ABSTRACT

Title of Dissertation: PARENT-OFFSPRING RECOGNITION AND ALLOPARENTAL CARE IN GREATER SPEAR-NOSED BATS

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Selection should insure that parents selectively care for their own offspring. Thus, alloparental care, or care of other's young, seems counterintuitive to evolutionary theory. Alloparental care is often attributed to: 1) mistaken identity, when individuals confuse their young with others or 2) cooperation, when the alloparent and young mutually benefit. Cooperative care, in turn, is often explained by kin selection, where animals selectively care for genetic relatives. In this dissertation, I examine these alternative explanations for alloparental care in greater spear-nosed bats (*Phyllostomus hastatus*). In this species, females form stable social groups of relatively unrelated individuals. Females give birth once a year to nonvolant pups that frequently fall from roost

sites in cave ceilings and likely perish unless retrieved by an adult. In this context, pups emit vocalizations, termed isolation calls, that are used in parent-offspring recognition.

I examine parent-offspring recognition in *P. hastatus* by examining isolation call variability and both detection and perception of isolation calls by adults. I found that pups emit individually distinctive calls but that pups from the same social group have more similar calls than pups from different social groups. Psychoacoustic experiments in the laboratory showed that greatest hearing sensitivity and frequency selectivity in adult *P. hastatus* is at the fundamental frequency of isolation calls. I found that this is a common phenomenon in bats using comparative phylogenetic methods. Finally, using psychoacoustic experiments I demonstrated that *P. hastatus* females could discriminate between pups' isolation calls regardless of the pups' social groups.

Next, I examine parental care in the natural habitat of *P. hastatus*. I found that females respond more frequently and spend more time visiting group mates' pups than non-group mates pups, even though many of these females are not missing pups of their own. These results, combined with the results from psychoacoustic studies, indicate that mistaken identity cannot explain this visiting behavior. By visiting group mates' pups, females protect them from non-group mates who attack and sometimes kill them. However, kin selection cannot explain this behavior because females are unrelated to group mates' pups that they visit.

PARENT-OFFSPRING RECOGNITION AND ALLOPARENTAL CARE IN GREATER SPEAR-NOSED BATS

by

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Preface

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To my parents Belle and John Bohn, and in loving memory of my grandfather Dr. Guy Weston Bohn.

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INTRODUCTION

Parental care is an important component of reproductive success because it can directly affect offspring survival. Parental care can be costly, particularly when young are born altricial and must be provisioned over an extended period. Because of these costs, selection should act to insure that animals direct parental care towards their own young. Alloparental care, or care of others' young, has received a great deal of attention because it appears contradictory to natural selection. Most cases of alloparental care have been attributed to 1) mistaken identity, when individuals confuse their young with others (McCracken 1984; Roulin 2002), or 2) cooperation, where animals care for the young of others but obtain benefits that offset the costs associated with this behavior (Riedman 1982; Packer et al. 1992; Clutton-Brock et al. 2000; Roulin 2002).

Parent Offspring Recognition

Most cases of mistaken identity occur in colonial species when the likelihood of confusing filial young with others is high. Selection against mistaken identity has resulted in the evolution of parent-offspring recognition systems. Acoustic signals facilitate offspring recognition in many colonial species (e. g. Trillmich 1981; Stoddard & Beecher 1983) including bats (e. g. Balcombe 1990). However, even with parent-offspring recognition systems in place, mistaken identity can still occur (reviewed in Roulin 2002).

Parent-offspring recognition can be broken down into two main components. First, young must emit signals that contain individual "signatures" (Beecher 1982). Signature signals must be highly repeatable within individuals but vary between individuals (Beecher 1982). For signals to be distinctive, the amount of information encoded in them must increase as the number of individuals increases (Beecher 1989). Thus, parents may not be able to recognize offspring if signals do not contain sufficient information (Loesche et al. 1991). The second component of parent-offspring recognition is that parents must be able to discriminate between signals and use signal information to recognize their young from others (Beecher 1982). If parents have insufficient resolution in the auditory system to discriminate among signals, increased recognition error can also result in mistaken identity (Job et al. 1995; McCulloch et al. 1999).

Cooperation

Many cases of alloparental care cannot be explained by mistaken identity. This is especially the case in cooperative breeders, when animals that do not have young of their own care for other's offspring. An alternative explanation for these behaviors is cooperation. Here I define cooperation as an interaction between individuals that results in mutual benefits (Dugatkin 1997). For alloparental care to evolve in a cooperative context, the costs of providing parental care must be counterbalanced by either immediate or future benefits.

Most commonly, cooperative care is explained by kin selection, where alloparents care for genetic relatives (Emlen & Wrege 1988; Creel et al. 1991;

Pusey & Packer 1994). In this case, individuals receive indirect benefits that offset the cost of alloparenting. If kin selection is acting, care should be selectively allocated so that Hamilton's rule is met: r*B > C, where r is the relatedness between the alloparent and young, B is the benefit the young receive and C is the cost of providing care (Hamilton 1964). Alternatively, the costs of caring for other's young can be balanced by delayed benefits the caregiver receives. For example, in cooperative breeders, helpers may benefit through future breeding opportunities (Richardson et al. 2002) or group augmentation (Rood 1990; Clutton-Brock et al. 2000). Finally, in some species, alloparents may also benefit through reciprocity, by alternately caring for each other's young (Owens & Owens 1984).

Greater Spear-Nosed Bats

In this study I investigate parental care and parent-offspring recognition in greater spear-nosed bats (*Phyllostomus hastatus*). *P. hastatus* is a large (70-100 g) omnivorous species that is widely distributed in the Neotropics (Santos et al. 2003). In Trinidad, West Indies, *P. hastatus* commonly roost in large colonies in caves. Colonies are composed of discrete clusters in specific depressions in cave ceilings (see Appendix I). Clusters contain groups containing either 18 adult females, on average, with a single male or males of all ages. Female social groups are highly stable with some females remaining together for 16 years or more (G. F. McCracken, G. S. Wilkinson & J. W. Boughman, unpublished data). Social groups are attended by a single harem male who fathers the majority of pups in a group but does not affect social group formation or maintenance

(McCracken & Bradbury 1977; McCracken & Bradbury 1981). During their first year, young bats of both sexes leave their natal social groups. Young females join either existing social groups or form new groups while males join bachelor groups (McCracken & Bradbury 1981).

Unlike most other group-living mammals, female social groups are typically comprised of unrelated individuals in *P. hastatus* (McCracken & Bradbury 1981; McCracken 1987). The benefits of maintaining such stable social relationships are likely cooperative. For example, *P. hastatus* forage cooperatively using group-specific vocalizations (Boughman 1997; Wilkinson & Boughman 1998). Socially-mediated birth synchrony also occurs within groups (Porter & Wilkinson 2001) which is commonly associated with cooperative care of young (Ims 1990). Thus, another benefit to forming stable social groups in *P. hastatus* may be related to parental care.

Female *P. hastatus* have high adult survival rates (90%, McCracken & Bradbury 1981) but low reproductive rates (one pup per year) and low infant survival (40-60%,Stern & Kunz 1998). Thus, parental care should strongly affect reproductive success. Pups are born non-volant and do not begin to fly until approximately 6 weeks of age (Stern & Kunz 1998). One important aspect of parental care in this species is pup retrieval. Non-volant pups sometimes fall from roosts in cave ceilings where they likely perish unless retrieved by an adult (McCracken & Bradbury 1981; G. S. Wilkinson & K. M. Bohn pers. obs.). When pups fall they emit vocalizations termed "isolation calls" (Gould et al. 1973). Females respond to these calls and carry young back to roosts. Previous

researchers have observed females respond to and sometimes retrieve group mates' fallen pups (T. S. Porter and J. W. Boughman pers. comm.).

Present Study

One possible reason for females to respond to group mates' pups is that they are simply making mistakes. Alternatively, given the social structure of this species, females may be cooperatively caring for young. In this dissertation, I examine these alternatives using a multidisciplinary approach. I use psychoacoustic experiments in the laboratory to examine parent-offspring recognition, field studies to investigate female behavior, and genetic analyses to determine relatedness between females and fallen pups.

In chapter one I examine auditory sensitivity and frequency selectivity in *P. hastatus* and compare auditory tuning to vocalization frequencies. *P. hastatus* provide an interesting case study for auditory tuning because they emit three distinctive signals at different frequency bands. First, they emit echolocation calls at ultrasonic frequencies,(40-80 kHz, Griffin & Novick 1955; Pye 1967). Second, they emit isolation calls with fundamental frequencies around 15 kHz. Third, they emit socially modified "screech calls" at 6-11 kHz that are used in group foraging (Boughman 1997; Boughman 1998). I find that hearing sensitivity and frequency selectivity matches the fundamental frequency of isolation calls.

Almost all species of bats hear at frequencies that are lower than their echolocation calls and emit isolation calls at similar frequencies. The results from chapter one raise the question as to whether low-frequency hearing has coevolved with isolation call frequencies in other echolocating bats. In chapter

two I investigate this possibility using a comparative phylogenetic approach with data from thirteen species of bats from five families. I test for correlated evolution between high-frequency hearing and echolocation calls, low-frequency hearing and high-frequency hearing, and low-frequency hearing and isolation calls. I find that not only is high-frequency hearing highly correlated with echolocation frequency but that it also affects low-frequency hearing. However, after controlling for these effects there is evidence of coevolution between isolation call frequency and low-frequency hearing. These results indicate that selection for detection of young has played an important role in the evolution of auditory tuning in echolocating bats.

In chapter three I examine production and perception of infant isolation calls. First, I examine isolation call variability and determine that isolation calls have sufficient variability between pups relative to within pups for individual identification. However, isolation calls are also more similar within social groups than between social groups, which could confound pup recognition. Second, I examine greater spear-nosed bats' perception of isolation calls. I find that females can discriminate among pups' calls regardless of social group. These results indicate that females should be able to recognize their pups using isolation calls and that mistaken identity is not a likely explanation for females responding to group mates' pups.

Finally, in the last chapter I use behavioral observations of wild *P. hastatus* to revisit the question as to why females visit group mates' pups. Bymaking individually distinctive marks on the backs of all females from seven

social groups, I was able to observe female behavior around fallen pups. I use these behavioral data combined with genetic analyses to further test whether females are making mistakes as well as examine the costs and benefits associated with visiting behavior. I find that females visit group mates' pups even when their own pups are not missing from their social groups, which is inconsistent with females making mistakes. Visiting group mates' benefit those pups because females guard them from non-group mates that attack and sometimes kill them. Furthermore, this behavior is likely costly for visiting females as they have pups of their own that remain unattended while females visit others. However, females are unrelated to the pups they visit, and therefore this behavior is not under kin selection. The most likely explanation for pup guarding is that females receive direct benefits from this behavior possibly in the form of improved thermoregulation for their pups in group crèches. However, because these females form long-lived stable social groups, it seems likely that females also recoup any costs of pup guarding by cooperating with group mates.

This dissertation adds to our understanding of parent-offspring communication and cooperation. In a wide variety of mammals, young use vocal signals in parent-offspring communication. Thus, the chapters on perception and coevolution are likely applicable to many mammalian species. Greater spear-nosed bats provide a rare example of alloparental care that cannot be attributed to either mistaken identity or kin selection. My dissertation presents evidence of behaviors that benefit other unrelated individuals, in a species where long-lived

females reside in stable social groups. These results raise new questions as to how cooperation might evolve in such structured societies.

CHAPTER I

Auditory Sensitivity and Frequency Selectivity in Greater Spear-Nosed Bats Suggest Specializations for Acoustic Communication

ABSTRACT

I investigated the relationship between auditory sensitivity, frequency selectivity, and the vocal repertoire of greater spear-nosed bats (*Phyllostomus hastatus*). *P. hastatus* commonly emit three types of vocalizations: group-specific foraging calls that range from 6 to 11 kHz, low amplitude echolocation calls that sweep from 80 to 40 kHz, and infant isolation calls from 15 to 100 kHz. To determine if hearing in *P. hastatus* is differentially sensitive or selective to frequencies in these calls, I determined absolute thresholds and masked thresholds using an operant conditioning procedure. Both absolute and masked thresholds were lowest at 15 kHz, which corresponds with the peak energy of isolation calls. Auditory and masked thresholds were higher at sound frequencies used for group-specific foraging calls and echolocation calls. Isolation calls meet

the requirements of individual signatures and facilitate parent-offspring recognition. Many bat species produce isolation calls with peak energy between 10 and 25 kHz, which corresponds with the frequency region of highest sensitivity in those species for which audiogram data are available. These findings suggest that selection for accurate offspring recognition exerts a strong influence on the sensory system of *P. hastatus* and likely on other species of group-living bats.

INTRODUCTION

For communication systems to function effectively, recognition signals or signatures must contain information about identity, and receivers must be able to detect, as well as discriminate among those signatures. Perception of individual signatures is believed to occur through a template-matching process, a mechanism by which a template of the target signal is formed in the memory of the receiver, and new signals are then compared with this template (Holmes & Sherman 1982; Lacy & Sherman 1983). The difficulty of this task depends on the number of entities in the recognition pool and the nature of the decision. As the number of entities increases, the amount of information that must be encoded by the signaler and decoded by the receiver must increase to insure accurate recognition (Beecher 1989). Thus, the ability to recognize a signaler depends on the task, the resolving power of the receiver, and the similarity between the template and novel signal.

Most empirical studies of signature systems have focused on a single perceptual task, such as offspring recognition by a parent, and have frequently demonstrated that sufficient information exists in the signal to permit accurate identification (birds, McArthur 1982; Stoddard & Beecher 1983; Nakagawa et al. 2001; seals, Trillmich 1981; Insley 2001; primates, Pereira 1986; dolphins, Smolker et al. 1993). An issue that has received considerably less attention is how a sensory system should be designed when more than one type of recognition problem must be solved. An ideal system would have sufficient sensitivity and resolving ability to enable accurate detection and discrimination of all possible signal variants. However, animals are constrained by the physics associated with signal production and transmission, as well as by physiological limitations imposed on the receiver (Bradbury & Vehrencamp 1998). Greater spear-nosed bats (*Phyllostomus hastatus*) present an important case for the study of signal production and reception because they use vocalizations for three different recognition problems: to recognize social group membership, to recognize offspring, and to recognize self-generated sonar vocalizations from echoes and calls produced by conspecifics.

P. hastatus roosts in stable social groups of, on average, 20 unrelated females (McCracken & Bradbury 1981) that appear to use group-specific "screech" calls to coordinate foraging (Boughman 1997; Boughman & Wilkinson 1998; Wilkinson & Boughman 1998). Auditory specializations might occur in the frequency range of screech calls because these low frequency (5-12kHz) signals can be modified by vocal learning (Boughman 1998). When separated from their

mothers, infant *P. hastatus* emit isolation calls that attract adult females and facilitate maternal retrieval of offspring. Isolation calls consist of a harmonic series of frequency modulated tones that range from 15 to 100 kHz (Gould 1975). Isolation calls contain sufficient variation in frequency and temporal characteristics to permit unambiguous assignment of calls to individuals (Lill and Wilkinson unpublished data). As in other species that roost in large colonies, recognizing and directing parental care towards young should be under strong selection (Beecher et al. 1981; Beecher 1982; Colgan 1983). Hearing in the frequency range of isolation calls should, therefore, also be under selection to the extent that it aids in detecting the calls of fallen offspring and discriminating among related and unrelated individuals.

P. hastatus emit short (1-3 ms), low amplitude echolocation calls which consist of high frequency (80-40 kHz), broad band sweeps (Griffin & Novick 1955; Pye 1967). *P. hastatus* are omnivorous, predominantly consuming fruit and large insects (Emmons 1997). It has been long recognized that *P. hastatus* use echolocation for orientation as do most frugivorous phyllostomids, however, recent studies have shown that *P. hastatus* also rely on echolocation to find fruit (Kalko & Condon 1998). Thus perception of sonar cries and returning echoes should also be under selection.

In bats, studies on hearing have focused mainly on the ultrasonic frequency range, even though many species are most sensitive to frequencies below those used for echolocation (reviewed in Neuweiler 1990, Moss & Schnitzler 1995). Low frequency hearing may be used for passive listening to

prey-generated noises (Ryan et al. 1983; Coles et al. 1989; Schmidt et al. 1991; reviewed in Neuweiler 1990), however, a correspondence between frequencies of highest auditory sensitivity and social vocalizations has been noted for some species (*Noctilio leporinus*, Wenstrup 1984; *Macroderma gigas* and *Nyctophilus gouldi*, Guppy & Coles 1988; *Phyllostomus discolor*, Esser & Daucher 1996). Except for work on *P. discolor* (Esser & Kiefer 1996) few studies have focused on possible auditory specializations related to conspecific vocal signals in bats.

In this study I examine auditory sensitivity and frequency selectivity in *P. hastatus* and compare these estimates with the spectral content of both social communication and echolocation signals. I use an operant conditioning paradigm to determine hearing sensitivity and frequency selectivity. I estimate frequency selectivity by measuring critical ratios from measurements of pure-tone thresholds embedded in broadband white noise. Critical ratios indirectly measure the frequency selectivity of the auditory system, which operates with a bank of overlapping band-pass filters or critical bands (Fletcher 1940). Estimates of critical bands from critical ratios are based on the following assumptions: 1) the detection of pure tones embedded in broadband noise are masked only by the noise within the critical band 2) critical bands are symmetrical and rectangular and 3) the energy level of the tone at threshold is equal to the energy level of the noise within the critical band (Fletcher 1940). From these assumptions it follows that the critical ratio in dB can be used to estimate the critical band at a given frequency by determining the bandwidth of the white noise that contains energy

equal to that of the level of the tone at threshold. Smaller critical ratios imply narrower frequency bands and higher frequency selectivity.

In my study I use the same experimental set up and positive reinforcement methods to generate an audiogram and a critical ratio function for four individual *P. hastatus.* Absolute hearing sensitivity measured in this study can be compared with published audiograms obtained using negative reinforcement (Koay et al. 2002) and neural recordings (Grinnell 1970). My data permit direct comparisons of hearing sensitivity and frequency selectivity because I use the same behavioral methods in the same individual bats. I then compare these measures to the spectral content of three common vocalizations: screech calls, isolation calls, and echolocation calls.

METHODS

Subjects

Four adult female *P. hastatus* were used in the experiments. The experimental animals came from groups captured in Trinidad, West Indies in 1993. During the study, bats were housed in a large cage (3.3 by 2.7 by 2.4 m) in a separate room at the University of Maryland, College Park. The room was maintained on an 8-hour light, 16-hour dark cycle at approximately 24° C and 30% humidity. Bats were maintained at a weight of 65 to 70 g during experiments (85-90% free-fed body weight) on a diet of fruit and marmoset food (Premium

Nutritional Products) supplemented with mealworms, which were provided as food rewards during test sessions.

Behavioral Training

All experiments were conducted in a single-wall acoustic chamber (Industrial Acoustics Company, Inc) lined with acoustic foam (Sonex). Bats were trained and tested using a V-shaped platform enclosed in a hardware-cloth cage (Fig. 1). Subjects were trained for a modified go/no-go procedure (Suthers & Summers 1980). A red light was used to signal the onset of a trial. During each trial either a pure tone was played (stimulus trial) or was not played (catch trial). The bats were rewarded with a mealworm at the end of the right arm of the platform (30 cm long by 13 cm wide) during stimulus trials and a mealworm at the starting position during catch trials. If the bats went to the end of the platform during catch trials, the light was extinguished and both verbal commands ("get back") as well as light tapping on the bottom of the cage were used to direct bats back into the starting position. If bats did not move to the end of the platform during stimulus trials, a 20-second time-out was given. If the bat failed to respond for three consecutive trials, the session was terminated.

Once the subjects learned the go/no-go task, a one-up/one-down staircase procedure was introduced to the stimulus levels (Niemiec & Moody 1995). The percentage of catch trials was varied in order to maximize correct responses to stimuli while keeping incorrect responses to catch trials at or below 20%. In order to be certain that the bats were accustomed to the procedure and performing reliably, I did not begin collecting audiogram measurements until the thresholds

for all four bats at 10 kHz were within 5 dB of each other over five consecutive sessions.

Threshold Determination

During test sessions, stimulus (65%) and catch (35%) trials were alternated at random. Sessions with greater than 25% response during catch trials were discarded, although false alarm rates were usually below 10%. If a bat responded correctly to a stimulus, the amplitude of the signal was reduced by 5 dB. If a bat failed to respond to a stimulus for two consecutive trials, the amplitude of the tone was increased by 5 dB. For each session, trials continued until six reversals occurred. A reversal was counted every time the direction of amplitude adjustment was changed. The first two reversals were discarded and the last four averaged to calculate a threshold as described in Niemiec and Moody (1995).

For the audiogram, thresholds were determined for each subject at 11 different frequencies (2.5, 5, 7.5,10, 15, 20, 30, 40, 60, 80 and 100 kHz). For critical ratio estimates, masked thresholds were determined for 7.5, 15, 30, 40 and 60 kHz with a noise spectrum level of 25 dB/Hz. I also measured thresholds at 7.5, 15 and 40 kHz with a noise spectrum level of 35 dB/Hz. Valid critical ratios should remain the same at different noise spectrum levels (Fletcher 1940). Threshold and masked threshold estimates were taken at least three times at each test frequency. Critical ratios were calculated as the amplitude (in dB) of the tone at threshold minus the spectrum level of the noise (in dB/Hz). Based on

assumptions outlined by Fletcher (1940), each critical ratio was converted to an equivalent filter bandwidth using the formula: critical ratio (Hz) = $10^{(\text{critical ratio dB}/10)}$.

Stimuli and Calibration

All pure tone signals were synthesized digitally at a sample rate of 250 kHz using SIGNAL (Version 3.0, Engineering Design). Every stimulus trial consisted of three pure tones with durations of 350 ms each, including 25 ms rise/fall times and 50 ms intervals between tones. Stimuli were played through two serially connected attenuators (Hewlett Packard 350D) that controlled amplitude in 5 dB steps. The signal was then band pass filtered (Krohn-Hite 3550), amplified (Harman Kardan AVR 100), and sent to a speaker (Pioneer PT-R) that was located 1 m from the subjects' starting position. White noise was created using a function generator (Stanford Research Systems, DS345), passed through a graphic equalizer (Rack Rider, RR-131) and filtered (Stanford Research Systems, SR650). With this system I created random white noise that was flat (± 3 dB) from three to 80 kHz. For the masked thresholds experiments, pure tones and noise were sent to a custom made mixer prior to being amplified and sent to the speaker.

Each day I recorded the pure tones and/or noise at five locations separated by 2 cm at the bats' starting position on the platform (Fig. 1). Sounds were recorded onto a laptop computer equipped with a high-speed data acquisition card (INEES, Daq508), which sampled 16 bits at 333 kHz, using a one-eighth inch microphone (Brüel & Kjær), connected to a preamplifier (Larson Davis 2200C) and amplifier (SHURE, FP-2). Time waveforms and power spectra

of stimuli were inspected daily for any distortions using Bat Sound Pro (Pettersson Electronik). I also recorded a calibration tone daily with a piston phone (Brüel and Kjaer type 4231). Sound levels were calculated by taking the root mean square of 10,000 samples of each waveform and then averaged over the five locations on the observation platform.

Vocalizations

All *P. hastatus* vocalizations, except for echolocation calls (see below), were recorded at Guanapo cave, Trinidad (McCracken and Bradbury 1981), in April 2001. I recorded screech calls from flying bats at the entrance of the cave using a shotgun microphone (Audio-Technica AT4071A) and phantom power supply (AKG Acoustics B18) connected to a laptop computer which sampled 16 bits at 44 kHz. This system had a flat response (± 5 dB) from 20 Hz to 20 kHz. Screech calls do not contain appreciable energy above 15 kHz (Boughman 1997).

I recorded isolation calls from ten individual pups that were captured with their mothers and briefly held outside Guanapo cave in April 2002. Isolation calls were recorded at a sample rate of 250 kHz using a high frequency microphone (Ultra Sound Advice M2) and the same equipment that was used for making recordings during psychoacoustic experiments.

The four bats studied in the psychoacoustic experiments were allowed to fly freely in a large room at the University of Maryland. Echolocation calls were recorded with a high frequency microphone (Ultra Sound Advice M2), band pass filtered (5-110 kHz, Stewart, VBF7), amplified, and digitized onto a laptop

computer using a high-speed analog-digital card which sampled 16 bits at 250 kHz (IOTECH Wavebook).

I calculated mean power spectra for 23 screech calls, 50 isolation calls (five calls/pup) and 50 echolocation calls using Bat Sound Pro (Pettersson Electonik). For each call type I determined the peak frequency and calculated the peak frequency range by determining frequencies above and below the peak frequency that were –3dB below the peak energy. For each call type I determined the relative amplitude of the power spectrum at each frequency for which I had measured an auditory threshold, except for screech calls where only frequencies equal to or below 20 kHz were included in the analysis. as this was the upper range of the microphone used to record these calls. For each call type I calculated a correlation coefficient between the spectral power of the vocalization and the mean of the lowest auditory thresholds measured in each bat. Because the data were not normally distributed and violated independence assumptions, I tested whether the correlation coefficients were different from zero using randomization tests (Manly 1991). For each call type, the order of one variable was randomized and a correlation coefficient was calculated. Correlation coefficients were calculated for all possible permutations for screech calls (N = 720) and for 10,000 permutations for both echolocation and isolation calls. I then determined the proportion of these correlation coefficients that had absolute values greater than the observed correlation to assign a two-tailed probability to the hypothesis that the observed coefficient was significantly different from zero.

RESULTS

Absolute Thresholds

All four bats responded to tones from 2.5 to 100 kHz. Pure-tone thresholds were similar for all four bats and were lowest at 15 kHz (Fig. 2a). Thresholds ranged from a maximum of 71 dB SPL at 2.5 kHz to a minimum of 13 dB SPL at 15 kHz. Hearing sensitivity increased at a rate of approximately 5 dB/kHz from five to 15 kHz and then decreased at a slower rate of approximately 0.4 dB/kHz from 15 to 100 kHz.

Masked Thresholds

Critical ratios were similar for all four bats and for the two noise spectrum levels tested (Fig. 2b, Fig. 3b). Critical ratio estimates were lowest at 15 kHz, increased by approximately 10 dB at 30 kHz, decreased slightly by 4 dB at 40 kHz, and then increased by another 10 dB at 60 kHz, where the highest estimates were obtained. Critical ratio values can be converted to equivalent frequency bands following Fletcher's (1940) assumptions. Calculations yield a minimum bandwidth of 209 Hz and maximum bandwidth of approximately 17kHz (Fig. 3b).

Spectral Characteristics of Species-Specific Vocalizations

Screech calls are broadband acoustic signals (Fig. 4a) with average peak energy at 9.5 kHz and a –3dB frequency range of 6 to11 kHz (Fig. 3a). Isolation calls are characterized by frequency-modulated syllables with multiple harmonics

(Fig. 4b). Maximum energy of these calls occurred in the fundamental at 15 kHz and the –3dB frequency range was 14 to 17 kHz (Fig. 3a). The peak frequency of the first harmonic of isolation calls was at 28 kHz with a –3dB frequency range of 26 to 29 kHz, which was 5 dB less than the peak frequency at 15 kHz. Echolocation calls consist of steep multi-harmonic sweeps (Fig. 4c) with peak energy at 46 kHz and a –3dB frequency range between 42 and 50 kHz (Fig. 3a). A second peak occurs at 62 kHz and had a –3 dB range of 59 to 65 kHz.

The peak energy of screech calls (6-11 kHz) lies in a frequency region where hearing sensitivity is comparatively poor (Fig. 3). The correlation between power spectra of screech calls and absolute auditory thresholds was not significant (Fig. 3, $r = 0.057$, randomization test $P = 0.911$, $n = 6$). In contrast, peak energy of isolation calls (14-17 kHz) is within the range of best hearing sensitivity, and I found a significant negative correlation between isolation call power spectra and auditory thresholds across sound frequency (Fig. 3, $r = -0.790$, randomization test $P = 0.004$, $N = 11$). There was no relationship between the spectral characteristics of echolocation calls and absolute hearing sensitivity ($r = -0.318$, randomization test $P = 0.356$, $N = 11$).

