RESEARCH ARTICLE

High duty cycle echolocation and prey detection by bats

Louis Lazure* and M. Brock Fenton

Department of Biology, University of Western Ontario, London, ON, Canada, N6A 5B7

*Author for correspondence (louis.lazure@gmail.com)

SUMMARY

There are two very different approaches to laryngeal echolocation in bats. Although most bats separate pulse and echo in time by signalling at low duty cycles (LDCs), almost 20% of species produce calls at high duty cycles (HDCs) and separate pulse and echo in frequency. HDC echolocators are sensitive to Doppler shifts. HDC echolocation is well suited to detecting fluttering targets such as flying insects against a cluttered background. We used two complementary experiments to evaluate the relative effectiveness of LDC and HDC echolocation for detecting fluttering prey. We measured echoes from fluttering targets by broadcasting artificial bat calls, and found that echo amplitude was greatest for sounds similar to those used in HDC echolocation. We also collected field recordings of syntopic LDC and HDC bats approaching an insect-like fluttering target and found that HDC bats approached the target more often (18.6% of passes) than LDC bats (1.2% of passes). Our results suggest that some echolocation call characteristics, particularly duty cycle and pulse duration, translate into improved ability to detect fluttering targets in clutter, and that HDC echolocation confers a superior ability to detect fluttering prey in the forest understory compared with LDC echolocation. The prevalence of moths in the diets of HDC bats, which is often used as support for the allotonic frequency hypothesis, can therefore be partly explained by the better flutter detection ability of HDC bats.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/214/7/1131/DC1

Key words: bat, echolocation, duty cycle, prey detection, evolution, foraging.

INTRODUCTION

The timing of production of echolocation calls separates bats using laryngeal echolocation into two categories: those signalling at high duty cycles (HDCs) and those signalling at low duty cycles (LDCs) (Fenton, 1999). The duty cycle of a periodic sound is defined as the proportion of time spent emitting signals in a given period of time. LDC echolocators separate pulse and echo in time and, to avoid self-deafening, do not simultaneously broadcast and receive. The search phase calls of LDC echolocators are typically short, separated by relatively long periods of silence, and consist of frequency-modulated sweeps of varying bandwidth. In contrast, bats using HDC echolocation avoid self-deafening by separating pulse and echo in frequency. The calls of HDC echolocators are long, separated by short periods of silence, and are typically dominated by a constant frequency component and begin and/or end with a short-frequency modulated sweep.

Physiologically, HDC echolocators have an acoustic fovea because their auditory systems are tuned to very narrow ranges of frequencies (Schuller and Pollak, 1979; Schnitzler et al., 1983; Schnitzler, 1987; Rübsamen et al., 1989; Kober and Schnitzler, 1990; Neuweiler, 1990). This narrow-band sensitivity enables these bats to readily detect moving prey as spectral variation around the carrier frequency. Flutter detection allows HDC bats to distinguish moving (usually referred to as fluttering because of the movement of prey wings) targets from stationary objects in the background (von der Emde and Schnitzler, 1986; Schnitzler, 1987; Neuweiler, 1990; Roverud et al., 1991). Furthermore, HDC bats ignore stationary or very slow fluttering prey (Goldman and Henson, 1977; Schnitzler and Henson, 1980; Bell and Fenton, 1984; Sum and Menne, 1988).

Some LDC bats can detect fluttering insect-like targets in the laboratory (Kober and Schnitzler, 1990; Roverud et al., 1991; Moss and Zagaeski, 1994; Grossetête and Moss, 1998) but their ability to do this under natural conditions has not been tested. In captivity, the performance of LDC bats in detecting flutter is usually inferior (Roverud et al., 1991) or, at best, comparable to that of HDC bats (Sum and Menne, 1988).

LDC echolocation is considered to be ancestral in bats whereas HDC echolocation is thought to be derived (Fenton et al., 1995; Simmons and Geisler, 1998; Schnitzler et al., 2004; Eick et al., 2005; Jones and Teeling, 2006). This interpretation implies that HDC echolocation offers an advantage over LDC echolocation, resulting in its having been selected for. Improved flutter detection by HDC bats relative to syntopic LDC bats might provide part of the advantage. However, the role of competition in bat assemblages remains unclear. Some authors suggest that competition might structure bat communities and influence patterns of echolocation (Schoeman and Jacobs, 2008), but several disagree (Arita, 1997; Stevens and Willig, 1999; Jiang et al., 2008; Meyer and Kalko, 2008).

Compared with the echolocation calls of LDC bats, HDC bats often use echolocation calls that are higher in frequency and dominated by a single frequency (Jones, 1999; Jacobs et al., 2007). Emphasis on a single frequency could make bats' echolocation calls more conspicuous to insects with bat-detecting ears (Yager, 1999), so hearing-based defences of prey could influence foraging success of echolocating bats (Novick, 1977; Fenton and Fullard, 1979; Jacobs et al., 2008; Surlykke and Kalko, 2008). The allotonic frequency hypothesis (Novick, 1977; Fenton and Fullard,

1979; Schoeman and Jacobs, 2003) proposes that relatively low and high calling frequencies in bats result from strong selection pressure to become less audible to tympanate prey. The allotonic frequency hypothesis is commonly invoked to explain the tendency for HDC bats using high-frequency echolocation calls to eat more moths than LDC bats (Jones, 1992). Maximum sensitivity of insect ears is typically 20–60 kHz (Fullard, 1987; Yager, 1999), but many African moths are sensitive to frequencies up to 110 kHz (Jacobs et al., 2008). This raises the question of why there is a prevalence of moths in HDC bats' diet, considering that many moths can still hear them.

