

INFORMATION CONTENT OF SONAR CALLS OF LITTLE BROWN BATS (*MYOTIS LUCIFUGUS*): POTENTIAL FOR COMMUNICATION

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We examined variability in bat sonar calls related to characteristics of the caller (i.e., individual identity, sex, state of lactation, age category, and colony membership) in 2 situations. If variation in call features reliably reflects characteristics of individuals, then there is potential for information transfer to conspecifics. Thus, sonar calls, in addition to their use in orientation and prey location, may function for communication. We obtained recordings of sonar calls from 66 little brown bats (*Myotis lucifugus*) captured from buildings at Chautauqua Institution, State University of New York at Fredonia campus, and surrounding areas in 2003–2005. Calls were recorded using a laptop computer, digitizing card, connector box, amplifier, and U30 bat detector while the bat crawled from hand to hand and while in flight. Calls were extracted and analyzed using custom computer programs to generate measurements for variables describing each call, which were then analyzed statistically. Our analysis revealed individual identity, state of lactation, and age category are reliably indicated by call variation of crawling bats, and individual identity information is contained in calls of bats in flight. To our knowledge these results are the 1st report of differences between sonar calls of lactating and nonlactating individuals for any bat species and individual differences in sonar calls for *M. lucifugus*. Individual identity information is available in calls produced within a situation; however, this information does not transfer well to the calls of the same bats produced in different situations. Our results suggest that the potential for communication of information via sonar calls depends on calling situation.

Key words: Chiroptera, echolocation calls, little brown bats, *Myotis lucifugus*, Vespertilionidae

It is well known that bats use sonar calls for orientation and prey location. In addition, there is evidence that sonar calls also play a role in communication between bats (Fenton 1985; Masters et al. 1995). Supporting evidence includes analysis of variation in calls of bats in the laboratory and field. Sonar calls contain variation related to the situation of the caller, for example, while handheld or in flight, open areas or clutter, with conspecifics or alone, or in different geographic locations (Barclay et al. 1999; Broders et al. 2004; Burnett et al. 2001; Ibáñez et al. 2004; Obrist 1995; Ratcliffe et al. 2004; Siemers and Kerth 2006). Research into call variation also suggests that variation is related to characteristics of the bat and may reveal the caller's species (Fenton and Bell 1981; Parsons and Jones 2000; Waters and Jones 1995), colony membership (Pearl and Fenton 1996), family affiliation (Masters et al. 1995), age (Jones and Kokurewicz 1994; Kazial et al. 2001; Masters et al.

1995; Moss et al. 1997), sex (Jones and Kokurewicz 1994; Neuweiler et al. 1987; Suga et al. 1987), or individual identity (Brigham et al. 1989; Fenton et al. 2004; Kazial et al. 2001; Obrist 1995; Siemers and Kerth 2006).

Additional evidence consistent with the use of sonar calls for communication comes from field and laboratory behavioral experiments. Early work by Möhres (1967) suggested that *Rhinolophus ferrumequinum* was able to recognize familiar roost mates by their vocalizations. Several playback studies document eavesdropping where bats respond to conspecific calls and calls of sympatric species when locating feeding sites, roosts, maternity colonies, and hibernacula (for a review see Fenton 2003). Playback studies also have demonstrated recognition of mothers' calls by young *Plecotus auritus* (de Fanis and Jones 1995). Together, these studies indicate that bats respond to sonar calls of other bats, supporting the idea that sonar is used for communication.

Bats may be able to hear calls of other bats up to 50 m away (Barclay 1982) and use information contained in them. In addition to incidental transmission of information resulting in fitness benefits to the receiver, calling bats may include information in sonar calls that can be considered use of signals

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for true communication (see Dusenbery 1992) with fitness benefits to the sender as well.

Our purpose was to examine variation in sonar calls in the context of individual identity, sex, state of lactation, age category, and colony membership for little brown bats (*M. lucifugus*) in 2 calling situations to determine whether calling situation affects the characteristics indicated by variation in sonar calls. We evaluated this quantitatively by recording the same bats in 2 calling situations with known individuals. Few studies have compared potential communicative characteristics in 2 calling situations.

We hypothesized that information would be carried in the sonar calls of bats because it could be used by conspecifics to locate potential mates (sex and age information) and roost sites (colony membership and individual identity information), and for young and juveniles to locate lactating females and mothers (state of lactation and individual identity information). We predicted there would be variation in sonar calls of crawling bats associated with caller characteristics. We hypothesized that sonar calls produced in flight would carry additional variation due to bats altering calls with stages of insect pursuit (Griffin 1958) and with changes in distance to obstacles (i.e., clutter effects—see Burnett et al. 2004), thereby decreasing the reliability of variation indicating the caller's characteristics. We predicted flight calls would have more variation, decreased signal consistency, and less association with caller characteristics. An additional current topic of interest we addressed is whether sonar call descriptor variables indicating individual identity in one calling situation would do so in another (addressed recently for *Myotis bechsteinii* by Siemers and Kerth [2006]).

