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HIGH DUTY CYCLE ECHOLOCATION AND PREY DETECTION ABILITY IN BATS

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HIGH DUTY CYCLE ECHOLOCATION AND PREY DETECTION ABILITY IN BATS

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by

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Graduate Program in Biology

A thesis submitted in partial fulfillment
of the requirements for the degree of
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The thesis by

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ABSTRACT

While most echolocating bats separate pulse and echo in time (low duty cycle bats), a few species separate pulse and echo in frequency (high duty cycle). The type of echolocation is defined by the pattern of signal emission, but many other call variables differ significantly between the two approaches. This study used two complementary experiments to understand the role of flying prey detection ability as a selective force that could explain the diversity of echolocation behaviour in extant bats. I collected data from acoustical measurements of artificial sonar echoes from fluttering targets, and field recordings of hunting behaviour of different bat species. I found that prey detection is improved with increased pulse length and duty-cycle of echolocation calls. Proportionally, high duty cycle bats approached fluttering targets more often (18.6 %) than low duty cycle bats (1.16 %). Pulse length used by low duty cycle bats is limited by functional constraints of their auditory system. The frequency used by a given bat species cannot be explain by prey detection ability. High and low duty cycle bats are syntopic in some habitats, despite the fact that high duty cycle bats are more efficient at detecting flying prey than low duty cycle bats. Competition for flying insects as a food resource does not seem to be shaping the bat assemblage.

Key words: bat, echolocation, evolution, duty cycle, prey detection, diversity

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CF	Constant frequency
FM	Frequency modulated
HDC	High duty cycle
LDC	Low duty cycle
PC	Principal component
PCA	Principal component analysis
SNR	Signal to noise ratio

GLOSSARY

Acoustic fovea	In the auditory system, area of high sensitivity to a specific, narrow range of frequency.
Auditory system	The anatomical system for the sense of hearing. Implicated in echolocation.
Bandwidth	Variation in frequency (max – min) within a single pulse.
Broadband	Describe an echolocation call with a relatively large bandwidth.
Clutter	Unwanted echoes received by the bats. For a bat foraging, echoes from anything else than a prey can be considered clutter (e.g. trees, ground, falling leaves).
Constant frequency	Describe part of an echolocation call with no frequency variation.
Detection	Perception that a target is present.
Doppler shift	Change in the apparent frequency of a wave as observer and source move relative to each other.
Duty cycle	Proportion of time sounds are emitted.
Frequency modulated	Describe part of an echolocation call with a bandwidth > 0 kHz.
Narrowband	Describe an echolocation call with a relatively small bandwidth.
Sweep Rate	Bandwidth of a pulse divided by its duration

CHAPTER 1. INTRODUCTION

1.1 Echolocation call designs

Microchiropteran bats fall into two categories with respect to echolocation, namely low and high duty cycle (respectively LDC and HDC; Fenton 1999). Low duty cycle echolocators separate pulse and echo in time and cannot simultaneously broadcast and receive. High duty cycle echolocators separate pulse and echo in frequency and can broadcast and receive at the same time. When using echolocation, LDC bats emit signals 5 to 20% of the total time, whereas HDC bats emit signals 30 to 80% of the time. When referring to bats or echolocation as LDC or HDC, it is important to note that I refer to the overall approach including the bat's auditory system, call design and behaviour.

Most microchiropteran bats (approximately 740 out of 900 species) use LDC echolocation. HDC echolocation is typical of ~160 species in two Old World families, Rhinolophidae and Hipposideridae. HDC echolocation has also evolved in *Pteronotus parnellii*, one species of New World Mormoopidae. HDC bats adjust the frequency of their emitted signal when flying to compensate for Doppler-shifted generated by their own motion, allowing these bats to receive echoes at the precise frequency to which a large region of the basilar membrane and part of the auditory cortex is highly sensitive (an acoustic fovea; Schuller and Pollak 1979). LDC bats also experience Doppler-shift, but appear unable to compensate for it (Simmons 1971, Kalko and Schnitzler 1993, but see Smotherman and Guillén-Servent 2008). In both approaches, the information on distance from prey and clutter is encoded in the time delay between the pulse and the echo.

The duty-cycle categories also differ in other respects. The calls of HDC echolocators are long and typically dominated by one frequency, while most LDC echolocators use frequency modulated (FM) sweeps of varying bandwidth (Figure 1). Calls emitted by HDC bats include short FM sweeps at the beginning and/or at the end of the pure tone element, and they often reach higher frequencies than LDC calls (Jones 1999).

Foraging bats need to detect, locate and identify prey, while avoiding obstacles (e.g., Fenton 1995, Neuweiler 1990, Obrist 1995, Schnitzler and Kalko 1998). An echolocation call can be analysed in term of a number of variables that each have different properties. All HDC bats possess an important constant frequency (CF) element in their call. A constant frequency call or one with a low sweep rate (bandwidth / pulse duration) improves the probability of detecting an echo and increases the detection range (Holderied *et al.* 2006, Boonman and Ostwald 2007). Because HDC auditory system is very sensitive to a very short range of frequencies (acoustic fovea), any spectral variation around the carrying frequency is readily detected (Neuweiler 1990).

LDC bats possess frequency modulated calls, with very varying bandwidth values. FM sweeps are important during obstacle avoidance and for distance discrimination (Vogler and Neuweiler 1983, Neuweiler *et al.* 1987, Tian and Schnitzler 1997). A high sweep rate improves acuity and localization as a broadband echo will cover a larger number of neural frequency channels, and provide bats with multiple measures (Schnitzler *et al.* 2003, Holderied *et al.* 2006, Boonman and Ostwald 2007). Short, broadband calls perform badly at detecting prey very close to clutter (Arlettaz *et al.* 2001). The presence of harmonics, in low or high duty cycle echolocation, can be a way to increase bandwidth while keeping the pulse short (Zbinden 1988). Higher frequency

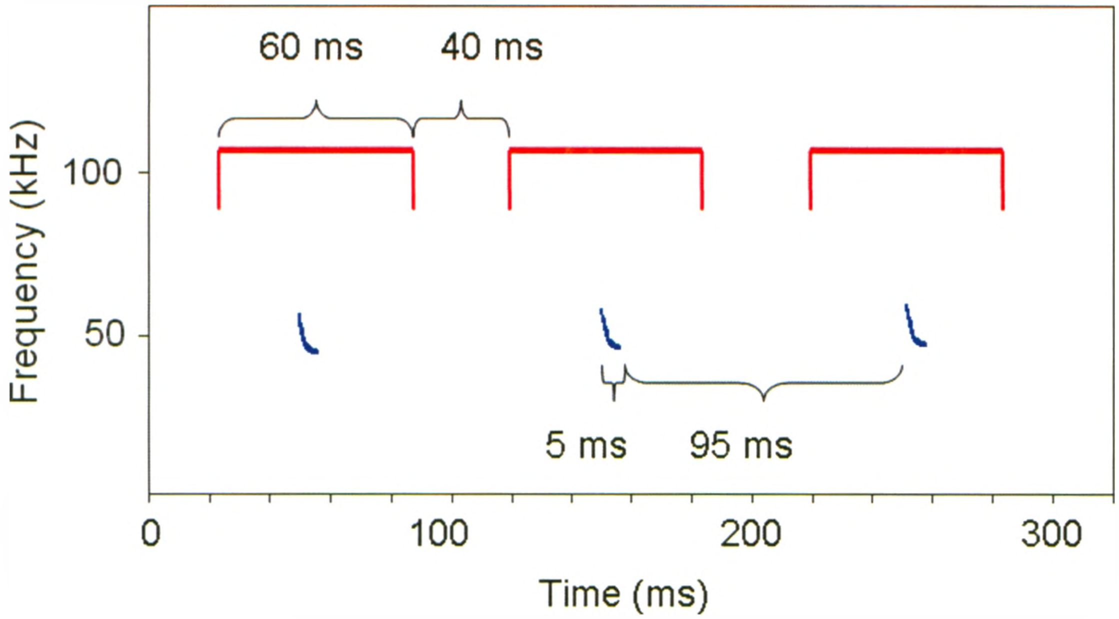

Figure 1. Spectrogram view of two calls representative of the high and low duty cycle approaches (red and blue respectively). In theses examples, duty cycles are respectively 60 % and 5 %. Low duty cycle bats need long period of silence between pulses because they cannot emit pulse and listen to the echo at the same time. Adapted from Fenton and Ratcliffe (2004).

echolocation signals are well suited for detecting a wide range of target sizes, but are limited to short ranges by atmospheric attenuation (Griffin 1971, Lawrence and Simmons 1982, Houston et al. 2004).

HDC echolocation behaviour allows simultaneous detection of fluttering (flying) prey and spatial orientation relative to clutter while avoiding self-deafening, because of their acoustic fovea (Fenton et al. 1995). HDC bats can discriminate small differences in frequency and amplitude associated with the movement of insect wings while detecting stationary objects in the background (Schuller and Pollak 1979, Schnitzler *et al.* 1983, von der Emde and Schnitzler 1986, Schnitzler 1987, Kober and Schnitzler 1990, Roverud *et al.* 1991). The amplitude glint from the wingbeat can be as high as 20 dB over the intensity of the stationary parts of the insect, and produce spectral broadening between one and three kHz (Schnitzler *et al.* 1983). Those glints encoded in the echo are a conspicuous signal for the bat indicating the presence of a flying insect. Furthermore, HDC bats ignore preys that are not fluttering (Schnitzler and Henson 1980, Bell and Fenton 1984, Sum and Menne 1988). Laboratory experiments have shown that LDC bats can also detect flutter (Kober and Schnitzler 1990, Roverud *et al.* 1991, Moss and Zagaeski 1994, Grossetête and Moss 1998) but their efficacy in the field needs to be tested. In the laboratory, LDC bat performance in detecting flutter was deemed comparable to the HDC bats (Sum and Menne 1988) or inferior (Roverud *et al.* 1991). In cluttered environments, HDC echolocation provides a clutter-rejection mechanism (Bell and Fenton 1984, Neumann and Schuller 1991) that should give bats a clear advantage in detecting flying insects. Some insects successfully fly closer to clutter to avoid predation by LDC bats (Andersson *et al.* 1998, Rydell 1998), but this should not work against HDC echolocators (Fenton 2004). To my knowledge, a direct comparison of echolocation performance of HDC and LDC bats in natural setting has not been done yet.