Critical ratios followed pattern similar to that of absolute thresholds with highest frequency selectivity at 15 kHz, corresponding with the peak frequency of isolation calls. Critical ratio measurements resulted in estimated bandwidths of approximately 5 kHz in the frequency range of screech calls, 200 Hz at the peak frequency of isolation calls, and between 2 and 17 kHz in the range of echolocation calls. A small decrease in critical ratios occurred at 40 kHz, which

was close to the peak frequency of echolocation calls. However, frequency selectivity at peak frequencies of screech and echolocation calls is considerably less than it is at the frequencies containing maximum energy in isolation calls.

DISCUSSION

Hearing Sensitivity and Frequency Selectivity

In this study I examined hearing sensitivity and frequency selectivity of *P. hastatus* using positive reinforcement and a go/no-go procedure. In my behavioral audiogram, the minimum absolute threshold was 13 dB SPL at 15 kHz. Minimum absolute thresholds in bats have been reported at 0 dB SPL or lower using conditioned avoidance (e.g. *Eptesicus fuscus*, Koay et al. 1997), two alternative forced choice (*Megaderma lyra*, Schmidt et al. 1983), and neural recording methods (e.g. *M. lyra*, Kossl 1992; *M. gigas*, Guppy & Coles 1988). The higher thresholds I obtained were likely due to my training procedures, as I did not use negative reinforcement. My audiogram showed greater sensitivity to low frequencies than the neurophysiological audiogram by Grinnell (1970). However, higher thresholds at frequencies below 25 kHz have been noted for most bat audiograms that were measured by neural recordings while animals were under anesthesia (Neuweiler 1990). The shape of my audiogram for *P. hastatus* is similar to a behavioral audiogram recently published by Koay et al. 2002), although they report a minimum threshold of 1.5 dB SPL at 20 kHz and a drop in threshold at 64 kHz, a frequency I did not test. Both absolute sensitivity

and critical ratio data collected in this study showed consistent thresholds across days and bats, suggesting that my findings are reliable. Furthermore, because I used positive reinforcement, my thresholds should be representative of amplitude levels that would elicit behavioral responses under natural circumstances.

In most mammals and birds critical ratios increase by approximately 3 dB/octave over the range of hearing (Fay 1988; Dooling et al. 2000). *P. hastatus* deviates from this pattern with a 14 dB/octave decrease in critical ratios from 7.5 kHz to 15 kHz, followed by an approximate 10 dB/octave increase in critical ratios from 15 kHz to 60 kHz. Deviations from the general pattern have been reported in some birds with smaller critical ratios in the frequency range of vocalizations and have been interpreted as possible specializations for conspecific communication (Okanoya & Dooling 1987; Dooling et al. 2000; Wright et al. 2003). Interestingly, although critical ratios have only been published for two species of bats, both species deviate from the 3 dB/octave pattern (*Rhinolophus ferrumequinum*, Long 1977; *Rousettus aegyptiacus*, Suthers & Summers 1980).

Critical ratios can be used to estimate auditory filter bandwidths. The smaller the estimated auditory filter bands, the greater the animal's ability to discriminate between sound frequencies. Frequency discrimination, or the ability to detect changes in pure-tone frequency, shows a parallel relationship with critical ratios in species for which both have been measured; however, critical ratios are usually 20 times larger than pure tone frequency discrimination thresholds (Long 1994). If this relationship holds in *P. hastatus*, then frequency

discrimination would be approximately 10 Hz, 250 Hz, and between 100 and 850 Hz in the range of isolation calls, screech calls and echolocation calls respectively.

Screech Calls

The spectral energy of screech calls between 6 and 11 kHz lies near the lower frequency limit of hearing in *P. hastatus.* Although the peak energy in these calls does not correspond with peak hearing sensitivity, some energy at higher frequencies of screech calls overlaps with lower thresholds in the audiogram. However, screech calls are emitted while bats forage, which requires lower frequencies for optimal propagation over long distances (Wiley & Richards 1982). Estimates of screech call amplitude are 75-79 dB SPL at 1 m (Boughman unpublished data), and when combined with hearing sensitivity and transmission loss (Marten et al. 1977), result in detection distances between 70 and 109 meters (for 5 and 10 kHz respectively).

Screech call discrimination should be an easier perceptual task than isolation call discrimination because the recognition problem requires less information. This is due to the fact that the number of groups likely encountered while foraging is fewer than the number of pups in a cave. Thus, heightened frequency selectivity may not be required to learn and decode these signals. Analysis of 161 calls from 28 bats in three groups revealed that seven acoustic features, including spectral, temporal, and relative amplitude variables exhibited significant variation among groups (Boughman 1997). Four of these variables involved frequency measurements and 14 to 34 percent of the variation in these

variables was accounted for by group identity. In contrast, group identity only explained between 0 and 13 percent of the variation in each of four temporal variables. The most informative variable was bandwidth, which ranged from 5 to 8 kHz – surprisingly close to my critical ratio estimate at this frequency range. The frequency at –12 dB below the second energy peak of these calls was the second most informative variable. This variable lies between 10 and 12 kHz. Although I did not measure critical ratios at these frequencies, given the form of my critical ratio function, better frequency discrimination would be expected at these frequencies when compared with the first peak of the calls that lies between 4 and 5 kHz (Boughman 1997).

Echolocation Calls

Greatest auditory sensitivity and frequency selectivity of *P. hastatus* did not correspond with the spectral peaks of echolocation calls at 42 and 60 kHz. Although there was a small decrease in critical ratios at 40 kHz, frequency selectivity was much poorer than at the peak frequency of isolation calls. This result does not preclude other auditory specializations related to sonar localization. *P. hastatus* echolocation calls are broadband sweeps, well suited for carrying spatial information about target range, direction in azimuth and elevation (Simmons 1973; Simmons & Stein 1980). Heightened sensitivity and frequency selectivity in the ultrasonic range may not be essential for these tasks. For example, echolocating bats use a temporal cue, the time delay between sonar cries and returning echoes, to determine the distance to targets. Species using broadband signals, such as *P. hastatus,* exhibit finer range resolution than

species using narrowband signals (Simmons 1973). In contrast, *R. ferrumequinum*, a bat that uses long constant frequency echolocation signals, has very low critical ratios in the frequency range of their sonar cries, indicating specialized frequency selectivity in that region (Long 1977). Long narrowband signals, such as those used by *R. ferrumequinum*, are well designed for spectral analysis but are poorly suited for temporal analysis (Simmons and Stein 1980).

Isolation Calls

Both sensitivity and frequency selectivity were highest at 15 kHz, which corresponds with the peak energy of the fundamental in isolation calls. Auditory sensitivity was also high across the peak energy range of the first harmonic (26-30 kHz). Although additional harmonics exist in these calls, these higher harmonics contain less energy and attenuate more rapidly, making them less reliable for isolation call detection and discrimination (Wilkinson 1995). Although I do not have absolute amplitude measurements of these calls, they are emitted at least 12 to 14 dB louder than echolocation calls (Gould 1977). Highest auditory sensitivity at the peak frequency of isolation calls should maximize adult detection of offspring.

An association between auditory sensitivity, frequency selectivity and isolation calls, as well as maternal directive calls, has been reported in the congener, *P. discolor* (Esser & Daucher 1996; Esser & Lud 1997). In this species, young bats appear to modify isolation calls to match maternal directive calls (Esser 1994). Maternal directive calls have unique sinusoidal frequency modulation patterns (Esser & Schmidt 1989; Esser & Lud 1997). Studies in *P.*

discolor on the minimum detectable frequency modulation (Esser & Kiefer 1996) and minimum detectable difference in modulation frequency (Esser & Lud 1997) were conducted at 18.5 kHz, the fundamental frequency of maternal directive calls. Results not only indicated sufficient spectral and spectro-temporal resolution to distinguish individuals but also enhanced frequency resolution when compared with other mammals (Esser & Kiefer 1996; Esser & Lud 1997).

Isolation call discrimination is likely among the most challenging acoustic tasks encountered by adult *P. hastatus* because they must discriminate among many isolation call signatures. The amount of variation among pups in acoustic features of isolation calls should reflect the magnitude of this problem (Wilkinson 2003). Nested analysis of variance on acoustic measurements of the first harmonic in 615 isolation calls recorded from 127 pups captured in 22 female groups, revealed that five frequency and five temporal variables exhibited significant variation among pups (Lill and Wilkinson unpublished data). After adjusting for age-related effects, variation among pups accounted for 38 to 51 percent of the total variance in each of the five frequency measures and 27 to 39 percent of the variance in each of the five temporal measures. Heightened frequency selectivity should enable females to identify pups using such large acoustic differences.

Highest hearing sensitivity and frequency selectivity occurred at the same frequency and corresponded with peak spectral energy of isolation calls. This finding is consistent with auditory specializations for detection and discrimination of individual vocal signatures and indicates that isolation calls are an essential

component of the vocal repertoire. Non-volant pups frequently fall from roosts and then emit isolation calls (Wilkinson and Bohn unpublished data). Isolation calls attract females who carry young back to the roost. *P. hastatus* have low reproductive rates (one pup per year) and high infant mortality (40-60%, McCracken & Bradbury 1981; Stern & Kunz 1998). Pup recognition, therefore, is essential for successful reproduction. Detection and discrimination of isolation calls is likely important for many bat species that roost in colonies and leave non-volant young behind while foraging. Isolation calls exhibit the requirements of individual signatures in many species of bats (e.g. *Tadarida brasiliensis*, Gelfand & McCracken 1986; *P. discolor*, Rother & Schmidt 1985; *Nycticeius humeralis,* Scherrer & Wilkinson 1993; *Plecotus auritus*, de Fanis & Jones 1995) and maternal recognition of individual isolation calls has been demonstrated in a few species (Rother and Schmidt 1985; Balcombe 1990; de Fanis and Jones 1995). Furthermore, overlap between regions of peak sensitivity and isolation call frequencies occurs in several other bat species (e. g. *Antrozous pallidus* Brown & Grinnell 1980; *P. auritus*, Coles et al. 1989; de Fanis & Jones 1995; *R. ferrumequinum*, Long & Schnitzler 1975; Matsumura 1979), as well as marsupials (*Dasyurus hallucatus*, Aitkin et al. 1994; *Monodelphis domestica*, Frost & Masterton 1994; Aitkin et al. 1997) and rodents (*Mus musculus*, Ehret 1989). Thus, detection and discrimination of offspring vocalizations may represent an important source of selection on hearing sensitivity in a variety of mammals. These findings indicate that perception of social vocalizations,

particularly those involved in parent-offspring communication, deserves further study.

ACKNOWLEDGEMENTS

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FIGURE CAPTIONS

Figure 1. Schematic of test apparatus (not to scale). During stimulus trials bats crawled to the end of the platform and were presented with a mealworm. During catch trials bats were presented with a mealworm for remaining at the start position. Grey circles indicate the five positions where stimulus levels were recorded

Figure 2. Average of the lowest two thresholds for each of the four bats: (**a)** absolute thresholds, **(b)** critical ratios at 25 dB/Hz. For critical ratios, estimates in decibels are on the left axis and equivalent critical ratio bands in hertz are shown on the right axis

Figure 3. **(a)** Average power spectra of screech (light dashed line), isolation (solid line) and echolocation calls (thick dashed line). **(b)** Average of the lowest absolute thresholds (solid line) and masked thresholds at 25 dB/Hz (dashed line) and 35 dB/Hz (grey circles) for all four bats

Figure 4. **(a)** Spectrogram of screech calls recorded in flight outside of Guanapo cave. **(b)** Spectrogram of isolation calls made by two pups at Guanapo cave. Two of the four call types: double-note and triple-note, are shown for each pup. **(c)** Spectrogram of echolocation calls recorded in a flight room.

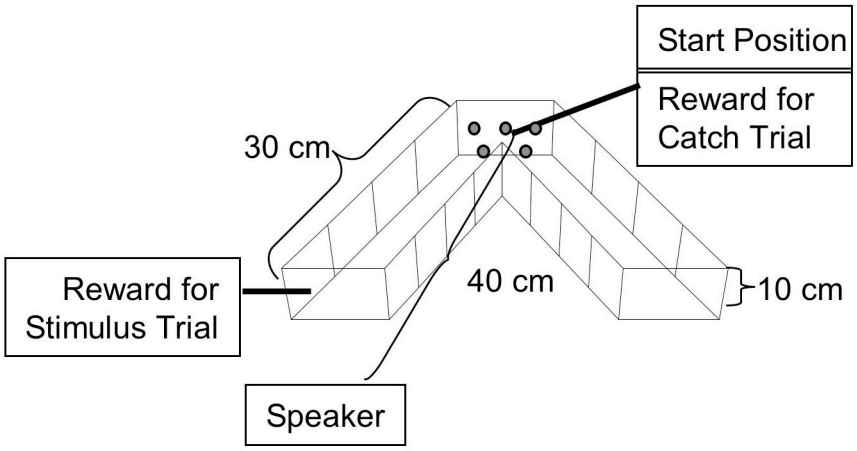

Start Position
Reward for
Catch Trial
30 cm
Reward for
Stimulus Trial
40 cm
10 cm
Speaker

Figure 1

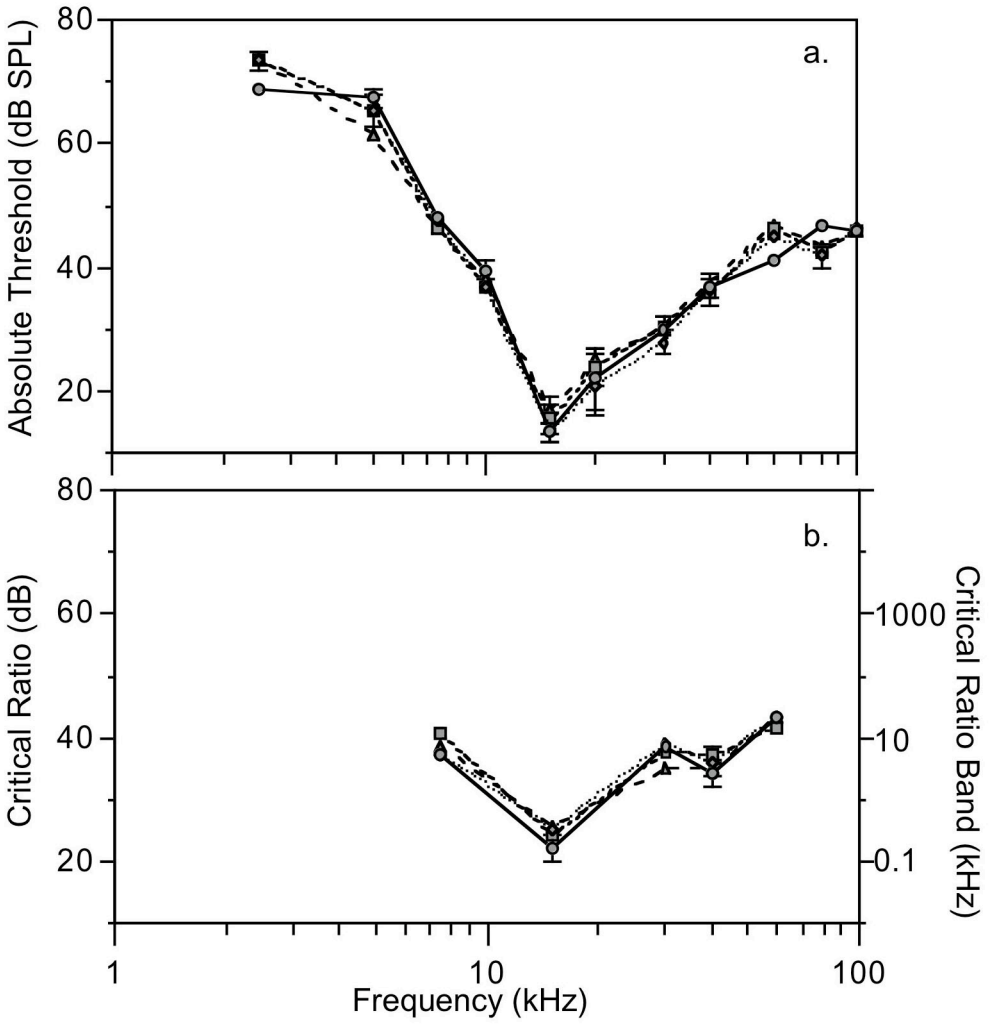

a.
b.
Absolute Threshold (dB SPL)
Critical Ratio (dB)
Critical Ratio Band (kHz)
Frequency (kHz)
80
60
40
20
1000
10
0.1
1
10
100

Figure 2

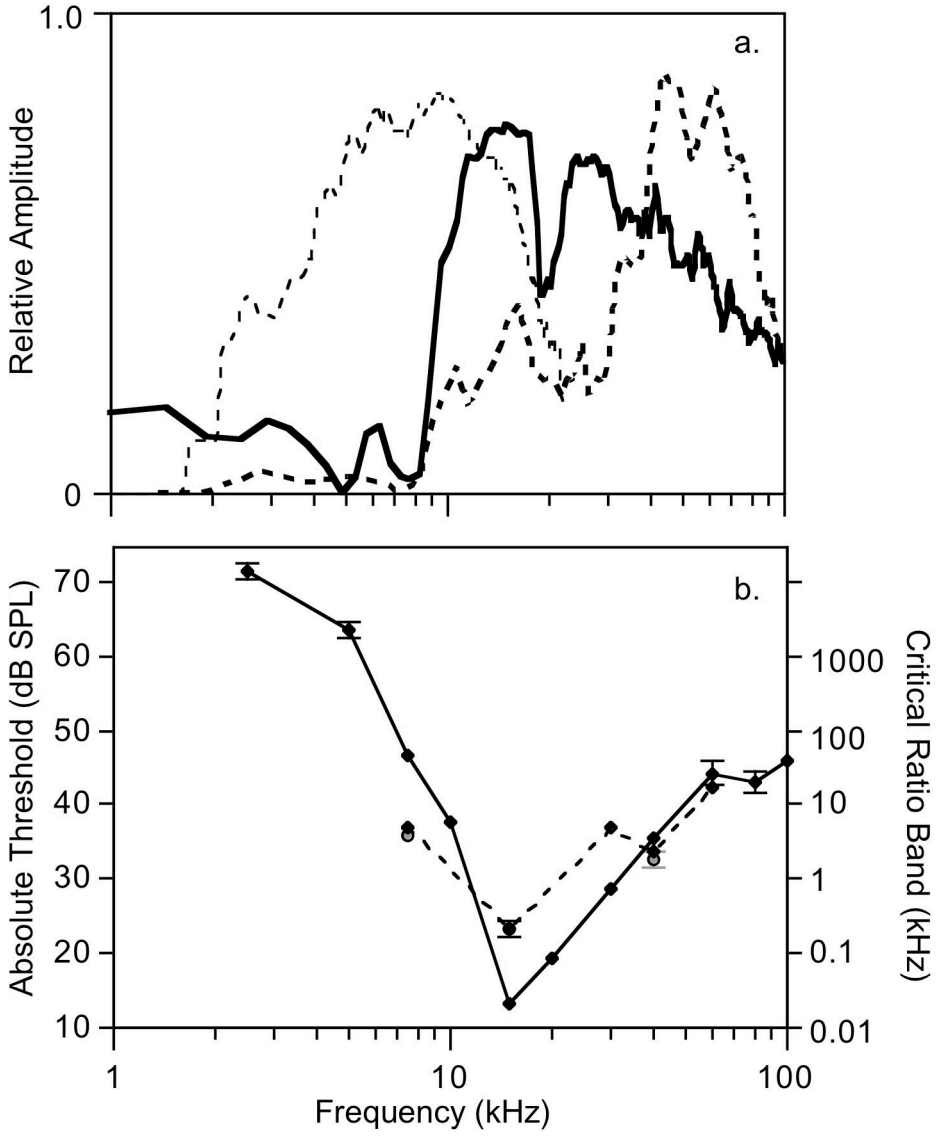

1.0
a.
Relative Amplitude
0
b.
Absolute Threshold (dB SPL)
70
60
50
40
30
20
10
1000
100
10
1
0.1
0.01
Critical Ratio Band (kHz)
1
10
100
Frequency (kHz)

Figure 3

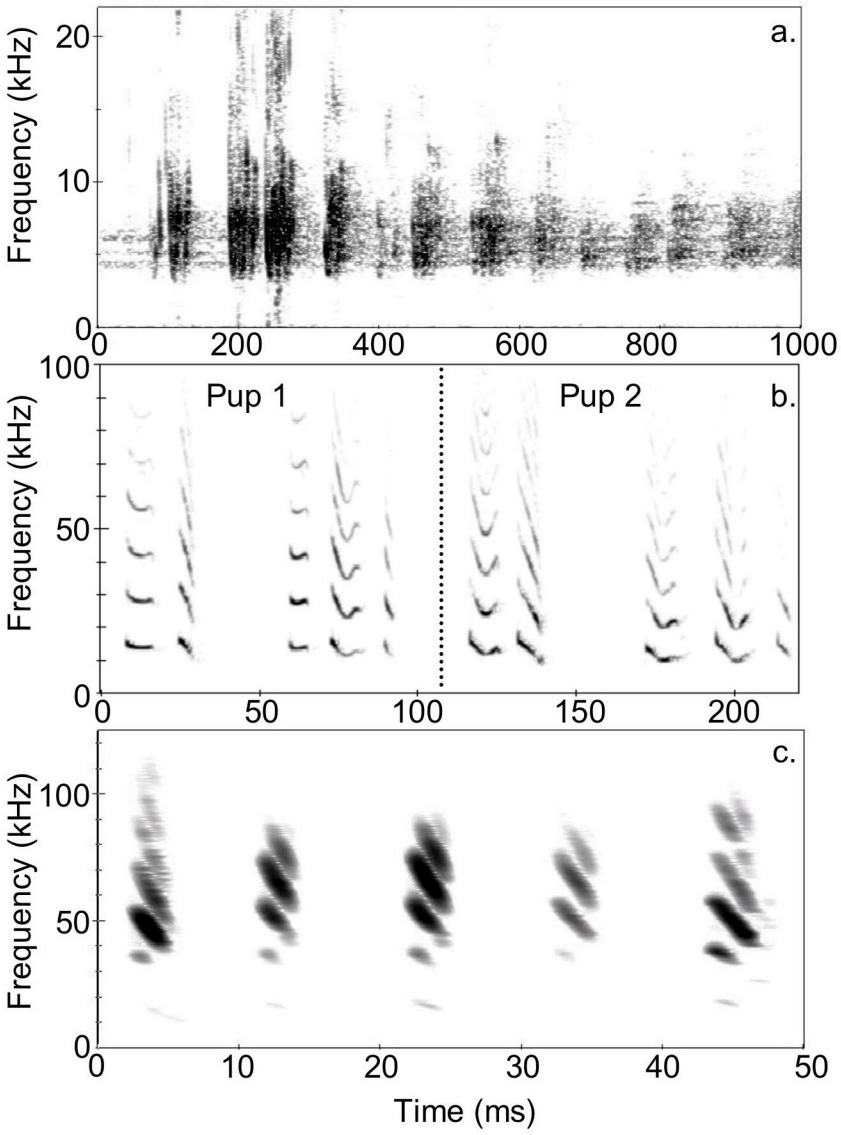

a.
Frequency (kHz)
20
10
0
0
200
400
600
800
1000
b.
Pup 1
Pup 2
Frequency (kHz)
100
50
0
0
50
100
150
200
c.
Frequency (kHz)
100
50
0
0
10
20
30
40
50
Time (ms)

Figure 4

CHAPTER II

Correlated Evolution Between Hearing Sensitivity and Communication Calls in Bats

ABSTRACT

Most bats have two regions of heightened hearing sensitivity. The first occurs at ultrasonic frequencies associated with echolocation calls, whereas the second is at frequencies below those used for echolocation. Although low-frequency hearing may be used for detection of prey in some species, pups of all species of bats emit vocalizations, termed isolation calls, at low frequencies when isolated from their mothers. In this study I tested whether low-frequency hearing exhibits correlated evolution with 1) body size, 2) high frequency hearing sensitivity or 3) isolation call frequency. Using published audiograms for 13 species of bats and a super-tree phylogeny, I found that low-frequency hearing sensitivity is not dependent on body size but is related to high frequency hearing sensitivity. After removing variation associated with high-frequency hearing sensitivity, I found that low frequency hearing sensitivity exhibits correlated evolution with isolation call frequency. Most bats have low reproductive rates,

non-volant altricial young, and must locate pups in roosts after foraging. Thus, detection and discrimination of isolation calls likely has been under strong selection. These results may apply to other species that use vocal signals for parent-offspring communication.

INTRODUCTION

Auditory systems are expected to be under selection to increase detection of signals that affect survival or reproduction. In the absence of physical or physiological constraints, such selection may result in correlated evolution between vocalizations and auditory tuning (Endler 1992; Webster et al. 1992; Bradbury & Vehrencamp 1998). Indeed, correlations between audiograms and vocalization frequency within species have been reported (e.g. Dooling et al. 1971; Brown & Waser 1984). However, only one study on frogs has attempted to incorporate the evolutionary history of multiple species, and in this case no evidence of correlated evolution between call frequency and hearing sensitivity was detected (Wilczynski et al. 2001).

Echolocating bats provide an interesting system for evolutionary study because they have two regions of heightened hearing sensitivity. Hearing in bats exhibits enhanced sensitivity not only to ultrasonic echolocation frequencies (Grinnell 1970; Vater 1987; Neuweiler 1990) but also to a second region below 30 kHz (reviewed in Neuweiler 1990; Moss & Schnitzler 1995). Low-frequency hearing sensitivity has often been attributed to a need for eavesdropping on prey-generated noises (Guppy & Coles 1988; Coles et al. 1989; reviewed in Neuweiler

1990). However, in all species of bats, young emit isolation calls with fundamental frequencies between 13 and 30 kHz (Gould et al. 1973, reviewed in Altringham & Fenton 2003). In at least one species, a correlation between the spectral energy of isolation calls and hearing sensitivity has been reported (Bohn et al. 2004). This result raises the possibility that low-frequency hearing may be the result of selection for detection of offspring rather than prey.

In this study I examine whether selection for detection of isolation calls has resulted in correlated evolution between auditory tuning and isolation call frequency. I also consider two alternative explanations for variation in low-frequency hearing. First, I examine the possibility that variation in body size may cause variation in low frequency hearing. In many species there is an inverse relationship between body size and both hearing (Koay et al. 1997; Heffner & Heffner 1998) and call frequency (Ryan & Brenowitz 1985; Hauser 1993; Jones 1999) due to the physics of sound production and reception. Second, I test whether low-frequency hearing depends on high-frequency hearing as has been observed in other mammals (Koay et al. 1997). If so, and if high-frequency hearing is tightly coupled with echolocation, then differences in low-frequency hearing may simply be due to of selection acting on the echolocation system.

METHODS

I gathered data from the literature for all species of bats where echolocation calls, isolation calls, and audiograms were available (Table 1). As an estimate of body size, I used the median of published forearm measurements.

For estimates of call frequencies, I used the median frequency of the fundamental for isolation calls and the median frequency of echolocation calls. Hearing sensitivity data came from published audiograms (Table 1). I did not use neural audiograms that were recorded from anesthetized bats as these show reduced or no sensitivity to low frequencies when compared with behavioral or neural audiograms from awake animals (Neuweiler 1990; Koay et al. 2002). Most bat audiograms have two regions of increased sensitivity separated by relatively insensitive regions (Fig. 5a). I used the frequency of greatest sensitivity in the two regions of heightened sensitivity. If two adjacent frequencies had the same hearing threshold, I took the midpoint between those two values (e. g. low frequency region, Fig. 5b). Bats that use constant frequency echolocation calls, like *R. ferrumequinum*, have three regions of increased sensitivity. For these species, I used the lowest and highest frequency regions (Fig. 5b). I did not include one species, *Phyllostomus discolor*, because I could not determine a specific value for the low frequency region due to variability among tested animals in the low frequency region (Esser & Daucher 1996).