MATERIALS AND METHODS

Animals.—We captured 66 bats by hand or net from attics of Chautauqua Institution residences (Chautauqua, New York), the State University of New York at Fredonia campus (Fredonia, New York), and surrounding areas during the summers of 2003–2005. Bats were treated in a humane manner, following guidelines of the American Society of Mammalogists (Gannon et al. 2007), permitted by a license to collect and possess issued by the New York State Department of Environmental Conservation and a protocol approved by the State University of New York at Fredonia Animal Care and Use Committee. Bats were housed individually in a ventilated plastic container for approximately 1 h and transported to Smith Wilkes Hall on the grounds of Chautauqua Institution for recording. For each bat we recorded mass using a spring scale (Pesola AG, Baar, Switzerland), sex, state of lactation (lactating or not), age category (adult or young of year), and colony membership (location from which the bat was obtained). Bats were classified as having lactated during the current season by a lack of fur around the nipples and determined to be young of year based on elongated, translucent finger joints versus the knobby, opaque finger joints of adults.

Recording.—We recorded calls using a U30 bat detector as an ultrasonic microphone (Ultra Sound Advice, London,

United Kingdom), a custom-built amplifier, a connector box, and a digitizing card (500-kHz sampling rate maximum; National Instruments Corporation, Austin, Texas) with a laptop computer. To have the release of bats coincide with their natural emergence time bats were recorded within 1 h before dusk. Bats were recorded individually and within sight. Because recordings were made before natural emergence, inadvertent recording of other bats was avoided. To record crawling bats we allowed them to crawl from 1 hand to the other at the same height, 4 m from the microphone, and 1 m above a concrete floor. To record flying bats, bats were released from the hand within Smith Wilkes Hall, a large amphitheater with tiered seating, a stage, a roof, and open to the outside on 3 sides (floor to ceiling height is approximately 7.6 m). Typically bats would circle the ceiling area before finding their way out through the side openings.

Call analysis.—We used a program written by S. C. Burnett in MATLAB (2002; The MathWorks, Inc., Natick, Massachusetts) to extract calls from the original data files and then analyzed calls using a 2nd MATLAB program (written by S. C. Burnett and W. M. Masters, updated from Masters et al. [1991]) to obtain measurements for 30 variables describing the temporal, frequency, and shape structure of each suitable call. If calls were recorded with echo overlap due to the recording setup they were excluded from the analysis. Among the variables describing call structure were the calculated fits of 6 different mathematical functions describing the time course of the fundamental frequency (describing call shape). The best-fitting function (i.e., the function having the lowest average mean-squared error) for calls produced while bats were crawling and flying was the power-3 sweep function (equation 8 from Parsons and Jones [2000]). We retained the 4 variables used by that function and excluded the 13 variables pertaining to other functions. Curvature was included to describe call shape (modified from Boonman and Schnitzler [2005]). Curvature values ranged from -1 to $+1$, with a negative value indicating a concave shape, 0 indicating a straight line between starting and ending frequencies (i.e., no curvature), and a positive value indicating a convex shape. We excluded maximum call amplitude because calls were recorded at different gains and source level was difficult to estimate in the field. The time of maximum frequency of the fundamental also was excluded given that it could not be calculated in a consistent manner. These exclusions reduced the variable set to 15 describing the fundamental frequency of each call (see Appendix I for descriptions of variables).

Statistical analysis.—Because recording situation affects the structure of calls, only bats recorded in Smith Wilkes Hall ($n = 56$) were used in subsequent analyses. Calls used in statistical analyses were obtained from a single recorded sequence while the bat was crawling or a single recorded sequence while the bat was in flight. Of the 56 bats recorded while crawling, 40 were adult and 16 were young of the year, 35 were female and 21 were male, and of the 35 females, 20 did not exhibit evidence of lactation whereas 15 did. Thirty-seven bats were recorded while flying (all except 1 of which were recorded while crawling). Of these, 29 were adult and 8 were young of the year, 26 were female and 11 were male, and of the 26

females, 13 had not lactated and 13 had. For inclusion in the analysis based on colony membership, bats needed to have been caught within a known colony roost site and at least 2 bats needed to have been recorded per colony. This left us with a total of 40 bats recorded while crawling and 37 bats recorded while in flight from the same 8 colonies for analysis. For 6 of the 8 colonies we confirmed the presence of lactating females, and for 1 additional colony, the presence of young. This supports the assumption that these locations were maternity colonies. Examination of the data of Neilson and Fenton (1994) supports the assumption that *M. lucifugus* uses buildings as maternity roosts at Chautauqua, reporting that >90% of recaptures occurred in the building where bats were originally caught. All colonies were found within the 1.88 km² of the Chautauqua Institution.

