



Discrimination of infant isolation calls by female greater spear-nosed bats, *Phyllostomus hastatus*

KIRSTEN M. BOHN*, GERALD S. WILKINSON* & CYNTHIA F. MOSS†

*Department of Biology, University of Maryland

†Department of Psychology, University of Maryland

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In colonial species, recognition of offspring should be under strong selection. For accurate identification to occur, offspring must produce individually distinctive signals and parents must be able to discriminate between signals. Female greater spear-nosed bats roost in stable social groups and use infant vocalizations, termed isolation calls, to locate and identify their young. In this study, we investigated both the production and perception of isolation calls in *P. hastatus*. First, we measured acoustic features of calls and found that, after controlling for ontogenetic effects, sufficient variation exists between pups for isolation calls to function as individual signatures. Moreover, calls of pups from the same social group were more similar in spectral and spectrotemporal features than were calls of pups from different social groups, indicating that these features are probably heritable. We used psychoacoustic experiments in the laboratory to determine whether adult females could discriminate between calls from pups in the same or different social group. Females discriminated between pups when faced with a template-matching task and their performance was correlated with the salience of spectral and spectrotemporal features. We found no difference in performance when females had to discriminate between pups from the same and different social groups. These results indicate that females should be able to accurately identify their young using isolation calls.

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The process of identifying offspring is expected to be under strong selection to ensure that parental care is confined to related individuals. For animals living in large groups, the probability of confusing related offspring with others can be high. Consequently, parent–offspring recognition systems have evolved in many colonial species (e.g. Trillmich 1981; Stoddard & Beecher 1983). For bats, offspring recognition can be a particularly vital but difficult task because mothers typically leave their pups behind in large colonies. Accurate offspring recognition requires fulfilment of two criteria: (1) offspring must produce individually distinctive signals and (2) parents must be able to discriminate

between these signals (Beecher 1982). Here, we examine these two components of parent–offspring recognition in greater spear-nosed bats.

In Trinidad, West Indies, *P. hastatus* form stable social groups of 8–40 adult females attended by one adult male (McCracken & Bradbury 1981). Unlike most other group-living mammals, females are typically unrelated to group members (McCracken & Bradbury 1981). Males have high reproductive control over harems (McCracken & Bradbury 1977), and socially mediated birth synchrony occurs within groups (Porter & Wilkinson 2001). Consequently, pups reside in clusters of predominantly paternal half-siblings of similar age from a single social group.

Most infant bats, including *P. hastatus*, produce frequency-modulated multiharmonic vocalizations known as isolation calls, which are used in parent–offspring recognition (Gould et al. 1973). In some species, isolation calls contain enough information to serve as individual signatures (e.g. Thomson et al. 1985; Gelfand &

Correspondence and present address: K. M. Bohn, Section of Neurobiology, University of Texas, Austin, TX 78712, U.S.A. (email: kbohn@mail.utexas.edu). G. S. Wilkinson is at the Department of Biology, University of Maryland, College Park, MD 20742-4415, U.S.A. C. F. Moss is at the Department of Psychology, University of Maryland, College Park, MD 20742-4411, U.S.A.

McCracken 1986; Scherrer & Wilkinson 1993) and often change as pups age (reviewed in Altringham & Fenton 2003). In *P. hastatus*, females sometimes visit and retrieve group members' pups that have fallen to the cave floor (K. M. Bohn & G. S. Wilkinson, personal observation). This observation raises the possibility that isolation calls may not contain sufficient information for a female to recognize her pup from others in her social group.

Females probably use a template-matching mechanism (Lacy & Sherman 1983) to recognize their own pups' calls. By template matching, females compare incoming isolation calls to a signal template represented in memory. Offspring recognition should then depend on a female's ability to form a template and discriminate among offspring signals. Psychoacoustic studies suggest that bats should be able to discriminate between isolation calls. For example, adult female *Phyllostomus discolor* can discriminate among frequency-modulated sounds similar to isolation calls (Esser & Lud 1997), and other studies have demonstrated maternal recognition of pup calls (Balcombe 1990; de Fanis & Jones 1996). No study has yet examined the acoustic features used by mothers to recognize their pups' calls.

Here we present a comprehensive study of parent-offspring recognition in *P. hastatus*. First, we investigated signal production. We examined how isolation calls change with pup age and then we controlled for age effects to assess how acoustic features vary among bats in different caves or social groups. Next, we used psychoacoustic experiments in the laboratory to examine signal perception. We tested whether females can discriminate between pup isolation calls in a template-matching procedure, which mimics the perceptual task faced by females in the wild. We then determined which acoustic features were correlated with female performance and compared the results with the analysis of isolation call variation to infer potentially salient cues. Finally, in a second perceptual experiment, we tested whether call similarity within social groups affects female discrimination of individual pups.

METHODS

Isolation Call Recordings

We recorded isolation calls from infant *P. hastatus* at Guanapo and Tamana caves (McCracken & Bradbury 1981) in Trinidad, West Indies, in April and May, 2002 and 2004. In the evening, we captured nonvolant pups by hand for measuring and recording and then returned them to their crèche before the adult bats returned from foraging. We banded each pup with numbered stainless steel bands (National Band and Tag, Newport, Kentucky, U.S.A.) and used callipers to measure forearm length (FA) to a tenth of a millimetre. We estimated age in days as $0.77 \times FA - 24.6$ (Stern & Kunz 1998). We recorded spontaneously produced isolation calls from pups that were placed in a cardboard box (approximately $0.75 \times 0.5 \times 0.5$ m) lined with acoustic foam (Sonex, Seattle, Washington, U.S.A.). Calls were digitized at a sample rate of 250 kHz into Bat Sound Pro (Pettersson Elektronik, Uppsala, Sweden) on a laptop computer

equipped with a data acquisition card (INEES, Daq508, 12 bits) using a bat detector as a high-frequency microphone (Ultra Sound Advice, S-25) and an external amplifier (SHURE, FP-2).