We set out to test the hypothesis that HDC echolocation confers advantages over LDC echolocation because of improved detection of fluttering targets. We predicted that HDC echolocation would generate more detectable echoes from fluttering targets than LDC echolocation. We also predicted that bats using HDC echolocation would be better able to detect fluttering prey than syntopic bats using LDC echolocation. Finally, we assessed the hypothesis that prey detection is a primary selective force influencing the echolocation features of bats. To test our hypotheses, we used two complementary experimental approaches. First, we created synthetic echolocation calls representing a wide selection of both HDC and LDC signals. We used these calls to ensonify a fluttering target and measured the influence of call variables on echo characteristics. Second, we exposed artificial fluttering prey to free-living syntopic HDC and LDC bats to assess bats' responses to these targets. We used sound and video recordings to monitor the bats' behaviour. From the field experiments we also assessed the relationship between natural call parameters and assumptions about the detection ability of various species.

MATERIALS AND METHODS
Synthetic calls

We digitally synthesised calls using MATLAB version 7.5 (The MathWorks Inc., Natick, MA, USA) and saved them as sound files (.wav format). We created a variety of calls (N=278) with a wide range of call designs, based on the echolocation calls of living bats (Table 1). Some synthetic calls resembled typical LDC calls (short pulse duration, large bandwidth), others HDC calls (long pulses, constant and high frequency). The rest were a combination of intermediate features. All calls had a constant sweep rate.

Recording setup and procedure

We constructed a custom fluttering target by attaching a piece of masking tape to a thin metal rod (1 mm diameter, 40 cm length) connected to a battery-powered 12 V DC motor (NexxTech 2730255, Orbyx Electronics, Concord, Ontario, Canada). We constructed a 'large' (1.2×2.2 cm wing area) target rotating at 70 Hz and a 'small'

Table 1. Variables used to create synthetic calls

Variable	Range	No. intervals
Pulse duration (ms)	1–60	6
Duty cycle (%)	1–80	5
Frequency (kHz)	20–110	19
Bandwidth (kHz)	0–70*	15
Harmonics (dimensionless)	1–4	4
Sweep rate[†] (kHz ms^{-1})	0–70*	14

The number of intervals is the number of different values used in the creation of the calls.

*0 kHz is a constant frequency signal.

[†]Dependent on pulse duration and bandwidth.

target (0.7×1.7 cm wing area) rotating at 95 Hz because smaller insects usually have faster wing-beat frequencies (Kober and Schnitzler, 1990). The rotation of the 'wing' produces an acoustic echo similar to that of a fluttering insect (Bell and Fenton, 1984; von der Emde and Schnitzler, 1986; Sum and Menne, 1988; Grossetête and Moss, 1998).

We played back simulated echolocation calls with a ScanSpeak ultrasonic loudspeaker (frequency range of 1–120 kHz; Avisoft Bioacoustics, Berlin, Germany) aimed at the fluttering target. Calls were digitised at a sampling rate of 250 kHz and a resolution of 16 bits with an Avisoft UltraSoundGate 116 and Recorder (version 2.9) software (Avisoft Bioacoustics), and stored as .wav files. Analog signals were generated by a digital-to-analog converter board (DAQCard 6062E, National Instruments, Austin, TX, USA) and amplified using an ultrasonic amplifier (Avisoft Bioacoustics) before delivery to the loudspeaker.

To detect echoes from the target, we mounted a condenser microphone (CM16, Avisoft Bioacoustics) 0.1 m from the speaker. To attenuate direct transmission of the sound, we put a plastic sheet and a piece of acoustic foam 0.02 m thick between the microphone and the speaker, which were 0.4 m from the target. Recording took place in a 45 m^2 room with a 2.9 m high ceiling.

Analysis of echoes

We quantified bats' potential to detect fluttering prey by measuring relative echo strengths (Houston et al., 2004). Prior to analysis, we filtered out frequencies below 18 kHz and above 120 kHz using custom MATLAB scripts. We assessed the likelihood of target detection as the signal-to-noise ratio given in decibel (SNR$_{db}$); specifically, the SNR$_{db}$ of the strongest glints (a sudden amplitude peak and spectral broadening) in the echo produced by the wingbeat in the echo. We marked the location of the glints in the file after plotting the data as an oscillogram in MATLAB. In the SNR$_{db}$ measurement, the glint is the signal. We calculated SNR$_{db}$ as follows:

$$\mathrm{SNR_{db}} = 20\log_{10}(A_{\mathrm{signal}} / A_{\mathrm{noise}}), \tag{1}$$

where A_{signal} and A_{noise} are the peak amplitudes of the signal and noise floor, respectively.

Field experiment locations

We conducted most of our field-based experiments in June and July 2008 at two sites in Yangminshan National Park, Taipei County, Taiwan (25°09′N, 121°31′E), and three sites in Hsin Chu County, Taiwan (24°36′N, 121°07′E). All sites were in forested areas where bats foraged, based on previous observations by researchers from the Institute of Ecology and Evolutionary Biology at the National Taiwan University (Taipei, Taiwan). Yangminshan Park has six species of LDC bats and three species of HDC bats, all insectivorous (Lee and Huang, 2007). We also performed experiments from 23 to 27 April 2009 at Lamanai, Orange Walk County, Belize (17°45.848′N, 88°39.128′W), where over 40 species of bats have been reported, 21 of them insectivorous bats (20 LDC species; one HDC species, *Pteronotus parnellii*) (Fenton et al., 2001). We set up the apparatus close to flight paths used by bats foraging in the forest understory. We changed sites every night, though all sites were less than a 30 min walk apart.

