



## Strong individual signatures and weaker group signatures in contact calls of Spix's disc-winged bat, *Thyroptera tricolor*

Erin H. Gillam<sup>a,\*</sup>, Gloriana Chaverri<sup>b,1</sup>

<sup>a</sup> Department of Biological Sciences, North Dakota State University

<sup>b</sup> Department of Biology, Boston University

### ARTICLE INFO

#### Article history:

Received 6 June 2011

Initial acceptance 26 July 2011

Final acceptance 6 October 2011

Available online 29 November 2011

MS. number: A11-00528R

#### Keywords:

call variation

individual signature

social call

Spix's disc-winged bat

*Thyroptera tricolor*

Spix's disc-winged bats, *Thyroptera tricolor*, form small, long-term social groups in which members are loyal to a patch of forest but move on a daily basis between highly ephemeral roosting sites (partly unfurled *Heliconia* leaves). This species has been shown to exchange social calls that facilitate contact with nearby bats and recruitment to roost sites. During flight, *T. tricolor* emits 'inquiry' calls that frequently elicit a response from individuals that have already entered a furled leaf. These 'response' calls are then followed by the flying bat entering the occupied leaf roost. In this study, we examined variation in the structure of inquiry and response calls, and assessed whether calls encode information about individual or group identity that would allow for acoustic discrimination to occur. We found that both inquiry and response calls were sufficiently consistent within individuals, and divergent between individuals, to permit separation of individual bats based on call structure. We also found some evidence for group-specific signatures, although these were less defined than differences observed between individuals. While this does not confirm that Spix's disc-winged bats can discriminate between individuals and groups based on call information, our results indicate that both call types have a broad enough parameter space for this to occur.

© 2011 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Social animals are regularly faced with the challenge of relocating group members after periods of separation. Communication is one of the most important mechanisms for maintaining group associations over time, and signals facilitating group cohesion, or 'contact calls', are common in many avian and mammalian species (Kondo & Watanabe 2009). If contact calls are variable across individuals and/or groups, animals can potentially use this discriminating signal information to identify groupmates (Tibbetts & Dale 2007).

The ability to encode information, such as individual or group identity, depends upon the characteristics of the communication signal and its associated information capacity (Beecher 1989; Searby & Jouventin 2004). For example, if an animal can be flexible over a wide frequency range for multiple spectral characteristics of an acoustic signal, this creates a broad parameter space that can be partitioned to create characteristic-specific signals (i.e. male and females use different call frequencies). While many signals encode information with a small number of possible states,

such as sex or reproductive condition, more complex discriminations, such as group or individual identification, are substantially more difficult since there may be insufficient parameter space to encode a unique signature for every individual or group in a population.

Individual and group signatures can be shaped by multiple underlying factors. Signatures conveying individual identities are likely to be a result of genetic differences (Scherrer & Wilkinson 1993), while group signatures can be more readily modified by social learning. In a variety of taxa, group members have been shown to converge on a shared group call structure (Farabaugh et al. 1994; Nousek et al. 2006), and changes in group composition can lead to adjustments in signal design (Boughman 1998).

Bats are an especially interesting group for examining whether communication signals encode information about individual or group identity. Bats are highly social animals, with the vast majority of species living in groups. Furthermore, bats produce a rich repertoire of communication signals, most of which are acoustic in nature. The most common acoustic signals of bats are echolocation calls. While echolocation is primarily used for orientation and prey detection (Griffin 1958), consistent call structures associated with sex (Neuweiler et al. 1987; Suga et al. 1987; Siemers et al. 2005) and age (Jones et al. 1992; Russo et al. 2001; Yoshino et al. 2006) have been observed. Thus, in some species, echolocation encodes

\* Correspondence: E. H. Gillam, Department of Biological Sciences, North Dakota State University, Stevens Hall, Room 218, NDSU Dept 2715, P.O. Box 6050, Fargo, ND 58108-6050, U.S.A.

E-mail address: [Erin.Gillam@ndsu.edu](mailto:Erin.Gillam@ndsu.edu) (E. H. Gillam).

<sup>1</sup> G. Chaverri is at the Department of Biology, Boston University, Boston, MA 02215, U.S.A.

information about the sender that can be detected and received by other bats, although it is unclear whether this is passive or active information transfer (Wilkinson 1995). Echolocation can also potentially encode information about social group affiliation (Hiryu et al. 2006) and individual identification (Suga et al. 1987). Yet, such information is unlikely to be useful since most bats alter call structure substantially in response to local environmental conditions (Kalko & Schnitzler 1993; Obrist 1995), making maintenance of a signature difficult (Siemers & Kerth 2006).

Unlike echolocation, social signals are not used for orientation, making the possibility of encoding group or individual signatures more plausible. Social calls of bats have received substantially less attention than echolocation, yet when researchers have investigated this topic they have found that many species produce a diverse repertoire of calls involved in facilitating a variety of behavioural interactions (Barclay et al. 1979; Porter 1979; Kanwal et al. 1994; Pfalzer & Kusch 2003; Melendez et al. 2006; Bohn et al. 2008). Social call functions include mother–pup recognition (Balcombe & McCracken 1992; Knornschild & von Helversen 2008), mate attraction (Behr & von Helversen 2004; Bohn et al. 2009), territory defence (Barlow & Jones 1997) and indication of distress (Russ et al. 2004) or aggression (Pfalzer & Kusch 2003; Bohn et al. 2008).