Infant *P. hastatus* produce multiple types of isolation calls composed of different numbers of notes. Double-note calls are the simplest and most frequently produced calls in *P. hastatus* and in many other species of bats (Gould et al. 1973). For both simplicity and consistency, we used double-note calls for all analyses and psychoacoustic experiments (see below).

Isolation Call Measurements

We used SIGNAL (version 3.0, Engineering Design, Belmont, Massachusetts, U.S.A.) to band-pass filter isolation calls between 5 kHz and 85 kHz, normalize amplitudes by dividing each signal by its peak amplitude, and measure 12 call features (Fig. 1). We measured three temporal features, eight spectral features and one spectrotemporal feature, the relative location of the frequency minimum of the first note (MNT1) using the formula $MNT1 = (\text{end time} - \text{time of minimum frequency})/\text{end time}$. In addition to these measurements, for perceptual experiments, we used SIGNAL to calculate spectral

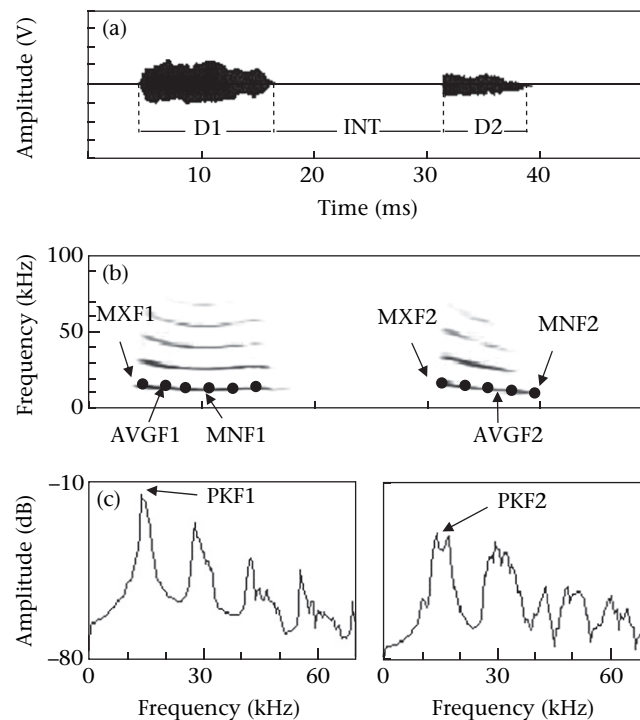


Figure 1. (a) Oscillogram, (b) spectrogram and (c) power spectra of a typical double-note isolation call. Measurements taken are first note duration (D1), second note duration (D2), interval between notes (INT), average frequency of the first (AVGF1) and second (AVGF2) notes, minimum frequency of the first (MNF1) and second (MNF2) notes, maximum frequency of the first (MXF1) and second (MXF2) notes, and peak frequency of the first (PKF1) and second (PKF2) notes. Large dots in (b) represent spectral contours, which were averaged over the duration of the calls to calculate AVGF1 and AVGF2. Spectral contours were calculated by determining the peak frequency at each point in time of the call (Beeman 1996).

cross-correlations between call pairs for each note (COR1, COR2). The spectral cross-correlation procedure slides two spectral contours across each other and calculates the maximum correlation between the two signals (Beeman 1996). Spectrograms were constructed using a transform length of 512 points, resulting in a temporal resolution of 2 ms and a frequency resolution of 500 Hz. The time between transforms was set at 0.15 ms, so that all signals had the same number of transformations per second but different total numbers of transforms.

Subjects

For the psychoacoustic experiments we used five (experiment 1) and four (experiment 2) adult female *P. hastatus*. The subjects were captured in Tamana Cave in 1993, except for one bat that was born in captivity in 1996. During the study, bats were housed in a cage (3.3 × 2.7 × 2.4 m) kept in a room maintained on an 8:16 h light:dark cycle at approximately 24°C and 30% RH. Bat weights were maintained between 60 and 70 g during experiments (minimum of 90% free-fed body weight) by feeding them a diet of fruit and marmoset food (Premium Nutritional Products, Mission, Kansas, U.S.A.). During experiments bats were rewarded with mealworms (*Tenebrio molitor*) and fruit.

Psychoacoustic Apparatus and Procedures

All experiments were conducted in a single-walled acoustic chamber (Industrial Acoustics Company, Inc., Bronx, New York, U.S.A.) lined with acoustic foam (Sonex). Bats were trained and tested using a V-shaped platform enclosed in a hardware-cloth cage (Bohn et al. 2004). A modified Go/No-go procedure was used for both experiments (Suthers & Summers 1980). During experimental trials, bats were trained to either (1) stay at the top of the platform ('No-go' trial) or (2) run to the end of the right arm of the platform ('Go' trial). Bats were rewarded at the starting position for correctly staying during 'No-go' trials and rewarded at the end of the right arm of the platform for correctly responding during 'Go' trials. For both experiments (see below), bat performance was assessed by two measurements: percentage of responses that were correct and response latency. Response latency was measured as the difference between the onset of a stimulus train and a bat's departure time using a real-time processor (Tucker Davis Technologies, RP 2.1, Alachua, Florida, U.S.A.). Bat departure times were recorded automatically using an infrared light-emitting diode (LED) and matching photosensor located at the top of the platform and triggered whenever the bat left the starting position.

