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Social and vocal behavior in adult greater tube-nosed bats (*Murina leucogaster*)

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ABSTRACT

Many studies have revealed the significant influence of the social nature and ecological niche of a species on the design and complexity of their communication sounds. The knowledge of communication sounds and particularly of the flexibility in their use among mammals, however, remains patchy. Being highly vocal and social, bats are well suited for investigating vocal plasticity as well as vocal diversity. Thus, the overall aim of this study was to test the presence of structural overlap between calls used in social communication and echolocation pulses emitted during foraging in greater tube-nosed bats (*Murina leucogaster*). Acoustic analysis and spectrotemporal decomposition of calls revealed a rich communication repertoire comprising 12 simple syllables and 5 composites with harmonics in the ultrasonic range. Simultaneous recording of vocal and social behavior in the same species yielded a strong correspondence between distinct behaviors and specific call types in support of Morton's motivation-structure hypothesis. Spectrographic analysis of call types also revealed the presence of modified components of echolocation pulses embedded within social calls. Altogether, the data suggest that bats can parse complex sounds into structurally simpler components that are recombined within behaviorally meaningful and multifunctional contexts.

1. Introduction

Yangochiropteran bats (Teeling et al., 2005) have weak vision and are well known for their ability to echolocate (Griffin et al., 1960; Jones and Teeling, 2006). They live in highly specialized ecological niches and use echolocation to forage within cluttered environments (Kunz and Fenton, 2003; Neuweiler, 2003). Many species live in complex societies and use sounds for communication within social contexts (Kanwal et al., 1994; Ma et al., 2006; Clement and Kanwal, 2012). This vocal ability is of significant interest to ecologists, ethologists and cognitive scientists, as well as geneticists and evolutionary biologists because use of communication sounds is widespread among many taxonomic groups. Yet, our understanding of many aspects of social communication in bats is incomplete because literature on comprehensive and in-depth, long-term studies in any single species is severely lacking.

Communication sounds are important for establishing and maintaining social bonds between individuals, group cohesion (Chaverri et al., 2013), roost selection (Clement and Castleberry, 2013), territoriality (Eckenweber and Knörnschild, 2013) and other social interactions (Bohn et al., 2008). The study of vocal communication in bats is beginning to open a window into a deeper understanding of the evolution of communication signals and language as well as brain mechanisms that govern call processing (Kanwal et al., 1994; Kanwal, 2012) and generation of social calls within appropriate behavioral contexts (Liu et al., 2013). Finally, the study of communication in bats along with other mammalian species is providing a deeper understanding of the perception and expression of emotions in animals and consequently their origin and organization in humans (Kanwal et al., 2013).

Much of the earlier work on social vocal communication in bats was restricted to mother–infant interactions because of their robust nature and the ease of recording vocalizations within a well-defined social context (Fanis and Jones, 1995). Over the last two decades, the database of communication sounds produced by different bat species as well as other mammalian species has been growing steadily. In bats, these advances, accompanied by neurophysiological analyses of call processing (Kanwal, 2012), have benefited from a taxonomy of social calls that is based on the patterns of frequency modulation visualized by spectrographic

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analysis of the acoustic structure rather than on anthropomorphic naming schemes (Kanwal et al., 1994; Ma et al., 2006). Developing the ability to connect specific vocalizations with well-defined behaviors, however, remains a daunting task because of the complexity of many social behaviors, their inherently unpredictable nature and timing within which they can be elicited, and their relatively rare occurrence within captive bat populations maintained within semi-natural environments. Nevertheless, relating social calls to natural behaviors remains an important goal of studies of social communication signals as they can test the correspondence of the acoustic structure of social calls in relation to particular ecological niches, which are rather unique in bats. So far this has only been done for echolocation vocalizations in bats (Sun et al., 2013). Similar to echolocation, species-level variation in the acoustic structure of social calls could emerge from informational, environmental and/or biological constraints. Alternatively, the acoustic diversity and complexity of social calls could provide a substrate for the elaboration of diversity of echolocation sounds produced by different species (Kanwal et al., 1994; Arch and Narins, 2008). To address these and other socially, ecologically, physiologically and possibly evolutionarily relevant phenomena, it is important to study the acoustic structure of social calls, including their spectrographic variation within and between different social behaviors using a well-defined and relatively objective acoustic classification system. This is a major goal of the present study that was focused on testing the correspondence between social and vocal behavior in the greater tube-nosed bat, *Murina leucogaster*.