When comparing traits across taxa, many species share values because of common descent, and therefore are not independent (Felsenstein 1985). Consequently, I used the Comparative Analysis by Independent Contrasts program (CAIC v. 2.0.0, Purvis & Rambaut 1995) to test for correlated evolution of independent contrasts calculated from recent phylogenies. Independent contrasts were generated from $\log_{10}$-transformed values. Relationships were tested using least-square regressions forced through the origin (Harvey & Pagel

1991). I tested whether 1) call or hearing frequencies exhibit correlated evolution with body size, 2) high-frequency hearing shows correlated evolution with echolocation call frequency, 3) low-frequency hearing shows correlated evolution with high-frequency hearing, and 4) low-frequency hearing displays correlated evolution with isolation call frequency. I used call frequencies as independent variables based on the supposition that the requirements of the task associated with a call, particularly echolocation calls, will affect call design (Simmons & Stein 1980; Schnitzler & Kalko 2001; Siemers & Schnitzler 2005), and that hearing sensitivity should then be under selection to adjust to these changes.

To determine whether results depended on the phylogenetic hypothesis used, I performed analyses on three sets of independent contrasts that had different tree topologies or different branch lengths. First, I calculated contrasts using a bat super-tree (Jones et al. 2002) with branch lengths (Jones et al. 2005, Fig 6a). This phylogeny was constructed using over 100 phylogenetic studies and included 900 species of bats. However, the relationships between the four vespertilionid species formed a polytomy in this phylogeny. A more recent molecular phylogeny developed from 2.6 kilobases of mitochondrial DNA resolved these relationships; *N. gouldi* and *E. fuscus* were sister taxa as were *A. pallidus* and *P. auritus* (Hoofer & Bussche 2003). Therefore, for the second analysis I incorporated these relationships into the Jones et al. (2002) phylogeny and set branch lengths equal. Finally, for the third analysis, I incoporated a recent molecular phylogeny (Teeling et al. 2005) with branch lengths from Jones et al. (2005). This topology differed from Jones et al. (2002) in the location of *R.*

aegyptiacus. In Teeling et al. (2005), *R. aegyptiacus* is a sister taxon to the Rhinolophidae species alone, whereas in Jones et al. (2002), it is a sister taxon to all other microchiropterans. I present the results from the phylogeny of Jones et al. (2002) with branch lengths because branch length data permit a more realistic model of evolutionary change (Felsenstein 1985). However, all three analyses gave similar results and I include the range of r^2 values from the other analyses.

RESULTS

Phylogenetic analyses resulted in ten independent contrasts for each comparison (Fig. 6a). Forearm length did not affect echolocation call frequency ($F_{1,9}$ = 1.77, P = 0.22, r^2 = 0.16, range = 0.19-0.26, Fig. 7a), high-frequency hearing sensitivity ($F_{1,9}$ = 0.02, P = 0.90, r^2 = 0.002, range = 0.007-0.01, Fig. 7b), isolation call frequency ($F_{1,9}$ = 0.23, P = 0.64, r^2 = 0.03, range = 0.0002-0.04, Fig. 7c), or low-frequency hearing sensitivity ($F_{1,9}$ = 0.007, P = 0.98, r^2 = 0.0001, range = 0.003-0.01 Fig. 7d). High frequency hearing sensitivity showed correlated evolution with echolocation call frequency ($F_{1,9}$ = 26.57, P = 0.0006, r^2 = 0.75, range = 0.51-0.75, Fig. 6b) and low-frequency hearing sensitivity exhibited correlated evolution with high-frequency hearing sensitivity ($F_{1,9}$ = 5.85, P = 0.04, r^2 = 0.39, range = 0.38-0.41, Fig. 6c). To remove the effects of high-frequency hearing sensitivity on low-frequency hearing sensitivity, I used residuals from that analysis to compare to contrasts in isolation call frequency. I found a significant positive relationship between change in isolation call

frequency and change in residual low-frequency hearing sensitivity ($F_{1,9}$ = 25.83, P = 0.0007, r^2 = 0.74, range = 0.66-0.74, Fig. 6d).

DISCUSSION

This study presents evidence of correlated evolution between auditory tuning and vocalizations at two frequency ranges in echolocating bats. These results did not depend on the phylogenetic topology or branch lengths used. Given that echolocation functions in autocommunication, it is not surprising that aural tuning matches echolocation call frequency, as has been noted before (Grinnell 1963; Grinnell 1970; Long & Schnitzler 1975; Vater 1987; Neuweiler 1990). Selection acting on echolocation also seems to affect low-frequency hearing. However, after removing these effects, there appears to be sufficient evolutionary flexibility in the auditory system to respond selectively to isolation calls.

Although these results suggest specializations for parent-offspring communication, in some species detection of prey also likely contributes to low-frequency hearing. For example, two gleaners that are known to use prey-generated sounds, *P. auritus* (Coles et al. 1989; Anderson & Racey 1991) and *N. gouldi* (Guppy & Coles 1988; Grant 1991) are sensitive to frequencies that are lower than their isolation calls and than other microchiropteran bats in this study (Table 1). In contrast, in frugivorous species, such as *C. perspicillata* and *A.*

jamaicensis, prey-detection should not influence selection for low-frequency hearing.

Correlated evolution between auditory tuning and infant isolation calls might be expected simply to maintain signal production. However, bats only emit isolation calls while they are young and hearing sensitivity changes during the course of development so that adult hearing does not always match that of young bats (Brown et al. 1978; Rubsamen et al. 1989; Sterbing 2002). In some species, pups begin vocalizing before they can even hear (Brown et al. 1978). Furthermore, isolation calls likely have a strong genetic component (Scherrer & Wilkinson 1993). Thus, auditory feedback may not be as crucial to the production of isolation calls as in other vocal communication systems, such as bird song (Marler & Sherman 1985).

Female bats give birth to nonvolant altricial young that are left in roosts while their mothers forage. As a result, females must frequently locate, and for group living species, recognize their offspring among others. Echolocation calls would not function well in this context because ultrasonic frequencies have very short transmission distances (Griffin 1971; Lawrence & Simmons 1982). Because most bats have low reproductive rates, infant survival should have a large impact on adult fitness (Barclay & Harder 2003). Correlated evolution between hearing and isolation calls likely reflects strong selection for detection of young. Although here we have focused on echolocating bats, young emit vocalizations that are used in parent-offspring communication in many mammals (e.g. seals, Trillmich 1981; dolphins, Smolker et al. 1993, primates Symmes & Biben 1985; pigs,

Illmann et al. 2002, rodents, Branchi et al. 2001). In some species hearing sensitivity also corresponds with the frequency of infant vocalizations (marsupials, Aitkin et al. 1994; Frost & Masterton 1994; Aitkin et al. 1997; rodents, *Mus musculus*, Ehret 1989). Thus, selection for detection of young may have a significant influence on the evolution of auditory tuning in many species.

ACKNOWLEDGEMENTS

I wish to thank U. Schmidt for sharing *Desmodus rotundus* vocalizations, J. J. Wenstrup for sharing *Nocitilio leporinus* vocalizations as well as audiogram data, G. Schuller for sharing *Rhinolophus rouxi* and *Hipposideros speoris* audiogram data and G. Koay for sharing *Carollia perspicillata* and *Eptesicus fuscus* data. Thanks to K. E. Jones who provided branch-length data for phylogenetic analyses.

Table 1. Echolocation call frequency, high frequency hearing sensitivity, isolation call frequency, and low frequency hearing sensitivity values for the thirteen species used in analyses. Number column refers to species in Figure 6.

number	species	echolocation call	high hearing	isolation call	low hearing	sources[a]
1	*Rousettus aegyptiacus*	41	45	14	10	LH, HH: Koay et al. 1998 IC: Herbert 1983 EC: Herbert 1985; Holland et al. 2004
2	*Rhinolophus ferrumequinum*	83	81.5	16	17.5	LH, HH, EC: Long & Schnitzler 1975 IC: Matsumura 1979
3	*Rhinolophus rouxi*	86	87	22.5	25	LH, HH, EC: Schuller 1980 IC: Rubsamen 1987
4	*Hipposideros speoris*	132	130	30	30	LH, HH: Schuller 1980 IC, EC: Habersetzer & Marimuthu 1986

number	species	echolocation call	high hearing	isolation call	low hearing	sources[a]
5	*Phyllostomus hastatus*	62.5	64	15	15	LH, IC, EC:Bohn et al. 2004 HH: Koay et al. 2002
6	*Artibeus jamaicensis*	55.5	56	18	16	LH, HH: Heffner et al. 2003 IC: Gould 1977 EC: Jennings et al. 2004
7	*Carollia perspicillata*	80	71	28	25	LH, HH: Koay et al. 2003 IC, EC: Sterbing 2002
8	*Desmodus rotundus*	72.5	74	13	15	LH, HH: Schmidt et al. 1991 IC, EC: U. Schmidt[1]
9	*Noctilio leporinus*	43.5	58	25	24	LH, HH: Wenstrup 1984 IC, EC: J. J. Wenstrup[1]

number	species	echolocation call	high hearing	isolation call	low hearing	sources[a]
10	*Eptesicus fuscus*	52.5	64	18	20	LH, HH: Koay et al. 1997 IC, EC: C. F. Moss[1]
11	*Nyctophilus gouldi*	47.5	33.5	16	11	LH, HH, IC, EC: Guppy & Coles 1988
12	*Antrozous pallidus*	53.5	40	15	15	LH, HH, EC: Brown et al. 1978; Brown & Grinnell 1979 IC: Brown 1976
13	*Plecotus auritus*	52	50	16.5	12	LH, HH, EC: Coles et al. 1989 IC, EC: de Fanis & Jones 1995

[a]Abbreviations are: HH= high hearing, LH = low hearing, IC = isolation call and EC = echolocation call.

[1] Provided recordings of calls that we measured

FIGURE CAPTIONS

Figure 5. Audiograms for two species of bats that show minimum sound pressure levels (SPL) that elicit behavioral responses. **(a)** *Eptesicus fuscus* from Koay et al. 1997. **(b)** *Rhinolophus ferrumequinum* from Long & Schnitzler 1975. Black arrows show low-frequency hearing sensitivity values and gray arrows show high-frequency hearing sensitivity values.

Figure 6. **(a)** Phylogenetic relationships of species used in the analysis based on Jones et al. 2002. Numbers correspond to species in Table 1. Independent contrasts were calculated for the nodes designated by black squares. Branch lengths are not drawn to scale. Relationships between **(b)** high-frequency hearing and echolocation call contrasts, **(c)** low-frequency hearing and high-frequency hearing contrasts, and **(d)** residual low-frequency hearing and isolation call contrasts.

Figure 7. Relationships between **(a)** echolocation call and forearm length contrasts, **(b)** high-frequency hearing and forearm length contrasts, **(c)** isolation call frequency and forearm contrasts and **(d)** low-frequency hearing and forearm contrasts.

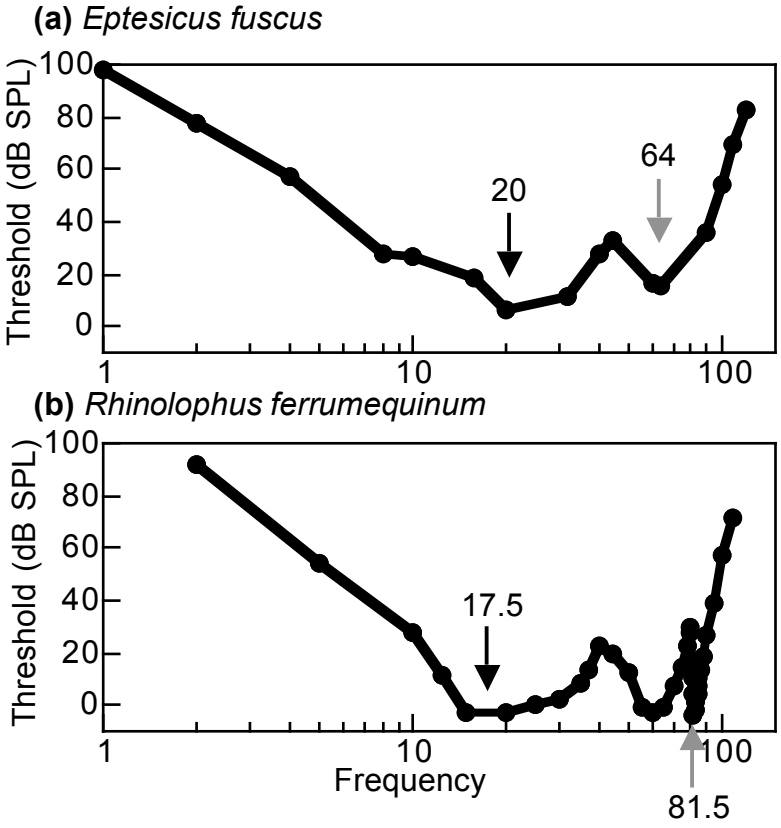
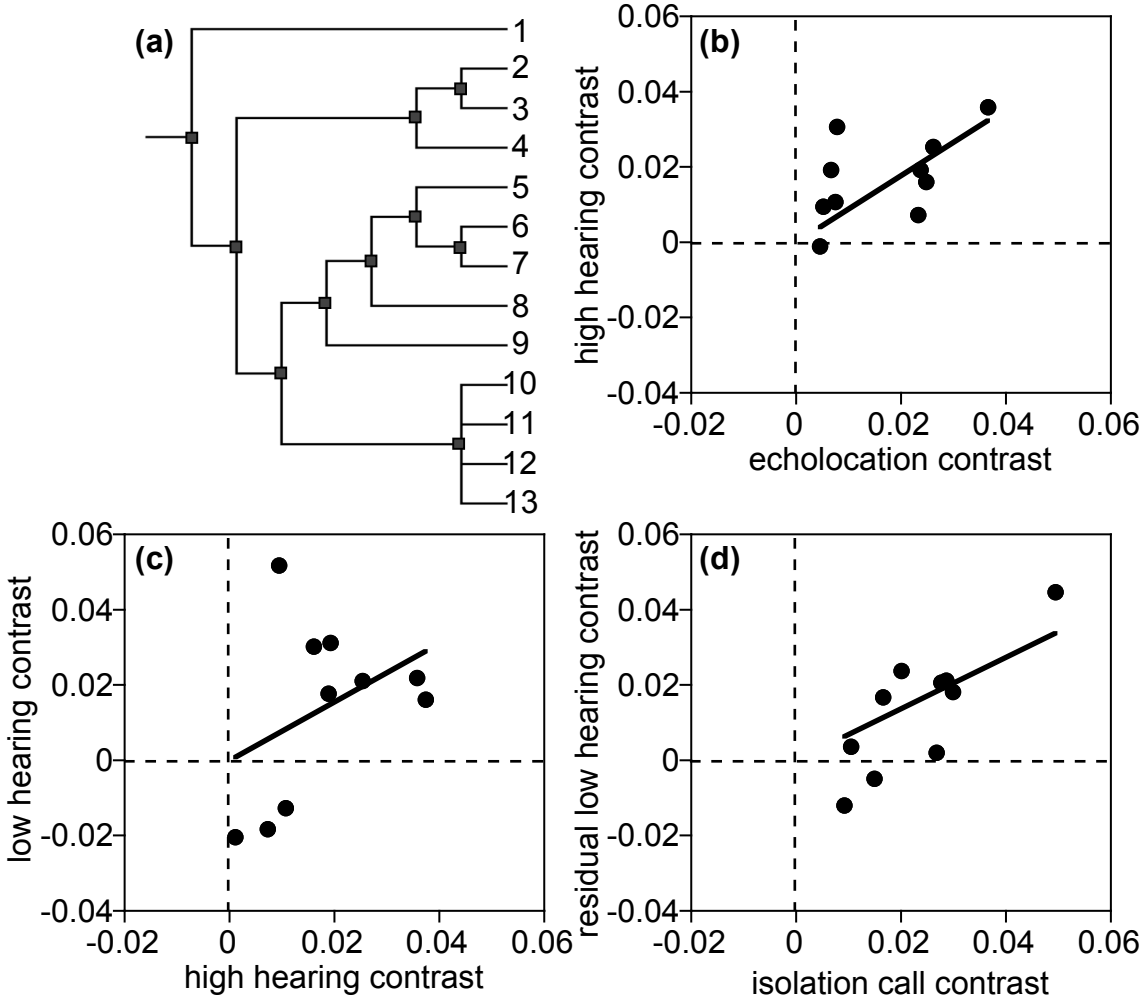

(a) Eptesicus fuscus
Threshold (dB SPL)
100
80
60
40
20
0
1
10
100
20
64
(b) Rhinolophus ferrumequinum
Threshold (dB SPL)
100
80
60
40
20
0
1
10
100
Frequency
17.5
81.5

Figure 5

.


(a)
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2
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11
12
13
(b)
high hearing contrast
echolocation contrast
0.06
0.04
0.02
0
-0.02
-0.04
-0.02
0
0.02
0.04
0.06
(c)
low hearing contrast
high hearing contrast
(d)
residual low hearing contrast
isolation call contrast

Figure 6

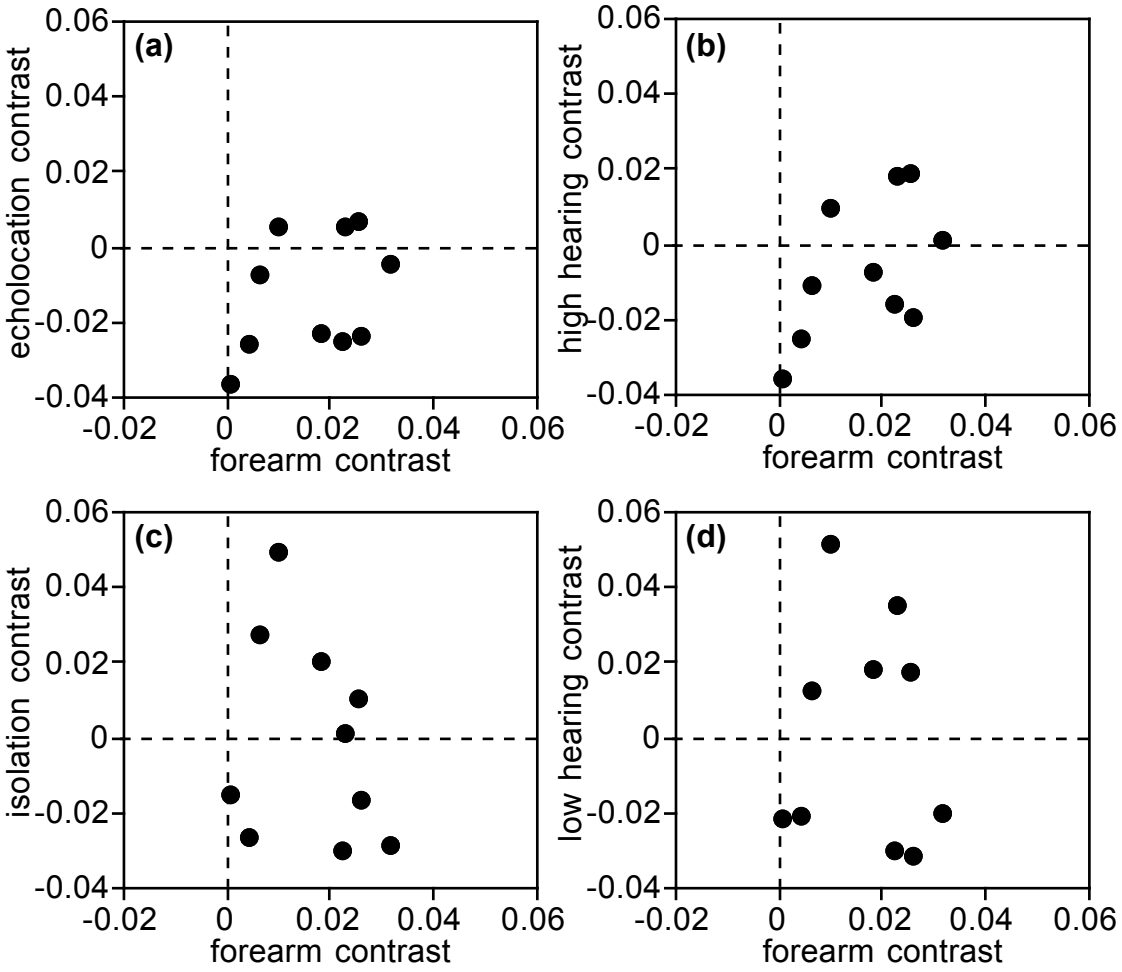
(a)
(b)
(c)
(d)
echolocation contrast
high hearing contrast
isolation contrast
low hearing contrast
forearm contrast
0.06
0.04
0.02
0
-0.02
-0.04
-0.02
0
0.02
0.04
0.06

Figure 7

CHAPTER III

Discrimination of Infant Isolation Calls by Female Greater Spear-Nosed Bats, *Phyllostomus hastatus*

ABSTRACT

In colonial species, recognition of offspring should be under strong selection. For accurate identification to occur offspring must emit individually distinctive signals and parents must be able discriminate between signals. Female greater spear-nosed bats roost in stable social groups and use infant vocalizations, termed isolation calls, to locate and identify their young. In this study, I investigate both the production and perception of infant isolation calls. I measured acoustic features of isolation calls and found that sufficient differences exist between pups for these calls to function as individual signatures. However, isolation calls have more similar spectral and spectro-temporal features when pups are from the same social group. I used psychoacoustic experiments in the laboratory to determine if adult female greater spear-nosed bats could discriminate between calls from pups in the same or different social group. I found that females discriminated between pups when faced with a template-

matching task and their performance correlated with spectral and spectro-temporal cues. I found no difference in performance when females had to discriminate between pups from the same and different social groups. These results indicate that females should be able to use isolation calls to accurately identify young.

INTRODUCTION

The process of identifying offspring is expected to be under strong selection to insure that parental care is confined to related individuals. For animals living in large groups the probability of confusing related offspring with others can be high. Consequently, parent-offspring recognition systems have evolved in many colonial species (e. g.Trillmich 1981; Stoddard & Beecher 1983). In colony forming bats, mothers typically leave their pups behind at night to forage, which makes offspring recognition a particularly vital but difficult task. Accurate offspring recognition requires fulfilment of two criteria: 1) offspring must emit individually distinctive signals and 2) parents must discriminate between these signals (Beecher 1982).

Infant bats produce frequency-modulated multi-harmonic vocalizations known as isolation calls (Gould et al. 1973). In some species isolation calls contain enough information to serve as individual signatures (e.g. Thomson et al. 1985; Gelfand & McCracken 1986; Scherrer & Wilkinson 1993; de Fanis & Jones 1995). Because pups can be left in crèches immediately after birth, the time

available for a female to learn her offspring's signature is short. Not surprisingly, at least in some species acoustic features of isolation calls are heritable (Scherrer & Wilkinson 1993), although they may also change in response to social cues as pups age (Esser 1994).

Psychoacoustic studies indicate that bats should be able to discriminate between isolation calls. For example, adult female *Phyllostomus discolor* can discriminate among frequency-modulated sounds similar to isolation calls (Esser & Kiefer 1996; Esser & Lud 1997). Other studies have demonstrated maternal recognition of pup calls (Rother & Schmidt 1985; Balcombe 1990; Rasmuson & Barclay 1992 de Fanis & Jones 1995; de Fanis & Jones 1996). However, no study has yet determined the acoustic features used by mothers to recognize their pup's calls.

Signal recognition is believed to occur through template matching (Lacy & Sherman 1983), a process by which a model or template is represented in the memory of the receiver and incoming signals are then compared with this template. By this process, offspring recognition should depend on a parent's ability to form a template and discriminate among offspring signals. Ideally, parents could use all of the information contained in offspring signals, discriminate among signatures, and then respond selectively to their own offspring. In contrast, low discrimination ability relative to signal similarity would cause high perceptual overlap and result in increased error by parents. In this study I examine both the variability in infant isolation calls and the perception of isolation calls by adult female greater spear-nosed bats, *Phyllostomus hastatus*.

In Trinidad, West Indies, *P. hastatus* form stable social groups of eight to 40 adult females attended by one adult male (McCracken & Bradbury 1981). Unlike most other group-living mammals, females are typically unrelated to group members (McCracken & Bradbury 1981; McCracken and Wilkinson 1987). Males have high reproductive control over harems (McCracken & Bradbury 1977) and socially mediated birth synchrony occurs within groups (Porter & Wilkinson 2001). Consequently, crèches of pups contain paternal half-siblings of similar age from a single social group. Previous studies indicate that isolation calls in *P. hastatus* possess individually distinctive acoustic features (Lill, unpub. thesis). However, they also exhibit some differences between social groups and with pup age (Lill, unpub. thesis). Thus, discrimination of pups within social groups is potentially difficult because pups within a group emit calls that are more similar than pups from different social groups. Furthermore, *P. hastatus* females sometimes visit and retrieve group members' fallen pups (Chapter Four). These observations raise the possibility that females are sometimes unable to recognize their own pups from others in their social group.

In this chapter I examine both components of the parent-offspring signature system in *P. hastatus*. First, I investigate signal production. I examine how isolation calls change as pups age and then control for age effects and examine how calls vary in spectral and temporal features between caves, groups and pups. Second, I use psychoacoustic experiments in the laboratory to test whether females can discriminate between pups' isolation calls in a template

matching procedure and identify the acoustic features they use for this task. Finally, I examine whether group–level similarity affects female discrimination.

METHODS

Isolation Call Recordings

I recorded isolation calls from infant *P. hastatus* at Guanapo and Tamana caves in Trinidad, West Indies, in April and May, 2002 and 2004. Non-volant pups were removed from caves in the evening while adults foraged. Pups were measured, sexed and banded with numbered stainless steel bands (National Band and Tag). Infants emitted isolation calls spontaneously when placed in a cardboard box (approximately 0.75 by 0.5 by 0.5 meters) lined with acoustic foam (Sonex). Isolation calls were recorded at a sample rate of 250 kHz into Bat Sound Pro (Pettersson Electronik) on a laptop computer equipped with a high-speed data acquisition card (INEES, Daq508, 12 bits), using a bat detector that functioned as a high frequency microphone (Ultra Sound Advice, S-25) and an external amplifier (SHURE, FP-2). I used calipers to measure the forearm length (FA) of each pup to a tenth of a millimeter. I used forearm measurements to estimate age, i.e. age = 0.77 x (forearm length) – 24.6, where age is in days and forearm length is in millimeters (Stern & Kunz 1998). This equation explained 96% of the variation in age for pups up to 35 days of age (Stern & Kunz 1998). I recorded isolation calls from 68 pups in 2002 and 82 pups in 2004.

Infant *P. hastatus* emit multiple types of isolation calls composed of different numbers of notes. Double-note calls are the simplest and most frequently emitted calls in *P. hastatus* and in many other species of bats (Gould et al. 1973). For both simplicity and consistency, I used double-note calls for all call analyses and both psychoacoustic experiments (see below). I used SIGNAL (version 3.0, Engineering Design), to band-pass filter isolation calls at 5 and 85 kHz and normalize amplitudes by dividing each signal by its peak amplitude.

To construct stimulus trains that would resemble unmanipulated pup calls, I calculated the average number of double-note calls in a calling bout and the average interval between calls within a bout for 30 pups. Bouts were separated by a minimum of 500 ms, whereas intervals between calls within bouts were much shorter (average = 57 ms, range 27.3 to 78.8 ms). Pups emitted, on average, four calls per bout (range 2 to 8 calls).

Isolation Call Measurements

I used SIGNAL to measure isolation call features. I measured three temporal features: the duration of the first and second notes (D1 and D2, respectively) and the interval between notes (INT). To examine spectral variation, for each note I measured the beginning (BF1, BF2), end (EF1, EF2), average (AVGF1, AVGF2), minimum (MNF1, MNF2), maximum (MXF1, MXF2) and peak frequencies (PKF1, PKF2) of the fundamental (Fig. 8). AVGF1 and AVGF2 were calculated by taking averages of spectral contours of the fundamental of each note. Spectral contours were calculated by determining the peak frequency at each point in time of the call (Beeman 1996). I measured one spectro-temporal

feature, the relative location of the frequency minimum of the first note (MNT1), using the formula: (end time – time of minimum frequency)/end time. MNT1 ranged from zero to one where zero represented a minimum at the end of the call and one represented a minimum at the beginning of the call. In addition to the above measurements, for perceptual experiments, I used SIGNAL to calculate spectral cross-correlations between call pairs for each note (COR1, COR2). The spectral cross-correlation procedure slides two spectral contours across each other and calculates the maximum correlation between the two signals (Beeman 1996). Spectrograms were constructed using a transform length of 512 points, resulting in a temporal resolution of 2 ms and frequency resolution of 500 Hz. The time step size or time between transforms was set at 0.15 ms, so that all signals had the same number of transformations per second but different total number of transforms.

