THE USE OF ULTRASOUND FOR COMMUNICATION BY THE BIG BROWN BAT (*EPTESICUS FUSCUS*)

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THE USE OF ULTRASOUND FOR COMMUNICATION BY THE BIG BROWN BAT (*EPTESICUS FUSCUS*)

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THE USE OF ULTRASOUND FOR COMMUNICATION BY THE BIG BROWN BAT (*EPTESICUS FUSCUS*)

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Matthew Earl Grilliot, son of Earl Hubert and Sherron (McGettigan) Grilliot, was born September 13, 1974, in Birmingham, Alabama. He graduated from Grissom High School, Huntsville, Alabama, in 1992. He graduated with a Bachelor of Science degree in Wildlife Science from Auburn University in December 1998. He married Amy Lynn Lucas, daughter of Dr. Aaron and Donna (Jones) Lucas, on May 20, 2000. He and his wife have a son, Matthew Alexander Grilliot, born on June 19, 2003; and a daughter, Vivian Lucas Grilliot, born on March 23, 2006.

DISSERTATION ABSTRACT

THE USE OF ULTRASOUND FOR COMMUNICATION BY THE BIG BROWN BAT (*EPTESICUS FUSCUS*)

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Communication signals are important regulators of mating behavior in many animals. Various pre- and post-copulatory mechanisms have been suggested to play a role in the reproductive success and mating strategies of many mammals. Recent studies have cited sperm competition as a possible post-copulatory mechanism of selection in bats, but few studies have examined which pre-copulatory mechanisms influence mate selection. Although it is generally accepted that bats emit vocalizations that function for communication purposes as well as the more universally recognized echolocation function, there is lack of actual empirical support for this idea. In this dissertation, I test the hypothesis that ultrasonic vocalizations of big brown bats are sexually dimorphic and differ contextually in the mating season. I used playback experiments to test the response of male and female big brown bats to variations in ultrasonic vocalizations of the opposite sex and to determine if ultrasonic vocalizations are used for mate selection. My data suggest that males were likely to select ultrasonic vocalization of frequently copulating females, but females did not select ultrasonic vocalizations of frequently copulating males over infrequently copulating males. These results suggest that mate selection of male big brown bats is influenced by ultrasonic vocalizations of females.

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INTRODUCTION

Communication has different meanings for researchers in different fields (Rogers and Kaplan 2000). Most agree that communication involves transfer of information, in the form of a chemical or physical signal, from a sender to a receiver. The receiver uses the information in formulating a response. Communication can be intentional, such as vocal interaction of an animal with its mate, as well as unintentional, such as eavesdropping on that interaction by a predator. The common thread in all definitions of communication is the idea of information transfer. No matter how communication is defined, it is used by most animals and may involve any of the senses.

Mammals use a combination of touch, vision, smell, and hearing to communicate; but the ecology of the species influence the modes of communication that are most important (Sales & Pye 1974; Fenton 1985). For example, visual communication signals are categorized as badges or displays. Badges are structural adaptations (i.e., antlers of male deer), and displays are behavioral adaptations (i.e., male elephant sways back and forth, stomps its feet, and flaps its ears). Visual communication signals are particularly important for many diurnal species during mate selection. Although the sender and receiver must be in the line of sight, visual communication in the form of displays and badges can provide accurate information about potential quality of a mate. Conversely, nocturnal species are active when use of vision is limited, and it is no surprise that olfactory and acoustic communication are important in these situations. Olfactory signals, in the form of pheromones, play an important role in mammalian reproductive behaviors. In mammals, pheromones are chemical signals released from a wide array of scent glands that act on conspecifics and function to regulate various social interactions (Bradbury & Vehrencamp 1998; Buck 2000; Bloss et al. 2002). Functional effects of pheromones can best be seen in male and female mating behaviors of rodents (Buck 2000). Animals use acoustic vocalizations for a variety of purposes, including individual recognition (Rendall et al. 1996), courtship and mating (Bosch et al. 2000), predator avoidance (Hauser 1988), and navigation and foraging (Fenton 1985). Although acoustic communication signals have their disadvantages (i.e., they may reveal location of sender to a predator, they may be energetically expensive to produce, and they rapidly attenuate), they have the advantage of high information content (i.e. a result of signal variation in form, function, and complexity) and are not limited by environmental barriers. Thus, acoustic signals (ranging from infrasonic [<20Hz to ultrasonic >20,000Hz]) are an important method of communication among many species particularly, insects, fish, amphibians, birds, and mammals (Bradbury & Vehrencamp 1998).

Sexual and seasonal variation in vocal behavior is well documented across a wide range of taxa in which acoustic communication signals are important regulators of mating behaviors (Catchpole 1982; Bosch et al. 2002). For example, spectral analysis of advertisement calls of male and female eared grebes (*Podiceps nigricollis*) look visually similar; however, calls of females are higher in frequency and duration, and playback studies have shown that male grebes can distinguish between vocalizations of unpaired males and unpaired females (Nuechterlein & Buitron 1992). In most song birds, temporal and spectral features of the song change as hormone levels increase in response to changes in photoperiod (i.e., season). For example, in male songbirds, rate of singing increases and "richness" of song improves as testosterone levels increase in the breeding season. Furthermore, many acoustic communication signals exhibit context-dependent variation influenced by the ecological (e.g., season, time of day) and social context (e.g., reproductive, territorial, predatory) of the situation (Catchpole 1979: Ehrlich et al. 1988; Ruiz-Miranda et al. 2002). For example, low-frequency signals sent by female African elephants (*Loxodonta africana*) change in response to social context, rank, and hormonal cycle (Leong et al. 2005).

While a variety of animals can produce ultrasonic signals, much less is known about the function of these high-frequency signals than about the function of lower-frequency signals. In general, ultrasonic vocalizations fall into one of two categories based on their intended purpose: 1) vocalizations emitted for social purposes and 2) vocalizations emitted for echolocation purposes. Ultrasonic vocalizations produced by some insects and lower vertebrates function for communicative purposes, but are not used for echolocation purposes. For example, evolution of tympanal sound receptors in moths set the stage for later evolution of ultrasonic courtship signals; and these signals are now used for species recognition, male-male competition for mates, and choice of mates by females (Conner 1999). There is recent evidence that ultrasonic vocalizations produced by concave-eared torrent frogs (*Amolops tormotus*) function for communication purposes. Feng et al. (2006) showed that male concave-eared torrent frogs hear and respond to ultrasonic components of advertisement calls of conspecifics.

In addition to some insects and lower vertebrates, many mammals are known to produce ultrasonic vocalizations that function for social purposes. Most insectivores (Class Mammalia, Order Insectivora) and rodents (Class Mammalia, Order Rodentia) live at least some part of their life in situations where light is limited; acoustic signals in the ultrasonic range play a fundamental role in the social system of these animals (Airapet'yants 1974). Infant rodents in the family Muridae emit ultrasonic distress calls in response to isolation from the nest, cold stress, and possibly hunger (Airapet'yants 1974; Elwood & Keeling 1981; Newman 1988). Ultrasonic signals also are used during aggressive encounters in rats, and may be important in dominance-subordination relationships (Airapet'yants 1974; Sales & Pye 1974). Shrews (family Soricidae) emit vocalization in the ultrasonic range that are suggested to be context dependent and have been categorized into calls used for alarm, defense, or aggression, calls used in mating behaviors, calls used in mother-young interactions, and calls used during exploration and foraging (Churchfield 1990).

While the intended function of these signals is not always clear, anatomical and behavioral studies of rodents (*Chinchilla laniger*, *Rattus norvegicus*, *Meriones unguiculatus*, *Mus musculus*, *Cavia porcellus*) and insectivores (Soricidae, Erinaceidae) suggest that these animals can hear well into the ultrasonic range (to 50 kHz) (Henson 1961; Airapets'yants & Konstantinov 1970; Fay & Popper 1994). Thus, in addition to ultrasonic vocalization for social purposes, some insectivores and rodents may produce ultrasonic vocalizations for echolocation. For example, studies on a variety of shrews determined that many of these animals produced ultrasonic pulse trains in foraging and orientation tasks that would be typical of echolocation signals (Gould 1969; Tomasi 1979; Forsman and Malmquist 1988; Feldehamer et al. 1999). Airapet'yants (1974) reported that voles (*Microtus socialis* and *M. gregalis*) that were blinded and had their vibrissae removed could perform orientation tasks in a darkened room while deafened voles could not perform these same tasks. The ability of blinded brown rats (*Rattus norvegicus*) to orient correctly in a Y-maze was suggested to provide evidence for echolocation in rodents (Bell et al. 1970; Chase 1980). Thomas and Jalili (2004) reported that brown rats produced pulses typical of echolocation only when placed in the dark. However, most insectivores and rodents have well-developed tactile (vibrissae) and olfactory communication systems that were not adequately controlled for in most of these studies; and more detailed studies are needed before conclusions can be made about the potential echolocation abilities of these animals (Thomas & Jalili 2004).

Cetaceans (Class Mammalia, Order Cetacea) and bats (Class Mammalia, Order Chiroptera) are the best known animals that use ultrasound (Sales & Pye 1974). While much has been learned about acoustic behavior of both of these mammalian groups, difficulties inherent in studying the social behavior of these animals in the field leaves much to be learned about the function of their vocalizations. Cetaceans and their sonar system evolved to function in the aquatic environment, whereas bats and their sonar system evolved to function in the terrestrial environment. Although there are differences in acoustic properties of sound in aquatic versus terrestrial environment, vocalizations of cetaceans and bats have broad functional similarities. For instance, cetaceans and bats produce vocalizations as a series of pulsed sounds, ranging from 30to 100 kHz in cetaceans and 12 to 200 kHz in bats and generally are believed to function for echolocation purposes (Herman & Tavolga 1980; Altringham 1996). Additionally, calls produced by cetaceans and bats are highly variable which indicates, at least fundamentally, the calls could be used for communication. In fact, species within each group (i.e. cetaceans and bats) use a repertoire of simple and complex calls suggested to be used for communication (Fenton 1985; Clark 1990). Although the intended function of these calls are not well described for either of these mammalian groups, simple and complex calls are typically low-frequency, narrowband or broadband signals that typically are produced in various social situations (i.e., aggressive encounters, sexually active groups, mother-young interactions) suggesting that the signals are used for communication (Thomas et al. 1979; Herman & Tavolga 1980; Balcombe 1990; Clark 1990).

Additionally, cetaceans produce calls that have been categorized as clicks, pulses, knocks, and grunts. These calls are typically short duration (<0.1s); but knocks and grunts fall in the low-frequency range (100-1000 Hz) and clicks and pulses fall in the high-frequency range (3-31 kHz). For some cetaceans, clicks and pulses may function for communication purposes. For example, when two or more fin whales (*Balaenoptera physalus*) approach they produce high-frequency calls that have been labeled as social and navigational (Edds 1988). Northern right whales (*Eubalaena glacialis*) produce high-frequency clicks and pulses when they are in sexually active groups, and it appears that only one whale calls at a time (Clark 1990). There is general agreement that most sounds produced by whales have a communicative function, but there also is disagreement among researchers in classifying the signals for a particular function (Herman & Tavolga 1980; Watkins & Wartzok 1985).

Although bats are well known for use of ultrasound for echolocation, there is limited evidence for its use in a social context. While little is known about evolution of echolocation in bats, it most likely evolved from signals used for acoustic communication (Fenton 1984; Obrist 1995). For example, studies by Jones et al. (1991) support the idea of overlap between sonic and ultrasonic vocalizations used for communication and those used for echolocation (Moss 1988). There appear to be vocalizations that specifically are used for communication, and transition between these sounds and those used for echolocation is gradual (Fenton 1984; Jones etal. 1991; Moss 1988). Furthermore, signals that are acoustically variable have the potential to communicate a great deal of information concerning the sender and situation. There is considerable variation in sonic and ultrasonic vocalizations both within and between species, and this lends support to the idea that ultrasonic vocalizations are used in communication (Fenton 1985). There is certainly inter-individual flexibility and inter-specific differences in ultrasonic vocalizations of bats, which suggests the possibility for use in communication (Kazial et al. 2001; Habersetzer 1981; Obrist 1995).

