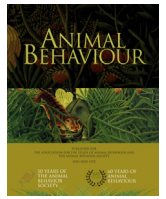




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journal homepage: www.elsevier.com/locate/anbehavMother–offspring recognition in the bat *Carollia perspicillata*Mirjam Knörnschild^{a, *}, Marion Feifel^a, Elisabeth K. V. Kalko^{a, b}^a Institute of Experimental Ecology, Faculty of Natural Sciences, University of Ulm, Germany^b Smithsonian Tropical Research Institute, Balboa, Panama

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Parental care is crucial for offspring survival in many taxa but its burden and costs are often not equally distributed between the sexes. In bats, the majority of parental care is provided by females, making examples of paternal support towards pups exceedingly rare. One exception to this general pattern seemed to be the polygynous Seba's short-tailed fruit bat *Carollia perspicillata*; two earlier studies suggested that paternal care occurs, i.e. that harem males prompt females to retrieve vocalizing pups. To corroborate this suggestion, we investigated the occurrence of maternal and paternal care in reaction to pup isolation calls in *C. perspicillata*. Acoustic measurements of 905 isolation calls from 17 pups revealed sufficient interindividual variation to encode an individual vocal signature. Correspondingly, mothers were capable of using this individual signature to discriminate between their own pups and age-matched pups from other females belonging to their colony. Maternal experience was positively correlated with the strength of response behaviour during playbacks. Thus, our results indicate that pup isolation calls were used to elicit maternal care and that mothers recognize their pups based on an individual signature in isolation calls. However, in contrast to the previous studies mentioned above, we found no evidence that harem males reacted to pup isolation calls by prompting the respective mothers to retrieve their vocalizing pups. Instead, our results demonstrate that harem males engaged in courtship activities that were unaffected by pup isolation calls.

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Parental care is found in many taxa (reviewed in Rosenblatt & Snowdon 1996) and consists of nutritional and non-nutritional support such as protection, transport and thermoregulation (reviewed in Rosenblatt 2003). Parental care can be crucial to offspring survival and therefore the reproductive success of caregivers (reviewed in Clutton-Brock 1991; Royle et al. 2012). Since parental care is costly, parents should avoid investment in offspring that are not their own (Clutton-Brock & Godfray 1991), a problem that is especially prevalent in gregariously breeding species (reviewed in Hepper 1986; Beecher 1991). Thus, different mechanisms allowing parents to discriminate between their own and alien offspring have evolved (reviewed in Halliday 1983; Beecher 1991). The sensory modalities used during parent–offspring recognition can be visual (e.g. Dasser 1988), olfactory (e.g. Porter 1999), acoustical (e.g. Briefer & McElligott 2011) or a combination of the above; in most species, olfactory and acoustical cues are crucial for recognition (reviewed in Hepper 1986; Beecher 1991).

Parent–offspring recognition mechanisms have been thoroughly studied to elucidate the faculty of individual recognition

across different taxa (reviewed in Sherman et al. 1997; Tibbetts & Dale 2007). In species with a single offspring, parental offspring recognition fulfils the criteria of 'true individual recognition' (sensu Tibbetts & Dale 2007; Tibbetts et al. 2008), whereas offspring recognition in species with larger litters is generally considered 'class-level recognition' (sensu Tibbetts & Dale 2007; i.e. the distinction between familiar 'own' and unfamiliar 'alien' offspring classes). However, evidence for true individual offspring recognition exists even in species with multiple offspring where parents treat individual offspring differently (e.g. Draganoiu et al. 2006). Individual parent–offspring recognition based on vocal signatures is predicted to occur predominantly in gregarious species (Beecher 1991; Sherman et al. 1997; Tibbetts & Dale 2007), especially in species that cannot rely exclusively on positional information to recognize offspring (e.g. because the offspring is already fairly mobile or separation periods are large). Thus, vocal signatures facilitating parent–offspring recognition have been found in diverse taxa such as colonial sea birds (e.g. king penguin, *Aptenodytes patagonicus*; Jouventin et al. 1999), herd-living ungulates (e.g. sheep, *Ovis aries*; Searby & Jouventin 2003), colonial pinnipeds (e.g. Australian sea lion, *Neophoca cinerea*; Pitcher et al. 2010), group-living cetaceans (e.g. bottlenose dolphins, *Tursiops truncatus*; Sayigh et al. 1999) and gregarious bats (e.g. Mexican free-tailed bat, *Tadarida brasiliensis*; Balcombe 1990).