1.2 Evolution of echolocation

1.2.1 Phylogeny of high duty cycle bats

The evolution of bat echolocation is a controversial topic because of conflicting views about its origin and the lack of data necessary to retrace the evolutionary lineages of bats. Much of the molecular evidence (using sequences of nuclear or mitochondrial DNA followed by phylogenetic analyses) supports the most recent classification of bats into the two clades Yinpterochiroptera and Yangochiroptera (Teeling *et al.* 2000, 2002, 2005, Eick *et al.* 2005). This classification still does not give a definite answer as whether echolocation (regardless of duty cycle) evolved only once or twice (Teeling 2009). If echolocation is monophyletic, there are clear indications that LDC echolocation is the ancestral form of echolocation and that HDC bats evolved from LDC ancestors (Fenton *et al.* 1995, Simmons and Geisler 1998, Schnitzler *et al.* 2004, Eick *et al.* 2005, Jones and Teeling 2006). If echolocation is diphyletic, LDC echolocation is still believed to be the ancestral form in Yangochiroptera (including the New World HDC bat), but whether LDC or HDC echolocation appeared first in Yinpterochiroptera (including Old World HDC bats) is still unknown (Eick *et al.* 2005). No matter what scenario best reflects the genealogical truth, HDC echolocation is at least diphyletic, having evolved once in the common ancestor the Old World Rhinolophidae and Hipposideridae (superfamily Rhinolophoidae) and again in the New World species *Pteronotus parnellii* (Mormoopoidae) (Figure 2). Although not identical, their echolocation behaviour is

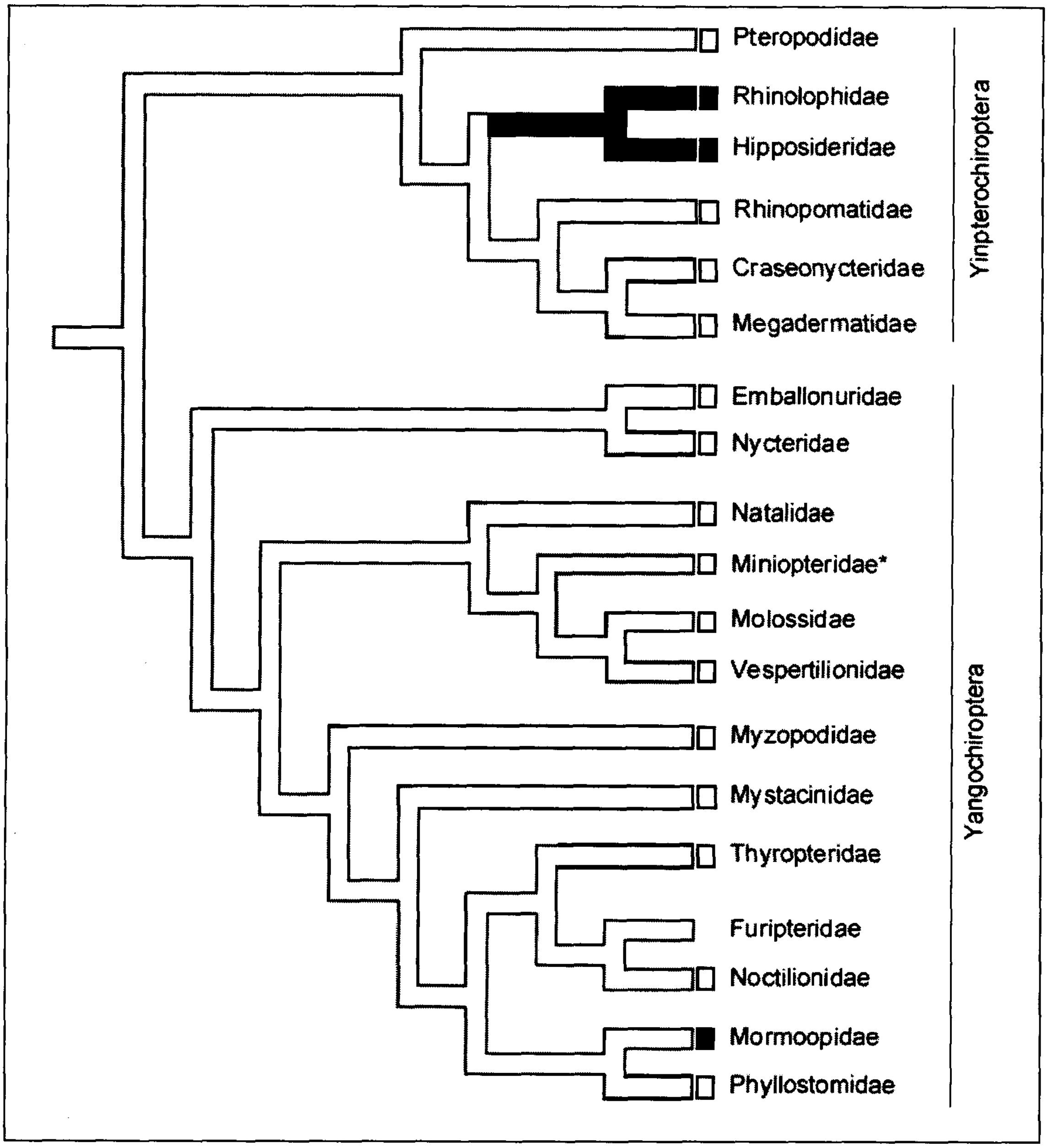

Figure 2. High duty-cycle bats mapped (black) onto a maximum likelihood tree from molecular data. Rhinolophidae and Hipposideridae are found in the Old World. Mormoopidae is a family of the New World. Modified from Jones and Teeling (2006).

strikingly similar and proves the convergent evolution of a highly specialized sensory system.

Rhinolophoidae includes 158 species in 10 genera (Simmons 2005). This is in stark contrast to the single HDC species in the New World. The explanation put forward by Simmons and Conway (2003) is that the time since the appearance of HDC echolocation in each taxa is very different: Old World bats acquired HDC echolocation 40 millions years ago (middle Eocene), whereas it possibly evolved within the past few million years (Pleistocene) in *Pteronotus* (Kössl *et al.* 1999). Also, the Neotropics were an ecologically crowded place for bats by the time HDC echolocation evolved in *P. parnellii.* The radiation of LDC bats foraging in narrow-space habitat (as phyllostomids) was already underway in the New World by the middle Miocene and those bats were occupying the 'foraging in clutter habitat' niche. As a result, New World bats supposedly faced greater competition, restricting their radiation. Competition is a process that structures bat communities and influences echolocation patterns (Schoeman and Jacobs 2008). But food competition might not be a significant factor in the evolution of echolocation behaviour, because species with similar echolocation can coexist at the landscape level when the relative abundance of prey is high (Jiang *et al.* 2008). More information on the competition between LDC and HDC bats are needed to better understand the situation.

1.2.2 Mechanisms of selection

Echolocation calls are malleable signals that evolve through continuous change in signal parameters (Schnitzler *et al.* 2004). Perceptual tasks exert a strong selective pressure on echolocation behaviour and call design (Schnitzler *et al.* 2003). Call design and echolocation behaviour are highly correlated to foraging behaviour and habitat, and are more structured by ecological factors than evolutionary history (Jones and Teeling 2006, Jones and Holderied 2007).

An efficient flutter detection mechanism may have opened new ecological niches by enabling HDC bats to hunt flying insects in dense forest close to vegetation or ground (Simmons and Conway 2003). Improved hunting efficiency would have been energetically favourable, making them better competitors in a cluttered environment. Schnitzler *et al.* (2004) suggested that the addition of a CF component to a previously broadband signal could have enabled a LDC ancestor to go from a passive foraging mode from perch (i.e., scanning from a perch before flying to catch the prey) to an active mode hunting of airborne fluttering target. The advantage of going from perch hunting to an active foraging mode is not obvious, as flight is an energetically costly activity. Echolocation alone accounts for 50-70 % of the cost of flight (Speakman *et al.* 1989), and HDC bats cannot possibly benefit from the wingbeat and pulse coupling found in LDC bats due to their long duration pulses. HDC bats also have stiffened rib cages (only rhinolophoids) and specialized abdominal muscles that allow them to produce intense calls at a relatively low cost when perched (Speakman *et al.* 2004). Furthermore, other researchers argue that the primitive foraging strategy is aerial hawking (Fenton *et al.* 1995, Simmons and Geisler 1998). Therefore the advantage of evolving HDC echolocation lies somewhere else than the start of flight hunting. An improved prey detection mechanism relative to syntopic LDC bats might provide part of the answer.

To visualize evolution, one can picture populations migrating through an adaptive landscape (Wright 1932, Simpson 1944), where LDC and HDC bats are each located on different adaptive peaks. Adaptive peaks, as opposed to adaptive valleys, are a combination of traits that have high adaptive value. The landscape itself would vary in time and with environmental factors. In the case of echolocation, the traits would be behavioural (which control the call characteristics) and morphological, which are themselves mainly determined by ontogeny (Boughman and Moss 2003). Natural selection will displace populations on that landscape toward the peaks. This could be a large scale pattern of disruptive selection, that could explain the echolocation dichotomy observed in extant bats (for a general overview on adaptive landscapes, see McGhee 2007).

1.3 Research objective, hypotheses, and predictions

My objective was to provide a better understanding of the role of prey detection as a potential selective pressure in echolocation evolution, and uncover if HDC behaviour really offers an advantage in prey detection for insectivorous bats. Schnitzler *et al.* (2004) rightly mentioned that to understand the adaptive value of echolocation, we must consider the complete set of tasks performed with echolocation. Although I agree with this statement, experimentally, it is necessary to examine one task at a time and find relationships that are potentially applicable to the bat's experience. There are so many factors correlated when it comes to foraging (echolocation variables, body size, wing shape, prey type, environment), that finding realistic precise correlations is unlikely. In this study, I traced the relationship between the bat's need to hunt efficiently, the prey detection ability, the call variables, the echolocation behaviour and the selective pressure those factors could exert over time and location. My hypothesis and predictions were as follows:

(1) Prey detection is one of the primary force influencing echolocation characteristics used by a given species (in contrast to the other perceptual tasks

aforementioned). An increase in detection ability leads to an improved foraging performance which is energetically beneficial. Thus I considered the ability to detect flying insects important in evaluating the adaptive value of a given call behaviour in a cluttered habitat. If prey detection is as important as I thought, I predicted that results from the artificial experiment (only measuring echo strength of a variety of call design) and from behavioural experiment (which combines more than one perceptual task for the bat) should give similar results.

(2) HDC echolocation confers a detection advantage during hunting compared to LDC echolocation, allowing HDC bats to occupy some niches previously occupied by LDC bats. I expected HDC bats to benefit from their flutter detection ability in the field, and show better detection ability than LDC bats.

(3) The impossibility of managing overlapping pulse and echo for many bats is an important constraint to selection pressures, as it limits the pulse duration and duty cycle LDC bats can use. If longer pulse duration is advantageous in prey detection, bats able to tolerate pulse-echo overlap will conserve and develop this character. I predicted that an increase in duty-cycle and pulse duration correlates with higher detection performance.

(4) There are adaptive peaks corresponding to call designs at both extremes of the echolocation spectrum, i.e. LDC high sweep rate FM sweeps, and HDC long CF pulses. We can see that the dichotomy in echolocation behaviour in clutter-habitat insectivorous is defined by a separation in echolocation behaviour and call design, meaning that there are very few intermediate forms represented among extant bats. If the low and high duty cycle approaches are the equivalent to adaptive peaks and intermediate forms are in an adaptive valley, selective forces will maintain this gap in the distribution. I predicted that

intermediate forms of echolocation behaviour will have lower detection ability, than call design of LDC and HDC bats.

CHAPTER 2. METHODS

I used two complementary experimental approaches. One approach involved tightly controlled variables in a fixed environment (involving artificial echolocation calls and prey) to measure the role of call variables on echo characteristics, as a mean to detect prey. Field experiments demonstrated if HDC bats have a better ability to detect prey than LDC bats, and correlate call variables to detection ability. Although artificial and controlled conditions allow much more refined experiments and analysis, echolocation performance is constrained under natural conditions, and only field experiments can show how abilities differ from the laboratory.

2.1 Synthetic Calls Experiment

2.1.1 Synthetic calls

I digitally synthesized calls using MatLab v.7.5 (The MathWorks Inc., Natick, USA) and saved them as sound files (.wav format; see Appendix 1). Different combinations of variables resulted in the variety of calls that I then projected on a target (described later). I created calls following two approaches.

