>5 m) detection (Neuweiler 1990; Neuweiler and Fenton 1988) because insect prey are generally widely dispersed and at low density. The dispersed distribution of prey also favors greater flight speeds and a consequent loss of maneuverability (Norberg and Rayner 1987), further necessitating long-range detection to maximize response time after detection of prey (Barclay 1986; Barclay and Brigham 1991). Calls are of low frequency, which reduces

attenuation (Barclay 1985, 1986; Lawrence and Simmons 1982; Neuweiler and Fenton 1988), and energy is concentrated in a narrow band of frequencies, which facilitates detection of weak echoes and acoustic glints (increases in amplitude of the echo created when an insect wing is perpendicular to the impinging sound wave—Kober and Schnitzler 1990; Neuweiler 1990; Schnitzler 1987). Long call durations further increase the likelihood of detecting glints.

This is the “typical” design of echolocation signals used by open-space foragers during search phase. However, a number of species alternate between calls that are relatively stereotypical in terms of structure of components, although they differ between types in frequency, duration, and harmonic structure. This call alternation has been reported in at least 8 species of vespertilio-

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nids (Ahlén 1981; Denzinger et al. 2001; Heller 1989; Jones and Corben 1993; Miller and Degn 1981; Obrist 1995; Waters et al. 1995; Weid and von Helversen 1987; Zingg 1990), 4 species of molossids (Fenton et al. 1998; Heller 1995; Kössl et al. 1999), 2 emballonurids (Barclay 1983; Kalko 1995; Pye 1973), *Rhinopoma hardwickei* (Haber-setzer 1981), and *Thyroptera tricolor* (Fenton et al. 1999).

A number of explanations for call alternation in bats have been proposed. It may play a social or antijamming role (Haber-setzer 1981; Kössl et al. 1999); provide a mechanism for increasing maximum detection distance (a key consideration for bats searching in open spaces for dispersed prey—Fenton et al. 1998; Heller 1989, 1995; Weid and von Helversen 1987); allow for detection of prey that differ in range and size (Heller 1989, 1995); provide a means by which calls facilitating long-range detection can be integrated with those providing localization information (Denzinger et al. 2001; Heller 1989; Parsons et al. 2001); or increase bandwidth to facilitate object identification (Heller 1995). We evaluate the applicability of these hypotheses to call alternation exhibited by 5 syntopic open-space species from Malaysia: *Chaerephon johorensis*, *Mops mops*, *Cheiromeles torquatus* (Molossidae); and *Pipistrellus stenopterus* and *Hesperoptenus blanfordi* (Vespertilionidae). Alternation of calls in *Che. torquatus* and *H. blanfordi* was described by Heller (1989, 1995), but calls of *Cha. johorensis* and *M. mops* have not been described, and previous recording of *P. stenopterus* has been made only under laboratory conditions (Sum and Menne 1988).

MATERIALS AND METHODS

The study was conducted from January 1996 to March 1997 at Kuala Lompat Research Station, Krau Wildlife Reserve, Pahang, Peninsular Malaysia (3°43'N, 102°10'E—see Kingston et al. 1999). Mist nets were set across 2 small rivers at the edge of the reserve (Sungai Lompat and Sungai Krau), and species were identified

following Medway (1982), Payne and Francis (1985), and Lekagul and McNeely (1977).

Recordings were made just before dusk to permit visual observations of recording conditions and to prevent confusion with other species during recording. Acoustic monitoring before release ensured that only the bat being recorded was active in the area. At take off, bats leaving a roost or being released by hand tend to use calls with a greater frequency-modulated (FM) component and more harmonics. To minimize this effect, recording was not begun until the bat had been free-flying, clear of the release point for ≥ 10 s. Recording conditions were classified in terms of height of flight above the ground or distance to clutter. Three categories were recognized: high altitude, >10 m above the ground clear of clutter (vegetation and buildings); low altitude, 5–10 m from the ground or clutter; cluttered, <5 m from the ground or clutter.

Recordings were made with a bat detector (model S-25, Ultra Sound Advice, London, United Kingdom) linked to a digital signal processor (model S-350, Ultra Sound Advice) sampling at 400 kHz. Time-expanded (10 \times) output was recorded on a cassette recorder (model WM-D6C, Sony, Tokyo, Japan). Time-expanded sequences were analyzed with a digital sonograph (model 5500, Kay Elemetrics Corporation, Pine Brook, New Jersey) giving a frequency resolution of 400 Hz on the real-time signal.