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In bats, maternal care is by far the predominating form of parental care, while paternal care is considered to be an exceedingly rare exception (Jones 2000; Kunz & Hood 2000). Most species selectively nurse their own offspring, and acoustically mediated mother–pup recognition is frequently used to prevent erroneous maternal investment (reviewed in: Fenton 1985; Kunz & Hood 2000; Wilkinson 2003). Thus, in many bat species, isolation calls produced by pups to solicit maternal care encode individual signatures (i.e. enough interindividual variation to facilitate offspring recognition by mothers; reviewed in Wilkinson 2003).

The Seba's short-tailed fruit bat, *Carollia perspicillata*, is a common frugivorous generalist that is widely distributed in the Neotropics from southern Mexico to southern Brazil (Cloutier & Thomas 1992). It is a gregarious species with resource defence polygyny as its mating system (Williams 1986; Fleming 1988). Females roost in the territories of harem males, which defend their territories vigorously against male competitors (Porter 1979a; Williams 1986). Several territories can be found in the same day-roost. Individual harems contain up to 18 females plus their current offspring (Williams 1986). Bachelor groups, solitary bachelors and mixed-sex subadult groups also occur in such collective day-roosts (Porter 1978, 1979a; Williams 1986). All pups of both sexes disperse from their natal harem after weaning (Porter 1978, 1979a). Dispersal from the natal colony seems to be slightly female biased; however, both sexes can also remain in their natal colony (more than 50% of females and 80% of males remain; Fleming 1988). Harem males are older and heavier but not necessarily larger than bachelor males (Williams 1986). They switch roost sites only when taking over a competitor's territory or after being displaced; nevertheless, displaced harem males sometimes establish a new territory elsewhere in the colony (Porter 1979a; Williams 1986). Male tenure as harem holders in the day-roost averages 277 days and can last up to 2 years (Williams 1986). Spatial fidelity is much lower in females than in males; transitions between harem territories occur frequently, on average every 17 days in captivity (Porter 1979a) and every 11 days in the wild (Williams 1986), and cannot be prevented by harem holders (Porter 1978; Williams 1986). Females can switch between up to 10 different harems per year but spend the majority of the time in one harem (their 'primary harem'; Fleming 1988). Thus, not all pups are sired by the harem male in whose territory they grow up (Porter & McCracken 1983). Harem males often return to the day-roost at night, presumably to defend their harem territories against intruders, whereas females and bachelor males generally do not return (Williams 1986). This indicates that harem groups do not forage together at night (Williams 1986). Female choice of the harem territory seems to be far more influenced by roosting site characteristics than by male characteristics (Williams 1986; Fleming 1988); however, the exact nature of female preferences for certain roosting sites characteristics remains unknown. Likely reasons for female grouping are predator avoidance and reduced metabolic energy expenditure (Williams 1986). Thus, the presence of harem males does not appear to be the cause for female grouping but merely a result. Correspondingly, free-living males have not been observed to actively recruit females in their territory (Williams 1986; Fleming 1988). However, observations on captive individuals suggest that harem males actively recruit females with hover flights and vocalizations (Porter 1979a). Additionally, Porter (1979a, b) proposed that harem males engage in parental care efforts by prompting females to reunite with their vocalizing pups (i.e. harassing and nudging them with folded wings) and suggested that females may choose harem males based on their "competency as caretakers" (Porter 1979a, page 415). If this is the case, then the behaviour of male *C. perspicillata* would represent one of the scarce examples of paternal care in bats (Kunz & Hood 2000). Moreover, it would mean that bats are capable of

recognizing so called 'third-party social relationships' (i.e. relationships in which the observer is not directly involved). Only a few species such as certain primates (reviewed in Tomasello & Call 1997; Seyfarth & Cheney 2003) and spotted hyaenas, *Crocuta crocuta* (Engh et al. 2005) have the faculty to recognize third-party social relationships (e.g. when associating mothers with their offspring: Cheney & Seyfarth 1980; or when assessing social alliances of conspecifics: Cheney & Seyfarth 1986). This faculty requires advanced cognitive abilities (reviewed in Tomasello & Call 1997) and, obviously, the ability to recognize individual conspecifics. Apart from the earlier studies mentioned above (Porter 1979a, b), no evidence we are aware of suggests the recognition of third-party social relationships in bats.

