



## Nonmutual vocal mother–pup recognition in the greater sac-winged bat

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We investigated the acoustical component of the recognition process leading to successful mother–pup reunions in the greater sac-winged bat, *Saccopteryx bilineata*, using both a statistical approach and playback experiments. Statistical evidence for individual distinctiveness was found in the isolation calls uttered by pups and, to a weaker degree, in the echolocation pulse trains emitted by mothers. In contrast to other bat species, isolation calls of *S. bilineata* pups were complex and multisyllabic, with most of the vocal signature information encoded in the composite syllables at the end of calls. Playback experiments with free-living bats revealed that mothers were able to discriminate between their own pup and an alien young on the basis of isolation calls alone, which confirms the results of the acoustical analysis on vocal signatures in isolation calls. Pups, on the other hand, indiscriminately vocalized in response to echolocation pulse trains from their own and alien mothers, rendering the mother–pup recognition process unidirectional. The one-sidedness of the vocal recognition process in *S. bilineata* as well as in other bat species might be explained by a lack of selection pressures that shape mutual vocal parent–offspring recognition in other species of mammals and birds. To our knowledge, this study is the first in which playbacks were used to elicit antiphonal calling behaviour between bat mothers and pups experimentally. We argue that vocal responses to playback stimuli are a more feasible and reliable response measure for conducting mother–pup recognition playbacks in bats than the phonotaxis behaviour used in the past.

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Evolutionary theory predicts that in gregariously breeding species it is vital for parents to discriminate between their own and alien offspring to direct parental care to their own descendants (Hepper 1986; Beecher 1991). It may be advantageous for both parents and offspring if the recognition process is mutual instead of unidirectional. If offspring can recognize their parents as well as vice versa, parent–offspring reunions could be facilitated which, in turn, would have advantages for both sides: misdirected parental care would be less likely, as would aggressive reactions of adults to the solicitations from alien young (Beer 1970; Pierotti & Murphy 1987). Mutual parent–offspring recognition has been shown for several birds (e.g. Falls 1982) and mammals (e.g. Fischer 2004) but in general unidirectional recognition is more prevalent (Insley 2001).

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Parents should have a vital interest in avoiding confusion over reproductive investment (Halliday 1983) but for offspring it could be beneficial to exploit parental care and try to nurse indiscriminately if such a behaviour is not punished severely (Trivers 1974; Porter 1987). Mutual recognition is likely to occur only when the recognition task is too complicated to be unidirectional (Insley 2001). Selection pressures driving mutual recognition include coloniality (Beecher 1990), offspring mobility (Insley et al. 2003) and time span of parental separation (Insley 1992). In the absence of these selection pressures unidirectionality prevails, resulting in a biased recognition task towards either the parents (e.g. Illmann et al. 2002) or the offspring (e.g. Beer 1969).

In most bat species studied to date, mothers selectively nurse only their own offspring (Fenton 1985) although pups of some species attempt to suckle indiscriminately but normally with little success (Hughes et al. 1989; Gebhard 1997). The vocal component of mother–pup

communication is well studied: pups utter so-called isolation calls when communicating their wish to nurse or, when still not volant, to be retrieved from their current location. Mothers, in turn, produce either so-called directive calls or echolocation pulses as a response to isolation calls (for an overview see Fenton 1985). To facilitate offspring recognition, isolation calls should contain enough individual variation to allow mothers to discriminate between their own and alien young (Beecher 1989). The existence of such a 'vocal signature' in isolation calls has been shown statistically for several bat species (for overviews see Kunz & Hood 2000; Wilkinson 2003). However, studies verifying their statistical results about individual distinctiveness through playback experiments, in which mothers have to discriminate between recorded isolation calls of different pups, are still fairly scarce (Rother & Schmidt 1985; Thomson et al. 1985; Balcombe 1990; DeFanis & Jones 1995, 1996; Bohn et al. 2007). Vocal signatures in maternal directive calls (Esser & Schmidt 1989; Balcombe & McCracken 1992) and in echolocation pulses (DeFanis & Jones 1995; Masters et al. 1995; but see Siemers & Kerth 2006) have also been reported, but playback experiments with pups are even scarcer than with mothers. To our knowledge, the potential occurrence of reciprocal mother–pup recognition has been studied in only four bat species from three families (Molossidae, Vespertilionidae and Phyllostomidae) and the playback results are somewhat ambiguous: for two species, mutual recognition could not be shown (*Tadarida brasiliensis*: Balcombe 1990; *Pipistrellus pygmaeus*: DeFanis & Jones 1996), whereas for two other species mutual recognition could be demonstrated but not statistically assured (*Plecotus auritus*: DeFanis & Jones 1995; *Phyllostomus discolor*: Esser 1998). We investigated a fifth species, the greater sac-winged bat, *Saccopteryx bilineata* (family Emballonuridae), to gather more data on the potential occurrence of mutual mother–pup recognition in bats. Taxonomic breadth is important because only with data from enough species that differ sufficiently in their way of life (e.g. in coloniality, roost fidelity and amount of maternal separation) can we ultimately understand the influence of selection pressures shaping mother–offspring recognition in bats.

