Behavioural strategies associated with using an ephemeral roosting resource in Spix's disc-winged bat

B. Karina Montero, Erin H. Gillam

Department of Biological Sciences, North Dakota State University, Fargo, ND, U.S.A.

Article history:

Received 19 March 2015

Initial acceptance 13 April 2015

Final acceptance 18 June 2015

Available online xxx

MS. number: A15-00223R2

* Correspondence: B. K. Montero, 218 Stevens Hall, NDSU Dept. 2715, P.O. Box 6050, Fargo, ND 58108-6050, U.S.A.

E-mail address: bianca.montero@ndsu.edu (B. K. Montero).

The limited availability of refuges may represent an important factor promoting the evolution of sociality, particularly in bats. Spix's disc-winged bats (*Thyroptera tricolor*) show highly specialized morphological adaptations that enable individuals to roost inside furled musoid leaves. This roosting ecology presents major challenges, as leaves rapidly unfurl, forcing bats to locate new roosts on a daily basis. Despite the reliance of *T. tricolor* on such ephemeral roosting resources, bats form stable group associations. This social stability has been attributed to the potential benefits that group members gain from searching for new roosts together versus alone. The purpose of our study was to characterize the behavioural patterns associated with assessing roosts and any accompanying communication strategies of Spix's disc-winged bat. We determined that bats regularly monitor furled leaves within their roosting range, during which they emit a number of distinct social calls. Roost assessment involved close inspection of leaf dimensions and surroundings, and was mostly performed by single individuals. *Thyroptera tricolor*'s call repertoire during flight indicates that group contact involves a combination of acoustic signals. Our approach in a natural setting provides valuable insight into the role of shelter stability in the evolution of the *T. tricolor* social system.

Keywords:

contact call

group interaction

roosting ecology

social stability

Thyroptera tricolor

Ecological factors, such as resource availability and predation pressure, can have major impacts on the evolution of social behaviour in animals (Hatchwell & Komdeur, 2000; Heg et al., 2004). Such ecological constraints are a result of reliance upon resources that fluctuate with time and space, and consequently vary in availability, distribution and predictability. This variability can shape social systems in many ways and lead to outcomes ranging from solitary individuals to highly stable group associations (Chapman et al., 1995; Smith et al., 2008; Wrangham et al., 1993). Thus, examining the influence of ecological parameters on social behaviour is critical for understanding the adaptive significance of sociality.

Food and shelter are among the most critical resources required by animals for effective survival and reproduction. Previous work has shown that the distribution and availability of food resources can have an important impact on patterns of group association. For instance, when food is rare or varies seasonally, group cohesion and group size decrease, mainly as a result of increased competition between conspecifics (e.g. African elephants, *Loxodonta Africana*: Wittemyer et al., 2005; hamadryas baboons, *Papio hamadryas*: Schreier et al., 2012; spotted hyaenas, *Crocuta crocuta*: Holekamp et al., 2012). Less attention has been given to the role of shelters in shaping sociality, although shelters are broadly recognized as an important resource for animals. Shelters provide physical protection from predators and adverse environmental conditions (Kumagai, 2008; Kunz & Lumsden, 2003), and may also facilitate efficient access to food and mates (Booksmythe et al., 2008; Chapman et al., 1989). As a result, the ability of an individual to acquire a shelter will impact its survival and likelihood of reproduction.

Among bat species, the availability of roosting resources has an important influence on the strength of group associations. In turn, distinct patterns of roosting behaviour have been observed in relation to the distribution of roosts in a habitat (Chaverri & Kunz, 2010; Kerth,

2008). For example, big brown bats, *Eptesicus fuscus*, living in buildings exhibit high roost fidelity (Brigham, 1991; Brigham & Fenton, 1986), while populations of the same species that roost in tree cavities regularly switch among a large number of roosts within their habitat (Kalcounis & Brigham, 1998; Vonhof & Barclay, 1996; Brigham et al., 1997). Despite a common pattern of low roost fidelity in many bat species, the presence of long-term associations between colony members has been observed (*Myotis bechsteinii*: Kerth & König, 1999; *Eptesicus fuscus*: Willis & Brigham, 2004; *Nyctalus lasiopterus*: Popa-Lisseanu et al., 2008). Roost-switching behaviour is even more pronounced in Spix's disc-winged bat, *Thyroptera tricolor*, compared to temperate cavity-roosting species. These bats use suction discs on the wrists and ankles to adhere to the smooth surface of developing furled leaves of *Heliconia* (Musaceae) and *Calathea* (Maranthaceae) plants that typically grow in the understory of tropical forests (Findley & Wilson, 1974; Riskin & Fenton, 2001). Developing leaves remain furled for up to 60 h, forcing bats to switch roosts daily (Vonhof & Fenton, 2004). Despite the reliance of *T. tricolor* on such an ephemeral roosting resource, bats form long-lasting stable social groups composed primarily of close kin (Buchalski et al., 2014; Chaverri, 2010). The specialized roosting ecology of *T. tricolor*, coupled with the cohesive social structure observed in roosting groups, suggest the presence of behavioural strategies that enable individuals to regroup after periods of separation. Previous experiments have identified two social calls that are involved in reuniting group mates when separated (Chaverri et al., 2010; Gillam & Chaverri, 2012). However, aside from the recent studies documenting the use of these two calls, we know little about the communication system of *T. tricolor*. Furthermore, nothing is known about the behavioural processes involved in how a group selects a new roost, a process that invariably happens every day.

Our objective in the current study was to characterize the behavioural strategies involved in locating and selecting new roosts. We used continuous video and acoustic monitoring to test the hypotheses that finding a suitable roost involves individuals (1) assessing the suitability of furled leaves within their roosting range and (2) exchanging social calls in a manner that facilitates coordination with group members during or after foraging bouts. While we initially assumed that any occupied leaf would remain so until the next evening (i.e. a standard day roost), we found that a large number of leaves were used exclusively as night roosts. As a result, our post hoc analyses focus on trying to understand how patterns of behaviour and social call production differ near night roosts, day roosts and unoccupied leaves. Studying free-ranging bats allowed us to gain a better understanding of how individuals select new roosts and the potential benefits of social cohesion in bats that rely on highly ephemeral resources.