Studies of communication between mother and infant microchiroptera (Class Mammalia, Order Chiroptera) has determined that mothers of many species emit special sonic and ultrasonic search calls upon returning to the cave after foraging, and young have specific replies (Fenton 1985; Matsumura 1979 & 1981). In both mother and young, these vocalizations include sonic and ultrasonic components. In young bats, vocalizations serve as precursors to ultrasonic vocalizations used for echolocation (Jones et al. 1991). Although it is as yet undetermined whether components of ultrasonic vocalizations are genetically inherited or learned (Jones et al. 1990; Obrist 1995), Esser and Schmidt (1989) have shown that social sonic calls of bats require acoustic learning. Jones and Ransome (1993) suggested that fine-tuning of echolocation is partly learned from the mother, and that learning may be important in development of ultrasonic vocalizations used for echolocation. If mother bats can recognize ultrasonic vocalizations of their young, then bats familiar to each other may experience some degree of ultrasonic recognition. Furthermore, if fine-tuning of echolocation is partly learned from the mother, then some form of communication probably is required. All of these factors create a bridge between ultrasonic vocalizations used for echolocation and those used for communication.

Although many scientists dismiss self-communication (i.e., echolocation) as a form of communication, it is possible that ultrasonic vocalizations used for echolocation also are used for other types of communicative purposes (i.e., transmitting information to other bats). However, there is relatively little information on function of ultrasonic vocalizations outside the realm of navigation and foraging. Although acoustic signals are documented to play an important role in choice of mates in a variety of taxa (particularly insects, frogs, and birds), much less is known about their role in this context in mammals. Additionally, while a variety of animals produce ultrasonic signals, much less is known about functions of these high-frequency signals than about functions of lower-frequency signals. There are a variety of mating systems exhibited by bats, but much less is known about mechanisms involved in courtship and mating. While recent studies have suggested the importance of post-copulatory (i.e., sperm competition, sperm choice) mechanisms that function in reproductive success of some bats, there are certainly precopulatory mechanisms (vocalizations, phermones) that influence the mating system of most bats (Wilkinson & McCracken 2003, Vonhof et al. 2006). I suggest the possibility that, in addition to echolocation, bats use their ultrasonic vocalizations for certain reproductive behaviors.

Ultrasonic signals produced by bats during mating encounters may serve a social function; but, few studies have investigated the potential role of sonic/ultrasonic communication in the mating season. Consistent advancements in technology have led to improved studies of acoustic communication in regards to animal behavior. However, much of the work on echolocation, as it pertains to communication, has been done in the lab with small numbers of bats and in the non-mating season when interactions between males and females may be limited. In addition, many experiments focused on echolocation signals used for target detection. Uses of echolocation go far beyond that of simply target detection. Presently, there is no evidence for use of ultrasonic signals for communication during courtship or mating in bats. Objectives of the following studies were to determine if ultrasonic signals of big brown bats, *Eptesicus fuscus*, communicate multiple messages to conspecifics. I tested for sex and season differences in ultrasonic vocalization of male and female big brown bats. Additionally, I used binomial-choice playbacks to experimentally test if ultrasonic vocalizations function as a pre-copulatory mechanism in reproductive behaviors of big brown bats.

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CHAPTER 1

SEX AND SEASONAL DIFFERENCES IN COMPONENTS OF ULTRASONIC VOCALIZATIONS IN *EPTESICUS FUSCUS* (CHIROPTERA: MAMMALIA)

ABSTRACT

Although a variety of animals produce ultrasonic signals, less is known about the function of these higher-frequency signals than about the function of lower-frequency signals. Bats are well known for use of ultrasound for navigation and foraging, but there is limited evidence for its use in social contexts. Although, there are studies that have investigated the possibility of sexual differences in echolocation (i.e., ultrasonic) calls of bats, few have explored its function in the context of courtship and mating. We recorded ultrasonic calls of adult male and female big brown bats (*Eptesicus fuscus*) during mating and non-mating seasons. Seven call variables were analyzed for sexual and seasonal differences. Call components related to time were significantly greater in males compared to females within the mating season. However, no call components were significantly sexually dimorphic in the non-mating season. Additionally, when call components of the same individuals were compared in non-mating versus mating season, males significantly increased call components related to time from non-mating to mating season, while call components of females remained unchanged from non-mating to mating season. In addition, there was a significant correlation between start of

fundamental frequency (i.e., h1start) and mating score for males. These results suggest there is seasonal alteration in ultrasonic signals of male big brown bats and this alteration may be important in sexual recognition and some aspect of mate choice.

INTRODUCTION

Acoustic signals ranging from infrasonic (<20Hz) to ultrasonic (>20,000Hz) are an important method of communication among many species including insects, fishes, amphibians, rodents, birds, and cetaceans (Bradbury and Vehrencamp 1998). Although acoustic signals are documented to play an important role in mate choice in a variety of taxa (particularly insects, frogs, and birds), less is known about their role in this context in mammals. Additionally, while a variety of animals produce ultrasonic signals, less is known about functions of these high-frequency signals than about functions of lower-frequency signals.

Across a wide range of taxa (e.g., rodents, insects, and mammals), vocalizations in the ultrasonic range have been documented to be used in intraspecific and interspecific communication for a variety of social purposes (Sales and Pye 1974). For example, infant rodents in the family Muridae emit ultrasonic distress calls in response to isolation from the nest, cold stress, and possibly hunger. Ultrasonic signals also are used during aggressive encounters in rats, and may be important in dominance-subordination relationships (Sales and Pye 1974). Conner (1999) suggests that ultrasonic cues may be involved in all aspects of reproductive behavior of moths. The evolution of tympanal sound receptors in moths set the stage for later evolution of ultrasonic courtship signals; and these signals are now used for species recognition, male-male competition for mates, and female mate choice (Conner 1999).

Communication signals are important regulators of mating behavior in most mammalian species (Bosch et al. 2002), but few studies have investigated the potential role of sonic/ultrasonic communication in the mating season. Furthermore, while numerous studies have investigated social function of vocalizations within the sonic range of bats, little has been done regarding social function of vocalizations in the ultrasonic range. While bats are well known for using ultrasound for echolocation, there is little known about its function in social contexts. Although, a variety of mating systems have been documented in bats, there are a limited number of detailed studies, especially in mechanisms involved in courtship and mating of this group (Bradbury 1977a).

Vocalizations are considerably variable in bats, thus providing the opportunity for communication. In fact, bats emit sonic vocalizations specifically used for communication and these signals have components that span into the ultrasonic range (Fenton 1985; Moss 1988; Jones et. al. 1990). Because there is variation in the ultrasonic component of bat vocalizations, these signals also could fulfill a social communication function, and they may be important in mating success and mate choice. Although ultrasonic signaling in bats has the potential for communication purposes, there is little information on function of ultrasonic vocalizations outside the realm of navigation and foraging. It has been shown that ultrasonic vocalizations of bats are important in mother-young interactions (Thomson et al. 1985; Balcombe 1992). In addition, Thomas et al. (1979) provided circumstantial evidence suggesting that adult bats can determine the sex of other adult bats from ultrasonic vocalizations they use for echolocation. Other studies have shown that males and females respond differently to ultrasonic vocalizations used for echolocation (Neuweiler et al. 1987; Suga et al. 1987; Jones et al. 1992; Kazial and

Masters 2003). There is recent evidence from studies of *Rhinolophus ferrumequinum* and *Myotis bechsteinii* that suggests echolocation calls may be a source of information for individual recognition (Siemers et al. 2005; Siemers and Kerth 2006). Although studies have not yielded conclusive evidence of sexual dimorphism in ultrasonic vocalizations of bats, there were several deficiencies with studies that could have led to those findings: 1) the studies were done without specifically comparing calls from mating and non-mating seasons, 2) they only used a small number of bats (usually individually caged) and not naturally interacting, and 3) many experiments focused on echolocation signals used for target detection; and not specifically in the context of mating behavior (e.g., Masters et al. 1995; Russo et al. 2001; Siemers et al. 2005).

Despite these deficiencies, circumstantial evidence suggests that there are sexual differences in ultrasonic vocalization (Thomas et al. 1979; Neuweiler et al. 1987; Suga et al. 1987; Jones et al. 1992; Kazial et al. 2003) and raises the question of whether acoustic signals are used by bats not only for identification of sexes but also for reproductive behaviors such as courtship and mating. In big brown bats, females form maternity colonies while males form separate colonies in the non-mating season, and interaction between males and females is limited. If ultrasonic vocalizations are useful in a social context, then sexual dimorphism in vocalizations may not be important at this time of year. Conversely, there would be selective pressure to identify sexes when males and females congregate at hibernacula in the mating season, and it is possible that it is in this season that vocalizations may differ by sex as well as provide information in the context of mating behavior. In this study, I investigated the potential for sexual dimorphism in ultrasonic vocalizations of big brown bats (*Eptesicus fuscus*), and whether these differences would exhibit a seasonal component, changing between mating and non-mating seasons. If ultrasonic vocalizations are sexually dimorphic within the mating season, this finding would suggest that vocalizations may also have a social context. I also made detailed observations of mating behavior and copulation frequency for all bats studied and compared whether specific components of male and female ultrasonic calls are associated with variation in copulation frequency.

MATERIALS AND METHODS

Study species

Big brown bats (*Eptesicus fuscus*, Vespertilionidae) were chosen to study because they are locally abundant, thrive in captivity, and can be observed during both mating and non-mating periods. Relatively little is known about mating system of big brown bats; but they, as well as other species of Vespertilionidae, have been suggested to be promiscuous and random maters, with males and females having multiple partners (Thomas et al. 1979; Fenton 1984; Wai-Ping and Fenton 1988). We know that big brown bats exhibit a dissociated pattern of reproduction, mating in the autumn and winter months while gonads are regressed (males) or in stasis (females) (Oxberry 1979). In summer, ovaries mature to a point of stasis. Stasis is maintained along with basal sex-steroid levels through the mating season. Ovulation occurs in spring when sex-steroid levels peak, and the egg is fertilized with stored sperm from this winter mating period (Oxberry 1979) (Fig. 1).

Housing

Bats used in this study were wild-caught males and females from Alabama and

Georgia, or were descendent offspring from wild-caught individuals. Bats were collectively housed in environmentally controlled chambers (2.1m x 4.0m x 2.1m, EJS Limited, Inc., Stafford, TX) and allowed to interact at will during the mating season. During the non-mating season, males and females were housed separately in groups of their own sex, similar to natural associations. Bats were maintained on a diet of mealworms and water *ad libitum*, supplemented with vitamins and minerals. Behavioral information and standard body measurements are maintained for all bats in the colony. All bats were individually tagged with wing bands and have pit tags implanted for identification.

Environmental chambers allow researchers to alter temperature and photoperiod to induce desired behaviors or conditions. Mendonça and Hopkins (1997) documented that arousal from hibernation is a potent stimulator of sexual behavior in bats. During spring and summer, chambers were kept at 23°C with natural photoperiod. In November, bats are exposed to periods (e.g., 4 days) of low temperature (5°C) to induce hibernation. These periods of exposure to low temperatures, with alternating 4-7 day periods of 23°C to induce arousal and subsequent degree of mating activity, ended in early spring (March/April). This temperature regime has been documented to stimulate high degree of mating behavior and mimics natural seasonal variation during these months in Alabama (Mendonça et al. 1996; Mendonça and Hopkins 1997). Every night during arousal and peak mating periods, I observed bats for <9 hours/night. During observation sessions, I kept detailed records of all mating activity and associated behaviors (eg., female-male aggression, female-female aggression, male-male aggression, attempted male-male copulations, etc.). Again, each bat was individually pit tagged, and when bats engaged in mating, they were captured and identified. This allowed calculation of a mating score (number of copulations) for each individual male and female in the colony.

Recordings

Most bats use a combination of frequency-modulated (FM) and constant-frequency (CF) signals (Altringham 1996). The echolocation signals of *Eptesicus fuscus* are strongly frequency modulated (FM). In the lab, these signals are typically 2-5 ms, and characterized by a steep downward sweep from the original frequency to another, usually from about 60 to 30 kHz (Altringham 1996) (Fig. 2).

To obtain accurate ultrasonic signals under controlled conditions, bats were recorded while in an experimental anechoic room (2.1m x 2.1m x 2.1m). The experimental room was lined on walls and ceiling with 12.7cm-thick mattress foam, and covered with 5.1cm-thick corrugated mattress padding. The floor is covered with 5.1cm-thick corrugated mattress padding. Preliminary recordings showed that this design sufficiently reduced outside noise interference. Bats were placed individually in the experimental room, and I recorded ultrasonic vocalizations that each individual made during at least a one hour period. Bats were recorded before peak feeding times because prior feeding tends to limit activity level and calls (Boughman and Wilkinson 1998). Bats were introduced to the experimental room and given a brief period of time (~10 minutes) to acclimatize. When a bat began emitting ultrasonic vocalizations, I oriented the microphone to face the bat. I made <5 recordings, 8 seconds in duration, from each bat (therefore, each bat was recorded for >40 seconds) to attain >200 calls for each bat. Data from these calls were averaged, and mean values were used in statistical analysis.

