Echolocation calls of Myotis lucifugus and M. leibii (Vespertilionidae) Flying Inside a Room and Outside

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The purpose of this study was to compare the echolocation calls of the same four individual *Myotis lucifugus* and *Myotis leibii* flying inside a closed room and when released outside. Echolocation calls were recorded using a Pettersson D980 bat detector, the high frequency output fed into a personal computer via an F2000 Control Filter and an Ines High speed card. Recorded as .wav files, recordings were analyzed with BatSoundPro. We measured call duration (DUR in ms), frequency with maximum energy (FMAX in kHz), highest frequency (HF in kHz), lowest frequency (LF in kHz), and inter-pulse interval (IPI in ms). Multivariate Analyses of Variance (MANOVA) indicated significant differences in call features between species, between settings, between species in each setting, and finally between settings for each individual. Discriminant Function Analyses (DFA) revealed that inside DUR was the most important parameter distinguishing *M. lucifugus* from *M. leibii*, with 66.3% correct classification, while outside, the two species were distinguished 78.8% of the time by LF. The data demonstrate that the same individuals flying in confined spaces change the details of their echolocation calls compared to when flying in the open. Calls produced inside are shorter in DUR and are produced at shorter IPIs than calls produced outside. FMAX differed most between the calls of *M. lucifugus* and *M. leibii* whether flying inside or outside. Differences between echolocation calls were more pronounced between setting (inside versus outside) than between species.

Key words: *Myotis lucifugus*, *M. leibii*, echolocation, inside, outside

INTRODUCTION

Self-recognition of a bat's echolocation calls is fundamental for echolocation and must be dynamic enough to cover the wide range of calls that an individual produces (e.g., Suthers, 1965; Simmons *et al*., 1979; Obrist, 1995; Schnitzler and Kalko, 2001). Bats that use echolocation to detect, track, and assess airborne prey (usually flying insects) adjust the features of their calls according to the situation, from search through approach and terminal phases of an attack (Griffin *et al*., 1960; Schnitzler and Kalko, 2001). Bats also may adjust their echolocation calls according to habitat (Obrist, 1995), social setting (Habersetzer, 1981) or local conditions such as those associated with clutter or with atmospheric attenuation (Lawrence and Simmons, 1982). Therefore it is no surprise that the details of echolocation call design vary according to acoustical constraints and areas where they forage (Kalko, 1995; Neuweiler *et al*.,

1987; Simmons *et al.*, 1979). Echolocating bats use different information-gathering strategies for flying and hunting in confined situations versus more open ones (Schnitzler and Kalko, 2001).

One clear demonstration of bats adjusting their echolocation calls according to situation is provided by changes in call design effected when bats fly in confined as opposed to open settings (Schumm *et al.*, 1991). The purpose of this study was to compare the echolocation calls of the same individual *Myotis lucifugus* and *Myotis leibii* flying in a room and in the open out-of-doors (Schumm *et al.*, 1991). We expected the bats' calls to differ between the two situations and wanted to determine if the situation differences were more pronounced than the differences between species. *Myotis lucifugus* and *M. leibii* take airborne prey and use echolocation calls dominated by broadband frequency modulated components. Both species are generalists, foraging in open areas (spaces over water) or in more confined spaces (within wooded areas) usually taking flying prey (Fenton and Barclay, 1980; Best and Jennings, 1997). Differences in echolocation calls according to settings have consequences for those who identify bats by their echolocation calls and include in their collections of reference calls recordings from bats flying indoors (e.g., Barclay, 1983; Jones *et al.*, 1993; O'Farrell and Miller, 1997; Rydell *et al.*, 2002).

MATERIALS AND METHODS

Between 1 and 6 September 2002, we studied the echolocation calls and behaviour of *M. lucifugus* and *M. leibii* in southeastern Ontario, Canada. We recorded bats flying in a 12 by 6 by 3 m room at the Queen's University Biological Station (44°15'N; 79°15'W). We had captured the bats in a Tuttle trap (Tuttle, 1974) set at the entrance of the Lafleche Caves in Quebec or the Renfrew mine near Renfrew, Ontario from 22:00 to 24:00 hrs. Four individuals of each species were brought back to the Biological Station and their calls recorded the day after capture. Each bat

was identified by a numbered piece of tape attached to its back before first flying them individually in the room that contained stationary objects such as tables and chairs. The walls of the room were made of drywall and contained many windows. In the room, the bats flew readily, circling the room repeatedly, first flying near the ceiling and progressing towards the floor. Whereever possible we used call sequences produced as bats approached a wall and were ≥ 2 m from it.

