



Twin siblings sound alike: isolation call variation in the noctule bat, *Nyctalus noctula*

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(Received 18 October 2006; initial acceptance 20 November 2006;
final acceptance 10 December 2006; published online 4 September 2007; MS. number: 9146)

We studied the individual variation in bat pups' isolation calls in the noctule bat, one of the few bat species in which twin births are common. Based on discriminant function analyses we showed that pups' isolation calls were individually distinct and therefore likely to bear vocal signatures suitable for acoustically mediated mother–offspring recognition. The comparison of individual variation of isolation calls in intratwin and intertwin pairs revealed that isolation calls of twin siblings were more similar to the calls of each other than to the isolation calls of unrelated pups of the same age. Therefore, isolation calls may not only encode individual identity but also affiliation to a certain social group (e.g. twin pairs). The distinctiveness of isolation calls increased with the age of the pups and calls of twin siblings remained similar to each other as the pups got older, whereas the calls of unrelated pups diverged, suggesting that vocal signatures become more distinct during ontogeny and that the similarity of twin siblings' isolation calls is persistent over time.

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Keywords: Chiroptera; isolation calls; noctule bat; *Nyctalus noctula*; twins; vocal ontogeny; vocal signatures

Mechanisms of parent–offspring recognition are generally expected to evolve in species with parental care, especially in gregarious breeders, to reduce confusion over reproductive investment (Halliday 1983). Parental care can be defined as any parental behaviour that increases offspring survival and ultimately the fitness of the parents (Hamilton 1964; Ridley 1978) and may depend on the costs and benefits associated with the respective behaviour (Trivers 1972; Clutton-Brock & Godfray 1991). Since newborn mammals are completely dependent on milk provided by their mothers, lactation is the first and most important component of parental care (Martin 1984; Lee 1997). The energetic costs of lactation impose physiological stress on mothers (Millar 1977; König et al. 1988) and may reduce the mother's survival and future reproductive success (Loudon et al. 1983; Clutton-Brock et al. 1989). Therefore, mothers should be reluctant to nurse alien offspring (Packer et al. 1992; but see Roulin 2002; Roulin & Hager 2003) and rely on some form of recognition to identify their offspring. Depending on the communication abilities of the species involved, mother–offspring recognition cues can be visual (Dasser 1988; Parr & de Waal

1999), olfactory (Porter 1999; Levy et al. 2004) or acoustical (Smolker et al. 1993; Charrier et al. 2002). Often a combination of different sensory modalities is used, with olfactory and acoustical cues being the most important ones (Ballard & Kovacs 1995).

Mothers of most bat species studied to date selectively nurse only their own offspring and the use of acoustic signals for mother–offspring recognition has been shown for a variety of species. Normally, infants utter so-called isolation calls when being separated from their mothers and in some species mothers also use a distinct directive call to summon their offspring (for overviews see Fenton 1985; Kunz & Hood 2000; Wilkinson 2003). Pups' isolation calls are generally much lower in frequency than echolocation pulses and are often uttered repeatedly in series (Gelfand & McCracken 1986; Jones et al. 1991). Individual variation in isolation calls ('vocal signatures') can help mothers to identify their offspring (Turner et al. 1972; Brown 1976; Kolb 1977; Barclay et al. 1979; Schmidt et al. 1981; Brown et al. 1983; Gelfand & McCracken 1986; Jones et al. 1991) and several studies verified this hypothesis through playback experiments, in which previously recorded isolation calls from different pups were broadcast to their respective mothers (Rother & Schmidt 1985; Thomson et al. 1985; Balcombe 1990; DeFanis & Jones 1996). The difficulty in the discrimination task faced by

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the mothers as well as the isolation call complexity depends on the social structure of the species involved and is expected to increase with group size (Beecher 1989; Medvin et al. 1993). Pups of species that roost in large colonies tend to have isolation calls encoding more information than pups of species that roost solitarily or in small colonies (Koehler & Barclay 1988; Wilkinson 2003). Only few studies attempted to assess the potential genetic and social components of the individual variation in pups' isolation calls (Esser & Schmidt 1989; Rasmuson & Barclay 1992; Scherrer & Wilkinson 1993).

In this study we recorded, measured and statistically analysed the isolation calls of captive noctule bat pups. The noctule bat is a middle-sized, insectivorous species common in Europe. Female noctule bats aggregate in maternal colonies with up to 100 individuals to give birth and raise their offspring. Like few other European bat species, the occurrence of twin births is common in noctule bats (Heise 1989; Gebhard 1997a). Females normally only nurse their own offspring, though pups sometimes attempt to suckle indiscriminately (Gebhard 1997b).