The aim of our study was to validate Porter's (1979a, b) interpretation of paternal care in *C. perspicillata* by investigating whether pup isolation calls elicit maternal and/or paternal care. We measured acoustic parameters of pup isolation calls to gather statistical evidence for an individual signature. Moreover, we conducted a playback experiment to test our hypothesis that mothers are capable of discriminating own pups and age-matched pups from other females based on isolation calls alone. Additionally, we tested whether the extent of maternal experience influences the strength of maternal response behaviour towards pup isolation calls. Using this playback experiment, we also aimed to corroborate the existence of paternal care in *C. perspicillata*, as suggested by Porter (1979a, b). If paternal care occurs, we expected harem males to react to pup isolation calls directly by approaching the speaker or indirectly by prompting the respective mothers to retrieve their vocalizing pups.

METHODS

Study Animals

We worked with a captive breeding colony of *C. perspicillata* housed in an indoor flight cage at the University of Ulm, Germany. The bats originated from a breeding colony of the Zoological Garden at Frankfurt, Germany. Our study colony consisted of 22–36 adult individuals (2010: 22, early 2011: 29; late 2011: 36) and occupied a 4.2 × 3 × 2 m wooden flight cage. Bats were kept at 25 °C ambient temperature and 70% humidity. They were provided with an ad libitum diet consisting of fresh fruit (banana, melon, cucumber, apple, grapes), vitamin enriched mash (semolina and fruit), honey and water. We maintained a reversed 12 h day and night cycle to facilitate observations (day: 0200–1400 hours; night: 1400–0200 hours). Individuals were banded with coloured split-plastic rings (A.C. Hughes Ltd, U.K., size X3) on their forearm, rendering them individually discernible from a distance. For the duration of playback trials, focal mothers were additionally marked with 1 × 1 cm pieces of adhesive reflective tape (Reflexia; T. Nacke, Germany) on their back and between the ears. The reflective tape fell off after approximately 60 min and did not have any obvious negative effects on the bats.

Female *C. perspicillata* normally bear a single offspring twice per year (Porter 1979a; Williams 1986; Fleming 1988). Parturition is highly synchronized (Porter 1978, 1979a; Fleming 1988) and females exhibit a postpartem oestrus 3–10 days after birth (Badwaik & Rasweiler 2000). Pups become volant at 2.5–4 weeks of age (Porter 1979a; Porter & McCracken 1983). We conducted playback experiments when pups were 15–20 days old. The rationale behind this was to use focal mothers with nonvolant or barely volant pups for playback experiments to make the scenario of a crashed pup vocalizing from the ground (mimicked by the speaker) plausible. Moreover, we wanted to perform playback experiments after the physiological oestrus of females to ensure that potential paternal

care behaviour would not be masked by male reproductive interests.

Sound Recordings

We recorded 905 isolation calls of 17 nonvolant pups (nine females, eight males) from three successive reproductive seasons (second season of 2010, first and second season of 2011). Isolation calls of *C. perspicillata* pups were partly audible, monosyllabic vocalizations that were normally produced in bouts of two to eight calls (Fig. 1). Subsequent bouts were separated by at least 30 s of silence. To elicit calling behaviour, pups were separated briefly from their mothers, hand-held and gently stroked. Immediately after recording, pups were returned to their mothers. Sound recordings were made with a high-quality ultrasonic recording set-up (300 kHz sampling rate and 16-bit depth resolution) consisting of an ultrasonic microphone (Avisoft USG 116Hme with condenser

microphone CM16; frequency range 1–200 kHz) connected to a laptop computer (JVC, MP-XP741DE) running the software Avisoft-Recorder v.4.2 (R. Specht, Avisoft Bioacoustics, Berlin, Germany). Only isolation calls with excellent signal-to-noise ratio were selected for analyses. To reduce temporal dependence among calls, we analysed isolation calls from at least 20 different call bouts per pup (905 isolation calls in total; 27–76 isolation calls per pup). During the first reproductive season in 2011, we performed playback experiments to test whether mothers could discriminate between own and alien pups based on isolation calls alone; isolation calls from the seven pups of the first reproductive season in 2011 were used as playback stimuli.