METHODS

Study Locations

The study was conducted from January to February 2011 and from March to April 2012 at three protected areas in Costa Rica. This period corresponds to the driest months at these sites and coincides with the breeding season of *T. tricolor*. Fieldwork during 2011 was carried out at Sirena Field Station (SIR, 8°28'48''N, 83°35'24''W), located in the south Pacific coast within Corcovado National Park. Fieldwork in 2012 was conducted at two sites in the northeast Atlantic region: Cuatro Esquinas Field Station at Tortuguero National Park (TOR, 10°32'24''N, 83°30'W), located in the northeastern coast, and Tapiria National Reserve (TAP, 10°31'48''N,

84°1'48''W), located in the foothills of the Cordillera Central mountain range. The study sites are characterized by the presence of primary and secondary tropical rainforest, with numerous streams draining throughout the study area. Plants used by *T. tricolor* as roosts are typically distributed along streams and forest gaps; however, the composition and abundance of these plants differed between our study sites; the study area at Sirena exhibited dense patches of *Calathea* and *Heliconia* spp., whereas at Tortuguero, patches of *Calathea* spp. were dominant, and at Tapiria, both *Heliconia* and *Calathea* spp. were highly scattered and usually not found in dense stands (B. K. Montero, personal observation).

Data Collection

Capture and marking techniques

During the day, we searched for bats roosting in furled *Heliconia* spp. and *Calathea* spp. leaves. Bats were captured by pinching the top of the leaf and directing individuals into a cloth holding bag. We recorded standard measurements (body mass, forearm length, sex, reproductive state and age) of all the individuals captured. Individual identification and group membership was assessed using two different methods. Bats at Sirena field site were marked with passive integrated transponders (9 mm PIT tags; Biomark Inc., ID, U.S.A.) that were implanted subcutaneously between the scapulae (tags represented <2 % of the average weight of bats). Individuals at Tortuguero and Tapiria were marked with coloured forearm rings (males and females were marked in the right and left forearm, respectively) (Avinet, Inc., Dryden, NY, U.S.A.). At these two sites, we also attached reflective tape to the dorsal pelage, which enabled us to recognize the individuals of a focal group monitored during the night. Reflective tape fell

off after approximately 5 days (B. K. Montero, personal observation). Only adults and subadults were marked during the study. Measurements and marking of a focal group lasted no more than 30 min. Bats were released simultaneously, which typically allowed individuals to regroup in close proximity to the previous day roost.

Recording methods

At each site we selected three to seven independent groups and monitored the approach behaviour of bats to known and potential furled leaf roosts using video and acoustic recordings. Focal groups were not selected at random; rather, we selected groups for which we had identified at least three roosting leaves and for which the estimated roosting range was greater than 50 m apart. This enabled us to obtain a preliminary estimate of the boundaries in which a group would roost (by means of minimum convex polygons based on georeferenced locations) and reduce the possibility of recording leaves in areas where adjacent groups show high spatial overlap. Day roosts were identified by positioning a mirror attached to an extending 2 m pole above an uncoiled leaf or by means of a PIT tag portable reader (PocketReaderEx, Biomark). Once the focal group was located, we placed a video and acoustic recording devices in front of the day roost if we predicted that the leaf would remain furled during the night. If the leaf would not remain furled, the recording devices were placed in front of a previously unoccupied suitable leaf. The recording set-up was the same for both previous roosts and suitable leaves. The following criteria were used to select the suitable leaf to be monitored on a given night: (1) we searched for suitable leaves with structural characteristics that roughly matched previous work describing roost dimensions (Vonhof & Fenton, 2004) and our own observations of occupied leaves in our field sites (5–7 cm diameter opening, 140–220 cm total height); (2) we identified

multiple furled leaves within the estimated roosting range and selected the closest leaf to the roost in which bats were found.

The recording system consisted of a night-shot camera (Sony HDR-CX550V) enclosed in a water-resistant case attached to an infrared light source (Wildlife Engineering, IRLamp6) and a microphone ultrasonic recording system (Avisoft Bioacoustics, Berlin, Germany). The video camera and lighting system were aimed at the distal end of the leaf, positioned about 3 m away from the focal leaf. The camera was zoomed in to obtain a viewing radius around the leaf entrance of approximately 2 m. A microphone (Avisoft condenser microphone CM16) was positioned at a distance of roughly 30 cm and oriented towards the leaf entrance to capture acoustic signals emitted by bats approaching, entering or exiting the roost. Video recordings started before dusk (1750 hours) and were continued until daylight (0530 hours). These times were selected based on previous field observations in which bats would depart and enter a roost respectively. Acoustic recordings at Sirena field station were conducted in two sessions: from 1750 to 2100 hours and from 0330 to 0530 hours. Acoustic recordings in Tortuguero and Tapiria were conducted continuously during the night. Because of the sensitivity of the microphones, we interrupted acoustic recordings in the event of rain bouts, but we continued recording once the rain ceased. Acquisition of sound data was made with an Avisoft Ultrasound Gate 416 and Avisoft Recorder running in a Toughbook laptop (Panasonic). Acoustic recording was conducted with a 16-bit resolution at a sampling rate of 250 kHz.

Video analysis

Behaviours observed on video near the furled leaf were classified using a customized ethogram (Table 1; see Supplementary Material for video files of behaviours observed near

furled leaves) that logged the type of approach behaviour, the number of bats approaching the leaf and the time of occurrence of each observation (i.e. time of behavioural event). We defined three categories for the type of leaf being monitored: (1) previous roost: leaf in which bats were found roosting during the day; (2) night roost: available leaf where bats entered during the night at least once; (3) unoccupied leaf: suitable leaf not used as a night roost by bats. As part of our post hoc analyses, we divided suitable leaves in two categories that reflected whether the bats entered a leaf but did not remain there through the morning (night roost) or whether the leaf remained unoccupied throughout the night (unoccupied leaf) despite exhibiting preferred structural characteristics. We scored behaviours by observing videos at $\frac{1}{8}$ playback speed. When present, simultaneous video and audio recordings allowed us to link behavioural observations with the acoustic signals emitted by bats, and we noted the presence and type of social calls associated with a behavioural observation. In our video recordings, bats within a group were not individually identifiable due to their rapid movements and small body size. Yet, our primary interest was in quantifying overall visits to each leaf, regardless of the identity of the visiting bats; hence, information about individual identity was not necessary. Behavioural data was obtained using the analysis software JWatcher v.1.0 (Blumstein et al., 2007). Video scoring was carried out by the same person.