Subjects

The experimental animals came from groups captured in Tamana Cave, Trinidad, West Indies in 1993, except for one bat that was born in captivity in 1996. During the study, bats were housed in a cage (3.3 x 2.7 x 2.4 m) kept in a room maintained on an 8L:16D cycle at approximately 24° C and 30% humidity. Bats weights were kept between 60 and 70 g during experiments (minimum of 90% free-fed body weight) by feeding them a diet of fruit and marmoset food (Premium Nutritional Products). During experiments bats were rewarded with mealworms and fruit.

Psychoacoustic Apparatus and Procedures

All experiments were conducted in a single-wall acoustic chamber (Industrial Acoustics Company, Inc) lined with acoustic foam (Sonex). Bats were trained and tested using a V-shaped platform enclosed in a hardware-cloth cage (Fig. 9). A modified go/no-go procedure was used for both experiments (Suthers & Summers 1980). During experimental trials, bats were trained to either a) stay at the top of the platform ("no-go" trial) or b) run to the end of the right arm of the platform ("go" trial). Bats were rewarded at the starting position for correctly staying during "no-go" trials and rewarded at the end of the right arm of the platform for correctly responding during "go" trials.

Response latency, or the time it took for the bats to respond, was determined for all trials. An infrared light-emitting diode (LED) and matching photosensor were positioned at the top of the platform and triggered whenever the bat left the starting position (Fig. 9). Bat departure times were coordinated with stimulus trains using a real-time processor (Tucker Davis Technologies, RP 2.1).

Playback Stimuli and Calibration

Isolation calls were played directly from a computer equipped with SIGNAL and a 250 kHz DA board (Data Translation, DT5727), band-pass filtered at 5 and 85 kHz (Krohn-Hite, 3550), amplified (Harman Kardan, AVR 100), and sent to a speaker (Pioneer, PT-R) that was located 1 m from the subjects' starting position. Stimuli were recorded daily onto a laptop computer equipped

with a 12-bit high-speed data acquisition card (INEES, Daq508), using a one-eighth inch microphone (Brüel & Kjær, type 4138) connected to a preamplifier (Larson Davis, 2200C). Time waveforms and power spectra of stimuli were inspected daily for any distortions using Bat Sound Pro. I also recorded a calibration tone daily with a piston phone (Brüel and Kjaer type 4231) and adjusted amplitudes so that all stimuli were presented at 75 dB SPL. For both psychoacoustic experiments, stimuli consisted of various numbers of call sets or bouts and 900 ms of silence between sets. Each call set was comprised of three isolation calls separated by 60 ms (Fig. 10 and Fig. 11) to match natural calling behaviour.

Experiment 1: Pup Discrimination

The goal of this experiment was to determine whether bats could discriminate between isolation calls emitted by different pups and if so, the acoustic features used by females for discrimination. Only pups recorded in 2002 were used in this experiment. Sixteen pup pairs were selected at random without replacement with the requirement that the difference in age between pups was no greater than 2 days for each pair. I imitated the recognition task females encounter in their natural environment by having subjects associate one pup's call with "go" trials and a different pup's call with "no-go" trials (Fig. 10). Females not only had to perceive a difference between calls, but they also had to store calls in memory in order to know which call was associated with which behaviour. For each pup pair, one pup was arbitrarily assigned as a "no-go" pup and the other was a "go" pup. Each trial stimulus consisted of five call sets (Fig. 10). Two

isolation calls were used for each pup and presented in random order. Before testing, bats were trained using the same procedures on pairs of highly distinctive pups. I selected two pairs of pups that differed by at least 10-days of age and whose calls were the most different in temporal, spectral and spectro-temporal features. Pup calls used for training were not used in experiments. Testing began when bats performed at or above 75% response accuracy for more than 3 days for each of the two pairs.

Each testing day consisted of 30 trials per subject. Trials were randomized with the restriction that no more than three trials of one type could occur consecutively (Gellerman 1933). For the first five trials of each day, I presented mealworms at the end of the platform ("go" pup) or at the top of the platform ("no-go" pup) during stimulus trains, to show the subjects the correct responses. For the remaining 25 trials bats were only rewarded at the end of trials if they performed correctly. Responses and response latencies were recorded for these last 25 trials. Each pair was tested for 2 days with 1 day of no testing between pairs. Of the 2-day testing period per pair, the day with the best performance (the highest percent correct) for each bat was used for analyses. To assess bat performance, I calculated the number of correct responses and the average response latency for each pair for each of the five bats. Equipment malfunction resulted in the loss of five response latency estimates.

Experiment 2: Group Discrimination

The goal of this experiment was to determine whether social group membership affected the ability of adult females to discriminate between pups'

isolation calls. For this experiment I used a modified Alternating Sound Task (Dooling & Okanoya 1995). During "go" trials bats were trained to stay at the starting position while call sets from one pup were played (the background) and move to the end of the ramp when call sets from a different pup (the target) were alternated with the background (Fig. 11). For training I used two pairs of pups that were from different social groups and of different ages. Calls used for training were not used in either psychoacoustic experiment. Unlike experiment 1, for this task the bats did not need to remember each call, they only needed to perceive a difference in the isolation calls to respond correctly. Using this procedure all stimuli could be presented daily, which controlled for day-to-day variation in the subjects' behaviour.

For this experiment a block design was used to increase power. I used 24 "go" stimuli, which consisted of two sets of twelve pairs. Calls from twelve different pups served as background and were each paired with calls from two different target pups: one pup from the same group as the background pup and one pup from a different group than the background pup. Pairs were selected at random without replacement as long as the difference in ages of the background and target pups was no greater than 2 days. I incorporated within pup variation in double note calls by randomly combining four to five calls from each pup into the stimulus sets. New random combinations were constructed for each day of testing.

Each trial consisted of three, four, or five call sets from the background pup, then two call sets from the target pup, and finally one call set from the

background pup (Fig. 11). As in experiment 1, calls were separated by 60 ms and call sets were separated by 900 ms. This procedure resulted in three repetition levels (3, 4 or 5) for each of the 24 “go” stimuli. Bats were rewarded immediately upon going, as long as they left after the first target call and prior to 500 ms after the last call of the trial. During "no-go" trials, bats were played six, seven or eight background call sets, again resulting in three repetition levels. Bats were rewarded at the starting position for staying until 500 ms after the last call. If the bat made an incorrect response, the trial ended immediately, and a 20-second “time out” was given during which no new stimuli were presented. If a bat did not respond during a "go" trial, the trial ended but no time out was given.

Bats were tested on all 24 "go" pairs and twelve "no-go" pairs on each of 9 days. All pairs were presented nine times, three times at each repetition level. The number of call sets was randomized across days. “No-go” and “go” trials were randomized within days, with a rule that not more than five trials of one type could occur consecutively. If bats left too early during a “go” trial, that stimulus was added to the end of the day’s trials to insure that a clear response was recorded for each day. If bats responded during “no-go” trials greater than 50% of the time or did not respond during “go” trials greater than 50% of the time, those days’ stimuli were rerun on a different day. For response latencies, one data point was missing because one bat did not respond correctly during any of the three trials at that repetition level.

Statistical Analyses

Variables were examined for normality using normal-probability plots and Shapiro-Wilk's tests. Any variable deviating from normality was transformed to satisfy assumptions for parametric tests (see Appendix for summary of transformations used). Statistical significance was evaluated using two-tailed tests with $\alpha = 0.05$. Analyses were performed in either JMP 5.0 or SAS 9.1 (SAS Institute Inc).

For analysis of isolation call variation, I used a subset of calls recorded in 2002 and 2004. Pups were included in the analysis if at least four double-note calls with high signal-to-noise ratio were recorded and if at least four pups from a social group were available. In total, I used 309 double-note calls from 63 pups in eight social groups. Pairwise correlation coefficients between average call measurements from each pup revealed a high degree of redundancy between beginning and end frequency of each note (BF1 and BF2; EF1 and EF2) and maximum and minimum frequency (MXF1 and MXF2; MNF1 and MNF2) respectively ($r > 0.98$, $N = 63$). Therefore, BF1, EF1, BF2 and EF2 were removed from all subsequent analyses.

First I used the average of each measurement for each pup and tested whether age, sex, or an age by sex interaction affected isolation call features using a multivariate analysis of variance (MANOVA). To reduce the number of variables and control for colinearity, I used a principal components analysis (PCA) and varimax factor rotation on the residuals of age on call measurements. Factors with eigenvalues greater than 1.0 were used in a MANOVA with cave,

group, and pup as nested random effects (PROC GLM, SAS Institute Inc.). I used restricted maximum likelihood to estimate the proportion of variation in the PCA factors explained at each nesting level.

For experiment 1, I calculated the average daily percent correct for each bat, compared these values with confidence intervals for "guessing" (50%, n = 25 trials per day), and conducted binomial tests on the pooled responses to all 400 trials. For each call feature, I calculated the absolute difference between call pairs. PCA and varimax factor rotations were used to reduce the number of independent variables associated with differences in call features. To test if call features correlated with discrimination ability, I used a logistic regression (PROC GLIMMIX, SAS) to examine response outcome (correct or incorrect) and an ANCOVA to analyze response latency. For both analyses a full model was tested that included bat as a random factor, order of pair presentation as a repeated measure, extracted factors as predictors and all bat by factor interactions. Repeated measures analysis was used to determine if responses to call pairs changed over time. The repeated measures variance components were zero, and all interaction effects were non-significant. Thus, these terms were removed from the final model for testing extracted factors.

For experiment 2, I used responses to all trials to calculate the percentage of correct responses for each bat. In order to assess performance, I calculated the probability of subjects responding correctly by chance. Unlike experiment 1 the probability of responding correctly was not 50%. In order to respond correctly bats not only had to "go" or "not go" but also had to depart

within acceptable time windows, which depended on the number of repetitions. For each of the possible time windows, I calculated the proportion of trials that would result in a correct response if the bat departed. These calculations resulted in a chance probability of 45% correct.

The "no-go" trials served to keep bats from giving "go" responses for all trials and to assess the frequency with which bats responded with a "go" even though they did not detect a difference (a false alarm response). Because there was only one "no-go" stimulus for both the within social group and between social group pairs, I only used "go" trials for testing whether group membership affected discrimination. For “go” trials, I counted the number of correct responses and the average response latency for each bat-stimulus-repetition combination. I then used a logistic regression (PROC GLIMMIX, SAS) on response outcome and an ANOVA on response latency. For both variables, a full model was first analyzed that included pup pair as a block, number of background repetitions, group (same or different), and number of background repetitions by group interaction as fixed effects, and bat and bat by group interaction as random effects. However, for both variables, interaction terms were non-significant and consequently removed from the final analysis.

RESULTS

Isolation Call Variation

Pup age had a significant effect on call features (MANOVA, Wilk's Lambda = 0.477, *df* = 12, 47, *P* < 0.0001). Call features were not affected by sex and there was no interaction between sex and age (Wilk's Lambda = 0.894 and 0.897 respectively, *df* = 12, 47, *P* = 0.92 and 0.93 respectively). Age had a negative effect on D1 and D2 and a positive effect on PKF1, PKF2, and MXF2 (Table 2). During ontogeny isolation calls become shorter and increase in frequency (Fig. 12).

Residuals from regressions of age on call features were used for the remaining analyses on isolation call variation. Factor analysis produced four factors that together explained 81% of the variation in the twelve call measurements. Spectral features loaded into the first factor, D2 loaded into the second factor, MNT1 and INT loaded into the third factor and D1 loaded into the fourth factor (Table 3). There was significant variation among pups (Wilk's Lambda = 0.01, *df* = 220, 973, *P* < 0.0001) and social groups (Wilk's Lambda = 0.49, *df* = 24, 183, *P* = 0.02). However, cave did not have a significant effect on the extracted factors (Wilk's Lambda = 0.92, *df* = 4, 3, *P* = 0.99). While all factors contributed to differences among pups, only the first factor (spectral features) and third factor (spectro-temporal features) contributed to differences among social groups (Table 4).

Experiment 1: Pup Discrimination

Bat performance averaged between 78% and 83% correct responses per day, well above the 95% confidence interval for guessing (Fig. 13). Across all pairs of call presentations, all bats performed above 50% (binomial tests, N = 400, all $P < 0.0001$). For each acoustic variable the absolute value of the difference between the two calls of each pair was calculated. A PCA on these differences resulted in four rotated factors that explained 79% of the variation. The first factor was correlated with differences in spectral characteristics of both notes (Table 5). The difference in MNF1, AVGF1 and COR1 loaded highly into the second factor. The third factor was associated with two temporal features (D2 and INT) and a spectro-temporal variable (COR2). Finally, the fourth factor corresponded with the spectro-temporal feature MNT1.

There were no interaction effects between bats and the four extracted factors for either correct responses (logistic regression: $\chi_4^2 \leq 6.25$, $P>0.18$) or response time (ANCOVA: $F_{4, 51} \leq 1.79$, $P>0.14$) and these terms were removed from the analyses. Bats did not vary in their overall number of correct responses (logistic regression:$\chi_4^2=6.95$, $P=0.14$). Only the second factor predicted whether or not bats responded correctly (Table 6). Response latency did differ between bats (ANCOVA: $F_{4,67}=3.22$, $P=0.02$). Only the first factor had a significant effect on response latency (Table 6). Thus, spectral and spectro-temporal features affected female ability to discriminate between isolation calls.

Experiment 2: Group Discrimination

The four bats' performance averaged between 79% and 80% correct responses per day, well above guessing (45%, Fig. 14). Performance across all trials was greater than 45% (binomial test, $N = 324$, all $P < 0.0001$). Both the highest false alarm rates (responding early or during a "no-go" trial) and the highest miss rates (not responding during a "go" trial) were under 15%. There were no interaction effects between the number of background repetitions and social group (logistic regression: $\chi_2^2 = 0.69$, $P = 0.71$) or bat and social group ($\chi_3^2 = 1.41$, $P = 0.70$) on correct responses so these two terms were removed from the model. Correct responses differed across bats ($\chi_3^2 = 13.73$, $P = 0.003$) and number of background repetitions ($\chi_2^2 = 12.76$, $P = 0.002$). However, there was no difference in the number of "go" responses between pups from the same group and pups from a different group ($\chi_1^2 = 1.37$, $P = 0.24$, Fig. 15a).

There were no interaction effects between the number of background repetitions and social group (ANOVA: $F_{2, 264} = 1.26$, $P = 0.29$) or between bat and social group ($F_{3, 264} = 1.38$, $P = 0.25$) on response latency. With these interaction terms removed, response latency differed between bats ($F_{3,269} = 7.79$, $P < 0.0001$), and between the number of background repetitions ($F_{2, 269} = 5.72$, $P = 0.004$). I did not detect a difference in response latency between pups from the same and different social groups ($F_{1, 269} = 3.31$, $P = 0.07$, Fig. 15b).

DISCUSSION

Isolation Call Variation

In *P. hastatus*, both temporal and spectral features of isolation calls change with pup age. After removing effects due to age, isolation calls exhibit sufficient variation among pups and groups to serve as both individual and group signatures. At the pup level, all PCA factors were informative. Duration of the first note correlated highly with the most informative factor and 71% of the variation in this factor contributed to variation among pups. However, at the group level only spectral and spectro-temporal features were informative. Twenty-one percent of the variation in the factor correlated with spectral features was attributed to variation among groups. This factor also showed the least amount of variation within pups indicating that spectral features show the highest degree of repeatability. The relative location of the minimum frequency (MNT1) varied significantly between groups and had the same degree of within pup stereotypy as temporal features. Spectral and to a lesser extent spectro-temporal features are possible candidates for group recognition features. I did not find any measurable differences in isolation calls emitted by bats from different caves. However, as there were only two caves and eight social groups, this result suffers from low statistical power and should be treated accordingly.

Experiment 1: Pup Discrimination

The bats successfully performed the first psychoacoustic experiment, which was designed to imitate a template-matching process. Unlike many

psychoacoustic procedures, where subjects only need to detect a difference in stimuli, in this experiment the bats had to store isolation calls in memory. The bats could have performed this task in two ways. Females could have learned and stored each call and its associated behavioural response during the first five trials and then compared each incoming call with the calls and associated behaviours stored in memory. Alternatively, bats may have stored the call and their own response in memory during each trial and then compared the following trial's call with the previous one. If those calls were the same, they performed the same behaviour as the previous trial. If those calls were different, they performed the opposite behaviour. In either case the bats must have compared a new call with one stored in memory. The main difference between these two behavioural strategies is the length of time between call acquisition and comparison with a new call.

Isolation call recognition was associated with spectral and spectro-temporal features. The lack of interaction effects between bats and extracted factors indicates that these findings were consistent across bats. Interestingly, spectral and spectro-temporal features are also more similar within social groups than between social groups. On the other hand, females did not appear to use temporal features for recognition even though these features should be informative for identification. However, isolation calls are emitted at loud amplitudes in roosting sites such as caves or hollow trees where reverberations are likely. Temporal features, such as call duration, are likely to be highly distorted by overlapping echoes in these environments (Bradbury & Vehrencamp

1998). Thus, selection may have acted on the auditory system for enhanced detection of features that suffer the least distortion during transmission.

Experiment 2: Group Discrimination

The multivariate analysis of isolation call features revealed that group mates emit calls with more similar spectral and spectro-temporal features than non-group mates. Experiment 1 showed that bats use these same features to discriminate pups. For experiment 2, I then examined whether this hierarchical signature system affected female discrimination discriminate between individual pups from the same or different group. For this experiment I incorporated between four and five isolation calls per pup. Consequently, bats had to discriminate between individuals even though calls varied within individuals. I found that group-level similarity did not affect discrimination ability as measured by the proportion of correct responses. This result was consistent across bats. Although there was significant variation across bats, all bats performed above guessing and there was no interaction effect between bats and social group. Response latencies were marginally non-significant ($P = 0.07$). This result raises the possibility that group-similarity may affect perception of isolation calls in an acoustic environment that is noisier than the anechoic chamber used for these experiments. However, critical ratio estimates for *P. hastatus* showed that they have highest frequency selectivity at the fundamental frequency of isolation calls (Bohn et al. 2004), which should enhance discrimination of spectral features of isolation calls. Furthermore, in the experiments described here, I only examined double-note calls, the simplest of isolation calls. Pups also emit isolation calls

with three or more notes, which likely provide more information for discrimination. I also confined age differences between pups to 2 days, which is at the lower end of age difference ranges within social groups in the wild (Porter & Wilkinson 2001). Thus, in this experiment the bats were likely faced with more similar calls than what would occur naturally. If *P. hastatus* use multiple spectral features, in addition to spectro-temporal features from multiple call types, they should be able to differentiate between most, if not all, pups' calls.

Isolation Calls and Parental Care

The high degree of isolation call discrimination found in *P. hastatus* indicates strong selection for accurate offspring recognition. *P. hastatus* give birth to only one pup per year, and infant mortality is high (Stern & Kunz 1998). Parental care is extensive as pups are altricial at birth and do not begin to fly until at least 6 weeks of age (Stern & Kunz 1998). Therefore, any trait that increases the likelihood of infant survival will strongly impact female reproductive success. Isolation calls are likely crucial to pup survival in *P. hastatus* because non-volant pups often fall from roost sites (Chapter Four). Fallen pups emit isolation calls that attract females who may bring pups back to their roost sites. If non-volant pups are not retrieved, they do not survive. Presumably, females accrue some costs by responding to other young as this may reduce care directed to filial young. However, based on the evidence presented in this study, mistaken identity is unlikely to be a plausible explanation for recurrent cases of female *P. hastatus* remaining near or retrieving fallen pups from their social group.

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Table 2. Results of regressions of age on average call variables. Intercepts and slopes from transformed variables (see Appendix).

	Variable	*F*†	Intercept	Slope
Temporal	D1	6.91*	13.7	-0.96
	D2	4.66*	0.90	-0.03
	INT	3.75	3.59	-0.16
Note 1 Spectral	MNF1	1.29	133,000	4240
	MXF1	3.68	14.1	0.28
	AVGF1	2.41	151,000	5,800
	PKF1	15.60***	11.3	0.64
Note 2 Spectral	MNF2	2.12	0.10	0.001
	MXF2	5.12*	220,000	9,990
	AVGF2	3.19	157,000	6,900
	PKF2	8.65**	12.5	0.54
Spectro-Temporal	MNT1	0.32	0.46	0.020

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

† $N = 63$

Table 3. Factor loadings after PCA and varimax rotation of isolation call measurements for analysis of call variation.

	Variable	Factor 1	Factor 2	Factor 3	Factor 4
Temporal	D1	-0.07	0.02	0.12	**0.93**
	D2	-0.03	**-0.92**	-0.03	0.01
	INT	0.01	0.48	**-0.69**	0.39
Note 1 Spectral	MNF1	**0.82**	-0.26	-0.13	-0.21
	MXF1	**0.76**	-0.10	-0.16	0.49
	AVGF1	**0.90**	-0.28	-0.19	-0.10
	PKF1	**0.77**	-0.08	-0.34	0.03
Note 2 Spectral	MNF2	**0.79**	0.35	0.06	-0.17
	MXF2	**0.87**	-0.03	0.05	0.16
	AVGF2	**0.90**	0.25	0.08	-0.02
	PKF2	**0.77**	0.20	0.07	-0.03
Spectro-Temporal	MNT1	-0.07	0.17	**0.83**	0.28

Call features with loadings of 0.65 or greater in bold.

Table 4. Univariate *F*-tests from nested ANOVAs on PCA factors from Table 3.

	Group		Pup		Call
Estimate	*F*	VCE†	*F*	VCE†	VCE†
N		8		63	309
Factor1	3.12*	0.18	19.68***	0.66	0.17
Factor2	0.94	0.00	8.58***	0.61	0.40
Factor3	2.59*	0.10	7.37***	0.50	0.40
Factor4	0.27	0.00	10.15***	0.64	0.37

* $P < 0.05$; *** $P < 0.001$

† Variance component estimates (VCE) show the proportion of variation explained by differences among groups within caves, pups within groups, and calls within pups.

Table 5. Factor loadings after PCA and varimax rotation for isolation call measurements used in the pup discrimination experiment (experiment 1).

	Variable	Factor 1	Factor 2	Factor 3	Factor 4
Temporal	D1	-0.31	0.59	0.57	0.01
	D2	-0.19	0.18	**0.73**	0.34
	INT	0.15	-0.20	**0.70**	-0.48
Note 1 Spectral	MNF1	-0.21	**-0.90**	0.06	0.01
	MXF1	**-0.86**	-0.06	0.04	-0.02
	AVGF1	-0.57	**-0.74**	0.06	-0.05
	PKF1	**-0.81**	-0.18	-0.28	-0.27
Note 2 Spectral	MNF2	**-0.70**	0.24	0.26	0.39
	MXF2	-0.53	-0.60	-0.20	-0.18
	AVGF2	**-0.69**	-0.25	0.32	0.30
	PKF2	-0.62	-0.45	0.28	0.26
Spectro-Temporal	MNT1	0.00	-0.13	0.00	**0.86**
	COR1	0.08	**-0.79**	-0.38	0.22
	COR2	0.01	-0.12	**-0.92**	0.01

Call features with loadings of 0.65 or greater in bold.

Table 6. Factors that predicted correct responses or response latencies in the pup discrimination experiment (experiment 1).

Variable	Factor Description	Response Outcome†	Response Latency§
Factor1	Spectral	0.43	5.09*
Factor2	Spectral & Spectro-temporal	7.01**	0.85
Factor3	Temporal & Spectro-temporal	0.35	0.53
Factor4	Spectro-temporal	1.29	0.07

† χ^2 from a logistic regression, *df* = 1

§ *F* statistic from an ANCOVA, *df* = 1, 67

*$P < 0.05$, **$P<0.01$

Appendix. Data transformations for the analysis of isolation call variation and the pup discrimination experiment (experiment 1). "None" indicates data were normally distributed. "NA" = not applicable, variable was not in analysis.

Variable	Call Variation	Experiment 1
D1	none	log *x*
D2	log *x*	none
INT	$\sqrt{x}$	log *x*
MNF1	x^2	log *x*
MXF1	x^2	$\sqrt{x}$
AVGF1	x^2	log *x*
PKF1	none	none
MNF2	$\sqrt{x}$	log *x*
MXF2	x^2	$\sqrt{x}$
AVGF2	x^2	x^2
PKF2	none	none
MNT1	arcsine $\sqrt{x}$	arcsine $\sqrt{x}$
COR1	NA	arcsine $\sqrt{x}$
COR2	NA	none
Age	$\sqrt{x}$	NA

FIGURE CAPTIONS

Figure 8. **(a)** Oscillogram, **(b)** spectrogram, and **(c)** power spectra of a typical isolation call. Measurements taken from isolation calls are: first note duration (D1), second note duration (D2), interval between notes (INT), beginning frequency of the first (BF1) and second (BF2) notes, end frequency of the first (EF1) and second (EF2) notes, average frequency of the first (AVGF1) and second (AVGF2) notes, minimum frequency of the first (MNF1) and second (MNF2) notes, maximum frequency of the first (MXF1) and second (MXF2) notes, and peak frequency of the first (PKF1) and second (PKF2) notes. Large dots represent spectral contours, which were averaged over the duration of the calls to calculate AVGF1 and AVGF2.

Figure 9. Schematic of test apparatus for psychoacoustic experiments. Bats begin the trial at the Start Position in between the delay sensor. The delay sensor is triggered when a bat leaves the starting position. Circles represent an LED light and photoreceptor that is triggered when the bat departs. During a "No Go" trial bats are rewarded at the starting position and during a "Go" trial bats are rewarded at the end of the right arm of the platform.

Figure 10. Stimuli for pup discrimination experiment (experiment 1). **(a)** “Go” trial of five call sets with 60 ms between calls and 900 ms between sets, all calls from a single pup, “Pup A”. **(b)** “No-go” trial, all calls from a single pup, “Pup B”. **(c)**

and **(d)** spectrograms of single isolation calls from Pup A and Pup B respectively.

Figure 11. Stimuli for group discrimination experiment (experiment 2). **(a)** "Go" trials consisted of three, four, or five background call sets from one pup (Pup A), two calls from a target pup (Pup B), and one call from the first pup (Pup A). Subjects were rewarded for going during the last three call sets. **(b)** "No-go" trials consisted of six, seven or eight call sets. Subjects were rewarded for staying during entire trial. (**c)** Example call set for Pup A. **(d)** Example call set for Pup B.

Figure 12. Effect of age on the duration of the first **(a)** and second **(b)** notes, and peak frequency of the first **(c)** and second notes **(d)**.

Figure 13. Plot of mean percent correct (±SE) for five bats per day (*N* = 16) in the pup discrimination experiment (experiment 1). Dashed line indicates 95% upper confidence interval for 50 % correct per day.

Figure 14. Plot of mean percent correct (±SE), per day (*N* = 9) for the four bats in the group discrimination experiment (experiment 2). Dashed line indicates 95% upper confidence interval for 45% correct per day.

Figure 15. Results of the group discrimination experiment. Mean (± SE) percent correct (a) and response latency (b) for pup pairs from the same and different social group.