Isolation Call Analyses

We used Avisoft-SASLab Pro (v.5.2, R. Specht, Berlin, Germany) for acoustic analyses. Measurements were taken from spectrograms

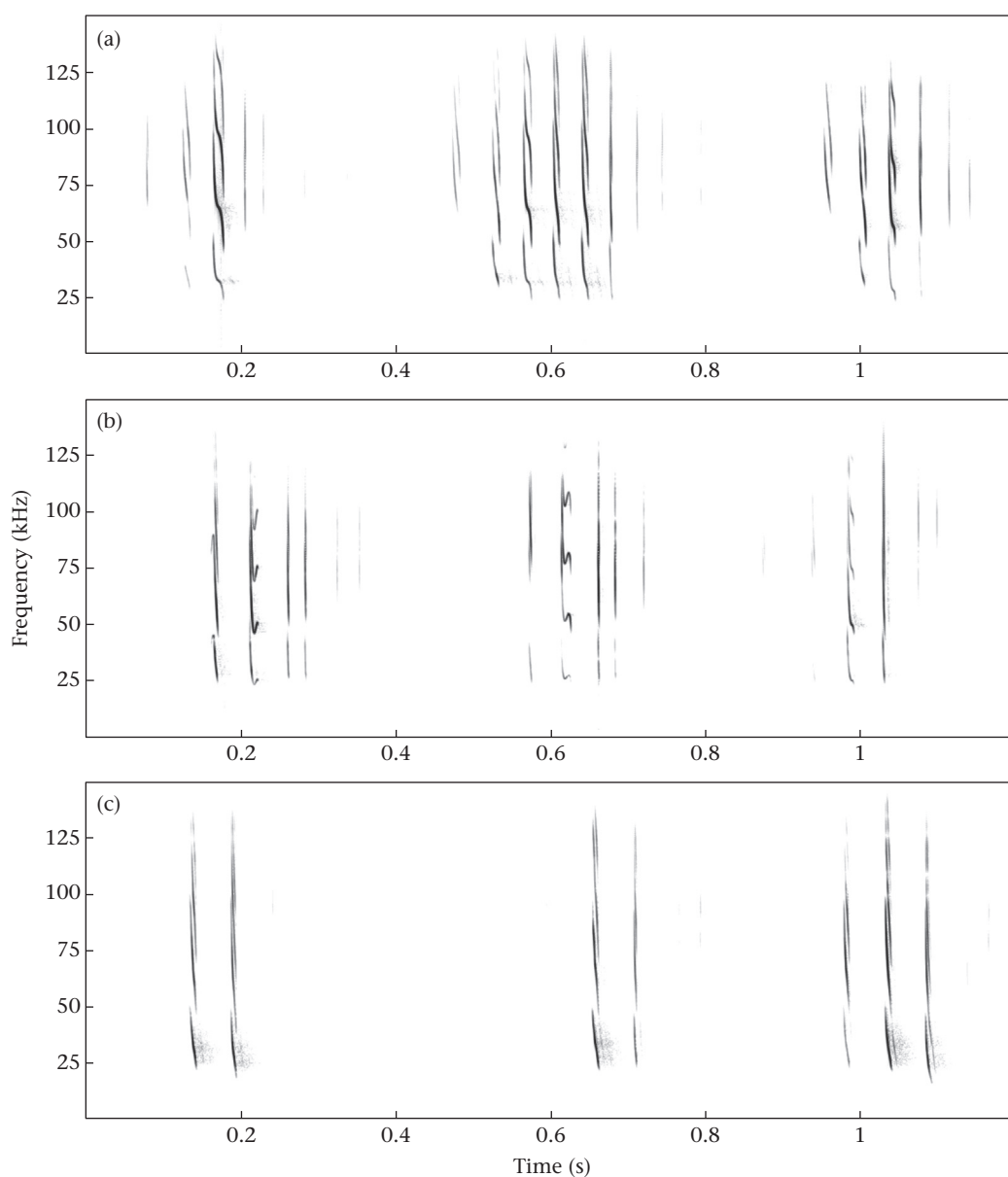


Figure 1. Isolation call bouts from three *C. perspicillata* pups, illustrating individual vocal signatures of a (a) 15-day-old female, (b) 17-day-old male and (c) 15-day-old male. The spectrograms depict frequency as a function of time and were generated using a 1024-point fast Fourier transform, a frame size of 100% and a Hamming window with 75% overlap. These settings resulted in a frequency resolution of 293 Hz and a temporal resolution of 0.8533 ms (files with 300-kHz sampling frequency and 16-bit depth).

using a Hamming window with 1024-point fast Fourier transform and 96.87% overlap, which resulted in a frequency resolution of 293 Hz and a time resolution of 0.1067 ms. Start and end of isolation calls were determined automatically (± 20 dB relative to the peak frequency of the signal). Isolation calls were multiharmonic, but we used only the fundamental frequency for measurements because it normally contained most of the sound energy. To characterize isolation calls, we measured five spectral parameters (peak frequency, minimum and maximum frequency, bandwidth, entropy) at 10 different locations distributed equally over the isolation call as well as averaged over the entire isolation call. Moreover, we measured three temporal parameters (duration, time to maximum amplitude, interval) and one waveform parameter (energy). In total, we obtained 59 acoustic parameters per isolation call.

We performed a principal component analysis with varimax rotation on these parameters and extracted seven principal components (with eigenvalues > 1), which explained 89.7% of the total variance. The principal component analyses fulfilled Kaiser–Meyer–Olkin (KMO) and Bartlett's test criteria. The KMO index measures sampling adequacy and was used together