An *S. bilineata* mother hides her nonvolant pup in various night roosts in the rainforest during the time she spends foraging (Tannenbaum 1975). For later retrieval, spatial memory alone is likely to be insufficient for a successful mother–pup reunion because the pup might have crawled to a different location (to avoid predation) or fallen down. It should be beneficial if the calling behaviour of pups was triggered by echolocation pulses of conspecifics flying by because a constantly vocalizing pup would attract predators. It is uncertain whether pups call in response to all conspecifics' echolocation pulses or whether they discriminate between their own and alien mothers. Maternal echolocation pulses might contain enough individual variation to facilitate individual recognition (e.g. by adult conspecifics), but whether this information is used by pups for discrimination depends on the exigency of such a behaviour. Pups might discriminate between their own and alien mothers based on echolocation pulses only if a unidirectional recognition process is insufficient for successful mother–pup reunions.

The aim of this study was to investigate whether acoustically mediated mother–pup recognition takes place in *S. bilineata*, and, if so, whether this recognition process is mutual or unidirectional. We studied the individual distinctiveness inherent in isolation calls uttered by pups and echolocation pulse trains produced by mothers, using both a statistical approach and playback experiments with free-living bats. We hypothesized that mothers are able to discriminate between their own and alien offspring based on isolation calls alone and that the calling behaviour of pups is triggered by conspecifics' echolocation pulses. We further tested whether pups could discriminate between their own and alien mothers based on echolocation pulses. To our knowledge, this is the first bat study in which both mothers and pups were tested with the same experimental playback design that was applied to elicit vocal responses belonging to the correct social context instead of initiating phonotaxis behaviour. This gave us the novel opportunity to elicit antiphonal calling behaviour between mothers and pups experimentally.

## METHODS

### Study Site and Animals

Sound recordings and playback experiments were conducted during four field seasons (June–July 2004 and 2005, June–August 2006 and 2007) at the Biological Station La Selva (Organisation for Tropical Studies, OTS) in Costa Rica (10°25'N, 84°0'W). The five study colonies of *S. bilineata* were located on the walls of buildings and each contained one or two harems with several lactating females and their offspring. Bats of all colonies were habituated to the presence of humans and sound recordings and behavioural observations in the day roost could be made without causing noticeable disturbance. All adult bats in our study colonies were marked with plastic bands on their forearms, rendering them individually discernible from a distance. Bats were captured with mist nets (Avinet, Inc., New York, U.S.A.) outside the roost when they emerged at dusk or returned at dawn and banded with a unique combination of coloured and numbered plastic bands (A.C. Hughes Ltd., Hampton Hill, Middlesex, U.K., size XCL). The banding procedure was already well established as part of a long-term study since 1996 (see Heckel et al. 1999 for details) and there were no negative effects on the bats' behaviour or health we were aware of. Nonvolant pups were first identified via their respective mothers and banded at a later stage. This is an accurate identification procedure since females bear only one pup per year and are aggressive towards alien pups. Vocalizations were analysed from nine pups in 2004 (when they were approximately 3–5 weeks old) and nine lactating females in 2005 and 2006. Playbacks were performed with nine lactating females in 2005 and nine pups in 2006 and 2007 (which were approximately 6 weeks old when tested). The nine focal animals used in each data set came from at least three different colonies. We used only individuals for which we had high-quality sound recordings. Females were probably unrelated because of female-biased natal