Field procedures

We presented the same artificial fluttering targets in the field that we had used to generate echoes in the laboratory. In the field, we mounted the targets so they could flutter and move (Fig. 1). The prey moved back and forth (along the plane of rail) at ~0.7 m s^{-1}.
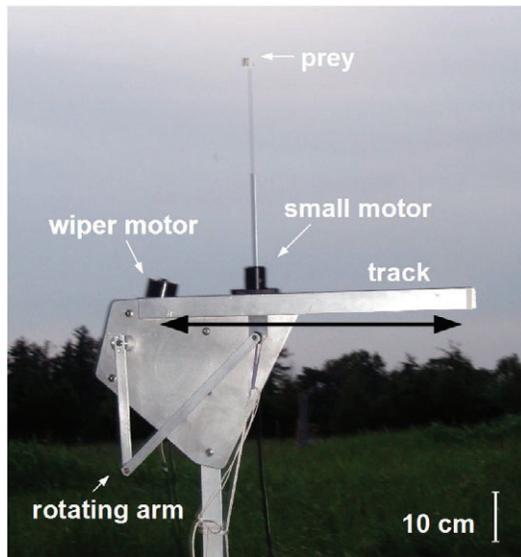
Fig. 1. Mechanical apparatus used for presenting a fluttering target in the field. The wingbeat of the prey is simulated by a piece of tape connected to the end of a thin metal rod rotated by a small motor. The black case enclosing the small motor can slide from left to right along the railing, animated by the motion of the rotating arm, powered by a wiper motor. The apparatus is not shown in the context under which experiments were conducted.

We remotely controlled the motors responsible for fluttering rate and movement from a distance of 4 m.

We varied prey wing size and wingbeat frequency, as well as the linear motion of the prey. We tested three wing sizes (0.58, 0.72 and 2.89 cm²), four wingbeat rates [0 (control), 20, 50 and 80 Hz] and whether the prey moved laterally (moving) or not (stationary control), resulting in a total of 24 possible combinations. This variation should reflect a diversity of prey types ranging from a small fast-flying insect like a fly to a large moth with slower wing beats.

We monitored approach and attack behaviour by bats during a 4 h period beginning at dusk. Bats were presented with various combinations of wingbeat flutter rate, prey size and motion. During 2 min recording trials we changed the wingbeat speed or motion every 20 s. Between each recording trial, we changed the target size. The order of presentation of different combinations of target characteristics was randomly determined prior to the experiment. We recorded bat calls with an ultrasound microphone (CM16, Avisoft Bioacoustics) connected to a laptop computer running Recorder software version 2.9 (Avisoft Bioacoustics). Sounds were digitised at a sampling rate of 250 kHz and a resolution of 8 bits, and stored as .wav files. We filmed bats as they approached the target using a video camera equipped with 'night vision' (DCR-SR46, Sony, Tokyo, Japan) and an infrared light source (IRLamp6, Wildlife Engineering, Tucson, AZ, USA). We recorded at 30 frames sec⁻¹. The field of view captured by the camera measured ~160×120 cm, with the target at the centre of the screen. The ultrasonic microphone and video camera were synchronised while running side-by-side 4 m from the target and 1.5 m above the ground.

Analysis of behaviour and echolocation

We counted the number of bat passes in our acoustic recordings. We defined a bat pass as the sequence of echolocation calls produced by a bat as it passed through the airspace sampled by the recording microphone (Fenton et al., 1998). We determined the following call variables using BatSound Pro version 3.31b (Petterson Electronik AB, Uppsala, Sweden): call duration (ms), peak spectral frequency (kHz), bandwidth (kHz), sweep rate (bandwidth/pulse duration; kHz ms⁻¹) and duty cycle [pulse duration/(pulse duration + interpulse interval); %]. We assumed that detection of prey would result in an observable change in both the bat's echolocation and flight behaviour. We assigned each acoustic pass to either: (1) approach calls coupled with a bat approaching the fluttering target on the video or (2) no approach when the bat did not alter its flight path and calling pattern. We used the video recordings to validate the categorisation of passes. We counted approaches to the prey when the bat changed its trajectory to face and then fly towards the target.

Statistics

We conducted a principal component analysis (PCA) on the data collected in the synthetic call experiment. This reduced the dimensionality of the synthetic call data and the potential for interaction effects associated with highly intercorrelated call variables. We then plotted the PC values against recorded target strength. We used regression analysis, and examined different relationships (linear, quadratic, inverse, logistic and logarithmic) to determine which best fit the data to identify significant relationships. Prior to these analyses, we transformed the data by adding 10 units to the PCA results, shifting the entire distribution by a positive constant, to accommodate functions that cannot handle negative values. We compared the coefficient of determination (R^2 values) to decide which regression model was the best fit. We compared the echo target strengths of the small fast-fluttering targets and large slow-fluttering targets using a two-sample t-test.

For data from the field experiment, we used Pearson's chi-square test to determine whether approach toward the fluttering target was paired with echolocation type, location or bat species. We used Fisher's exact test when it was more appropriate (2×2 contingency table and small samples). We compared HDC bats with LDC bats, LDC bats from Taiwan with LDC bats from Belize, and further compared taxa within LDC and HDC bats. To assess whether the number of approaches was influenced by prey characteristics (wing size, wingbeat frequency and prey movement), we performed Pearson's chi-square tests. We performed these analyses separately for LDC and HDC bats. To examine call variables separately, we conducted binary logistic regressions considering a binary response variable ('approach' and 'no approach') for each call variable. We only used data from Taiwan for logistic regression analysis because of the large sample size and we wanted to avoid bias stemming from differences in sampling effort and geography.

RESULTS
Synthetic calls experiment

The strength of echoes from larger and more slowly fluttering targets was stronger than that from smaller and faster fluttering targets (t=4.47, d.f.=136, P<0.001).