with Bartlett's test to examine the appropriateness of our factor analysis. We performed discriminant function analyses (DFA) on the seven principal components to test for an individual signature in isolation calls. All principal components were included simultaneously in the respective DFAs. The DFAs used a leave-one-out-cross-validation procedure that classified each call based on discriminant functions established with all calls except the call being classified. DFAs were performed for all 17 pups as well as separately for pups from different reproductive seasons (second season 2010: three pups; first and second season 2011: seven pups each) to resemble the respective natural situation in our breeding colony. The significance of the classification success was estimated by using one-tailed binomial tests (following Mundry & Sommer 2007). In DFA signal space, the distance between centroids (i.e. mean canonical score for every individual) is a good indicator of acoustic similarity (Boughman 1998; Knörnschild et al. 2007, 2010, 2012), with similarly sounding individuals clustering together. We calculated the squared Mahalanobis distance between centroids of 17 pups in the seven-dimensional DFA signal space to investigate whether pup sex influenced isolation call variation. With this analysis, we wanted to assess whether sex could function as a factor that helps females to identify their pups. For each pup, we calculated squared Mahalanobis distances between itself and individuals of the same or opposite sex. Subsequently, we calculated means for same-sex and opposite-sex distances for each pup and compared these distances using a Wilcoxon test. Statistical tests were conducted using SPSS v.17.0 (SPSS Inc., Chicago, IL, U.S.A.) and STATISTICA v.10 (Statsoft, Tulsa, OK, U.S.A.) and, whenever nonsignificant *P* values occurred, G*Power v.3.1.6 for post hoc power analyses (Franz Faul, University of Kiel, Kiel, Germany; Faul et al. 2007).