First, I created a wide variety of calls (n = 278) loosely exploring all the possible call designs based on the limits seen in extant bats (what I refer to as the 'all-inclusive' approach). Some were typical LDC calls (short pulse duration, large bandwidth, low duty cycle), others HDC calls (constant and high frequency, long pulses, high duty cycle), while the rest are a combination of intermediate features. All calls are linear with a constant sweep rate. The specifics of the calls are detailed in Table 1.

Second, as a way to simply visualize an adaptive landscape, I explored possible

Table 1. Variables used to create synthetic calls. The number of intervals is the number of different values used in the creation of the calls.

Variables	**Range**	**Number of Intervals**
Pulse duration	1-60 ms	6
Duty Cycle	1-80 %	5
Frequency	20-110 kHz	19
Bandwidth	0-70 kHz *	15
Harmonics	1-4	4
Sweep Rate †	0-70 kHz/ms *	14

* Zero being a constant frequency pulse.

† Dependent on pulse duration and bandwidth.

intermediate call designs based on likely evolutionary history (the 'evolutionary sequence' approach). Starting with the echolocation of LDC bats, I synthesized intermediate calls up to the echolocation of extant HDC bats. The species modeled are described in Appendix 2.

2.1.2 Setup and recording

A series of echolocation calls was projected from a high-frequency speaker (frequency range of 1-120 kHz; ScanSpeak ultrasound speaker, Avisoft, Berlin, Germany) aimed at the target. The signal was generated by a digital-to-analog conversion board (DAQCard 6062E, National Instrument, Austin, USA) ran on a Pentium-III computer. This analog electrical signal was amplified using an ultrasonic amplifier (frequency range of 1-125 kHz; Avisoft) before delivery to the loudspeaker. I mounted a microphone (frequency range of 10-200 kHz; UltraSoundGate CM16, Avisoft) 10 cm next to the speaker and directed both microphone and speaker toward the prey by eye. Recordings were digitized using the software Avisoft Recorder USG v.2.9 (Avisoft) at a sampling rate of 250 kHz and a resolution of 16 bits, and stored as .wav files. To attenuate direct transmission of the sound, a small plastic sheet and a piece of acoustic foam 2 cm thick separated the microphone and the speaker. The microphone and speaker were 0.4 m from the target.

Recording took place in a room 7.5 m long, 6 m wide and 2.9 m high (room 112, Collip building, The University of Western Ontario campus). There is little furniture in this room, and floor and ceiling offer relatively smooth surfaces. Considering the intensity of the pulses emitted by the speaker and the distance sound had to travel, practically no echo from the room was recorded or stood out from noise level.

2.1.3 Description of the target

The target for the experiment was a piece of masking tape attached to a thin metal rod (diameter = 1 mm, length = 40 cm). The metal rod was connected to a small 12V DC motor (NexxTech 2730255, Orbyx Electronics, Concord, Canada) powered by four D batteries. I used two different target types: a larger one with surface area of 1.2 x 2.2 cm rotating at 70 Hz, and a smaller one measuring 0.7 x 1.7 cm rotating at 95 Hz. The targets loosely imitated a big slow flying prey wingbeat (like a moth) and a small fast flying insect wingbeat (like a fly). Previous behavioural experiments with bats have successfully used this kind of apparatus (Goldman and Henson 1977, Bell and Fenton 1984, von der Emde and Schnitzler 1986, Sum and Menne 1988, Grossetête and Moss 1998). The rotation of the 'wing' produces similar, but not identical, acoustic echo to a fluttering insect, as the shape, number, wingbeat, and stiffness are not consistent between the artificial target and between all insect species.

2.1.4 Recording Analysis

I measured relative target strengths to quantify the bat's potential to detect fluttering prey (Houston et al. 2004). Prior to analysis, I filtered sounds below 18 kHz and above 120 kHz using a program written with MatLab (The MathWorks Inc.). I measured target strength as the signal to noise ratio (SNR). I specifically measured the SNR of the strongest glints produced by the wingbeat in the echo. Glints are sudden amplitude peaks and spectral broadenings on the recording caused by Doppler shifting of the echo when the wing is moving perpendicularly toward or away of the microphone. Strong glints are a conspicuous indication to the bat that a target is present, even if the complete echo is more complex. I localized the glints in the file after plotting the data as an oscillogram in

MatLab (The MathWorks Inc.). In the SNR measurement, the glint is the signal and the background noise is the floor noise. SNR is calculated using the following equations:

$$A_{noise} = \mathrm{std}\ (a{:}b);$$

$$A_{signal} = \mathrm{std}\ (c{:}d) - A_{noise};$$

$$SNR = 20 \log_{10} (A_{signal} / A_{noise})$$

where A stands for amplitude, and (a:b) and (c:d) are the time frames within the file when the noise and signal need to be calculated, as defined by myself after examining the oscillogram of the recording generated by the software. Because some energy was transferred directly from the speaker to the microphone, there was sometimes an overlap between outgoing pulse and returning echo. The presence of the pulse was detected if it produced sounds above the noise level. To control for this I subtracted the signal intensity of the pulse when no target was present, then calculated the SNR.

2.1.5 Statistical Analyses

For the 'all-inclusive' approach, I used principal component analysis (PCA) to reduce the dimensionality of the data and view the relationship between call variables (Appendix 3). I used SAS v.6.12 (SAS Institute, Cary, USA) to do the PCA, and SPSS v.16 (SPSS Inc., Chicago, USA) for all the remaining analyses. To observe the relationship between PCs and target strength, I used regression analysis, and tested different equations (linear, quadratic, inverse and logarithmic) to find the one that best described the relationship, if significant. To decide which regression model was the best fit, I compared their coefficient of correlation (R^2). Prior to the analysis, I transformed my data by adding 10 units to my PCA results, shifting all my distribution into positive numbers, to allow the logarithmic and inverse regression to be done. This does not affect the relationship. In addition, I compared the target strength from small fast flying and large slow flying preys using a paired-sample t-test.

For the 'evolutionary sequence' approach, I performed an ANOVA comparing target strength between each call design. I then performed a Tukey post-hoc test to find which designs were significantly different.

2.2 Field Experiment

2.2.1 Study area

I conducted most of my experiment in June and July 2008 at two sites in the 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 students and researchers from the Institute of Ecology and Evolutionary Biology at the National Taiwan University (NTU; Taipei, Taiwan). I selected this region because northern Taiwan harbours both LDC and HDC bats, and specific sites were easy to select and access with the assistance of the members of the NTU. Taiwan has 31 species of LDC bats, and four species of HDC bats, all of them insectivorous, as well as two frugivorous non-echolocating bats (Hsu Chao-Lung, Bat Association of Taiwan, pers. com.).

I also performed the experiment from 23 to 27 April 2009 at Lamanai, Orange Walk County, Belize (17°45.848'N; 88°39.128'W). Lamanai is host to *P. parnellii*, the only known HDC bat species in the New World, and to at least 36 other species, including 21 insectivorous bats (Fenton *et al.* 2001). I changed sites every night, but they were all less than half-hour walking distance from each other. All sites were on the side of paths that could be used by bats while foraging in the forest understory.

2.2.2 Description of the artificial prey

The same artificial apparatus used in the previous experiment was fixed on a custom-made aluminium stand consisting of a 1.5 m stand, to which was attached a 54 cm rail, an automotive wiper motor (12 V, 1 ampere) and a rotating arm. The arm was attached to a plastic case containing the small DC motor and the rest of the prey sticked out from the case (Figure 3). When the wiper motor was activated, the small case slid on the support resulting in a back-and-forth motion (at a speed of ~ 0.7 m/s).

To control for prey choice, I tested different combination of variables associated with the prey. I varied prey wing size and wingbeat rate, as well as linear movement. I tested three wing sizes: 0.58 cm^2, 0.723 cm^2, and 2.89 cm^2; four wingbeat rate: immobile, 20 Hz, 50 Hz, and 80 Hz; and the prey was either moving or not, for a total of 18 possible combinations. This variation accounted for a diversity of potential prey from a small fast flying insect like a fly to a large moth with slower wingbeats (Kober and Schnitzler 1990). I controlled the wingbeat rate and the lateral movement from a distance with a remote control, minimising observer interference. The two motors emitted sound with most energy under 20 kHz, a frequency range of low sensibility in most bats. I included control treatments, with one or both motors turned off, in my experiment.

2.2.3 Recordings

I monitored approach and attack behaviour commencing at dusk, as soon as the first bats started flying, for a period lasting up to four hours after dawn, as long as the batteries powered the equipment. I was recording two minutes at a time. Within those two minutes, I would change the wingbeat speed or motion every 20 s. Between recordings, I was changing target size. The order of presentation of the different combinations of target characteristics was randomly determined before the beginning of the experiment.

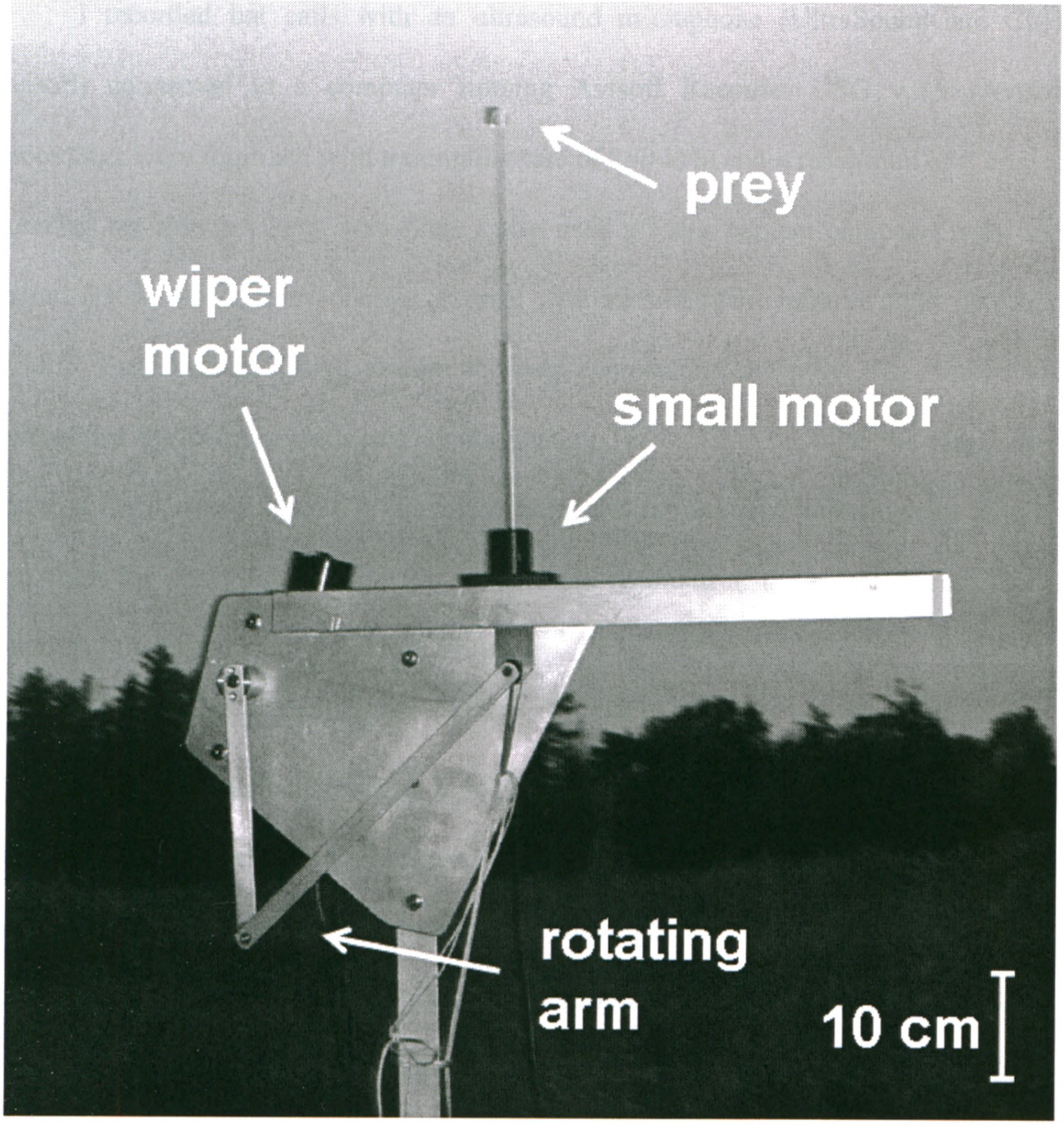

Figure 3. Experimental apparatus used on the field.