Following a PCA on call variables, we chose the first three PCs that accounted for >75% of the variation, defining biologically relevant groups of variables (see supplementary material Tables S1 and S2). The relationship between spectral variables (PC1) and target strength was best described by a quadratic equation ($F_{2,273}$=105.30, P<0.001, R^2=0.44; Fig. 2A). Weaker target echoes were significantly associated with increasing call frequency, but only the decreasing
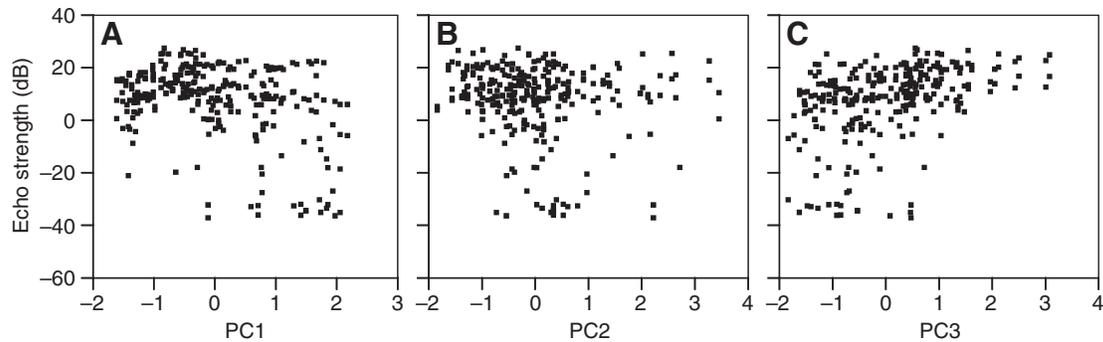
Fig. 2. Echo strength (measured as the signal to noise ratio) from a fluttering target in relation to varying call variables. Principal components (PC) represent (A) spectral variables (maximum, minimum and frequency of maximal energy), (B) signal bandwidth and sweep rate and (C) temporal variables (pulse duration and duty cycle). Greater values of a PC score correspond to increased values of the call variables.

portion of the curve fitted within a realistic range of signalling frequencies. Bandwidth, sweep rate variables (PC2) and target strength were significantly related ($F_{2,273}$=40.22, P<0.001; Fig. 2B), with a best-fit quadratic equation (R^2=0.23). The weakest target strength occurred in the mid range of the distribution. Finally, temporal acoustic variables (PC3) and target strength were also significantly related ($F_{2,273}$=62.66, P<0.001; Fig. 2C). A quadratic function had the best fit (R^2=0.32), with all values in the increasing portion of the curve, indicating a stronger target strength with increasing duration and duty cycle.

Field experiment
During 23 nights of sampling in Taiwan, we recorded 2727 passes and echolocation calls from three species of HDC bats (*Rhinolophus monoceros*, *Hipposideros armiger* and *R. formosae*). We were unable to identify all LDC bats to species level because of the similarity of echolocation calls among species and intraspecific variability in call features. Almost all approaches to the target (442 of 446) were by HDC echolocators. The mean approach rate was 18.6% (*N*=2382 passes) for HDC bats and 1.2% for LDC bats (*N*=345). The four LDC bats that approached fluttering targets were vespertilionids in the subfamily Murininae and/or Kerivoulinae. These bats have distinctive echolocation calls characterised by short durations (~1 ms), high sweep rates (21–26 kHz ms^{-1}) and broad bandwidths (>60 kHz).

In Belize, we recorded five passes of the HDC bat *P. parnellii* and 370 passes of LDC species over five sampling nights. The relative percentage of HDC individuals over the total number of passes we recorded (1.3%) is less than the percentage of HDC bats caught by Fenton et al. in the same location (3.5%) (Fenton et al., 2001). Among the LDC bats recorded, 134 were members of the genus *Pteronotus* (*Pteronotus personatus* and *P. davyi*). Three of the LDC *Pteronotus* spp. passes included an approach to the fluttering target, yielding an approach rate of 2.2%. We saw no approaches from other LDC species (vespertilionids, emballonurids and molossids) in Belize.

The likelihood of approach to prey was related to echolocation call type (χ^2=135.5, d.f.=1, P<0.001; Figs 3, 4). Hipposiderids and rhinolophids differed significantly in their approach rate (χ^2=302.6, d.f.=1, P<0.001), with rhinolophids approaching more often than hipposiderids (30.3 *versus* 2.2%, respectively). The approach rate for the LDC bats in Taiwan did not differ significantly from that for LDC bats in Belize (Fisher's exact test, P=0.72). We further examined the data from Belize, comparing LDC *Pteronotus* spp.

with all other LDC bats, and found no relationship between species and approach rate (Fisher's exact test, P=0.08). LDC bats exhibited a low approach rate to fluttering targets that was not influenced by wingbeat frequency (χ^2=1.16, d.f.=2, P=0.56; Fig. 3A), wing size (χ^2=2.12, d.f.=2, P=0.35) or the presence of lateral movement (Fisher's exact test, P=0.46; Fig. 4). However, the approach rate of HDC bats was significantly influenced by wingbeat frequency (χ^2=20.24, d.f.=2, P<0.001; Fig. 3) and prey size (χ^2=52.77, d.f.=2, P<0.001; Fig. 3B) but not by lateral prey movements (Fisher's exact test, P=0.18).