From September 2003 to March 2004, I recorded ultrasonic signals of adult male and adult female big brown bats (see experiments for number of individuals used) in two different physiological states (sample period I and II). In sample period I (Aug.-Nov.), bats had undergone gonadal development but were not mating. In sample period II (Dec.-Feb.), bats were exposed to an alternating 5°C and 22°C temperature regime to stimulate mating activity (Fig. 1).

Each bat was recorded individually in an experimental anechoic chamber (2.4m x 2.4m x 2.4m). Echolocation calls were detected with a U30 bat detector (sensitivity typically 10 dB SPL at 50 kHz; >20 dB SPL 20-120 kHz). A custom built 6x amplifier (designed by W.M. Masters, The Ohio State University, Columbus, OH) was used to amplify output of the microphone. I used a program called CBDisk (Engineering Design, Belmont, MA) that allowed the user to acquire high-frequency signals direct-to-disk at sample rates <330 kHz. I used a Toshiba laptop with a Pentium III processor, 128MB of RAM, and a 6.4 GB hard disk. Recordings consisted of a single file that contained many echolocation calls. These individual calls were extracted from the large files so that they could be analyzed individually (Burnett and Masters 2001). After extraction, calls were analyzed with a custom program written in Matlab (Version 6.5 Mathworks, Inc., Natick, MA) that automatically measured the value of 34 different variables that were used to describe calls (7 variables that were direct descriptors and 27 variables that used mathematical equations to describe the call) (Burnett and Masters 2001). After calls were analyzed, I analyzed 7 variables (Table 1, Fig. 2) that were direct descriptors of the call and have been used to discriminate individual bats (Burnett et al. 2001). Because I was using untrained bats that could move about the anechoic chamber at will, directional sensitivity of the U30 bat detector prevented me from using amplitude as one of my variables. Requirements for choosing calls were as follows: signals must be loud enough to start computer acquisition, signals must be of sufficient length to be useful, and signals must contain enough information to determine the value for 7 descriptors (Burnett et al. 2001).

I investigated seasonal differences in ultrasonic vocalizations of male and female bats by comparing recordings from the same bats taken in the non-mating season (sample period I) and the mating season (sample period II). To investigate sexual differences, I compared recordings of ultrasonic vocalizations of males and females within each season. Additionally, for all recorded, I related each mating score to ultrasonic vocalization of the bat.

Seasonal differences (sample period I & II) in ultrasonic vocalization

I recorded adult male (2003: n=8; 2004: n=10) and female (2003: n=8; 2004: n=8) big brown bats in the non-mating season (sample period I), and recorded the same bats again in the mating season (sample period II).

Sexual differences in ultrasonic vocalizations within season

In the non-mating season (sample period I), I recorded adult male (2003: N=8; 2004: N=16) and female (2003: N=8; 2004: N=17) big brown bats. In the mating season (sample period II), I recorded different adult male and female big brown bats (2003: male:female N=8:8; 2004: male:female N=12:14). Calls did not differ between years for each season; thus, data were combined (see Data Analysis section).

Data analysis

I analyzed 7 call descriptors (Table 1) that have been used to discriminate individual bats (Burnett et al. 2001). I performed a principal components analysis on ultrasonic vocalizations because it was likely that certain call characteristics were correlated, and second, this analysis allowed me to reduce the number of call variables into a more manageable number (from seven to two; see results). I used the 75% variance rule as my (note: you are the author of the dissertation, so use singular descriptors such as I, me, my, throughout) extraction criteria for number of components that would be retained in principal components analysis. The first principal component (PC1) explained characteristics of the call related to frequency, and the second principal component (PC2) explained characteristics of the call related to time. Calls were collected in multiple years; therefore, we tested difference between call components (PC1 and PC2) obtained in 2003 and 2004. For males and females, there was no significant difference between years for call components in the non-mating (PC1: df=31, p=0.89, F=0.02; PC2: df=31, p=0.31, F=1.07) and mating seasons (PC1: df=40, p=0.46, F=0.55; PC2: df=40, p=0.35, F=0.88). Thus, we combined data between years for further analysis of these variables. We used separate ANOVAs on PC1 and PC2 to test for sexual differences within season. In addition, we used a repeated-measures ANOVA to test for seasonal differences (i.e., non-mating and mating season) to determine which factors significantly varied with sex. We then used a simple regression to compare each factor with the mating score. In addition, we used a simple regression to compare the dominant call variable within each factor (i.e., the call variable within each factor with the highest loading) with the mating score. We compared variables statistically using StatView for Windows (SAS Institute Inc., version 5.0.1).

RESULTS

Ultrasonic vocalization of 20 male and 17 female big brown bats was reduced to two principal components that explained 79% (Eigenvalues; 0.516 and 0.284, respectively) of variation among these variables (Table 2). The first principal component (PC1) explained characteristics of the call related to frequency, receiving strong loadings from h1start, middle and end, and h1maxa (loadings; 0.98, 0.75, 0.90, respectively). The second principal component (PC2) explained characteristics of the call related to time, receiving strong loadings for duration and time to reach calls maximum amplitude (loadings; 0.87 and 0.83, respectively).

In males, there was a significant seasonal difference based on components of call related to time (PC2: df=17, p=0.001, F=14.62) (Fig. 3), but no significant seasonal difference based on components of call related to frequency (PC1: df=17, p=0.86, F=0.03). In females, there was no significant seasonal difference based on components of call related to frequency (PC1: df=16, p=0.09, F=3.1) or time (PC2: df=16, p=0.12, F=14.6).

Call components related to time (PC2: df=40, p=0.008, F=7.7) were significantly sexually dimorphic (with all measures elevated in males) in the mating season, but call components related to frequency (PC2: df=40, p=0.056, F=3.9) were not significantly sexually dimorphic (Fig. 4). There was no significant sexual dimorphism in the non-mating season based on components of call related to frequency (i.e., PC1: df=31, p=0.38, F=0.8) or time (i.e., PC2: df=31, p=0.40, F=0.7).

Mating

Each bat was individually pit tagged, and observed nightly through the mating season. When bats engaged in mating, they were captured and identified. This allowed us to calculate a mating score (number of copulations) for the entire mating season for each individual in the colony. In males and females, there was no significant correlation between mating success and PC1 (males: n=20, p=0.15, r^2=0.109 & females: n=21, p=0.67, r^2=0.009) or PC2 (males: n=20, p=0.71, r^2=0.008 & females: n=21, p=0.73, r^2=0.006). However, in males there was a positive trend between mating success and PC1 (i.e., call components related to frequency). Because duration and h1start explained the variance of their respective principal components, we used a simple regression analysis to investigate the relationship between each of these variables and the mating score (number of copulations/bat). Mating score was significantly correlated with h1start for males (n=20, p=0.02, r^2=0.27) (i.e., males that had a greater number of copulations also had a higher starting fundamental frequency)(Fig. 5). There was no significant correlation between mating score and duration (n=20, p=0.43, r^2=0.034).

DISCUSSION

I found evidence that big brown bats use ultrasound in sexual communication. Males significantly increased components of their ultrasonic call related to time (PC2) between the non-mating and mating season. Additionally, components of ultrasonic call related to time (PC2) differed significantly between sexes within the mating season. Although, we did not find a significant correlation between mating success and PC1 or PC2, the dominant variable of PC1 (i.e., the start of fundamental frequency -- h1 start) was significantly correlated with copulation frequency in males.

In the non-mating season, there were no significant difference in ultrasonic call components related to frequency (PC1) or time (PC2) between males and females, a result that may relate to the mating system of this species. Males and females roost separately in the non-mating season, and interaction between males and females is limited. Therefore, if ultrasonic vocalizations are useful for reproductive assessment (i.e., male condition, female receptivity, etc.) of an individual, then differences in ultrasonic vocalizations may not be important at this time of year. Another possible explanation could be that other variables (i.e., some sonic component, call rate, and/or temporal pattern, etc) that we did not measure were being used at this time of year. For instance, Kazial and Masters (2003) reported that female big brown bats decreased their calling rate when they heard a female, while calling rate remained the same when they heard a male. It is likely that other factors may distinguish male and female bats, but our data showed no sexual dimorphism in descriptive variables we measured within the non-mating season.

In the mating season, when there would be selective pressure to identify gender, ultrasonic vocalizations of big brown bats was sexually dimorphic. In males, variables associated with time (PC2-duration and tcmax) of ultrasonic call increased significantly in the mating season when compared to calls of individuals in the non-mating season. Additionally, components of the ultrasonic call related to frequency was just at the edge (PC1-h1start, middle, end, and h1maxa: p=0.056), and may well be significant. Change in these call components may not only identify the sender as male, but have the potential to provide some information on condition or reproductive status of the male. For example, the significant increase in average duration of a call would suggest that the signal is not being used for echolocation because of the potential for overlap in signals that are sent and received by the emitter when signal duration increases. For most bats, overlap in pulse and echo must be avoided due to neural mechanisms used for interpretation of echoes (Altringham 1996). This raises the question of what use are high-frequency vocalizations if signals are not being used for echolocation.

In physical theory, increasing variables associated with time and frequency would be more energetically costly. For example, cost of producing echolocation calls can be 9.5x resting metabolic rate (Speakman et al. 1989). In addition, for some taxa (particularly birds and amphibians), increasing duration, frequency, and/or call rate proportionally increases energy expenditure (Taigen and Wells, 1985; Ryan, 1988; Andersson, 1989; Eberhardt, 1994). During winter hibernation, one would expect bats to conserve as much energy as possible. Therefore, it may be that producing an energetically expensive signal suggests to a potential female that a male is of high quality and may be a potential mate. An example is seen in male baboons (*Papio cynocephalus ursinus*) that produce loud ‘contest’ calls during many social behaviors, and it has been shown that rate and duration of calling is associated with male quality (Fischer et al. 2004). However, in our study, it is interesting that differences in call variables related to time (PC2) that differed significantly from females in the mating season do not correlate with copulation frequency. Instead, another variable, the starting point of fundamental frequency (h1start), was significantly correlated with mating frequency suggesting that other information may be being transferred. In a recent study on *Myotis bechsteinii*, Siemers and Kerth (2006) reported that the starting point of fundamental frequency (h1start) was the call characteristic that provided the best discrimination among individuals. Because Speakman et al. (1998) showed that producing echolocation signals was energetically expensive for a resting bat; then with all other components equal, energetic cost of producing a signal should increase as frequency increases. Therefore, it could be that starting at a higher frequency (h1start), in conjunction with an increase in other call variables that were sexually dimorphic in the mating season (PC2; duration and tcmax), increases energy associated with producing the signal and suggests to a female that a male would make a good mate. We did not test whether call rate or other temporal aspects of the signal were associated with male quality in bats and this aspect should be investigated in the future. Furthermore, although time and frequency components of the call provide a good indirect measure of energy in the call, it is possible to measure energy of the call more directly (Waters and Jones 1995; Holland et al. 2004). We detected seasonal differences in call variables in males, but not females. Jones and Ransome (1993) noted that resting frequency of greater horseshoe bats (Rhinolophus ferrumequinum) was affected by season, and suggested this association was due to variation in body temperature from summer to winter. However, differences that we detected were related to time (PC2 -- duration and tcmax), and are not necessarily affected by seasonal constraints. Furthermore, one would expect average values of these call components to decrease in winter and increase in summer, especially because there are energetic costs associated with producing the signal (Jones and Ransome 1993). However, our results are contrary to what would be expected. We determined that duration and tcmax (PC2) increased from the non-mating to the mating season in males.

Bats primarily are active at night or in situations where use of vision is limited; therefore, one would expect acoustic signals to be an important system for communication. Big brown bats are well known for their use of ultrasound for navigation and foraging. Our research suggests that ultrasonic signaling in bats also is important in mating. *Eptesicus fuscus* calls are sexually dimorphic within the mating season. Principal components analysis showed that various ultrasonic call components related to time are associated with sexual and seasonal differences in big brown bats. Additionally, one call variable (h1 start) is correlated with mating frequency. However, further investigations are needed to determine if the observed sexual and seasonal differences are used by bats in a social context. Use of playback experiments to test individual responses to ultrasonic vocalizations in mating and non-mating seasons would be an informative technique for evaluating sexual and seasonal differences.

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FIGURE LEGENDS

Figure 1: Annual dissociated cycle of reproduction pattern exhibited in big brown bats, *Eptesicus fuscus*. Vertical bars represent bouts of arousal from hibernation when bats are mating. Sample period I coincides with recordings taken in the non-mating period, and Sample II coincides with recordings taken in the mating season.

Figure 2: Sonogram and power spectrum of a typical ultrasonic call of *Eptesicus fuscus*. Five of seven call descriptors are labeled for descriptive purposes (see Table 1 for description of tcmax and curvature).