Acoustic repertoire

We visually examined social signals and categorized them according to the patterns observed in the call structure. We identified six call types (Fig. 1): (1) downward-modulated frequency (INQ) (previously described as an ‘inquiry call’ by Chaverri et al., 2010); (2) short quasiconstant frequency (SQCF): short element of dominant frequency within 5 kHz of the start

frequency; (3) quasiconstant frequency (QCF): dominant frequency within 5 kHz of the start frequency; (4) long upward-modulated (LUM) frequency: initial upward sweep followed by a quasiconstant frequency section; usually occurring in bouts of two to five calls; (5) short upward (SUP) frequency: a concave-downward sweep with positive slopes; and (6) short upward-modulated (SUM) frequency: concave-upward sweep with positive slopes and step-like increases. Echolocation sequences correspond to low-intensity broadband signals (Fig. 1g). We characterized call structure by measuring signal parameters from power spectra, waveform and spectrograms. Visual examination of social calls and acoustic measurements were done using SasLab Pro v.5.2.07 (Avisoft Bioacoustics). Call duration was measured from the waveform. Spectral properties were measured with a FlatTop window, 1024 point fast Fourier transform and 75% overlap, resulting in a frequency resolution of 244 Hz. Frequency parameters included: start (F_{start}), end (F_{end}), peak (F_{peak}), minimum (F_{min}) and maximum (F_{max}) frequency. Frequency parameter data were collected from the spectrogram using the automatic parameter measurements function. Peak frequency (defined as the frequency of highest intensity within the signal) was obtained from the power spectrum. We limited our measurements to the fundamental frequency in those social calls that exhibited multiple harmonics. To characterize call structure, we also included calls that were recorded in the vicinity of a focal leaf but where no bats were observed in our video. Only calls with high signal-to-noise ratio were included in the analysis.

Statistical Analysis

To test whether the behaviour observed in the vicinity of a leaf varied between leaf type, time or site, we fitted generalized estimation equations (GEE). Since we recorded multiple

observations throughout the night for each leaf monitored, our data for each group lacked independence. For analysis, we excluded those leaves where video recordings were interrupted due to extreme weather conditions or equipment failure, resulting in unequal sample sizes across groups. We accounted for differences in sampling effort, as well as lack of independence of multiple observations made at each leaf, in our GEE models. GEE allowed us to account for the spatial and temporal correlation structure of the behaviour recorded in the vicinity of furled leaves by grouping observations into clusters that could subsequently be considered statistically independent. We assumed an autoregressive correlation structure and allowed repetition of focal leaf. In addition, we included group size as an offset term and acoustic recording duration (log sampling duration) to account for the variation of sampling effort between groups. The following variables were used as response terms: (1) number of visits per night, (2) duration of the approach behaviour, which was defined as circling, hover or overall approach (sum of circling and hover), (3) the presence of social calls when approaching a leaf and (4) the rate of social calls recorded in the vicinity of a focal leaf. We estimated call rate by quantifying the total number of social calls recorded in the vicinity of monitored leaves, including those where bats were not captured in our video recordings. We used a Poisson error structure for all of the response terms except presence of social calls, for which we used a binomial error structure. Leaf type (previous roost, unoccupied leaf or night roost), site (SIR, TOR or TAP) and time (observations grouped in 1 h time categories from 1730 to 0530 hours) were used as explanatory variables. Models were fitted using the 'geeglm' function of the geepack package (Halekoh, Højsgaard, & Yan, 2006) in R (R Core Team, 2014).

For those visits in which we recorded the presence of a social call, we examined whether the frequency of a particular call type was related to (1) the number of bats visiting the leaf, (2)

leaf type or (3) time of visit. The effect of each of these factors (call type, number of bats, leaf type and time) on the variability in number of calls recorded was examined using generalized linear models with a Poisson distribution.

Ethical Note

The marking techniques used in this study allowed us to identify focal groups within a roost without disturbing individuals. Furthermore, during the length of our study we did not identify any individual that showed signs of infection after implanting the PIT tags or attaching forearm bands, and all bats were observed to conduct normal flight behaviour after being released. This study was approved by the Costa Rican authorities (Ministry of Environment and Energy, MINAE; National System of Conservation Areas, SINAC; reference no. ATM-ACOSA-001-01, 034-2012) and by the NDSU Animal Care and Use Committee (Protocol numbers A11022, A12052).

RESULTS

Approach Behaviour

We characterized the approach behaviour of 14 groups of bats and 38 leaves using 456 h of video and 297 h of acoustic recording (see Supplementary Table S1 for details on sample size). We recorded a total of 398 visits across all sampled leaf types and sites. We determined that the majority of visits were made by single individuals (76%); visits made by pairs of

individuals were less common (17%) while visits of more than two bats flying together were rarely observed. Out of the 96 visits made by multiple individuals, 88% of these visits were characterized by the presence of a chase, where typically one bat leads the flight throughout (Table 1, Supplementary Videos S2, S3). The majority of these chase events (70%) occurred when bats approached a night roost. Although we were not able to obtain reliable information about individual identity, the presence of a forearm band was visible in 13% of the visits. From this limited sample, we were able to confirm that both females and males approached leaves.