I recorded bat calls with an ultrasound microphone (UltraSoundGate CM16, Avisoft) connected to a computer running Avisoft Recorder USG v.2.9 (Avisoft). Recordings were digitized with a sampling rate of 250 kHz and a resolution of 8 bits, and stored as six minutes long .wav files. I also used a video camera equipped with 'night vision' (DCR-SR46, Sony, Tokyo, Japan), with an additional infrared light (IRLamp6, Wildlife Engineering, USA), to film the bats approaching the prey. The 'screen area' covered by the camera measured approximately 160 x 120 cm, with the target at the center of the screen (Figure 4). The microphone and camera were installed side to side at 4 m from the prey, 1.5 m from the ground, and recorded at the same time so that the sound and the visual were synchronized.

2.2.4 Recordings Analysis

From the sound recordings, I counted the passes and determined the following variables from the calls using BatSound Pro v.3.31b (Petterson Electronik AB, Uppsala, Sweden): pulse duration, peak frequency, bandwidth, sweep rate (bandwidth / pulse duration), and duty cycle (pulse duration / [pulse duration + inter pulse interval]). Species are not systematically identified as it is not necessary to my experiment as long as the call variables can be measured from the recording.

I assumed that the detection of prey would result in a change in the behaviour of the bat to obtain more information on the target, not necessarily to attack it. I assigned each acoustic pass to one of two categories. Calls coupled with a bat seen approaching the prey on the video are identified 'approach', otherwise it is 'no approach'. To find when a pass was concluded by an approach, I used the video recordings, slowed down at 30 frames / sec. I considered that there was an approach when the bat flight was directed

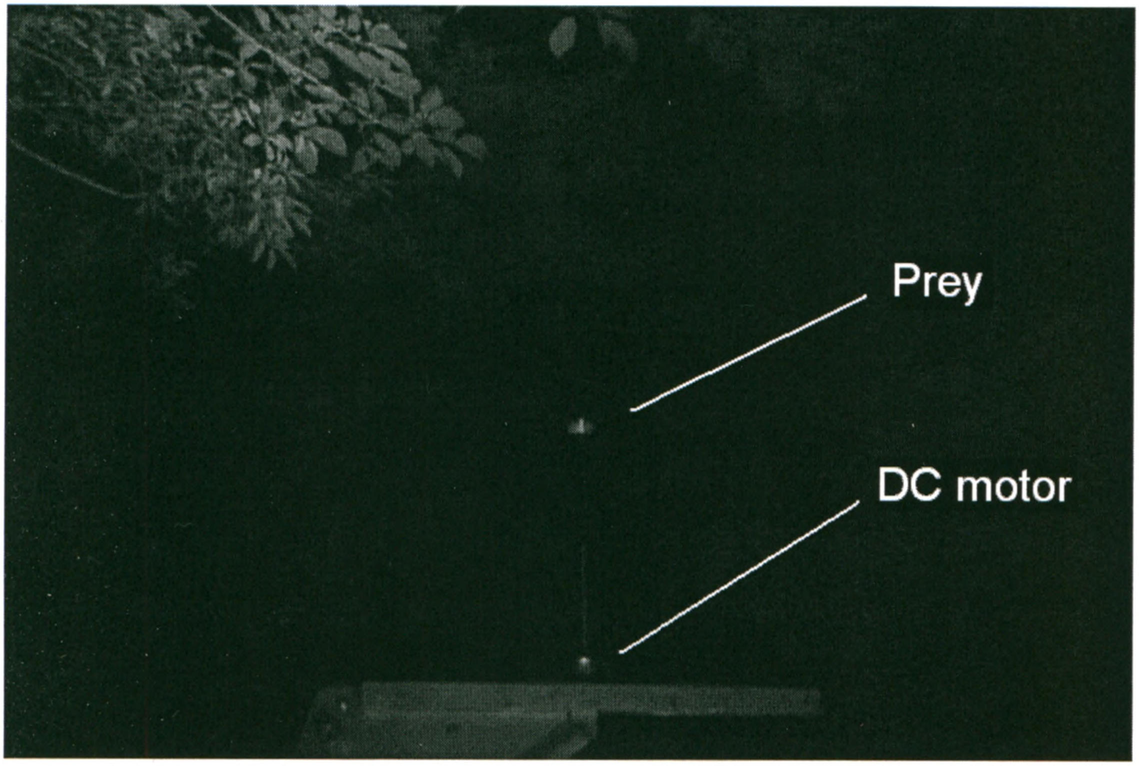

Figure 4. Full field of view covered by the camera. Screenshot of the apparatus in action.

toward the prey and, most of the time, this was after changing its trajectory to face the target.

2.2.5 Statistical Analysis

I used Pearson's chi-square tests to determine if approach toward the prey was paired with echolocation type, location or species. I used the Fisher's exact test when it was more appropriate (i.e. when I had a 2 x 2 contingency table and a small sample size). I compared HDC bats to LDC bats, compared LDC bats from Taiwan and Belize, and did the same within LDC and HDC bats. For all the tests, a p-value <0.05 is considered statistically significant. To see if the number of approaches is influenced by the prey characteristics, I also performed Pearson's chi-square tests, to determine if approach rate is paired with any of the prey characteristics (wing size, wingbeat rate or prey movement). I did these analyses separately for LDC and HDC bats.

To examine call variables separately, I also conducted multiple binary logistic regressions between the cases without any approach and all the cases where there was an approach observed ('approach' and 'no approach' as the two possible outcomes of the dependant variable). I only used Taiwan data to examine call variables, because it had the most voluminous database and because I wanted to avoid including a bias from different sampling effort and potential geographical differences. I performed the statistical analyses using SPSS v.16 (SPSS Inc.).

CHAPTER 3. RESULTS

3.1 Synthetic Calls Experiment

3.1.1 'All-inclusive' approach

The PCA on call variables yielded three principal components: PC1 is frequency related (peak frequency, minimum and maximum frequencies), PC2 is bandwidth related (bandwidth and sweep rate), PC3 is time related (pulse duration and duty cycle) (Appendix 3).

The significant relationship between PC1 and target strength ($F_{2,273} = 105.299$, $p < 0.001$) was best described by a quadratic equation ($R^2 = 0.435$), but only the decreasing portion of the curve fit within a realistic range of frequencies, depicting weaker target strength as PC1 increases (Figure 5). PC2 and target strength were related ($F_{2,273} = 40.219$, $p < 0.001$), and the best fit was with the quadratic equation ($R^2 = 0.228$), with maximum target strength at the extremities of the distribution. Finally, PC3 and target strength were also related ($F_{2,273} = 62.656$, $p < 0.001$). The quadratic function had the best fit ($R^2 = 0.315$) with all values in the increasing portion of the curve, depicting a slightly stronger target strength as the PC3 increased.

Target echo strength from larger and slower prey was stronger than for the smaller and faster one ($t = 4.471$, $df = 136$, $p < 0.001$; Figure 6), meaning the wing reflective surface has more impact on echo strength than wingbeat speed.

3.1.2 'Evolutionary sequence' approach

In the Old-World scenario, there was a significant difference in target strengths as indicated by the ANOVA results ($F_{6,116} = 90.9084$, $p < 0.001$). The Tukey post-hoc test results showed that the call of Hipposideridae yielded weaker target strength than any

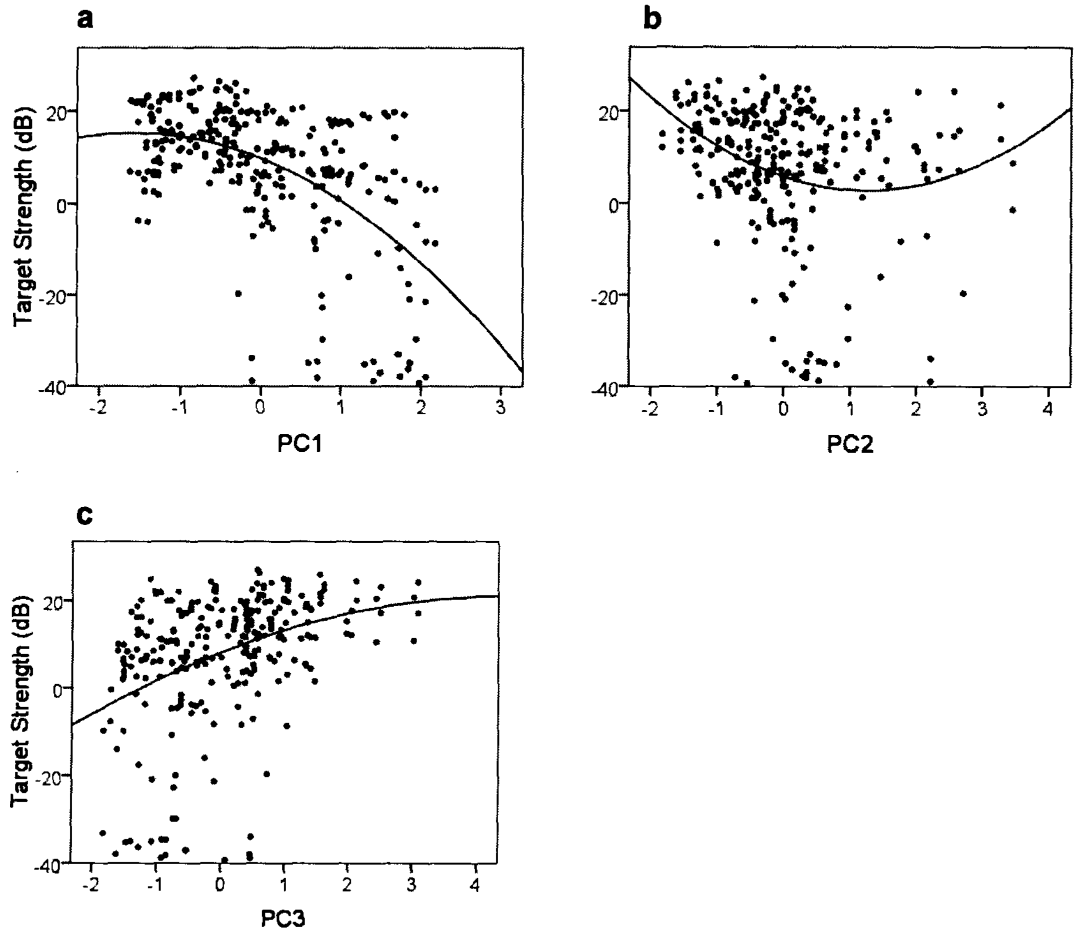

Figure 5. Target strengths (measured as the sound to noise ratio) of the echoes from a fluttering target in relationship with varying call variables. Principal components (PC) represent a) frequency related variables, b) bandwidth and sweep rate, and c) time related variables. Moving to the right on the x-axis correspond to increased values of the call variables. The best fitting regression line is shown.