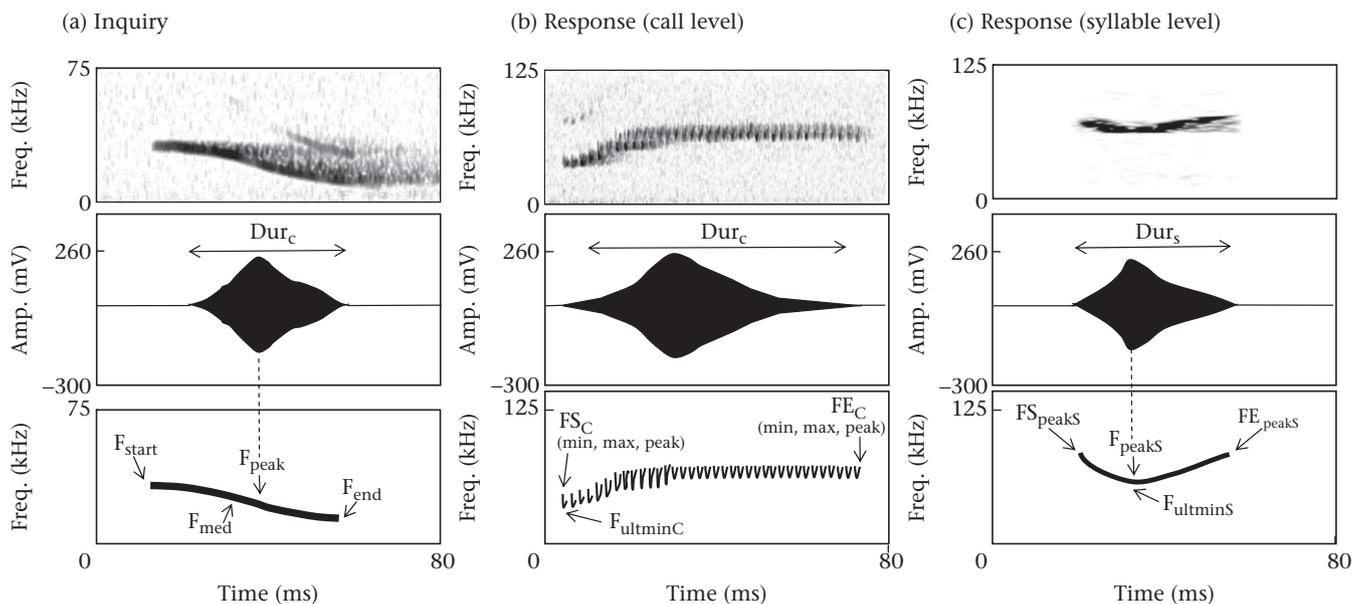
Social calls in bats are also used to maintain contact with adult conspecifics and facilitate group cohesion. Encoding information about individual or group identity should be especially valuable for contact calls, since potentially costly behavioural decisions by the receiver are likely to depend upon the identity of the signaller (Tibbetts & Dale 2007). Yet, we know relatively little about contact calls in bats, and even less about the information capacity of these signals. Female greater spear-nosed bats, *Phyllostomus hastatus*, produce group-specific screech calls when exiting a roost and at foraging sites; these calls attract groupmates to the caller's location, which presumably facilitates group foraging (Boughman & Wilkinson 1998). White-winged vampire bats, *Diademus youngi* (Carter et al. 2008, 2009) and pallid bats, *Antrozous pallidus* (Arnold & Wilkinson 2011) are the only species that have been shown to

exchange consistent, individual-specific social calls that facilitate maintenance of contact with conspecifics. Although contact calls can be difficult to study since they are often produced by bats during flight, they have the potential to influence the social structure and the behavioural interactions within populations.

We sought to determine whether contact calls produced by a leaf-roosting bat show group or individual signatures. Spix's disc-winged bats, *Thyroptera tricolor*, are small (3–4 g) insectivores that roost in furled, tubular leaves, using specialized suction discs on the wrists and ankles (Riskin & Fenton 2001). Tubular leaf roosts are highly ephemeral, with leaves unfurling and becoming unsuitable for bats in 5–60 h (Vonhof et al. 2004). Roosting groups vary from 2 to 12 individuals (Vonhof & Fenton 2004), and group stability is very high, with individuals remaining together for up to 22 months (Chaverri 2010). Such stability is especially striking given that bats must locate new roosts on an almost daily basis.

This species has been shown to exchange social calls that facilitate contact with nearby bats and recruitment to roost sites. Flying individuals actively searching for a roost emit 'inquiry' calls; roosting conspecifics in the area rapidly answer with a 'response' call, which is followed by the flying bat entering the occupied leaf roost (Chaverri et al. 2010). Inquiry calls are simple downward frequency-modulated (FM) sweeps, while response calls are more complex, consisting of multiple syllables that together form a signal with a short upward FM sweep followed by a longer constant frequency (CF) component (Chaverri et al. 2010; Fig. 1). Furthermore, inquiry calls are emitted individually, with intercall intervals of several seconds, while response calls are generally produced in a rapid bout.

Here, we examined variation in the structure of inquiry and response calls, and assessed whether calls encode identity information that would facilitate acoustic discrimination. Differentiation between group members and nongroup members could occur if contact calls have individual signatures, group signatures, or both. We hypothesized that the contact calls of *T. tricolor* primarily encode information about individual identity because (1)



**Figure 1.** Depiction of primary sound measurements taken on inquiry calls (a) and response calls (b, c) of Spix's disc-winged bats. Response calls were analysed at the level of the call (b) and the syllable (c). For each set (a–c), the top graph depicts a spectrogram of the respective acoustic signal, the middle graph is a stylized depiction of the signal waveform, and the bottom graph is a stylized version of the signal spectrogram. For inquiry calls (a), measures of slope ( $S_{\min, \text{med}, \text{max}}$ ) and concavity ( $C_{\min, \text{med}, \text{max}}$ ) are not shown. For response analyses at the call level (b), measures of syllable number (Syll) are not shown. For response analyses at the syllable level (c), measures of intersyllable interval (ISI) and peak frequency at call centre ( $FC_{\text{peaks}}$ ) are not shown.

groups contain only a small number of individuals, (2) groups are highly stable, with limited interaction among groups in a roosting context and (3) groups are spatially separated from each other, occupying distinct roosting home ranges (Vonhof et al. 2004). As a result, only a relatively narrow parameter space should be necessary for all individuals in a group to have a unique signature. Thus, we predicted that variance in call structure between individuals would be significantly higher than variance within individuals. We further predicted that group signatures would be relatively weak in the contact calls of *T. tricolor*, since social learning of a group call is redundant if bats can identify the individual signatures of all group members. Alternatively, high natal philopatry and strong genetic relatedness within groups (Vonhof 2001; Chaverri & Kunz 2011) may lead to call similarity within a group if contact calls are genetically determined.

## METHODS

### Field Trials

Data were collected in March and December 2009, in La Gamba, southwestern Costa Rica. Groups, defined as bats roosting in the same leaf, were located and captured by searching suitable leaves in areas where *T. tricolor* had been found previously. Upon capture, bats were housed in cloth bags and fitted with numbered metal wing bands. Basic measurements, including mass, forearm length, sex, age and reproductive condition, were taken for each individual. For each group, we found a suitable tubular leaf near the capture site to perform experimental trials. If a nearby suitable leaf was not available, we cut an unoccupied tubular leaf from another site and planted it near the capture site. We conducted all trials in areas where bats would have enough space for manoeuvrability and access to the experimental leaf. Two to four condenser microphones (CM16, Avisoft Bioacoustics, Berlin, Germany) mounted on tripods were placed in the study area and used to record any calls produced by bats during experimental trials. Microphone recordings were captured on a Dell Latitude laptop using Avisoft's Ultrasound Gate 416H and Avisoft Recorder software (sampling rate 384 kHz, 16-bit resolution). All protocols for capturing and handling bats were approved by the Costa Rican government (permit number R-008-2009-OT-CONAGEBIO) and the North Dakota State University Institutional Animal Care and Use Committee (Protocol Number A0928).