Figure 3: Seasonal comparison of PC2 (i.e., ultrasonic call components related to time) in males.

Figure 4: PC1 (i.e., ultrasonic call components related to frequency) and PC2 (i.e., ultrasonic call components related to time) of a principal components analysis showing there is significant sexual dimorphism within the mating season.

Figure 5: Regression analysis between dominant variable of PC1 (i.e. h1start) and mating score in males.

Table I

Seven descriptors used to characterize each ultrasonic signal (from Burnett et al. 2001).

CALL VARIABLE
1. Duration
2. H1start = starting frequency of fundamental (kHz)
3. H1mid = middle frequency of fundamental (kHz)
4. H1end = ending frequency of fundamental (kHz)
5. H1maxa = frequency at maximum amplitude (kHz)
6. Tcmax = time to reach maximum call amplitude (ms, relative to beginning of call
7. Curvature (ranges between 0 and 1) = describes the shape of the call; a measure of the drop in the call from the starting frequency to middle frequency compared to a linear curve with the same starting and ending frequency

Table II

Eignevectors for first and second principal component (PC1 and PC2) of a principal component analysis on seven call variables for male and female big brown bats.

Call variable	Component 1	Component 2
Duration	-0.198	0.873
h1start	0.978	0.142
h1middle	0.753	0.456
h1end	0.897	-0.026
h1maxa	0.901	0.218
tcmax	-0.248	0.830
curvature	-0.556	0.496

Figure 1

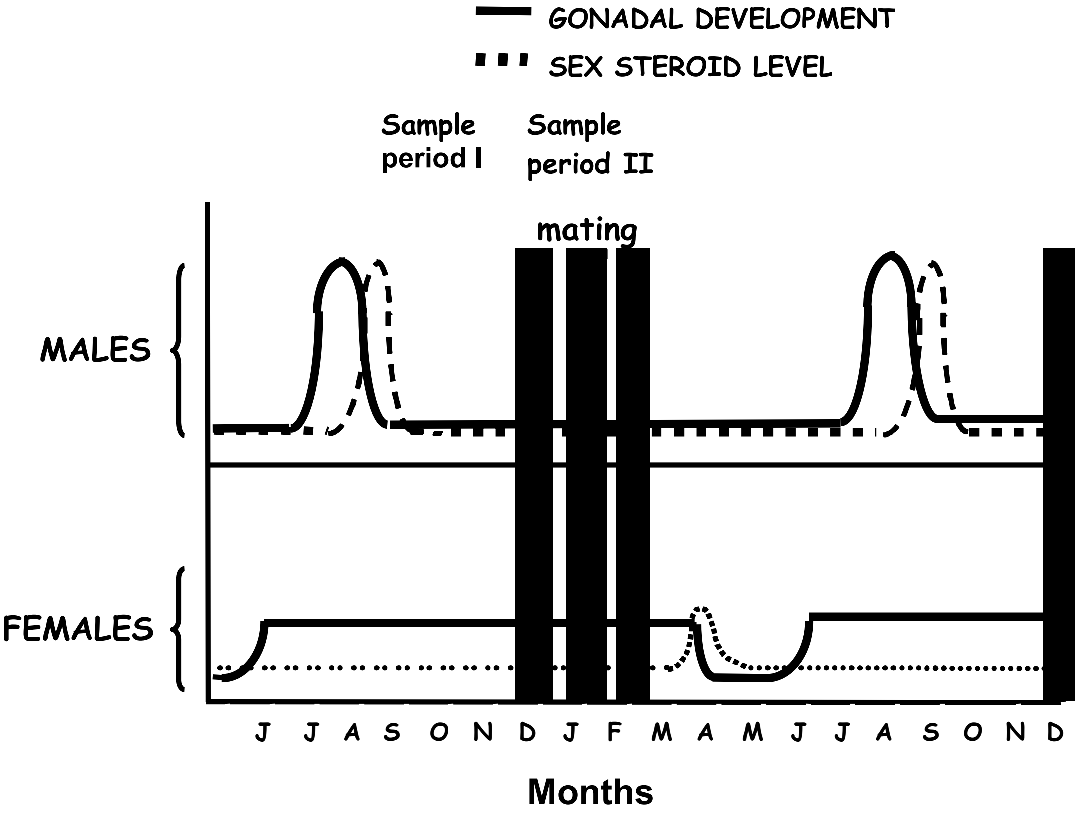

GONADAL DEVELOPMENT
SEX STEROID LEVEL
Sample period I
Sample period II
mating
MALES
FEMALES
J
J
A
S
O
N
D
J
F
M
A
M
J
J
A
S
O
N
D
Months

Figure 2

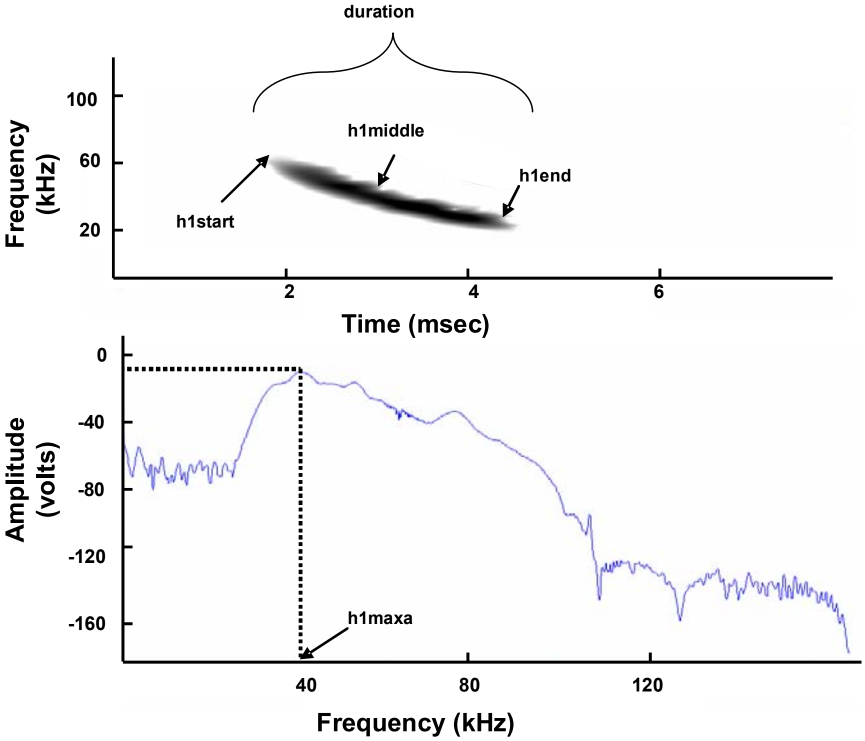
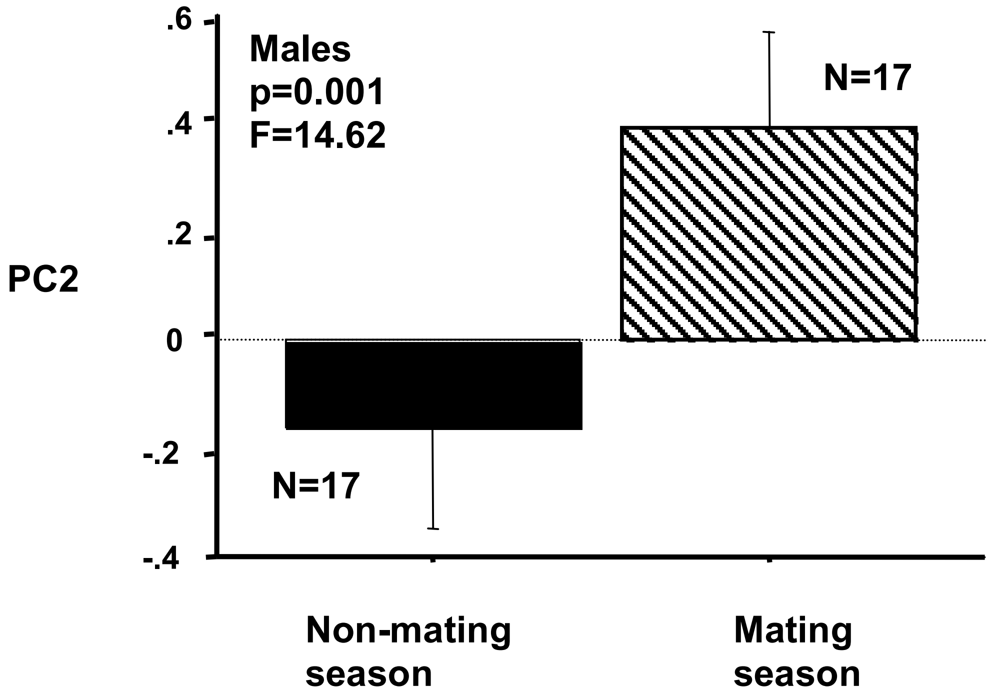

duration
100
60
20
Frequency (kHz)
h1middle
h1start
h1end
2
4
6
Time (msec)
0
-40
-80
-120
-160
Amplitude (volts)
h1maxa
40
80
120
Frequency (kHz)

Figure 3



Males
p=0.001
F=14.62
PC2
.6
.4
.2
0
-.2
-.4
N=17
N=17
Non-mating season
Mating season

Figure 4

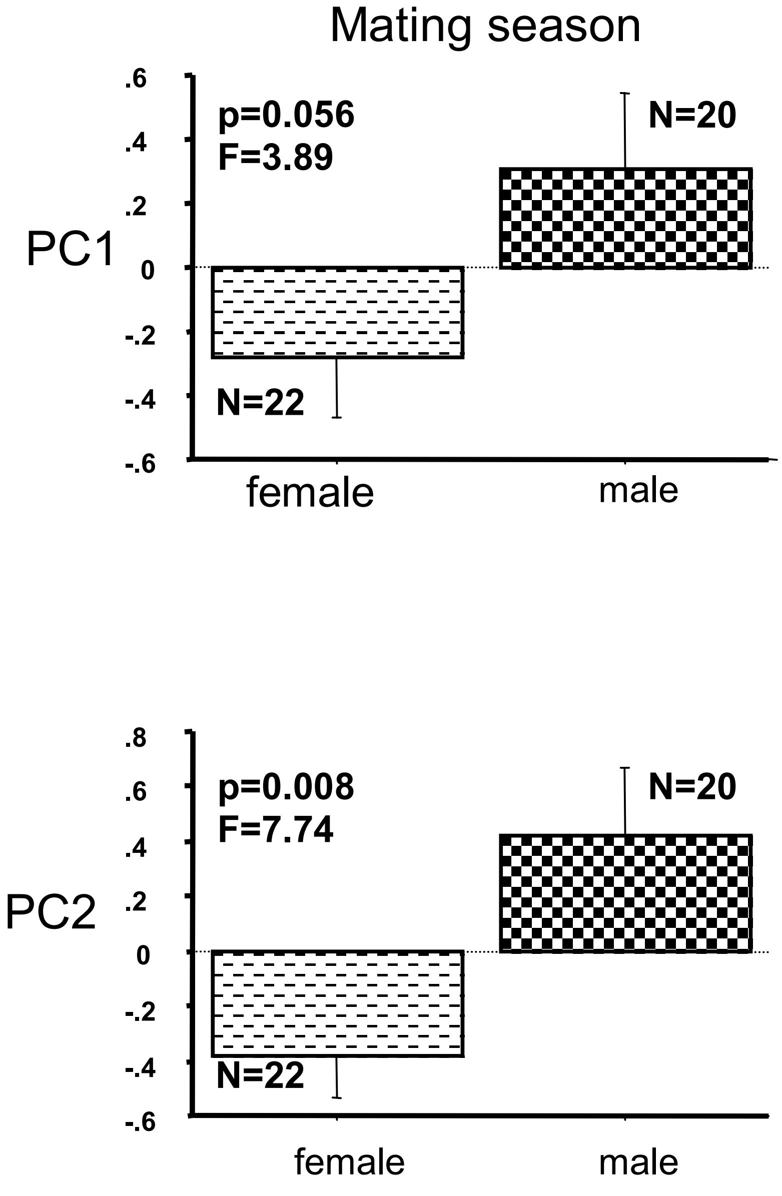
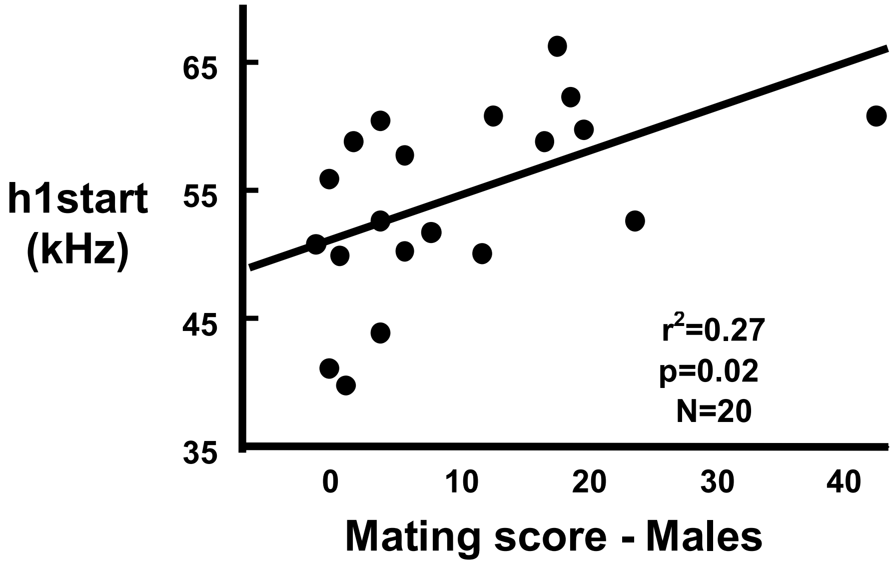