Parameters of calls (Fig. 1) were measured from calls with high signal-to-noise ratio for each individual. Up to 7 calls of each call type per sequence were measured. Power spectra were used to derive 3 measures of sound frequency (in kHz) for the fundamental harmonic: starting frequency, terminal frequency, and peak frequency. Peak frequency is that exhibiting the greatest relative power on the power spectrum. Starting and terminal frequencies were measured from the points on the power spectrum at which relative power began to consistently rise above the background noise on either side of the peak frequency, which were cross-checked visually against the sonogram. Duration of each call (in ms) was measured from the waveform. The interval between 2 calls was measured as time between the start of 1 call and start of the next (pulse interval, in ms). Field recordings varied with respect to both distance to microphone and atmospheric conditions, precluding a quantitative assessment of the distribution of energy

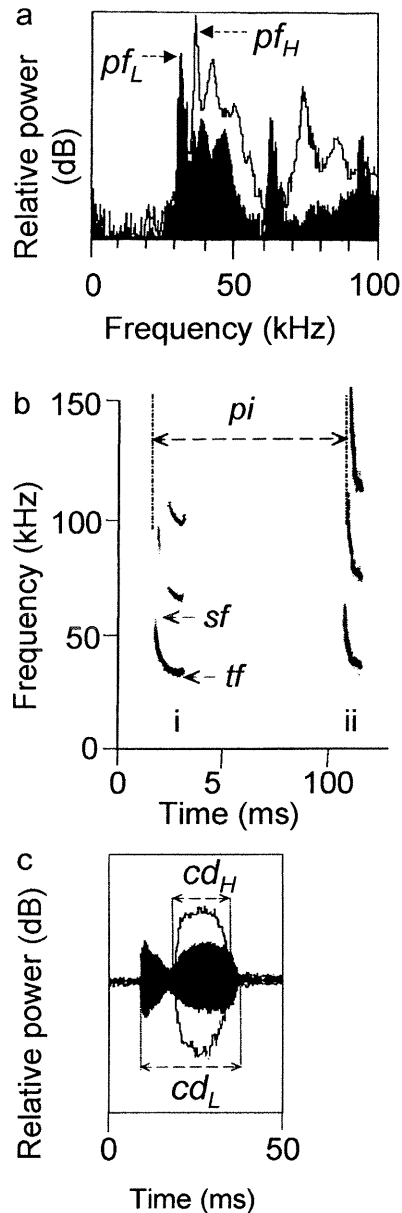


FIG. 1.—Structure of echolocation calls of *Pipistrellus stenopterus* recorded at altitude >10 m and clear of clutter. a) Power spectra (filled trace, low-frequency call; open trace, high-frequency call), b) sonograms of low-frequency call (i) and high-frequency call (ii), and c) waveforms (filled trace, low-frequency call; open trace, high-frequency call). Parameters measured: *sf*, starting frequency (kHz); *tf*, terminal frequency (kHz); *pf*, peak frequency (kHz); *cd*, call duration (ms); *pi*, pulse interval (ms). Subscripts indicate low (*L*-) or high (*H*-) frequency calls.

across call types from sound pressure levels. Instead, the direction of change in sound pressure level at peak frequency during the transition between successive call types was recorded.

Multivariate analysis of variance (MANOVA), with individual nested within call type to guard against pseudoreplication, was used to test for differences in call structure (as described by starting frequency, terminal frequency, peak frequency, and duration of call) between the high- and low-frequency alternating calls within each species. Analysis of variance (ANOVA) was used to test for variation in call parameters in *H. blanfordi*, *P. stenopterus*, and *Che. torquatus* (as small sample sizes precluded testing of the *M. Mops* and *Cha. johorensis*). We used a chi-square test to determine whether the direction of change in sound pressure level was independent of direction of change in peak frequency. All analyses were conducted using SAS for Unix version 6.12 (SAS Institute, Inc. 1994).