Methods for experimental trials differed during the two periods of data collection. In March 2009 (Chaverri et al. 2010), two microphones were focused on the experimental leaf (< 5 cm from leaf surface and oriented towards the leaf) at different heights close to where the bat would be roosting. A third microphone was placed on the path between the release site and the leaf. Release sites were located at approximately 5 m from the experimental leaf. Trials began by placing one individual from the captured group inside the leaf (i.e. roosting bat), and then placing a second bat from the same group in the researcher's hand at the release site (i.e. flying bat). The cloth bag with the remaining bats was placed inside a waterproof bag to avoid acoustic interference. Recordings began when we released the flying bat, and ended when the bat entered the experimental leaf or after the flying individual left the area and was not visible for more than 1 min.

In December 2009, microphone positions differed depending upon whether we were collecting calls from the roosting or flying bat. When recording the flying bat (inquiry calls), four microphones were placed at varying distances from the leaf, in an attempt to capture the calls of the focal individual as it flew around the study area. Trials began by putting one individual in the experimental leaf (roosting bat) and placing a circular piece of mesh in the upper

portion of the leaf to prevent escape. In addition, a cloth bag housing one to five other bats was hung from each microphone tripod. Since bats in a bag produce response calls similar to bats inside a leaf, these animals served as an additional attractant to keep the flying individual in the recording area for an extended period, which allowed us to record multiple inquiry calls per bat. Bats were released and recorded during flight as described above.

When recording response calls from the roosting bat, we placed one individual inside the experimental leaf, with the mesh in place to prevent escape. Two microphones were positioned within 5 cm from the leaf's surface and oriented towards the leaf to capture any calls produced by the roosting bat. We broadcast previously recorded inquiry calls to the roosting bat through a Dell Inspiron laptop and an Avisoft Ultrasound Gate Player 116 (product number 70111) attached to an Avisoft speaker magnet (product number 60101) positioned 2 m from the leaf. Playback allowed us to increase the number of inquiry calls to which an individual was exposed, and consequently the number of response calls that were produced and recorded. Upon broadcast of an inquiry call, the bat was given 30 s to respond; if no response was recorded, an additional inquiry call was broadcast. If the bat did not respond after five inquiry broadcasts, we ceased broadcasting calls for 2 min, then played an additional set of five calls. If the bat did not call after 10 inquiry broadcasts, it was considered unresponsive and removed from the leaf. When bats responded acoustically to the broadcast, we played an additional inquiry signal 10 s after the bat finished a calling bout. We continued this pattern of broadcast for 3–5 min, or until the bat stopped producing response calls. Recordings were started before the initial inquiry broadcast and stopped at the end of the trial. Recordings were done with 16-bit resolution and a 300 kHz sampling rate.

### Sound Analysis

For both inquiry and response calls, we selected calls for analysis that had a high signal-to-noise ratio. For inquiry calls, we often recorded multiple signals in the same recording period; calls were generally separated by long periods of silence (10+ s), so we included multiple calls from the same session in our analysis. Response calls are also produced in distinct bouts containing multiple signals. To determine whether calls produced within a bout were more similar to each other than calls from separate bouts, we measured the contribution of bat and bout to the variance of the call characteristics using the restricted maximum likelihood estimation of variance components (Searle et al. 2009). Results indicated that most of the variability was attributed to bat (variance component estimates: bout: 0.001–0.028; bat: 0.300–0.495), so we chose to include multiple calls from the same bout in our analysis.

Characteristics of both call types were measured using Avisoft SASLab Pro (Avisoft Bioacoustics). Spectrograms were generated using a 1024-point fast Fourier transform (93.75% overlap). For the 300 kHz sampling rate we used, this gave a 293 Hz frequency resolution and 0.21 ms temporal resolution. For inquiry calls (Fig. 1a), we measured call duration ( $Dur_c$ ), minimum frequency at call start ( $F_{start}$ ), minimum frequency at call end ( $F_{end}$ ), minimum frequency at the position of greatest amplitude in the call ( $F_{peak}$ ) and median frequency of the entire call ( $F_{med}$ ). Although it would have been preferable to take other spectral measurements on inquiry calls, such as maximum frequency, this was not feasible because of the abundant echoes present in our recordings; even though echoes did not overlap with the original call, they made it difficult for Avisoft's 'Automated Parameters Measurement' feature to detect other parameters, and filtering efforts did not fix this problem. Despite this, we are confident that minimum frequency

measurements are more than sufficient for describing the spectral structure of these simple, tonal frequency sweeps. For each call, we also created frequency contours by measuring minimum frequency every ( $\text{Dur}_c/50$ ) ms, which partitioned the signal into 49 sections. From these contours we calculated values of call slope and concavity. Slope was calculated as  $(\text{freq at } t_1 - \text{freq at } t_0)/(t_1 - t_0)$ , while concavity was calculated as  $(\text{slope at } t_1 - \text{slope at } t_0)/(t_1 - t_0)$ , where  $t_0$  is the start time of a given section and  $t_1$  is the start time of the following section. For each inquiry call, we calculated minimum, maximum and median slope ( $S_{\min}$ ,  $S_{\max}$ ,  $S_{\text{med}}$ ) and concavity ( $C_{\min}$ ,  $C_{\max}$ ,  $C_{\text{med}}$ ).

Response calls were composed of 6–46 individual syllables, so we conducted separate analyses at the level of the call and the syllable (Fig. 1b, c). Although we measured all individual syllables in a call, the terms below refer to average syllable structure for a given call, as only these mean values were used in statistical analyses. We measured syllable duration ( $\text{Dur}_s$ ), intersyllable interval (ISI) and peak frequency at four temporal positions in the syllable: start ( $\text{FS}_{\text{peaks}}$ ), end ( $\text{FE}_{\text{peaks}}$ ), centre ( $\text{FC}_{\text{peaks}}$ ) and maximum amplitude ( $\text{FM}_{\text{peaks}}$ ). In addition, we measured the minimum frequency of the entire syllable ( $\text{F}_{\text{ultminS}}$ ) and the location of  $\text{F}_{\text{ultminS}}$  ( $\text{Loc}_{\text{ultminS}}$ ). At the call level, we assessed the number of syllables (Syll), call duration ( $\text{Dur}_c$ ) and minimum, maximum and peak frequency at the start ( $\text{FS}_{\min C}$ ,  $\text{FS}_{\max C}$ ,  $\text{FS}_{\text{peakC}}$ ) and end ( $\text{FE}_{\min C}$ ,  $\text{FE}_{\max C}$ ,  $\text{FE}_{\text{peakC}}$ ), the lowest frequency in the entire call ( $\text{F}_{\text{ultminC}}$ ) and the location of the  $\text{F}_{\text{ultminC}}$  ( $\text{Loc}_{\text{ultminC}}$ ).