Information on individual identity in calls requires signal consistency within individuals. Information on individual identity necessarily contains variation associated with sex, state of lactation, age category, and colony membership of the individual. These characteristics were considered separately in other analyses. We analyzed signal consistency using reliability analysis (2-way random model) for the variables describing each call (SPSS 2004; SPSS, Inc., Chicago, Illinois). We performed the analysis using 5 calls for each of the 56 bats recorded while crawling and 37 bats recorded in flight. We report both single-measure and average-measure intraclass correlation coefficients (r_1). A coefficient approaches 1 when the between-bat variation in calls is large relative to the within-bat variation and a coefficient has a 0 value when the within-bat variation in calls equals the between-bat variation. The coefficient can be negative when the within-bat variation in calls is greater than the between-bat variation. Hence, the coefficient is a useful measure of the reliability of individual identity information within calls.

Single-measure intraclass correlation coefficients indicate the reliability of the value from a single call for a particular descriptive variable. Average-measure intraclass correlation coefficients reflect the reliability of the mean of values from several calls for a particular descriptive variable. Both single-measure and average-measure intraclass correlation coefficients are informative, because a bat may use a single call to assess the individual identity of a calling bat it hears, or more likely, may use several calls to determine individual identity.

We used discriminant function analysis (DFA) to examine the classification success of calls to individual bats. All DFAs employed 13 variables, excluding pow3f0 and pow3f1 (Table 1) because of their failure in tolerance tests (see Appendix I for descriptions of variables). We examined classification success for 18 calls assigned to individuals using cross-validation (leave-one-out method in SPSS), as well as the success achieved using the original set of 18 randomly chosen calls to generate the discriminant function, and a new set of 18 randomly chosen calls to classify. Because we also were interested in how useful calls produced in 1 situation (e.g., crawling) would be for identifying a flying bat, a 3rd and 4th DFA used calls of crawling bats to generate the discriminant function and calls of flying bats to classify and vice versa. These DFAs used 18 calls from 36 bats recorded in both the crawling and flying situations.

TABLE 1.—Descriptive statistics for 36 sonar calls of 36 bats (*Myotis lucifugus*) recorded while crawling and in flight at Chautauqua Institution, Chautauqua, New York, 2003–2005. Mean, standard error (*SE*), and coefficient of variation (*CV*) are given for 15 variables describing sonar calls. See Appendix I for descriptions of variables.

Variable	\bar{X}	<i>SE</i>	<i>CV</i>
Temporal structure			
duration	2.25	0.02	39.05
t50	0.81	0.01	30.16
Frequency structure			
h1start	73.17	0.30	14.90
h1mid	51.17	0.12	8.63
h1end	38.48	0.09	8.57
h1maxf	48.14	0.11	7.89
l3fh1	52.38	0.12	8.18
u3fh1	44.32	0.09	7.48
l10fh1	61.09	0.23	13.39
u10fh1	40.44	0.08	7.52
pow3f0	73.18	0.30	14.88
pow3f1	38.46	0.09	8.58
Shape			
pow3a	20.22	1.43	255.40
pow3c	−21.85	1.18	−194.17
curvature	0.26	0.003	43.83

To avoid pseudoreplication introduced by including >1 call from a single bat in our analyses involving calling situation, sex, state of lactation, age category, and colony membership, we used an average value for each variable based on 18 calls from each bat. We avoided multiple testing and correlated descriptive variables by using principal component analysis on the variable averages to obtain principal components (PCs) that were used in subsequent statistical tests (SPSS 2004). We used binary logistic regression to assess whether call variation described by PCs could predict situation, sex, state of lactation, and age category. To determine which descriptor variables were most likely to contain information about bat characteristics we used logistic regression on the descriptor variables that loaded most heavily (>0.7) on a significant PC. We used a general linear model to test whether colony membership of a bat was significantly associated with differences in PCs describing calls. We used DFA to determine classification success of calls for characteristics that were significant in previous statistical tests.

RESULTS

Descriptive statistics for 36 calls from 36 bats that were recorded in both flying and crawling situations are included in Table 1. Spectrograms show within-bat variation including example calls produced by the same bat while crawling and in flight (Fig. 1).