RESULTS

As is typical for open-space, aerial-hawking bats, all 5 species produced narrowband calls of long duration (\bar{X} , 6–21 ms) and low peak frequency (\bar{X} , 16–44 kHz). Alternation in type of call was heard in each species flying at high altitude (>10 m), and call types differed in spectral and temporal composition in *P. stenopterus* (MANOVA: Wilks' $\lambda = 0.002$, *d.f.* = 28, 99, $P < 0.001$), *H. blanfordi* (Wilks' $\lambda = 0.001$, *d.f.* = 12, 48, $P < 0.001$), *Cha. johorensis* (Wilks' $\lambda = 0.013$, *d.f.* = 12, 14, $P < 0.01$), and *Che. torquatus* (Wilks' $\lambda = 0.001$, *d.f.* = 44, 166, $P < 0.001$; Table 1; Figs. 1–3). Small sample sizes precluded multivariate analysis of *M. mops*. Individual variation within call type was significant for all parameters in the 3 species tested (*P. stenopterus*, *H. blanfordi*, and *Che. torquatus*; ANOVA: $P < 0.01$ in each case) with the exception of starting frequency of low-frequency calls of *H. blanfordi* ($F = 3.913$, *d.f.* = 1, 10, $P > 0.05$).

In all species the high-frequency calls were characterized by higher starting frequencies, terminal frequencies, and peak frequencies (Figs. 1, 2, and 4) but with sub-

TABLE 1.—Parameters of calls for 5 species of bats from Krau Wildlife Reserve, Malaysia. Altitude indicates height above ground or vegetation: high, >10 m; low, 5–10 m; clutter, <5 m. Pulse interval was measured between successive calls irrespective of call type. Number of bats recorded follows the altitude descriptor. High-frequency (“High calls”) and low-frequency (“Low calls”) calls are compared, with asterisks indicating level of significant differences (Ryan–Eimot–Gabriel–Welsch multiple range test for comparison of means following multivariate analysis of variance). Small sample sizes precluded analysis of *Mops mops* data.

Species and situation	Call frequency (kHz)						Call timing (ms)					
	Starting		Terminal		Peak		Duration		Pulse interval		n	
	$\bar{X} \pm SE$	n	$\bar{X} \pm SE$	n	$\bar{X} \pm SE$	n	$\bar{X} \pm SE$	n	$\bar{X} \pm SE$	n		
<i>Pipistrellus stenopterus</i>												
High calls, high altitude (4)	56.0 ± 1.81	18	32.9 ± 0.28	18	37.0 ± 0.38	18	9.7 ± 0.21	18	102.0 ± 3.98	18	43	
Low calls, high altitude (4)	42.8 ± 2.01	20	28.0 ± 0.40	20	31.0 ± 0.49	20	13.8 ± 0.42	20		20		
Significance of difference	***		***		***		***					
Calls in clutter (1)	68.7 ± 0.78	6	34.1 ± 0.16	6	38.6 ± 0.20	6	8.3 ± 0.16	6	74.4 ± 6.9	6	12	
<i>Hesperoptenus blanfordi</i>												
High calls, high altitude (2)	60.3 ± 1.78	13	40.3 ± 1.05	13	43.8 ± 0.75	13	6.6 ± 0.28	13	81.7 ± 1.43	13	50	
Low calls, high altitude (2)	55.1 ± 0.72	12	33.2 ± 0.40	12	36.4 ± 0.43	12	8.3 ± 0.52	12		12		
Significance of difference	***		***		***		***					
<i>Chaerephon johorensis</i>												
High calls, high altitude (2)	31.4 ± 1.83	5	17.4 ± 1.07	5	21.0 ± 0.89	5	15.7 ± 1.25	5	361.8 ± 31.02	5	10	
Low calls, high altitude (2)	21.8 ± 0.53	7	14.1 ± 0.53	7	16.7 ± 0.21	7	17.5 ± 1.29	7		7		
Significance of difference	***		***		***		NS ^a					
Calls at low altitude (2)	37.6 ± 0.57	15	19.9 ± 0.57	15	26.2 ± 0.46	15	8.5 ± 0.39	15	107.1 ± 5.64	15	32	
<i>Mops mops</i>												
High calls, high altitude (2)	36.0 ± 1.23	3	17.3 ± 1.96	3	23.3 ± 1.14	3	15.5 ± 1.04	3	327.0 ± 11.77	3	8	
Low calls, high altitude (2)	25.9 ± 0.78	8	15.5 ± 0.57	8	18.5 ± 0.28	8	16.7 ± 1.03	8		8		
<i>Cheiromeles torquatus</i>												
High calls, high altitude (6)	42.8 ± 1.26	31	25.3 ± 0.43	31	28.9 ± 0.50	31	21.7 ± 1.02	31	187.0 ± 11.93	31	46	
Low calls, high altitude (6)	32.0 ± 0.65	27	18.7 ± 0.35	27	24.1 ± 0.35	27	21.1 ± 1.10	27		27		
Significance of difference	***		***		***		NS ^a					
Calls in clutter	41.0 ± 0.28	26	11.9 ± 0.35	26	26.7 ± 0.51	26	10.6 ± 0.33	26	84.3 ± 5.88	26	50	