### Statistical Analysis

To determine whether variation in call structure was attributed to differences between bats, groups, or both, we ran a nested one-way MANOVA in SPSS v.17.0 (SPSS, Chicago, IL, U.S.A.). We also estimated the contribution of each independent variable to the variance of the call characteristics using the restricted maximum likelihood method. Before running MANOVAs, we first eliminated correlations among the dependent variables using the principal components analysis (PCA) extraction method, with varimax rotation and Kaiser normalization (Jolliffe 2002). Eigenvalues greater than one were used to determine the number of factors retained, and rotated scores were saved using the regression method in SPSS. After running MANOVAs with the retained factors, we calculated the proportion of variance in call structure accounted for by individual or group membership using partial  $\eta^2$ . Based on values of significance and partial  $\eta^2$  estimated from MANOVA (which is comparable to  $R^2$  in regression analyses; Levine & Hullett 2002) and on the contribution of each independent variable to the variance in call structure, we decided whether the variation in call characteristics was primarily due to differences between bats, groups or both.

We ran a stepwise discriminant function analysis (DFA) in SPSS and a permuted discriminant function analysis (pDFA; Mundry & Sommer 2007) in R v.2.11 (The R Foundation for Statistical Computing, Vienna, Austria), to explain the differences in call structure between bat (DFA) and group (pDFA) based on the entire suite of call measurements. We used the permuted version of the DFA for the analysis of group signatures to account for the fact that conventional DFAs only allow analysis of one factor at a time, leading to large numbers of replicated observations per subject (i.e. group), which could result in pseudoreplication and hence violation of the assumption of independence (Mundry & Sommer 2007). To increase the power of the tests and to reduce the disparity in sample sizes, which can lower the effectiveness of the DFA in distinguishing among categories (McGarigal et al. 2000), in the analysis of bat signatures, we included only those individuals for which we had more than 10 calls. Likewise, in the analysis of

group signatures, we included only those individuals for which we had at least four calls, and we included groups with a minimum of three bats. These data selection rules represent a compromise between maximizing the number of bats and groups in the analyses and maximizing the number of calls within bats and groups to make the analyses viable.

Before running the DFAs for both call types (inquiry and response), we determined whether the assumptions of equality of variance–covariance matrices, normality of canonical scores and multicollinearity were met using methods described in McGarigal et al. (2000). We assessed the first assumption using Box's  $M$  test, the second by running a Kolmogorov–Smirnov test on canonical scores, and the third by calculating pairwise correlations among call variables using a Pearson product-moment correlation coefficient. Canonical scores were normally distributed, but the assumption of within-group equality of covariance matrices was not met; hence, we used the individual within-group covariance matrices in the classification criterion. Also, because multicollinearity issues were found among discriminant variables, we tested variables involved in high pairwise correlations (i.e.  $r \geq 0.7$ ) using a univariate one-way ANOVA with the grouping variable (i.e. bat or group) as the main effect. Variables retained for the DFA were the ones with the largest  $F$  value. We also used Cohen's kappa (Cohen 1960; Titus et al. 1984) to assess the improvement in classification accuracy over that expected by random assignment, with values near zero indicating no improvement over chance, and values near one indicating perfect assignment. Because this procedure is considered biased when the samples used in calculating the classification function are the ones being classified, we also used a randomized sample validation to gauge the reliability of the classification criterion and to validate the discriminant functions (McGarigal et al. 2000). For this, we first randomized our call data and then derived the classification functions and estimated the correct classification rate using the classification matrix. We repeated this process 50 times and compared the random permutation distribution to the correct classification rate of the original data. The difference between the randomized and the original classification rates shows the power of the classification functions, with small differences indicating that the classification function power may be primarily attributed to sample biases.

## RESULTS

### General Behaviour

We found that *T. tricolor* produced inquiry and response calls under a variety of experimental conditions. Bats placed inside leaves readily responded to the inquiry calls produced by conspecifics and playbacks of inquiry calls. While there was variation in the tendency of individuals to respond, it was common for bats to answer an inquiry call rapidly, often responding repeatedly to a series of inquiry calls that were separated by several seconds. Bats also produced response calls when hanging in the cloth bag; despite the artificial environment, detection of an inquiry call led to production of response calls. Such patterns of call production suggest that group formation is important in this species, as individuals continue to produce contact calls even in a partly artificial experimental situation.

### Inquiry Calls

We analysed 557 inquiry calls from 104 bats and 30 groups. After PCA, three factors were retained for analysis and explained 69.7% of the total variance. The first factor had loadings higher than

0.70 for components related to duration and call shape (i.e. slope and concavity), while the second factor had high loadings for components related to call frequency (Table 1). There was a significant effect of bat and group on inquiry call structure (Table 2), but bat explained a greater proportion of the variance in call structure ( $\eta^2 = 0.3$ ) than group ( $\eta^2 = 0.23$ ; Table 2). Bat also explained a greater proportion of the variance in call structure than group for two of the three factors considered (Table 3).

For the discriminant function analysis on individual signatures, we analysed 265 inquiry calls from 18 bats, with an average of 14.72 calls per bat (range 10–25). The final variables included in the DFA, after removing variables involved in high pairwise correlations, were  $Dur_c$ ,  $F_{start}$ ,  $F_{end}$ ,  $F_{peak}$ ,  $S_{max}$ ,  $S_{med}$ ,  $C_{max}$  and  $C_{med}$ . We found that 75.1% of calls were correctly assigned to individuals, and a Cohen's  $\kappa$  of 0.87 indicated that classification based on the discriminating variables was 87% better than chance assignment. Furthermore, the mean randomized classification rate (50 permutations) was 42.49% (range 29.8–50.6), indicating that the randomized rate was smaller than the classification rate from the original data. Taken together, these results indicate that the predictive power of the derived discriminant functions was greater than that of random functions. A stepwise DFA indicated that the most useful subset of discriminating variables were  $Dur_c$ ,  $F_{start}$ ,  $F_{end}$ ,  $F_{peak}$ ,  $S_{max}$  and  $C_{max}$ . After adding these variables to the model,  $S_{med}$  and  $C_{med}$  did not contribute significantly to individual separation. The first discriminant function derived from DFA accounted for 44.3% of the total discriminatory power of the eight variables used in the analysis, and the variable that contributed most to this function was  $Dur_c$ . The second discriminant function explained 22.7% of the total discriminatory power, and the variable that contributed most to this function was  $F_{start}$ . These findings signify that individuals can be differentiated based on the structure of inquiry calls.