Playback Stimuli and Calibration

Isolation calls were played directly from a computer equipped with SIGNAL and a 250-kHz DA board (Data Translation, DT5727), band-pass filtered at 5 and 85 kHz (Krohn-Hite, 3550), amplified (Harman Kardon, AVR 100), and sent to a loudspeaker (Pioneer, PT-R), which was 1 m from the subject's starting position. Stimuli

were recorded daily onto a laptop computer and inspected for distortion. We recorded a calibration tone daily with a piston phone (Brüel & Kjaer type 4231) and adjusted amplitudes so that all stimuli were 75 dB SPL at the bats' starting position. For both psychoacoustic experiments, stimuli presentations were determined from measurements of natural calling behaviour. Each call set was separated by 900 ms of silence and contained three calls, each separated by 60 ms of silence (Figs 2, 3).

Experiment 1: Pup Discrimination

The goals of this experiment were to determine whether bats could discriminate between isolation calls produced by different pups and to identify the acoustic features that

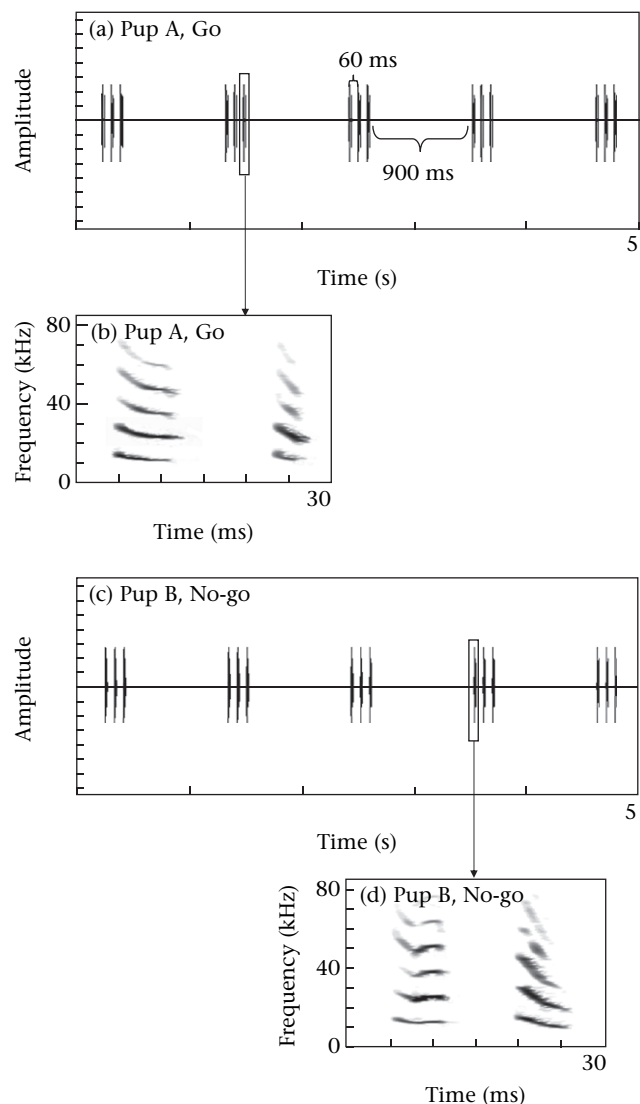


Figure 2. Stimuli used in experiment 1 for pup discrimination. (a) 'Go' trial of five call sets and (b) spectrogram of a single isolation call from one pup, 'Pup A'. (c) 'No-go' trial of five call sets and (d) spectrogram of a single isolation call from one pup, Pup B. In (a) and (d), calls were separated by 60 ms and sets were separated by 900 ms.

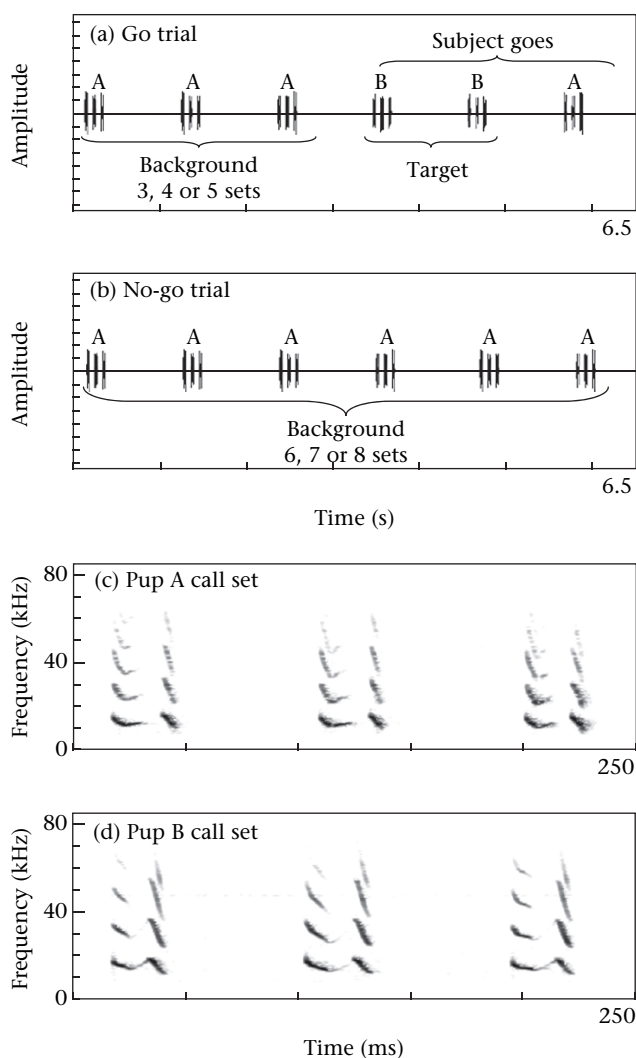


Figure 3. Stimuli used in experiment 2 for group discrimination. (a) 'Go' trials consisted of three, four or five background call sets from one pup (Pup A), two calls from a target pup (Pup B), and one call from the first pup (Pup A). Subjects were rewarded for responding during the last three call sets. (b) 'No-go' trials consisted of six, seven or eight call sets. Subjects were rewarded for staying during the entire trial. (c) Example call set for Pup A. (d) Example call set for Pup B.