^a NS = not significant.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

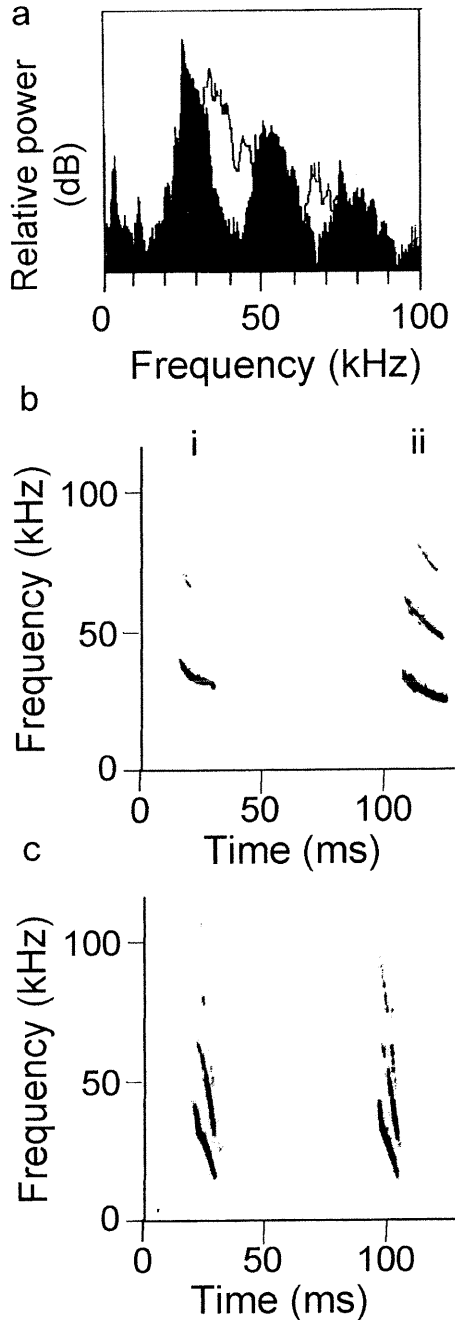


FIG. 2.—Structure of echolocation calls of *Cheiromeles torquatus*. a) Power spectra recorded at altitude >10 m and clear of clutter (filled trace, low-frequency call; open trace, high-frequency call), b) sonograms recorded at altitude >10 m (i, high-frequency call; ii, low-frequency call), and c) sonograms of calls recorded <5 m from the ground or clutter.

stantial overlap of frequencies used between call types. Bandwidth was greater in the high-frequency calls in all species except *H. blanfordi*. Low-frequency calls were of significantly longer duration in *P. stenopterus* and *H. blanfordi* but did not differ significantly in the molossids. Across species there was a positive relationship between the frequency separation of alternating calls and the peak frequency of the low-frequency call (Fig. 5). Therefore, species that called at higher frequencies tended to emit higher calls at a greater frequency separation from the lower call than did species calling at lower frequencies.

There was a significant association between direction of change in sound pressure level and the direction of change in frequency in 3 species (Fig. 6). *P. stenopterus*, *H. blanfordi*, and *M. mops* all exhibited a reduction in sound pressure level in shifting from a low call to a high call and an increase in sound pressure level in the transition from high to low frequency. No such association was found in *Che. torquatus*. Small sample sizes precluded testing of such associations in *Cha. johorensis*.

None of the species used call alternation when close to the ground or vegetation (within 5 m). In the molossids, there was a characteristic decrease in duration of call, increase in FM, and addition and overlap of harmonics (Fig. 2c), modifications to signals that are typical of species approaching obstacles (clutter—Simmons et al. 1978). Calls of a single *P. stenopterus* flying <5 m from the ground or clutter comprised a steep FM sweep with a single harmonic. Structure of the calls resembled that of the fundamental of high-frequency calls from the alternating sequence (terminal frequency, peak frequency, and duration of call) but with a slightly higher starting frequency (increasing bandwidth). These calls are similar to those described for *P. stenopterus* under laboratory conditions (Sum and Menne 1988).