Out of the 38 suitable leaves monitored, 21 leaves were never occupied by bats during the night, seven represented previous roosts and 10 were used as night roosts, two of which were also selected as day roosts. Bats spent an average of 8.5 min inside furled leaves selected as night roosts. Of the 52 times that bats were observed to enter a leaf, the majority of night roost behaviour corresponded to single bats (40%). We also observed that typically bats entered and exited the leaf simultaneously in those events in which more than one bat was observed using a night roost (92% and 85% of enter and exit events performed by multiple bats). When comparing the number of visits made to each leaf type, bats were observed to visit night roosts more frequently than previous roosts or unoccupied leaves (GEE: Wald = 6.41, $P = 0.011$; Fig. 2). Furthermore, variation in visit frequency was more pronounced among unoccupied leaves selected as night roosts (Fig. 2a). Out of the 52 total visits observed at night roosts, only 16 visits were observed to occur when a bat was roosting inside the furled leaf. Bats at Sirena and Tapiria were observed to visit every recorded furled leaf within their roosting range at least once, with the most frequent visits to night roosts (up to 77 visits per night). The total number of visits differed significantly between sites (GEE: Wald = 6.25, $P = 0.012$). Tortuguero exhibited the lowest visit frequency; half of the leaves monitored, including previous roosts and suitable

leaves, were never visited. Furthermore, the number of visits was independent of time (GEE: Wald = 0.914, $P = 0.40$). Visiting behaviour suggests that leaves are visited on multiple occasions during the night (Fig. 2b).

When visiting a furled leaf, approach behaviour typically consisted of a circling flight followed by a short hover in close proximity to the leaf opening (Supplementary Video S1). The time spent in an approach event did not differ significantly between sites (GEE: Wald = 5.56, $P = 0.062$); however, bats at Tortuguero spent less time flying within the proximity of furled leaves, whereas bats at Tapiria spent more time during an approach event (Fig. 3). In addition, we found a significant interaction between leaf type and time category (GEE: Wald = 6.61, $P = 0.01$). Approach to night roosts took longer early in the night, while approach duration at suitable leaves was similar throughout the night. The time bats spent hovering on the leaf entrance did not differ significantly between sites or leaf type (Fig. 3b).

Calling Behaviour

We analysed a total of 722 social calls, which were visually classified into six call types. We excluded from the analysis the call type SQCF, as it was rarely recorded with sufficient signal-to-noise ratio. The vocalizations recorded were tonal signals with most energy from 10 to 32 kHz (Table 2).

The most common vocalization types were INQ and LUM, which represented 42 and 33% of the total calls recorded, respectively. Overall, from the 156 events in which we recorded the presence of a social call, most signals were emitted by bats during a direct approach to the leaf (69%) or during a pass by behaviour (28%). When examining whether the presence of a

particular call type was associated with the leaf type visited, we found that LUM calls were most commonly recorded around unoccupied leaves (GLM: $P = 0.045$), while no significant differences were detected for the other call types (Fig. 4). The vast majority of social calls were recorded during visits in which an individual bat approached a leaf (90%), and no significant difference between call types was evident with respect to the number of bats that visited a leaf. We did not find any significant pattern in the presence of a particular call type with respect to time category; all call types were recorded throughout the night, although the number of calls increased between 0230 and 0430 hours.

The presence of social calls during a visit differed between leaf types. Based on our model, the probability of recording a call was higher near unoccupied leaves (GEE: Wald = 3.89, $P = 0.049$) than near night roosts and previous roosts. We estimated that the likelihood of a social call being made during a visit was 76%, 6% and less than 1% at unoccupied leaves, night roosts and previous roosts, respectively. There was no significant interaction between time and leaf type. At previous roosts and night roosts, the probability of recording a social call was similar throughout the night; however, at unoccupied leaves, this probability increased as the night progressed (Fig. 5).

Overall, call rate was similar between leaf types (GEE: Wald = 0.86, $P = 0.649$) and across sites (GEE: Wald = 4.83, $P = 0.09$). Call rate was significantly influenced by time (GEE: Wald = 9.70, $P < 0.001$). The number of calls recorded increased throughout the night, particularly at unoccupied leaves (Fig. 6).

DISCUSSION

The purpose of our study was to characterize the patterns of roost assessment and the associated communication strategies of Spix's disc-winged bat. Our approach of continuously monitoring roosting resources allowed us to (1) examine the behaviour of *T. tricolor* in the vicinity of furled leaves and (2) characterize the vocal repertoire of free-ranging bats during roost exploration. Because *T. tricolor* must locate new roosts daily, we hypothesized that individuals visit furled leaves within their group's roosting range to assess their suitability as a day roost. In our study, the bats approached furled leaves, but the frequency of these visits varied considerably across sites and types of leaves monitored. Given the social stability observed in *T. tricolor*, we also hypothesized that the bats would produce acoustic signals in the vicinity of furled leaves as a behavioural strategy that would enable them to regroup at some point during the night. Here we report the presence of distinct social calls and expand on our current knowledge of *T. tricolor*'s acoustic repertoire. Although our approach in a natural setting was labour intensive, it provides the most realistic picture of natural patterns of behaviour and, thus, is invaluable for understanding the role of shelter stability in the evolution of *T. tricolor*'s social system.

Monitoring of Furled Leaves

Our results show that one behavioural mechanism permitting *T. tricolor* to exploit a highly ephemeral roosting resource is continual assessment of potentially suitable roosts during nightly foraging bouts. We found that a suitable focal leaf was visited an average of seven times during the night. Because the dimensions of furled leaves do not remain constant (Montero, n.d.), multiple visits within and between nights should allow individuals to evaluate the quality of a leaf as it develops. Alternatively, multiple visits may represent independent assessment from

different individuals within a group. Although we were not able to reliably identify individuals, we did observe females and males approaching a focal leaf. This offers some evidence that multiple individuals visit furled leaves independently.

Approach behaviour consisted of a close examination of both the furled leaf and its surroundings. The duration of the overall approach was dominated by a repeated circling flight around the leaf; on 87% of approach events, we also observed bats conducting a relatively short hover above the leaf opening (Supplementary Videos S1, S3). Both the inspection of leaf dimensions and the surrounding vegetation presumably aids in the process of selecting the most appropriate roost on a given night. Bats most likely choose leaves that will remain furled during the day and preferably those that offer less risk of predation. During the length of our study, we only observed two occupied leaves that were partially unfurled by the end of the day, suggesting that in most cases, bats choose a leaf that will remain furled for at least 24 h.