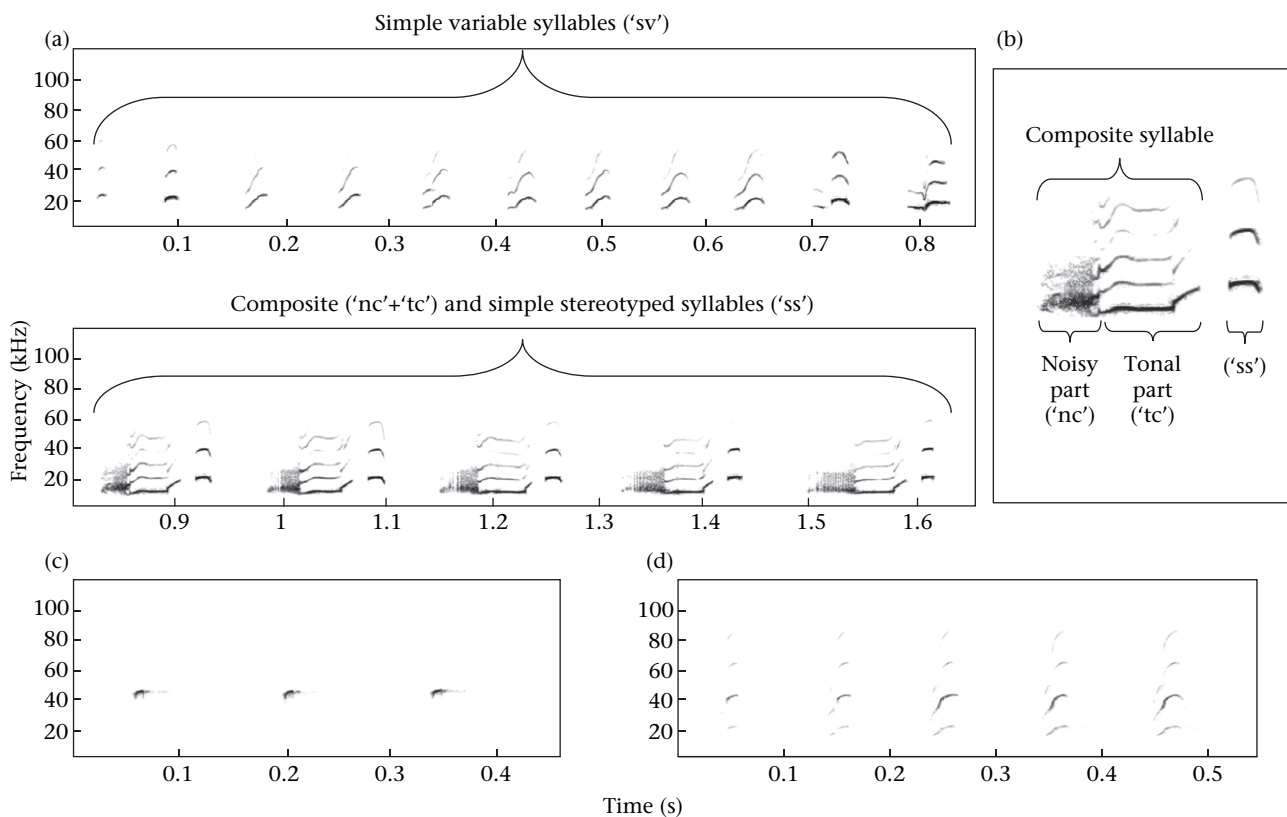
dispersal (Nagy et al. 2007) but pups from the same colony in the same year could be paternal half-siblings. When we conducted playback experiments, pups were already volant (but not weaned yet) and we were therefore able to catch a lactating female or her respective pup separately with a mist net close to the day roost and thus minimize disturbance in the colonies. Playbacks were conducted out of earshot of the focal bat's day roost so that the respective pup or mother belonging to the focal bat being tested could remain undisturbed in its social group and did not have to be caught as well. Since pups were already volant and hence partly independent when we conducted playbacks, a short separation from their mothers did not pose a threat for their health nor had any other negative effects we were aware of. Immediately after the playbacks, focal bats were released close to their day roost. In all cases, focal mothers returned directly to their respective pups and vice versa. All field work was approved by the Costa Rican Ministerio del Ambiente y Energía (MINAE).

### Sound Recording and Playback Equipment

Sound recordings were made to analyse statistically the potential individual distinctiveness of isolation calls from pups (Fig. 1a, b) and echolocation pulse trains from mothers (Fig. 1c) and to provide stimuli for our playback

experiments. Isolation calls were recorded from pups actively pursuing their mothers to nurse and echolocation pulses were recorded from perched females responding to their respective pup's vocalizations. In addition, we gathered behavioural data on successful mother–pup reunions under natural circumstances in the day roost. The vocalizations were digitally recorded with a high-quality ultrasonic recording set-up (400 kHz sampling rate and 12 bit depth resolution) consisting of a  $\frac{1}{4}$  inch Bruel & Kjaer microphone (type 4939; free field response  $\pm 2$  dB from 4 to 100 kHz), a GRAS preamplifier (type 26 AB), a GRAS power module (type 12 AA; 20 dB amplification), a National Instruments A/D DAQCard 6062E, and a notebook computer running Avisoft-Recorder software version 2.9 (R. Specht, Berlin, Germany). The directional characteristic of the microphone was focused by a 0.33 m diameter parabolic reflector mounted on a tripod with a dimmed laser pointer attached to aim at focal animals. This set-up permitted individual recordings even if other bats were vocalizing in the vicinity.

Playback signals (300 kHz sampling rate) were fed into an ultrasonic amplifier (Avisoft Ultrasonic Power Amplifier; frequency response of  $\pm 1$  dB from 0.1 to 100 kHz) and an ultrasonic speaker (Avisoft Ultrasonic Speaker Scan-Speak R2904-700000; frequency response of  $\pm 8$  dB from 4 to 115 kHz). Amplitudes of playback stimuli were adjusted to 100 dB sound pressure level at a distance of 0.1 m. Vocal



**Figure 1.** Spectrograms of (a, b) a multisyllabic isolation call from a 30-day-old pup, (c) three maternal echolocation pulses and (d) five maternal directive calls. Simple but variable syllables (sv-syllables) at the beginning of the isolation call gradually merge into composite syllables consisting of a noisy (nc-syllables) and a tonal part (tc-syllables) which are followed by simple, but stereotyped syllables (ss-syllables). The spectrograms depict frequency as a function of time and were generated using a 1024 point fast Fourier transform and a Hamming window with 75% overlap.

responses to playbacks were recorded with a sampling rate of 100 kHz using the set-up described above.