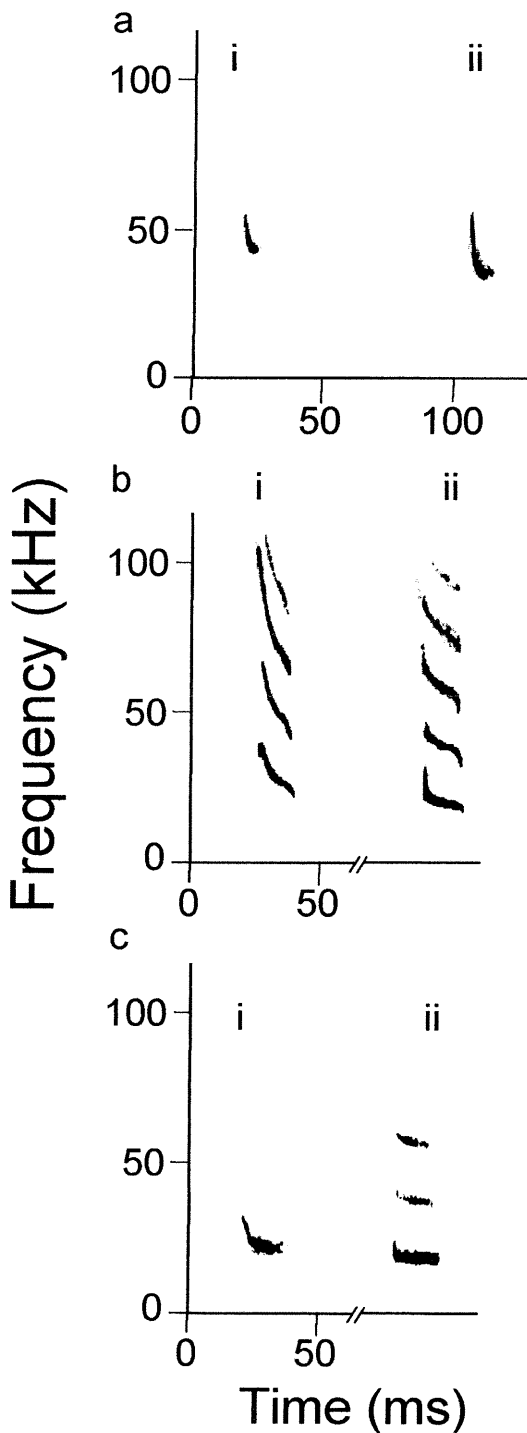


FIG. 3.—Sonograms of echolocation calls produced at altitude >10 m of a) *Hesperoptenus blanfordi*, b) *Mops mops*, and c) *Chaerephon johorensis* (i, high-frequency call; ii, low-frequency call).

DISCUSSION

Several considerations arise from the present study in evaluating possible functions of call alternation in bats flying in open spaces. First, all 5 species described used alternating calls when they flew at high altitudes (>10 m) and were clear of clutter, as in *Nyctalus noctula* (Jones 1995; Miller and Degn 1981) and *Lasiurus borealis* and *L. cinereus* (Obrist 1995) but in contrast to *Barbastella barbastellus* (Denzinger et al. 2001). Thus, modifications to the signal are unlikely to represent the typical plastic response to increasing clutter. At low altitudes (5–10 m) and near clutter, the bats did not alternate calls but changed call structure to increase bandwidth and decrease duration of call, presumably to obtain more detailed information about background clutter and to prevent pulse–echo overlap. Second, any hypothesis about function of call alternation should explain the relevance of frequency differences between alternating calls and the increase in this difference as call frequency increases across species. Third, 3 of the 5 species exhibited a decrease in sound pressure level when making the transition from low- to high-frequency calls and an increase in sound pressure level in switching from high- to low-frequency.