Mating season
PC1
.6
.4
.2
0
-.2
-.4
-.6
p=0.056
F=3.89
N=20
N=22
female
male
PC2
.8
.6
.4
.2
0
-.2
-.4
-.6
p=0.008
F=7.74
N=20
N=22
female
male

Figure 5


h1start (kHz)
65
55
45
35
0
10
20
30
40
Mating score - Males
r²=0.27
p=0.02
N=20

CHAPTER 2

SEXUAL DIMORPHISM IN ULTRASONIC VOCALIZATIONS OF BIG BROWN BATS (*EPTESICUS FUSCUS*) IS CONTEXT DEPENDENT

ABSTRACT

Acoustic signals are important regulators of mating behavior in most mammalian species. Much of the work done on the function of these signals has focused on those signals within the sonic range, but less is known about their function in the ultrasonic range. Although bats are well known for their use of ultrasound for echolocation, there is limited evidence for its use in a social context. In this study, we tested whether ultrasonic vocalizations in bats were contextually sexually dimorphic (when signals were used for purposes other than navigation and foraging). During the reproductive season, we recorded ultrasonic signals of captive adult male and female big brown bats (*Eptesicus fuscus*) that were allowed to fly on tether lines in the field; and compared these signals to ultrasonic vocalizations that males and females made while roosting.

Principal components analysis reduced 7 ultrasonic descriptors to two components that correlated with components related to frequency (PC1) and time (PC2). During the mating season, while bats were roosting, ultrasonic components of calls related to time and frequency were both sexually dimorphic, being significantly higher in males in each instance. However, when bats were recorded while flying at the same time

of year, these same components of calls were no longer sexually dimorphic. This finding suggests that bats are changing their ultrasonic calls in relation to functional context, making them monomorphic and utilitarian for an activity like foraging and navigation, but dimorphic in a situation when mating activity is likely.

INTRODUCTION

Acoustic signals, ranging from infrasonic (<20Hz) to ultrasonic (>20,000Hz), are an important method of communication among many species including insects, fish, amphibians, birds, and mammals (review in Bradbury & Vehrencamp 1998). Additionally, it is well documented that many communication signals exhibit context-dependent variation influenced by ecological (e.g. season, time of day, geographic location; Baptista & King 1980; Rotella & Ratti 1988) and social context (e.g., reproduction, territorial defense, predation) of the situation (Catchpole 1979; Ehrlich et. al. 1988; Ruiz-Miranda et. al. 2002). Some examples of these context-dependent changes include: Calling behavior and acoustic structure of calls exhibiting differences related to seasonal variation in function of call in the gray partridge (*Perdix perdix*); (Rotella and Ratti 1988), calls of male yellow baboons (*Papio cynocephalus*) functioning as alarm calls or contest calls depending on the situation (i.e., aggressive encounters, male-male calling bouts; Fischer et. al. 2004), and male zebra finches (*Taeniopygia guttata*) altering song tempo in the presence of females versus when alone (Cooper & Goller 2006).

Use of vocalizations in the ultrasonic range have been documented in intraspecific and interspecific communication for a variety of social purposes and may also be context dependent (Sales & Pye 1974). For example, infant rodents in the family Muridae emit ultrasonic distress calls in response to isolation from the nest, cold stress, and possibly hunger (Sales & Pye 1974). Ultrasonic signals also are used during aggressive encounters in rats, and may be important in dominance-subordination relationships (Sales & Pye 1974). Conner (1999) suggested that ultrasonic cues may be involved in all aspects of reproductive behavior of moths ranging from species recognition, male-male competition for mates, and female mate choice (Conner 1999). Additionally, there is recent evidence for ultrasonic communication in frogs; Feng et. al. (2006) have shown that male concave-eared torrent frogs (*Amolops tormotus*) hear and respond to ultrasonic components of advertisement calls of other males.

Bats are well known for their use of ultrasound for navigation and foraging, but there is less information on the function of ultrasonic vocalizations for other purposes, especially in regards to mating. Although bats exhibit a wide diversity of mating systems little is known about the mechanisms involved in courtship and mating. Evidence does suggest that there are sex differences in ultrasonic vocalizations of at least some species of bats (Thomas et al. 1979; Neuweiler et al. 1987; Suga et al. 1987; Jones et al. 1992; Siemers et al. 2005; Siemers & Kerth 2006), which raises questions about the functional significance that these differences might serve.

Thomas et al. (1979) noted circumstantial evidence suggesting that adult bats can determine the sex of other adult bats from ultrasonic vocalizations they use for echolocation. More recent studies have shown that males and females respond differently to ultrasonic vocalizations used for echolocation (Neuweiler et al. 1987; Suga et al. 1987; Jones et al. 1992; Kazial & Masters 2004). Additionally, there is recent evidence from work on *Rhinolophus ferrumequinum* and *Myotis bechsteinii* that suggests echolocation calls may be a source of information for individual recognition (Siemers et al. 2005; Siemers & Kerth 2006). Recently, Grilliot et al. (submitted) demonstrated that the ultrasonic vocalizations of big brown bats differ between sexes seasonally, and that these differences are correlated with copulation success.

During autumn and winter, big brown bats congregate at the hibernacula and engage in mating activity. In these conditions of little to no light, it has been suggested that vocalizations would be important for sex recognition. One way to assess the functional significance of sexually dimorphic vocalizations is to measure vocalizations used in different situations. We hypothesize that components of ultrasonic calls of big brown bats are sexually dimorphic in the mating season when they are being used in a social context. To test our hypothesis, we investigated if sexual dimorphism in characters of ultrasonic calls of big brown bats was context dependent. To accomplish this objective, we recorded the ultrasonic vocalizations of adult male and female big brown bats to test whether components of the signal would differ when bats were flying in the field (i.e, echolocating) versus when bats were roosting in a lab colony setting (i.e. social context).

METHODS

Study species

Big brown bats were selected as the model species because they are locally abundant, thrive in captivity, and can be observed year round. Relatively little is known about the mating system of big brown bats; but they, as well as other species of Vespertilionidae, have been suggested to be promiscuous and random maters, with males and females having multiple partners (Thomas et al. 1979; Fenton 1984; Wai-Ping & Fenton 1988). Big brown bats exhibit a dissociated pattern of reproduction, mating in autumn and winter months while the gonads are regressed (males) or in stasis (females) (Oxberry 1979).

All bats used in this study were adult wild-caught males and females from Alabama and Georgia that had been acclimated to captivity for months pior to the experiment (see description below). All bats were individually tagged with wing bands, and implanted with PIT tags for identification. Bats were maintained on a diet of mealworms and water *ad libitum*, supplemented with vitamins and minerals.

Bats were collectively housed in environmentally controlled chambers (2.1m x 4.0m x 2.1m, EJS Limited, Inc., Stafford, TX), and allowed to interact at will during the mating season. The environmental chambers allowed researchers to alter temperature and photoperiod to induce desired behaviors or conditions. During spring and summer months, chambers were kept at 23°C with natural photoperiod. In November, bats were exposed to periods (i.e., 4 days) of low temperature (5°C) to induce hibernation, followed by 4-7 day periods of 23°C. The alternating temperature pattern ended in early spring (March/April). This temperature regime has been documented to stimulate a high degree of mating behavior and mimics natural seasonal variation during these months in Alabama (Mendonça et al 1996; Mendonça and Hopkins 1997).

Recordings

Each bat was recorded individually in an experimental anechoic chamber (2.4m x 2.4m x 2.4m) and while flying outside on a tether line (for description see Szewczak 2000). Ultrasonic calls were detected with a U30 bat detector (sensitivity typically 10 dB SPL at 50 kHz; better than 20 dB SPL 20-120 kHz). A custom built 6x amplifier (designed by W.M. Masters, The Ohio State University, Columbus, OH) was used to amplify output of the microphone. We used CBDisk (Engineering Design, Belmont, MA) to acquire high-frequency signals direct-to-disk at sample rates up to 330 kHz. We used a Toshiba laptop with a Pentium III processor, 128MB of RAM, and a 6.4 GB hard disk. The recordings consist of a single file that contains many different calls. The calls were extracted from the large files so they could be analyzed individually (Burnett & Masters 2001). The individually extracted calls were analyzed with a custom program written in Matlab (Version 6.5 Mathworks, Inc., Natick, MA) that automatically measured the value of different variables that were used to describe the calls (i.e. duration, starting frequency; Burnett & Masters 2001). After individual calls were extracted and analyzed by the Matlab program, we picked seven variables (Table 1, Fig. 1) that are direct descriptors of calls and have been used to discriminate individual bats (Burnett et al. 2001). Requirements for choosing calls were as follows: signals must be loud enough to start computer acquisition, signals must be of sufficient length to be useful, and signals must contain enough information to determine the value for the seven descriptors (Burnett et al. 2001).

Experiment 1: Ultrasonic signals used when not flying

We recorded individual adult male and female bats in 2003 (n=8 and 8, respectively) and 2004 (n=12 and 14, respectively) reproductive seasons (Jan-Mar). There was no significant difference in calls between years (see below); therefore, data were combined for analysis. To obtain accurate ultrasonic signals under controlled conditions, bats were recorded while in an experimental anechoic room (2.4m x 2.4m x 2.4m). The experimental room is lined on the walls and ceiling with 12.7-cm-thick mattress foam, and covered with 5.1-cm-thick corrugated mattress padding. The floor was covered with 5.1-cm-thick corrugated mattress padding. Bats were placed individually in the experimental room, and we recorded ultrasonic vocalizations that each individual made during the experimental period. Bats were recorded before peak feeding times because prior feeding tends to limit activity level and calls (Boughman & Wilkinson 1998). Bats were introduced to the experimental room and given a brief period of time (~10 minutes) to acclimatize. When a bat began emitting ultrasonic vocalizations, we oriented the microphone to face the bat. We recorded at least 5 files, 8 seconds in duration, from each bat (therefore, each bat was recorded for >40 seconds) to attain >150 calls for each bat. Data from these calls were averaged and mean values were used in statistical analyses.

Experiment 2: Ultrasonic signals used when flying

In the 2004 reproductive season (Jan-Mar), we recorded a different set of big brown bats (16 adult males, 16 adult females) while flying singly on a tether line in the field. The tether-line system provided the opportunity to acquire semi-natural field recordings of ultrasonic vocalizations while flying. Recordings took place just after sunset when bats typically would be leaving the roost to forage. The tether line consisted of a 25m run line with a 10m zip line. The 25m run line was placed running north to south because preliminary trials showed that bats consistently flew in this direction. Therefore, we placed the microphone and recording apparatus at the southern end of the 25m run line to record echolocation signals emitted by the subject. A collar was placed around the bats neck and then attached to the 10m zip line. All bats were released from the same point and facing the direction of the recording microphone. Bats were hand released and recorded while flying alone. We recorded >5 files, 15 seconds in duration, from each bat (therefore, each bat was recorded for >75 seconds) to attain >150 calls for each bat. Data from these calls were averaged and mean values were used in statistical analyses.

Data analysis

We analyzed seven call descriptors (Table 1) that have been used to discriminate individual bats (Burnett et al. 2001). Then, we performed a principal components analysis on average call values (i.e. calls from an individual bat were averaged) from all bats used in the study (i.e. anaechoic chamber plus flying: N=36 male; N=38 female) because it was likely that certain characteristics of calls were correlated, and because this analysis allowed us to reduce the number of variables into a more manageable number (from seven to two; see results). We used the 75% variance rule as our extraction criteria for the number of components that would be retained in the principal components analysis. Calls recorded in the anechoic chamber were collected in multiple years; therefore, we tested if there was a difference between call components obtained in 2003 and 2004. We used separate ANOVAs on components of the PCA to test for variation in ultrasonic vocalizations associated with sex and situation. Separate ANOVAs were justified because PCA axes are independent. We conducted statistical analyses using StatView for Windows (SAS Institute Inc., version 5.0.1).