## Playback Design

We used the same experimental design (Fig. 2) to investigate the vocal responses of both mothers and pups to the different playback situations. In a first set of playback experiments, lactating females were exposed to isolation calls of their own versus an alien pup; in a second set, pups were exposed to echolocation pulse trains of their own versus an alien mother. Vocal responses were defined as either isolation calls (from tested pups) or echolocation pulse trains (from tested mothers). No other vocalization types were ever recorded during playbacks. A playback started once the focal animal had habituated to the playback cage (15 x 25 cm and 15 cm high, consisting of a wire frame covered with soft mesh). The bat could move freely within the cage but normally perched on one vertical wall and appeared calm (e.g. started grooming) less than 10 min after it had been caught and transferred to the playback cage. Each playback trial had a total duration of 9 min and consisted of a preobservation period (2 min), the first stimulus presentation period (2 min), an observation period (1 min), the second stimulus presentation period (2 min) and a postobservation period (2 min). Isolation calls or echolocation pulse trains in the stimulus presentation periods were either from the focal animal's own pup (or mother) or from an alien one and the order in which they were broadcast was pseudorandomized. Vocalizations of focal animals were recorded not only in the stimulus presentation periods but also in the observation periods to control for changes in motivation throughout the playback experiment.

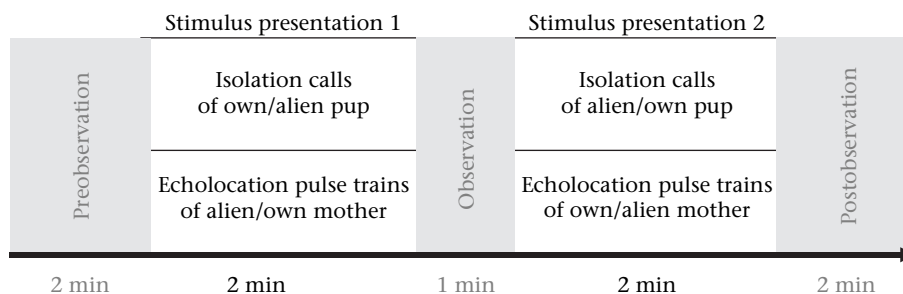
## Playback Stimuli

To avoid pseudoreplication (McGregor et al. 1992), 10 different isolation calls (or echolocation pulse trains) were chosen as stimuli from each pup (or mother). The stimuli were selected from different vocalization bouts to minimize temporal dependence among vocalizations uttered in succession. The time interval between recordings of stimuli and playback experiments was less than a week

in all cases. After filtering for background noise, we combined the different stimuli into a single sound file interspaced with silent intervals of 8, 10 or 12 s. Every sound file started with 5 s of silence and was 2 min long. Sound files were generated with Cool Edit 2000 (Syntrillium, Phoenix, AZ, U.S.A.). Every sound file was unique because of the random order of stimuli and silent interval lengths and was used only once. If stimuli were used both as 'own stimuli' for the respective mother (or pup) and 'alien stimuli' for a different female (or pup), new sound files were generated. We used isolation calls and echolocation pulse trains from colony members as 'alien stimuli' in the playbacks because we wanted to test for individual recognition and not for an effect of familiarity (i.e. colony member versus noncolony member).

## Acoustical Analyses

We analysed vocalizations with Avisoft-SASLab Pro version 4.1 (R. Specht, Berlin, Germany). Measurements were taken from spectrograms generated using a 1024 point fast Fourier transform and a Hamming window with 75% overlap, which resulted in a frequency resolution of 390 Hz and a time resolution of 0.64 ms. To characterize echolocation pulses uttered by mothers, we measured two temporal (duration; distance from start to maximum amplitude of the pulse) and six spectral parameters (peak frequency at start, middle and end of the pulse; minimum, maximum and peak frequency averaged over the entire pulse). Pups' isolation calls were multisyllabic and consisted of simple syllables that gradually merged into composite syllables (Fig. 1a, b). Several different syllable types were categorized: simple, but variable syllables ('sv-syllables'; at the beginning of calls), composite syllables (at the end of calls, consisting of a noise-like beginning followed by a tonal part; 'nc-syllables and tc-syllables'), and simple, but stereotyped syllables ('ss-syllables'; immediately following the composite syllables). To characterize isolation calls, several different spectral parameters were measured according to the respective syllable type: peak frequency at start and end as well as number of frequency modulations for sv- and tc-syllables, bandwidth over the entire length for nc-syllables, and peak frequency at start and end for ss-syllables. Duration and number of syllables per call were measured for all syllable types. This resulted



**Figure 2.** Experimental design for playbacks used to test both mothers and pups. Observation periods alternated with stimulus presentation periods over the course of 9 min. During the three observation periods, spontaneous vocalizations of the focal animal were recorded. During the two stimulus presentation periods, stimuli were presented and the elicited vocal response of the focal animal recorded. The order in which stimuli belonging to the focal animal's own versus alien pup/mother were broadcast was pseudorandomized between the two stimulus presentation periods.

in a total of 17 spectral and temporal parameters used for characterizing isolation calls. To minimize temporal dependence among vocalizations uttered in succession, we used echolocation pulses and isolation calls from different vocalization bouts on different days.