When comparing the monitoring behaviour of bats at different sites, we found that visit frequency and approach duration were lower at Tortuguero than at Sirena and Tapiria. Although our criteria for selecting focal leaves remained constant during the study, plant density and composition differed across sites. While we mainly monitored *Heliconia* plants at Sirena and Tapiria, all focal leaves at Tortuguero were *Calathea*; patches of *Calathea* stands dominated Tortuguero's study site and *Heliconia* plants were rare. When estimating the rate at which leaves unfurl, Vonhof and Fenton (2004) found that *Calathea* were available as roosts for shorter periods than *Heliconia* spp. The high abundance of rapidly unfurling roosts at Tortuguero may lead to a different optimal monitoring strategy there than at other sites, where leaves suitable for roosting are available at higher densities. Specifically, if more low-quality leaves must be visited

to find a suitable roost at Tortuguero, a natural side effect would be fewer visits to, and less time spent at, each leaf.

In our study we monitored furled leaves that we predicted would be suitable roosts. Half of these leaves were selected as night roosts by individuals of the focal group, two of which were also selected as day roosts. The use of night roosts is fairly common among bats (Kunz & Lumsden, 2003), as they tend to offer strategic locations where animals are close to feeding locations, while also providing resting sites between foraging bouts (Knight & Jones, 2009). In general, night roosts were mostly used by one bat at a time, but about 37% of night roosts were occupied by two or three individuals simultaneously. While we cannot exclude the possibility that bats were attracted to night roosts because of the presence of other bats already within the leaf, this is unlikely, as bats were mostly observed approaching unoccupied leaves. Night roosts are potentially important in allowing individuals to regroup and engage in social interactions during the night.

We originally anticipated that any selected roost would be occupied until the next evening (i.e. as a day roost), but this was not the case. Two of our monitored leaves were used as day roosts, but the vast majority were used as night roosts. Although this post hoc discovery is not ideal for characterizing day roost selection, it provides new insight into the nightly roosting patterns of this species, as communal night roosting has not previously been reported. This finding also indicates that factors other than basic roost dimensions may be important for predicting day roost use.

Groups of *T. tricolor* typically have small roosting home ranges (~0.4 ha), and previous work suggests that bats remain faithful to a habitat patch (Chaverri & Kunz, 2011; Vonnhoff et al., 2004). Our results suggest that bats regularly visit leaves within their roosting range, including

previous roosts that may no longer be suitable. This assessment of roosting resources offers some evidence that maintaining access to a minimum number of plants that will supply enough suitable roosting sites for both night and day shelters is a relevant aspect of *T. tricolor*'s roosting ecology, as suggested by previous studies (Chaverri & Kunz, 2011; Vonhof & Fenton, 2004).

Communication Strategies

The social stability of *T. tricolor* suggests the presence of a mechanism that allows individuals to encounter each other at some point during the night. In our study, we recorded a variety of distinct social calls emitted by bats that approached a furled leaf (Fig. 1). These calls were emitted by bats during flight, which suggests that the calls are important in establishing or maintaining contact with conspecifics during nightly foraging and roost reconnaissance trips. While the fast flight behaviour of *T. tricolor* means that tracking individuals and their associated patterns of call emission is particularly difficult, we can gain insight into the behavioural function of these calls by examining their temporal and spectral structure (Kime et al., 2000; Morril et al., 2013).

One critical factor in understanding the communication signals of any species is the environment in which the animal lives (Bradbury & Vehrencamp, 2000; Ryan & Brenowitz, 1985). Groups of *T. tricolor* are usually found in clearings of secondary forests, where the surrounding vegetation is dense. Such an environment is challenging in terms of acoustic communication, as sound propagation will be hampered due to attenuation from scattering and deflection of acoustic signals (Wiley & Richards, 1978). From a signal design perspective, elements of *T. tricolor*'s vocal repertoire are well suited for long-range communication in such

environments. Two calls in particular are likely candidates for locating group members during flight, namely QCF and LUM. These calls are relatively low in frequency and narrowband, which is ideal for longer transmission distance (Morton, 1975; Wiley & Richards, 1978). Also, the low-frequency modulation of the LUM call coupled with its production in trill-like bouts (two to five calls) may enhance the receiver's ability to estimate the distance of the signaller (Naguib, 1995, 2003). Overall, the LUM call type was the second most common signal recorded in our study, which probably reflects its relevance in facilitating group interactions during night activity.

Inquiry calls were the most common call emitted by bats during our study. Previous work has demonstrated that this call is part of a two-signal system that permits individuals to locate roosting group-mates (Chaverri et al., 2010). Experiments have also revealed that inquiry calls encode individual signatures that can be discriminated by bats in flight (Gillam & Chaverri, 2012). Despite the common detection of inquiry calls, we did not record 'response' calls, which are the second part of the signalling system that facilitates recruitment to roosts (Chaverri et al., 2010). Because previous studies have only assessed diurnal calling behaviour, use of the inquiry–response contact call system may be a strategy used only to relocate a roost within a brief period after a disturbance event. Yet, we do not exclude the possibility that this calling system is used when selecting a day roost, since the number of day roosts we were able to record was small (two day roosts).

The acoustic repertoire we describe in this study reveals that *T. tricolor* produces multiple structurally distinct signal types during flight (Fig. 1, Table 2). While some elements of this calling system were commonly detected, others were extremely rare. Given the observational nature of our study, we were not able to definitively link call types with specific behaviours,

which would give insight into call function. All call types were recorded when bats flew in close proximity to a focal leaf; in general, social calls were recorded less often in association with other behaviours, such as entering or exiting a roost. Finally, there was no association between the number of bats present and the types of calls produced (i.e. no calls were exclusively produced by solitary individuals or by bats within a group). Further investigations, potentially employing playback experiments of social calls, might be valuable for elucidating the function of rare call types.

Despite the relatively small areas of habitat that we sampled acoustically, bats regularly emitted social calls during the night (Figs 4, 5), indicating that actual call production rates across a group's entire home range are probably higher than those reported in our study. One possibility is that extensive social calling may facilitate group cohesion by advertising the location of suitable roosting sites within the group's home range. The use of contact calls to advertise roost location is common among bat species (e.g. tree-dwelling bats: Furmankiewicz et al., 2011; Schöner et al., 2010; crevice-roosting bats: Arnold & Wilkinson, 2011; foliage-roosting bats: Gillam et al., 2013). Previous work on noctule bats has shown that locating a suitable shelter is not a trivial task, and that social cues aid in the process of roost selection (Ruczyński et al., 2007). Similarly, the monitoring behaviour of *T. tricolor* that we observed indicates that locating and selecting a suitable roost may be energetically costly and that social behaviour may greatly influence the ability of individuals to acquire roosting resources.