The aim of this study was to assess individual variation in pups' isolation calls to investigate whether acoustically mediated mother-offspring recognition could occur in noctule bats. We compared the isolation calls of individual pups with the calls of their twin sibling and with the calls of unrelated pups to test whether individual variation is smaller for intratwin pairs than for intertwin pairs, which may be due to genetic and/or social influences. We also investigated how isolation call parameters changed with increasing age and whether pups' isolation calls became more individually distinct over time. Individual distinctiveness of isolation calls may become more important with increasing age of the pups because older pups are more mobile and mothers cannot rely on spatial memory alone to locate their offspring in the roost.

METHODS

Study Subject

A colony of captive noctule bats was kept in a large aviary at the University of Erlangen-Nuremberg where water and meal-worms enriched with vitamin and mineral supplements were provided ad libitum. Due to previous injuries in the wild, these bats were unable to fly but showed normal behaviour otherwise. All volant pups born in captivity were released after weaning in a nearby forest, where food and potential roosts were abundant. To allow individual recognition by the observers, captive females were individually banded with split plastic rings on their forearms (Hughes, XCL). After parturition, pups were banded in the same way. The pups analysed in this study were born in 1990 (six twin pairs) and 1996 (three twin pairs).

Recordings

Pups were separated from their mothers for a maximum of 15 min and placed in a sound proof chamber where

their isolation calls were recorded using a half-inch Bruel & Kjaer microphone (type 4136) and stored on a Racal Store 4D tape recorder (upper frequency limit: 125 kHz). The range between the focal pup and the microphone was 5–30 cm. The temperature in the chamber was high enough to keep the isolated pups warm during the recording sessions (20–25°C).

The six twin pairs in 1990 were recorded at three to four different age categories (1, 3, 9 and >19 days of age), whereas the three twin pairs in 1996 were only recorded at one age category (9 days of age). Therefore, the sample size differed among analyses conducted in this study. To test for individual variation and to compare the distinctiveness of isolation calls of intratwin and intertwin pairs, we combined the data from 1990 and 1996 (nine twin pairs; 55 calls/individual). To test for age-dependent differences in statistical distinctiveness of isolation calls, we only used data from 1990 (six twin pairs; 25 calls/individual and date).

Call Analysis

Recordings were digitized from Racal tapes with a 500-kHz sampling rate and 16-bit depth using custom-made software (DSP Control FAU Erlangen-Nuremberg, Erlangen, Germany). Isolation calls were randomly chosen from a larger set of recorded calls, which had been preselected based on recording quality. To minimize temporal dependence among calls within a series, calls were taken from different series within a recording sample. Acoustic analysis of calls was performed in Avisoft-SASLab Pro (version 4.1, R. Specht, Berlin, Germany). Isolation calls were often multiharmonic in structure, but we used only the first harmonic (fundamental frequency) for measurements because it contained most of the sound energy. Two temporal (duration and interval) and 12 spectral parameters were measured and used for statistical analysis. Peak frequency (frequency of the maximum amplitude), minimum frequency and maximum frequency were measured at three different regions within each call (start, centre and end of call) and over the entire call (mean: parameters derived from the averaged spectrum of the entire call). Measurements were taken from spectrograms. Spectrograms were generated using a 1024-point fast Fourier transform and a Hamming window with 75% overlap.

Statistical Analyses

We performed discriminant function analyses (DFAs) to assess the individual variation in isolation calls. DFAs generate canonical discriminant functions representing the linear combinations of original variables (acoustic properties of isolation calls) that optimally separate groups (individual pups) in multidimensional signal space. All 14 variables were included simultaneously into the DFAs. The mean values of the discriminant functions for every group are represented by group centroids, which can be depicted in a multidimensional scatter plot defined by the discriminant functions. All original variables are correlated with the different discriminant functions and the canonical

coefficients representing these correlations reveal the contribution of each original variable to every discriminant function. Therefore, it can be assessed, which variables convey information on group membership.

Based on the discriminant functions generated in a DFA, calls are classified into separate groups. Classification success depends on the number of separate groups (individual pups), the number of cases per group (isolation calls per pup), and the number of acoustic parameters measured per call (original variables). In general, classification success decreases with increasing group number and increases with increasing case and variable numbers (Beecher 1989; Bee et al. 2001).