We performed a multiple binary regression with call characteristics as predictor variables and echo detection (yes/no) as the dependant variable. We conducted the regression using pulse duration, peak frequency and bandwidth to avoid multicollinearity. An increase in approach rate was related to an increase in pulse duration (*W*=25.74, P<0.001; Fig. 4A) and peak frequency (*W*=130.99, P<0.001; Fig. 4B), but not bandwidth (*W*=0.61, P=0.44; Fig. 4C). The midpoint of the logistic curve for peak frequency was 120 kHz, corresponding to an approach rate of 0.5.

DISCUSSION
Prey detection
Our data demonstrate that, in forest understory habitats, where hunting echolocating bats receive multiple echoes from objects other than insect prey, HDC bats approached fluttering targets significantly
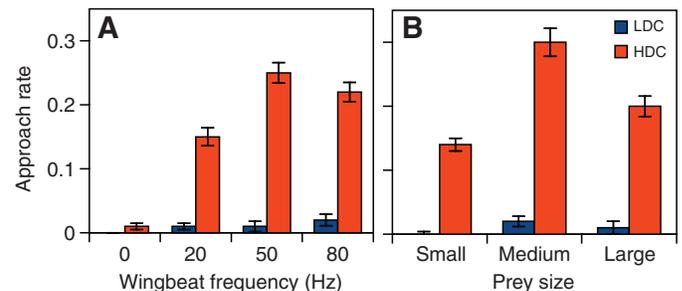

Fig. 3. (A) Approach rate (proportion of bats that approached the target over the total number of bats recorded acoustically in proximity; mean ± s.e.m.) of low duty cycle (LDC) and high duty cycle (HDC) bats presented with a mechanical prey target fluttering at different wingbeat frequencies (Hz). (B) Approach rate of LDC and HDC bats presented with artificial prey of different sizes: small (0.58 cm^2), medium (0.723 cm^2) and large (2.89 cm^2).
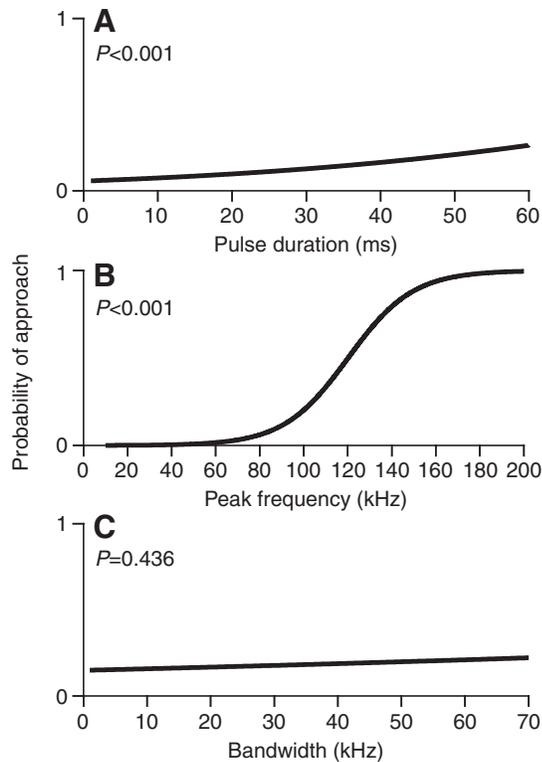
Fig. 4. Relationships between the probabilities of approach toward the target by bats in the field and three call variables: (A) pulse duration, (B) peak frequency and (C) bandwidth. Probability of approach is determined from binary data: 0=target not approached by the bat, 1=target approached. The range of values presented for the call variables are within reasonable ranges found in extant bats.

more often than LDC bats. Our data confirm that HDC echolocation is better than LDC echolocation for detecting fluttering prey in cluttered habitat (von der Emde and Schnitzler, 1986; Schnitzler, 1987; Neuweiler, 1990; Roverud et al., 1991). Our results also indicate that rhinolophids may be more efficient at detecting fluttering targets than hipposiderids. In previous experiments, rhinolophid bats showed higher sensitivity to echoes containing flutter information compared with hipposiderids, indicating that they were more effective at detecting flutter (von der Emde and Schnitzler, 1986; Roverud et al., 1991).

The prevalence of moths in the diet of rhinolophid bats cannot entirely be explained by the allotonic frequency hypothesis (Jacobs et al., 2008; Fullard et al., 2008). The diets of rhinolophids and hipposiderids may reflect their superior performance in flutter detection than their LDC counterparts. Our data suggest that, among HDC echolocators, rhinolophids are more effective flutter detectors than hipposiderids, a prediction that is testable in the field.

Size of target and rate of flutter influenced approach rate by HDC bats, but our data set did not allow us to determine whether this reflects differences in detection ability and/or prey preference. For both wingbeat frequency and prey size, targets in the mid range were most often approached. Our experiments using synthetic calls showed that larger prey reflected stronger echoes than smaller prey, supporting Waters et al. (Waters et al., 1995), who used LDC calls and dried immobile insects. The most common HDC bats recorded in Taiwan were the small *R. monoceros* (36–40 mm forearm length)

and increased approaches to medium-sized prey could reflect a compromise between bats' ability to detect and then catch and handle prey of different sizes.

Bats in the subfamilies Murininae and Kerivoulinae occasionally approached the fluttering targets in the forest understory. This response may be a function of their distinct echolocation calls and behaviour (Kingston et al., 1999). Their range of target detection is likely less than that of HDC bats because of the low-intensity and high-frequency echolocation calls (Murininae calls sweep from 180 to 43 kHz and Kerivoulinae calls sweep from 152 to 86 kHz) (Griffin, 1971; Kingston et al., 1999). In Belize, some LDC bats use a very different strategy. The two LDC *Pteronotus* spp. we recorded, and other species in this genus, emit relatively long calls for LDC bats, with some narrowband elements, and *P. personatus* accomplishes partial Doppler shift compensation (Smotherman and Guillén-Servent, 2008). However, their reaction to the fluttering prey did not differ from that of other LDC bats in the present study.