We consider a social or antijamming role (Habersetzer 1981; Kössl et al. 1999) unlikely because bats in the present study used call alternation in the absence of conspecifics. The 2nd hypothesis, in which the use of frequency alternation extends the range of echolocation during search phase, assumes that a bat can discriminate between successive echoes and cross-match the pulse sequence with the echo sequence. Alternating call frequencies could provide pulse–echo separation in the frequency domain, and this would enable the bat to increase both duration of call and pulse interval (thereby increasing maximum detection distance) while minimizing danger of signal overlap and circumventing constraints on pulse emission rates imposed by

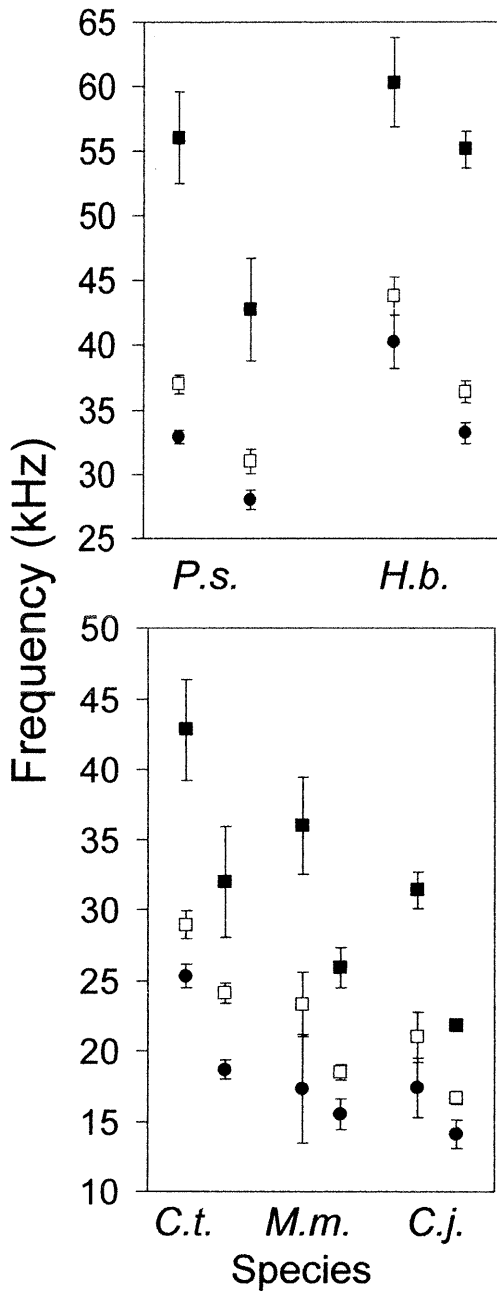


FIG. 4.—Frequencies of high- and low-frequency calls emitted during flight at high altitude as shown by starting frequency (filled squares), peak frequency (open squares), and terminal frequency (filled circles). Symbols indicate means. Error bars indicate 95% confidence interval. Species: *P.s.*, *Pipistrellus stenopterus*; *H.b.*, *Hesperoptenus blanfordi*; *M.m.*, *Mops mops*; *C.j.*, *Chaerephon johorensis*; *C.t.*, *Cheiromeles torquatus*.

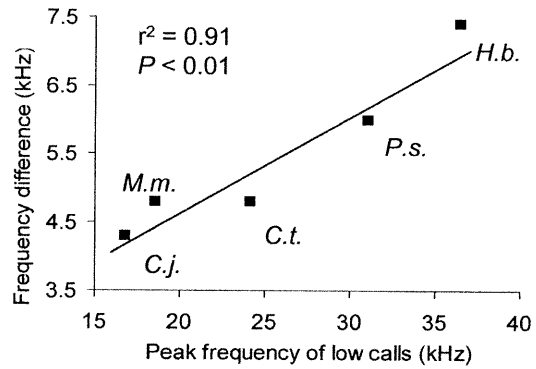


FIG. 5.—Relationship between frequency separation of alternating calls and peak frequency of low-frequency calls in 5 open-space species of bats at Krau Wildlife Reserve, Malaysia. Species abbreviations as in Fig. 4.

the wing beat frequency (Fenton et al. 1998; Heller 1995). Three predictions arise from this interpretation (Fenton et al. 1998). First, the overlap in frequency between alternating calls will be minimal to maximize