For the pDFA on group signatures, we analysed 253 inquiry calls from 26 bats and seven groups. We analysed an average of 9.73 inquiry calls per bat (range 4–25) and 36.14 inquiry calls per group (range 27–54). The final variables used in the pDFA on group signatures were the same as the ones retained for the analysis of individual signatures, namely  $FM_{peakS}$ ,  $Dur_s$ ,  $ISI$ ,  $FS_{peakS}$ ,  $F_{ultminS}$ ,  $Loc_{ultminS}$ ,  $Syll$ ,  $FE_{minC}$  and  $Loc_{ultminC}$ . Results of the pDFA with 1000 permutations indicated that 18% of calls were correctly assigned to groups. The proportion of randomized data sets with the number of correctly classified calls being at least as large as the average number of correctly classified calls of the unrandomized data was 0.09 (which was also the  $P$  value for the test). Although there was

**Table 1**  
Call component loadings for the three factors retained during PCA for inquiry calls of Spix's disc-winged bats, with the percentage of variance explained by each factor after varimax rotation

Call component	Factor		
	1	2	3
$Dur_c$	<b>0.708</b>	0.187	0.282
$F_{start}$	0.239	<b>0.709</b>	-0.525
$F_{end}$	-0.346	<b>0.704</b>	0.238
$F_{med}$	-0.008	<b>0.893</b>	-0.312
$F_{peak}$	0.069	<b>0.725</b>	0.080
$S_{min}$	<b>0.806</b>	-0.113	0.351
$S_{max}$	- <b>0.741</b>	0.150	0.222
$S_{med}$	0.138	-0.110	<b>0.908</b>
$C_{min}$	<b>0.936</b>	0.029	0.131
$C_{max}$	- <b>0.922</b>	-0.024	-0.179
$C_{med}$	0.110	0.012	0.247
Variance	33.09	21.86	14.74

Bold values indicate the highest loading associated with a particular component (see Methods for a description of call components).

**Table 2**  
Summary statistics of the one-way MANOVAs with the three and five retained factors (for inquiry and response calls of Spix's disc-winged bats, respectively) as dependent variables and bat and group as the independent variables

Call type	Variable	Wilk's $\lambda$	$F$	$ndf, ddf$	$P$	$\eta^2$
Inquiry	Bat	0.33	2.65	222, 1353	<0.001	0.30
	Group	0.45	4.71	87, 1350	<0.001	0.23
Response	Bat	0.25	8.82	75, 1972	<0.001	0.23
	Group	0.45	11.81	30, 1646	<0.001	0.22

Partial  $\eta^2$  values, which are comparable to  $R^2$  values in measuring effect size, indicate significant effects of bat and group for both inquiry and response calls.

no significant discriminability in inquiry call structure among groups, the borderline significance of this test indicates that group signatures may be biologically significant, but are clearly weaker than the observed individual signatures.

### Response Calls

We analysed 618 response calls from 34 bats and 14 groups. Five factors were retained for analysis after PCA and they explained 82.6% of the total variance. The first two factors had high loadings for components related to call and syllable frequencies, while the last three factors had high loadings primarily for temporal characteristics of both calls and syllables (Table 4). There were significant and comparable effects of bat ( $\eta^2 = 0.23$ ) and group ( $\eta^2 = 0.22$ ) on response call structure (Table 2), although bat explained a greater proportion of the variance in call structure than group for all five factors considered (Table 3).

For the DFA on individual signatures, we analysed 618 calls from 34 bats, with an average of 18.18 calls per bat (range 10–34). The final variables used in the analysis, after removing variables involved in high pairwise correlations, were  $FM_{peakS}$ ,  $Dur_s$ ,  $ISI$ ,  $FS_{peakS}$ ,  $F_{ultminS}$ ,  $Loc_{ultminS}$ ,  $Syll$ ,  $FE_{minC}$  and  $Loc_{ultminC}$ . The classification matrix showed that 86.2% of calls were correctly assigned, and a Cohen's  $\kappa$  of 0.74 indicated that classification based on the discriminating variables was 74% better than chance assignment. The mean randomized classification rate (50 permutations) was 41.65% (range 36.4–44.8), indicating that the randomized rate was smaller than the classification rate from the original data. Overall, these findings indicate that discrimination of individuals via response call structure is possible based on the measured variables.

The most useful subset of discriminant variables, based on a stepwise DFA, were  $Syll$ ,  $F_{ultminS}$ ,  $FE_{minC}$ ,  $FM_{peakS}$ ,  $Dur_s$ ,  $ISI$ ,  $FS_{peakS}$  and  $Loc_{ultminS}$ . Thus, after accounting for the separation attributed to the former variables,  $Loc_{ultminC}$  did not contribute significantly to individual separation. The first discriminant function derived from DFA accounted for 30.3% of the total discriminatory power of the eight variables used in the analysis, and the variable that

**Table 3**  
Contribution of each independent variable, bat and group, to the variance of the three and five retained factors (for inquiry and response calls of Spix's disc-winged bats, respectively) using the restricted maximum likelihood estimation of variance

Call type	Factor	Variance component estimates	
		Bat ( $N=104$ )	Group ( $N=30$ )
Inquiry ( $N=557$ )	1	0.10	0.23
	2	0.29	0.05
	3	0.28	0.12
Response ( $N=618$ )		Bat ( $N=34$ )	Group ( $N=14$ )
	1	0.33	0.18
	2	0.31	0.09
	3	0.29	0.02
	4	0.20	0.10
	5	0.29	0.03

**Table 4**

Call component loadings for the five factors retained during PCA for response calls of Spix's disc-winged bats, with the percentage of variance explained by each factor after varimax rotation

Call component	Factor				
	1	2	3	4	5
Syll	0.001	0.032	<b>0.978</b>	-0.047	-0.119
Loc <sub>ultminC</sub>	-0.001	-0.079	-0.001	<b>0.766</b>	-0.012
Dur <sub>C</sub>	-0.035	-0.025	<b>0.972</b>	-0.002	0.004
F <sub>ultminC</sub>	<b>0.891</b>	0.208	-0.026	-0.070	-0.019
F <sub>SpeakC</sub>	<b>0.944</b>	0.077	-0.067	0.124	-0.015
F <sub>SminC</sub>	<b>0.942</b>	0.093	-0.053	0.085	-0.014
F <sub>SmaxC</sub>	<b>0.929</b>	0.046	-0.064	0.147	-0.028
F <sub>EpeakC</sub>	0.091	<b>0.955</b>	-0.039	-0.041	-0.019
F <sub>EminC</sub>	0.079	<b>0.946</b>	-0.033	-0.070	0.003
F <sub>EmaxC</sub>	0.108	<b>0.934</b>	-0.050	-0.038	-0.058
Dur <sub>S</sub>	0.119	0.071	-0.147	-0.344	<b>0.747</b>
ISI	-0.118	-0.300	-0.266	0.236	<b>0.633</b>
F <sub>SpeakS</sub>	<b>0.710</b>	<b>0.614</b>	0.123	-0.029	-0.017
F <sub>EpeakS</sub>	<b>0.637</b>	<b>0.673</b>	0.094	-0.217	0.023
F <sub>CpeakS</sub>	<b>0.659</b>	<b>0.668</b>	0.128	-0.108	-0.076
F <sub>MpeakS</sub>	<b>0.676</b>	<b>0.660</b>	0.133	-0.090	-0.064
F <sub>ultminS</sub>	<b>0.628</b>	<b>0.686</b>	0.129	-0.176	-0.073
Loc <sub>ultminS</sub>	0.143	-0.105	-0.067	<b>0.777</b>	0.100
Variance	30.08	26.50	11.15	8.37	6.51