Selective forces on call design and behaviour

In the laboratory, measures of echo strength indicated that the best bandwidths and sweep rates for detecting fluttering targets occurred at both ends of the distribution (either narrowband or very broadband), but we found no clear relationship in field experiments. Bandwidth, sweep rate and even pattern of frequency change over time were not directly related to detection ability. Previous experiments showed that bandwidth and sweep rate were related to angular localization, reduction of ranging error and feature extraction (Boonman et al., 2003; Schnitzler et al., 2003; Holderied et al., 2006; Boonman and Ostwald, 2007). Therefore, bandwidth is probably not a call characteristic shaped by selection for improved detection of prey. Reducing signal bandwidth is a way to achieve increased call intensity when total power is constant. If signal power is conserved, every 10× reduction in call bandwidth would increase power spectral density at the call resonant frequency by a factor of 10, or 10 dB (Oppenheim and Schafer, 1989). The obvious advantage of a narrowband echolocation signal is an increase in call energy with no overall increase of energy expenditure in signal production.

Field and laboratory data showed that longer pulse durations and a higher duty cycle translated into higher flutter detection performance because temporal variables are important to aerial-hawking bats in forested habitat. At higher duty cycles, it may be easier to detect, track and lock onto a fluttering target because the silent period between calls is dramatically reduced. Enhanced tracking of flying targets by HDC bats is a function of their approach to avoiding self-deafening (separating pulse and echo in frequency). Additionally, longer signals are better for encoding target movements because of relatively uninterrupted modulations in echo amplitude and frequency (Schnitzler et al., 2003). Although increased pulse length can be advantageous for LDC bats, the constraint imposed by pulse–echo overlap limits call duration.

Results from our synthetic call experiment matched field data for temporal but not spectral variables. In the field, the midpoint of approach rate calculated for peak frequency occurred at 120 kHz (Fig. 4B). Species with echolocation calls that have maximum energy at 120 kHz approached fluttering targets more often than those using lower frequencies with maximum energy. Higher frequencies might permit better flutter detection by HDC bats, but suffer increased attenuation (Lawrence and Simmons, 1982). Houston et al. (Houston et al., 2004) showed that lower frequencies (20–30 kHz) yielded weaker target strengths from smaller insects, because of Rayleigh scattering (Pye, 1993). Surlykke and Kalko reported that LDC bats

calling at higher frequencies emitted stronger calls to compensate for increased atmospheric attenuation, resulting in similar ranges at which prey were detected (Surlykke and Kalko, 2008). Focusing energy at one frequency in the calls of HDC bats increases the operational range of their calls (Waters and Jones, 1995). Intraspecific variation in the frequencies dominating the echolocation calls of HDC bats may reflect some combination of environmental factors or interspecific competition. Furthermore, the acoustic communication hypothesis states that social interactions among rhinolophids impose selection pressure on peak frequency in these HDC bats (Möhres, 1967; Heller and von Helversen, 1989; Kingston et al., 2000) through intraspecific communication and identification, and avoidance of interspecific competition.

In conclusion, our results support the hypothesis that bats using HDC are more effective at detecting fluttering targets than LDC bats. We found no clear relationship between prey detection ability and frequency-dominating echolocation calls likely because many selective forces act on frequency. Prey detection is unlikely to influence the use of particular bandwidth or sweep rate. However, longer pulses and higher duty cycle improve the bats' ability to detect fluttering prey, and could have been a major selective force for an increased pulse duration and duty cycle in HDC bats.

REFERENCES

Arita, H. T. (1997). Species composition and morphological structure of the bat fauna of Yucatan, Mexico. *J. Anim. Ecol.* **66**, 83-97.

Bell, G. P. and Fenton, M. B. (1984). The use of Doppler-shifted echoes as a flutter detection and clutter rejection system: the echolocation and feeding behaviour of *Hipposideros ruber* (Chiroptera: Hipposideridae). *Behav. Ecol. Sociobiol.* **15**, 109-114.

Boonman, A. and Ostwald, J. (2007). A modelling approach to explain pulse design in bats. *Biol. Cybern.* **97**, 159-172.

Boonman, A. M., Parsons, S. and Jones, G. (2003). The influence of flight speed on the ranging performance of bats using frequency modulated echolocation pulses. *J. Acoust. Soc. Am.* **113**, 617-628.

Eick, G. N., Jacobs, D. S. and Matthee, C. A. (2005). A nuclear DNA phylogenetic perspective on the evolution of echolocation and historical biogeography of extant bats (Chiroptera). *Mol. Biol. Evol.* **22**, 1869-1886.

Fenton, M. B. (1999). Describing the echolocation calls and behaviour of bats. *Acta Chiropt.* **1**, 411-422.

Fenton, M. B. and Fullard, J. H. (1979). The influence of moth hearing on bat echolocation strategies. *J. Comp. Physiol. A* **132**, 77-86.

Fenton, M. B., Audet, D., Obrist, M. K. and Rydell, J. (1995). Signal strength, timing and self-deafening: the evolution of echolocation in bats. *Paleobiology* **21**, 229-242.

Fenton, M. B., Portfors, C. V., Rautenbach, I. L. and Waterman, J. M. (1998). Compromises: sound frequencies used in echolocation by aerial-feeding bats. *Can. J. Zool.* **76**, 1174-1182.

Fenton, M. B., Bernard, E., Bouchard, S., Hollis, L., Johnston, D. S., Lausen, C. L., Ratcliffe, J. M., Riskin, D. K., Taylor, J. R. and Zigouris, J. (2001). The bat fauna of Lamanai, Belize: roosts and trophic roles. *J. Trop. Ecol.* **17**, 511-524.