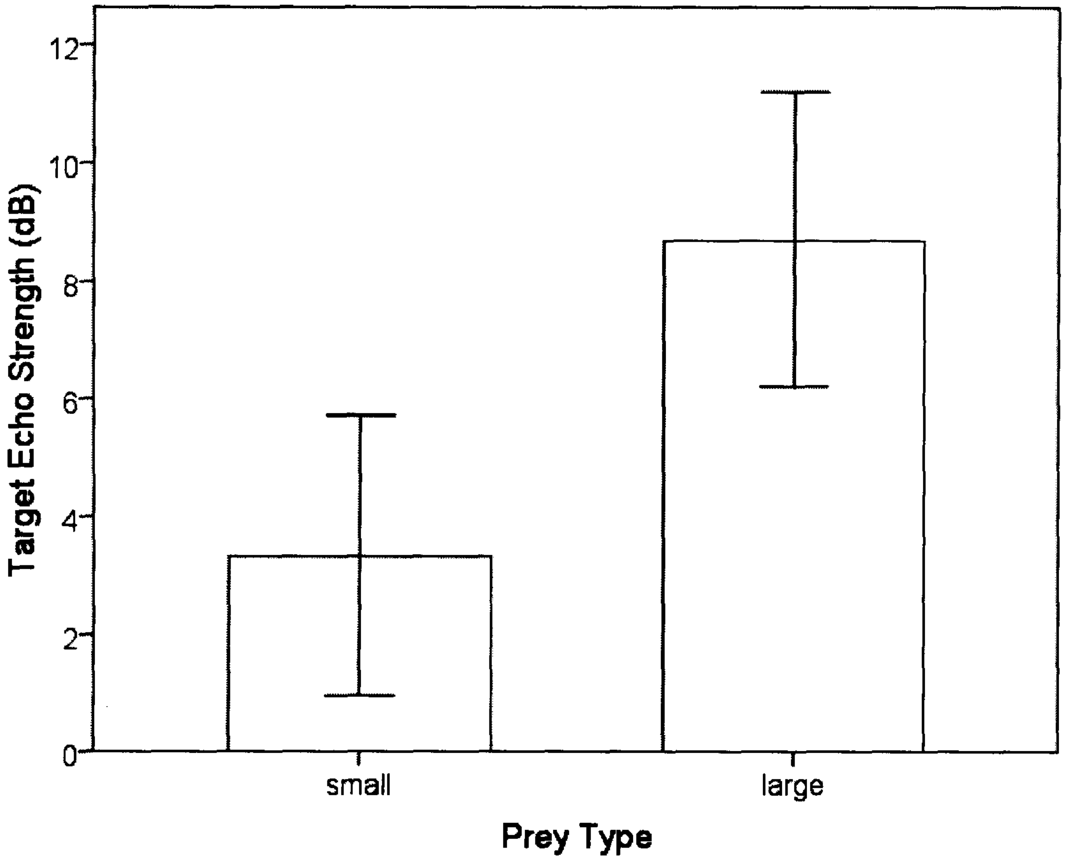

Figure 6. Relative target echo strength of the two targets used in synthetic calls experiments (±2 SE). The small one had a surface area of 0.7 x 1.7 cm and rotated at 95 Hz. The large one had a surface area of 1.2 x 2.2 cm and rotated at 70 Hz.

other, with stronger target strength at both ends of the spectrum (Figure 7).

In the New-World scenario, there was a significant difference in target strengths as indicated by the ANOVA results ($F_{4,95} = 50.910$, $p < 0.001$). According to the post-hoc test, target strength resulting from *P. parnellii* calls, was significantly higher than all other calls (Figure 7).

3.2 Field experiment

In Taiwan, I recorded over 2,000 passes during 23 nights of sampling, with great variability between nights. I recorded the calls of three species of HDC bats (*Rhinolophus monoceros*, *Hipposideros armiger* and *R. formosae*). I could not identify all LDC bat species due to the similarity between species calls and intra-species variability, but I identified *Pipistrellus abramus* and *Murina puta* among the LDC bats. The mean approach rate calculated for HDC bats was 18.56% (n = 2382) and for LDC bats it was 1.159% (n = 345). Almost all (442 / 446) bats that approached the target were HDC bats. The four LDC bats that approached the target had calls with high sweeping rate (21-26 kHz/s) with a low duty cycle (5%), the opposite of the HDC approach. They belonged to one or more species from the sub-families Murininae or Kerivoulinae.

In Belize, I only recorded five passes of *P. parnellii* over five recording nights, which was insufficient to analyse on its own. I recorded 370 passes of LDC species, among which 134 low duty cycle from the genus *Pteronotus* (*P. personatus* and *P. davyi*, hereafter referred to as LDC *Pteronotus* spp.). Three of the LDC *Pteronotus* spp. passes resulted in an approach toward the prey, none from other species. Their approach rate was 2.24 %.

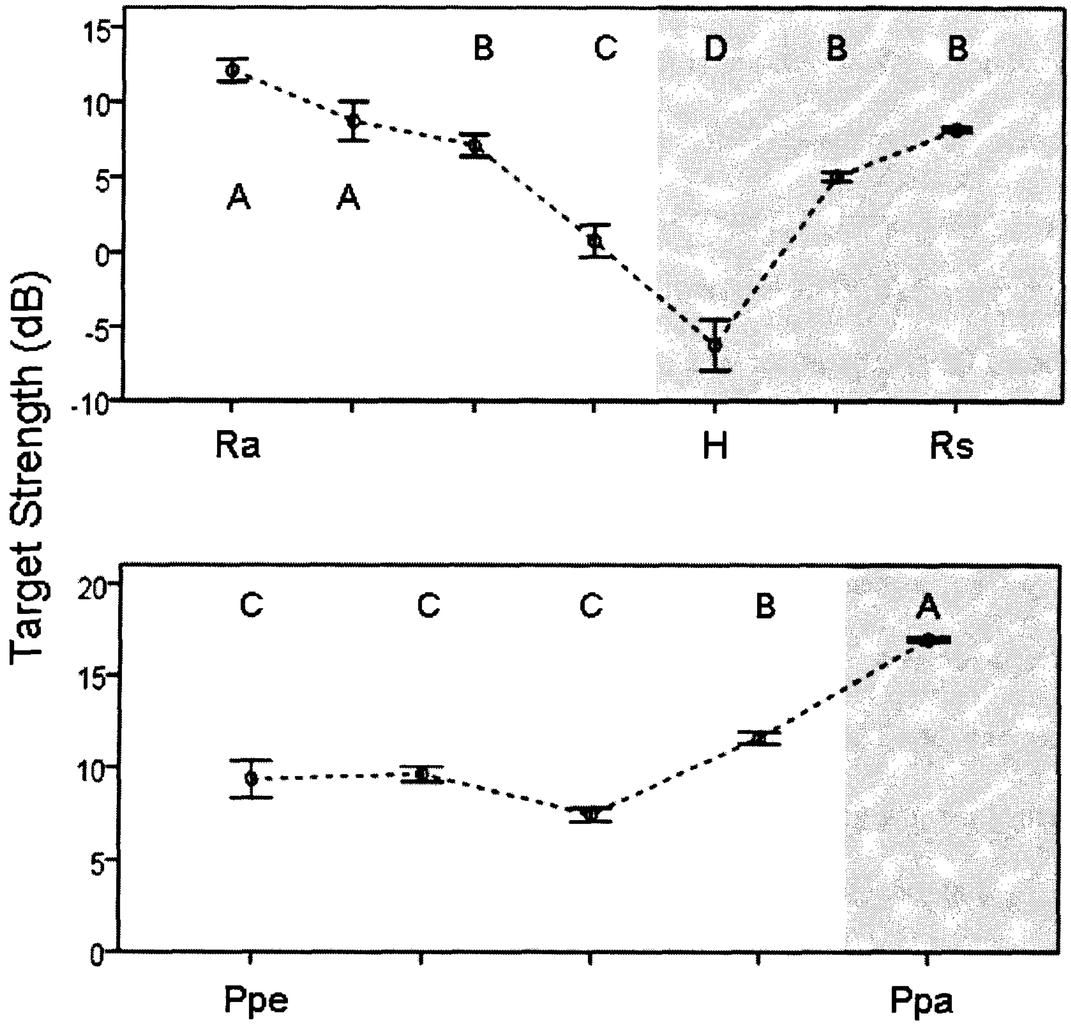

Figure 7. Mean target strength (±1 SE) measured in the synthetic calls experiment using hypothetical evolutionary sequence of calls. Sequences go from low duty-cycle bat (left) to high duty-cycle bats (right, in grey areas), with intermediate forms in between. Upper graph presents Old World bats: *Rhinopoma* sp. (Ra), Hipposideridae (H), Rhinolophidae (Rs). Lower graph presents New World bats: *Pteronotus personatus* (Ppe), *Pteronotus parnellii* (Ppa). Unlabeled ticks are intermediate forms I created. Letters at the top of each graph show the results of the Tukey post-hoc tests. n=20 per 'species', except 2nd, 4th and 5th in upper panel (n=15).

Approach toward the prey was correlated with echolocation type ($\chi^2 = 135.5$, df = 1, p <0.001; Figures 8 and 9). Comparing hipposiderids and rhinolophids, approach was paired with family ($\chi^2 = 302.6$, df = 1, p <0.001), rhinolophids approaching more often (30.31% approach rate compared to 2.21%). Looking only at LDC bats, comparing Taiwan to Belize, approach was not paired with location (p = 0.719, Fisher's exact test). Then, I examined bats from Belize, comparing LDC *Pteronotus* spp. to all other LDC bats, and there was no relation between species and approach (p = 0.078, Fisher's exact test).

LDC bats had a low approach rate that was not influenced by any prey characteristics (Figures 8 and 9), whether it was wingbeat speed ($\chi^2 = 1.158$, df = 2, p = 0.561), size ($\chi^2 = 2.124$, df = 2, p = 0.346), or movement (p = 0.458, Fisher's exact test). On the other hand, the approach rate of HDC bats was influenced by wingbeat speed ($\chi^2 = 20.24$, df = 2, p <0.001; Figure 8) and prey size ($\chi^2 = 52.77$, df = 2, p <0.001; Figure 9) but not by prey movement (p = 0.175, Fisher's exact test). When testing for wingbeat speed, the immobile treatment was left out of the analysis, as there was a minute amount of approach, and it would increase the chance of type I error. I then split the HDC bats into *R. monoceros* and *H. armiger*, the two HDC species with a big enough sample size, to look for an association between detection and prey characteristics within those species. In *R. monoceros*, there was a significant association between detection and both size ($\chi^2 = 9.716$, df = 2, p = 0.008) and wingbeat speed ($\chi^2 = 24.65$, df = 2, p <0.001). *R. monoceros* presented the highest approach rates toward medium size prey and the medium wingbeat speed. The *H. armiger* data also presented an association between detection and size ($\chi^2 = 30.59$, df = 2, p <0.001) and wingbeat speed ($\chi^2 = 10.36$, df = 2,