## Statistical Analyses

To test for individual distinctiveness of isolation calls and echolocation pulse trains, we performed discriminant function analyses (DFAs) that allowed us to separate individuals in a multidimensional signal space defined by the acoustic parameters measured before. The DFAs were calculated for isolation calls and echolocation pulse trains separately. All acoustic parameters were included simultaneously in the respective DFAs. We used a subset validation procedure to assign isolation calls and echolocation pulse trains to different individuals. This procedure randomly assigns calls to a ‘training’ set and a ‘test’ set (50% of all calls per set) and uses the training set to calculate discriminant functions with which the test set is then classified. The classification success depends on the number of individuals, vocalizations and acoustic parameters measured per syllable. In general, the classification success decreases with increasing number of individuals and increases with increasing numbers of vocalizations per individual and acoustic parameters measured per syllable (Beecher 1989). After testing for normality, we analysed results from playback experiments using two-tailed parametric tests ( $\alpha = 0.05$ ). For all statistical tests we used SPSS version 11.5 (SPSS Inc., Chicago, IL, U.S.A.).

## RESULTS

### Vocal Mother–Pup Communication

During all mother–pup reunions in the day roost, pups always uttered multisyllabic isolation calls in which simple syllables gradually merged into composite syllables (see Fig. 1a,b). When pups approached their mothers, isolation calls were interspaced with silent intervals (mean 8.53 s, range 1.5–36.1 s;  $N = 9$  pups) that decreased in length towards the end of the solicitations (Wilcoxon signed-ranks test; matched pairs were the first and second half of successful solicitation periods:  $T = 3$ ,  $N = 9$ , exact  $P = 0.020$ ). Mothers reacted with echolocation pulse trains (Fig. 1c), which they uttered while perched in the roost. In addition, mothers produced so-called directive calls (Fig. 1d), barely audible vocalizations that resembled the sv-syllables of isolation calls. However, these directive calls were rarely uttered, mainly when pups were still very young and left their mother’s teat for the first few times. As soon as pups were volant (but not weaned), we never recorded any more directive calls from their mothers. Therefore, they are not part of the analyses presented here.

### Distinctiveness of Isolation Calls

All pups could be distinguished statistically based on the acoustical parameters extracted from isolation calls

(Table 1). A DFA with 90 isolation calls of nine pups (10 calls each) classified 62% of all calls to the correct individual, which was significantly higher than expected by chance alone (11.11%; Wilcoxon signed-ranks test for matched pairs:  $T = 0$ ,  $N = 9$ , exact  $P = 0.005$ ). The first three discriminant functions together accounted for 85.3% of the observed variation. A DFA using only the parameters extracted from the composite syllables at the end of calls (nc- and tc-syllables) still assigned 53% of all calls to the correct pup. The classification success obtained was again significantly higher than expected in a random

**Table 1.** Statistical evidence for a vocal signature character in isolation calls and echolocation pulse trains of *S. bilineata* pups and mothers

Acoustic parameters	DF1	DF2
<b>Vocal signatures in isolation calls (9 pups, 90 calls)</b>		
sv-Syllables		
Number	0.00	0.13
Duration	−0.04	0.05
Peak frequency (start)	0.12	0.02
Peak frequency (end)	0.18	0.18
Frequency modulations	0.05	0.16
tc-Syllables		
Number	0.01	0.18
Duration	0.12	0.14
Peak frequency (start)	0.03	0.00
Peak frequency (end)	0.17	0.35
Frequency modulations	0.26	0.07
nc-Syllables		
Number	−0.22	0.14
Duration	−0.02	−0.24
Bandwidth	−0.07	−0.19
ss-Syllables		
Number	0.08	0.13
Duration	0.19	0.02
Peak frequency (start)	0.05	0.10
Peak frequency (end)	0.07	0.09
Assessment of model fit		
Eigenvalue	10.562	4.523
Percentage of variation	50.9	21.8
Wilk’s $\lambda$	0.0001	0.005
Chi-square (all $P < 0.001$ )	238.093	162.212
<b>Vocal signatures in echolocation pulse trains (9 mothers, 90 trains)</b>		
Duration	0.28	−0.42
Distance to maximum amplitude	−0.18	0.61
Peak frequency (start)	0.07	0.09
Peak frequency (centre)	0.04	−0.20
Peak frequency (end)	0.02	−0.25
Peak frequency (entire pulse)	0.04	−0.19
Minimum frequency (entire pulse)	0.13	0.14
Maximum frequency (entire pulse)	−0.15	−0.31
Assessment of model fit		
Eigenvalue	6.280	2.133
Percentage of variation	58.5	19.9
Wilk’s $\lambda$	0.007	0.053
Chi-square (all $P < 0.001$ )	174.620	104.146