Isolation Call Playbacks

Playbacks were performed on seven mothers during the first reproductive season of 2011. To avoid pseudoreplication (McGregor et al. 1992), 30 different isolation calls from each of the seven nonvolant pups were used as playback stimuli. The stimuli were selected from 20 different isolation call bouts to minimize temporal dependence among calls uttered in succession. No more than two stimuli originated from the same isolation call bout. Isolation calls used as stimuli in a playback trial were recorded in the morning and the playback trial was performed later that same day. All playback stimuli were normalized (maximum amplitude to 0.0 dB). For each playback trial, we mimicked natural pup calling behaviour by

combining 30 different stimuli from one pup interspaced with silent intervals of 2–13 s into a single 3 min sound file. 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 (created with the random number generator in Microsoft Excel 2010) and was used only once. If vocalizations from an individual pup were used both as 'own stimuli' for the respective mother and as 'alien stimuli' for a different female, new sound files were generated. Since we used isolation calls from age-matched, cohabitating pups (from the same reproductive season) as 'alien stimuli' in the playbacks, we were able to test for individual discrimination and not for an effect of familiarity (since mothers should be at least somewhat familiar with isolation calls of cohabitating pups). Each playback trial had a total duration of 15 min and consisted of five 3-minute periods: pre-observation, first stimulus presentation, in-between observation, second stimulus presentation, and post-observation period. Each female was used only once as a focal animal in our playbacks. Isolation calls in both stimulus presentation periods came from the focal animal's own pup and from an alien one; the broadcasted order was pseudorandomized. Thus, females heard isolation calls from both their own pup and an alien pup during a single playback.

Playback stimuli (300 kHz sampling rate and 16-bit depth resolution) were broadcast with an ultrasonic amplifier (Avisoft Ultrasonic Power Amplifier; frequency response of ± 1 dB from 0.1 to 100 kHz) and an ultrasonic speaker (Avisoft Ultrasonic Speaker Scan, Speak R2904-700000; frequency response of ± 8 dB from 4 to 115 kHz) connected to a laptop computer (Panasonic CF-73, Panasonic Corp.) running the software Avisoft-Recorder v.4.2 (R. Specht, Avisoft Bioacoustics, Berlin, Germany). Amplitudes of playback stimuli were adjusted to 80 dB SPL at a distance of 0.1 m. The ultrasonic speaker was placed on the floor in one corner of the flight cage to mimic a crashed pup soliciting maternal care. A 1 m area around the ultrasonic speaker and the night-roost were monitored with a digital video camera with night-shot function (Sony Handycam DCR-SR32) and two infra-red lights (Sony HVL-IRM). Additionally, the night-roost was monitored with the ultrasonic recording equipment described above (300 kHz sampling rate). A detailed layout of the experimental set-up is provided in Fig. S1 in the [Supplementary material](#). Videos were analysed using the VLC-media-player (v.1.1.5; VideoLAN Organization, Paris, France) by an observer that was unaware which playback stimuli were being broadcast. Sound recordings were analysed with Avisoft-SASLab Pro (v.5.2, R. Specht, Berlin, Germany).

An hour before each playback trial (at 1330 hours), a mother–pup pair was removed from the colony in the flight cage using a custom-made hand-net. Immediately before the trial, the pup was separated from its mother and kept in a box lined with soft cloth ($20 \times 30 \times 20$ cm) at 25 °C ambient temperature for the duration of the trial. The focal mother was marked with adhesive reflective tape and released back into the flight cage (at 1430 hours). A playback trial started once the released focal mother had habituated and appeared calm (e.g. entered the night-roost and started grooming). The experimenter was positioned in room 1 and conducted the playback trial in room 2 from there (see [Supplementary Fig. S1](#)). Three different harem males, each with two to five adult females (10 adult females in total, seven reproductively active), occupied the three day-roosts in room 2. All three social groups used the night-roost in room 2, but the harem males never roosted in it simultaneously. Harem males were individually identified based on their coloured rings.