Greater tube-nosed bats have distinctive tubular nostrils and are the most geographically widespread and abundant species of *Murina*, which is one of the widely dispersed genera of Vespertilionidae in China (Koopman, 1993). Studies combining behavioral and acoustic analyses of social calls are lacking for *Murina*, which are only found in caves. Given their evolutionary adaption to dark environments where sounds are critical for facilitating social interactions, we hypothesized that this species has a rich repertoire of communication sounds that, as in some other bat species (Clement and Kanwal, 2012), might be used in more than one context. We also hypothesized that this species, like the Mexican free-tailed bat (Bohn et al., 2008), uses echolocation pulses within a purely communicative context. A test of both of these hypotheses required a detailed examination of the acoustic structure of social calls and the behavioral context in which they are produced.

Here, as a first step towards testing call diversity predicted by motivational-structural rules (Morton, 1977) within the context of social behaviors, we identify and describe specific behavioral contexts, including flight, contact, distress, approach and aggression, appeasement, mating and grooming behaviors in *Murina*, including associated vocalizations that were emitted by adult bats. In describing social communication, we discovered two context-specific call types that have not been described previously in bats. Using a nomenclature based on a quantitative analysis of the spectrotemporal features as well as the scope of their variation in adult greater tube-nosed bats, we hope to provide a deeper understanding of the function and evolution of social vocalizations in *Murina*, an important model species representative of Vespertilionidae as a whole.

2. Materials and methods

2.1. Acquisition and maintenance of animals

A total of 14 greater tube-nosed bats, *M. leucogaster* (7 ♀, 7 ♂), from Dalazi cave (125°50′9.9″ E, 41°3′55.8″ N) in Ji'an, Jilin province, China, were marked and housed in free-flight husbandry rooms (5 m × 8 m × 3 m each), which were connected with a sliding door (1.5 m × 2 m). A 12-h light/dark cycle was maintained using an astronomical light timer adjusted to the natural photoperiod at the study location. The temperature and humidity were controlled according to ambient conditions, with temperatures ranging between 25 and 30 °C (40–60% relative humidity) during the light period, and between 15 and 20 °C (50–80% relative humidity) during the dark period. The bats could crawl and roost on the waterproofed and roughened walls and ceiling, as well as in separate areas (1–2 m^2) of the ceiling with black tarp that reached down to approximately 50 cm above the floor. When left undisturbed, the bats roosted in the simulated "cave-like" environment during most of the light period. We also randomly hung numerous artificial plants from the ceiling or attached to the walls to enrich the environment. Bats were given ad libitum access to water and mealworms. All husbandry and experimental procedures were approved by the Wildlife Conservation Office at Jilin Forestry Department, China, and were in accordance with the guidelines for animal experiments of Northeast Normal University.

2.2. Acoustic recordings

Data acquisition took place from May to November 2012, including the breeding season. Recording sessions started at the beginning of the light period and lasted for about 12 h so as to include the period of maximal social vocal activity.

Sound recordings were made using Avisoft UltraSoundGate 116 H (Avisoft Bioacoustics, Berlin, Germany) with a condenser ultrasound microphone (CM16/CMPA, with a flat frequency response between 10 Hz and 200 kHz (±3 dB); Avisoft Bioacoustics). Sounds were stored and analyzed using a sampling rate of 500 kHz at 16 bits/sample on a notebook computer and each sound file was a maximum of 1 min in length. The gain was adjusted appropriately to ensure that enough high quality signals were recorded. Simultaneously, an activated night-shot camcorder (HDR-CX 760E; Sony Corp., Tokyo, Japan) was used to record social behaviors. The microphone and the camcorder were aligned on the same tripod so that they were always aimed toward the bats. The recording was conducted in the bat clusters, near the feeding stations and during free flight. Recordings were made in the same room for all recording sessions. In addition, up to four bats were separated in varying combinations of individuals (i.e., one male + one female, one male + two females, two males, two females, two males + one female, and all four bats together) and placed in a cage (1.5 m × 1 m × 1 m) in the center of a different room. The sliding door was closed to prevent any disturbance by roosting bats. This allowed us to perform recordings focused on details of social and vocal behaviors. We assigned a call to a certain bat by matching its mouth opening, as well as the head and body movements according to the recorded vocalizations based on either or both a frame-by-frame and slow motion analysis of the recorded video.

2.3. Analysis and terminology of vocalizations

We used Avisoft-SASLab Pro version 5.1 (Avisoft Bioacoustics) for sound analysis. Sounds with a good signal-to-noise ratio were selected for measurement of those acoustic parameters that facilitated classification of syllables into different types. To reduce the influence of pseudoreplication, we selected the same syllable types measured in different call sequences. A high-pass filter with a cut-off frequency at 1 kHz was used to reduce background noise outside the relevant frequency band. The amplitude of each syllable was normalized to 0.75 V. Measurement of acoustic parameters was based upon a 1024 fast Fourier transform (FFT) window (Hamming window), 75% frame size and 93.75% temporal overlap yielding frequency spectra and spectrograms (frequency resolution: 488 Hz, temporal resolution: 0.128 ms). Peak frequency was