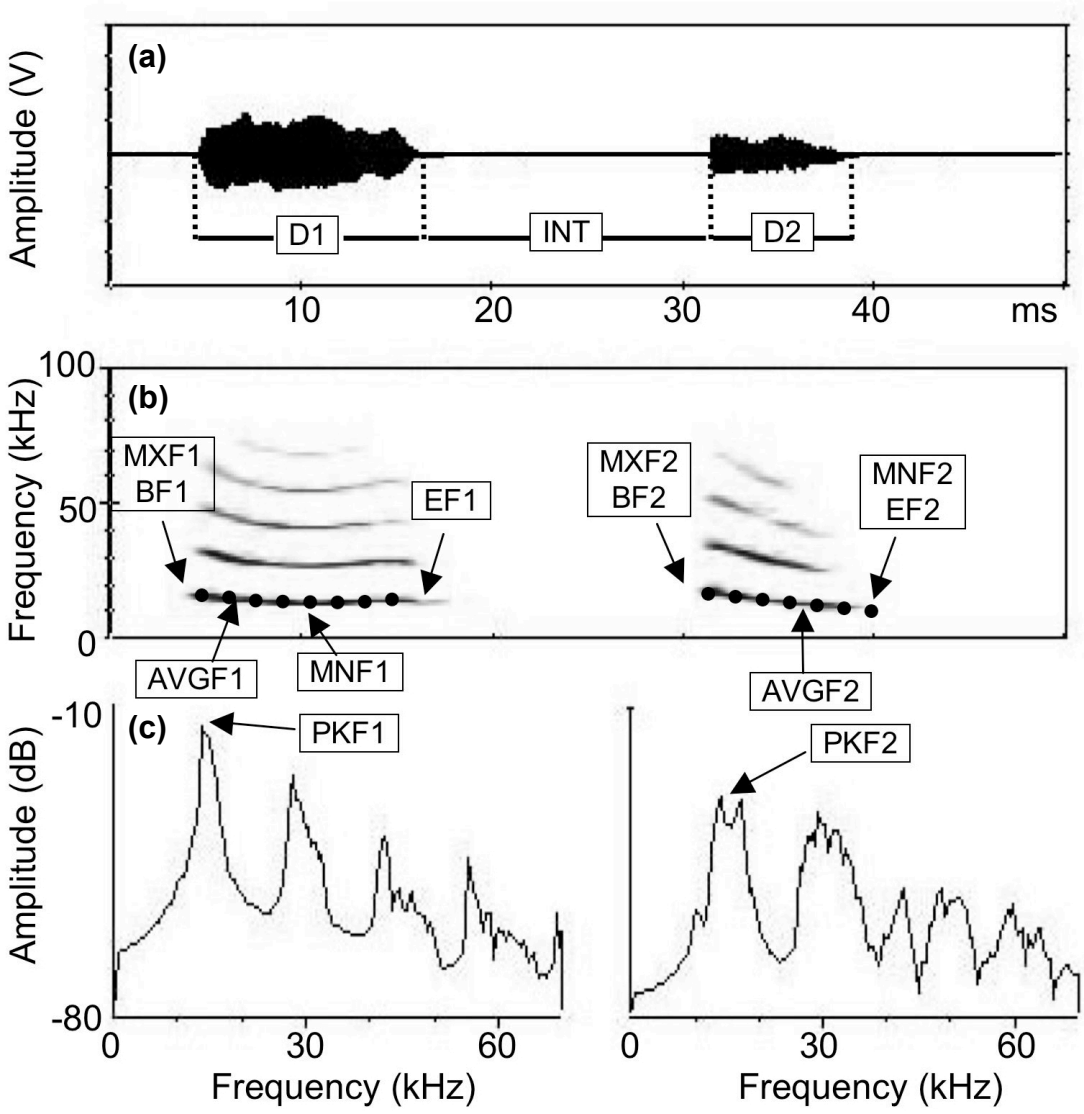
(a)
Amplitude (V)
D1
INT
D2
10
20
30
40
ms
(b)
Frequency (kHz)
100
50
0
MXF1
BF1
EF1
MXF2
BF2
MNF2
EF2
AVGF1
MNF1
AVGF2
(c)
Amplitude (dB)
-10
-80
PKF1
PKF2
0
30
60
Frequency (kHz)
0
30
60
Frequency (kHz)

Figure 8

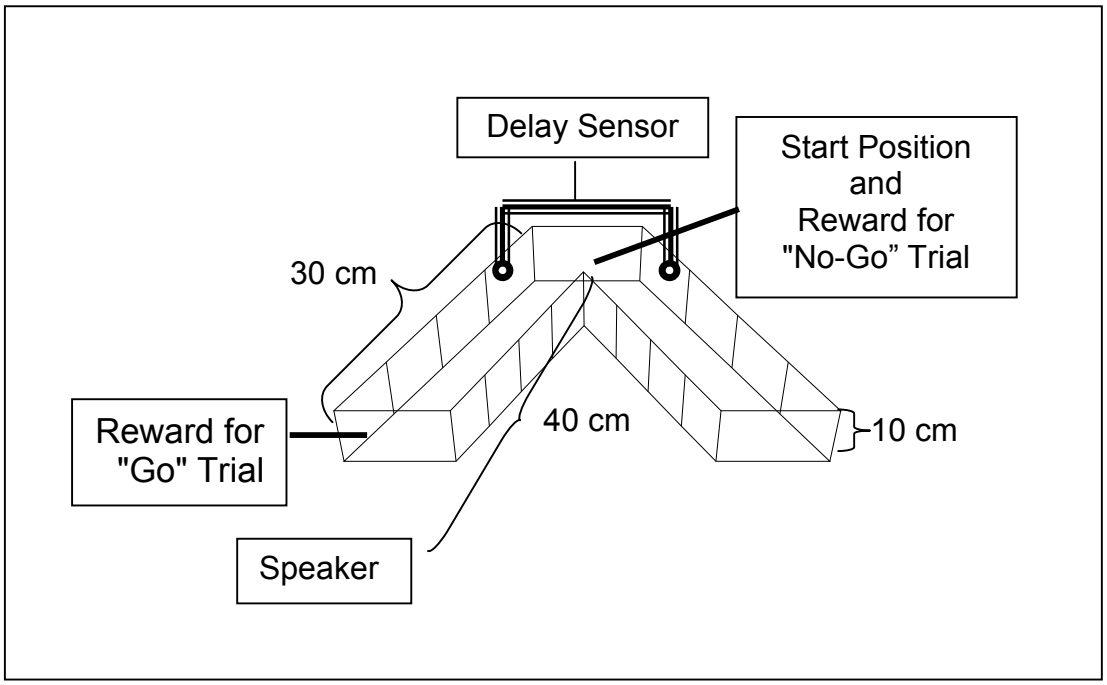
Delay Sensor
Start Position
and
Reward for
"No-Go" Trial
30 cm
Reward for
"Go" Trial
40 cm
10 cm
Speaker

Figure 9

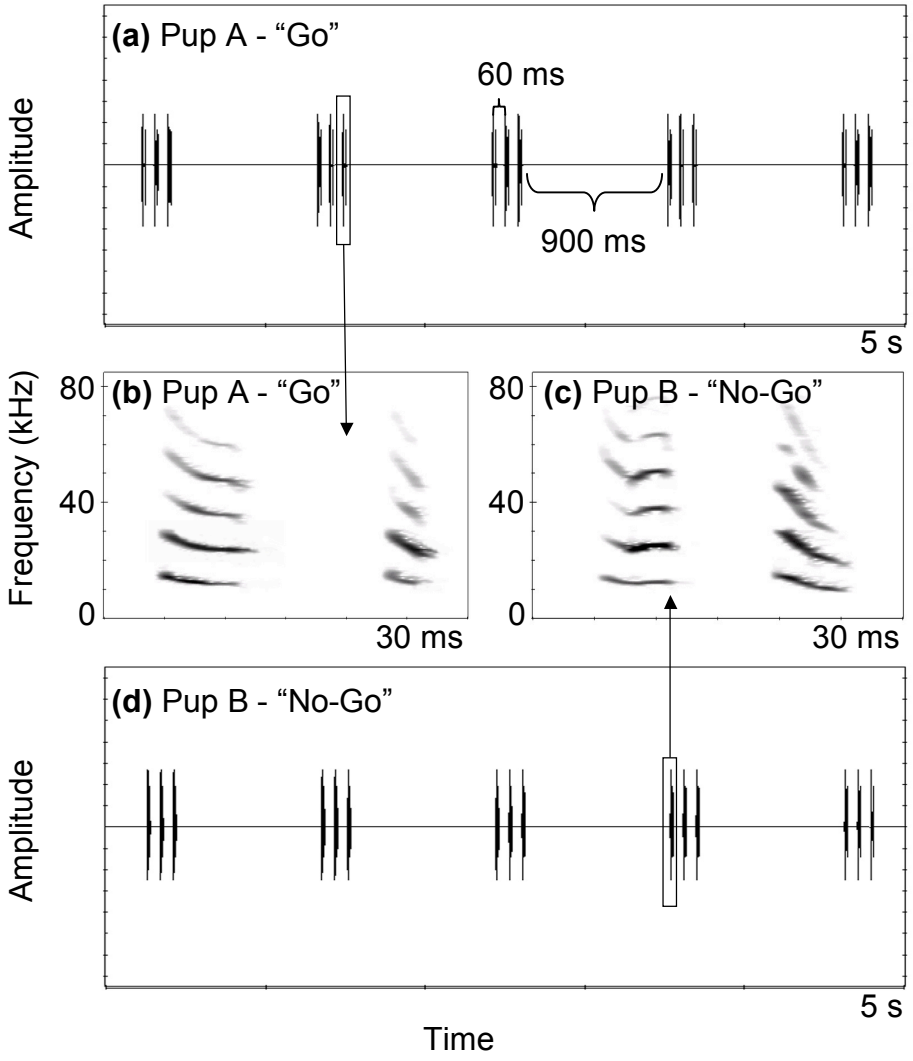
(a) Pup A - "Go"
60 ms
900 ms
5 s
Amplitude
Frequency (kHz)
80
40
0
(b) Pup A - "Go"
30 ms
(c) Pup B - "No-Go"
(d) Pup B - "No-Go"
Time

Figure 10

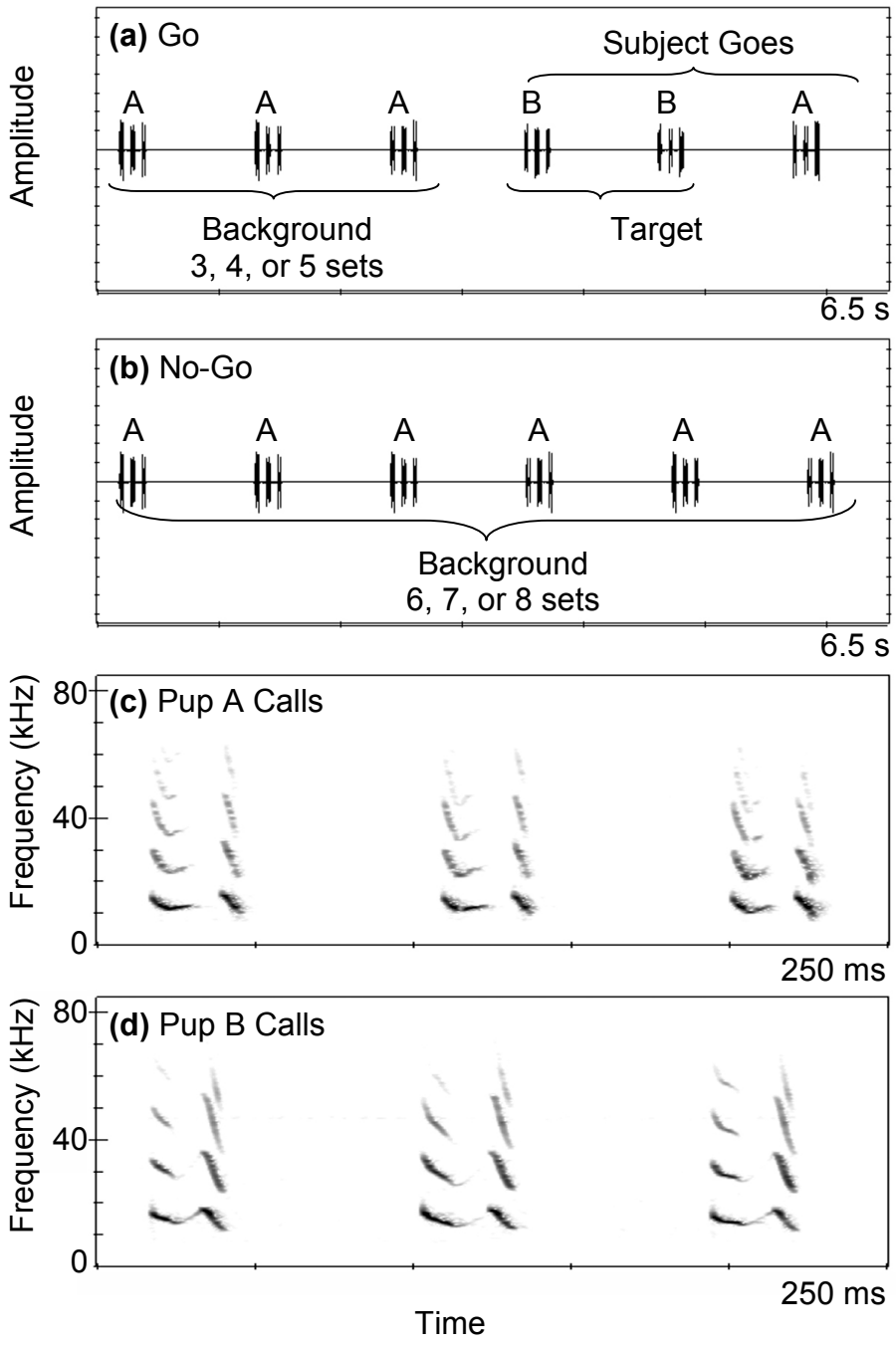
(a) Go
Subject Goes
A
A
A
B
B
A
Amplitude
Background
3, 4, or 5 sets
Target
6.5 s
(b) No-Go
A
A
A
A
A
A
Amplitude
Background
6, 7, or 8 sets
6.5 s
Frequency (kHz)
80
40
0
(c) Pup A Calls
250 ms
Frequency (kHz)
80
40
0
(d) Pup B Calls
250 ms
Time

Figure 11

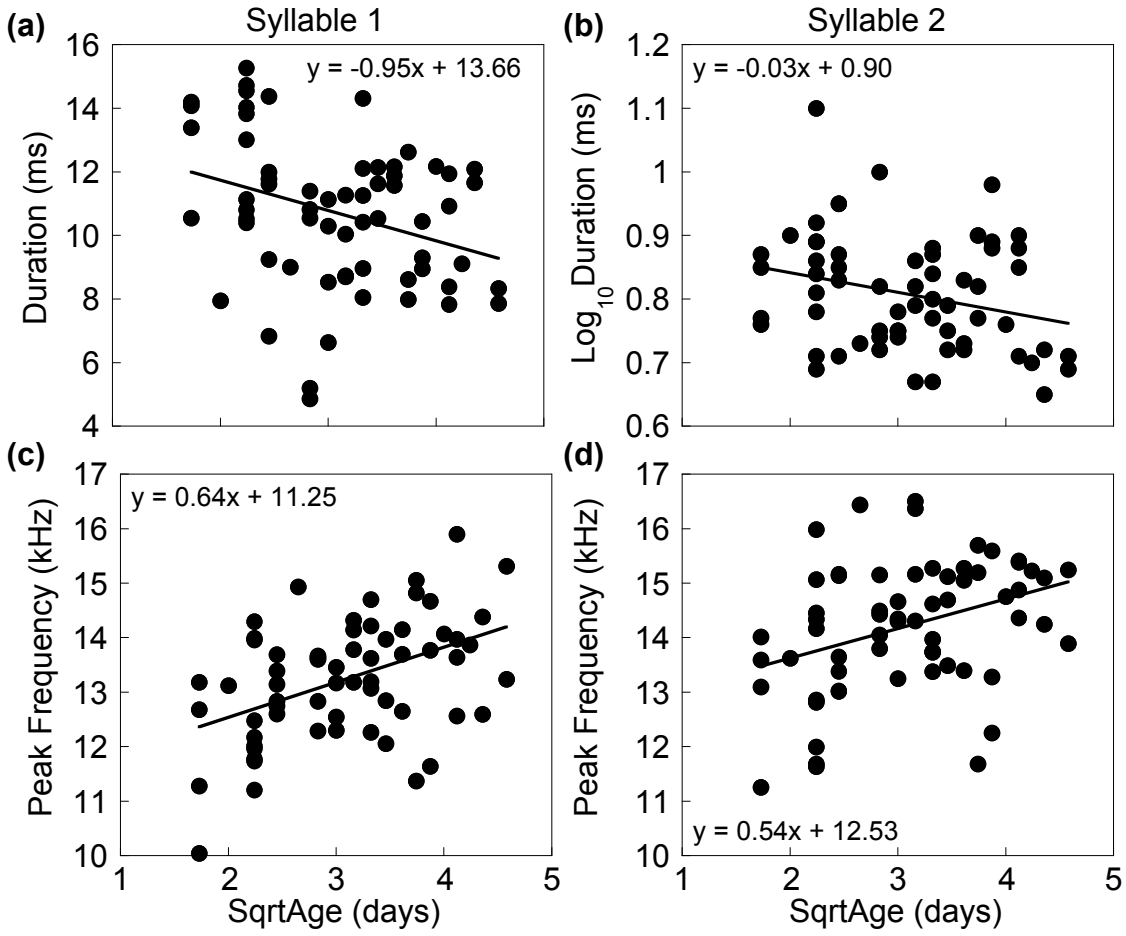
(a)
Syllable 1
y = -0.95x + 13.66
Duration (ms)
(b)
Syllable 2
y = -0.03x + 0.90
Log10 Duration (ms)
(c)
y = 0.64x + 11.25
Peak Frequency (kHz)
SqrtAge (days)
(d)
y = 0.54x + 12.53
Peak Frequency (kHz)
SqrtAge (days)

Figure 12

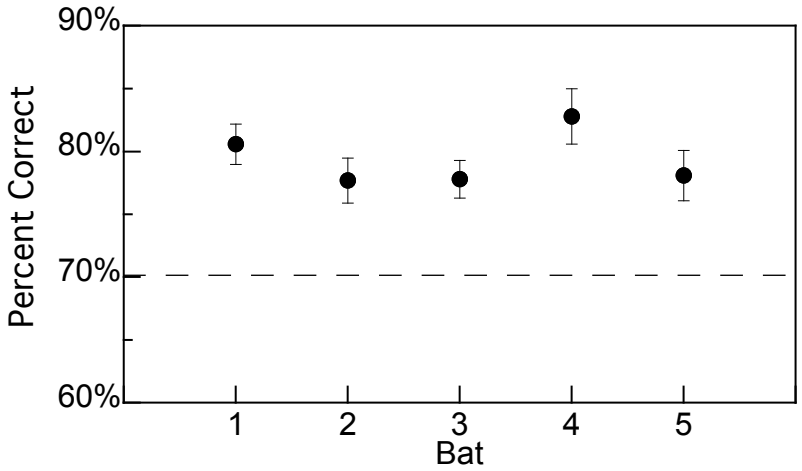

Figure 13

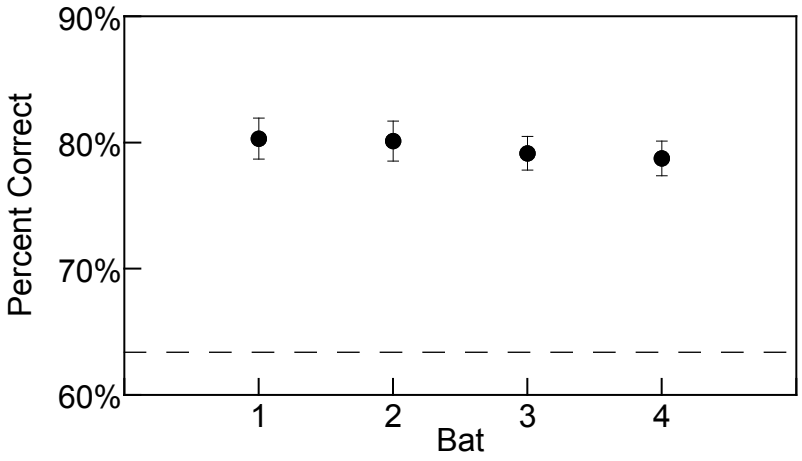

Figure 14

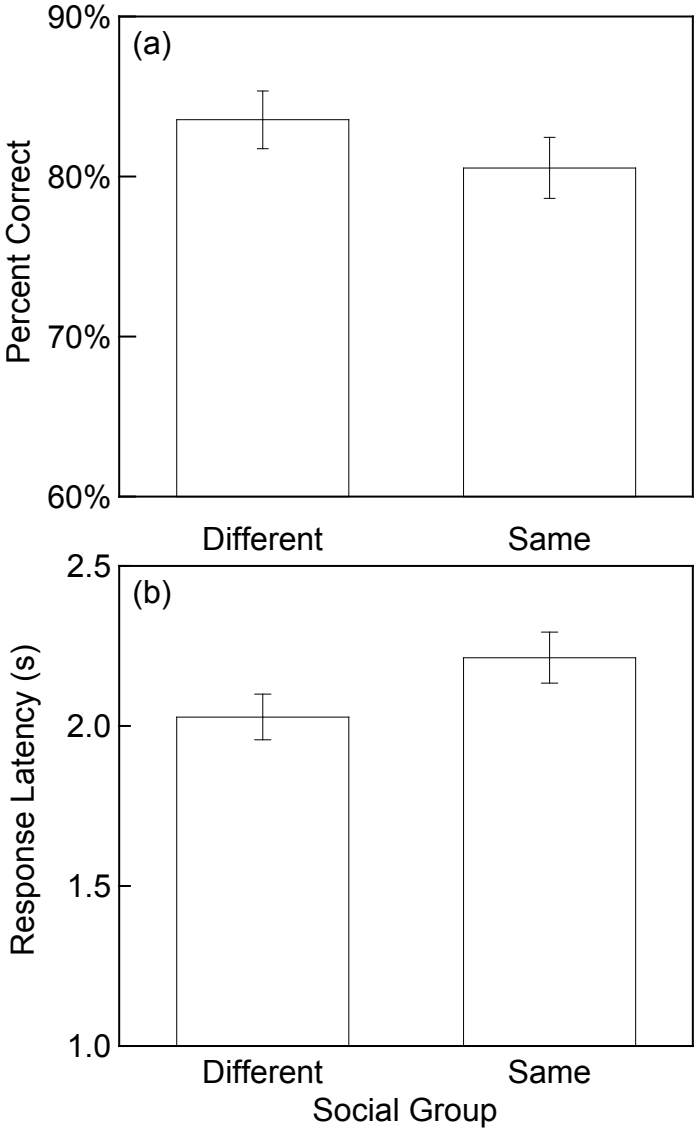
90%
(a)
Percent Correct
80%
70%
60%
Different
Same
2.5
(b)
Response Latency (s)
2.0
1.5
1.0
Different
Same
Social Group

Figure 15

CHAPTER IV

Cooperative Care of Young by Unrelated Greater Spear-Nosed Bats

ABSTRACT

Alloparental care, or care of others' young, appears contradictory to Darwinian natural selection. Most instances of alloparental care have been attributed to: 1) mistaken identity, when individuals confuse their young with others (McCracken 1984; Roulin 2002), or 2) cooperation (Riedman 1982; Packer et al. 1992; Clutton-Brock et al. 2000; Roulin 2002). Cooperative care, in turn, is often explained by kin selection, where animals receive indirect benefits by selectively caring for genetic relatives (Emlen & Wrege 1988; Creel et al. 1991; Pusey & Packer 1994). In greater spear-nosed bats (*Phyllostomus hastatus*) non-volant pups often fall from cave ceilings and risk death unless retrieved by an adult female. Here I show that females frequently visit group mates' fallen pups when they are not missing pups of their own, which is inconsistent with mistaken identity. Female visits appear to protect fallen pups

from marauding females who attack and sometimes kill them. However, kin selection cannot explain cooperative care in *P. hastatus*, because analysis of microsatellite genotypes indicates that females are not related to the pups they guard. Although females that guard pups may receive direct benefits associated with group pup crèches, given the stable social structure of this species, they may also receive benefits through cooperative behaviours with group mates.

MAIN TEXT

P. hastatus are large, omnivorous bats that commonly inhabit caves in Trinidad, West Indies (McCracken & Bradbury 1981). Reproductive females roost in the ceilings of caves in social groups of eight to 40 unrelated individuals that are attended by a single harem male (McCracken & Bradbury 1981; McCracken 1987). Female social groups are highly stable with some females remaining together for 16 years or more (G. F. McCracken, G. S. Wilkinson & J. W. Boughman, unpublished data). Benefits derived from cooperative behaviours may play a role in maintaining such long-term associations. For example, *P. hastatus* social groups forage cooperatively using learned group-specific vocalizations (Boughman 1998; Wilkinson & Boughman 1998). Moreover, females within groups exhibit socially mediated birth synchrony (Porter & Wilkinson 2001), which is often associated with cooperative care of young (Ims 1990). Alloparental care may, therefore, provide another advantage of high group fidelity in this species.

P. hastatus give birth to only one pup per year and 40% of pups die in their first year (Stern & Kunz 1998). Pups do not begin to fly until at least 6-

weeks of age (Stern & Kunz 1998). Previous researchers have observed females visiting and retrieving group mates' fallen pups other than their own (T. A. Porter, pers. comm.). In the context of a dark cave, females must rely on pup vocalizations, termed isolation calls (Gould et al. 1973), when deciding if they should visit a fallen pup. Thus, females may mistakenly visit pups other than their own if they cannot discriminate different pups' isolation calls. However, I have found that *P. hastatus* have their best hearing sensitivity and frequency selectivity at the fundamental frequency of isolation calls (Chapter 1). Furthermore, in the laboratory, females can discriminate pup isolation calls regardless of social group (Chapter 3). Thus, although visiting others' pups may be due to females mistaking these pups for their own, another explanation is that females are cooperatively caring for group mates' young. Here I test these two alternatives by marking females from seven social groups and examining their visits to fallen pups in their natural environment (see Methods) I observed 155 pup falls, 86 of which were from social groups of individually marked females, and 2,887 female visits to fallen pups.

First, I conducted maternal exclusion tests using five microsatellite markers to determine if females were retrieving their own pups. Seventy-one of the 86 marked pups were retrieved, eight were captured and seven others were ignored (see Methods and below). Females involved in eight (12.7%) out of the 63 retrievals for which I had genetic information could be excluded as mothers, with two of those cases involving females from different social groups than the pups.

Within two weeks of birth, on average, 4 ± 2% (standard error of the mean, s.e.m.) of pups naturally fell from roost sites in the cave each night. Fallen pups were visited on average 17.0 ± 2.6 (s.e.m) times (range 1-342 visits). However, visits by females that retrieved pups only accounted for 3.9% of these visits (113 of 2,887 visits to marked and unmarked pups). To determine if the other bats that visited pups did so independently of their social group, I excluded all visits when a female picked up a pup. I found that females visited pups from their own social group more frequently than expected (Fig. 16).

Next, I examined visiting behaviour to test whether mistaken identity could explain visits to group mates' pups. For each night that I conducted experiments, I removed multiple pups from each social group thereby creating known females whose pups had been removed. I also identified all pups that fell naturally and considered these pups "removed" as well (see Methods). Each female that visited a pup during the course of a night was then classified as to her own pup's status: removed or not removed. Because *P. hastatus* social groups roost in site-specific locations (McCracken & Bradbury 1981), females should be able to locate pups that are in their roost sites. Thus, if females are making mistakes, they should only visit others' pups when their own pups had not been removed. However, I found that 395 (69%) of the 576 visits by marked females occurred when the female's pup had not been removed. I then examined three aspects of visiting behaviour: the time spent visiting a pup, the proportion of visits where a female inspected a pup, and the number of return visits to a pup. If females are all searching for their own pups when visiting others, then there should be no

difference in female behaviour during visits with respect to pup status (removed or not removed). Although time spent visiting pups and the proportion of visits where females inspected pups were not affected by pup status, there was an interaction between pup status and social group on the number of return visits to a pup (Fig. 17). Females whose pups had not been removed returned to visit group mates' pups more frequently. These results indicate that many females that visit group mates' pups are not simply searching for their own pups.

Although females initially use isolation calls to identify pups, females inspect pups by actively smelling them when they visit. Presumably, females are using scent to confirm the identity of pups once they have landed. If females visit group mates' pups because they are more uncertain about their identity, then they should inspect these pups more frequently. However, females inspect group mates' pups less frequently than non-group mates' pups (Fig 17c). Thus, taking all of the evidence taken together, I conclude that mistaken identity cannot explain all of the visits to group mates' pups.