We recorded the behaviour of focal animals (mothers and their respective harem males) in the night-roost and in the vicinity of the ultrasonic speaker throughout the entire trial (i.e. during stimulus presentation periods but also during the silent observation periods

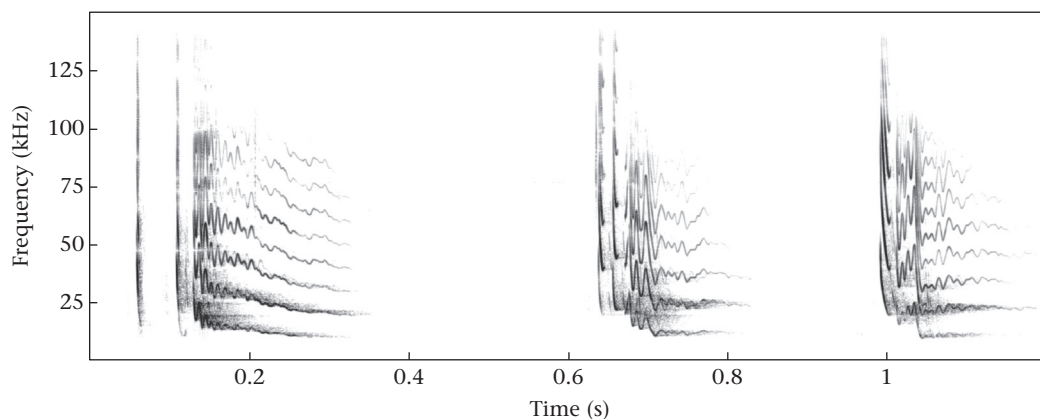


Figure 2. Vocal courtship behaviour of *C. perspicillata* males. Harem males produced sequences of trills with a variable structure when courting females in the night-roost. Together with stereotypic movements (see [Supplementary Video S1](#)), trills represented the conspicuous male courtship behaviour that was presumably misinterpreted by an earlier study ([Porter 1979b](#)) as males prompting females to retrieve calling pups. The spectrogram depicts frequency as a function of time and was generated using a 1024-point fast Fourier transform, a frame size of 100% and a Hamming window with 75% overlap (300-kHz sampling frequency, 16-bit depth; frequency resolution: 293 Hz; temporal resolution: 0.8533 ms).

to serve as a control for changes in motivation throughout the playback experiment). We defined maternal motivation as the females' willingness to approach the speaker and thus search for their pups. The behaviour of focal mothers in the vicinity of the ultrasonic speaker was classified into three categories that were ranked with regard to the intensity of the bats' approach behaviour (rank 1–3): pass (bats flew past the speaker without slowing down, rank 1), search flight (bats circled the area around the speaker, rank 2) and hover flight (bats hovered in mid-air over the speaker, rank 3). We noted the frequency of occurrence for all three behaviours (when focal bats left the 1 m area around the ultrasonic speaker, we considered the respective behaviour to be terminated). These values were combined into one response variable by multiplying them with their respective rank and subsequently adding them (number of passes \times 1 + number of search flights \times 2 + number of hover flights \times 3). We performed a Friedman test on the combined response variable to compare the reaction of focal mothers between the five playback phases. Harem males were never observed in the vicinity of the speaker. Since our sample size was relatively small ($N = 7$), we calculated exact instead of asymptotic P values (following [Mundry & Fischer 1998](#)).

The seven mothers tested during playbacks differed in their experience with rearing young. We ranked their relative experience (rank 1–5) based on the number of offspring each female had conceived prior to the one used in the playback, ranging from naïve (no pups) to very experienced (four pups). Naïve females had a rank of 1, very experienced females a rank of 5. The rationale behind this was to test whether the pup-rearing experience of mothers influenced their responses towards isolation call playbacks. We performed a linear regression to test for a relation between female experience and response strength to playbacks. A Shapiro–Wilk test was used to ascertain that residuals did not deviate significantly from a normal distribution.

We observed the behaviour of harem males in the night-roost and noted how harem males interacted with the focal mothers and other harem females during playback trials. Behavioural interactions consisted of hovering (hovering in mid-air directly in front of the female), sniffing (sniffing the female with body arched forward, sometimes accompanied by rapid wing beats), repeated wing poking (poking the female with one or both folded wings), and producing trill calls ([Fig. 2](#)), all of which were elements of male courtship behaviour (see [Supplementary material, Video S1](#)) which, in the case of oestrous females, eventually results in copulations (see [Supplementary Video S2](#)). We noted the number and total

duration of male courtship behaviour and used Friedman tests with exact P values ([Mundry & Fischer 1998](#)) to compare them between the five playback phases. Statistical tests were conducted using SPSS v.17.0 (SPSS Inc., Chicago, IL, U.S.A.) and, whenever nonsignificant P values occurred, G*Power v.3.1.6 for post hoc power analyses (Franz Faul, University of Kiel, Kiel, Germany; [Faul et al. 2007](#)). The pairwise post hoc comparisons for the Friedman test were calculated following the instructions provided by [Zar \(1999\)](#).