Individual variation in sonar calls of crawling bats.—Call variables generally had a high level of repeatability indicating individual identity information within calls (Table 2). All temporal and frequency measures of calls were significantly repeatable, as were measures of call shape, except for pow3a.

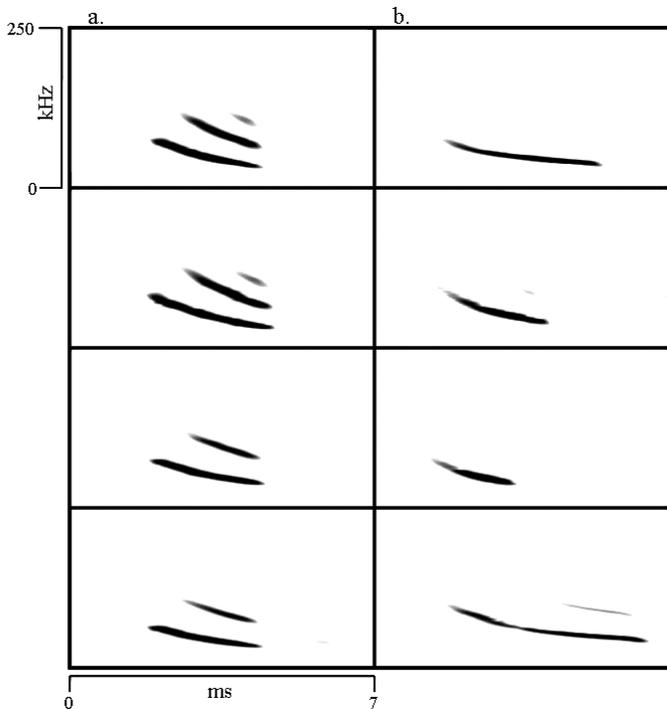


FIG. 1.—Spectrograms of example sonar calls from a young-of-the-year female *Myotis lucifugus* recorded at the Chautauqua Institution, Chautauqua, New York, while crawling from hand to hand (column a) and while in flight (column b). Only the fundamental frequency was considered in our analyses. Time in milliseconds is on the x axis and frequency in kHz is on the y axis.

As expected, intraclass correlation coefficients based on average measures were greater than coefficients based on single measures. There was a negative intraclass correlation coefficient for pow3a, meaning that variation within calls exceeded variation between calls of different bats. DFA correctly classified calls of crawling bats to individuals with 27.4% success, above the 2% expected by chance for 56 individuals (functions 1–13, Wilks' $\lambda = 0.01$, $d.f. = 715$, $P < 0.0005$). Classification success of calls by crawling bats to the level of individual was 28.6% (2% expected by chance) when a 1st set of 18 calls per bat was used to derive the discriminant function and a 2nd set of 18 calls produced by the same bats was classified (functions 1–13, Wilks' $\lambda = 0.01$, $d.f. = 715$, $P < 0.0005$).

Individual variation in sonar calls of flying bats.—Call variables from flying bats were also significantly repeatable (Table 3) for all temporal and frequency measures of calls, and most measures of call shape, with the exception of pow3c. Again, as expected, average-measures intraclass correlation coefficients were greater than single measures. Compared to crawling bats, intraclass correlation coefficients of calls produced by flying bats were lower for all variables, except for pow3a. DFA classified calls of flying bats to individuals with 23.0% success, above the 3% expected by chance for 37 individuals (functions 1–13, Wilks' $\lambda = 0.05$, $d.f. = 468$, $P < 0.0005$). Classification success of calls by flying bats to individuals was 27.2% (3% expected by chance) when a 1st set

TABLE 2.—Repeatability of 15 variables describing sonar calls of crawling individuals (*Myotis lucifugus*) recorded at Chautauqua Institution, Chautauqua, New York, 2003–2005. See Appendix I for descriptions of variables. For all tests $d.f. = 55$, 220. r_1 = intraclass correlation coefficient.

Variable	Single-measures r_1	Average-measures r_1	F
Temporal structure			
duration	0.59	0.88	8.10**
t50	0.61	0.88	8.65**
Frequency structure			
h1start	0.41	0.78	4.44**
h1mid	0.55	0.86	6.98**
h1end	0.59	0.88	8.19**
h1maxf	0.44	0.80	4.96**
l3fh1	0.34	0.72	3.57**
u3fh1	0.52	0.85	6.50**
l10fh1	0.37	0.75	3.96**
u10fh1	0.56	0.86	7.23**
pow3f0	0.41	0.78	4.44**
pow3f1	0.59	0.88	8.25**
Shape			
pow3a	−0.01	−0.04	0.96 ^a
pow3c	0.20	0.55	2.21**
curvature	0.41	0.77	4.42**

^a $P = 0.56$.