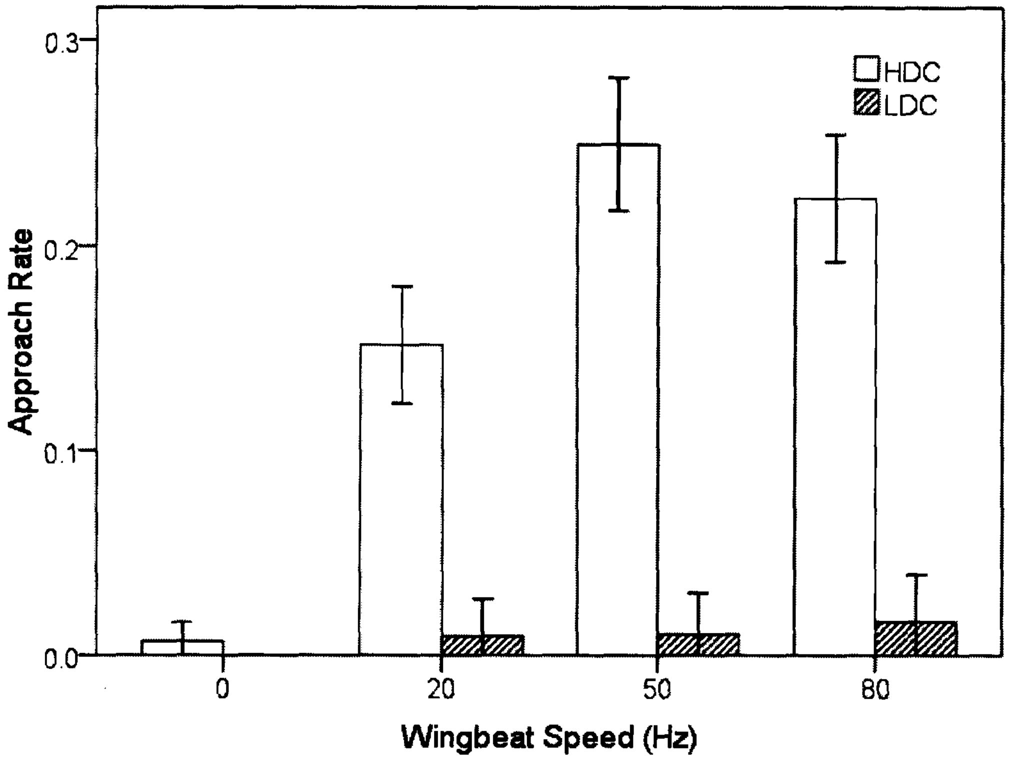

Figure 8. Approach rate (proportion of bats that approached the target over the total number of bats recorded acoustically in proximity) of low and high duty-cycle bats presented with prey fluttering at varying speed (Hz, ±2 SE).

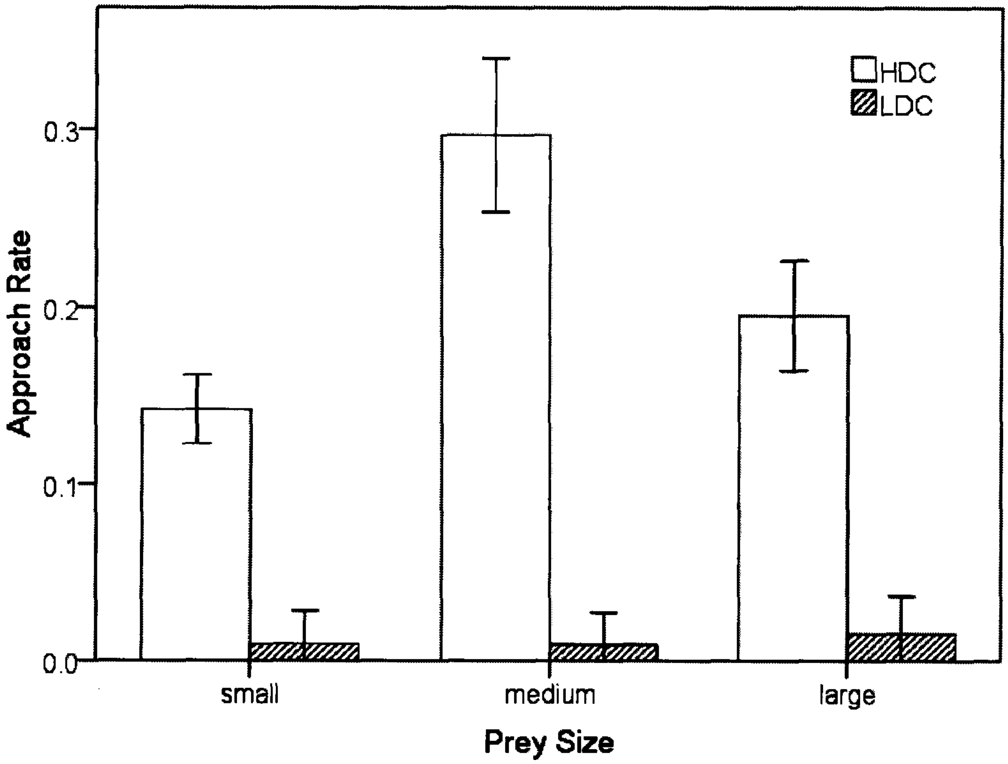

Figure 9. Approach rate (proportion of bats that approached the target over the total number of bats recorded acoustically in proximity) of low and high duty-cycle bats presented with prey of different sizes (±2 SE). Small size (0.58 cm^2), medium (0.723 cm^2) and large (2.89 cm^2).

p = 0.006). *H. armiger* approaches were more frequent on medium-sized prey and less frequent on smaller ones. They also approached more often the faster the prey was fluttering.

I performed a multiple binary regression with the call characteristics as predictor variables and detection (yes or no) as dependant variable. I did the multiple regression using only three call variables to avoid multicollinearity: pulse duration, peak frequency and bandwidth (Figure 10). An increase in approach rate was related to an increase in pulse duration ($W = 25.744$, $p <0.001$) and in the peak frequency ($W = 130.99$, $p <0.001$), but not bandwidth ($W = 0.607$, $p = 0.436$).

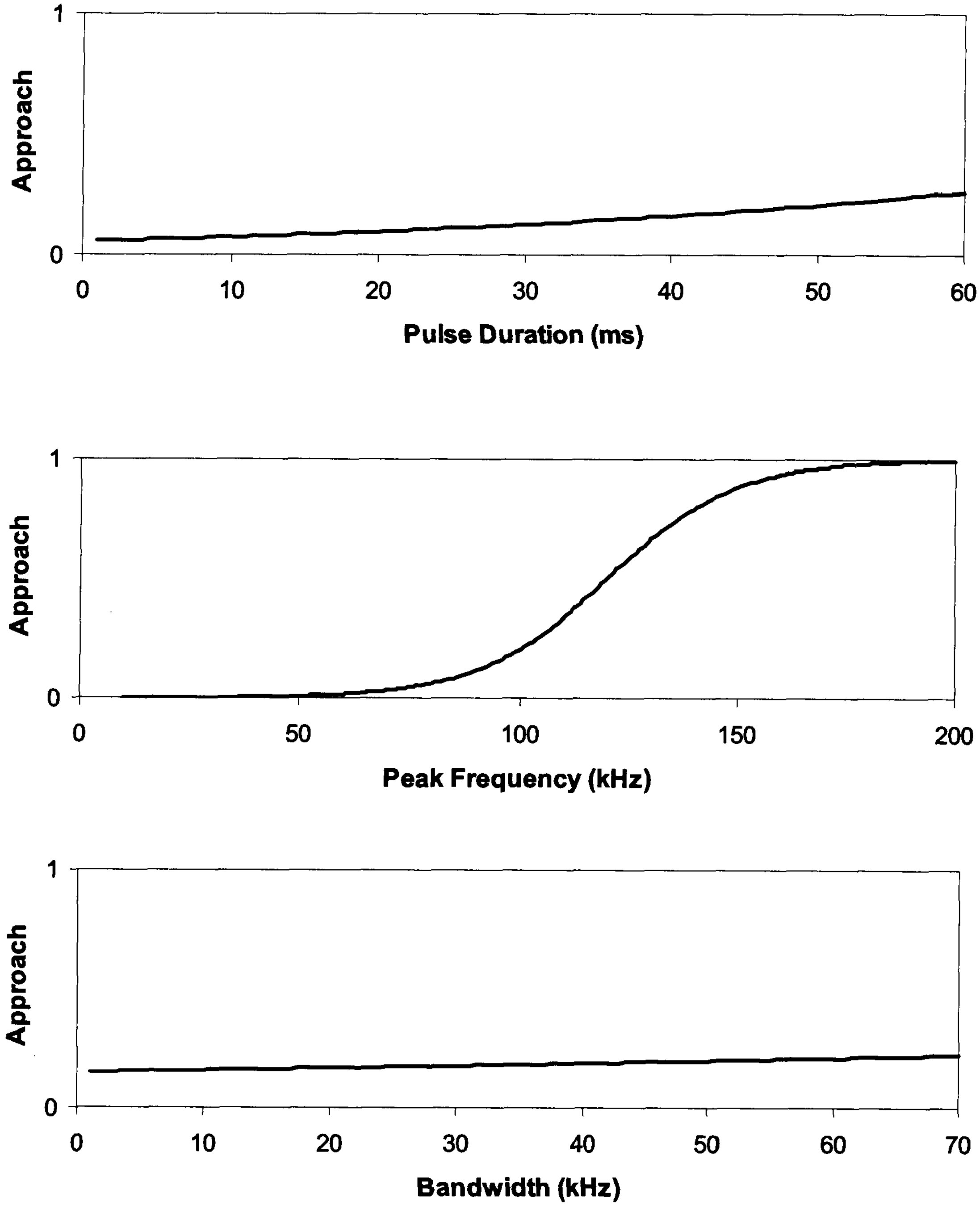

Figure 10. Relationships between three call variables and approaches toward the target presented to bats in the field. The dependant variable 'approach' is binary: 0 = target not approached by the bat, 1 = target approached. The range of value presented for the call variables is within reasonable ranges found in extant bats.

CHAPTER 4. DISCUSSION

Two of my hypotheses were validated. Considering insectivorous bats in cluttered habitat, HDC bats had higher approach rates than LDC bats, showing that it is an efficient call design and that they make good use of their flutter detection ability. Pulse-echo overlap was a constraint on time-related variables because longer pulse and higher duty cycle equal higher detection performances, based on field and laboratory results. My two other hypotheses were partially supported. Prey detection is potentially only a minor selective pressure on call design. Results from the synthetic calls experiment measuring target strength did not match exactly the results from the field looking at call characteristics, except for time-related variables. Finally, detection ability did not fit an adaptive landscape as predicted.

4.1 Prey detection

I found that long pulses and high duty cycle, that are clear HDC echolocation attributes, provided better detection ability than short pulses emitted at a low duty cycle (figures 5, 10). Behavioural observations showed that HDC bats approached all the prey types presented much more often than LCD bats (as the proportion of total bats in the surrounding area monitored acoustically), demonstrating that HDC bats were better at detecting prey. The advantage of HDC echolocation to detect fluttering prey was underlined by the results. Although one could argue that the approaches recorded on the field might be a matter of prey choice, the different targets presented to the bats (with varying size, wingbeat, and movement) would likely have satisfied any food preference.

No specific type of prey seems to be preferentially hunted for by HDC or LDC bats in Taiwan (Lee and Lee 2005, Lee *et al.* 2005).

In accordance with the previous observations, results from the evolutionary sequence experiment show that the *P. parnellii* HDC calls yielded much stronger echoes than its hypothetical predecessors. On the contrary, in the Old World scenario, LDC calls showed the strongest target echoes. When looking at the calls used (Figure A.1 in the appendices), target strength seemed to be highly correlated to the peak frequency of the calls in Old World calls, whereas frequencies were more constant for New World calls. My other measurements of target strength show that an increase in frequency decreases target strength, and I am inclined to attribute most of the variation along the Old World sequence to frequency changes. Intensity of calls can be controlled by the bats (Surlykke and Kalko 2008), and the acuity of the hearing ultimately depends on the number of neurons activated in the inner ear. Also, my microphone obviously did not have an acoustic fovea similar to the HDC bats. For these reasons, the huge differences seen in target strength in that experiment might not translate directly to live bats in natural conditions.