Fullard, J. H. (1987). Sensory ecology and neuroethology of moths and bats: Interactions in a global perspective. In *Recent Advances in the Study of Bats* (ed. M. B. Fenton, P. A. Racey and J. M. V. Rayner), pp. 244-272. Cambridge: Cambridge University Press.

Fullard, J. H., Ratcliffe, J. M. and Jacobs, D. S. (2008). Ignoring the irrelevant: auditory tolerance of audible but innocuous sounds in the bat-detecting ears of moths. *Naturwissenschaften* **95**, 241-245.

Goldman, L. J. and Henson, O. W., Jr (1977). Prey recognition and selection by the constant frequency bat, *Pteronotus p. parnellii. Behav. Ecol. Sociobiol.* **2**, 411-419.

Griffin, D. R. (1971). The importance of atmospheric attenuation for the echolocation of bats. *Anim. Behav.* **47**, 55-61.

Grossetête, A. and Moss, C. F. (1998). Target flutter rate discrimination by bats using frequency-modulated sonar sounds: Behavior and signal processing models. *J. Acoust. Soc. Am.* **114**, 2167-2176.

Heller, K.-G. and von Helversen, O. (1989). Resource partitioning of sonar frequency bands in rhinolophoid bats. *Oecologia* **80**, 178-186.

Holderied, M. W., Jones, G. and von Helversen, O. (2006). Flight and echolocation behaviour of whiskered bats commuting along a hedgerow: range-dependent sonar signal design, Doppler tolerance and evidence for 'acoustic focusing'. *J. Exp. Biol.* **209**, 1816-1826.

Houston, R. D., Boonman, A. M. and Jones, G. (2004). Do echolocation signal parameters restrict bats' choice of prey? In *Echolocation in Bats and Dolphins* (ed. J. A. Thomas, C. F. Moss and M. Vater), pp. 339-345. Chicago, IL: The University of Chicago Press.

Jacobs, D. S., Barclay, R. M. R. and Walker, M. H. (2007). The allometry of echolocation call frequencies of insectivorous bats: why do some species deviate from the pattern? *Oecologia* **152**, 583-594.

Jacobs, D. S., Ratcliffe, J. M. and Fullard, J. H. (2008). Beware of bats, beware of birds: the auditory responses of eared moths to bat and bird predation. *Behav. Ecol.* **19**, 1333-1342.

Jiang, T., Feng, J., Sun, K. and Wang, J. (2008). Coexistence of two sympatric and morphologically similar bat species *Rhinolophus affinis* and *Rhinolophus pearsoni. Prog. Nat. Sci.* **18**, 523-532.

Jones, G. (1992). Bats vs moths: studies on the diet of rhinolophid and hippposiderid bats support the allotonic frequency hypothesis. In *Prague Studies in Mammalogy* (ed. I. Horábeck and V. Vohralik), pp. 87-92. Prague: Charles University Press.

Jones, G. (1999). Scaling of echolocation calls parameters in bats. *J. Exp. Biol.* **202**, 3359-3367.

Jones, G. and Teeling, E. C. (2006). The evolution of echolocation in bats. *Trends Ecol. Evol.* **21**, 149-156.

Kingston, T., Jones, G., Akbar, Z. and Kunz, T. H. (1999). Echolocation signal design in Kerivoulinae and Murininae (Chiroptera: Vespertilionidae) from Malaysia. *J. Zool.* **249**, 359-374.

Kingston, T., Jones, G., Zubaid, A. and Kunz, T. H. (2000). Resource partitioning in rhinolophid bats revisited. *Oecologia* **124**, 332-342.

Kober, R. and Schnitlzer, H.-U. (1990). Information in sonar echoes of fluttering insects available for echolocating bats. *J. Acoust. Soc. Am.* **87**, 882-896.

Lawrence, B. D. and Simmons, J. A. (1982). Measurements of atmospheric attenuation at ultrasonic frequencies and the significance for echolocation by bats. *J. Acoust. Soc. Am.* **71**, 585-590.

Lee, L.-L. and Huang, C.-C. (2007). Status of bat diversity in the Yangmingshan National Park. *J. Natl. Park* **17**, 1-15.

Meyer, C. F. J. and Kalko, E. K. V. (2008). Bat assemblages on Neotropical land-bridge islands: nested subsets and null model analyses of species co-occurence patterns. *Divers. Distrib.* **14**, 644-654.

Möhres, F. P. (1967). Communicative characters of sonar signals in bats. In *Animal Sonar Systems* (ed. R. Busnel), pp. 939-945. Brussels: NATO Advanced Study Institute.

Moss, C. F. and Zagaeski, M. (1994). Acoustic information available to bats using frequency-modulated sounds for the perception of insect prey. *J. Acoust. Soc. Am.* **95**, 2745-2756.

Neuweiler, G. (1990). Auditory adaptations for prey capture in echolocating bats. *Physiol. Rev.* **70**, 615-641.

Novick, A. (1977). Acoustic orientation. In *Biology of Bats,* Vol. 3 (ed. W. A. Wimsatt), pp. 73-287. New York: Academic Press.

Oppenheim, A. V. and Schafer, R. W. (1989). *Discrete-Time Signal Processing.* Englewood Cliffs: Prentice-Hall, Inc.

Pye, J. D. (1993). Is fidelity futile? The 'true' signal is illusory, especially with ultrasound. *Bioacoustics* **4**, 271-286.

Roverud, R. C., Nitsche, V. and Neuweiler, G. (1991). Discrimination of wingbeat motion by bats, correlated with echolocation sound pattern. *J. Comp. Physiol. A* **168**, 259-263.