RESULTS

The ultrasonic vocalizations of 36 adult males and 38 adult females (i.e., the combined bats from the two situations- anaechoic chamber and flying) were reduced to two principal components that explained 77% (Eigenvalues; 0.46 and 0.31, respectively) of the variation among the seven call variables (Table 2). The first principal component (PC1) correlated with characteristics of the call related to frequency, receiving strong loadings from h1start, middle and end, and h1maxa (loadings; 0.71, 0.98, 0.79, and 0.94, respectively). The second principal component (PC2) correlated with characteristics related to time and shape, receiving strong loadings for duration, time to reach calls maximum amplitude, and curvature (loadings; 0.95, 0.84, and 0.80, respectively).

Calls recorded in the anechoic chamber were collected during two mating seasons (bats were kept in the same reproductively stimulatory conditions for both years); therefore, we tested if there was a difference between call components (PC1 and PC2) obtained in 2003 and 2004. For males and females, there was no significant difference between years for any of the call components in the mating seasons (PC1: df=40, p=0.46, F=0.55; PC2: df=40, p=0.35, F=0.88). Thus, we combined data between years for further analysis of these variables.

We tested whether there was sexual dimorphism between PC1 and PC2 during the reproductive season in the contexts of "roosting" (i.e., in an anechoic chamber) versus flying (i.e., on a tether line in the field). PC1 (i.e., variables correlated with frequency) was significantly higher in males (df=40, p=0.05, F=3.91; Fig. 2A) when bats were recorded in the anechoic chamber, but not when flying on the outside tether line (df=30, p=0.11, F=2.76; Fig. 2B). Additionally, PC2 (variables correlated with time components) also was significantly higher in males when bats were recorded in the anechoic chamber (df=40, p=0.01, F=7.76; Fig.3A), but not when they were flying (df=30, p=0.94, F=0.01; Fig. 3B).

DISCUSSION

This study suggests that ultrasonic vocalizations produced by big brown bats within the mating season are sexually dimorphic depending on context of the situation. When bats kept in reproductively stimulatory conditions were "roosting" in the anechoic chamber and ultrasonic vocalizations could potentially be used in a social signaling context, we determined that there was sexual dimorphism in variables associated with frequency (PC1) and time/shape (PC2) components (Fig 2). Conversely, when bats held under the same reproductively stimulating conditions flew on the tether line, presumably using their ultrasonic vocalizations for navigation purposes, we detected no significant sexual dimorphism between these same components of calls (Fig 3).

There are several lines of evidence supporting the hypothesis that bats may be modulating their ultrasonic vocalizations for a reproductive or social context rather than only for echolocation. First, a recent study in our laboratory documented that when bats were recorded in the same “roosting” context as described above during the non-mating season, the frequency and time parameters of calls were not dimorphic. When the same bats were re-recorded during the mating season, males had significantly higher values for frequency and time components of their vocalizations than females (Grilliot et al. submitted). The appearance of the dimorphism only within the mating season suggests it has a reproductive role. Seasonal variation in vocal behavior is known to play a role in mating behaviors in a variety of taxa (Catchpole 1982; Bosch et al. 2002). In the non-mating season, female big brown bats form maternity colonies while males form separate colonies, so interaction between males and females is limited. In the mating season, male and female big brown bats congregate at hibernacula to engage in reproductive behaviors and to hibernate. If ultrasonic vocalizations are useful in a social context, then sexual dimorphism in vocalizations should be important at this time of year.

Additionally, in the “roosting” situation, the fact that males were significantly higher for PC2, the component associated with time, suggests that signals are not being used for echolocation because of the potential for signal overlap. If ultrasonic vocalization is being used for navigation and foraging, then duration of signal is limited because of the need to prevent overlap of the emitted pulse and returning echo (Altringham 1996). For most bats, overlap in emitted pulse with the returning echo must be avoided due to neural mechanisms used for interpretation of the echoes (Altringham 1996). We suggest that the signal used in the “roosting” situation is being used for social purposes, and would eliminate the need to interpret the returning echo. Support for the preceding statement comes from the results (Fig. 3A and B) showing that when bats are flying males and females are using their signal for similar purposes (i.e. navigation); and there is no apparent difference in ultrasonic components related to time/shape (i.e. PC2). However, between the situations (flying vs roosting) we discovered that females decreased time/shape components more than males. Therefore, although components were shorter when bats were "roosting" than when bats were flying, relative difference was still higher, so that the signal of males was longer and possibly more expensive than females. These differences in components of calls might not only identify the sender as male, but have the potential to provide some information on condition or reproductive status of the male

Finally, relative to the situation, the fact that males do not decrease variables associated with time and frequency to the same extent as females may indicate that the "roosting" male is incurring an energetic cost. For example, cost of producing echolocation calls can be 9.5x resting metabolic rate (Speakman et al. 1989). In addition, for some taxa (particularly birds and amphibians), increasing duration, frequency, and/or rate of call proportionally increases energy expenditure (Taigen and Wells 1985; Ryan 1988; Andersson 1989; Eberhardt 1994). During winter hibernation (i.e. mating season), one would expect bats to conserve as much energy as possible. Therefore, when ultrasonic signals are being used for navigation and foraging the signals are non-dimorphic and utilitarian in an effort to be energetically efficient. Conversely, when ultrasonic vocalization is being used in a social context, it may be that producing more energetically expensive signal suggests to a potential female that a male is of high quality and may be a suitable mate.

Our study provides support for the possibility that ultrasonic vocalization commonly regarded to function in navigation and foraging, may also play a role in social communication. It is known that there is considerable variation in sonic and ultrasonic vocalizations both within and among species, and signals that are acoustically variable have the potential to communicate a great deal of information concerning the sender and the situation (Fenton 1985). Furthermore, noticeable differences in structure of calls among species suggest that bats might recognize when conspecifics are present (Fenton 1985) and studies indicate that ultrasonic vocalizations emitted by one bat could be heard by other individuals at moderate distances (5-15m) as in a roost situation (Barclay 1982; Obrist 1995. Additionally, Kazial & Masters (2004) noted that female big brown bats could differentiate between echolocation signals of males and females, although they did not observe sexual dimorphism in characteristics of calls (in that study, animals were not housed communally nor under reproductively stimulatory conditions). If a variety of information is carried in the ultrasonic vocalizations of bats, then the signals could function in a social context.

In conclusion, our results indicate that there is contextual variation in ultrasonic vocalizations of male and female big brown bats in a manner that suggests that this variation is used to signal the sex of the bat and, potentially, some aspect of male condition or quality. Indeed, further experiments using playbacks confirm differential response between sexes to specific components of the ultrasonic vocalization (Grilliot et al. unpublished data). Evidence from this study suggests that there are acoustic differences between vocalizations of male and female big brown bats, but much work remains to be done in regards to the function of high-frequency vocalizations in situations other than for navigation and foraging.

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FIGURE LEGEND

Figure 1: Sonogram of a typical ultrasonic call of a big brown bat. Four of seven descriptors of calls are labeled for descriptive purposes (see Table 1 for description of h1maxa, tcmax and curvature).

Figure 2: Comparison of average ultrasonic-frequency components (i.e., PC1) for male versus female big brown bats when signals were used in a social context (A) vs when signals were used for navigation (B). Error bars represent standard error.

Figure 3: Comparison of average ultrasonic components of time (i.e., PC2) for male versus female big brown bats when signals were used in a social context (A) vs. when signals were used for navigation (B). Error bars represent standard error.

Table 1. Components of ultrasonic vocalization analyzed.

The seven descriptors used to characterize each ultrasonic signal (directly from Burnett et al. 2001)
1. duration
2. h1start = starting frequency of fundamental (kHz)
3. h1mid = middle frequency of fundamental (kHz)
4. h1end = ending frequency of fundamental (kHz)
5. h1maxa = frequency at maximum amplitude (kHz)
6. tcmax = time to reach maximum call amplitude (ms, relative to beginning of call
7. curvature (ranges between 0 and 1) = describes the shape of the call; a measure of the drop in the call from the starting frequency to middle frequency compared to a linear curve with the same starting and ending frequency

Table 2. Factor loadings for the first and second principal component (PC1 – 46% of the variance and PC2 – 31% of the variance) of a principal component analysis on seven call variables of the combined male and female big brown bats used in the study (i.e. anaechoic chamber and flying, n=74).

Call variable	Component1	Component 2
duration	0.001	0.948
h1start	0.713	0.249
h1middle	0.982	-0.190
h1end	0.788	-0.120
h1maxa	0.937	-0.196
tcmax	0.049	0.843
curvature	-0.355	0.795

Figure 1

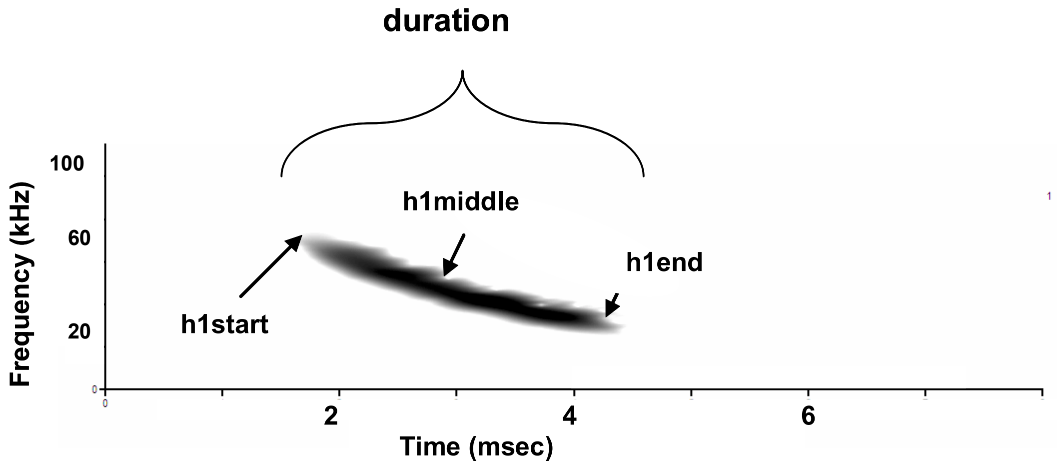


Figure 2

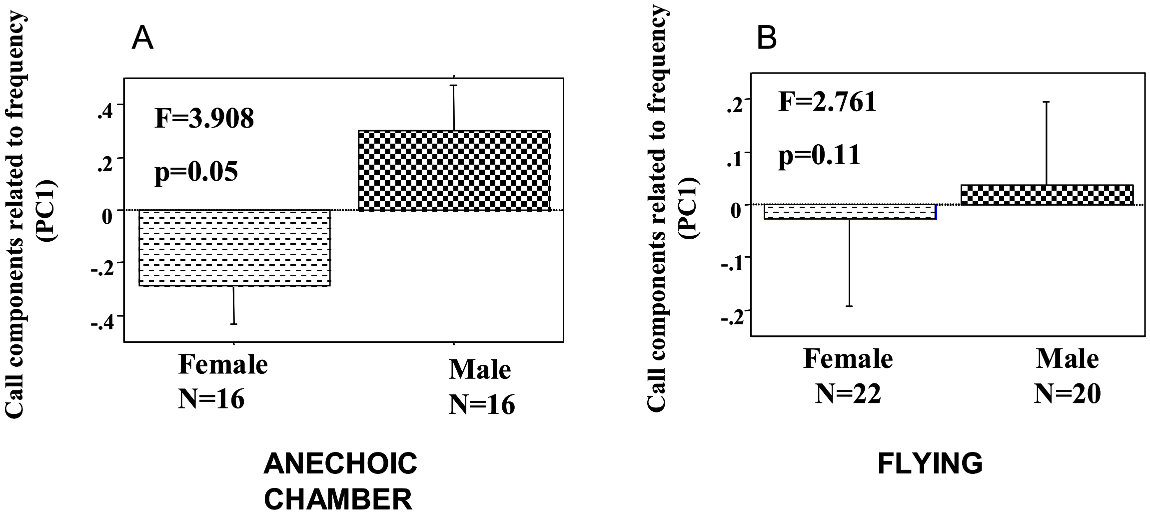
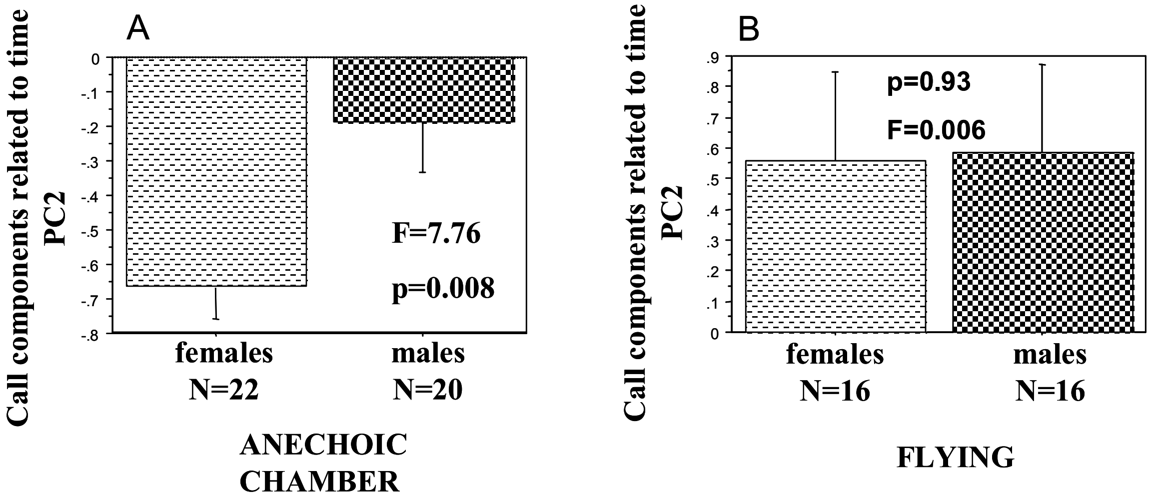