If visits by group mates are intentional and cooperative, visits must provide benefits to pups. Pups do not benefit from retrievals since females rarely retrieve group mates' pups. However, 10% of fallen pups that were picked up were captured i.e. carried off in the teeth of the visiting female (see Methods). In five cases, captured pups were subsequently found outside of the cave or heard vocalizing as the perpetrator left the cave with the pup. Pup captures only occurred when the visiting female was not a group mate, whereas the majority of retrievals (where the pup was nursing when the female departed) occurred when

females were from the same social group as the pup (Fisher's Exact Test; P<0.0001; N=8 captures, 71 retrievals). Visiting females also bit pups and I found three dead pups in the cave with obvious bites on them. Ninety-eight percent of bites occurred when females were from a different social group than the pup, significantly more than expected when compared to the frequency of visits (χ_1^2=8.59, P= 0.003, N =46 bites, 1688 total visits, only marked pups included). Male pups were captured and bitten more frequently than expected (χ_1^2=7.31, P=0.007, 100% of captured pups were male; χ_1^2=6.24, P=0.01, 77% of bitten pups were male, 49 female and 58 male fallen pups). Interestingly, reproduction is highly skewed for males in *P. hastatus*, with relatively few harem males fathering the majority of young in the cave (McCracken & Bradbury 1977). Females, therefore, may be increasing the likelihood of their sons' reproductive success by eliminating other males.

Females spent more time visiting group mates' pups (Fig. 17a) even though females inspected these pups less frequently (Fig. 17c). In fact, group mates often perched near pups without interacting with them for long periods, often staying until the pups' mothers arrived and retrieved them. However, these females did fight with other visiting females and fights occurred almost exclusively (96%) between non-group mates, significantly more than expected when compared with the frequency of encounters (χ_4^2=16.6 P<0.0001, N=113 fights, 448 total interactions involving marked females). Prolonged visits by group mates, aggression towards pups and fights involving non-group mates, are

consistent with "pup-guarding", where females protect group mates' pups from non-group mates that attack and sometimes kill them.

For cooperative behaviour to evolve, aid givers, in this case visiting females, must receive benefits that outweigh the costs of the behaviour. Visiting females appear to lessen the costs associated with alloparental care. First females return to visit group mates' pups more frequently when their own pup is already in the roost (Fig. 17b). Second, females rarely retrieve pups that are not their own. Retrievals are costly because the only way to carry a pup is in a nursing position. However, pup guarding likely has some costs. Females that respond to group mates' pups have pups of their own. Therefore, females leave their own pups unattended while expending time and energy on others' pups. Also, females risk injury from fights with other females. I tested whether visiting others' pups affected female condition (see Methods). I found no difference in female condition at the end of experiments for females that did or did not visit pups ($F_{1,91}$= 0.002, P=0.97), but a negative relationship between female condition and the amount of time spent visiting others' pups (Fig. 18). Given that there are some costs to this behaviour, the next question is what benefits counterbalance these costs.

Most examples of cooperation are explained by kin selection, where animals receive indirect benefits by preferentially assisting genetic relatives. For kin selection to operate, Hamilton's rule, $rB–C>0$ must be met, where in this case r is the relatedness between the visiting female and pup, B is the benefit to the pup, and C is the cost of visiting (Hamilton 1964). If kin selection is operating,

females should selectively care for pups they are related to. To test this I examined relatedness between females and pups they retrieved (retrievals), females and pups they visited from the same social group (same visits), and females and pups they visited from different social groups (different visits). Mean relatedness (± s.e.m.) for retrievals was r=0.42± 0.03, which is consistent with females preferentially retrieving their own pups (see Methods, Fig. 18). On the other hand, relatedness for visits by females from the same social group was only r=-0.02±0.03, which is no different than zero. Therefore, females do not selectively guard kin. I also confirmed that adult females are unrelated within social groups (r = 0.01±0.01). Relatedness between females and pups they visit from different social groups was r=–0.12±0.04, which is lower than zero and lower than visits by females to pups from the same social group (Fig. 18). These results indicate that attacking females can preferentially select unrelated pups.

Most commonly, alloparents receive indirect benefits by directing care towards genetic relatives (Emlen & Wrege 1988; Manning et al. 1992; Pusey & Packer 1994; Gemmell 2003). On the other hand, direct benefits, may also play a role in alloparental care. For example, in cooperative breeders, helpers may benefit directly by increasing their group size (Rood 1990; Kokko et al. 2000; Clutton-Brock et al. 2000). In *P. hastatus*, females cannot benefit from group augmentation because pups disperse from their natal groups during their first year (McCracken & Bradbury 1981). Alternatively, a possible direct benefit might be maintenance of crèche size if larger crèches provide thermoregulatory benefits or reduce predation risk. However I found that pup condition decreased

as crèche size increased (Fig. 20) and I never observed pup predation at roost sites in the cave ceiling.

Pup guarding benefits both pups and pup mothers. Thus, females may also receive benefits from adult group mates. While in some species alloparents benefit through reciprocity by alternately caring for each other's young (Owens & Owens 1984), in *P. hastatus*, fallen pups' mothers are rarely present when group mates visit them, making reciprocal pup guarding improbable. However, given that these females reside in long-lived stable social groups, it seems likely that females recoup the costs associated with pup guarding through other cooperative behaviours with group mates. For example, the frequency of group foraging has a positive effect on pup condition (J. W. Boughman unpubl.). Thus, pup guarding may also benefit females if it increases the likelihood that they are included in group foraging or other cooperative activities.

METHODS

Pup Retrievals

Retrieval experiments were conducted at Guanapo Cave, Trinidad in 2001, 2002 and 2004. In 2001, every night I divided the number of natural pup falls by the number of pups in the cave to determine the percentage of pups that fell per night. I captured one, two and four social groups in 2001, 2002 and 2004 respectively (133 females, 8 males). I sexed, banded, took wing-membrane samples for genetic analyses, and made individually distinctive marks on the

backs of adults using hair bleach (see Appendix I). Groups were re-captured and measured at the end of each field season.

While adults were foraging in the evenings, I measured, banded, sexed, and took wing-membrane samples from one to four pups after hand capture from roost sties of bleach-marked groups. For both females and pups I used the residuals of forearm length (mm) on body weight (grams) as an estimate of condition. To emulate natural pup falls, I placed one pup at a time on the wall of the cave and recorded visits with a digital camcorder equipped with an infrared light (Sony DCR-TRV460). For each visit, I determined the identity of the female if marked, the length of the visit in seconds, and whether the female inspected the pup. An inspection was defined as a female actively smelling the pup. I also noted females that bit pups and fights between visiting females. I categorized each pup pick-up as either 1) a capture if the pup was carried in the teeth or 2) a retrieval if the pup was in a nursing position when the female departed. If pups were not picked up after 45 minutes, I returned them to their roost sites at the end of the night.

Guanapo cave is sufficiently small that I could detect all fallen pups while I was in the cave. For each of the 39 nights that I observed retrievals, I placed camcorders with infrared lights at the roost sites of groups that were used in experiments and recorded any pup falls or females returning with pups. In 2002 and 2004, I removed two pups from two groups per night and alternated the order of pups being placed on the walls. In 2004, I also alternated between the four groups used for experiments each night. Any pups that fell naturally during

retrieval experiments were immediately removed, sampled, measured, and banded. I then placed them on the wall of the cave and recorded visits when no other pups were being monitored.

Statistical Analyses

I analyzed nominal data using contingency tables and tested significance using Chi-squared tests unless any cells had expected values less than five, in which case I used Fisher's Exact tests. I combined visits to natural and experimental pup falls after finding no difference in the number of female visits (Mann-Whitney U test, U=3,169 P=0.48, N =155), time spent visiting by females (U=2,760, P=0.44, N=155), or the proportion of visits where a female inspected a pup (U=3559, P=0.52, N=131). For marked females, I examined the total time spent visiting a pup, the proportion of visits where a female inspected a pup, and the number of return visits to a pup for each visit that involved a unique female/pup combination. Each female/visited-pup combination was categorized by social group (same or different) and pup status (removed or not removed). Although each female/pup combination was unique, some pups and females were repeated within the dataset. This non-independence precluded the use of parametric statistics, therefore I examined social group, pup status, and interaction effects using randomization tests that paralleled a two-way analysis of variance with 10,000 permutations (Edington 1995).

Maternal Exclusion

Wing-membrane samples were stored in 95% ethanol and DNA was extracted using DNeasy Tissue kits (Qiagen). I tested six *Artibeus jamaciensis* (Ortega et al. 2002) and seven *Lophostoma silvicolum* (Dechmann et al. 2002) microsatellite primer pairs on *P. hastatus*. For screening primer pairs, I performed polymerase chain reactions (PCRs) using unlabelled primers and both temperature gradient and touchdown programs on a PTC-200 Programmable Thermal Cycler (MJ Research). The temperature gradient program varied annealing temperatures between 45°C and 65°C while the touchdown program had an initial annealing temperature of 65°C and decreased by 0.7°C ending with a final temperature of 45°C. Amplification products were examined using agarose gels.

Of the thirteen primers tested, three *A. jamaciensis* and two *L. silvicolum* amplified consistently and were polymorphic. For these five loci I performed PCR with one of each primer pair fluorescently labelled (Integrated DNA Technologies) in 10 *u*l volumes containing 0.5 *u*l DNA, 2.5 mM $MgCl_2$, 0.5 *u*M of each primer, 1X PCR Buffer (Invitrogen), and 0.25 U *Taq* polymerase (Invitrogen). The PCR program consisted of 5 min at 95°C, 30 cycles of 45 s at 95°C, 45 s at annealing temperature, and 1 min at 72°C, and 5 min of extension at 72°C (Table 7). Fluorescently labelled PCR products were separated on a 3100 DNA Analyzer (Applied Biosystems) and evaluated with Genescan 3.1.2 software (Applied Biosystems). I used Genotyper 2.5 to size and score alleles (Applied Biosystems).

I tested whether each locus was in Hardy-Weinberg equilibrium using GENEPOP (Raymond & Rousset 1995). Loci had between two and fourteen alleles and observed heterozygosity ranged from 0.55 to 0.77. All loci were in Hardy-Weinberg Equilibrium (Table 7). I excluded females as mothers if they did not match the pup at any of the five loci. I determined relatedness within social groups using RELATEDNESS 5.0.8 (Queller & Goodnight 1989). I estimated relatedness for three sets of female-pup pairs: females and pups they retrieved, females and pups they visited from the same social group, and females and pups they visited from different social groups. To test for significance, I compared each of these estimates with the distribution of means from 10,000 random samples of the same size as the estimate, taken from all female-pup pairs for which I had genetic information (*N*=132 females, *N*=121 pups). I tested whether the relatedness of females that visited pups from the same social group differed from the relatedness of females that visited pups using a randomization test similar to an independent t-test (Edington 1995).

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Table 7. Microsatellite loci. The number of alleles, annealing temperature, allele sizes and observed heterozygosity for the five microsatellite loci.

Locus	No. Alleles	Temp (°C)[c]	Sizes	Obs. Het.[d]
AjA185[a]	2	54	86-88	0.55
AjA74[a]	6	50	145-155	0.77
AjA84[a]	16	50	93-131	0.60
Tsil2Ca1[b]	7	56	111-127	0.68
Tsil3Ca2[b]	8	48	186-204	0.70

[a] Loci from Ortega et al. 2002.

[b] Loci from Dechmann et al. 2002.

[c] Annealing temperature for PCR reaction.

[d] Observed heterozygosity. None of the loci showed significant deviation from Hardy-Weinberg expectations as calculated by GENEPOP Raymond & Rousset 1995.

FIGURE CAPTIONS

Figure 16. Females visit fallen pups from their own social group more frequently than expected. Association between the social group of fallen pups and the social group of visiting females tested each year with Chi-squared contingency tables. "Observed" bars show the mean ± s.e.m. observed proportions along the diagonal of the contingency table (when pup group equals female group). "Expected" bars show the overall expected visiting frequency based on the number of pup groups. 2001: two groups, $\chi_1^2 = 16.8$, $P < 0.0001$, N=1155 visits; 2002: three groups $\chi_4^2 = 39.6$, $P < 0.0001$, N =352 visits; 2004: five groups, $\chi_{16}^2 = 406$, $P < 0.0001$, N =1248 visits. For each year, all pups and females that were not from marked groups were treated as if they were from a single group. Visits where females picked up pups are excluded.

Figure 17. Effects of pup status and social group on visiting behaviour. **a**, Females spend more time visiting pups from the same social group regardless of pup status (social group, $P = 0.007$, pup status and pup status*social group $P > 0.45$). **b,** The number of return visits to a pup exhibits an interaction between social group and pup status (P=0.01, social group and pup status $P > 0.05$). **c**, Females inspect pups during fewer visits when pups are from the same social group regardless of pup status (social group, $P < 0.001$, pup status and pup

status* social group $P > 0.40$). Error bars are ± s.e.m. Visits when females picked up pups are excluded. Sample sizes for all tests shown in **a**.

Figure 18. Relationship between time spent visiting fallen pups and female condition. Female condition decreased as more time was spent visiting others' pups (linear regression, $F_{1,49} = 4.36$, $P = 0.04$, $r^2 = 0.08$). Time spent visiting was $\log_{10}$ transformed to meet normality requirements. Visits when females picked up pups are excluded.

Figure 19. Relatedness between females and the pups they retrieve or visit. "Actual" represents mean ± s.e.m relatedness between females and pups they retrieved, females and pups they visited from the same social group, and females and pups they visited from different social groups. Actual values were compared with random samples. "Random" represents the median and 95% upper and lower bounds of 10,000 means from *N* randomly selected samples from all female-pup pairs, where *N*=sample size of the actual estimate. Same and different visits were also tested against each other using a randomization test. * < 0.05, ** < 0.01, **** < 0.0001.

Figure 20. Relationship between group size and pup condition. Pup condition decreased as the number of pups in a group increased (regression, $F_{1,6} = 27.7$, $P = 0.003$, $r^2 = 0.85$) Pup condition was $\log_{10}$ transformed.

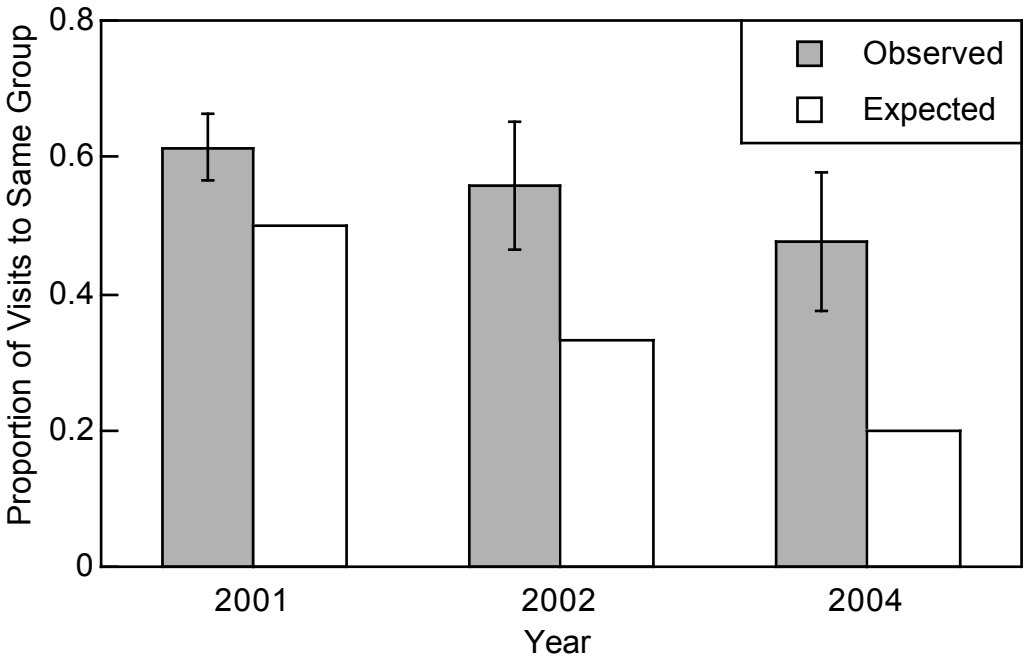

Proportion of Visits to Same Group
0.8
0.6
0.4
0.2
0
Observed
Expected
2001
2002
2004
Year

Figure 16

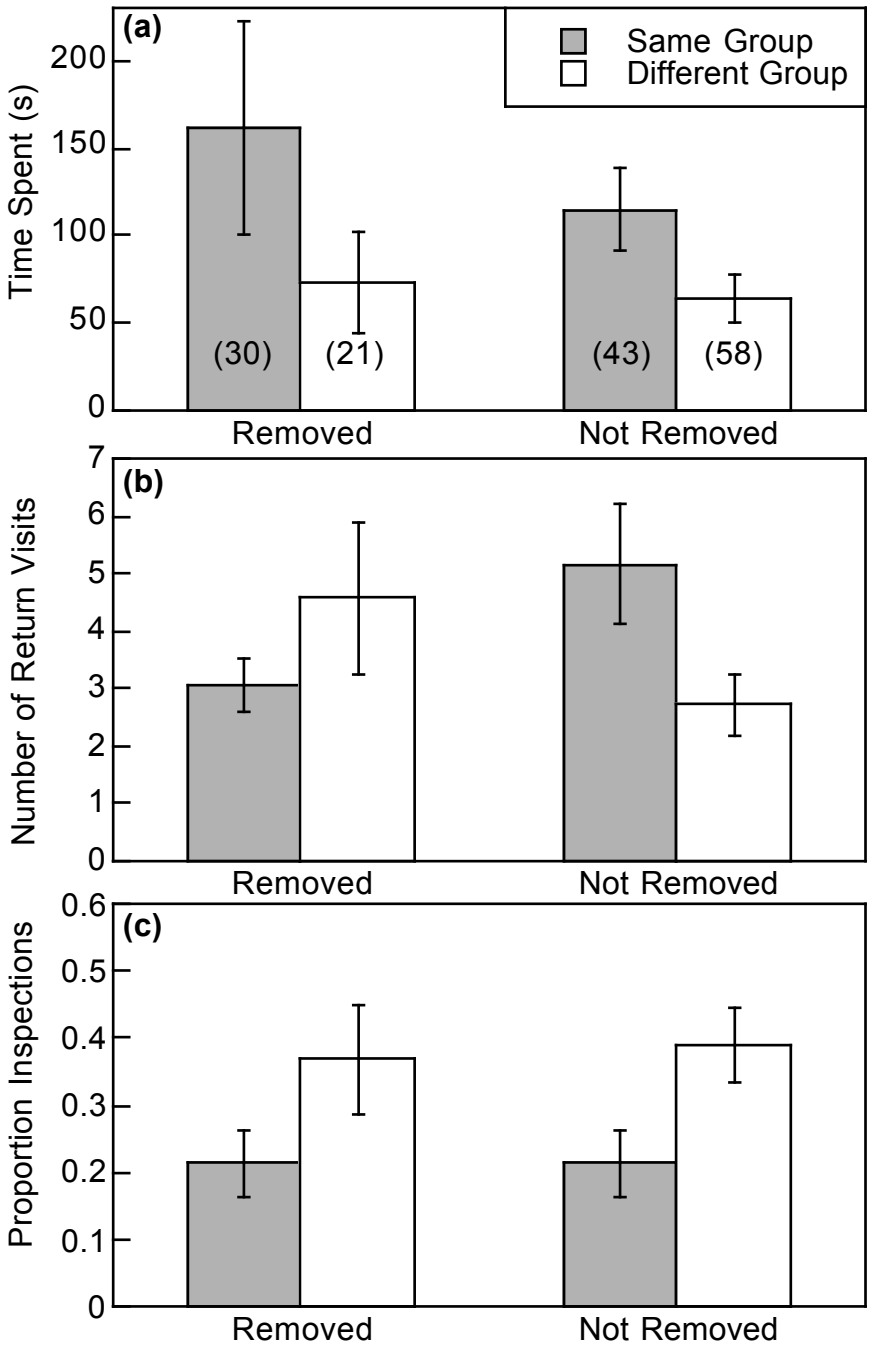

(a)
Same Group
Different Group
Time Spent (s)
200
150
100
50
0
(30)
(21)
(43)
(58)
Removed
Not Removed
(b)
Number of Return Visits
7
6
5
4
3
2
1
0
Removed
Not Removed
(c)
Proportion Inspections
0.6
0.5
0.4
0.3
0.2
0.1
0
Removed
Not Removed

Figure 17

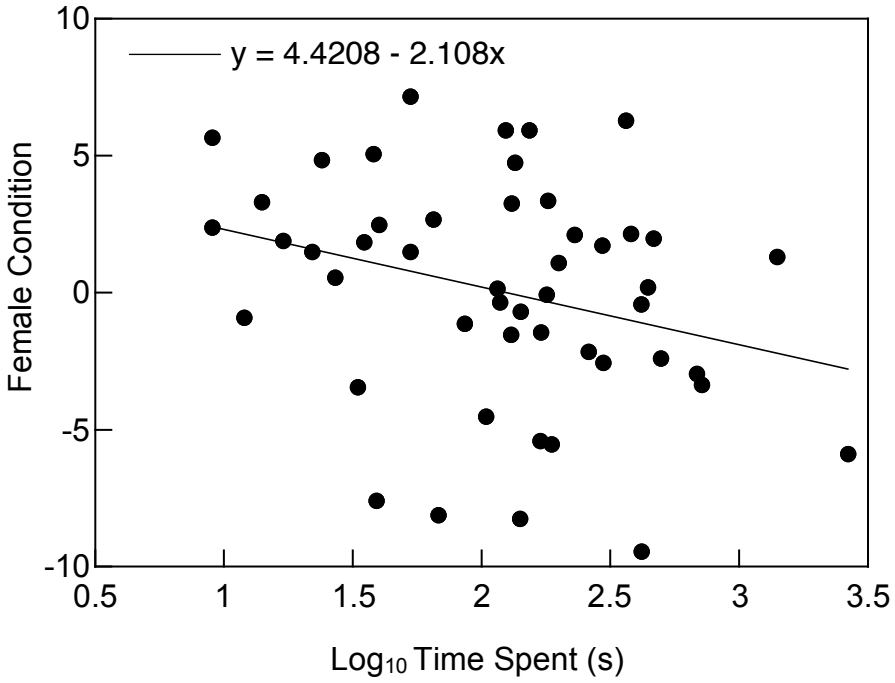

y = 4.4208 - 2.108x
Female Condition
10
5
0
-5
-10
0.5
1
1.5
2
2.5
3
3.5
Log10 Time Spent (s)

Figure 18

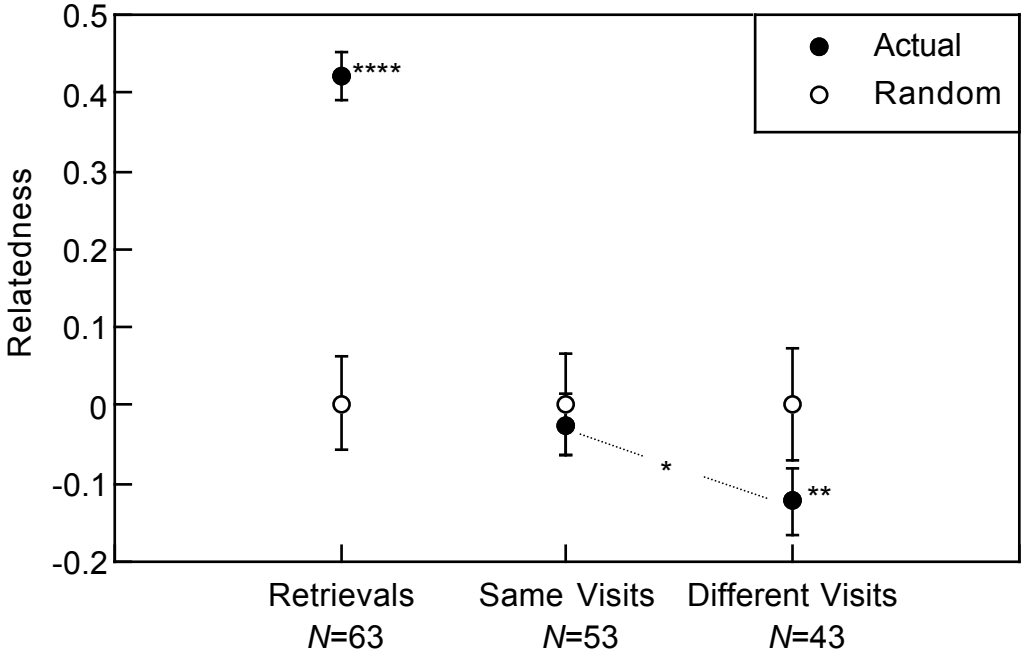

0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
Relatedness
Actual
Random
****
*
**
Retrievals
N=63
Same Visits
N=53
Different Visits
N=43

Figure 19

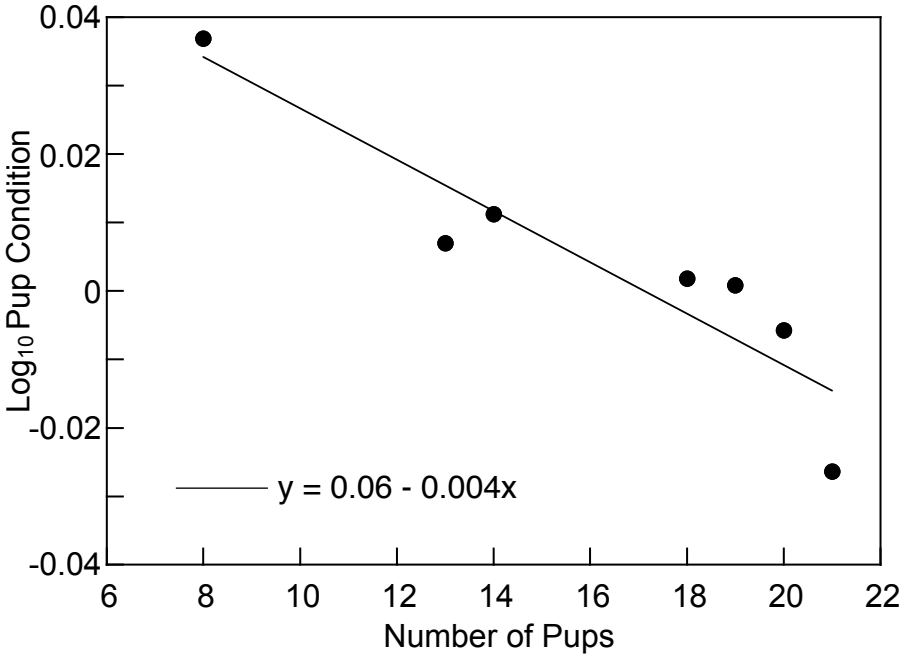

0.04
0.02
0
-0.02
-0.04
Log10 Pup Condition
y = 0.06 - 0.004x
6
8
10
12
14
16
18
20
22
Number of Pups