Before release outside at night, we light-tagged (Hovorka *et al.*, 1996) each bat and released them one at a time. Outside we recorded the calls of bats released in open areas at least 40 m from the nearest trees either at the abandoned mine near Renfrew, Ontario or at the Biological Station. In either setting, released bats normally circled the clearing once or twice before disappearing from view. All recordings were digitized and analyzed in the same way as the inside recordings.

We recorded the bats' echolocation calls using a Pettersson Electronic AB (Tallbacksvagen 51, S-756 45 Uppsala, Sweden) equipment and software. Specifically, a D980 bat detector was used to record the bats' vocalizations using a high frequency output of a D980 through an F 2000 Control Filter (Pettersson Elektronik AB, Uppsala, Sweden) and an Ines DAQ i508 high-speed card to a Dell Latitude PC running BatSoundPro (Pettersson Elektronik AB). The sampling frequency was 250 kHz, 16 bits. We recorded 60 s intervals separated by 10 s periods for resetting the system. The recordings were analyzed with Bat-SoundPro.

For each echolocation call, we measured duration (DUR – ms), highest frequency (HF – kHz), lowest frequency (LF – kHz) and frequency with most energy (FMAX – kHz) as well as inter-pulse interval (IPI – ms). We measured time features from the time-amplitude displays, and frequency features from the Fast Fourier Transform (FFT) power spectrum (size 512 Hanning Window), HF and LF at -10 dB from FMAX. For recordings inside and outside we analyzed two sequences for each bat (4 *M. lucifugus* and 4 *M. leibii*; = 4 sequences per bat). Call sequences were at least 60 s apart and each sequence consisted of 10 sequentially-produced echolocation calls. For each bat (4 *M. lucifugus*, 4 *M. leibii*) recorded inside and outside we analyzed 2 sequences, choosing call sequences with uniform time-amplitude displays and high signal-to-noise ratios (signal > 20% above background noise). For each analysis we first used a Multiple Analysis of Variance (MANOVA) to assess variation between sequences using DUR, HF, LF, FMAX and IPI as criterion variables and species, setting, species in each setting and individual calls in each

setting as respective factors. We then proceeded with Discriminant Function Analyses (DFA) to determine the parameters most important in discriminating the calls, as well as the accuracy with which call sequences could be classified to group. We used DUR, HF, LF, FMAX and IPI as predictors and species, setting, species in each setting and individual calls in each setting as group membership variables. We found that the variance of our dependent variables was not equally distributed across groups but Q-Q plots and the skew of each dependent variable indicated that variables approached normality, and DFA is relatively robust to departures from normality (Dillon and Goldstein, 1984). We conducted Box's tests that suggested that the covariance of our dependent variables were not equal across groups. However, Lachenbruch (1975) asserted that DFA is relatively robust even when there are violations of these assumptions. We report cross-validated classification results, obtained using the leave-one-out method to assess the generalizability of the models to calls outside our sample (Olden and Jackson, 2002). Prior probabilities were set to equal. The statistical tests were done using SPSS 11.0.1 (for Windows, 15 November 2001).

RESULTS

There were significant differences between the call sequences of *M. lucifugus* and *M. leibii* (Table 1) and inside or out, FMAX was the most important parameter distinguishing the two species, but only 67.2% of calls were correctly classified to species (Table 2). There were significant differences between the call sequences produced inside and outside (Table 1), with DUR as the most important parameter, and 90.9% of call sequences correctly classified (Table 2). In testing for differences between species within each setting, we found that inside, DUR was the most important parameter distinguishing *M. lucifugus* from *M. leibii*, with 66.3% correct classification, while outside, the two species were distinguished 78.8% of the time by LF (Table 2). The bats' calls outside were longer, composed of short, steep, broadband frequency modulated (FM) components followed by longer, narrowerband FM components (Fig. 1). For each individual, inside recordings differed significantly from those recorded outside (Table 1), and DUR was the most often the most important parameter distinguishing between settings (Table 2). Individual bats' calls were classified according to setting with at least 85% correct classification (Table 2). Finally, we ran an analysis using each sequence of calls as a separate group and found only 39.1% correct classification by sequence (Table 2). The data support the prediction that vespertilionids flying in confined spaces change the details of their