Rübsamen, R., Neuweiler, G. and Marimuthu, G. (1989). Ontogenesis of tonotopy in inferior colliculus of a hipposiderid bat reveals postnatal shift in frequency-place code. *J. Comp. Physiol. A* **165**, 755-769.

Schnitzler, H.-U. (1987). Echoes of fluttering insects: information for echolocating bats. In *Recent Advances in the Study of Bats.* (ed. M. B. Fenton, P. Racey and J. M. V. Rayner), pp. 226-243. Cambridge: Cambridge University Press.

Schnitzler, H.-U. and Henson, O. W., Jr (1980). Performance of airborne animal sonar systems: I. Microchiroptera. In *Animal Sonar Systems* (ed. R. G. Busnel and J. F. Fish), pp. 109-181. New York: Plenum Press.

Schnitzler, H.-U., Menne, D., Kober, R. and Heblich, K. (1983). The acoustical image of fluttering insects in echolocating bats. In *Neuroethology and Behavioral Physiology* (ed. F. Huber and H. Markl), pp. 235-250. Berlin: Springer-Verlag.

Schnitzler, H.-U., Moss, C. F. and Denzinger, A. (2003). From spatial orientation to food acquisition in echolocating bats. *Trends Ecol. Evol.* **18**, 386-394.

Schnitzler, H.-U., Kalko, E. K. V. and Denzinger, A. (2004). Evolution of echolocation and foraging behavior in bats. In *Echolocation in Bats and Dolphins* (ed. J. A. Thomas, C. F. Moss and M. Vater), pp. 331-338. Chicago: The University of Chicago Press.

Schoeman, M. C. and Jacobs, D. S. (2003). Support for the allotonic frequency hypothesis in an insectivorous bat community. *Oecologia* **134**, 154-162.

Schoeman, M. C. and Jacobs, D. S. (2008). The relative influence of competition and prey defenses on the phenotypic structure of insectivorous bat ensembles in Southern Africa. *PLoS One* **3**, e3715.

Schuller, G. and Pollack, G. (1979). Disproportionate frequency representation in the inferior colliculus of Doppler-compensating greater horseshoe bats: evidence of an acoustic fovea. *J. Comp. Physiol. A* **132**, 47-54.

Simmons, N. B. and Geisler, J. H. (1998). Phylogenetic relationships of *Icaronycteris, Archaeonycteris, Hassianycteris,* and *Palaeochiropteryx* to extant bat lineages, with comments on the evolution of echolocation and foraging strategies in Microchiroptera. *Bull. Am. Mus. Nat. Hist.* **235**, 1-182.

Smotherman, M. and Guillén-Servent, A. (2008). Doppler-shift compensation behaviour by Wagner's mustached bat, *Pteronotus personatus. J. Acoust. Soc. Am.* **123**, 4331-4339.

Stevens, R. D. and Willig, M. R. (1999). Assortment in New World bat communities. *J. Mammal.* **80**, 644-658.

Sum, Y. W. and Menne, D. (1988). Discrimination of fluttering targets by the FM-bat *Pipistrellus stenopterus*? *J. Comp. Physiol. A* **163**, 349-354.

Surlykke, A. and Kalko, E. K. V. (2008). Echolocating bats cry out loud to detect their prey. *PLoS One* **3**, e2036.

von der Emde, G. and Schnitzler, H.-U. (1986). Fluttering target detection in hipposiderid bats. *J. Comp. Physiol. A* **159**, 765-772.

Waters, D. A. and Jones, G. (1995). Echolocation call structure and intensity in five species of insectivorous bats. *J. Exp. Biol.* **198**, 475-489.

Waters, D. A., Rydell, J. and Jones, G. (1995). Echolocation call design and limits on prey size: a case study using the aerial-hawking bat *Nyctalus leisleri. Behav. Ecol. Sociobiol.* **37**, 321-328.

Yager, D. D. (1999). Structure, development, and evolution of insect auditory systems. *Microsc. Res. Tech.* **47**, 380-400.

Table S1. Eigenvalues of the correlation matrix of the principal component analysis

PC	Eigenvalue	Difference	Cumulative	Proportion
1	2.89277185	1.15532347	0.3616	0.3616
2	1.73744838	0.24561962	0.2172	0.5788
3	1.49182876	0.68593583	0.1865	0.7653
4	0.80589293	0.20103049	0.1007	0.8660
5	0.60486244	0.13767157	0.0756	0.9416
6	0.46719087	0.46718611	0.0584	1.0000
7	0.00000477	0.00000477	0.0000	1.0000

PC1 accounts for 36.16% of the variation in the data, PC2 accounts for 21.72% and PC3 accounts for 18.65%. Those three PCs together account for 76.53% of the variation and they are the only ones with eigenvalues >1.

Table S2. Relative contribution of call variables to the principal components

Call variable	PC1	PC2	PC3
Pulse duration	0.090632	−0.151037	0.620713
Duty cycle	0.186921	−0.109060	0.578823
Sweep rate	0.000463	0.615816	−0.204116
Maximum frequency	0.549517	0.202780	−0.060987
Minimum frequency	0.513313	−0.280338	−0.243356
Peak frequency	0.572671	−0.039919	−0.163150
Bandwidth	0.058025	0.648559	0.242518

For PC1, the spectral variables peak frequency, maximum frequency and minimum frequency have higher factor loading (>0.51) than the other variables (<0.18). PC2 is defined by sweep rate and bandwidth with factor loadings >0.62, in comparison to <0.20 for the others. Finally, PC3 includes the time-related variables pulse duration and duty cycle, which show factor loadings >0.58 (other variables <0.24).