Figure 20

APPENDIX I

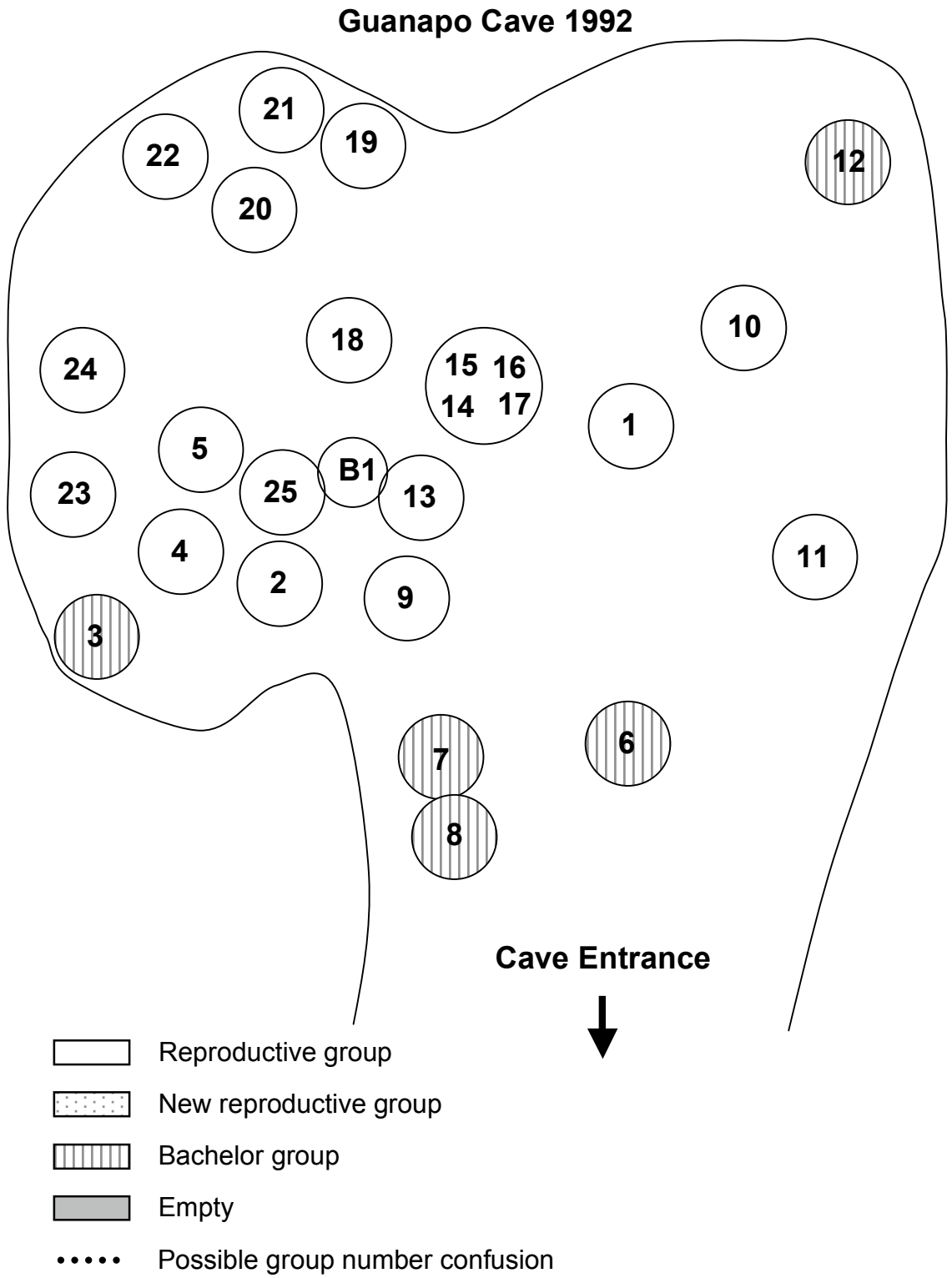
Diagrams of Roost Sites in Guanapo Cave

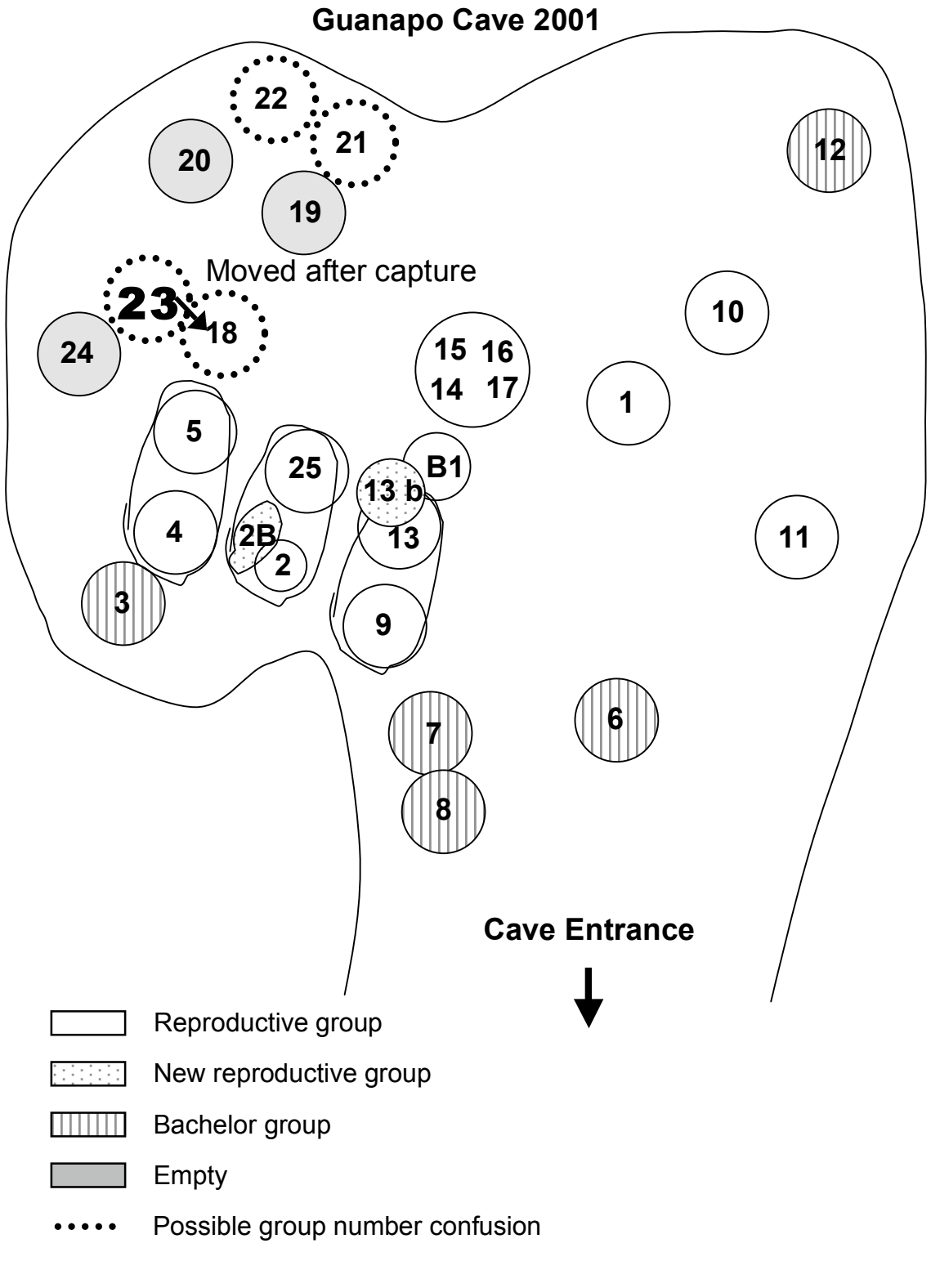
Figure 21. Guanapo Cave 1992. Diagram from G. S. Wilkinson.

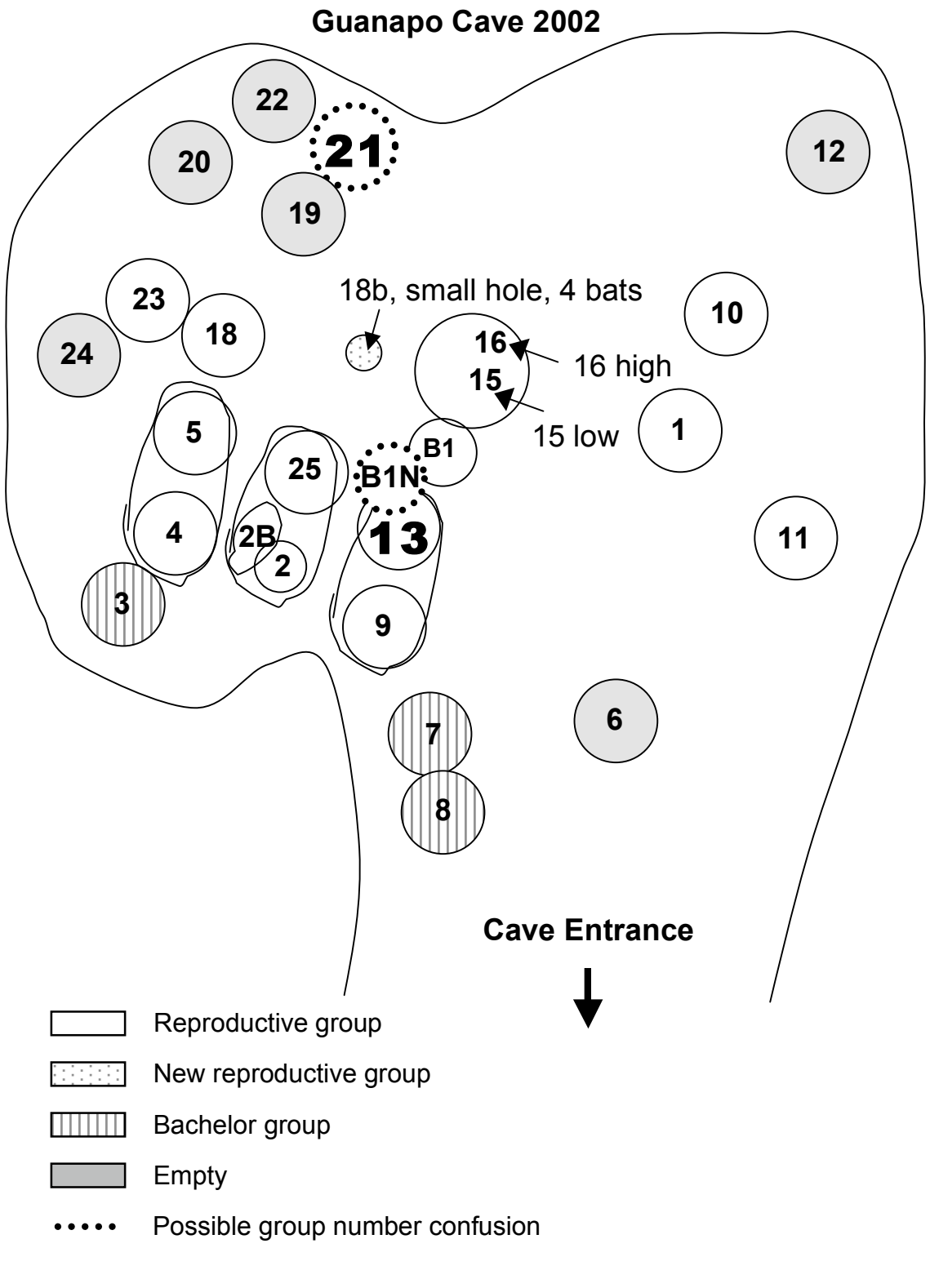
Figure 22. Guanapo Cave 2001. One group was bleach marked (23) and is designated by large bold type.

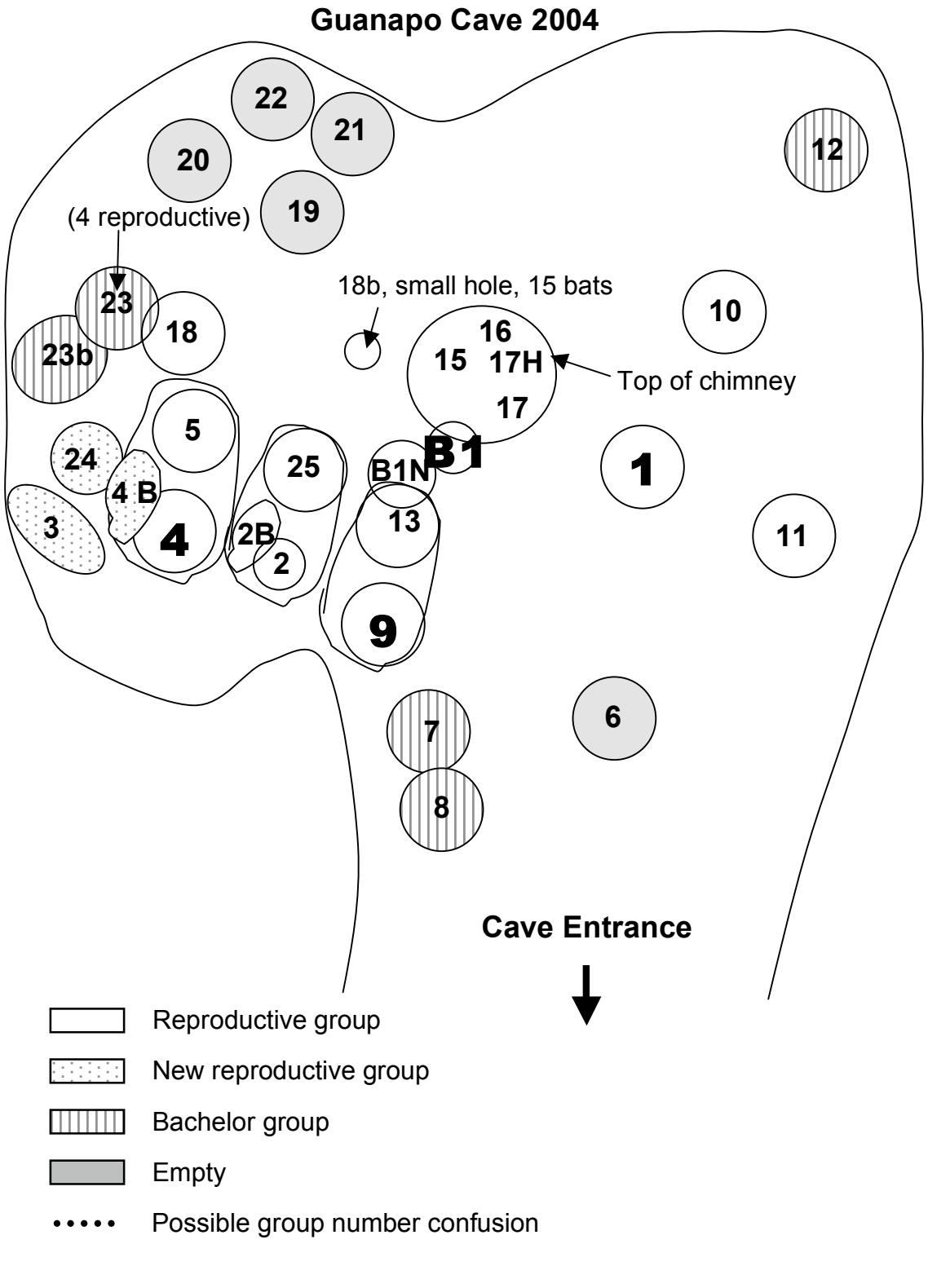
Figure 23. Guanapo Cave 2002. Two groups were bleach marked (13 and 21) and are designated by large bold type.

Figure 24. Guanapo Cave 2004. Four groups were bleach marked (1, 4, 9, B1) and are designated by large bold type.


Guanapo Cave 1992
21
19
22
20
12
18
10
24
15 16
14 17
1
5
B1
23
25
13
4
11
2
9
3
7
6
8
Cave Entrance
Reproductive group
New reproductive group
Bachelor group
Empty
Possible group number confusion


Guanapo Cave 2001
22
21
20
19
12
Moved after capture
23
18
24
10
15 16
14 17
1
5
25
B1
13 b
4
2B
13
11
2
3
9
7
6
8
Cave Entrance
Reproductive group
New reproductive group
Bachelor group
Empty
Possible group number confusion


Guanapo Cave 2002
22
21
20
19
12
18b, small hole, 4 bats
23
18
10
24
16
16 high
15
15 low
1
5
B1
25
B1N
4
2B
13
11
2
3
9
7
6
8
Cave Entrance
Reproductive group
New reproductive group
Bachelor group
Empty
Possible group number confusion


Guanapo Cave 2004
22
21
20
19
12
(4 reproductive)
18b, small hole, 15 bats
23
18
10
23b
16
15
17H
17
Top of chimney
5
24
25
B1N
B1
1
4 B
3
4
2B
2
13
11
9
7
6
8
Cave Entrance
Reproductive group
New reproductive group
Bachelor group
Empty
Possible group number confusion

Literature Cited

Introduction

Balcombe, J. P. 1990. Vocal recognition of pups by mother Mexican free-tailed bats, *Tadarida brasiliensis mexicana*. *Anim. Behav.* 39: 960-966.

Beecher, M. D. 1982. Signature systems and kin recognition. *Am. Zool.* 22: 477-490.

Beecher, M. D. 1989. Signaling systems for individual recognition - an information theory approach. *Anim. Behav.* 38: 248-261.

Boughman, J. W. 1997. Greater spear-nosed bats give group-distinctive calls. *Behav. Ecol. Sociobiol.* 40: 61-70.

Boughman, J. W. 1998. Vocal learning by greater spear-nosed bats. *Proc. R. Soc. Lond. B* 265: 227-233.

Clutton-Brock, T. H., Brotherton, P. N. M., O'Riain, M. J., Griffin, A. S., Gaynor, D., Sharpe, L., Kansky, R., Manser, M. B. & McIlrath, G. M. 2000. Individual contributions to babysitting in a cooperative mongoose, *Suricata suricatta*. *Proc. R. Soc. Lond. B* 267: 301-305.

Creel, S. R., Monfort, S. L., Wildt, D. E. & Waser, P. M. 1991. Spontaneous lactation is an adaptive result of pseudopregnancy. *Nature* 351: 660-662.

Dugatkin, L. A. 1997. *Cooperation Among Animals: An Evolutionary Perspective*. Oxford: Oxford University Press.

Emlen, S. T. & Wrege, P. H. 1988. The role of kinship in helping decisions among white-fronted bee eaters. *Behav. Ecol. Sociobiol.* 23: 305-315.

Gould, E., Woolf, N. K. & Turner, D. C. 1973. Double-note communication calls in bats - occurrence in 3 families. *J. Mammal.* 54: 998-1001.

Griffin, D. R. & Novick, A. 1955. Acoustic orientation of neotropical bats. *J. Exp. Zool.* 130: 251-300.

Hamilton, W. D. 1964. The genetical evolution of social behaviour. I and II. *J. Theor. Biol.* 7: 1-52.

Ims, R. A. 1990. The ecology and evolution of reproductive synchrony. *Trends Ecol. Evol.* 5: 135-140.

Job, D. A., Boness, D. J. & Francis, J. M. 1995. Individual variation in nursing vocalizations of Hawaiian monk seal pups, *Monachus schauinslandi* (Phocidae, Pinnipedia), and lack of maternal recognition. *Can. J. Zool.* 73: 975-983.

Loesche, P., Stoddard, P. K., Higgins, B. J. & Beecher, M. D. 1991. Signature versus perceptual adaptations for individual vocal recognition in swallows. *Behaviour* 118: 15-25.

McCracken, G. F. 1984. Communal nursing in Mexican free-tailed bat maternity colonies. *Science* 223: 1090-1091.

McCracken, G. F. 1987. Genetic structure of bat social groups. In: *Recent Advances in the Study of Bats* (Ed. by M. B. Fenton, P. Racey & J. M. V. Rayner), pp. 281-298. Cambridge: Cambridge University Press.

McCracken, G. F. & Bradbury, J. W. 1977. Paternity and genetic-heterogeneity in the polygynous bat, *Phyllostomus hastatus*. *Science* 198: 303-306.

McCracken, G. F. & Bradbury, J. W. 1981. Social organization and kinship in the polygynous bat *Phyllostomus hastatus*. *Behav. Ecol. Sociobiol.* 8: 11-34.

McCulloch, S., Pomeroy, P. P. & Slater, P. J. B. 1999. Individually distinctive pup vocalizations fail to prevent allo-suckling in grey seals. *Can. J. Zool.* 77: 716-723.

Owens, D. D. & Owens, M. J. 1984. Helping behavior in brown hyenas. *Nature* 308: 843-845.

Packer, C., Lewis, S. & Pusey, A. 1992. A comparative analysis of non-offspring nursing. *Anim. Behav.* 43: 265-281.

Porter, T. A. & Wilkinson, G. S. 2001. Birth synchrony in greater spear-nosed bats (*Phyllostomus hastatus*). *J. Zool.* 253: 383-390.

Pusey, A. E. & Packer, C. 1994. Non-offspring nursing in social carnivores: minimizing the costs. *Behav. Ecol.* 5: 362-374.

Pye, A. 1967. Structure of cochlea in chiroptera.3. microchiroptera - phyllostomatoidea. *J. Morphol.* 121: 241-254.

Richardson, D. S., Burke, T. & Komdeur, J. 2002. Direct benefits and the evolution of female-biased cooperative breeding in Seychelles warblers. *Evolution* 56: 2313-2321.

Riedman, M. L. 1982. The evolution of alloparental care and adoption in mammals in birds. *Q. Rev. Biol.* 57: 405-435.

Rood, J. P. 1990. Group size, survival, reproduction and routes to breeding in dwarf mongooses. *Anim. Behav.* 39: 566-572.

Roulin, A. 2002. Why do lactating females nurse alien offspring? A review of hypotheses and empirical evidence. *Anim. Behav.* 63: 201-208.

Santos, M., Aguirre, L. F., Vazquez, L. B. & Ortega, J. 2003. *Phyllostomus hastatus*. *Mammal. Spec.* 772: 1-6.

Stern, A. A. & Kunz, T. H. 1998. Intraspecific variation in postnatal growth in the greater spear-nosed bat. *J. Mammal.* 79: 755-763.

Stoddard, P. K. & Beecher, M. D. 1983. Parental recognition of offspring in the cliff swallow. *Auk* 100: 795-799.

Trillmich, F. 1981. Mutual mother-pup recognition in Galapagos fur seals and sea lions - cues used and functional significance. *Behaviour* 78: 21-42.

Wilkinson, G. S. & Boughman, J. W. 1998. Social calls coordinate foraging in greater spear-nosed bats. *Anim. Behav.* 55: 337-350.

Chapter I

Aitkin, L., Cochran, S., Frost, S., Martsi-McClintock, A. & Masterton, B. 1997. Features of the auditory development of the short-tailed Brazilian opossum, *Monodelphis domestica*: evoked responses, neonatal vocalizations and synapses in the inferior colliculus. *Hear. Res.* 113: 69-75.

Aitkin, L. M., Nelson, J. E. & Shepherd, R. K. 1994. Hearing, vocalization and the external ear of a marsupial, the northern quoll, *Dasyurus hallucatus*. *J. Comp. Neurol.* 349: 377-388.

Balcombe, J. P. 1990. Vocal recognition of pups by mother Mexican free-tailed bats, *Tadarida brasiliensis mexicana*. *Anim. Behav.* 39: 960-966.

Beecher, M. D. 1982. Signature systems and kin recognition. *Am. Zool.* 22: 477-490.

Beecher, M. D. 1989. Signaling systems for individual recognition - an information theory approach. *Anim. Behav.* 38: 248-261.

Beecher, M. D., Beecher, I. M. & Hahn, S. 1981. Parent-offspring recognition in bank swallows (*Riparia riparia*).2. Development and acoustic basis. *Anim. Behav.* 29: 95-101.

Boughman, J. W. 1997. Greater spear-nosed bats give group-distinctive calls. *Behav. Ecol. Sociobiol.* 40: 61-70.

Boughman, J. W. 1998. Vocal learning by greater spear-nosed bats. *Proc. R. Soc. Lond. B* 265: 227-233.

Boughman, J. W. & Wilkinson, G. S. 1998. Greater spear-nosed bats discriminate group mates by vocalizations. *Anim. Behav.* 55: 1717-1732.

Bradbury, J. W. & Vehrencamp, S. L. 1998. *Principles of Animal Communication*. Sunderland: Sinauer Associates.

Brown, P. E. & Grinnell, A. D. 1980. Echolocation ontogeny in bats. In: *Animal sonar systems* (Ed. by R.-G. Busnel & J. F. Fish), pp. 355-377. New York: Plenum Press.

Coles, R. B., Guppy, A., Anderson, M. E. & Schlegel, P. 1989. Frequency sensitivity and directional hearing in the gleaning bat, *Plecotus auritus* (Linnaeus 1758). *J. Comp. Physiol. A* 165: 269-280.

Colgan, P. 1983. *Comparative Social Recognition*. New York: John Wiley.

de Fanis, E. & Jones, G. 1995. Postnatal-growth, mother-infant interactions and development of vocalizations in the vespertilionid bat *Plecotus auritus*. *J. Zool.* 235: 85-97.

Dooling, R. J., Lohr, B. & Dent, M. L. 2000. Hearing in birds and reptiles. In: *Comparative Hearing In Birds And Reptiles* (Ed. by R. J. Dooling, R. R. Fay & A. N. Popper), pp. 308-359. New York: Springer-Verlag.

Ehret, G. 1989. Hearing in the mouse. In: *The Comparative Psychology Of Audition, Perceiving Complex Sounds* (Ed. by R. J. Dooling & S. H. Hulse), pp. 3-32. Hillsdale: Lawrence Erbaum Associates.

Emmons, L. H. 1997. *Neotropical Rainforest Mammals*. Chicago: The University of Chicago Press.

Esser, K.-H. 1994. Audio-vocal learning in a nonhuman mammal - the lesser spear- nosed bat *Phyllostomus discolor*. *Neuroreport* 5: 1718-1720.

Esser, K.-H. & Daucher, A. 1996. Hearing in the FM-bat *Phyllostomus discolor*: a behavioral audiogram. *J. Comp. Physiol. A* 178: 779-785.

Esser, K.-H. & Kiefer, R. 1996. Detection of frequency modulation in the FM-bat *Phyllostomus discolor*. *J. Comp. Physiol. A* 178: 787-796.

Esser, K.-H. & Lud, B. 1997. Discrimination of sinusoidally frequency-modulated sound signals mimicking species-specific communication calls in the FM-bat *Phyllostomus discolor*. *J. Comp. Physiol. A* 180: 513-522.

Esser, K.-H. & Schmidt, U. 1989. Mother-infant communication in the lesser spear nosed bat *Phyllostomus discolor* (Chiroptera, Phyllostomidae) - evidence for acoustic learning. *Ethol.* 82: 156-168.

Fay, R. R. 1988. *Hearing In Vertebrates: A Psychophysics Databook*. Winnetka: Hill-Fay Associates.

Frost, S. B. & Masterton, R. B. 1994. Hearing in primitive mammals *Monodelphis domestica* and *Marmosa elegans*. *Hear. Res.* 76: 67-72.

Gelfand, D. L. & McCracken, G. F. 1986. Individual variation in the isolation calls of Mexican free-tailed bat pups (*Tadarida brasiliensis mexicana*). *Anim. Behav.* 34: 1078-1086.

Gould, E. 1975. Neonatal vocalizations in bats of 8 genera. *J. Mammal.* 56: 15-29.

Gould, E. 1977. Echolocation and communication. In: *Special Publications the Museum of Texas Tech University, Biology of Bats of the New World Family Phyllostomatidae. Part II.* (Ed. by R. J. Baker, J. K. Jones & D. C. Carter), pp. 247-279. Lubbock: Texas Tech Press.

Griffin, D. R. & Novick, A. 1955. Acoustic orientation of neotropical bats. *J. Exp. Zool.* 130: 251-300.

Grinnell, A. D. 1970. Comparative auditory neurophysiology of neotropical bats employing different echolocation signals. *Z. Verg. Physiol.* 68: 117-153.

Guppy, A. & Coles, R. B. 1988. Acoustical and neural aspects of hearing in the Australian gleaning bats, *Macroderma gigas* and *Nyctophilus gouldi*. *J. Comp. Physiol. A* 162: 653-668.

Holmes, W. G. & Sherman, P. W. 1982. The ontogeny of kin recognition in two species of ground squirrels. *Am. Zool.* 22: 491-517.

Insley, S. J. 2001. Mother-offspring vocal recognition in northern fur seals is mutual but asymmetrical. *Anim. Behav.* 61: 129-137.

Kalko, E. K. V. & Condon, M. A. 1998. Echolocation, olfaction and fruit display: how bats find fruit of flagellichorous cucurbits. *Funct. Ecol.* 12: 364-372.

Koay, G., Bitter, K. S., Heffner, H. E. & Heffner, R. S. 2002. Hearing in American leaf-nosed bats. 1: *Phyllostomus hastatus*. *Hear. Res.* 171: 96-102.

Koay, G., Heffner, H. E. & Heffner, R. S. 1997. Audiogram of the big brown bat (*Eptesicus fuscus*). *Hear. Res.* 105: 202-210.

Kossl, M. 1992. High-frequency distortion products from the ears of two bat species, *Megaderma lyra* and *Carollia perspicillata*. *Hear. Res.* 60: 156-164.

Lacy, R. C. & Sherman, P. W. 1983. Kin recognition by phenotype matching. *Am. Nat.* 121: 489-512.

Long, G. R. 1977. Masked auditory thresholds from the bat, *Rhinolophus ferrumequinum*. *J. Comp. Physiol.* 116: 247-255.

Long, G. R. & Schnitzler, H. U. 1975. Behavioral audiograms from the bat, *Rhinolophus ferrumequinum*. *J. Comp. Physiol.* 100: 211-219.

Manly, B. F. 1991. *Randomization And Monte Carlo Methods In Biology*. London: Chapman and Hall.