** $P < 0.0005$.

of 18 calls per bat was used to derive the discriminant function and a 2nd set of 18 calls produced by the same bats was classified (functions 1–13, Wilks' $\lambda = 0.05$, $d.f. = 468$, $P < 0.0005$). Classifying individuals using a novel set of calls from each bat produced within the same situation demonstrated the same level of success as the cross-validation approach.

Situation and individual.—We used logistic regression to assess whether variation in calls predicted calling situation for 36 bats (recorded crawling and in flight) but there was no significant ability to predict for any PC (Wald statistic: $W_1 < 0.0005$, $P > 0.994$). This is despite the longer mean call duration (2.71 ms) given in flight (with increased distance to obstacles) compared with calls produced while crawling (1.80 ms; Fig. 1). DFA correctly classified calls of these same 36 bats to individuals with 17.8% success (3% expected by chance; functions 1–13, Wilks' $\lambda = 0.17$, $d.f. = 455$, $P < 0.0005$). However, this indicates the success in classifying calls to individual across situations is lower than when classifying individuals within a calling situation.

Our ability to classify calls of flying bats based on calls produced by crawling bats was no greater than chance (5.4% compared with 3% expected by chance; functions 1–13, Wilks' $\lambda = 0.01$, $d.f. = 455$, $P < 0.0005$). Similarly, we had no greater than chance ability to classify calls of crawling bats based on calls produced by flying bats (3.1% compared with 3% expected by chance; functions 1–13, Wilks' $\lambda = 0.05$, $d.f. = 455$, $P < 0.0005$).

Principal component analysis.—We identified 3 PCs that described a total of 84.5% of the total variation in descriptor variables for crawling bats (Table 4). Frequency variables loaded most heavily on PC1, temporal variables and 2 shape

TABLE 3.—Repeatability of 15 variables describing sonar calls of flying individuals (*Myotis lucifugus*) recorded at Chautauqua Institution, Chautauqua, New York, 2003–2005. See Appendix I for descriptions of variables. For all tests *d.f.* = 36, 144. *r*₁ = intraclass correlation coefficient.

Variable	Single-measures <i>r</i> ₁	Average-measures <i>r</i> ₁	<i>F</i>
Temporal structure			
duration	0.40	0.77	4.26**
t50	0.47	0.82	5.46**
Frequency structure			
h1start	0.32	0.70	3.34**
h1mid	0.50	0.83	5.98**
h1end	0.45	0.80	5.10**
h1maxf	0.24	0.61	2.58**
l3fh1	0.30	0.68	3.09**
u3fh1	0.27	0.65	2.82**
l10fh1	0.28	0.66	2.95**
u10fh1	0.42	0.78	4.59**
pow3f0	0.32	0.70	3.35**
pow3f1	0.44	0.80	4.99**
Shape			
pow3a	0.19	0.53	2.14*
pow3c	0.02	0.08	1.09 ^a
curvature	0.33	0.71	3.41**

^a *P* = 0.359.

* *P* = 0.001; ** *P* < 0.0005.

variables loaded most heavily on PC2, and 1 shape variable (curvature) loaded most heavily on PC3.

Four PCs described a total of 87.5% of the total variance in call descriptor variables for flying bats (Table 5). Frequency variables and 1 shape variable, pow3c, loaded most heavily on PC1 and PC2. Two shape variables loaded most heavily on PC3, pow3a and curvature, and the 2 temporal variables loaded most heavily on PC4.

Sex variation.—Sex was not predicted by variation in any PC for calls of crawling bats (*W*₁ < 3.19, *P* > 0.07) or flying bats (*W*₁ < 2.60, *P* > 0.11).

State of lactation variation.—State of lactation was predicted by variation in PC1 for calls of crawling bats with marginal significance (*W*₁ = 3.85, *d.f.* = 1, *P* = 0.05). PC1 is loaded with call variables describing the frequency structure of calls. Logistic regression analysis with call descriptor variables loaded most heavily on PC1 showed that h1maxf (*Score*₁ = 6.55, *P* = 0.01), l3fh1 (*Score*₁ = 10.37, *P* = 0.001), and l10fh1 (*Score*₁ = 5.90, *P* = 0.02) were most important in predicting lactation. DFA performed using these 3 variables classified the calls of crawling bats to the correct state of lactation with 74.3% success (50% expected by chance for 2 groups; Wilks' λ = 0.68, *d.f.* = 3, *P* = 0.007). State of lactation was not predicted by variation in any PC for calls of flying bats (*W*₁ < 1.79, *P* > 0.18).