Bold values indicate the highest loading associated with a particular component (see Methods for a description of call components).

contributed most to this function was F<sub>SpeakS</sub>. In addition, the second discriminant function explained 20.8% of the total discriminatory power, and the variable that contributed most to this function was ISI.

For the DFA on group signatures, we analysed 437 response calls from 22 bats (mean = 19.86, range 10–34) and seven groups (mean = 62.43, range 33–108). The final variables used in the pDFA on group signatures were Dur<sub>S</sub>, ISI, F<sub>CpeakS</sub>, Loc<sub>ultminS</sub>, Dur<sub>C</sub>, F<sub>ultminC</sub>, Loc<sub>ultminC</sub>, F<sub>EminC</sub> and F<sub>MpeakS</sub>. Results of the pDFA with 1000 permutations indicated that 26% of calls were correctly assigned to groups. The proportion of randomized data sets with the number of correctly classified calls being at least as large as the average number of correctly classified calls of the unrandomized data was 0.07 (*P* value). Although there was no significant discriminability in response call structure among groups, as for inquiry calls, the *P* value was sufficiently small to indicate that groups may show biologically significant differences in call structure, but these differences are clearly less well defined than the observed individual signatures.

## DISCUSSION

We examined individual and group variation in the structure of *T. tricolor* inquiry and response calls, which are involved in maintaining contact with conspecifics and forming roost groups (Chaverri et al. 2010). Results of our analyses support our hypothesis that both inquiry and response calls are sufficiently consistent within individuals and divergent between individuals to permit separation of bats based on call structure. Furthermore, we found some evidence of group-specific call structures and, as we hypothesized, these signatures appear to be less defined than individual signatures. While this does not confirm that the bats themselves can discriminate among individuals and groups based on call information, our results certainly suggest that both call types have a broad enough parameter space for this to occur.

Our findings are part of a growing body of literature documenting that contact calls not only function in mediating group formation, but also regularly encode information about group and/or individual identity. Contact calls that contain individual

signatures have been found in a wide variety of birds and mammals, including dolphins (Sayigh et al. 2007), nonhuman primates (Cheney et al. 1996), parakeets (Cortopassi & Bradbury 2006) and corvids (Kondo et al. 2010), among others. In some cases, contact calls contain information about group and individual identity (Crockford et al. 2004; Nousek et al. 2006), with different call features encoding separate information about social affiliation. Such information is likely to be important in mediating social interactions within groups, and future work focused on how animals use signature information to make behavioural decisions will inform us about the multiple functions of contact calls.

## Call Structure

We found that certain signal characteristics were especially important for separating individuals. For inquiry and response calls, both spectral and temporal factors were involved in discrimination. This same pattern of encoding individual identity with specific combinations of spectral and temporal cues has been shown in many mammals, ranging from bats (Scherrer & Wilkinson 1993; Bohn et al. 2007) to humans (Lavner et al. 2000; Anward 2002), among others. Peak frequency ( $F_{\text{peak}}$ ) was the dominant spectral characteristic defining individual signatures in both inquiry and response calls. This is not surprising, as  $F_{\text{peak}}$  is relatively consistent across calls compared to other variables that are frequently adjusted to fit environmental conditions (i.e.  $F_{\text{min}}$ ) or become degraded due to atmospheric attenuation (i.e.  $F_{\text{max}}$ ).

## Evolution of Contact/Recruitment Calls

Spix's disc-winged bats form small (Vonhof & Fenton 2004), highly stable social groups (Chaverri et al. 2010) that are loyal to a very small patch of forest (0.18 ha home range; Vonhof et al. 2004). This social structure may explain why the inquiry–response calling system shows stronger differences between individuals than between groups. If bats are able to remember the distinctive signals of the few individuals in their group, there would be less need for individuals to learn a call encoding a distinct group identity marker. If the individual signature is known, the flying bat is identified as a group member; if the signature is not known, the flying bat is identified as a nongroupmate. Hence, individual signatures would be important for mediating intragroup interactions, while the weaker group signatures could be useful for classifying individuals ranging over wide areas to specific groups. While social learning of group-specific call structures has been shown in bats (Boughman 1998), similarities observed among group members could be solely explained by call convergence due to the high levels of offspring retention within groups and high genetic relatedness among group members (Vonhof 2001; Chaverri & Kunz 2011). Further studies documenting (1) the link between call structure and genetic relatedness and (2) the impact of social learning on group signatures are necessary to better understand this system.

On the contrary, the social 'screech' calls of greater spear-nosed bats exhibit group-specific, but not individual specific, signatures, and these group calls are socially learned instead of genetically determined (Boughman 1998). Yet, from an information capacity standpoint, this group coding makes sense; *P. hastatus* roost in caves that can contain more than 800 bats, and during emergence, the period when screech calls are most commonly produced, large numbers of conspecifics are exiting the roost over a short time (Boughman & Wilkinson 1998). Even if the parameter space to create individual signatures were available, it is unlikely that a bat could retain the hundreds of associations between identity and call structure that makes individual identification possible. Alternatively,

associating call structure with group identity would be substantially more plausible because of the reduced number of groups and associated signatures (Wilkinson & Boughman 1998).

The ability to discriminate individuals and groups based on the structure of their inquiry calls may be especially beneficial from an evolutionary perspective. *Thyroptera tricolor* groups show high relatedness due to strong all-offspring philopatry, leading to the presence of multiple generations in a single group (Vanhof 2001). As a result, helping groupmates may increase an individual's inclusive fitness (Hamilton 1964). Roosting bats that respond to the inquiry call of a flying bat are advertising the location of a usable leaf, and the flying bat can use this information to locate a roost more quickly. This may result in a reduction of predation risk and energy spent during flight, increasing fitness for flying individuals. If roosting bats recognize group from nongroup members, and selectively respond to the former, they would increase the chances of survival and reproduction of a close relative, thus deriving indirect fitness benefits.