Correlations between the standardized canonical coefficients and the variables in the DFAs (subset validation) for the first two discriminant functions (i.e. DF1 and DF2). The higher the correlation, the more important is the respective variable in shaping an axis. For an explanation of syllable abbreviations and acoustic parameters see [Methods](#).

classification (11.11%; Wilcoxon signed-ranks test for matched pairs:  $T = 1$ ,  $N = 9$ , exact  $P = 0.01$ ), suggesting that most vocal signature information is encoded in the composite syllables.

### Distinctiveness of Echolocation Pulse Trains

Mothers could be statistically distinguished based on the acoustical parameters of echolocation pulse trains (Table 1). A DFA with 90 echolocation pulse trains of nine mothers (10 trains each; mean of six pulses per train in the analysis) classified 40% of all trains to the correct mother, which was significantly higher than expected by chance alone (11.11%; Wilcoxon signed-ranks test for matched pairs:  $T = 3$ ,  $N = 9$ , exact  $P = 0.020$ ) but considerably weaker than the result obtained for isolation calls, making it difficult to identify individuals reliably. The first three discriminant functions together accounted for 87.9% of the observed variation.

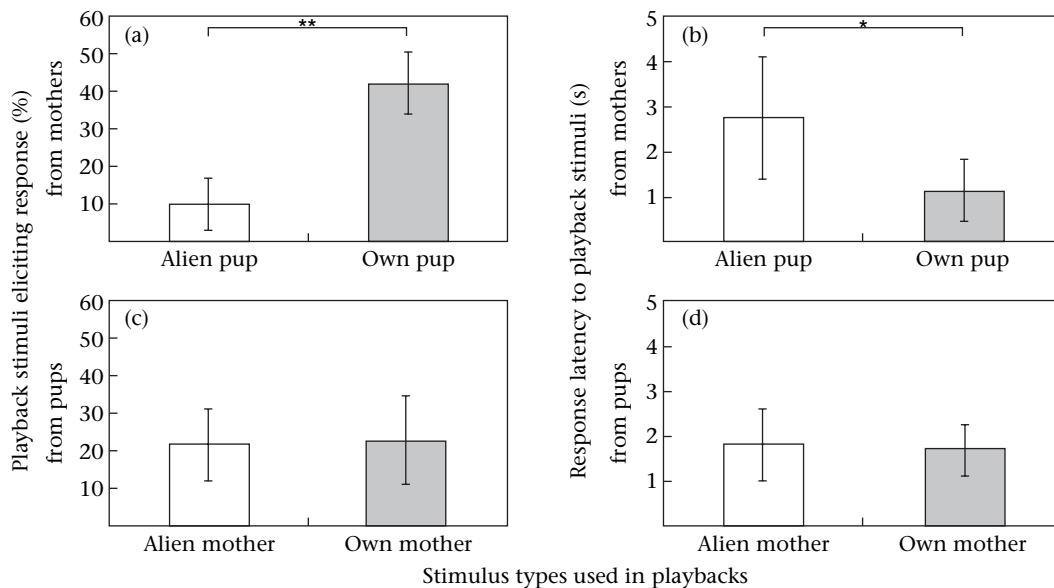
### Playbacks with Isolation Calls

Mothers could clearly discriminate between their own and alien offspring in our playbacks. Stimuli from the focal mother's own pup elicited vocal responses significantly more often than stimuli from an alien pup (paired  $t$  test:  $t_8 = -14.50$ ,  $P < 0.0001$ ; Fig. 3a). Additionally, the response latency was significantly shorter for the 'own pup' stimuli than for the 'alien pup' stimuli ( $t_6 = 2.785$ ,  $P = 0.032$ ; Fig. 3b). A one-factorial repeated measures ANOVA revealed a tendency towards significant differences between spontaneous calling behaviour in different observation periods ( $F_{2,16} = 2.932$ ,  $P = 0.082$ , partial  $\eta^2 = 0.268$ ) which was due to significantly more echolocation pulses being recorded during the preobservation period than during the other two observation periods

(pairwise comparisons of estimated marginal means; mean difference = 3.111, 95% confidence interval = 0.248–5.974,  $P = 0.034$ ; other mean differences were nonsignificant). The difference between spontaneous vocalizations before and during/after the playback (number of vocal responses: preobservation:  $\bar{X} \pm SD = 1.4 \pm 0.5$ ; observation:  $0.8 \pm 0.7$ ; postobservation:  $0.8 \pm 0.7$ ;  $N = 9$  mothers) might be the result of habituation but the short response latency ('own' stimuli:  $\bar{X} \pm SD = 1.16 \pm 0.66$  s; 'alien' stimuli:  $2.76 \pm 1.35$  s;  $N = 9$  mothers) showed that focal bats still readily responded to the playback stimuli.