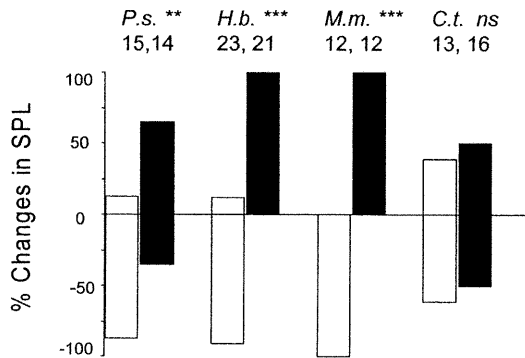


FIG. 6.—Direction of change in sound pressure levels (SPL) during transition between successive call types. Open bars—percentage of changes in sound pressure level during the transition from high- to low-frequency calls. Filled bars—percentage of changes in sound pressure level during the transition from low- to high-frequency calls. Results of chi-square test to determine whether the direction of change in sound pressure level was independent of the direction of change in peak frequency are indicated (ns, not significant; $P > 0.05$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$) and number of transitions measured (low-high, high-low). Species abbreviations as in Fig. 4.

separation of pulse and echo. Second, bats using low-frequency echolocation calls will be most likely to use frequency alternation because above 15 kHz atmospheric attenuation determines maximum detection distances. Third, during the approach and attack on prey, alternation will be minimal because there is less need to maximize effective range. Our findings provide mixed support for this hypothesis. All species in the present study used echolocation calls >15 kHz, although *M. mops* and *Cha. johorensis* were both <20 kHz. Although there was substantial overlap of alternating fundamental frequencies when the entire bandwidth was considered, individual parameters (starting frequency, terminal frequency, and peak frequency) never overlapped within a species (Fig. 4), and this lack of overlap may be sufficient for the bat to discriminate between successive calls. A single recording of *Che. torquatus* showed no alternation during the attack sequence; calls produced during approach were most like those of high-frequency calls (T. Kingston, in litt.), although this is in contrast to alternation observed in the approach phase in *Che. torquatus* recorded by Heller (1995).

Heller (1989, 1995) proposed that low-frequency calls may enable the bat to detect more distant but larger prey items, whereas high-frequency calls might permit the detection of smaller, nearer, prey items. Sound attenuation is frequency-dependent (Lawrence and Simmons 1982; Pye 1993); low-frequency calls experience less attenuation and favor detection over longer distances, but small prey items with diameters less than the wavelength of the emitted signal may not be detected due to Rayleigh scattering (Houston et al. 2003; Pye 1993). Target resolution is improved by the use of higher frequencies (so smaller prey are detectable), but atmospheric attenuation reduces the operating range (Simmons and Stein 1980). The lower sound pressure level associated with high-frequency calls of some species certainly suggests that opera-

tional range of the 2 call types will differ as predicted, and this hypothesis also offers a possible explanation for the relationship observed in Fig. 5. Wavelength is inversely proportional to frequency, and thus increases faster as frequency decreases. Therefore the frequency separation between alternating calls must be greater at higher frequencies to preserve a given difference in wavelength. For example, to achieve a 5 mm separation between 2 alternating calls, a bat calling at 20 kHz (wavelength 17.4 mm) would need to call at 28.0 kHz (a difference of 8 kHz), whereas a bat calling at 30 kHz would need to call at 53.6 kHz, a difference of 22.6 kHz. Conversely, a 4.8-kHz mean separation in call frequency achieves a wavelength difference of 3.9 mm in *M. mops* and 2.2 mm in the higher-frequency *Che. torquatus*. The difference in call wavelength between high- and low-frequency calls ranged from 1.6 mm in *H. blanfordi* (based on mean peak frequency values) to 4.3 mm in *Cha. johorensis*. Only *N. noctula* is known to exceed this (with a 4.5 mm difference in wavelength), but many species that use alternating calls show considerably less separation of wavelength (e.g., *B. barbastellus* and *Molossus molossus* 1.5 mm, *N. leisleri* 1.2 mm, *Chalinolobus gouldii* 0.9 mm, *T. tricolor* and *Saccopteryx bilineata* 0.3 mm, and *S. leptura* 0.2 mm). Whether such differences are sufficient to influence minimum detectable prey size is uncertain. Jones and Barlow (in press) found that a 1.4-mm wavelength difference had little influence on target strengths from different sized insects, and prey of different sizes may be detected with equal efficiency using a range of frequencies (Houston et al. 2003). Furthermore, dietary analyses confirm that bats with narrowband, low-frequency echolocation calls may feed on insects smaller than expected (Houston et al. 2003; Rydell and Yalden 1997; Waters et al. 1995).