Our results indicate that Spix's disc-winged bats have the potential to encode individual and group identification into the inquiry and response calls involved in contacting conspecifics and forming roosting groups. In the future, it will be necessary to conduct experimental playback trials using both call types to determine whether bats actually have the ability to make such discriminations. As we delve deeper into this interesting communication system, we hope to better understand the role social calls play in maintaining stable social groups despite the extreme ephemerality of leaf-roosting habitats and the associated daily movement between leaves.

## Acknowledgments

This research was funded by the North Dakota State University Department of Biological Sciences and College of Science and Mathematics, and by travel and LEAP subawards from a National Science Foundation ADVANCE Institutional Transformation Grant (HRD-0811239) awarded to the North Dakota State University Advance FORWARD program. We thank Maria Sagot and Karina Montero for field assistance, and Kirsten Bohn and Mark Skowronski for advice on sound analysis. We also thank Jose Manuel Quirós, ACOSA, and Jose Alfredo Hernández, CONAGEBIO, for research permits. Finally, we thank Dr Brock Fenton, Gerry Carter, Mirjam Knörnschild and an anonymous referee for comments on an earlier version of this manuscript.

## References

- Anward, J. 2002. Other voices, other sources. In: *Jagen och rösterna: Goffman, Viveka och samtalet* (Ed. by P. Linell & K. Aronsson), pp. 127–148. Linköping: University of Linköping.
- 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**, 1581–1593.
- Balcombe, J. P. & McCracken, G. F. 1992. Vocal recognition in Mexican free-tailed bats: do pups recognize mothers? *Animal Behaviour*, **43**, 79–87.
- Barclay, R. M. R., Fenton, M. B. & Thomas, D. W. 1979. Social behavior of the little brown bat, *Myotis lucifugus*. 2. Vocal communication. *Behavioral Ecology and Sociobiology*, **6**, 137–146.
- Barlow, K. E. & Jones, G. 1997. Function of pipistrelle social calls: field data and a playback experiment. *Animal Behaviour*, **53**, 991–999.
- Beecher, M. D. 1989. Signaling systems for individual recognition: an information theory approach. *Animal Behaviour*, **38**, 248–261.
- Behr, O. & von Helversen, O. 2004. Bat serenades: complex courtship songs of the sac-winged bat (*Saccopteryx bilineata*). *Behavioral Ecology and Sociobiology*, **56**, 106–115.
- Bohn, K. M., Wilkinson, G. S. & Moss, C. F. 2007. Discrimination of infant isolation calls by female greater spear-nosed bats, *Phyllostomus hastatus*. *Animal Behaviour*, **73**, 423–432.
- Bohn, K. M., Schmidt-French, B., Ma, S. T. & Pollak, G. D. 2008. Syllable acoustics, temporal patterns, and call composition vary with behavioral context in Mexican free-tailed bats. *Journal of the Acoustical Society of America*, **124**, 1838–1848.
- Bohn, K. M., Schmidt-French, B., Schwartz, C., Smotherman, M. & Pollak, G. D. 2009. Versatility and stereotypy of free-tailed bat songs. *PLoS ONE*, **4**, e8.
- Boughman, J. W. 1998. Vocal learning by greater spear-nosed bats. *Proceedings of the Royal Society B*, **265**, 227–233.
- Boughman, J. W. & Wilkinson, G. S. 1998. Greater spear-nosed bats discriminate group mates by vocalizations. *Animal Behaviour*, **55**, 1717–1732.
- Carter, G. G., Skowronski, M. D., Faure, P. A. & Fenton, M. B. 2008. Antiphonal calling allows individual discrimination in white-winged vampire bats. *Animal Behaviour*, **76**, 1343–1355.
- Carter, G. G., Fenton, M. B. & Faure, P. A. 2009. White-winged vampire bats (*Diaemus youngi*) exchange contact calls. *Canadian Journal of Zoology*, **87**, 604–608.
- Chaverri, G. 2010. Comparative social network analysis in a leaf-roosting bat. *Behavioral Ecology and Sociobiology*, **64**, 1619–1630.
- Chaverri, G. & Kunz, T. H. 2011. All-offspring natal philopatry in a Neotropical bat. *Animal Behaviour*, **82**, 1127–1133.
- Chaverri, G., Gillam, E. H. & Vanhof, M. J. 2010. Social calls used by a leaf-roosting bat to signal location. *Biology Letters*, **6**, 441–444.
- Cheney, D. L., Seyfarth, R. M. & Palombit, R. 1996. The function and mechanisms underlying baboon 'contact' barks. *Animal Behaviour*, **52**, 507–518.
- Cohen, J. 1960. A coefficient of agreement for nominal scales. *Educational and Psychological Measurement*, **20**, 37–46.
- Cortopassi, K. A. & Bradbury, J. W. 2006. Contact call diversity in wild orange-fronted parakeet pairs, *Aratinga canicularis*. *Animal Behaviour*, **71**, 1141–1154.
- Crockford, C., Herbinger, I., Vigilant, L. & Boesch, C. 2004. Wild chimpanzees produce group-specific calls: a case for vocal learning? *Ethology*, **110**, 221–243.
- Farabaugh, S. M., Linzenbold, A. & Dooling, R. J. 1994. Vocal plasticity in budgerigars (*Melospittacus undulatus*): evidence for social factors in the learning of contact calls. *Journal of Comparative Psychology*, **108**, 81–92.
- Griffin, D. R. 1958. *Listening in the Dark*. New Haven, Connecticut: Yale University Press.
- Hamilton, W. D. 1964. Genetical evolution of social behaviour I. *Journal of Theoretical Biology*, **7**, 1–16.
- Hiryu, S., Katsura, K., Nagato, T., Yamazaki, H., Lin, L. K., Watanabe, Y. & Riquimaroux, H. 2006. Intra-individual variation in the vocalized frequency of the Taiwanese leaf-nosed bat, *Hipposideros terasensis*, influenced by conspecific colony members. *Journal of Comparative Physiology A, Sensory Neural and Behavioral Physiology*, **192**, 807–815.
- Jolliffe, I. T. 2002. *Principal Component Analysis*. New York: Springer-Verlag.
- Jones, G., Gordon, T. & Nightingale, J. 1992. Sex and age differences in the echolocation calls of the horseshoe bat, *Rhinolophus hipposideros*. *Mammalia*, **56**, 189–193.
- Kalko, E. K. V. & Schnitzler, H. U. 1993. Plasticity in echolocation signals of European pipistrelle bats in search flight: implications for habitat use and prey detection. *Behavioral Ecology and Sociobiology*, **33**, 415–428.
- Kanwal, J. S., Matsumura, S., Ohlemiller, K. & Suga, N. 1994. Analysis of acoustic elements and syntax in communication sounds emitted by moustached bats. *Journal of the Acoustical Society of America*, **96**, 1229–1254.
- Knörnschild, M. & von Helversen, O. 2008. Nonmutual vocal mother–pup recognition in the greater sac-winged bat. *Animal Behaviour*, **76**, 1001–1009.
- Kondo, N. & Watanabe, S. 2009. Contact calls: information and social function. *Japanese Psychological Research*, **51**, 197–208.
- Kondo, N., Izawa, E. I. & Watanabe, S. 2010. Perceptual mechanism for vocal individual recognition in jungle crows (*Corvus macrorhynchos*): contact call signature and discrimination. *Behaviour*, **147**, 1051–1072.
- Lavner, Y., Gath, I. & Rosenhouse, J. 2000. The effects of acoustic modifications on the identification of familiar voices speaking isolated vowels. *Speech Communication*, **30**, 9–26.
- Levine, T. R. & Hullett, C. R. 2002. Eta squared, partial eta squared, and misreporting of effect size in communication research. *Human Communication Research*, **28**, 612–625.
- McGarigal, K., Cushman, S. & Stafford, S. 2000. *Multivariate Statistics for Wildlife and Ecology Research*. New York: Springer-Verlag.
- Melendez, K. V., Jones, D. L. & Feng, A. S. 2006. Classification of communication signals of the little brown bat. *Journal of the Acoustical Society of America*, **120**, 1095–1102.
- Mundry, R. & Sommer, C. 2007. Discriminant function analysis with non-independent data: consequences and an alternative. *Animal Behaviour*, **74**, 956–976.
- Neuweiler, G., Metzner, W., Heilmann, U., Rubsam, R., Eckrich, M. & Costa, H. H. 1987. Foraging behavior and echolocation in the rufous horseshoe bat (*Rhinolophus rouxi*) of Sri Lanka. *Behavioral Ecology and Sociobiology*, **20**, 53–67.
- Nousek, A. E., Slater, P. J. B., Wang, C. & Miller, P. J. O. 2006. The influence of social affiliation on individual vocal signatures of northern resident killer whales (*Orcinus orca*). *Biology Letters*, **2**, 481–484.
- Obrist, M. K. 1995. Flexible bat echolocation: the influence of individual, habitat and conspecifics on sonar signal design. *Behavioral Ecology and Sociobiology*, **36**, 207–219.
- Pfalzer, G. & Kusch, J. 2003. Structure and variability of bat social calls: implications for specificity and individual recognition. *Journal of Zoology*, **261**, 21–33.
- Porter, F. L. 1979. Social behavior in the leaf-nosed bat, *Carollia perspicillata*. 2. Social communication. *Zeitschrift für Tierpsychologie*, **50**, 1–8.