### Playbacks with Echolocation Pulse Trains

In our playbacks, pups did not discriminate between their own and alien mothers on the basis of echolocation pulse trains. We found no significant difference in the percentage of stimuli eliciting vocal responses (paired  $t$  test:  $t_8 = 0.667$ ,  $P = 0.524$ ; Fig. 3c) or the response latency ( $t_7 = 0.847$ ,  $P = 0.425$ ; Fig. 3d) between the two stimulus types. No difference in spontaneous calling behaviour between playback observation periods was found (repeated measures ANOVA:  $F_{2,16} = 1.143$ ,  $P = 0.344$ ), not even a trend towards habituation throughout the playback (number of vocal responses: preobservation:  $\bar{X} \pm SD = 0.7 \pm 0.5$ ; observation:  $0.3 \pm 0.5$ ; postobservation:  $0.7 \pm 0.5$ ;  $N = 9$  pups). Although they failed to demonstrate discrimination between own and alien mothers, we are certain that our playbacks were valid for two reasons. First, pups called significantly more often during stimulus presentation periods than during observation periods (stimulus presentation periods:  $\bar{X} \pm SD = 2.4 \pm 0.9$  calls; observation periods:  $0.6 \pm 0.3$  calls; paired  $t$  test:  $t_8 = 5.679$ ,  $P < 0.0001$ ), suggesting that our stimuli indeed elicited calling behaviour. Second, pups did not call randomly during stimulus presentation periods but responded to the presented stimuli after only a short



**Figure 3.** (a, c) Percentage of playback stimuli eliciting vocal responses and (b, d) response latency to playback stimuli from focal mothers (a, b) or pups (c, d) for the two different stimulus types used in the playbacks ('alien' and 'own'). Means are shown  $\pm$  SD. \* $P < 0.05$ ; \*\* $P < 0.0001$ .

latency ( $\bar{X} \pm SD = 1.77 \pm 0.56$  s after the beginning of the silent intervals between stimuli;  $N = 9$  pups).

## DISCUSSION

### Statistical Evidence for Vocal Signatures

Our results suggest that both isolation calls uttered by pups and echolocation pulse trains uttered by mothers contain enough individual variation to allow for statistical discrimination better than expected by chance alone. However, there are differences in the strength of the vocal signature character inherent in the two vocalization types. The vocal signature of pups' isolation calls seems to be rather strong, indicating that vocal offspring recognition by mothers is likely to occur. Since we could not control for the pups' precise age in our analysis as some other studies did (Jones et al. 1991; Scherrer & Wilkinson 1993; Knörnschild et al. 2007), age effects could have contributed to or masked differences between pups in our study (Scherrer & Wilkinson 1993). Isolation calls of *S. bilineata* are among the most complex infant vocalizations reported for bats and most signature information is encoded in the composite end syllables of isolation calls. Perhaps the function of the simple syllables at the beginning of calls, which contribute only very little to individual distinctiveness, lies in attracting the attention of a female passing by in search of her pup, whereas the composite syllables are then used to communicate the identity of the vocalizing pup. The vocal signature inherent in maternal echolocation pulse trains was considerably weaker than the one in isolation calls, making it debatable whether echolocation pulses could be used by pups to identify their respective mothers. Several studies have reported vocal signatures in echolocation pulses encoding group identity, age, sex, individual identity, or all of the above (Jones et al. 1992; Masters et al. 1995; Pearl & Fenton 1996; but see Siemers et al. 2005; Siemers & Kerth 2006), and in most cases their classification success was higher than ours.

### Experimental Evidence for Vocal Signatures

Mothers could clearly discriminate between their own and alien offspring solely on the basis of isolation calls and responded more often and with shorter latency to their own young. Several other studies have also verified vocal offspring recognition by mothers through playback experiments (*P. discolor*: Rother & Schmidt 1985; *T. brasiliensis mexicana*: Balcombe 1990; *P. pygmaeus*: DeFanis & Jones 1996; *Phyllostomus hastatus*: Bohn et al. 2007) but used phonotaxis behaviour or, in psychoacoustic experiments, the Go/No-go procedure to evaluate the recognition abilities of the focal bats. To our knowledge, this study is the first to use vocal responses to playback stimuli as a measure of mother–offspring recognition in bats.