Information about lactation state also may indicate age and be associated with body mass because young-of-the-year females would not be lactating and would be lighter than adult females. Mass and age information contributes to a separation of bats by state of lactation based on a decrease in significance

TABLE 4.—Loadings and percentage variance explained by 3 principal components of call variables describing sonar calls of crawling bats (*Myotis lucifugus*) recorded at Chautauqua Institution, Chautauqua, New York, 2003–2005. See Appendix I for descriptions of variables. The greatest loading for each variable is indicated with italics.

Variable	Component		
	1	2	3
Temporal structure			
duration	-0.17	<i>0.91</i>	0.00
t50	-0.12	<i>0.91</i>	-0.25
Frequency structure			
h1start	<i>0.74</i>	0.58	0.25
h1mid	<i>0.93</i>	0.26	-0.05
h1end	<i>0.91</i>	-0.24	-0.03
h1maxf	<i>0.89</i>	0.14	-0.06
l3fh1	<i>0.93</i>	0.15	-0.03
u3fh1	<i>0.95</i>	-0.03	-0.11
l10fh1	<i>0.79</i>	0.43	0.17
u10fh1	<i>0.96</i>	-0.12	-0.13
pow3f0	<i>0.74</i>	0.57	0.25
pow3f1	<i>0.91</i>	-0.24	-0.03
Shape			
pow3a	-0.12	<i>-0.49</i>	-0.06
pow3c	-0.10	<i>-0.85</i>	-0.07
curvature	-0.14	-0.01	<i>0.96</i>
% variance	53.39	23.24	7.82

of PC1 to a trend (*P* < 0.09) when mass was included in the logistic regression and when a 2nd principal component analysis and logistic regression was conducted with data for adult females only.

Age variation.—Age could be predicted by variation in PC3 for calls of crawling bats (*W*₁ = 4.53, *d.f.* = 1, *P* = 0.03). PC3 is loaded most heavily with 1 shape call descriptor variable, curvature. Logistic regression analysis confirms curvature is important in predicting age (*Score*₁ = 7.03, *P* = 0.01). DFA using curvature classified calls of crawling bats to age with 67.9% success (50% expected by chance for 2 groups; Wilks' λ = 0.88, *d.f.* = 1, *P* = 0.007). Age could not be predicted by variation in any PC for calls of flying bats (*W*₁ < 1.98, *P* > 0.16).

Colony variation.—A 1-way multivariate analysis of variance found no significant differences among roosts on the 3 PCs describing calls of crawling bats (Wilks' λ = 0.66, *F* = 0.66, *d.f.* = 21, 86.69, *P* = 0.86) or of bats in flight (Wilks' λ = 0.35, *F* = 1.11, *d.f.* = 28, 91.56, *P* = 0.35). We conducted additional tests using only the calls of females produced while crawling (7 colonies, *n* = 26) and in flight (7 colonies, *n* = 24). We also conducted tests including only colonies (3 colonies) where we had 5 or 6 females recorded. Again, in none of these tests was there evidence of colony-specific variation.

DISCUSSION

We found a high level of repeatability in individual identity information extracted from the sonar calls of crawling and

TABLE 5.—Loadings and percentage variance explained by 4 principal components of call variables describing sonar calls of flying bats (*Myotis lucifugus*) recorded at Chautauqua Institution, Chautauqua, New York, 2003–2005. See Appendix I for descriptions of variables. The greatest loading for each variable is indicated with italics.

Variable	Component			
	1	2	3	4
Temporal structure				
duration	–0.14	0.12	0.43	<i>0.85</i>
t50	–0.10	0.04	0.02	<i>0.97</i>
Frequency structure				
h1start	0.26	<i>0.90</i>	0.30	0.06
h1mid	0.58	<i>0.76</i>	–0.05	–0.08
h1end	<i>0.88</i>	0.30	0.18	–0.07
h1maxf	<i>0.70</i>	0.57	–0.18	–0.16
l3fh1	<i>0.61</i>	0.57	–0.09	0.12
u3fh1	<i>0.80</i>	0.46	–0.20	–0.22
l10fh1	0.43	<i>0.76</i>	0.18	0.19
u10fh1	<i>0.86</i>	0.40	0.06	–0.14
pow3f0	0.26	<i>0.90</i>	0.29	0.06
pow3f1	<i>0.88</i>	0.30	0.18	–0.08
Shape				
pow3a	–0.23	–0.12	–0.85	–0.13
pow3c	–0.60	0.02	–0.34	–0.41
curvature	–0.10	0.20	<i>0.88</i>	0.18
% variance	52.07	20.44	7.59	7.37