We used a subset-validation procedure for classifying each call, which randomly groups calls into a 'training' set and a 'test' set (50% of all calls per set). The discriminant functions used for classifying the calls in the 'test' set are generated with the calls in the 'training' set. This procedure provides an unbiased estimate of the correct classification rate and is thought to be more conservative than other procedures (e.g. cross-validation) and therefore expecting a higher error rate due to misclassified calls. Statistical tests were conducted using SPSS version 11.5 (SPSS Inc., Chicago, IL, U.S.A.).

All 14 acoustic variables were included into the DFAs. However, to assess the change in isolation call parameters over time in a correlation analysis, we only used one spectral parameter (peak frequency at the start of the call) and both temporal call parameters (duration and interval). Since all spectral parameters were intercorrelated, this selection of variables seemed justified.

RESULTS

Isolation Call Parameters

Calls recorded from pups at the age of 19 days or older were not used in our analyses, because they did not

resemble isolation calls any more, even though they were uttered while the pups were separated from their mothers. Calls uttered by pups at more than 19 days of age strongly resembled adult social calls previously described for this species (call type b in Pfalzer 2001). Figure 1 shows a series of isolation calls from a 9-day-old pup and a series of adult-like social calls from the same pup at more than 19 days of age.

Values for measured parameters of isolation and social calls were summarized in Table 1. Means of parameters measured for 25 calls/individual and date were used in the correlation analysis and every individual was used only once. We found a significant positive correlation between the calls' peak frequency and the age of the pups (Pearson correlation: $r_{11} = 0.853$, $P < 0.0001$) and a significant negative correlation between the age of the pups and calls' duration and interval (duration: $r_{11} = -0.709$, $P = 0.01$; interval: $r_{11} = -0.680$, $P = 0.015$). The reported results were still significant after performing a Bonferroni correction for multiple testing ($\alpha = 0.016$ instead of $\alpha = 0.05$) and showed that isolation calls got higher in frequency and shorter in duration and interval with increasing age of the pups.

Individual Distinctiveness of Isolation Calls

All pups could be statistically distinguished by call parameters. A DFA subset-validation procedure with 990 isolation calls of 18 pups (nine twin pairs; all 9-day-old) classified 64% of all calls to the correct individual, which was significantly higher than expected by chance alone (5.55%; Wilcoxon signed-ranks test: $Z = -3.724$, $P < 0.0001$). The first two discriminant functions together accounted for 78.7% of the observed variation. Call duration was the most important parameter contributing to variation in discriminant function axis 1, whereas different frequency parameters were important in shaping axis 2 ('individual distinctiveness': Table 2).

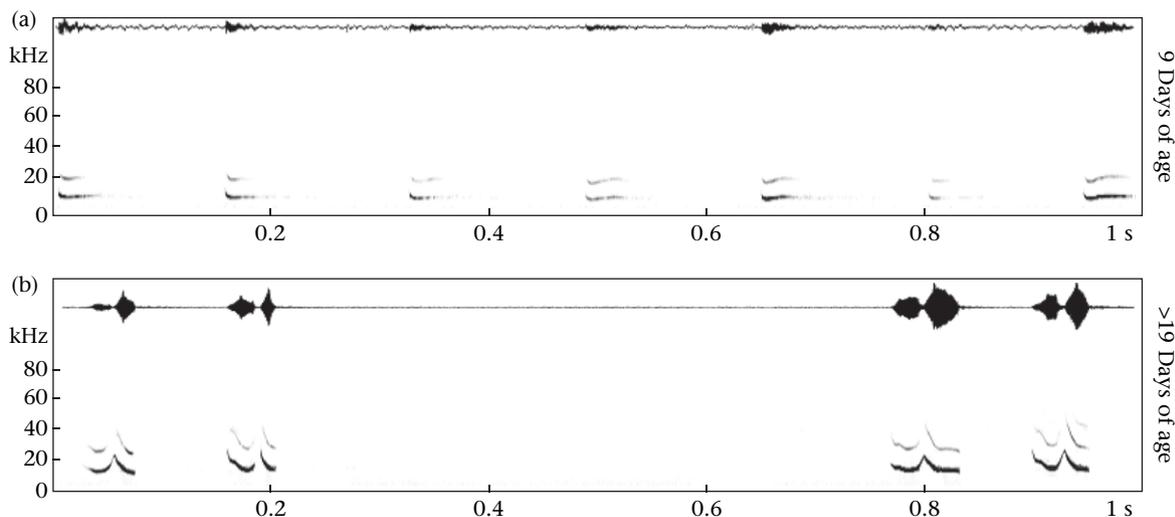


Figure 1. (a) Spectrogram of a series of isolation calls from a 9-day-old pup. (b) Spectrogram of adult-like social calls from the same pup at more than 19 days of age. Spectrograms depict frequency over time and were generated using a 1024-point fast Fourier transform and a Hamming window with 75 % overlap.