We argue that vocalizations are a more feasible response measure than phonotaxis behaviour for conducting mother–pup recognition playbacks in bats. Phonotaxis through flight normally covers distances that are too large

to be easily provided. Accordingly, most bird studies using phonotaxis as a response measure have conducted playbacks with free-ranging animals or provided large flight cages (Searcy 1992). In spite of being more practicable, phonotaxis through crawling nevertheless demands an experimental set-up properly adjusted to normal bat behaviour. Crawling around in the roost can occur along both the vertical and horizontal axes but the only horizontal axis bats commonly crawl along is the ceiling and not the floor. Therefore, we believe that forcing bats to show phonotaxis behaviour while crawling on a horizontal surface (in contrast to under it) constitutes a highly unnatural situation. For pups, falling to the floor is a potentially life-threatening situation because of the increased risk of predation, which they normally try to overcome by hectically crawling around until they find a vertical surface to gain elevation, and even adult bats do not appear very comfortable on the floor. We doubt that such a situation would make them very responsive to a discrimination task. This is true for the majority of species even though there are exceptions such as the vampire bat, *Desmodus rotundus*, which normally crawls towards its sleeping prey and therefore performs well in a conventional Y-maze set-up (Gröger & Wiegrebe 2006). This dilemma could be overcome by constructing Y-mazes or circular arenas which are either large enough to permit flight or in which the focal bats can crawl in a more natural way (i.e. under a horizontal surface or along a vertical one). When testing temporarily captive bats under field conditions this might not be feasible, whereas the use of vocalizations as a response measure constitutes a convenient alternative. In addition to the fact that phonotaxis in a conventional two-speaker design does not allow avoidance and preference of playback stimuli to be distinguished (Gerhardt 1992), vocalizations belonging to the correct social context (i.e. mother–pup reunions) are easier to interpret as reactions to the playback stimuli than movements that could be either phonotaxis behaviour or escape attempts. This is especially true when negative results are obtained. Ideally, more than one response variable should be measured (McGregor 1992) and most playback studies on birds, anurans and insects do so. We argue that in cases where this is not feasible, vocal responses should be preferred over phonotaxis behaviour to assess acoustically mediated mother–pup recognition in bats.

Our playbacks demonstrated clearly that the production of pups' isolation calls was triggered by echolocation pulses from adult conspecifics. Under natural circumstances, this antiphonal calling behaviour is probably very useful to make the pup's current location known to any female passing by that could be its mother. Apparently there is no adaptive advantage for *S. bilineata* pups to discriminate further between their own and alien mothers, which corresponds to the results of other studies (Balcombe 1990; DeFanis & Jones 1996; but see DeFanis & Jones 1995; Esser 1998). There could be several reasons for this. First, the pups we tested might have been too young to perform a successful discrimination task (e.g. because of incomplete hearing development, as suggested by Thomson et al. 1985). This seems unlikely in our case, since we used pups of approximately 6 weeks of age in our

playbacks and hearing should be fully developed in volant pups because of the need to orient by echolocation. Second, there might be no strong selection pressures for mutual mother–pup recognition in this species.

### Selection Pressures on Parent–Offspring Recognition

Mutual parent–offspring recognition is well documented for several birds (penguins: Jouventin et al. 1999; alcids: Jones et al. 1987; Lefevre et al. 1998; Insley et al. 2003) and mammals, especially otariid pinnipeds (Trillmich 1981; Insley 2001; Charrier et al. 2003), dolphins (Sayigh et al. 1998) and ungulates (reindeer, *Rangifer tarandus*: Espmark 1971, 1974; domestic sheep *Ovis aries*: Searby & Jouventin 2003). In pinnipeds and penguins, mutual recognition might be essential to facilitate parent–offspring reunions because they breed in very large colonies and parents leave their young for long periods to forage, making the recognition task possibly too difficult to be unidirectional. In reindeer and sheep, neonates follow their mothers immediately after parturition and mutual vocal recognition should be beneficial to retain contact over a distance in a moving herd. Ungulates in which neonates hide between nursing periods only show unidirectional vocal recognition, either by the mother (goat, *Capra hircus*: Terrazas et al. 2003; pig, *Sus scrofa*: Illmann et al. 2002) or the offspring (fallow deer, *Dama dama*: Torriani et al. 2006), suggesting that the species-specific differences in neonate mobility may have influenced the direction of vocal mother–offspring recognition. Accordingly, mutual vocal recognition in alcids is influenced by offspring mobility; in species with extended parental care at sea, unidirectional recognition might be insufficient to ensure successful parent–offspring reunions (Jones et al. 1987; Insley et al. 2003). Dolphins form long-lasting social bonds and therefore selection pressure on mutual vocal recognition is likely to be strong, especially because they cannot use additional olfactory cues as pinnipeds and ungulates do (Insley 2001; Searby & Jouventin 2003).

The fact that vocal mother–offspring recognition is unidirectional in all bat species studied to date (but see DeFanis & Jones 1995 and Esser 1998) might be because of low selection pressure on mutual recognition, either because recognition errors are innocuous or because the discrimination task is simple enough to be unidirectional: long-lasting mother–pup bonds are absent, pups are fairly immobile for their first few weeks after parturition, and other sensory modalities such as olfaction and spatial memory can also be applied, especially when breeding aggregations are large.

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