Social calls could also serve a role in delimiting the territory of a group. Because bats are probably dependent on a minimum amount of plants that will guarantee a reliable supply of roosts (Chaverri & Kunz, 2011; Vonnhof et al., 2004), a benefit of grouping may be expressed by the ability of a social unit to monopolize a high-quality habitat (Wilkinson & Boughman, 1998).

Playback experiments would potentially help elucidate whether acoustic signalling plays a role in group spatial delimitation.

Both our observations near potential roosts and the acoustic behaviour of *T. tricolor* suggest that a behavioural mechanism that facilitates information transfer about suitable roosts is likely to be present in this species. We observed multiple bats approaching a leaf, seemingly as a chase (Supplementary Videos S2, S4). This behaviour occurred throughout the night, providing evidence that bats encounter each other and possibly fly in close proximity repeatedly. Such encounters at suitable leaves may serve to assess roost quality, a process that may be mediated by group decision making. In addition, our results from those leaves in which continuous acoustic recording was available indicate that the overall rate of social call production increased with time, with more calls recorded closer to dawn. This pattern may be related to the timing in which individuals regroup and select a day roost. Future research is necessary to address how bats decide which furled leaf to select as a day roost and whether this process is done as a group.

Acknowledgments

We thank Genuar Núñez and Jairo Moya for assistance in the field, and Julian Schmid, Jackie Wrage and Nicholas Johnson for their valuable help in video analysis. Maria Sagot, Bernal Rodriguez-Herrera, Gloriana Chaverri and Gilbert Barrantes provided constructive input regarding animal sociality and communication. We are especially grateful to Corcovado and Tortuguero National Park rangers and the Aguilar family for housing and support in the field. This research was supported by National Science Foundation Grant No. HRD-0811239 to the

North Dakota State University (NDSU) Advance FORWARD program, start-up funds from the Department of Biological Sciences and College of Science and Mathematics at NDSU (E.H.G.), the Latin American field research awards of the American Society of Mammalogists (B.K.M.) and an NDSU graduate school dissertation fellowship (B.K.M.).

Supplementary Material

Supplementary Material associated with this article is available, in the online version, at doi:

References

- Arnold, B.D., & Wilkinson, G.S. (2011). Individual specific contact calls of pallid bats (*Antrozous pallidus*) attract conspecifics at roosting sites. *Behavioral Ecology and Sociobiology*, 65(8), 1581–1593. doi:10.1007/s00265-011-1168-4.
- Blumstein, D.T., Daniel, J.C., & Evans, C.S. (2006). JWatcher 1.0. <http://www.jwatcher.ucla.edu>.
- Bradbury, J.W., & Vehrencamp, S.L. (2000). Economic models of animal communication. *Animal Behaviour*, 59, 259–268. doi:10.1006/anbe.1999.1330.
- Booksmythe, I., Detto, T., & Backwell, P.R.Y. (2008). Female fiddler crabs settle for less: the travel costs of mate choice. *Animal Behaviour*, 76, 1775–1781. doi:10.1016/j.anbehav.2008.07.022.
- Brigham, R.M., & Fenton, M.B. (1986). The influence of roost closure on the roosting and

- foraging behaviour of *Eptesicus fuscus* (Chiroptera: Vespertilionidae). *Canadian Journal of Zoology*, 64, 1128–1133.
- Brigham, R.M. (1991). Flexibility in foraging and roosting behaviour by the big brown bat (*Eptesicus fuscus*). *Canadian Journal of Zoology*, 69, 117–121.
- Brigham, R., Vonhof, M., & Barclay, R. (1997). Roosting behavior and roost-site preferences of forest-dwelling California bats (*Myotis californicus*). *Journal of Mammalogy*, 78(4), 1231–1239.
- Buchalski, M.R., Chaverri, G., & Vonhof, M.J. (2014). When genes move farther than offspring: gene flow by male gamete dispersal in the highly philopatric bat species. *Molecular Ecology*, 23(2), 464–480. doi:10.1111/mec.12593.
- Chapman, C., Chapman, L., & McLaughlin, R. (1989). Multiple central place foraging by spider monkeys: travel consequences of using many sleeping sites. *Oecologia*, 79(4), 506–511.
- Chapman, C., Chapman, L., & Wrangham, R. (1995). Ecological constraints on group size: an analysis of spider monkey and chimpanzee subgroups. *Behavioral Ecology and Sociobiology*, 36, 59–70.
- Chaverri, G. (2010). Comparative social network analysis in a leaf roosting bat. *Behavioral Ecology and Sociobiology*, 64(10), 1619–1630.
- Chaverri, G., & Kunz, T.H. (2010). Ecological determinants of social systems: perspectives on the functional role of roosting ecology in the social behaviour of tent-roosting bats. *Advances in the Study of Behavior*, 42, 275–318. doi:10.1016/s0065-3454(10)42009-4.
- Chaverri, G., & Kunz, T.H. (2011). Response of a specialist bat to the loss of a critical resource. *PLoS One*, 6(12), e28821. doi:10.1371/journal.pone.0028821.
- Chaverri, G., Gillam, E.H., & Kunz, T.H. (2013). A call-and-response system facilitates group