Table 1. Means and standard deviations of isolation call properties used in DFAs (day 1, 3, and 9) and social calls (day +19)

	Day 1 (N=150)	Day 3 (N=300)	Day 9 (N=300)	Day +19 (N=300)
Call property				
Duration (s)	0.04±0.01	0.03±0.01	0.03±0.01	0.02±0.01
Interval (s)	0.25±0.15	0.26±0.19	0.21±0.25	0.15±0.70
Peak frequency (start) (Hz)	13 419±2704	14 649±2478	16 600±3030	30 130±9339
Min. frequency (start) (Hz)	11 317±2494	12 519±2546	15 004±3033	28 373±8984
Max. frequency (start) (Hz)	15 654±2634	16 801±2411	18 748±3090	31 985±9397
Peak frequency (end) (Hz)	10 922±1443	11 465±1295	11 542±1276	15 730±4054
Min. frequency (end) (Hz)	9 547±1489	10 228±1328	10 458±1305	14 746±3970
Max. frequency (end) (Hz)	12 621±1499	13 280±1340	13 682±1591	17 704±4159
Peak frequency (centre) (Hz)	10 046±1766	11 119±1507	12 131±1355	16 067±3826
Min. frequency (centre) (Hz)	8 981±1765	10 042±1508	11 060±1433	14 942±3813
Max. frequency (centre) (Hz)	12 657±2035	13 534±1636	14 363±1506	18 233±3981
Peak frequency (mean) (Hz)	10 641±1925	11 625±1588	12 403±1512	15 669±3556
Min. frequency (mean) (Hz)	9 053±1750	10 112±1485	10 789±1297	14 090±3127
Max. frequency (mean) (Hz)	13 804±1981	14 622±1776	15 695±1895	18 888±3427

Temporal parameters: duration (from start to end of each call) and interval (duration of preceding call plus silence); spectral parameters: peak frequency (frequency of the maximum amplitude), minimum frequency and maximum frequency at three different regions within each call (start, centre and end of call) and over the entire call (mean: parameters derived from the averaged spectrum of the entire call).

Similarity of Twin Siblings' Isolation Calls

A scatter plot of group centroids representing individual pups in a two-dimensional signal space defined by the first two discriminant functions revealed that twin siblings were close together in signal space (Fig. 2). We calculated the Euclidean distance between group centroids for twin siblings (intratwin distance) and nonrelated pups (intertwin distance). Intratwin and intertwin distances were

significantly different (paired *t* test: $t_8 = -8.738$, $P < 0.0001$), showing that the calls of twins resembled the calls of their respective siblings more than the calls of unrelated pups (Fig. 3a). Also, significantly more calls were wrongly classified to the respective twin sibling than to the average of the 16 unrelated pups in the analysis (Friedman test: $\chi^2 = 18.000$, $P < 0.001$; post hoc test: all $P < 0.01$; to avoid pseudoreplication, only one pup per twin pair was used; Fig. 3b). This further corroborated our

Table 2. Correlations between the standardized canonical coefficients and the variables in the DFAs