Marten, K., Quine, D. & Marler, P. 1977. Sound transmission and its significance for animal vocalization.2. tropical forest habitats. *Behav. Ecol. Sociobiol.* 2: 291-302.

Matsumura, S. 1979. Mother infant communication in a horseshoe bat (*Rhinolophus ferrumequinum nippon*) - development of vocalization. *J. Mammal.* 60: 76-84.

McArthur, P. D. 1982. Mechanisms and development of parent-young vocal recognition in the pinon jay (*Gymnorhinus cyanocephalus*). *Anim. Behav.* 30: 62-74.

McCracken, G. F. & Bradbury, J. W. 1981. Social organization and kinship in the polygynous bat *Phyllostomus hastatus*. *Behav. Ecol. Sociobiol.* 8: 11-34.

Moss, C. F. & Schnitzler, H. U. 1995. Behavioral studies of auditory information. In: *Hearing By Bats* (Ed. by A. N. Popper & R. R. Fay), pp. 87-145. New York: Springer-Verlag.

Nakagawa, S., Waas, J. R. & Miyazaki, M. 2001. Heart rate changes reveal that little blue penguin chicks (*Eudyptula minor*) can use vocal signatures to discriminate familiar from unfamiliar chicks. *Behav. Ecol. Sociobiol.* 50: 180-188.

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

Niemiec, A. J. & Moody, D. B. 1995. Constant stimulus and tracking procedures for measuring sensitivity. In: *Methods In Comparative Psychoacoustics*

(Ed. by G. M. Klump, R. J. Dooling, R. R. Fay & W. C. Stebbins). Switzerland: Birkhauser Verlag Basel.

Okanoya, K. & Dooling, R. J. 1987. Hearing in passerine and psittacine birds - a comparative study of absolute and masked auditory thresholds. *J. Comp. Psychol.* 101: 7-15.

Pereira, M. E. 1986. Maternal recognition of juvenile offspring coo vocalizations in Japanese macaques. *Anim. Behav.* 34: 935-937.

Pye, A. 1967. Structure of cochlea in chiroptera.3. microchiroptera - phyllostomatoidea. *J. Morphol.* 121: 241-&.

Rother, G. & Schmidt, U. 1985. Ontogenetic development of vocalizations in *Phyllostomus discolor* (Chiroptera). *Z. Saugetierkd.* 50: 17-26.

Ryan, M. J., Tuttle, M. D. & Barclay, R. M. R. 1983. Behavioral responses of the frog-eating bat, *Trachops cirrhosus*, to sonic frequencies. *J. Comp. Physiol.* 150: 413-418.

Scherrer, J. A. & Wilkinson, G. S. 1993. Evening bat isolation calls provide evidence for heritable signatures. *Anim. Behav.* 46: 847-860.

Schmidt, S., Turke, B. & Vogler, B. 1983. Behavioural audiogram from the bat, *Megaderma lyra* (Geoffroy, 1810; Microchiroptera). *Myotis* 21/22: 62-66.

Schmidt, U., Schlegel, P., Schweizer, H. & Neuweiler, G. 1991. Audition in vampire bats, *Desmodus rotundus*. *J. Comp. Physiol. A* 168: 45-51.

Simmons, J. A. 1973. Resolution of target range by echolocating bats. *J. Acoust. Soc. Am.* 54: 157-173.

Simmons, J. A. & Stein, R. A. 1980. Acoustic imaging in bat sonar - echolocation signals and the evolution of echolocation. *J. Comp. Physiol.* 135: 61-84.

Smolker, R. A., Mann, J. & Smuts, B. B. 1993. Use of signature whistles during separations and reunions by wild bottle-nosed dolphin others and infants. *Behav. Ecol. Sociobiol.* 33: 393-402.

Stern, A. A. & Kunz, T. H. 1998. Intraspecific variation in postnatal growth in the greater spear-nosed bat. *J. Mammal.* 79: 755-763.

Stoddard, P. K. & Beecher, M. D. 1983. Parental recognition of offspring in the cliff swallow. *Auk* 100: 795-799.

Suthers, R. A. & Summers, C. A. 1980. Behavioral audiogram and masked thresholds of the megachiropteran echolocating bat, *Rousettus*. *j. Comp. Physiol.* 136: 227-233.

Trillmich, F. 1981. Mutual mother-pup recognition in Galapagos fur seals and sea lions - cues used and functional significance. *Behaviour* 78: 21-42.

Wenstrup, J. J. 1984. Auditory sensitivity in the fish-catching bat, *Noctilio leporinus*. *J. Comp. Physiol.* 155: 91-101.

Wiley, R. H. & Richards, D. G. 1982. Adaptations for acoustic communication in birds: sound transmission and signal detection. In: *Acoustic Communication in Birds* (Ed. by D. E. Kroodsma, E. H. Miller & H. Ouellet), pp. 131-181. New York: Academic Press.

Wilkinson, G. S. 1995. Information transfer in bats. *Symp. Zool. Soc. Lond.* 67: 345-360.

Wilkinson, G. S. 2003. Social and vocal complexity in bats. In: *Animal Social Complexity* (Ed. by F. B. M. de Waal & P. L. Tyack), pp. 322-341. Cambridge, Massachusetts: Harvard University Press.

Wilkinson, G. S. & Boughman, J. W. 1998. Social calls coordinate foraging in greater spear-nosed bats. *Anim. Behav.* 55: 337-350.

Wright, T. F., Cortopassi, K. A., Bradbury, J. W. & Dooling, R. J. 2003. Hearing and vocalizations in the orange-fronted conure (*Aratinga canicularis)*. *J. Comp. Psychol.* 117: 87-95.

Chapter II

Aitkin, L., Cochran, S., Frost, S., Martsi-McClintock, A. & Masterton, B. 1997. Features of the auditory development of the short-tailed Brazilian opossum, *Monodelphis domestica*: evoked responses, neonatal vocalizations and synapses in the inferior colliculus. *Hear. Res.* 113: 69-75.

Aitkin, L. M., Nelson, J. E. & Shepherd, R. K. 1994. Hearing, vocalization and the external ear of a marsupial, the northern quoll, *Dasyurus hallucatus*. *J. Comp. Neurol.* 349: 377-388.

Altringham, J. D. & Fenton, M. B. 2003. Sensory ecology and communication in the chiroptera. In: *Bat Ecology* (Ed. by T. H. Kunz & M. B. Fenton), pp. 90-127. Chicago: The University of Chicago Press.

Anderson, M. E. & Racey, P. A. 1991. Feeding behavior of captive brown long-eared bats, *Plecotus auritus*. *Anim. Behav.* 42: 489-493.

Barclay, R. M. R. & Harder, L. D. 2003. Life histories of bats: life in the slow lane. In: *Bat Ecology* (Ed. by T. H. Kunz & M. B. Fenton), pp. 209-253. Chicago: University of Chicago Press.

Bohn, K. M., Boughman, J. W., Wilkinson, G. S. & Moss, C. F. 2004. Auditory sensitivity and frequency selectivity in greater spear-nosed bats suggest specializations for acoustic communication. *J. Comp. Physiol. A* 190: 185-192.

Bradbury, J. W. & Vehrencamp, S. L. 1998. *Principles of Animal Communication*. Sunderland: Sinauer Associates.

Branchi, I., Santucci, D. & Alleva, E. 2001. Ultrasonic vocalisation emitted by infant rodents: a tool for assessment of neurobehavioural development. *Behav. Brain. Res.* 125: 49-56.

Brown, C. H. & Waser, P. M. 1984. Hearing and communication in blue monkeys (*Cercopithecus mitis*). *Anim. Behav.* 32: 66-75.

Brown, P. 1976. Vocal communication in the pallid bat, *Antrozous pallidus*. *Z. Tierpsych.* 41: 34-54.

Brown, P. E. & Grinnell, A. D. 1979. Echolocation ontogeny in bats. In: *Animal Sonar Systems* (Ed. by R. G. Busnel & J. F. Fish), pp. 355-377. New York: Plenum Press.

Brown, P. E., Grinnell, A. D. & Harrison, J. B. 1978. Development of hearing in the pallid bat, *Antrozous pallidus*. *J. Comp. Physiol.* 126: 169-182.

Coles, R. B., Guppy, A., Anderson, M. E. & Schlegel, P. 1989. Frequency sensitivity and directional hearing in the gleaning bat, *Plecotus auritus* (Linnaeus 1758). *J. Comp. Physiol. A* 165: 269-280.

de Fanis, E. & Jones, G. 1995. Postnatal growth, mother-infant interactions and development of vocalizations in the vespertilionid bat *Plecotus auritus*. *J. Zool.* 235: 85-97.

Dooling, R. J., Mulligan, J. A. & Miller, J. D. 1971. Auditory sensitivity and song spectrum of the common canary (*Serinus cararius*). *J. Acoust. Soc. Am.* 50: 700-708.

Ehret, G. 1989. Hearing in the mouse. In: *The Comparative Psychology Of Audition, Perceiving Complex Sounds* (Ed. by R. J. Dooling & S. H. Hulse), pp. 3-32. Hillsdale: Lawrence Erbaum Associates.

Endler, J. A. 1992. Signals, signal conditions, and the direction of evolution. *Am. Nat.* 139: S125-S153.

Esser, K. H. & Daucher, A. 1996. Hearing in the FM-bat *Phyllostomus discolor*: a behavioral audiogram. *J. Comp. Physiol. A* 178: 779-785.

Felsenstein, J. 1985. Phylogenies and the comparative method. *Am. Nat.* 125: 1-15.

Frost, S. B. & Masterton, R. B. 1994. Hearing in primitive mammals *Monodelphis domestica* and *Marmosa elegans*. *Hear. Res.* 76: 67-72.

Gould, E. 1977. Echolocation and communication. In: *Biology of Bats of the New World Family Phyllosomatidae. Part II. Special Publications of the*

Museum Texas Tech University. No. 13 (Ed. by R. J. Baker, J. K. Jones & D. C. Carter), pp. 247-279. Lubbock: Texas Tech Press.

Gould, E., Woolf, N. K. & Turner, D. C. 1973. Double-note communication calls in bats - occurrence in 3 families. *J. Mammal.* 54: 998-1001.

Grant, J. D. A. 1991. Prey location by two Australian long-eared bats, *Nyctophilus gouldi* and *N. geoffroyi*. *Aust. J. Zool.* 39: 45-56.

Griffin, D. R. 1971. The importance of atmospheric attenuation for the echolocation of bats (Chiroptera). *Anim. Behav.* 19: 55-61.

Grinnell, A. D. 1963. The neurophysiology of audition in bats: intensity and frequency parameteres. *J. Physiol.* 167: 38-66.

Grinnell, A. D. 1970. Comparative auditory neurophysiology of neotropical bats employing different echolocation signals. *Z. Verg. Physiol.* 68: 117-153.

Guppy, A. & Coles, R. B. 1988. Acoustical and neural aspects of hearing in the Australian gleaning bats, *Macroderma gigas* and *Nyctophilus gouldi*. *J. Comp. Physiol. A* 162: 653-668.

Habersetzer, J. & Marimuthu, G. 1986. Ontogeny of sounds in the echolocating bat *Hipposideros speoris*. *J. Comp. Physiol. A* 158: 247-257.

Harvey, P. H. & Pagel, M. D. 1991. *The comparative method in evolutionary biology*. New York: Oxford University Press.

Hauser, M. D. 1993. The evolution of nonhuman primate vocalizations: effects of phylogeny, body weight, and social context. *Am. Nat.* 142: 528-542.

Heffner, H. E. & Heffner, R. S. 1998. Hearing. In: *Comparative Psychology: A Handbook* (Ed. by G. Greenberg & M. Haraway). New York: Garland.

Heffner, R. S., Koay, G. & Heffner, H. E. 2003. Hearing in American leaf-nosed bats. III: *Artibeus jamaicensis*. *Hear. Res.* 184: 113-122.

Herbert, H. 1983. Vocal communication in the megachiropteran bat *Rousettus aegyptiacus*: development of isolation calls during postnatal ontogenesis. *Z. Saugetierkunde* 48: 187-189.

Herbert, H. 1985. Echolocation behavior in the megachiropteran bat, *Rousettus aegytiacus*. *Z. Saugetierkunde* 50: 141-152.

Holland, R. A., Waters, D. A. & Rayner, J. M. V. 2004. Echolocation signal structure in the Megachiropteran bat *Rousettus aegyptiacus* Geoffroy 1810. *J. Exp. Biol.* 207: 4361-4369.

Hoofer, S. R. & Bussche, V. D. 2003. Molecular phylogenetics of the chiropteran family Vespertilionidae. *Acta Chiropt.* 5: S1-S63.

Illmann, G., Schrader, L., Spinka, M. & Sustr, P. 2002. Acoustical mother-offspring recognition in pigs (*Sus scrofa domestica*). *Behaviour* 139: 487-505.

Jennings, N. V., Parsons, S., Barlow, K. E. & Gannon, M. R. 2004. Echolocation calls and wing morphology of bats from the West Indies. *Acta Chiropt.* 6: 75-90.

Jones, G. 1999. Scaling of echolocation call parameters in bats. *J. Exp. Biol.* 202: 3359-3367.

Jones, K. E., Bininda-Emonds, O. R. P. & Gittleman, J. L. 2005. Bats, clocks and rocks: diversification patterns in chiroptera. *Evolution* 59: 2243-2255.

Jones, K. E., Purvis, A., MacLarnon, A., Bininda-Emonds, O. R. P. & Simmons, N. B. 2002. A phylogenetic supertree of the bats (Mammalia: Chiroptera). *Biol. Rev.* 77: 223-259.

Koay, G., Bitter, K. S., Heffner, H. E. & Heffner, R. S. 2002. Hearing in American leaf-nosed bats. I: *Phyllostomus hastatus*. *Hear. Res.* 171: 96-102.

Koay, G., Heffner, H. E. & Heffner, R. S. 1997. Audiogram of the big brown bat (*Eptesicus fuscus*). *Hear. Res.* 105: 202-210.

Koay, G., Heffner, R. S., Bitter, K. S. & Heffner, H. E. 2003. Hearing in American leaf-nosed bats, II: *Carollia perspicillata*. *Hear. Res.* 178: 27-34.

Koay, G., Heffner, R. S. & Heffner, H. E. 1998. Hearing in a megachiropteran fruit bat (*Rousettus aegyptiacus*). *J. Comp. Psychol.* 112: 371-382.

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

Long, G. R. & Schnitzler, H. U. 1975. Behavioral audiograms from the bat, *Rhinolophus ferrumequinum*. *J. Comp. Physiol.* 100: 211-219.

Marler, P. & Sherman, V. 1985. Innate differences in singing behaviour of sparrows reared in isolation from adult conspecific song. *Anim. Behav.* 33: 57-71.

Matsumura, S. 1979. Mother-infant communication in a horseshoe bat (*Rhinolophus ferrumequinum nippon*) development of vocalization. *J. Mammal.* 60: 76-84.

Moss, C. F. & Schnitzler, H. U. 1995. Behavioral studies of auditory information. In: *Hearing by Bats* (Ed. by A. N. Popper & R. R. Fay), pp. 87-145. New York: Springer-Verlag.

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

Purvis, A. & Rambaut, A. 1995. Comparative analysis by independent contrasts (CAIC):an Apple Macintosh application for analyzing comparative data. *Computer Applic. Biosci.* 11: 247-251.

Rubsamen, R. 1987. Ontogeny of the echolocation system in the rufous horseshoe bat, *Rhinolophus rouxi* (audition and vocalization in early postnatal development). *J. Comp. Physiol. A* 161: 899-913.

Rubsamen, R., Neuweiler, G. & Marimuthu, G. 1989. Ontogenesis of tonotopy in the inferior colliculus of a hipposiderid bat reveals postnatal shift in frequency-place code. *J. Comp. Physiol. A* 165: 755-769.

Ryan, M. J. & Brenowitz, E. A. 1985. The role of body size, phylogeny, and ambient noise in the evolution of bird song. *Am. Nat.* 126: 87-100.

Scherrer, J. A. & Wilkinson, G. S. 1993. Evening bat isolation calls provide evidence for heritable signatures. *Anim. Behav.* 46: 847-860.

Schmidt, U., Schlegel, P., Schweizer, H. & Neuweiler, G. 1991. Audition in vampire bats, *Desmodus rotundus*. *J. Comp. Physiol. A* 168: 45-51.

Schnitzler, H. U. & Kalko, E. K. V. 2001. Echolocation by insect-eating bats. *Biosci.* 51: 557-569.

Schuller, G. 1980. Hearing characteristics and doppler-shift compensation in south Indian CF-FM bats. *J. Comp. Physiol.* 139: 349-356.

Siemers, B. M. & Schnitzler, H. U. 2005. Echolocation signals reflect niche differentiation in five sympatric congeneric bat species. *Nature* 429: 657-661.

Simmons, J. A. & Stein, R. A. 1980. Acoustic imaging in bat sonar - echolocation signals and the evolution of echolocation. *J. Comp. Physiol.* 135: 61-84.

Smolker, R. A., Mann, J. & Smuts, B. B. 1993. Use of signature whistles during separations and reunions by wild bottle-nosed dolphin others and infants. *Behav. Ecol. Sociobiol.* 33: 393-402.

Sterbing, S. J. 2002. Postnatal development of vocalizations and hearing in the phyllostomid bat, *Carollia perspicillata*. *J. Mammal.* 83: 516-525.

Symmes, D. & Biben, M. 1985. Maternal recognition of individual infant squirrel monkeys from isolation call playbacks. *Am. J. Primatol.* 9: 39-46.

Teeling, E. C., Springer, M. S., Madsen, O., Bates, P., O'Brien, S. J. & Murphy, W. J. 2005. A molecular phylogeny for bats illuminates biogeography and the fossil record. *Science* 307: 580-584.

Trillmich, F. 1981. Mutual mother-pup recognition in Galapagos fur seals and sea lions - cues used and functional significance. *Behaviour* 78: 21-42.

Vater, M. 1987. Narrow-band frequency analysis in bats. In: *Recent Advances in the Study of Bats* (Ed. by M. B. Fenton, P. Racey & J. M. V. Rayner), pp. 200-225. Cambridge: Cambridge University Press.

Webster, D. B., Popper, A. N. & Fay, R. R. 1992. *The evolutionary biology of hearing*. New York: Springer-Verlag.

Wenstrup, J. J. 1984. Auditory sensitivity in the fish-catching bat, *Noctilio leporinus*. *J. Comp. Physiol.* 155: 91-101.

Wilczynski, W., Rand, A. S. & Ryan, M. J. 2001. Evolution of calls and auditory tuning in the *Physaleaemus pustulosus* species. *Brain Behav. Evol.* 58: 137-151.

Chapter III

Balcombe, J. P. 1990. Vocal recognition of pups by mother Mexican free-tailed bats, *Tadarida brasiliensis mexicana*. *Anim. Behav.* 39: 960-966.

Beecher, M. D. 1982. Signature systems and kin recognition. *Am. Zool.* 22: 477-490.

Beeman, K. 1996. *SIGNAL/RTSD User's Guide*. Belmont: Engineering Design.

Bohn, K. M., Boughman, J. W., Wilkinson, G. S. & Moss, C. F. 2004. Auditory sensitivity and frequency selectivity in greater spear-nosed bats suggest specializations for acoustic communication. *J. Comp. Physiol. A* 190: 185-192.

Bradbury, J. W. & Vehrencamp, S. L. 1998. *Principles of Animal Communication*. Sunderland: Sinauer Associates.

de Fanis, E. & Jones, G. 1995. Postnatal growth, mother-infant interactions and development of vocalizations in the vespertilionid bat *Plecotus auritus*. *J. Zool.* 235: 85-97.

de Fanis, E. & Jones, G. 1996. Allomaternal care and recognition between mothers and young in pipistrelle bats (*Pipistrellus pipistrellus*). *J. Zool.* 240: 781-787.

Dooling, R. J. & Okanoya, K. 1995. Pscyhophysical methods for assessing perceptual categories. In: *Methods in Comparative Psychoacoustics* (Ed. by G. M. Klump, R. J. Dooling, R. R. Fay & W. C. Stebbins), pp. 307-318. Basel: Birkhåuser Verlag.

Esser, K.-H. 1994. Audio-vocal learning in a nonhuman mammal - the lesser spear- nosed bat *Phyllostomus discolor*. *Neuroreport* 5: 1718-1720.

Esser, K.-H. & Kiefer, R. 1996. Detection of frequency modulation in the FM-bat *Phyllostomus discolor*. *J. Comp. Physiol. A* 178: 787-796.

Esser, K.-H. & Lud, B. 1997. Discrimination of sinusoidally frequency-modulated sound signals mimicking species-specific communication calls in the FM-bat *Phyllostomus discolor*. *J. Comp. Physiol. A* 180: 513-522.

Gelfand, D. L. & McCracken, G. F. 1986. Individual variation in the isolation calls of Mexican free-tailed bat pups (*Tadarida brasiliensis mexicana*). *Anim. Behav.* 34: 1078-1086.

Gellerman, L. W. 1933. Chance orders of alternating stimuli in visual discrimination experiments. *J. Gen. Psych.* 42: 206-208.

Gould, E., Woolf, N. K. & Turner, D. C. 1973. Double-note communication calls in bats - occurrence in 3 families. *J. Mammal.* 54: 998-1001.

Lacy, R. C. & Sherman, P. W. 1983. Kin recognition by phenotype matching. *Am. Nat.* 121: 489-512.

McCracken, G. F. & Bradbury, J. W. 1977. Paternity and genetic-heterogeneity in the polygynous bat, *Phyllostomus hastatus*. *Science* 198: 303-306.

McCracken, G. F. & Bradbury, J. W. 1981. Social organization and kinship in the polygynous bat *Phyllostomus hastatus*. *Behav. Ecol. Sociobiol.* 8: 11-34.

Porter, T. A. & Wilkinson, G. S. 2001. Birth synchrony in greater spear-nosed bats (*Phyllostomus hastatus*). *J. Zool.* 253: 383-390.

Rasmuson, T. M. & Barclay, R. M. R. 1992. Individual variation in the isolation calls of newborn big brown bats (*Eptesicus fuscus*) - is variation genetic? *Can. J. Zool.* 70: 698-702.

Rother, G. & Schmidt, U. 1985. Ontogenetic development of vocalization in *Phyllosomus discolor* (Chiroptera). *Z. Saugetierkunde* 50: 17-26.

Scherrer, J. A. & Wilkinson, G. S. 1993. Evening bat isolation calls provide evidence for heritable signatures. *Anim. Behav.* 46: 847-860.

Stern, A. A. & Kunz, T. H. 1998. Intraspecific variation in postnatal growth in the greater spear-nosed bat. *J. Mammal.* 79: 755-763.

Stoddard, P. K. & Beecher, M. D. 1983. Parental recognition of offspring in the cliff swallow. *Auk* 100: 795-799.

Suthers, R. A. & Summers, C. A. 1980. Behavioral audiogram and masked thresholds of the megachiropteran echolocating bat, *Rousettus*. *J. Comp. Physiol.* 136: 227-233.

Thomson, C. E., Fenton, M. B. & Barclay, R. M. R. 1985. The role of infant isolation calls in mother infant reunions in the little brown bat, *Myotis lucifugus* (Chiroptera, Vespertilionidae). *Can. J. Zool.* 63: 1982-1988.

Trillmich, F. 1981. Mutual mother-pup recognition in Galapagos fur seals and sea lions - cues used and functional significance. *Behaviour* 78: 21-42.

Chapter IV

Boughman, J. W. 1998. Vocal learning by greater spear-nosed bats. *Proc. R. Soc. Lond. B* 265: 227-233.

Clutton-Brock, T. H., Brotherton, P. N. M., O'Riain, M. J., Griffin, A. S., Gaynor, D., Sharpe, L., Kansky, R., Manser, M. B. & McIlrath, G. M. 2000. Individual contributions to babysitting in a cooperative mongoose, *Suricata suricatta*. *Proc. R. Soc. Lond. B* 267: 301-305.

Creel, S. R., Monfort, S. L., Wildt, D. E. & Waser, P. M. 1991. Spontaneous lactation is an adaptive result of pseudopregnancy. *Nature* 351: 660-662.

Dechmann, D. K. N., Garbely, E., Kerth, G. & Garner, T. W. J. 2002. Highly polymorphic microsatellites for the study of the round-eared bat, *Tonatia silvicola* (d'Orbigny). *Conserv. Genet.* 3: 455-458.

Edington, E. S. 1995. *Randomization Tests, Third Edition, Revised and Expanded*. New York: Marcel Dekker.

Emlen, S. T. & Wrege, P. H. 1988. The role of kinship in helping decisions among white-fronted bee eaters. *Behav. Ecol. Sociobiol.* 23: 305-315.

Gemmell, N. J. 2003. Kin selection may influence fostering behaviour in Antarctic fur seals (*Arctocephalus gazella*). *Proc. R. Soc. Lond. B* 270: 2033-2037.

Gould, E., Woolf, N. K. & Turner, D. C. 1973. Double-note communication calls in bats - occurrence in 3 families. *J. Mammal.* 54: 998-1001.

Hamilton, W. D. 1964. The genetical evolution of social behaviour. I and II. *J. Theor. Biol.* 7: 1-52.

Ims, R. A. 1990. The ecology and evolution of reproductive synchrony. *Trends Ecol. Evol.* 5: 135-140.

Kokko, H., Johnstone, R. A. & Clutton-Brock, T. H. 2000. The evolution of cooperative breeding through group augmentation. *Proc. Natl. Acad. Sci. U. S. A.* 268: 187-196.

Manning, C. J., Wakeland, E. K. & Potts, W. K. 1992. Communal nesting patterns in mice implicate MHC genes in kin recognition. *Nature* 360: 581-583.

McCracken, G. F. 1984. Communal nursing in Mexican free-tailed bat maternity colonies. *Science* 223: 1090-1091.

McCracken, G. F. 1987. Genetic structure of bat social groups. In: *Recent Advances in the Study of Bats* (Ed. by M. B. Fenton, P. Racey & J. M. V. Rayner), pp. 281-298. Cambridge: Cambridge University Press.

McCracken, G. F. & Bradbury, J. W. 1977. Paternity and genetic-heterogeneity in the polygynous bat, *Phyllostomus hastatus*. *Science* 198: 303-306.

McCracken, G. F. & Bradbury, J. W. 1981. Social organization and kinship in the polygynous bat *Phyllostomus hastatus*. *Behav. Ecol. Sociobiol.* 8: 11-34.

Ortega, J., Maldonado, J. E., Arita, H. T., Wilkinson, G. S. & Fleischer, R. C. 2002. Characterization of microsatellite loci in the Jamaican fruit-eating bat *Artibeus jamaicensis* and cross-species amplification. *Mol. Ecol. Notes* 2: 462-464.

Owens, D. D. & Owens, M. J. 1984. Helping behavior in brown hyenas. *Nature* 308: 843-845.

Packer, C., Lewis, S. & Pusey, A. 1992. A comparative analysis of non-offspring nursing. *Anim. Behav.* 43: 265-281.

Porter, T. A. & Wilkinson, G. S. 2001. Birth synchrony in greater spear-nosed bats (*Phyllostomus hastatus*). *J. Zool.* 253: 383-390.

Pusey, A. E. & Packer, C. 1994. Non-offspring nursing in social carnivores: minimizing the costs. *Behav. Ecol.* 5: 362-374.

Queller, D. C. & Goodnight, K. F. 1989. Estimating relatedness using genetic markers. *Evolution* 43: 258-275.

Raymond, M. & Rousset, F. 1995. Genepop (Version-1.2) - population-genetics software for exact tests and ecumenicism. *J. Hered.* 86: 248-249.

Riedman, M. L. 1982. The evolution of alloparental care and adoption in mammals in birds. *Q. Rev. Biol.* 57: 405-435.

Rood, J. P. 1990. Group size, survival, reproduction and routes to breeding in dwarf mongooses. *Anim. Behav.* 39: 566-572.

Roulin, A. 2002. Why do lactating females nurse alien offspring? A review of hypotheses and empirical evidence. *Anim. Behav.* 63: 201-208.

Stern, A. A. & Kunz, T. H. 1998. Intraspecific variation in postnatal growth in the greater spear-nosed bat. *J. Mammal.* 79: 755-763.

Wilkinson, G. S. & Boughman, J. W. 1998. Social calls coordinate foraging in greater spear-nosed bats. *Anim. Behav.* 55: 337-350.