were correlated with performance. Only calls recorded from Guanapo pups in 2002 were used in this experiment. We selected calls from 16 pup pairs at random without replacement, but the difference in age between pups was no greater than 2 days for each pair. Two isolation calls were used for each pup and presented in random order. For each pup pair, the calls from one pup were arbitrarily assigned as a 'No-go' stimulus and the other was a 'Go' stimulus (Fig. 2). This procedure required signal recognition by the subjects because females had to store calls in memory to determine which call was associated with which behaviour. Before testing, bats were trained using the same procedure on calls from two pairs of pups whose ages differed by at least 10 days and whose calls were the most different in temporal, spectral and spectrotemporal features. Calls used for training were not used in experimental trials.

Each testing day consisted of 30 trials per subject. Trials were randomized but no more than three trials of one type occurred consecutively (Gellerman 1933). For the first five trials of each day, we presented mealworms at the end of the platform ('Go' stimulus) or at the top of the platform ('No-go' stimulus) to show the subjects the correct responses. For the remaining 25 trials, bats were only rewarded at the end of trials if they performed correctly. Responses and response latencies were only recorded for the last 25 trials. Each stimulus pair was used for 2 days of testing with 1 day without testing between pairs. For each 2-day testing period, the day with the best performance (the highest percentage of correct responses) for each bat was used for analyses. Equipment malfunction resulted in the loss of five response latency estimates.

Experiment 2: Group Discrimination

The goal of this experiment was to determine whether social group membership affected discrimination of pup isolation calls. For this experiment we used a modified Alternating Sound Task (Dooling & Okanoya 1995). During 'Go' trials bats were trained to stay at the starting position while call sets from one pup were played (the background) and move to the end of the ramp when call sets from a different pup (the target) were alternated with the background (Fig. 3). During 'No-go' trials, only the background pup was played. Using this procedure, all stimuli were presented in each test session, which controlled for day-to-day variation in the subjects' performance.

Bats were trained using calls from two pairs of differently aged pups from different social groups. Calls used for training were not used in experimental trials. For testing, calls from 12 pups served as background and were each paired with calls from two target pups: one pup from the same group as the background pup and one pup from a different group than the background pup. Pairs were selected at random without replacement as long as the difference in ages of the background and target pups was no greater than 2 days. We incorporated within-pup variation in double-note calls by randomly combining four to five calls from each pup into the stimulus sets daily.

Bats were tested using calls from all 24 'Go' pairs and 12 'No-go' pairs on each of 9 days. All pairs were presented nine times, three times at each repetition level or number of initial background sets. The number of call sets was randomized across days. 'No-go' and 'Go' trials were randomized within days but no more than five trials of one type could occur consecutively. If a bat made an early start during a 'Go' trial, that stimulus was added to the end of the day's trials to ensure that a clear response was recorded for each day. For response latencies, one data point was missing because one bat did not respond correctly during any of the three trials.

Statistical Analyses

Variables were examined for normality using normal-probability plots and Shapiro-Wilk's tests. Any variable

deviating from normality was transformed to satisfy assumptions for parametric tests (see Appendix, Table A1 for a summary of transformations used). Statistical significance was evaluated using two-tailed tests with $\alpha = 0.05$. Analyses were performed in either JMP 5.0 or SAS 9.1 (SAS Institute, Cary, North Carolina, U.S.A.).

For analysis of isolation call variation, we used pups if we had at least four double-note calls with high signal-to-noise ratio and at least four pups from a social group. First, we used the average of each measurement for each pup and tested whether age, sex, or age by sex interactions affected isolation call features using a multivariate analysis of variance (MANOVA). Next, to reduce the number of variables and to control for collinearity, we used a principal component analysis (PCA) and varimax factor rotation on age-corrected residuals for the measurements on each call. Factors with eigenvalues greater than 1.0 were used in a MANOVA, with cave, group and pup as nested random effects (PROC GLM, SAS Institute). We used restricted maximum likelihood to calculate variance component estimates (VCEs); that is, the proportion of variation in the PCA factors explained at each nesting level. We then calculated repeatability as follows: $VCE_{\text{pup}} / (VCE_{\text{pup}} + VCE_{\text{call}})$.

For experiment 1, we calculated the average daily percentage of correct response for each bat, compared these values with confidence intervals for 'guessing' (50%), and conducted binomial tests on the pooled responses to all 400 trials. For each call feature, we calculated the absolute difference between call pairs. PCA and varimax factor rotations were calculated on call differences. To test whether call differences correlated with performance, we used a logistic regression (PROC GLIMMIX, SAS) to examine response outcome (correct or incorrect) and an ANCOVA to analyse response latency for factors with eigenvalues greater than one. For both analyses, we tested a full model, with bat as a random factor, order of pair presentation as a repeated measure, extracted factors as predictors and all bat*factor interactions. The repeated measures variance components did not differ from zero, and all interaction effects were nonsignificant. Thus, these terms were removed from the final model.