Call property	Individual distinctiveness		Age-dependent differences in individual distinctiveness					
	Day 9 (18 pups, 990 calls)		Day 1 (Six pups, 150 calls)		Day 3 (12 pups, 300 calls)		Day 9 (12 pups, 300 calls)	
	<i>df</i> 1	<i>df</i> 2	<i>df</i> 1	<i>df</i> 2	<i>df</i> 1	<i>df</i> 2	<i>df</i> 1	<i>df</i> 2
Duration	1.05	0.17	0.31	0.66	0.01	-0.64	1.08	0.46
Interval	0.04	0.01	-0.07	0.01	0.07	0.15	0.03	-0.31
Peak frequency (start)	-0.26	-0.36	0.24	-2.11	0.49	0.36	0.13	0.38
Min. frequency (start)	0.00	-0.03	-0.84	2.23	0.95	0.55	-0.63	0.64
Max. frequency (start)	-0.15	-0.05	-0.06	-0.49	-0.78	-0.31	-0.26	-0.79
Peak frequency (end)	-0.35	0.00	-1.02	-1.03	-0.08	0.37	-0.11	-0.05
Min. frequency (end)	0.03	-0.06	-0.43	0.30	0.21	1.07	-0.10	0.54
Max. frequency (end)	0.11	0.11	0.70	0.96	-0.44	-0.57	0.43	-0.92
Peak frequency (centre)	-0.17	0.62	-0.16	-0.18	0.04	-0.61	-0.26	-0.24
Min. frequency (centre)	0.24	-0.10	-0.66	-0.14	0.32	-0.45	0.43	1.12
Max. frequency (centre)	0.15	0.41	0.58	-0.09	-0.28	0.29	0.04	0.19
Peak frequency (mean)	0.04	0.52	1.30	0.37	-0.17	-0.16	0.15	-0.45
Min. frequency (mean)	0.50	-0.22	0.73	-0.36	1.18	-0.48	0.27	-0.15
Max. frequency (mean)	-0.31	0.08	0.14	0.42	-0.59	0.47	-0.07	0.46
Assessment of model fit								
Eigenvalue	8.389	3.288	1.785	0.675	2.85	1.267	4.414	2.71
Proportion of variation (%)	56.5	22.2	53.4	20.2	49	21.8	38.8	23.8
Wilk's λ	0.002	0.023	0.101	0.282	0.027	0.104	0.003	0.014
Chi-square (all $P < 0.01$)	2869.852	1803.835	139.761	77.287	451.662	283.162	745.212	534.097

Bold face indicates values greater than 1. The higher the correlation, the more important is the respective variable in shaping an axis. For call property abbreviations see Table 1.

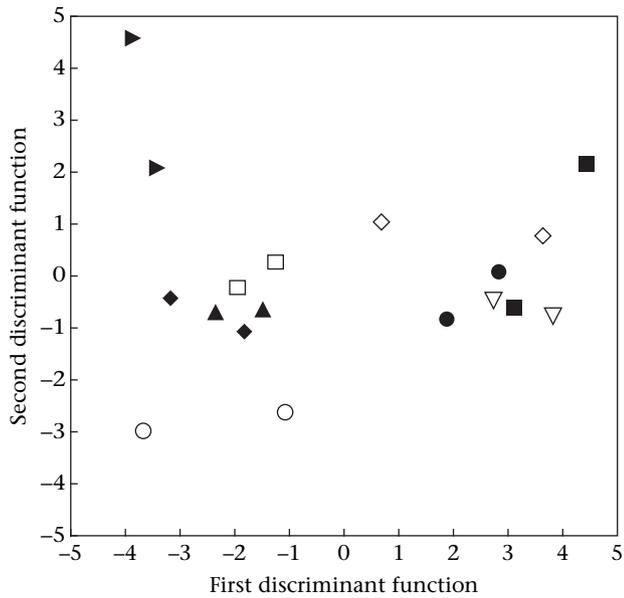


Figure 2. Plot of a two-dimensional signal space defined by the first two discriminant functions. Symbols represent group centroids of 18 pups (55 calls/individual; ●: twin pair 1; □: twin pair 2; ▲: twin pair 3; ◇: twin pair 4; ◆: twin pair 5; ▽: twin pair 6; ■: twin pair 7; ○: twin pair 8; ►: twin pair 9). Note that the group centroids of twin siblings are close together in signal space.

finding that the calls of twins were more similar than those of unrelated pups.

Age-dependent Differences in Isolation Call Distinctiveness

A DFA of 750 isolation calls from six to 12 pups (three twin pairs at day 1, six twin pairs at day 3 and 9; 25 calls/pup and date) showed that the amount of correctly classified calls increased with pups' age. The first two discriminant functions accounted for 73.6% of the observed variation on day 1, 70.7% on day 3 and 62.6% on day 9. The contribution that different acoustic parameters made to shape discriminant function axis 1–2 varied over time ('age-dependent differences': Table 2). At day 1, 39.7% of all isolation calls were correctly classified, 36% at day 3 and 52.8% at day 9, which was significantly higher than expected by random classification alone (16.66% for day 1, 8.33% for day 3 and 9) for all age categories (Wilcoxon signed-ranks test: day 1: $Z = -1.992$, $P = 0.046$; day 3: $Z = -3.062$, $P = 0.002$; day 9: $Z = -3.059$, $P = 0.002$). To understand why the reported value for correct classification success was lower for day 3 than for day 1, one has to take into account that on day 3 twice as many pups were included in the analysis than on day 1. Therefore, the correct classification success expected by random classification alone was twice as high for day 1 than for day 3 (16.66% versus 8.33%), resulting in a higher number of correctly classified calls. To illustrate this, we calculated a factor of correct classification better than random by dividing the percentage of correct classification success by the percentage of chance level for correct classification success expected in a random