Figure 3


A
Call components related to time PC2
0
-.1
-.2
-.3
-.4
-.5
-.6
-.7
-.8
F=7.76
p=0.008
females N=22
males N=20
ANECHOIC CHAMBER
B
Call components related to time PC2
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
p=0.93
F=0.006
females N=16
males N=16
FLYING

CHAPTER 3

SELECTION OF MATES BY MALE BIG BROWN BATS (*EPTESICUS FUSCUS*) IS INFLUENCED BY ULTRASONIC VOCALIZATIONS OF FEMALES

ABSTRACT

It generally is accepted that bats emit ultrasonic vocalizations that function for echolocation purposes as well as for communication, but there is lack of empirical data to support this idea. While numerous studies have shown that calls emitted by bats exhibit considerable variation both within and among species, few studies have tested whether bats respond to this variation. I determined whether male or female big brown bats responded to variation in ultrasonic vocalizations of the opposite sex in a manner that would suggest the vocalizations are used in a mating context. I presented 32 female and 10 male big brown bats with ultrasonic playbacks of differentially mating (i.e., high frequency copulators = HM vs. low-frequency copulators = LM) individuals of the opposite sex. I measured 1) which side of the arena each subject selected first (HM vs. LM), and 2) duration spent (seconds) on each side of the arena (HM vs. LM). For both of these measures (i.e., first choice and duration) male subjects were more likely to select the ultrasonic vocalization of HM females, but the same respective tests determined that female subjects did not select ultrasonic vocalizations of frequently copulating males over infrequently copulating males. My results support the possibility that ultrasonic

vocalizations of big brown bats function in the mating season for communication purposes, and may be a precopulatory mechanism of mate selection.

INTRODUCTION

The majority of mammal species studied to date have been classified as having polygynous mating systems, where a single male mates with multiple females (Kleiman 1977; Clutton-Brock 1989; McCracken and Wilkinson 2000). Bats make up >25% of the world's mammals, and yet for most species of bats there are relatively few detailed studies of mating systems and mechanisms controlling reproductive behaviors (Altringham 1996, McCracken and Wilkinson 2000). In large part, this circumstance is because bats can be difficult to study because they are nocturnal and often roost in places that make them inaccessible (Vonhoff et al. 2006). In the limited situations where there has been direct observation or genetic evidence, bats appear to conform to the general mammalian pattern; i.e., a large majority being polygynous, and few being monogamous, polyandrous, or promiscuous (Burland and Worthington Wilmer 2001; McCracken and Wilkinson 2000).

Contrary to what previously has been described, many species of bats may better be described as exhibiting a polygamous mating system, where males and females have multiple mating partners. Studies from the lab and field have revealed that females of many species, especially those that can store sperm, copulate with multiple males (Mendonca et al. 1996; Rossiter et al. 2000; Racey and Entwistle 2000; Vonhoff et al. 2006). However, adding to the challenge of studying mating systems of bats, in particular for temperate species, is that males and females exhibit some form of delayed reproduction (Oxberry 1979; Racey 1982; McCracken and Wilkinson 2000) in which storage of sperm by males and females can occur for periods ≤6 months (Racey 1973b; Uchida & Mori 1987). These factors make it difficult to determine reproductive success of individual bats and, thus, tease apart the precopulatory and postcopulatory mechanisms that are likely to influence mating strategies of bats.

Although mating strategies of many mammals are determined by the extent that males can monopolize females or resources (Clutton-Brock 1989), studies have indicated that choice of mates by female bats is important in some mating systems (McCracken and Wilkinson 2000; Rossiter et al. 2000; Vonhoff et al. 2006). In most temperate bats, males assume little to no parental responsibility (Kunz and Hood 2000); therefore, males would be expected to mate in a more widespread and indiscriminate manner while females would be the “choosy” sex (Emlin and Oring 1977; Bateson 1983). Numerous studies have cited the possibility for sperm competition (a type of male-male competition) as a postcopulatory mechanism of selection (Fenton 1984; Hosken 1997; Hosken 1998b; Wilkinson and McCracken 2003; Vonhoff et al. 2006), but the possibility of cryptic choice of mates by females cannot be ruled out (Thornhill 1983, 1984; Birkhead 1988).

In addition to mechanisms that may control postcopulatory selection of a mate, there are mechanisms that are likely to influence precopulatory selection of a mate. In fact, various communication signals (i.e., acoustic, olfactory, and tactile) have been cited as important in social interactions of bats (Fenton 1985), and are likely to have potent effects on reproductive success (Bronson 1989). Acoustic vocalizations play an important role in choice of mates within many taxa including insects, amphibians, birds, and mammals (Bradbury and Vehrencamp 1998; Bosch et al. 2002). It has been widely suggested that ultrasonic vocalizations of bats can function for social purposes in addition to being used for navigation and foraging (Fenton 1985), but empirical support for this statement is lacking. There is considerable variation in these calls both within and among species that would make it possible for bats to communicate information (Fenton 1985; Obrist 1995; Waters and Jones 1995). Additionally, there are individual differences in ultrasonic vocalizations of several species of bats (Masters et al. 1991, 1995; Obrist 1995; Burnett et al. 2001; Kazial et al. 2001), but behavioral studies that tested whether bats respond to this variation have provided mixed results (Pearl 1994; Kazial and Masters 2004). All of these observations suggest that some bats have the potential to recognize and respond to vocalizations of other bats in a manner that would indicate the signals are useful for communication.

Although little is known about mating system of this diverse group of mammals, current evidence suggests that there are differences between sexes in response to ultrasonic vocalizations (Kazial & Masters 2004). However, few studies have provided more than speculative support for the idea that sexual dimorphism in ultrasonic vocalizations function in social interactions of bats, and may influence reproductive success of males and females. Recently, it has been suggested that females have an established dominance hierarchy that is communicated in their ultrasonic vocalizations to other bats (Rydell 1986; Kazial and Masters 2004). Previous work has provided evidence to support the hypothesis that bats may be employing their ultrasonic vocalizations for a reproductive or social context rather than just for echolocation (Grilliot et al. submitted, Chapter 1 & 2). First, my data suggest that calls are sexually dimorphic only within the mating season. Second, my data suggest that there is contextual variation in ultrasonic vocalizations of big brown bats, making them monomorphic and utilitarian for an activity like navigation, but dimorphic in a situation when mating activity is likely (Grilliot et al. submitted, Chapter 2). Although the aforementioned studies suggest that there is context dependent variation in vocalizations, controlled laboratory experiments are needed to determine if either sex responds to the variation.

Here I focus on the function of ultrasonic vocalizations in social interactions of big brown bats (*Eptesicus fuscus*), a temperate species belonging to the family Vespertilionidae. Like other temperate species of bats, big brown bats are known to exhibit a dissociated pattern of reproduction, mating in the autumn and winter months, during periods of arousal from hibernation, while gonads are regressed (males) or in stasis (females)(Oxberry 1979). Behavioral observations suggest that big brown bats mate promiscuously; and within each sex, males and females exhibit different levels of mating activity, such that individuals can be described as mating frequently and others as mating infrequently (Mendonca et al. 1996). Recently, Vonhoff et al. (2006) demonstrated multiple paternity in big brown bats, and suggested that the mating system was complex in terms of mechanisms of postcopulatory and precopulatory choice of mates.

Male and female big brown bats exhibit different levels of mating activity (Mendonca et al. 1996) and recent studies suggest that this variation may be influenced by ultrasonic calls that an individual emits (Grilliot, Chapter 1 & 2). If there are differences in ultrasonic vocalization that would be useful to the opposite sex for mate assessment, we predict that bats can recognize and respond to this variation. To investigate this prediction, I tested whether male or female bats respond preferentially to ultrasonic vocalizations of differentially mating individuals of the opposite sex.

METHODS

Bats

Adult male and female big brown bats used in this study were part of a colony that was collectively housed in environmentally controlled chambers that allowed researchers to alter temperature and photoperiod to induce desired behaviors or conditions (for description of housing regimen see Mendonca & Hopkins 1997; Grilliot et al. submitted Chapter 1). Bats were reproductively active and observed throughout the mating season to assess mating frequency.

Experimental Protocol

During the mating season (January-March 2006) sequences of ultrasonic calls from big brown bats were presented to reproductively active adult male and female big brown bats. Male and female big brown bats were presented with binomial-choice playback experiments designed to test response to variation in the ultrasonic vocalizations between frequently copulating (i.e., High Mating = HM) versus infrequently copulating (i.e., Low Mating = LM) bats of the opposite sex.

Initial Recordings and Playback Sequences

In the mating season, I recorded number of copulations for each bat. I classified bats into two groups; frequent copulators (>10 copulations = High Maters or HM) and infrequent copulators (1-3 copulations = Low Maters or LM). Individual bats must have ≥1 observed copulation to be included in my classification system. I recorded ultrasonic vocalizations of these bats, and used them to build playback sequences (see Playback stimuli for details).

Ultrasonic calls were recorded using a U30 bat detector (Ultrasound Advice, London, U.K.) (sensitivity typically 10 dB SPL at 50 kHz; >20 dB SPL 20-120 kHz). Calls were obtained under controlled conditions in an experimental anechoic room (2.1 x 2.1 x 2.1m). Each bat was placed in the experimental room and given a brief period of time (~10 minutes) to acclimate. When a bat began emitting ultrasonic vocalizations, I oriented the microphone to face the bat. I made >5 recordings, 8 seconds in duration, from each bat (therefore, each bat was recorded for >40 seconds). I obtained >200 calls for each bat.

Playback sequences were constructed on a Dell computer by S. Burnett (unpublished software) using previously recorded ultrasonic vocalizations of HM and LM bats. To construct each playback recording, 10 calls were randomly selected from a series of vocalizations made by a single individual. These 10 calls were filtered in SIGNAL (Engineering Design, Belmont, MA) so they only contained the fundamental harmonic. Calls were then adjusted so that each call had the same amplitude of its loudest point. These calls were assembled into playback sequences with approximately 200 ms between each call. These sequences were produced with lengths of either 30 or 60 seconds as needed for different parts of the playbacks.

Playback Experiments

Playback trials took place in a circular arena (46cm diameter by 7.5cm height) inside an anechoic chamber (i.e. the same chamber that was used for the initial recordings). The base of the arena was plastic, and top and sides were 6.35 mm wire mesh. The center of the arena contained a removable wire-mesh partition (Fig. 1). Two speakers (Advent AV570, Audiovox corporation, Hauppauge, New York; frequency response -3dB points at 40Hz and 20kHz) were situated 5cm outside of the arena, one on the left and one on the right, and facing the center of the arena. Speakers were connected to a personal computer that was located outside the chamber. "Model" bats were placed next to each speaker to serve as visual stimuli (Balcombe 1990).