TABLE 1. Multivariate analysis of variance results, computed using $\alpha = 0.0125$

Analysis	Wilks' λ	*F*-value	Hypothesis *d.f.*	Error *d.f.*	*P*-level
M. lucifugus versus *M. leibii*	0.90	7.09	5.00	314.00	0.001
Inside vs. outside	0.39	97.47	5.00	314.00	0.001
M. lucifugus vs. *M. leibii* inside	0.75	4.96	5.00	74.00	0.001
M. lucifugus vs. *M. leibii* outside	0.54	12.66	5.00	74.00	<0.001
Individual bats' calls, inside versus outside					
M. lucifugus 1	0.17	32.50	5.00	34.00	0.001
M. lucifugus 2	0.10	59.30	5.00	34.00	<0.001
M. lucifugus 3	0.12	48.72	5.00	34.00	<0.001
M. lucifugus 4	0.19	28.90	5.00	34.00	<0.001
M. leibii 1	0.23	22.77	5.00	34.00	<0.001
M. leibii 2	0.31	15.30	5.00	34.00	<0.001
M. leibii 3	0.21	25.98	5.00	34.00	<0.001
M. leibii 4	0.25	20.61	5.00	34.00	<0.001
Each call sequence as separate group	0.01	12.67	155.00	1410.48	<0.001

TABLE 2. Summary of discriminant function analysis results, where FMAX is frequency with most energy (in kHz), DUR is duration (in ms), LF is lowest frequency (in kHz), and IPI is interpulse interval

Analysis	Most important parameters distinguishing groups (Wilks' λ)	% Correlation between parameter and function 1 used in analysis	% Correct classification (cross-validated)
M. lucifugus versus *M. leibii*	FMAX	78.4	67.2
Inside vs. outside	DUR	90.0	90.9
M. lucifugus vs. *M. leibii* inside	DUR	75.7	66.3
M. lucifugus vs. *M. leibii* outside	LF	88.3	78.8
Individual bats' calls, inside vs. outside			
M. lucifugus 1	DUR	62.2	95
M. lucifugus 2	DUR	82.9	100
M. lucifugus 3	DUR	70.5	97.5
M. lucifugus 4	DUR	88.4	95
M. leibii 1	DUR	76.3	90
M. leibii 2	IPI	73.5	95
M. leibii 3	DUR	87.1	92.5
M. leibii 4	DUR	82.2	85
Each call sequence as separate group	DUR	91.5*	39.1

* — this analysis used five functions to discriminate groups

echolocation calls compared to the same bats flying outside. Calls produced inside are shorter in duration and are produced at shorter inter-pulse intervals than calls produced outside (Table 3; Fig. 1). FMAX was the most important parameter separating the calls of *M. lucifugus* from those of *M. leibii* (higher) whether flying inside or outside. In this study, differences between calls were more marked between settings (indoors versus outdoors) than between species.
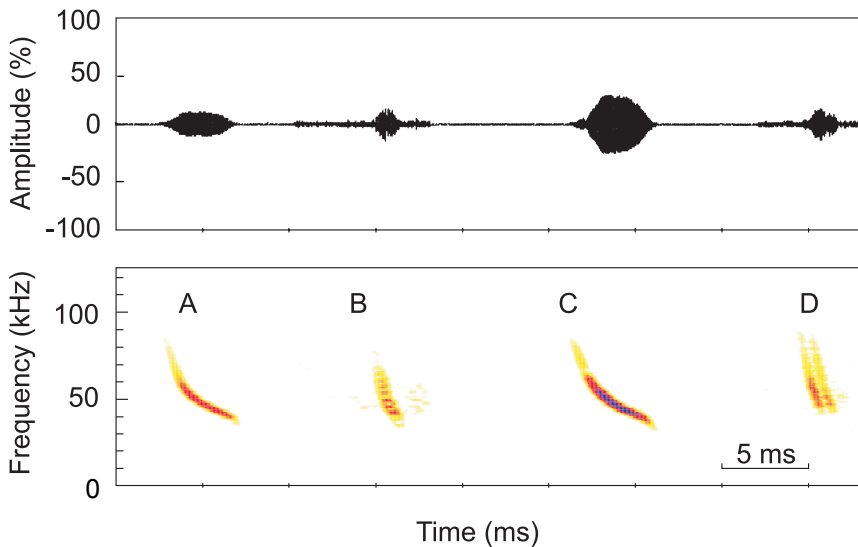