Using FM calls and dried immobile insects, Waters *et al.* (1995) found that target echo strength increases with prey size. I came to the same conclusion with my results on prey size, that bigger preys have higher target strength, but that did not translate in the field. Overall, the HDC bats approached the medium-sized prey more than the smallest or biggest ones (Figure 9). When looking at the two most common HDC bats recorded in Taiwan, the small *R. monoceros* (forearm length: 3.6 – 4 cm) and the large *H. armiger* (forearm length: 8.6 – 10.3 cm) do not show clear difference relative to prey size.

Rhinolophids show higher detection ability than hipposiderids in the experiments. *H. armiger* approached the prey more often the faster it was fluttering. *R. monoceros* also seemed to approach the slow fluttering target less often, but it was more obvious in *H. armiger*. In previous experiments, rhinolophid bats showed higher sensitivity to echo containing flutter information compared to hipposiderids bats, showing that their flutter detection performance is better (von der Emde and Schnitzler 1986, Roverud *et al.* 1991). Rhinolophids have more neurons tuned to their best frequency in their inferior colliculus than hipposiderids, which allows a more detailed detection of flying insects (Neuweiler *et al.* 1980).

The sub-families Murininae and Kerivoulinae (the only LDC bats that approached the prey in Taiwan) in the Old World possess extremely broadband frequency modulated sweeps of short duration that appears to be another approach to cope with foraging in clutter environment (Kingston *et al.* 1999). Although they are able to detect the prey, it still seems that they might only detect a limited amount of prey, restricted to a small airspace in front of them.

4.2 Selective forces on call design and behaviour

If prey detection is the primary force driving selection in echolocation behaviour, meaning that selection favours some call design because they improve prey detection, call characteristics that yield the strongest target echo in the lab should be defining the echolocation call of the bats that have the highest approach rate on the field. But the results from the field and from the synthetic calls experiment differed in many aspects, diminishing the apparent contribution of prey detection as a selective force.

I found that an increasing frequency created weaker target strength in the acoustical experiment (figure 5). On the contrary, the logistic relationship between frequency and approach rate in the field was positive (figure 10). Houston *et al.* (2004), showed that lower frequencies (20-30) yield weaker target strength on smaller insects, because of Rayleigh scattering (Pye 1993). But when considering sound attenuation in the air, the maximum target detection distance where obtained at frequencies above 30 kHz and was relatively constant above that level. Another study reported no clear relationship between target strength and the frequency of the pulse (Waters *et al.* 1995). Surlykke and Kalko (2008) discovered a trend that could explain why high frequency emitting bats can perform as well as low frequency bats in the field. Bats calling at higher frequencies emit stronger calls to compensate atmospheric attenuation, resulting in similar prey detection ranges. However, that does not cancel the advantage of emitting at a lower frequency to detect prey, as there is probably a cost at emitting sounds many decibels higher than another species of the same size.

Other selective pressures can influence frequency. A variety of nocturnal insects possess ultrasound-sensitive ears and ultrasound-induced avoidance and escape behaviours (see Yager 1999 for a review). Maximum sensitivity of those ears is most commonly between 20-60 kHz (Fullard 1987, Yager 1999). The allotonic frequency hypothesis (Novick 1977, Fenton and Fullard 1979) proposed that relatively low and high peak frequencies are the results of strong selection to become less audible to eared insects. That can explain the discrepancy seen in my results, with bats potentially using higher frequency to avoid being detected by their prey. Using a lower frequency to detect prey is an opposite selection force to the need to stay undetected by their prey.

Different populations of the same species sometimes present huge differences in peak frequency, up to 10 kHz (Heller and von Helversen 1989, Jones *et al.* 1994, Francis and Habersetzer 1998, Guillén *et al.* 2000). These differences could be attributable to environmental factors or different level of competition. Bats in drier habitats have higher frequency than those living in wet environments because high frequencies are severely attenuated by high humidity (Heller and von Helversen 1989, Jones *et al.* 1993). The acoustic communication hypothesis states that social interactions in rhinolophids impose selection pressure on peak frequency in HDC bats (Möhres 1967, Heller and von Helversen 1989, Kingston *et al.* 2000) to allow intra-specific communication and identification, and avoid inter-specific competition. The hypothesis that detection is the primary force in selection of echolocation attributes does not fit when considering frequency, as too many factors might influence the frequency used by a given species.

The best bandwidth and sweep rate for detection seem to be at both ends of the distribution based on the acoustic experiments, but no relationship was found in the field. Bandwidth, sweep rate and even curvature are not directly related to detection ability, but rather with angular localization, reduction of ranging error and features extraction (Boonman *et al.* 2003, Schnitzler *et al.* 2003, Holderied *et al.* 2006, Boonman *et al.* 2007). Thus, bandwidth is probably not a call characteristics shaped by selection for improved detection.

Time-related variables are important to the aerial-hawking bats in forested habitat. Longer pulses and higher duty cycle consistently had higher detection in both experiments. It is likely that improved detection ability given by longer calls has shaped the echolocation calls of HDC bats. Of course, the main constraint is the overlap of emitting sound and echo (self-deafening). Although there is an advantage in increasing the length of the pulse that is emitted, selective pressures were counteracted by neurological constraints for million of years. An ontogenic study of cochlear anatomy of the HDC bats *Rhinolophus* showed that the vocalizations are influenced by the cochlear fine tuning, and not the inverse (Rübsamen 1987, Vater 2000). HDC echolocation behaviour developed as a consequence of a physiological change in the auditory system. It is suggested that the auditory system adaptation that allow for efficient Doppler-shift compensation in *P. parnellii* is not the result of evolutionary driving forces (Kössl *et al.* 1999, Neuweiler 2003). It could have resulted from a change in the ontogenetic programs controlling the development of the cochlea, a rather sudden and important change. This would have given HDC bats ancestors, the necessary processing tool to overcome the pulse-echo overlap constraint. From then, their behaviour and call design could have evolved toward the HDC approach to echolocation we see nowadays.

My fourth hypothesis was about the existence of an adaptive landscape resulting in disruptive evolution toward LDC and HDC behaviours, away from intermediate approaches. My results do not fit an adaptive landscape as predicted. While it is easy to visualize a two (as in this study) or three-dimensional landscape, theoretically there is no limit to the number of characters (dimensions). Increasing the number of characters in the model will give a more realistic portrait (McGhee 2007).

The Old World scenario from my evolutionary sequence experiment, depicts a clear drop in target strength in the sequence. The significantly lowest target strength corresponded to the echolocation calls of hipposiderids, contradicting all my other results that underline the advantage of HDC echolocation for fluttering prey detection. Looking at this, it is hardly understandable how a LDC ancestor could have evolved HDC behaviour. But as mentioned earlier, issues related to frequency used for these calls suggest that the results of this experiment might not translate directly to live bats in natural conditions. Nevertheless, it appears that Rhinolophidae perform better than Hipposideridae. In the field, rhinolophids approached the prey significantly more often than hipposiderids. Hipposideridae and Rhinolophidae are sister taxa (Hand and Kirsch 1998, Eick *et al.* 2005, Teeling *et al.* 2005, Li *et al.* 2008) and are both able to do Doppler shift compensation, emitting pulse and listening to the echo at the same time. Therefore, there is no functional constraint in the evolution of an even higher duty-cycle in hipposiderids, more similar to rhinolophids, if it is truly advantageous.

In the New World scenario, the strongest target echo was found at the HDC end of the sequence (Figure 7). It seems possible that an intermediate call behaviour appeared by neutral evolution, or more likely by selection on an echolocation task other than prey detection. *P. personatus, P. davyi* and the other species of the genus could be such examples of intermediate forms, with some CF elements and relatively long calls for a LDC bat. *P. personatus* is even able to accomplish partial Doppler shift compensation (Smotherman and Guillén-Servent 2008). The field observations demonstrate that LDC *Pteronotus* spp. do not perform significantly better than other LDC bats.

4.3 Diversity patterns in the Old World and New World

The evolution of HDC echolocation probably occurred in response to similar pressures in both locations – among those the fact highlighted by my results that individuals using HDC echolocation were more efficient at detecting a fake flying prey than those using LDC echolocation – and in both cases it became a successful adaptation. Then, why is HDC echolocation not more common in the New World?

One explanation put forward by Simmons and Conway (2003) is that newly evolved HDC bats in the New World faced greater competition than their HDC equivalent in the Old World millions of year before. The most likely competitor to HDC bats are LDC bats that forage in clutter habitat. The Old World Kerivoulinae and Murininae are superficially similar to the New World phyllostomines in echolocation call design and are possibly competing for the same food resource as HDC bats (Simmons and Conway 2003, Kingston *et al.* 2000). A LDC species, *Pipistrellus pipistrellus*, is believed to dominate the competition with the HDC *Rhinolophus hipposideros* in Europe (Arlettaz *et al.* 2000), but in that case, LDC bats appeared to have taken advantage of artificial lights, which is not a factor in ancestral tropical forests. My results show that regardless of taxon and location, HDC echolocation has a significant advantage over LDC echolocation in prey detection. The diversity of rhinolophoids in the Old World and my observations also demonstrate the possibility for many HDC bats to coexist. Therefore, I support the idea that a longer evolutionary history has given the time for rhinolophoids to diversify (Simmons and Conway 2003), in comparison to *Pteronotus*, but the competition hypothesis is not plausible. Competition for flying insects as a food resource does not seem to be shaping the bat assemblage, because otherwise cluttered habitats would be dominated by HDC bats. Another explanation would be that LDC bats have another advantage that compensates a worst detection ability. This could be the use by many LDC bats of prey-generated sounds, or maybe the use of vision to complement echolocation.

In the tropical Old World, rhinolophids seem to be able to perform better prey detection than hipposiderids. For some reason, members of the two families are able to coexist and are both diverse and abundant (Kingston *et al.* 2003). Rhinolophids show more pronounced HDC characters than hipposiderids although the latter use higher frequencies (Jones 1999), and the families also possess slightly different acoustic foveae (Neuweiler 1984). That leads Jacobs *et al.* (2007) to suggest that those two groups are not part of the same guild and therefore do not influence each other's evolution. Hipposiderids are on average larger than rhinolophids (Jones 1999) and food resource partitioning might explain the coexistence of both families. Prey detection ability is not an important contributor in niche partitioning among HDC families, nor is it a factor in competition.

HDC bats in the New World provide a slightly different scenario. Although the HDC *P. parnellii* is much more efficient at detecting flying prey (based on laboratory experiments) than LDC bats, other species of *Pteronotus* show what could be considered an intermediate approach (Kössl *et al.* 1999). The abundance of HDC bats in the Old World suggests that there is still place for the evolution of more HDC bats in the New World, and their efficiency to hunt flying insects would facilitate their insertion in the bat assemblage. This could happen through two mechanisms. First, *P. parnellii* already has the functional mechanisms for HDC echolocation. Therefore, new species of HDC bats could evolve from *P. parnellii* that already possess that character. Secondly, HDC echolocation could evolve *de novo*. The other *Pteronotus* species, showing intermediate characters, are the most likely candidates.