classification. This factor represented a value not affected by the number of pups in the analysis and was used to compare isolation call distinctiveness among the three different age categories. The factor of correct classification better than random increased with pup's age (day 1: 2.38; day 3: 4.32; day 9: 6.34), suggesting that the statistical distinctiveness of isolation calls got better as pups matured.

Development of Twin Siblings' Isolation Call Similarity over Time

We examined whether the similarity of isolation calls among twin siblings changed during ontogeny by calculating the distance between group centroids in a two-dimensional signal space for twin siblings (intratwin distance) and nonrelated pups (intertwin distance) within the different age categories (1, 3 and 9 days of age). Again, sample size was three twin pairs for day 1 and six twin pairs for day 3 and 9. To compare values for Euclidean distances between group centroids of different age classes, we used the data from day 9 to calculate discriminant functions defining the signal space and used the same discriminant functions to separate the data from day 1, 3 and 9 into groups (pups). This enabled us to compare intratwin and intertwin distances between group centroids of different age classes in the same signal space (Fig. 4). A two-factorial repeated measures ANOVA revealed no significant changes over time for intratwin distances, but significant changes over time for intertwin distances (age: $F_{1,2} = 8.872$, $P = 0.034$; distance: $F_{1,2} = 87.354$, $P = 0.011$; age*distance: $F_{1,2} = 31.506$, $P = 0.004$). On day 9, the intertwin distance was greater than on day 1 or 3, suggesting that calls of unrelated pups became less similar with increasing age, whereas intratwin similarity did not change over time.

DISCUSSION

Isolation Call Parameters

Isolation calls recorded from the same individuals at 1, 3 and 9 days of age increased in frequency and decreased in duration and interval as pups matured. Similar findings were reported for the ontogeny of echolocation pulses (Konstantinov 1973; Brown & Grinnell 1980; Brown et al. 1983; Habersetzer & Marimuthu 1986; RübSamen 1987; Moss 1988; Jones et al. 1992; Moss et al. 1997) and isolation calls in some species (*Tadarida brasiliensis*: Gelfand & McCracken 1986; *Phyllostomus discolor*: Esser & Schmidt 1989; *Pipistrellus pipistrellus*: Jones et al. 1991; *Nycticeius humeralis*: Scherrer & Wilkinson 1993). In other species, however, isolation call parameters did not change during postnatal development (*Desmodus rotundus*: Schmidt et al. 1981; *Pteropus alecto*: Parijs & Corkeron 2002). When pups' isolation calls change during ontogeny, the mother's template of the acoustic identity of her offspring must change as pups mature (as suggested by Gelfand & McCracken 1986; Jones et al. 1991). In some species, echolocation pulses and isolation calls develop independently (Brown &