- Riskin, D. K. & Fenton, M. B.** 2001. Sticking ability in Spix's disk-winged bat, *Thyroptera tricolor* (Microchiroptera: Thyropteridae). *Canadian Journal of Zoology-Revue Canadienne de Zoologie*, **79**, 2261–2267.
- Russ, J. M., Jones, G., Mackie, I. J. & Racey, P. A.** 2004. Interspecific responses to distress calls in bats (Chiroptera: Vespertilionidae): a function for convergence in call design? *Animal Behaviour*, **67**, 1005–1014.
- Russo, D., Jones, G. & Mucedda, M.** 2001. Influence of age, sex and body size on echolocation calls of Mediterranean and Mehely's horseshoe bats, *Rhinolophus euryale* and *R. mehelyi* (Chiroptera: Rhinolophidae). *Mammalia*, **65**, 429–436.
- Sayigh, L. S., Esch, H. C., Wells, R. S. & Janik, V. M.** 2007. Facts about signature whistles of bottlenose dolphins, *Tursiops truncatus*. *Animal Behaviour*, **74**, 1631–1642.
- Scherrer, J. A. & Wilkinson, G. S.** 1993. Evening bat isolation calls provide evidence for heritable signatures. *Animal Behaviour*, **46**, 847–860.
- Searby, A. & Jouventin, P.** 2004. How to measure information carried by a modulated vocal signature? *Journal of the Acoustical Society of America*, **116**, 3192–3198.
- Searle, S. R., Casella, G. & McCulloch, E.** 2009. *Variance Components*. New York: J. Wiley.
- Siemers, B. M. & Kerth, G.** 2006. Do echolocation calls of wild colony-living Bechstein's bats (*Myotis bechsteinii*) provide individual-specific signatures? *Behavioral Ecology and Sociobiology*, **59**, 443–454.
- Siemers, B. M., Beedholm, K., Dietz, C., Dietz, I. & Ivanova, T.** 2005. Is species identity, sex, age or individual quality conveyed by echolocation call frequency in European horseshoe bats? *Acta Chiropterologica*, **7**, 259–274.
- Suga, N., Niwa, H., Taniguchi, I. & Margoliash, D.** 1987. The personalized auditory-cortex of the moustached bat: adaptation for echolocation. *Journal of Neurophysiology*, **58**, 643–654.
- Tibbetts, E. A. & Dale, J.** 2007. Individual recognition: it is good to be different. *Trends in Ecology & Evolution*, **22**, 529–537.
- Titus, K., Mosher, J. A. & Williams, B. K.** 1984. Chance-corrected classification for use in discriminant analysis: ecological applications. *American Midland Naturalist*, **111**, 1–7.
- Vonhof, M.** 2001. Habitat availability, population size, and the composition, stability, and genetic structure of social groups of Spix's disk-winged bat, *Thyroptera tricolor*. Ph.D. thesis, York University.
- 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.
- Vonhof, M. J., Whitehead, H. & Fenton, M. B.** 2004. Analysis of Spix's disc-winged bat association patterns and roosting home ranges reveal a novel social structure among bats. *Animal Behaviour*, **68**, 507–521.
- Wilkinson, G. S.** 1995. Information transfer in bats. *Symposium of the Zoological Society of London*, **67**, 345–360.
- Wilkinson, G. S. & Boughman, J. W.** 1998. Social calls coordinate foraging in greater spear-nosed bats. *Animal Behaviour*, **55**, 337–350.
- Yoshino, H., Matsumura, S., Kinjo, K., Tamura, H., Ota, H. & Izawa, M.** 2006. Geographical variation in echolocation call and body size of the Okinawan least horseshoe bat, *Rhinolophus pumilus* (Mammalia: Rhinolophidae), on Okinawa-Jima Island, Ryukyu Archipelago, Japan. *Zoological Science*, **23**, 661–667.