4.4 Conclusions

1. Prey detection is not the primary selective force shaping echolocation design.
    a. There is no clear relationship between prey detection ability and the frequency used by a given species. Prey detection is only one of many selective forces acting on frequency.
    b. Prey detection is unlikely to have influenced the use of particular bandwidth or sweep rate.
    c. Increasing time-related variables improve the ability to detect fluttering prey. Prey detection might have been a major selective force for an increased pulse duration and duty-cycle in HDC bats.
2. HDC bats have higher approach rate than LDC bats to a variety of prey, hinting that they have a superior ability to detect fluttering prey. Detection ability also varies within HDC bats. Competition between species, if any, is not based on detection ability.
3. The auditory system of LDC bats limit the duty cycle and pulse duration they can use. Because longer pulses and higher duty-cycle improve detection, functional constraints block the advantage conferred by higher duty-cycle echolocation.
4. There is no convincing evidence that intermediate call designs represent a region of depressed detection performance. The genus *Pteronotus* (other than *P. parnellii*) from the New World might represent an intermediate form between LDC and HDC bats.

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Appendix 1

```
% Mark Skowronski, March 17, 2008


clear all; format compact; close all;


% Parameters:

fs = 400e3; % Hz, sampling rate

bitRes = 16; % bits, resolution of WAV file

fmStartAll = [18.8]*1e3; % Hz, starting frequency of FM calls

fmEndAll = [14.6]*1e3; % Hz, end frequency of FM calls (if
fmStart==fmEnd --> will make a CF call)

durAll = [5.1]*1e-3; % sec, all call durations

harmonicEnergy = [-21.1 0 -20.8 -27.3]; % dB, energy per harmonic.
First entry is for fundamental frequency and is ALWAYS zero (number of
entries = number of harmonics)

gapBetweenCalls = 0.102; % sec, gap from beginning of one call to
beginning of next call

numRep = 5; % number of repetitions of each FM-dur combo


% Note about harmonics:

% harmonicEnergy is a length-M vector denoting the *relative* energy of
M harmonics of a call.

% Total call amplitude is scaled to fit inside the WAV file range of [-
1,+1].

% Examples: harmonicEnergy = [0 0 0] creates a fundamental and two other
harmonics, all with the

% same amplitude.  [0 -100 0] creates a fundamental and third harmonic
along with a second

% harmonic that is 100 dB in energy below the other harmonics.  The
fundamental would have unity

% amplitude while the second harmonic would have an amplitude of 1E-5.
[0 -10 -20 -30] creates

% a fundamental and three harmonics, each 10 dB below the next lowest
harmonic.  Amplitudes
```

```
% would be [1 1/sqrt(10) 1/10 1/sqrt(1000)].

harmonicEnergy = harmonicEnergy - harmonicEnergy(1); % Normalize to
unity fundamental


% Sanity check variables:

if length(fmStartAll)~=length(fmEndAll),

   error('ERROR: fmStartAll and fmEndAll must be same length.');

   return;

end;


% Create output variable:

x = randn(fs,.1); % start w/ 1 sec of white noise

x = [x;zeros(round(.2*fs),1)]; % 200ms of silence before first call


maxAmplitude = 0; % keep track of max amplitude of x1 for final scaling

for p1=1:length(fmStartAll),

   fmStart = fmStartAll(p1);

   fmEnd = fmEndAll(p1);

   for p2=1:length(durAll),

      dur = durAll(p2); % sec


      % Create next call, add harmonics, eliminate aliasing:

      t = [0:round(dur*fs)-1]/fs; % sec

      phi = fmStart*t+1/2*(fmEnd-fmStart)*t.^2/dur;

      x1 = sin(2*pi*phi+0)'; % COLUMN vector, zero phase

      fRange = linspace(fmStart,fmEnd,length(t)); % Hz

      x1(fRange>fs/2) = 0; % remove aliasing while preserving phase

      for p3=2:length(harmonicEnergy), % add harmonics if present
```

```
         xH = 10^(harmonicEnergy(p3)/20)*sin(2*pi*(p3*phi)+0)'; % zero
phase, scaled amplitude

         fRange = linspace(p3*fmStart,p3*fmEnd,length(t)); % Hz

         xH(fRange>fs/2) = 0; % remove aliasing while preserving phase

         x1 = x1 + xH;

      end;

      x1 = x1.*hanning(length(x1)); % amplitude modulate, taper to zero

      x1 = [x1;zeros(round(gapBetweenCalls*fs)-length(x1),1)]; % zero-
pad to beginning of next call


      % Check to see if x1 creates new maxAmplitude:

      x1Max = max(abs(x1));

      if x1Max > maxAmplitude,

         maxAmplitude = x1Max; % maxAmplitude depends on interaction of
harmonics

      end;


      % Copy and save:

      x = [x;repmat(x1,numRep,1)];

   end;

end;


% Include end noise:

x = x/maxAmplitude*.999; % scale to fit inside +/-1, except for white
noise parts


% Write to WAV file:

wavwrite(x,fs,bitRes,'1.wav');
```

Appendix 2

In this appendix, I present the reasoning I followed to create the calls used in the evolutionary sequence approach to my synthetic calls experiments. First, I identified from the literature species or genera that echolocate in a way that presumably resembles that of the LDC ancestor of the first HDC bats (Schnitzler *et al.* 2004). For the Old World evolution of HDC echolocation, I chose the calls of bats in the genus *Rhinopoma* as the ancestor call model. This genus is part of the same clade (Yinptochiroptera) as Hipposideridae and Rhinolophidae. *Rhinopoma* species sometime use narrowband pulses (Habersetzer 1981) and it is also argued that a species (*R. hardwickei*) is in the process of evolving an acoustic fovea, typical of HDC bats (Neuweiler 1984).

In the New World, I chose a modified version of the call from *Pteronotus personatus* (family Mormoopidae) as the ancestor model to *P. parnellii.* Both species originate from the two most basal nodes in the evolutionary history of the genus *Pteronotus* (Van Den Bussche and Weyandt 2003; Dávalos 2006) and Smotherman and Guillén-Servent (2008) showed that *P. personatus* can perform some Doppler-shift compensation. Although being a LDC species, *P. personatus* does have some CF element in its call, that I omitted from my synthetic calls to accentuate the difference between the two approaches. The resulting call is then reminiscent of *Mormoops* calls, which is the other genus from the Mormoopidae, keeping it a reasonable estimate of a LDC ancestor call.

For each evolutionary event (Old and New World), I synthesized the LDC call, the HDC call(s), and three or four intermediate calls. I designed the intermediates simply by choosing values in between the extant calls for each call variables. For example, *P. personatus* emit calls that are on average 4.8 ms long and *P. parnellii* emit for 19.7 ms.

The pulse duration for the three intermediates are 8.5, 12.3, and 16 ms. I also took in consideration the number of harmonics as well as the dominant harmonic.

The series of calls are presented in figure A.1. The values for the *Pteronotus* and *Rhinopoma* species were taken from recordings at hand, while the values for Rhinolophidae and Hipposideridae are from Jones (1999).

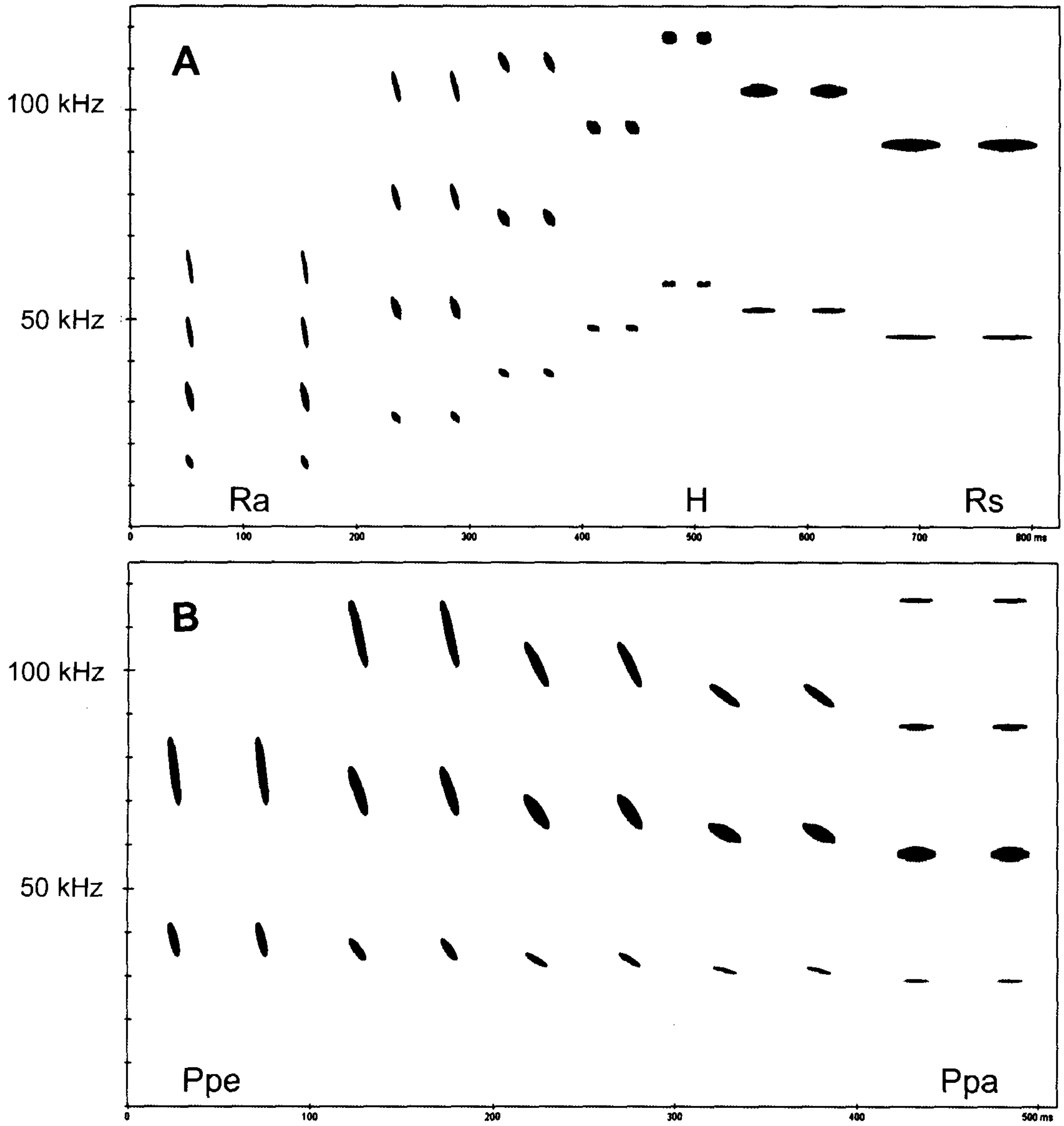

Figure A.1. Spectrograms showing series of calls (two pulses each) used in the synthetic calls experiment exploring the transition from low to high duty cycle bats. (A) Old World bats: *Rhinopoma* sp. (Ra), Hipposideridae (H), Rhinolophidae (Rs). (B) New World bats: *Pteronotus personatus* (Ppe), *Personatus parnellii* (Ppa). Unlabeled calls are hypothetical intermediate forms.

Appendix 3

I performed a principal component analysis (PCA) on the call variables of the synthetic calls experiments. From that analysis, PC1 accounts for 36.16% of the variation in the data, PC2 accounts for 21.72%, and PC3 for 18.65%. Those three PCs together account for 76.53% of the variation and they are the only ones with eigenvalues >1. They are also biologically relevant and well-defined. For PC1, the 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).

Table A.1. 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

Table A.2. Relative contribution of call variables to the principal components.

	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