flying bats, in support of our hypothesis. The repeatability increased when using average-measures intraclass correlation coefficients, which is likely akin to a bat using several calls it hears to make a determination of individual identity. DFA performed well when classifying calls to individual within each situation, and less well when calls produced in both situations were included in the data set. Classifying individuals using a novel set of calls from each bat produced within the same situation had a similar level of success as the cross-validation approach. In contrast, DFA resulted in no better than chance classification success in classifying calls produced in one situation based on calls produced by the same bats in the other. Although this suggests the possibility of situation-level variation in the calls, surprisingly, we did not find that variation in calls could predict calling situation.

State of lactation and age were reliably indicated by variation in calls of crawling bats. There was an indication that sex information may be present (although not statistically significant). None of these characteristics were evident in the calls of flying bats. This is consistent with our hypothesis that sonar calls produced in flight carry additional variation due to the bat altering calls during insect pursuit (Griffin 1958) and with changing distance to obstacles (i.e., clutter effects—Burnett et al. 2004). This additional variation decreases the reliability of variation indicative of the characteristics of the caller. Our result is important because it suggests there is a decrease in the potential transmission of information via sonar calls produced during flight compared with calls produced while crawling (e.g., in roosts). We found no evidence supporting the

hypothesis that colony membership information occurs in calls produced by bats either crawling or in flight.

Call variation for *M. lucifugus* has been reported in the context of species differences (Broders et al. 2004; Fenton and Bell 1981; Mukhida et al. 2004), clutter differences (Broders et al. 2004; Mukhida et al. 2004), habitat differences (Wund 2005), age differences (Buchler 1980; Gould 1971; Moss et al. 1997; Pearl and Fenton 1996), and colony differences (Pearl and Fenton 1996). To our knowledge, we are the 1st to report individual differences in calls of *M. lucifugus* and the 1st to find differences as a function of the state of lactation for any bat species. Our finding that calls convey information about age supports previous studies of *M. lucifugus*.

Sex differences in calls have been reported for other species. We found a nonsignificant trend for this information in calls of crawling bats. Sex differences in calls would allow sex recognition, which could be important for locating potential mates. Thomas et al. (1979) reported that male *M. lucifugus* emit sonarlike calls from crevices to which females were attracted. Information about age contained in the calls of crawling bats (which we found) also would contribute to location of potential mates because young-of-the-year males are sexually immature whereas young-of-the-year females may be sexually mature (Thomas et al. 1979).

Our results suggest that information about the state of lactation (coded in frequency variation) occurs in the calls of crawling females and is most evident when combined with information about age (i.e., evaluating all females, adults, and young of the year) and associated body mass. This describes a behaviorally relevant situation in maternity colonies where adult and juvenile females coexist, producing sonar calls while crawling about the roost. Information about the state of lactation could be used by young to locate lactating females, their mothers or others, to nurse. There is evidence that young attempt to suckle from females other than their mother (Thomson et al. 1985). Individual identity information in calls also would contribute to maternal recognition in maternity roosts.

We found that information about age is contained by the curvature variation in calls of crawling bats. The difference in curvature of the frequency sweep may reflect changes in the vocal tract of developing young. Information about age in calls may contribute to mother–offspring recognition and mate recognition (young-of-the-year males do not mate). It also may help newly volant young to follow experienced colony members to feeding and roosting sites (Wilkinson 1992), although examination of our data indicates that information about age occurs only in calls produced by crawling bats.

Information about colony membership, in addition to information about individual identity, contained in the calls of conspecifics could be useful for locating roosts and hibernacula. We found no evidence supporting colony variation in calls recorded at the same location. Pearl and Fenton (1996) described colony differences in sonar calls of *M. lucifugus* at Chautauqua Institution using different colonies than we studied; however, they pointed out that the set of calls providing the most evidence in support of colony differences

was unable to be separated from calling situation (i.e., location). Pearl and Fenton (1996) reported colony differences in the calls of females recorded in the same situation for 1 call variable, bandwidth.

Our reliability analysis indicates there is consistency within individuals and discrimination between individuals for calls produced within the same situation. Although classification success was better than chance levels, our analysis suggests a bat would be correct in identification 23–27% of the time within situation, and correct 18% of the time across situations. Whether this level of success would be beneficial to bats is unknown. However, additional information would be available to a bat including the use of multiple calls (which we addressed with reliability analysis) and harmonics over short distances such as in a roost (we did not include harmonics in our analysis). Individual differences would allow calls to function in communication, jamming avoidance, or both. Communication of identity information among conspecifics could enable cooperation among colony members, following others to colony, roost, and feeding locations, and in mother–offspring recognition. It also has been suggested that individual differences in sonar calls allow bats to recognize their own echoes among the calls and echoes of conspecifics (i.e., jamming avoidance—see Masters et al. 1991, 1995).