For experiment 2, we calculated the percentage of correct responses for each individual. In this experiment, unlike in experiment 1, the probability of responding correctly by chance was not 50%. In order to respond correctly, the bat had to depart during 'Go' trials within acceptable time windows depending on the number of initial background repetitions. For each of the possible time windows, we calculated the proportion of trials that would result in a correct response if the bat had departed. These calculations resulted in a chance probability of 45% correct.

Because there was only one 'No-go' stimulus for both the within- and between-social group pairs, we only used 'Go' trials for testing whether group membership affected discrimination. For 'Go' trials, we counted the number of correct responses and the average response latency for each bat-stimulus-repetition combination. We then used a logistic regression (PROC GLIMMIX, SAS) on response

outcome and an ANOVA on response latency. For both variables, a full model was first analysed with pup pair as a random block, number of background repetitions, group (same or different), and number of background repetitions*group interaction as fixed effects, and bat and bat*group interaction as random effects. For both variables, interaction terms were nonsignificant and consequently removed from the final model.

RESULTS

Isolation Call Variation

Pup age had a significant effect on call features (MANOVA: Wilk's lambda = 0.477, $df = 12, 47, P < 0.0001$). Call features were not affected by sex and there was no interaction between sex and age (Wilk's lambda = 0.894 and 0.897, respectively, $df = 12, 47, P = 0.92$ and 0.93 , respectively). Age had a negative effect on temporal features (D1 and D2) and a positive effect on spectral features (PKF1, PKF2, MXF2; Table 1). Spectrotemporal features were not affected by age (Fig. 4).

Residuals from regressions of call features on age were used for the remaining analyses on isolation call variation. Factor analysis produced four factors, which together explained 81% of the variation in the 12 call measurements. Spectral features loaded onto the first factor, D2 loaded onto the second factor, MNT1 and INT loaded onto the third factor and D1 loaded onto the fourth factor (Table 2). There was significant variation among pups (Wilk's lambda = 0.01, $df = 220, 973, P < 0.0001$) and social groups (Wilk's lambda = 0.49, $df = 24, 183, P = 0.02$) but not among caves (Wilk's lambda = 0.92, $df = 4, 3, P = 0.99$). While all factors contributed to differences between pups, only the first factor (spectral features) and the third factor (spectrotemporal features) contributed to differences between-social groups (Table 3).

Table 1. Results of regressions of age on average call variables for 63 greater spear-nosed bat pups

Variable	F	Intercept	Slope
Temporal			
D1	6.91*	13.7	-0.96
D2	4.66*	0.90	-0.03
INT	3.75	3.59	-0.16
Spectral note 1			
MNF1	1.29	133 000	4240
MXF1	3.68	14.1	0.28
AVGF1	2.41	151 000	5800
PKF1	15.60***	11.3	0.64
Spectral note 2			
MNF2	2.12	0.10	0.001
MXF2	5.12*	220 000	9990
AVGF2	3.19	157 000	6900
PKF2	8.65**	12.5	0.54
Spectrotemporal			
MNT1	0.32	0.46	0.020

Intercepts and slopes from transformed variables (see Appendix). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

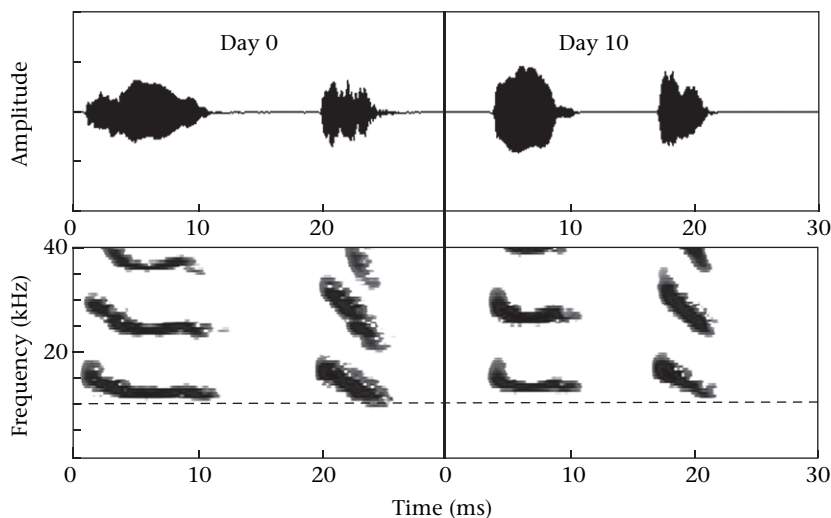


Figure 4. Oscillogram (top) and spectrogram (bottom) for isolation calls from a pup just after parturition and at 10 days of age. Note that the spectral shape remains constant while duration decreases (first note durations = 10 and 6 ms) and frequency increases (first note peak frequencies = 11.9 and 14.1 kHz).

Experiment 1: Pup Discrimination

On average, the five bats performed between 78% and 83% correct responses per day, well above the 95% confidence interval for guessing (Fig. 5a). Across all pairs of call presentations, all five bats performed above 50% (binomial tests: $N = 400$, all $P < 0.0001$). A PCA on acoustic differences between call pairs resulted in four rotated factors, which together explained 79% of the variation. The first factor was correlated with spectral differences, the second factor with spectral and spectrotemporal differences, the third factor with temporal differences, and the fourth factor with differences in the spectrotemporal feature MNT1 (Table 4).

Bats did not differ in the number of correct responses (logistic regression: $\chi^2_4 = 6.95$, $P = 0.14$). Only the second

rotated factor predicted whether or not bats responded correctly (Table 5). Response latency did differ between bats (ANCOVA: $F_{4,67} = 3.22$, $P = 0.02$). Only the first factor had a significant effect on response latency (Table 5). Thus, spectral and spectrotemporal features correlated with female discrimination of isolation calls (Tables 4, 5).