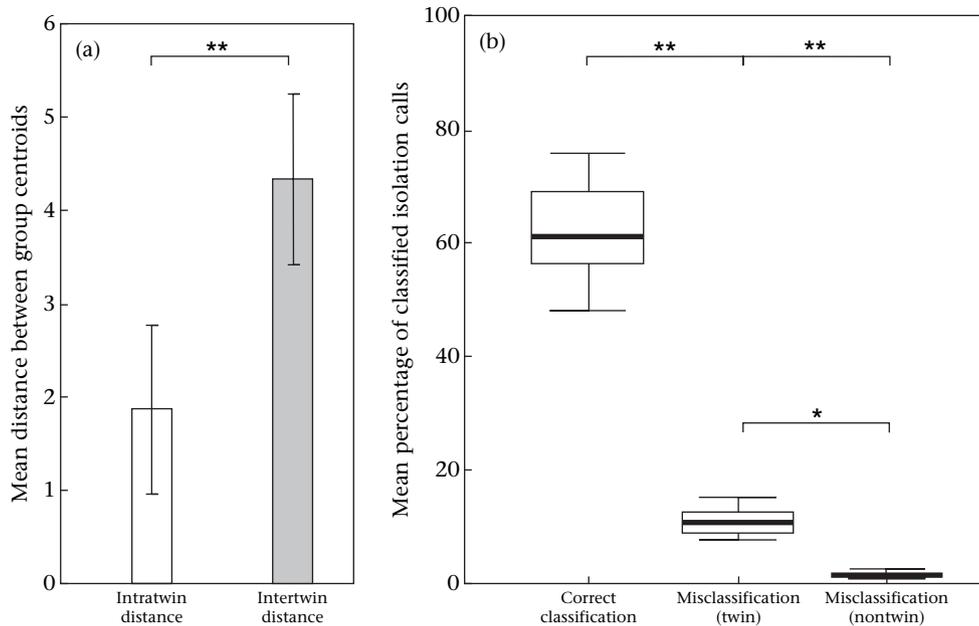


Figure 3. (a) Euclidean distance between group centroids in a two-dimensional signal space for twin siblings (intratwin distance) and nonrelated pups (intertwin distance). A paired t test revealed a significant difference between intratwin and intertwin distances ($**P < 0.001$). Means and standard deviations are shown. (b) Mean percentage of calls classified in the DFA (correct classification: misclassification (twin): wrongly classified to the twin sibling; misclassification (nontwin): wrongly classified to the average of the 16 unrelated pups). Significantly more calls were correctly classified than misclassified but if misclassification occurred, more calls were wrongly classified to the respective twin sibling than to the average of unrelated pups in the analysis (Friedman post hoc test: $*P < 0.01$, $**P < 0.001$). Median, interquartile range (25–75%) and whiskers (0–100%) are shown.

Grinnell 1980; Brown et al. 1983; Rother & Schmidt 1985; Moss 1988), whereas in other species echolocation pulses derive from communication signals (Möhres 1953; Gould 1971, 1977; Matsumura 1979; DeFanis & Jones 1995). Pups in our study uttered isolation calls and echolocation pulses from the first day of age, showing that isolation calls were not a precursor to echolocation pulses in noctule bats but occurred independently.

Individual Distinctiveness of Isolation Calls

Isolation calls of noctule bat pups were distinguishable on the basis of call parameters using DFAs. Individual differences in the structure of isolation calls were reported for several bat species (for overviews see Kunz & Hood 2000; Wilkinson 2003) and some studies verified vocal offspring recognition by mothers through playback experiments (*P. discolor*: Rother & Schmidt 1985; *Myotis lucifugus*: Thomson et al. 1985; *T. brasiliensis*: Balcombe 1990; *P. pipistrellus*: DeFanis & Jones 1996). The differentiation of individuals by the DFA was markedly better at distinguishing pups than expected by random classification, suggesting the existence of vocal signatures that allow the discrimination of individual pups. It is not certain that mothers use the parameters we measured, but our analyses showed that there is sufficient information for acoustically mediated mother–offspring recognition in noctule bats. Our results are a conservative measurement of the information available to the mother, since it is possible that mothers use parameters not included in our DFA and identify their offspring based on whole isolation call

series and not single calls, which should facilitate the identification process. In addition, olfactory cues are likely to be used in offspring recognition as well (Gustin & McCracken 1987; Kunz & Hood 2000).

Similarity of Twin Siblings' Isolation Calls

In colony-forming bat species, there is evidence for both genetically determined differences between vocal signatures of isolation calls (Scherrer & Wilkinson 1993, but see also Rasmuson & Barclay 1992) as well as effects of learning (Esser & Schmidt 1989; Esser 1994). A genetically

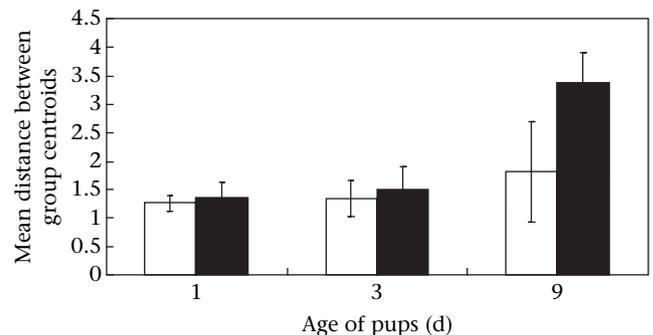


Figure 4. Euclidean distance between group centroids in the same two-dimensional signal space for twin siblings (□: intratwin distance) and nonrelated pups (■: intertwin distance) of three different age categories (1, 3 and 9 days of age). A two-factorial repeated measures ANOVA revealed a significant difference over time for the intertwin distances, but not for the intratwin distances. Means and standard deviations are shown.