Only 2 studies (laboratory-based [Burnett et al. 2001] and field-based [Siemers and Kerth 2006]) have compared individual variation within and across calling situations by including recordings from the same individuals. Collection of our data allowed us to assess whether individual variation exists within (supported) and or between (supported, but less reliably) situations and whether the same call variables indicate individual variation in the 2 situations (not supported). Burnett et al. (2001) examined calls by the same *Eptesicus fuscus* in 3 situations (i.e., handheld, perched on a platform, or flying in an anechoic chamber). Consistent with our results, they found discrimination of calls by recording situation was unsuccessful but that correct classification of calls to individual within recording situation was higher than across situations. This is consistent with variability due to situation obscuring individual variability. Siemers and Kerth (2006) recorded *M. bechsteinii* returning to 2 maternity colonies after foraging bouts and showed situation variability in call structure (in contrast to our results, albeit different situations) but weak evidence for information about individual identity.

We argue that information about individual identity available in a given situation is different from information about individual identity available in another situation (i.e., coded by different aspects of the calls). Results of Siemers and Kerth (2006) support this, because they were unable to identify a single variable that conveyed caller identity in different situations. We achieved similar success in classifying individuals using a novel set of calls recorded in the same situation (opposite to Siemers and Kerth [2006]). Bats may use multiple calls and calls produced as part of different call sequences that they hear produced by the same individuals to allow them to recognize other bats. This scenario is logical and should be beneficial because we found average intraclass correlation coefficients to be greater than

single measures. In addition, in cases where bats could determine the calling situation, they could then separate situation from individual-specific variation. This scenario also seems plausible given that situation-specific variation in *M. bechsteinii* (Siemers and Kerth 2006) occurred in temporal variables describing call structure, such as call duration, whereas frequency variables were relatively unchanged.

It is not necessary for a connection to exist between the variation in sonar calls and the ability of bats to perceive and respond to this variation (i.e., the behavioral relevance of variation). However, determination of the existence of variation tied to communicative characteristics of the call leads to the potential for communication. Although Pearl and Fenton (1996) found evidence for colony differences in calls of *M. lucifugus*, Pearl (1994) was unable to evoke a differential response of subject bats to calls of bats from the same and different colonies in playbacks. Even though Kazial et al. (2001) were unable to identify sonar call descriptor variables that indicate sex in *E. fuscus*, playbacks showed that females discriminated between the calls of male and female conspecifics (Kazial and Masters 2004). The obvious next step is to examine behavioral responses of bats to call variation.

The potential for communication by bats is in need of additional exploration. Questions remain about the extent to which sonar calls are involved in communication in comparison with the role of social calls and other signaling modalities. Harmonics should be assessed for a role in communication because they could be perceived by bats crawling in roosts. The question of the transferability of information coded in sonar call features to more than 1 calling situation remains unanswered. In addition to an examination of the variation and potential for information carried in sonar calls, testing bats perceptive ability within social contexts for using this information is needed.

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APPENDIX IDescription of variables used to evaluate the information content of calls of little brown bats (*Myotis lucifugus*).

Variable code (units)	Description ^a
duration (ms)	Duration of call
t50 (ms)	Time to reach middle frequency of call
h1start (kHz)	Starting frequency
h1mid (kHz)	Middle frequency
h1end (kHz)	Ending frequency
h1maxf (kHz)	Maximum frequency
l3fh1 (kHz)	Frequency of call spectrum at 3-dB bandwidth lower bound
u3fh1 (kHz)	Frequency of call spectrum at 3-dB bandwidth upper bound
l10fh1 (kHz)	Frequency of call spectrum at 10-dB bandwidth lower bound
u10fh1 (kHz)	Frequency of call spectrum at 10-dB bandwidth upper bound
pow3f0 (kHz) ^b	Starting frequency at $t = 0$ (used in power-3 sweep function)
pow3f1 (kHz) ^b	Asymptotic frequency approached as t becomes large (used in power-3 sweep function)
pow3a ^b	Decay constant used in power-3 sweep function
pow3c ^b	Decay constant used in power-3 sweep function
curvature ^c	Curvature of frequency sweep from starting to ending

^a Frequency measurements were taken on the fundamental.^b Obtained from Parsons and Jones (2000:2644, equation 8).^c Modified from Boonman and Schnitzler (2005).