- cohesion among disc-winged bats. *Behavioral Ecology*, 24(2), 481–487.
doi:10.1093/beheco/ars188.
- Chaverri, G., Gillam, E.H., & Vonhof, M.J. (2010). Social calls used by a leaf-roosting bat to signal location. *Biology Letters*, 6(4), 441–444. doi:10.1098/rsbl.2009.0964.
- Funakoshi, K., Katahira, R., & Ikeda, H. (2009). Night-roost usage and nocturnal behaviour in the Japanese house-dwelling bat. *Mammal Study*, 34(3), 131–139.
doi:10.3106/041.034.0302.
- Furmankiewicz, J., Ruczyński, I., Urban, R., & Jones, G. (2011). Social calls provide tree-dwelling bats with information about the location of conspecifics at roosts. *Ethology*, 117(6), 480–489. doi:10.1111/j.1439-0310.2011.01897.x.
- Gillam, E.H., & Chaverri, G. (2012). Strong individual signatures and weaker group signatures in contact calls of Spix's disc-winged bat, *Thyroptera tricolor*. *Animal Behaviour*, 83, 269–276. doi:10.1016/j.anbehav.2011.11.002.
- Gillam, E.H., Chaverri, G., Montero, K., & Sagot, M. (2013). Social calls produced within and near the roost in two species of tent-making bats, *Dermanura watsoni* and *Ectophylla alba*. *PLoS One*, 8(4), e61731. doi:10.1371/journal.pone.0061731.
- Halekoh, U., Højsgaard, S., & Yan, J. (2006). The R Package geepack for generalized estimating equations. *Journal of Statistical Software*, 15(2), 1–11.
- Hatchwell, B., & Komdeur, J. (2000). Ecological constraints, life history traits and the evolution of cooperative breeding. *Animal Behaviour*, 59, 1079–1086.
- Heg, D., Bachar, Z., Brouwer, L., & Taborsky, M. (2004). Predation risk is an ecological constraint for helper dispersal in a cooperatively breeding cichlid. *Proceedings of the Royal Society B: Biological Sciences*, 271(1555), 2367–2374.

doi:10.1098/rspb.2004.2855.

- Kalcounis, M., & Brigham, R. (1998). Secondary use of aspen cavities by tree-roosting big brown bats. *Journal of Wildlife Management*, 62(2), 603–611.
- Kerth, G. (2008). Causes and consequences of sociality in bats. *Bioscience*, 58(8), 737–746.
- Kime, N., Turner, W., & Ryan, M. (2000). The transmission of advertisement calls in Central American frogs. *Behavioral Ecology*, 11(1), 71–83.
- Knight, T., & Jones, G. (2009). Importance of night roosts for bat conservation: roosting behaviour of the lesser horseshoe bat *Rhinolophus hipposideros*. *Endangered Species Research*, 8, 79–86. doi:10.3354/esr00194.
- Kumagai, N.H. (2008). Role of food source and predator avoidance in habitat specialization by an octocoral-associated amphipod. *Oecologia*, 155(4), 739–749. doi:10.1007/s00442-007-0941-6.
- Kunz, T.H., & Lumsden, L.F. (2003). Ecology of cavity and foliage roosting bats. In T. H. Kunz & M. B. Fenton (Eds.), *Bat ecology* (pp. 3–87). Chicago, IL: University of Chicago Press.
- Lewis, S. (1996). Low roost-site fidelity in pallid bats: associated factors and effect on group stability. *Behavioral Ecology and Sociobiology*, 39(5), 335–344.
- Montero, B.K. (n.d.). [Leaf-roosting bats monitor changes in roost dimensions during night activity]. Unpublished raw data.
- Morton, E. (1975). Ecological sources of selection on avian sounds. *American Naturalist*, 109(965), 17–34.
- Naguib, M. (1995). Auditory distance assessment of singing conspecifics in Carolina wrens: the role of reverberation and frequency-dependent attenuation. *Animal Behaviour*, 50, 1297–1307.

Naguib, M. (2003). Reverberation of rapid and slow trills: implications for signal adaptations to long-range communication. *Journal of the Acoustical Society of America*, 113(3), 1749–1756.

McCracken, G., & Wilkinson, G. (2000). Bat mating systems. In E. G. Crichton & P. H. Krutzsch (Eds.), *Reproductive biology of bats* (pp. 321–362). San Diego, CA: Academic Press.

Morrill, R.J., Thomas, A.W., Schiel, N., Souto, A., & Miller, C.T. (2013). The effect of habitat acoustics on common marmoset vocal signal transmission. *American Journal of Primatology*, 75(9), 904–916. doi:10.1002/ajp.22152.

R Core Team. (2014). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. <http://www.R-project.org/>.

Riskin, D.K., & Fenton, M.B. (2001). Sticking ability in Spix's winged bat, *Thyroptera tricolor* (Microchiroptera: Thyropteridae). *Canadian Journal of Zoology*, 79, 2261–2267.

Ruczyński, I., Kalko, E.K., & Siemers, B.M. (2007). The sensory basis of roost finding in a forest bat, *Nyctalus noctula*. *Journal of Experimental Biology*, 210(20), 3607–3615. doi:10.1242/jeb.009837.

Ryan, M., & Brenowitz, E. (1985). The role of body size, phylogeny, and ambient noise in the evolution of bird song. *American Naturalist*, 126(1), 87–100.

Schöner, C.R., Schöner, M.G., & Kerth, G. (2010). Similar is not the same: social calls of conspecifics are more effective in attracting wild bats to day roosts than those of other bat species. *Behavioral Ecology and Sociobiology*, 64(12), 2053–2063. doi:10.1007/s00265-010-1019-8.

Schreier, A.L., & Swedell, L. (2012). Ecology and sociality in a multilevel society: ecological

- determinants of spatial cohesion in hamadryas baboons. *American Journal of Physical Anthropology*, 148(4), 580–588. doi:10.1002/ajpa.22076.
- Smith, J.E., Kolowski, J.M., Graham, K.E., Dawes, S.E., & Holekamp, K.E. (2008). Social and ecological determinants of fission–fusion dynamics in the spotted hyaena. *Animal Behaviour*, 76, 619–636. doi:10.1016/j.anbehav.2008.05.001.
- Vonhof, M.J., & Fenton, M.B. (2004). Roost availability and population size of *Thyroptera tricolor*, a leaf roosting bat, in north-eastern Costa Rica. *Journal of Tropical Ecology*, 20, 291–305.
- Findley, J.S., & Wilson, D.E. (1974). Observations on the Neotropical disk-winged bat, *Thyroptera tricolor* Spix. *Journal of Mammalogy*, 55(3), 562–571.
- Wiley, R. (2006). Signal detection and animal communication. *Advances in the Study of Behaviour*, 36, 217–247.
- Wiley, R., & Richards, D. (1978). Physical constraints on acoustic communication in the atmosphere: implications for the evolution of animal vocalizations. *Behavioral Ecology and Sociobiology*, 3, 69–94.
- Wilkinson, G.S., & Boughman, J.W. (1998). Social calls coordinate foraging in greater spear-nosed bats. *Animal Behaviour*, 55, 337–350.
- Willis, C.K., & Brigham, R.M. (2004). Roost switching, roost sharing and social cohesion: forest-dwelling big brown bats, *Eptesicus fuscus*, conform to the fission–fusion model. *Animal Behaviour*, 68, 495–505. doi:10.1016/j.anbehav.2003.08.028.
- Wittemyer, G., Douglas-Hamilton, I., & Getz, W.M. (2005). The socioecology of elephants: analysis of the processes creating multitiered social structures. *Animal Behaviour*, 69, 1357–1371. doi:10.1016/j.anbehav.2004.08.018.