Individual bats were introduced to the arena and allowed to acclimate for ~10 minutes the day before they were tested. At the beginning of the playback experiment, each individual was placed in the center of the arena with the partition in place for 90 seconds. During this time, each subject was presented first with 30 seconds of silence (which would only consist of electronic noise produced by the playback system) from both speakers, 30 seconds of either HM or LM ultrasonic stimulus (type of stimulus was chosen randomly) from either the left or right speaker (chosen randomly), and 30 seconds of the opposite stimulus from the opposite speaker. Recordings were broadcasted using Sony Soundforge (Sony Creative Software, Inc.) and a PCI soundcard (Chaintech 7.1, Chaintech USA, sampling rate up to 192kHz). After 90 seconds, the partition was removed, during which time both HM and LM stimuli were played from respective speakers, simultaneously. After the partition was removed, I recorded 1) which side of the arena each subject selected first (HM vs. LM), and 2) duration spent (seconds) on each side of the arena (HM vs. LM). If a subject failed to make a choice after the partition was removed, that individual was eliminated from the experiment. The apparatus was cleaned with 100% ethanol between trials.

Trials of females were conducted using playback stimuli from males and were designed to test response to HM versus LM vocalizations. Trials of males were conducted in the same manner, except using playback stimuli of females.

Statistical Analysis

For males and females, I used a paired t-test (Sokal & Rolf 1995) to compare duration spent on each side of the arena and to determine if subjects were more likely to select either the left or right side. For each sex, I used a paired t-test to compare duration spent in the side of the arena where HM vocalizations were played vs. the side of the arena where LM vocalizations were played. I predicted that males and females would select the HM side of the arena first. A binomial test commonly is used in non-parametric statistics when sample size is small (<100) and the null hypothesis is that two categories have an equal chance of occurring (Collani & Dräger 2001). My experiment met the criteria for a binomial test and was used to evaluate my prediction that males and females would select the HM side of the arena first.

RESULTS

A paired t-test determined that male (df = 9, t = 0.27, p = 0.79) and female (df = 30, t = 1.72, p = 0.10) subjects did not select the left or ride side of the arena more often. Male bats spent significantly more time in the respective zone where vocalizations were played of HM females versus LM females (df = 9, t = 3.15, p = 0.01)(Fig. 2A). Of the 10 reproductively active males that were tested, 9 selected ultrasonic vocalizations of females that were frequent copulators (HM) and 1 selected ultrasonic vocalization of a female that was an infrequent copulator (LM) (two-tailed binomial test; n = 10, p = 0.02)(Table 1).

Females did not spend significantly more or less time in the respective zone where vocalizations were being played of a HM male versus LM male ($df = 30$, $t = 1.72$, $p = 0.10$)(Fig. 2B). Of the 31 reproductively active females that were tested, 12 selected ultrasonic vocalizations of males that were frequent copulators (HM), and 19 selected ultrasonic vocalizations of males that mated infrequently (LM) (two-tailed binomial test; $n = 31$, $p = 0.28$)(Table 1).

DISCUSSION

Playback experiments confirmed there was differential response between sexes to specific components of ultrasonic vocalizations of the opposite sex. Playback trials revealed that male big brown bats selected (first choice, time spent) ultrasonic vocalizations of females that mated frequently. These results provide support for my prediction that male big brown bats respond to variation in ultrasonic vocalizations of the opposite sex in a reproductive context. However, my finding also indicates that females did not choose particular males, but this was to be expected given that current molecular evidence suggests choice of mates by females occurs postcopulation (Vonhoff et al. 2006).

Given the limited evidence reported on the mating strategy of big brown bats (McCracken and Wilkinson 2000), we can only speculate about why males show a preference, but females do not show a preference in the context of our study. Recently, it was suggested that females have established dominance hierarchies that are communicated in their ultrasonic vocalization to other bats (Rydell 1986; Kazial and Masters 2004). Similarly, female African elephants live in a female-bonded hierarchical society and signal their reproductive condition to distant males via low-frequency vocalizations (Leong et al. 2005). If ultrasonic vocalizations of female big brown bats are important in this regard, then I would expect males to pay particular attention to vocalizations of females in terms of their “rank.” In fact, I determined that males selected vocalizations of females that copulated more frequently than others. If I assume male bats cannot determine the “mating score” (i.e., number of copulations) of a female, it is likely that males are choosing females based on an established dominance hierarchy that may be revealed in ultrasonic vocalizations of female bats. It stands to reason, that if a male cannot ascertain the “mating score” of a female, but simply that she is a high-ranking female, then it would be selectively advantageous for him to solicit a high-ranking female over a low-ranking female for potential copulations.

In the context of my experiments, ultrasonic vocalizations of female big brown bats communicated information to male bats that may be useful for assessment of mates. Bats engage in mating activity in early autumn upon arrival at the hibernaculum, but continue to engage in mating activity during periods of arousal from hibernation that occur throughout winter. In most observational studies of bats exhibiting this mating strategy, males solicit females for copulations and have been seen to “force” copulations (Clutton-Brock 1989; McCracken and Wilkinson 2000).

Arousal from hibernation can be energetically costly to a bat in terms of using its winter fat stores (Speakman & Racey 1989); therefore, it would be advantageous to find a willing mate as quickly as possible to prevent the possibility of using up fat stores before spring emergence. Additionally, in the mating season, males have access to a potentially large number of females, but it would be energetically expensive to mate with all females during this limited time period. There would be a selective advantage for males that could mate with many females and waste little energy in failed attempts. Acoustic signals would provide an ideal avenue for communication in the aforementioned situations.

Bats primarily are active at night or in situations where use of vision is limited; therefore, one would expect acoustic signals to be an important system of communication. Although, olfactory cues are important for communication in low-light situations, this type of signal is not suited to identify location of the sender unless a bat is close or in direct contact. Additionally, acoustic signals provide higher signal-directionality than olfactory signals and localization would be more reliable in crowded situations as would be the case in the hibernaculum. For the previously mentioned reasons, it follows that acoustic signals would be reliable for quickly localizing the sender (i.e., female), but once a bat is found it could be that olfactory signals are important to provide further information about a potential mate.

There are several lines of evidence to support the idea that ultrasonic vocalizations of females advertently or inadvertently provide information that male bats use to solicit females. First, studies of bats indicate that ultrasonic vocalizations can reveal identity, age, and sex of an individual, and this information would be useful at the hibernaculum for assessment of mates. Second, it is possible that females emit ultrasonic vocalizations indicating they are a potential mate, and active males respond and solicit those females for copulations. A case in point, female African elephants send low-frequency communication signals that change due to social context, rank, and hormonal cycle. Low-frequency signals sent by female elephants may be received by males and inadvertently function as advertisement signals (Leong et al. 2005). Similarly, it is possible that male bats are soliciting females that are reproductively active because ultrasonic vocalizations of females indicate their reproductive status or condition.

Along with the present study, current evidence suggests that choice of mates by females does not occur in the social sense, but as the genetic evidence suggests choice by females occurs postcopulation (i.e., sperm competition, sperm choice---Birkhead 1998; Vonhoff et. al. 2006). Behavioral observations of big brown bats at the hibernaculum indicate that the majority of copulations are initiated by males (personal observation). Kazial and Masters (2004) reported that female big brown bats responded to vocalizations of other females, but not males. Additionally, we determined that females did not select ultrasonic vocalizations of males that copulated more frequently. In the non-mating season, female big brown bats form maternity colonies while males form separate colonies, so interaction between males and females is limited. In the mating season, male and female big brown bats congregate at the hibernaculum to engage in reproductive behaviors and to hibernate. If ultrasonic vocalizations are useful in a social context, then sexual dimorphism in vocalizations should be important at this time of year. In fact, a recent study found that male bats change their call seasonally and contextually in a manner that would be energetically costly, but female bats did not select males on the basis of ultrasonic variation (Grilliot et al. submitted, Chapter 1 & 2). These results suggest that male big brown bats may be changing their vocalization to signal other males, possibly for male-male competition.

Although my study excluded the possibility for bats to choose based on other communication cues (i.e., olfactory, visual, tactile), I cannot exclude the importance of other signals in the social system of bats. Nevertheless, binomial-choice, playback trials revealed that male big brown bats selected (first choice, time spent) ultrasonic vocalizations of females that mated frequently. These results provide support for my prediction that male big brown bats respond to variation in ultrasonic vocalizations of the opposite sex in a reproductive context. Additionally, my finding that females did not choose particular males is consistent with other behavioral studies and further is supported by molecular evidence suggesting that choice of mates by females occurs postcopulation. Although I cannot verify what particular aspect of the vocalization that male bats used to assess females, I know that (time and shape) components of ultrasonic vocalizations of frequent copulators differs from infrequent copulators.

In my study, male big brown bats showed a significant preference for ultrasonic vocalizations of females that mated the most frequently. However, female big brown bats did not show an apparent preference for ultrasonic vocalizations of males that mated frequently versus ultrasonic vocalizations of males that mated infrequently. Although it has been suggested that ultrasonic vocalizations play a social role in adult bats, there are few empirical studies that provide conclusive support for this statement. Studies have shown that there are individual differences in ultrasonic vocalizations of several species of bats (Masters et al. 1991, 1995; Obrist 1995; Burnett et al. 2001; Kazial et al. 2001), but behavioral studies that tested whether bats respond to this variation have provided mixed results (Pearl 1994; Kazial and Masters 2004). This is the first study to show that male big brown bats respond to ultrasonic vocalizations of females with differential levels of mating activity; and shows that bats respond to variation in ultrasonic vocalizations of other bats in a reproductive context. My experiments provide a new avenue to investigate a previously unexplored means of communication that has implications in the evolution of this species mating system.

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FIGURE LEGENDS

Figure 1. A, B, & C represents a typical ultrasonic playback sequence played from speakers placed outside of the playback arena. Playback sequences were designed to randomly assign calls to the left or right speaker, and randomly determine which speaker would play first. Each playback sequence was 120 seconds in length.

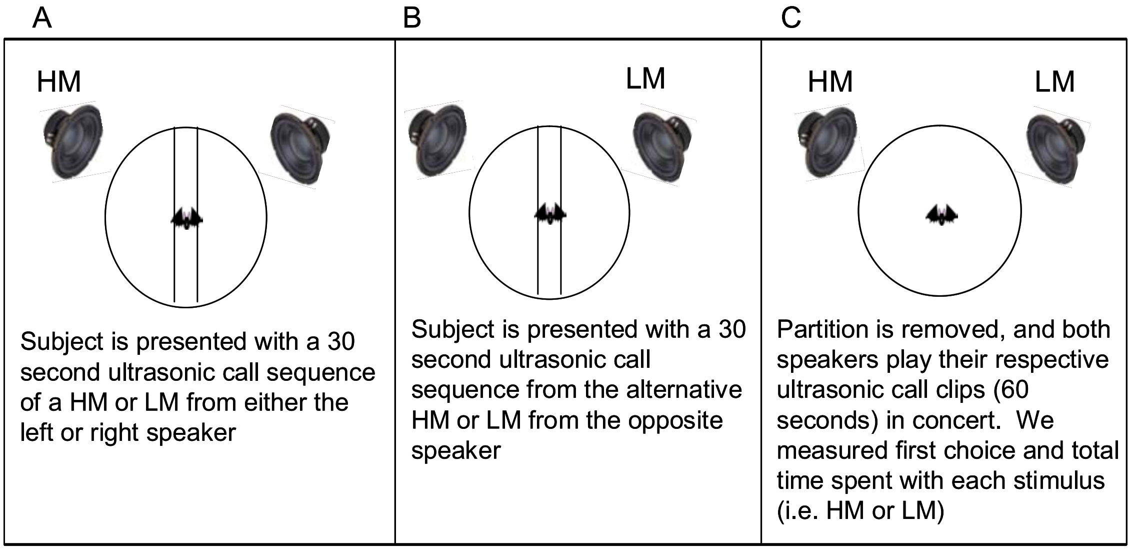
Figure 2: Results of playback experiments for HM vs. LM ultrasonic vocalizations. (A) time (seconds) that male subjects spent on each side of the arena (HM vs. LM). (B) time (seconds) that female subjects spent on each side of the arena (HM vs LM).

Table I. Summary of male and female choice (i.e. first choice) in binomial playback experiments

	Stimulus pair		P (two-tailed binomial)
	HM	LM	
Males	9	1	0.02
Females	12	19	0.28

HM = ultrasonic vocalizations from the opposite sex that were frequent copulators. LM = ultrasonic vocalizations from the opposite sex that were infrequent copulations.

Figure 1



Subject is presented with a 30 second ultrasonic call sequence of a HM or LM from either the left or right speaker

Subject is presented with a 30 second ultrasonic call sequence from the alternative HM or LM from the opposite speaker

Partition is removed, and both speakers play their respective ultrasonic call clips (60 seconds) in concert. We measured first choice and total time spent with each stimulus (i.e. HM or LM)

Figure 2