Heller (1989) also suggested that frequency alternation may enable *H. blanfordi* to combine long-ranging low-frequency

calls with the better target resolution of high-frequency calls, a hypothesis developed further by Parsons et al. (2001) and Denzinger et al. (2001) with the incorporation of call duration, bandwidth, and sound pressure level. For maximum long-range detection, low-frequency signals should be associated with narrower bandwidth and longer duration, contrasting with localization or ranging calls in which high frequencies are linked with greater bandwidth and shorter duration. The results of the present study provide some support for this. In the 2 vespertilionid species, high-frequency calls were of shorter duration, as predicted for a broadband localization signal and also facilitating the avoidance of pulse-echo overlap at higher frequencies. High-frequency calls were of greater bandwidth in *P. stenopterus* and the 2 molossid species tested (*Che. torquatus* and *Cha. johorensis*). High-frequency calls were not of shorter duration in the molossids.

This hypothesis also provides an explanation for the association of lower sound pressure level with high-frequency calls because the spectral and temporal structure of localization calls should reduce their operational range. Not only do higher frequencies attenuate faster, but the emitted sound energy is spread across a greater range of frequencies, and the calls are of short duration. Amplitude alternation may also indicate that the bat is directing calls in different directions to increase the search volume. This has been suggested for *Myotis nigricans*, which does not alternate in frequency (Siemers et al. 2001), and *B. barbastellus*, in which it is the high-frequency calls that are of greater amplitude (Denzinger et al. 2001).

We consider the hypothesis that the alternation in call frequencies may be a mechanism by which bandwidth is increased (Heller 1995). Heller (1995) noted that upper harmonics were frequently present in calls of free-flying *Che. torquatus*, that the frequency content of calls (including the 2nd harmonics) showed little over-

lap, and that successive signals provided a very broad, uninterrupted frequency band. Increasing the bandwidth of a single call could provide the same coverage, but this would spread the sound energy per pulse through more frequencies and reduce the operating range substantially. By alternating calls, the bat achieves a wide bandwidth without compromising detection distances. This assumes that bats can form images of their surroundings using information from several sequential echoes that return not just from a single pulse (Dear et al. 1993; Simmons et al. 1995 for review) but also from sequential pulses, as may be the case when the bat is in motion or detecting moving prey (Jensen et al. 2001; Wilson and O'Neill 1998).

Support for this hypothesis comes from the frequency relationship between alternating call types in the present study (Fig. 4), which further suggests that it is the inclusion of complementary harmonics (Figs. 1a and 2a) that affects the increase in bandwidth. Ideally, harmonics of high-frequency calls should minimize overlap with those of low-frequency calls (as suggested by Figs. 1a and 2a). The most intuitive expectation is that the high-frequency fundamental should be equidistant between the low-frequency fundamental and its 2nd harmonic (at 36.2 kHz in *Che. torquatus*). However, this would place the 2nd harmonic of the high-frequency call in the same frequency range as the 3rd harmonic of the low-frequency call (both at 72.3 kHz in *Che. torquatus*). Instead it appears that the peak frequencies of the fundamentals are arranged so that the 2nd harmonic of the high-frequency calls falls between the 2nd and 3rd harmonic of the low-frequency calls. This arrangement can be described as predicted peak frequency of high-frequency calls = ((observed peak frequency of low-frequency calls \times 5)/4) and predicts the peak frequency of high-frequency calls for *Che. torquatus* to be 30.1 kHz. This is just over 1 kHz above the observed value of 28.9 kHz. A similarly good fit between the predicted

and observed value of high-frequency calls was found for the other 4 species: *H. blanfordi*—predicted 45.5 kHz cf. observed 43.8 kHz; *Cha. johorensis*—20.9 kHz cf. 21.0 kHz; *M. mops*—23.1 kHz cf. 23.3 kHz; *P. stenopterus*—38.8 kHz cf. 37.0 kHz. Thus, as the fundamental frequency gets higher across species, the frequency difference between the high- and low-frequency calls increases. This relationship is particularly remarkable because it is defined by bats of very different sizes and belonging to 2 families. Harmonic inclusion was suggested by several recordings in the present study (Figs. 1a and 2a). However, further research is needed to obtain a more standard assessment of the presence or absence of harmonics during flight in open spaces and to obtain more detailed measurements.

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