determined signal structure is frequently observed if discrimination must occur immediately after birth. In birds, signature signals develop when young start to intermingle (Hepper 1986), but in most bat species intermingling between pups occurs only hours after parturition, leaving very little time to learn the respective vocal signatures necessary for mother–offspring recognition. We reported a greater similarity in isolation call parameters between twin siblings than between unrelated pups, suggesting that individual differences in isolation calls could be driven by genetic determination. However, social components influencing the similarity of twin siblings' isolation calls might exist as well, for example, a maternal preference for certain isolation call parameters.

Scherrer & Wilkinson (1993) suggested that half-sibling studies could provide a better estimate of the genetic contribution to isolation call variation by investigating paternal influences on vocal signatures. In noctule bats, some twins are half-siblings due to multiple mating of a female with different males (Mayer 1997). From the nine twin pairs included in our study, genetic tests were conducted on the six twin pairs in 1990, revealing the occurrence of one half-sibling twin pair (twin pair 6 in Fig. 3) and five fullsibling twin pairs (twin pair 1–5 in Fig. 3). Even though the sample size was not big enough to perform statistical analyses, we can state that the special status of twin pair 6 did not seem to influence the observed similarity of twin siblings' isolation calls. It remains unclear, whether the observed similarity of twin siblings' isolation calls in noctule bats is a result of genetic or cultural transmission, a problem that other studies reporting the similarity of vocal signals within families face as well (isolation calls: Scherrer & Wilkinson 1993; echolocation pulses: Jones & Ransome 1993; Masters et al. 1995).

Age-dependent Differences in Isolation Call Distinctiveness

The amount of correctly classified calls increased with the pup's age, showing that vocal signatures became more distinctive as pups grew older. Pups become more mobile in the roost as they mature and therefore spatial memory alone might not be adequate for mothers to locate their offspring. At close range, olfactory cues may be sufficient for mother–pup recognition, whereas acoustic cues provide longer range signals and may be important in general orientation. With increased pup mobility, the discrimination task faced by the mothers becomes more difficult, and therefore it might be adaptive to develop more distinct vocal signatures as pups get older. Evidence for this was found in vespertilionid bats (*P. pipistrellus*: Jones et al. 1991). In contrast, isolation calls produced by flying foxes appear to remain unchanged during maturation (*P. alecto*: Parijs & Corkeron 2002).

Development of Twin Siblings' Isolation Call Similarity over Time

Even though isolation calls became more individually distinguishable with increasing age of the pups, the

similarity of twin siblings' calls did not change over time, whereas the calls of unrelated pups became less similar during ontogeny. Are these findings a result of social or genetic influence? If isolation call distinctiveness was not heritable but learned, acquisition of vocal signatures could occur in three different ways. First, pups could adapt their isolation calls to vocal signals of their mothers (e.g. to the maternal directive call, as shown for *P. discolor* by Esser & Schmidt 1989); this has not been investigated for noctule bats. Second, pups could imitate the calls of their respective siblings, resulting in a decrease in call variation between twins as pups mature; this is unlikely for noctule bats, because the similarity of twin siblings' isolation calls stayed similar over time. Third, pups could gain acoustic identity by actively avoiding overlap in signal space with other young. This trial-and-error learning (as suggested by Scherrer & Wilkinson 1993) is thought to increase call variation among pups over time. Our results did not show an increase in isolation call variation over time among twins, but the calls of unrelated pups diverged with pup's age, suggesting that trial-and-error learning might play a role in separating twin pairs (not individual pups) in signal space. On the other hand, if isolation call distinctiveness was heritable, little change in call variation over time among related individuals would be expected. Genetic transmission could therefore explain the observed similarity of twin siblings' isolation calls during ontogeny. Possibly, a combination of genetic and social components is influencing isolation call variation in noctule bat pups, resulting in twin siblings' calls to stay similar and unrelated pups' calls to diverge over time.

Acknowledgments

This manuscript was greatly improved by the thoughtful comments of two anonymous referees, and by discussions with M. Bee, O. Behr, K. Gerow, M. Metz, M. Nagy, and B. Pfeiffer. This work was supported by a grant from the German Merit Foundation to M. K.

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