Experiment 2: Group Discrimination

Each of the four bats averaged close to 80% correct responses each test day (Fig. 5b). Across all trials their performance was greater than the guessing value of 45% (binomial test: $N = 324$, all $P < 0.0001$). The proportion of correct responses differed across bats ($\chi^2_3 = 13.73$, $P = 0.003$) and number of background repetitions ($\chi^2_2 = 12.76$, $P = 0.002$). We detected no difference in the proportion of 'Go' responses (percentage correct) when bats listened to calls of pups from the same social group and those from a different group ($\chi^2_1 = 1.37$, $P = 0.24$; Fig. 6a).

Table 2. Factor loadings after PCA and varimax rotation of isolation calls for analysis of call variation in greater spear-nosed bats

Variable	Factor 1	Factor 2	Factor 3	Factor 4
Temporal				
D1	-0.07	0.02	0.12	0.93
D2	-0.03	-0.92	-0.03	0.01
INT	0.01	0.48	-0.69	0.39
Spectral note 1				
MNF1	0.82	-0.26	-0.13	-0.21
MXF1	0.76	-0.10	-0.16	0.49
AVGF1	0.90	-0.28	-0.19	-0.10
PKF1	0.77	-0.08	-0.34	0.03
Spectral note 2				
MNF2	0.79	0.35	0.06	-0.17
MXF2	0.87	-0.03	0.05	0.16
AVGF2	0.90	0.25	0.08	-0.02
PKF2	0.77	0.20	0.07	-0.03
Spectrotemporal				
MNT1	-0.07	0.17	0.83	0.28

Call features with loadings of 0.70 or greater in bold.

Table 3. Univariate F tests from nested ANOVAs on PCA factors from Table 2

Estimate	Group		Pup		Call	Repeatability
	F	VCE†	F	VCE†	VCE†	
N		8		63	309	
Factor 1	3.12*	0.17	19.68***	0.66	0.17	0.80
Factor 2	0.94	0.00	8.58***	0.61	0.39	0.61
Factor 3	2.59*	0.10	7.37***	0.50	0.40	0.56
Factor 4	0.27	0.00	10.15***	0.63	0.37	0.63

* $P < 0.05$; *** $P < 0.001$.

†Variance component estimates (VCE) show the proportion of variation explained by differences between groups within caves, pups within groups, and calls within pups for greater spear-nosed bats.

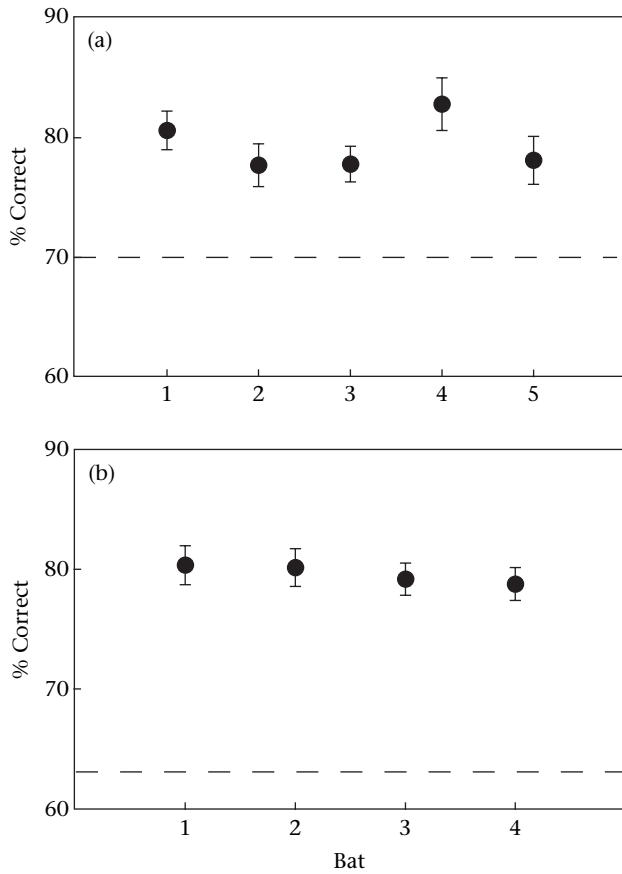


Figure 5. Mean \pm SE percentage of correct responses by bat and day for (a) pup discrimination (experiment 1, $N = 16$ days) and (b) group discrimination (experiment 2, $N = 9$ days). Dashed lines indicate 95% upper confidence intervals for guessing (50% experiment 1, 45% experiment 2).

Table 4. Factor loadings after PCA and varimax rotation on isolation call variables measured in experiment 1

Variable	Factor 1	Factor 2	Factor 3	Factor 4
Temporal				
D1	-0.31	0.59	0.57	0.01
D2	-0.19	0.18	0.73	0.34
INT	0.15	-0.20	0.70	-0.48
Spectral note 1				
MNF1	-0.21	-0.90	0.06	0.01
MXF1	-0.86	-0.06	0.04	-0.02
AVGF1	-0.57	-0.74	0.06	-0.05
PKF1	-0.81	-0.18	-0.28	-0.27
Spectral note 2				
MNF2	-0.70	0.24	0.26	0.39
MXF2	-0.53	-0.60	-0.20	-0.18
AVGF2	-0.69	-0.25	0.32	0.30
PKF2	-0.62	-0.45	0.28	0.26
Spectrotemporal				
MNT1	0.00	-0.13	0.00	0.86
COR1	0.08	-0.79	-0.38	0.22
COR2	0.01	-0.12	-0.92	0.01

Call features with loadings of 0.70 or greater in bold.