Wrangham, R., Gittleman, J., & Chapman, C. (1993). Constraints on group size in primates and carnivores: population density and day-range as assays of exploitation competition. *Behavioral Ecology and Sociobiology*, 32, 199–209.

Table 1

Ethogram of scored behaviours of Spix's disc-winged bats towards furled leaves

Behaviour	Description
Pass by	Flies across the recording view but does not approach furled leaf
Approach	Flies towards leaf; the offset of an approach was recorded when a bat flew to within 1 m of the focal leaf
Hover	Flies towards the tip of the furled leaf and performs a suspended flight above the leaf, directing the snout towards the roost entrance
Circling	Flies either from the base of the furled leaf or from above performing a circling flight usually in the form of a spiral, directing the snout towards the leaf
Chase	Two or more individuals fly in close proximity, typically trailing one behind the other
Enter	Individual lands on the tip of the leaf and uses its wrist and ankle discs to move towards the base of the leaf
Exit	Individual uses its wrist and ankle discs to move towards the tip of the leaf, then pushes its body, using its four discs, towards the back and begins to fly

Table 2

Percentage occurrence and mean $\pm$ SE duration and frequency (F_{start} , F_{end} and F_{peak}) of six social calls of Spix's disc-winged bats recorded at suitable roosting sites

Call type	% Occurrence ^a	Duration	F_{start}	F_{end}	F_{peak}
INQ	42.4	39.0 $\pm$ 0.6	31.7 $\pm$ 0.3	15.9 $\pm$ 0.2	32.1 $\pm$ 0.2
LUM	33.2	175.5 $\pm$ 1.7	15.9 $\pm$ 0.2	22.8 $\pm$ 0.3	16.3 $\pm$ 0.2
QCF	9.6	374.1 $\pm$ 13.9	10.5 $\pm$ 0.2	12.9 $\pm$ 0.3	10.7 $\pm$ 0.2
SUP	6.8	47.1 $\pm$ 3.0	17.1 $\pm$ 0.4	20.3 $\pm$ 0.3	17.7 $\pm$ 0.6
SUM	6.5	27.5 $\pm$ 1.2	11.2 $\pm$ 0.2	20.4 $\pm$ 0.4	11.6 $\pm$ 0.2
SQCF	1.5	79.2 $\pm$ 3.7	9.9 $\pm$ 0.9	11.1 $\pm$ 0.9	10.2 $\pm$ 0.9

INQ: downward-modulated frequency ('inquiry'); LUM: long upward-modulated frequency;

QCF: quasiconstant frequency; SUP: short upward frequency; SUM: short upward-modulated frequency; SQCF: short quasiconstant frequency.

^a Occurrence was estimated as a percentage of 722 recordings with high signal-to-noise ratio.

Figure 1. Spectrograms of the six social calls (a–f) and echolocation sequence (g) emitted by Spix’s disc-winged bats near furled leaves. Frequency is plotted against time using a 1024 fast Fourier transform and a FlatTop window with 75% overlap. (a) INQ: downward-modulated frequency (‘inquiry’); (b) SQCF: short quasiconstant frequency; (c) QCF: quasiconstant frequency; (d) LUM: long upward-modulated frequency; (e) SUP: short upward frequency; (f) SUM: short upward-modulated frequency; (g) ECH: echolocation sequence.

Figure 2. Monitoring frequency of focal leaves. (a) Variation in visit frequency between leaf type categories. Values for total visit frequency were averaged (mean $\pm$ SE) over leaf type category and pooled across sites ($N = 21$ unoccupied leaves, 10 night roosts and 7 previous roosts). (b) Number of individual bat visits per hour at the three study sites. Number of visits per hour were averaged (mean $\pm$ SE) over leaf category for each site: Sirena Field Station (SIR), $N = 13$; Tapiria National Reserve (TAP), $N = 9$; Cuatro Esquinas Field Station, Tortuguero National Park (TOR), $N = 7$.

Figure 3. Duration of an approach event when bats visited focal leaves. Approach was scored as (a) circling or (b) hover. Duration estimates of an approach event were averaged (mean $\pm$ SE) over leaf type category ($N = 13$ unoccupied leaves, 10 night roosts) at the three sites sampled. Abbreviations as in Fig. 2.

Figure 4. Proportion of bat social calls recorded at each leaf type. Different shades represent the frequencies in which call types were recorded. Call frequencies were pooled across sites. INQ: downward-modulated frequency ('inquiry'); LUM: long upward-modulated frequency; QCF: quasiconstant frequency; SUM: short upward-modulated frequency; SUP: short upward frequency.

Figure 5. Estimated probability of recording a bat social call when bats visited a focal leaf as the night progressed. Presence of social calls were estimated from bat visits in which continuous acoustic recording was available. Values were averaged (mean $\pm$ SE) over leaf type category across sites ($N = 12$ unoccupied leaves, 4 previous roosts, 5 night roosts).

Figure 6. Frequency of bat social calls recorded at focal leaves in which continuous acoustic recording throughout the night was available. Number of calls recorded per hour was averaged over leaf type category ($N = 12$ unoccupied leaves, 4 previous roosts, 5 night roosts) and pooled across sites. Symbol sizes reflect the numbers of focal leaves.