FIG. 1. Time-amplitude and frequency change over time representations of 'typical' echolocation calls of *Myotis lucifugus* produced when flying outside (A) and inside (B), and of *Myotis leibii* flying outside (C) and inside (D)

TABLE 3. Mean (± SD) values of 10 calls from two sequences per individual in each setting for four *M. lucifugus* and four *M. leibii*. Sample size was always 40 calls, DUR is duration, FMAX is frequency with maximum energy, HF is highest frequency, LF is lowest frequency, and IPI is interpulse interval

Parameter	*M. lucifugus* inside	*M. lucifugus* outside	*M. leibii* inside	*M. leibii* outside
DUR (ms)	2.4 ± 1.5	6.6 ± 2.2	2.8 ± 0.8	5.3 ± 1.6
FMAX (kHz)	51.5 ± 6.1	45.9 ± 3.3	51.1 ± 4.8	48.5 ± 4.5
HF (kHz)	62.1 ± 8.5	53.9 ± 5.2	60.2 ± 8.0	63.4 ± 10.4
LF (kHz)	45.4 ± 5.4	42.5 ± 2.1	44.6 ± 4.4	46.6 ± 3.2
IPI (ms)	51.3 ± 19.5	86.4 ± 18.0	55.1 ± 22.0	93.2 ± 24.6

DISCUSSION

Our results demonstrate that species and setting influence the structure of echolocation calls. We confirm that it is possible to distinguish *M. lucifugus* and *M. leibii* by their calls whether flying inside or outside, but only with 67% correct classification to species (Table 2). We found that echolocation calls recorded indoors provide little diagnostic information for using calls to distinguish between *M. lucifugus* and *M. leibii* flying in the field. Inside, echoes from walls, ceilings and other objects in a room influence the calls as bats shortened DUR and IPI in response to rapidly returning echoes in relatively confined spaces (Obrist, 1995). As reported from other high intensity echolocating bats, both *M. lucifugus* and *M. leibii* use shorter calls to obtain more information about background close objects (Schnitzler and Kalko, 2001).

In indoor studies where bats show normal hunting and echolocation behaviour (e.g., Suthers, 1965; Britton and Jones, 1999; Siemers and Schnitzler, 2000; Ratcliffe and Dawson, 2003), recordings provide an indication of part of the range of calls bats may use in the field. Our data suggest that recordings made in rooms are dominated by short, broadband FM signals, missing longer, narrowerband components. Short, broadband, FM signals (Fig. 1) are well suited for short-range spatial orientation, where a precise characterization of background targets is necessary for recognizing landmarks and avoiding collisions (Denzinger *et al*., 2001). Outside longer signals with narrowband components provide greater operational range, allowing bats to detect and characterize prey (Denzinger *et al*., 2001). Significant differences in echolocation signals of both species suggest flexibility in foraging and echolocation behaviour as suggested (e.g., Fenton, 1990) or demonstrated (e.g., Schum *et al*., 1991) for other species.

Our analyses raise questions about the use of discriminant function analysis (DFA) in the identification of bats by their echolocation calls. Depending upon the situation, our DFAs correctly assigned echolocation calls to circumstance between 39% and 100% of the time (Table 2). Other studies involving DFA have produced a similar range of results (e.g., Obrist, 1995; Jones *et al*., 2000; Kazial *et al*., 2001; Russo and Jones, 2002). No probability values associated with classification by DFA leaving open the question of what levels of classification are appropriate. Of particular note is our finding that calls were more accurately identified by recording situation (inside versus outside) than by species (Table 2).

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