Table 5. Results from a logistic regression on the percentage of correct responses and from an ANOVA on response latencies in experiment 1

Variable	Factor description	Response outcome†	Response latency‡
Factor 1	Spectral	0.43	5.09*
Factor 2	Spectral and Spectrotemporal	7.01**	0.85
Factor 3	Temporal and Spectrotemporal	0.35	0.53
Factor 4	Spectrotemporal	1.29	0.07

* $P < 0.05$; ** $P < 0.01$.

† χ^2 , $df = 1$.

‡ F statistic, $df = 1, 67$.

Response latency also differed between bats ($F_{3,269} = 7.79$, $P < 0.0001$), and between the number of background repetitions ($F_{2, 269} = 5.72$, $P = 0.004$). We did not detect a difference in response latency between calls of pups from the same and different social groups ($F_{1, 269} = 3.31$, $P = 0.07$; Fig. 6b); however, response latency tended to be shorter in response to calls from different social groups.

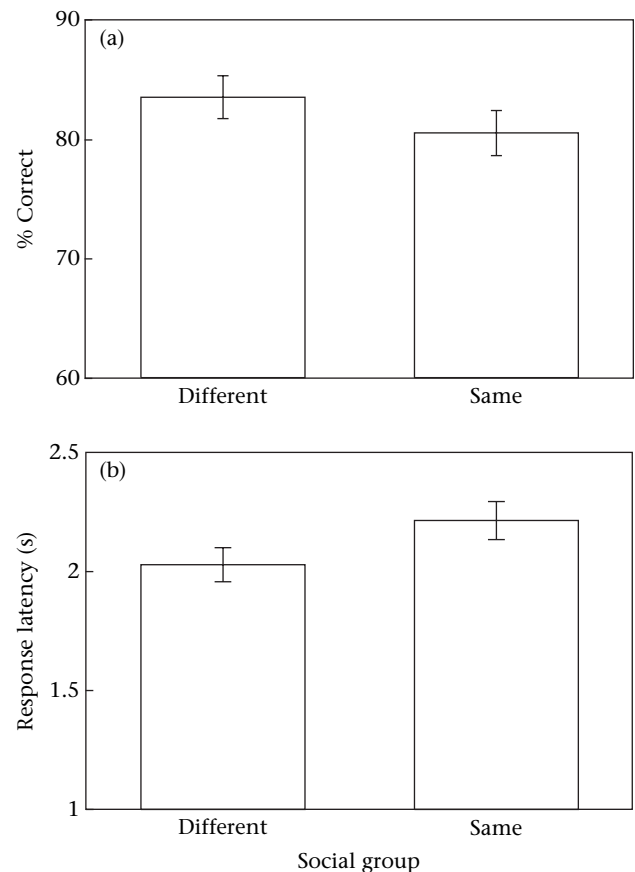


Figure 6. Group discrimination (experiment 2) results: (a) mean \pm SE percentage of correct responses and (b) mean \pm SE latency to respond to calls of pup pairs from the same and different social groups.

DISCUSSION

Isolation Call Variation

In *P. hastatus*, isolation calls become shorter and increase in frequency with age. Such age-related changes probably result from vocal tract development (Suthers & Fattu 1973; Gould 1975). Nevertheless, the spectrotemporal shape of each pup's isolation call appears to remain constant for at least the first week or two after parturition (Fig. 4), as has been noted for other species of bats (Scherrer & Wilkinson 1993).

After removing age effects, isolation calls differed significantly between pups and social groups according to multivariate analyses. Among pups, all four acoustic factors were significant and explained between 50% and 66% of the variation in isolation calls. The factor associated with spectral features showed the greatest variation between pups and the least variation within pups (i.e. had the highest repeatability; Table 3). Among social groups, only nontemporal factors were significant, and explained 10–17% of the variation. Assuming that all pups within a group share a common father and have unrelated mothers (i.e. are paternal half-siblings; McCracken & Bradbury 1977), the heritability (percentage of variation attributable to additive genetic effects) of call components can be estimated as four times the proportion of variance explained by social group. Thus, our analyses indicate that the first and third factors (i.e. spectral and spectrotemporal variables) have significant heritabilities of 0.68 and 0.40, respectively. The existence of heritable isolation calls in *P. hastatus*, as in other bat species (Scherrer & Wilkinson 1993), could facilitate individual and, potentially, sib-group recognition of pups by females.

Experiment 1: Isolation Call Discrimination

The bats successfully performed the first psychoacoustic experiment, which was designed to test a template-matching process. Unlike many psychoacoustic procedures, in which subjects only need to detect a difference in stimuli, in this experiment the bats had to store isolation calls in memory. The bats could have performed this task in two ways. Females could have compared calls from a trial with calls that they had learned and stored in memory during the first five trials. Alternatively, females could have compared calls from a trial with those of the previous trial. In either case they had to compare a new call with one stored in memory.

Although we did not directly test which acoustic features are used for isolation call recognition, positive correlations between acoustic dissimilarity and discrimination performance indicate that spectral and spectrotemporal features were important in discrimination. These findings were consistent across bats, as shown by the lack of statistical interactions between bats and extracted factors for percentage of correct responses and response latency data. On the other hand, temporal features of pup isolation calls were not correlated with performance, although they should carry useful information for identification. One possible explanation for this result is that

isolation calls are given at high signal amplitudes in roosting sites, such as caves or hollow trees, where temporal features, such as call duration, probably suffer distortions from reverberation (Bradbury & Vehrencamp 1998). Thus, selection may have acted on the auditory system for enhanced discrimination of call features that suffer the least distortion during transmission.

Experiment 2: Group Discrimination

The multivariate analysis of isolation call features revealed that groupmates produce calls with more similar spectral and spectrotemporal features than do nongroupmates. Experiment 1 showed that bats use these same features to discriminate pups. For experiment 2, we then examined whether this hierarchical signature system affected female discrimination of pups from the same or different group. For this experiment we incorporated between four and five isolation calls per pup. Consequently, bats had to discriminate between individuals even though calls varied within individuals. We found that group-level similarity did not affect discrimination ability, as measured by the proportion of correct responses. This result was consistent across bats, as shown by all bats performing above chance levels and the lack of statistical interactions between bats and social group. Our results indicate that although pups from the same social group have similar calls and call discrimination is correlated with call similarity, on average, calls differ enough between pups from the same social group for females to discriminate between them. Moreover, while the difference in the percentage of correct responses to same and different social group calls was not statistically significant, performance tended to be higher for calls from different social groups, suggesting that the percentage of correct responses may not be sufficiently sensitive to detect differences in perceptual encoding of social signals by bats performing in this task. Response latency appears to be a more sensitive measure of perceptual discrimination of calls from same and different social groups; however, this response measure yielded marginally nonsignificant ($P = 0.07$) differences.

Our findings from the group discrimination experiment demonstrate that *P. hastatus* can discriminate well among isolation calls of individuals from the same and different social groups but raise the possibility that group similarity may affect isolation call recognition in a natural acoustic environment that is noisier than our anechoic chamber. On the other hand, the discrimination tasks in our experiments were more difficult than those faced by females in the wild because we only used double-note calls, the simplest of isolation calls. Pups also produce isolation calls with three or more notes, which probably provide more information for discrimination. Furthermore, we confined age differences between pups to 2 days, which is at the lower end of the range for age differences within social groups in the wild (Porter & Wilkinson 2001). Thus, in this experiment, the bats were probably faced with more similar calls than what would occur naturally. In this context, it is worth noting that, based on critical ratio

estimates, *P. hastatus* shows the highest frequency selectivity at the fundamental frequency of isolation calls (Bohn et al. 2004), which should enhance discrimination of spectral features of isolation calls. If *P. hastatus* females use spectral and spectrotemporal features from multiple call types, they should be able to differentiate between most, if not all, pup calls in the wild.

The high performance in isolation call discrimination found in *P. hastatus* is consistent with strong selection for accurate offspring recognition. *Phyllostomus hastatus* give birth to only one pup per year, and infant mortality is high (Stern & Kunz 1998). Parental care is extensive because pups are altricial at birth and they do not begin to fly until they are at least 6 weeks old (Stern & Kunz 1998). Traits that increase the likelihood of infant survival will strongly impact female reproductive success. Isolation calls are probably crucial to pup survival in *P. hastatus* because nonvolant pups produce these calls when they fall from roost sites (K. M. Bohn & G. S. Wilkinson, personal observation), and these isolation calls attract adult females, who bring the pups back to their roost sites. If nonvolant pups are not retrieved, they do not survive. Presumably, females accrue some energetic costs by responding to calls of nonfilial young because this may reduce care directed to filial young. Based on the evidence presented in this study, mistaken identity is unlikely to be a plausible explanation for recurrent cases of female *P. hastatus* remaining near or retrieving groupmates' fallen pups (K. M. Bohn & G. S. Wilkinson, personal observation).

Parent–Offspring Recognition in Mammals

Infant vocalizations that are used in parent–offspring communication are common in mammals and often contain enough information for individual identification (e.g. rodents: Tokumaru et al. 2004; seals: Job et al. 1995; primates: Hammerschmidt & Todt 1995; ungulates: Terrazas et al. 2003; carnivores: Sieber 1986). Although maternal recognition has been demonstrated in some species (e.g. seals: Insley 2001; pigs: Illmann et al. 2002; primates: Symmes & Biben 1985), a lack of evidence for maternal recognition has also been reported even when vocalizations contain signature information (seals: Job et al. 1995; McCulloch et al. 1999). In these cases it was impossible to determine whether similar responses to offspring and nonfilial young were the result of perceptual or behavioural mechanisms. Here, we isolated the perceptual aspect of offspring recognition by performing experiments out of a natural context.

Our finding that spectral rather than temporal acoustic cues appear most important for offspring recognition may be part of a larger mammalian pattern. In many mammals, temporal features of infant vocalizations have greater intraindividual variation than do spectral features and are therefore less informative for recognition (bats: Gelfand & McCracken 1986; seals: Insley 1992; primates: Masataka & Symmes 1986). Unfortunately, few studies have examined the ability of female mammals to discriminate among infants using vocalizations; although, spectral and

spectrotemporal cues were found to be important in offspring recognition in seals (Charrier et al. 2002). Given the widespread use of infant vocalizations in mammals, perception of these acoustic signals deserves further study.

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Appendix

Table A1. Data transformations for the multivariate analysis on isolation call variation in experiment 1

Variable	Call variation	Experiment 1
D1	None	Log x
D2	Log x	None
INT	\sqrt{x}	Log x
MNF1	x^2	Log x
MXF1	x^2	\sqrt{x}
AVGF1	x^2	Log x
PKF1	None	None
MNF2	\sqrt{x}	Log x
MXF2	x^2	\sqrt{x}
AVGF2	x^2	x^2
PKF2	None	None
MNT1	Arcsine \sqrt{x}	Arcsine \sqrt{x}
COR1	NA	Arcsine \sqrt{x}
COR2	NA	None
Age	\sqrt{x}	NA

'None' indicates that the data were normally distributed. 'NA' indicates that the variable was not applicable to the analysis.