RESULTS

Statistical Evidence for an Individual Signature in Pup Isolation Calls

Pups could be distinguished statistically based on acoustical parameters extracted from their isolation calls ([Fig. 1](#)). A DFA with 905 isolation calls of 17 pups classified 55.4% of all calls to the correct individual ([Table 1](#)), which was significantly higher than expected by chance (5.9%; binomial test: $P < 0.0001$). The DFA classification success remained better than a chance classification (binomial tests: $P < 0.0001$ in all cases) when pups from different reproductive seasons were analysed separately, resembling the natural situation in the breeding colony. In 2010, 93.2% of all calls were classified to the correct individual (three pups, 220 isolation calls; chance: 33.3%). In early 2011, 54.1% of all calls were classified to the correct individual (seven pups, 399 isolation calls; chance: 14.3%) and in late 2011, 66.4% of all calls were classified to the correct individual (seven pups, 286 isolation calls; chance: 14.3%).

Despite the statistical evidence for an individual signature, no evidence for a sex-specific signature in pup isolation calls could be found. The squared Mahalanobis distance between pup centroids suggested that pups of the same sex did not cluster in DFA signal space (Wilcoxon test: $Z = -1.207$, $N = 17$, $P = 0.243$, power = 0.327) and, hence, exhibited no pronounced acoustic similarities in their isolation calls.

Experimental Evidence for an Individual Signature in Pup Isolation Calls

Mothers readily discriminated between isolation calls of their own pup and of an age-matched pup from another female. Maternal approach behaviour towards the speaker was significantly higher when isolation calls of a female's own pup were broadcast than during the three control phases or when isolation calls from another pup were broadcast (Friedman test:

Table 1
Statistical evidence for an individual signature in isolation calls of *C. perspicillata* pups

Data sets used in DFAs	Assessment of model fit	DF1	DF2
All 3 seasons (2010–2011) 17 pups 905 isolation calls	Eigenvalue	9.546	2.857
	% Variation	67.1	20.1
	Wilks' λ	0.006	0.065
	χ^2 (all $P < 0.0001$)	4541.524	2440.218
2010, 2nd season 3 pups 220 isolation calls	Eigenvalue	2.779	1.322
	% Variation	67.8	32.2
	Wilks' λ	0.114	0.431
	χ^2 (all $P < 0.0001$)	464.784	180.289
2011, 1st season 7 pups 399 isolation calls	Eigenvalue	1.142	0.405
	% Variation	58.9	20.9
	Wilks' λ	0.230	0.493
	χ^2 (all $P < 0.0001$)	574.504	276.624
2011, 2nd season 7 pups 286 isolation calls	Eigenvalue	8.693	1.132
	% Variation	84.9	11.1
	Wilks' λ	0.033	0.322
	χ^2 (all $P < 0.0001$)	946.054	314.604

DFA: discriminant function analysis.

$\chi^2_4 = 16.743$, $N = 7$, exact $P = 0.0001$; pairwise post hoc comparisons: 'own pup' versus all other phases: $Q > 4.1$, $N = 7$, $P < 0.001$; all other comparisons: $Q < 1.7$, $P > 0.5$; Fig. 3) Since all mothers were probably familiar with the isolation calls of pups from other females within their colony, the experimental task did not consist of discriminating between familiar and unfamiliar calls but rather between the calls of two familiar individuals. Females' motivation to search for their pups did not change significantly during the three observation phases of the playback trial (Friedman test pairwise post hoc comparisons, all comparisons: $Q < 1.7$, $P > 0.5$), suggesting that females did not habituate during the playback (i.e. showed less motivation to search for their missing pups).

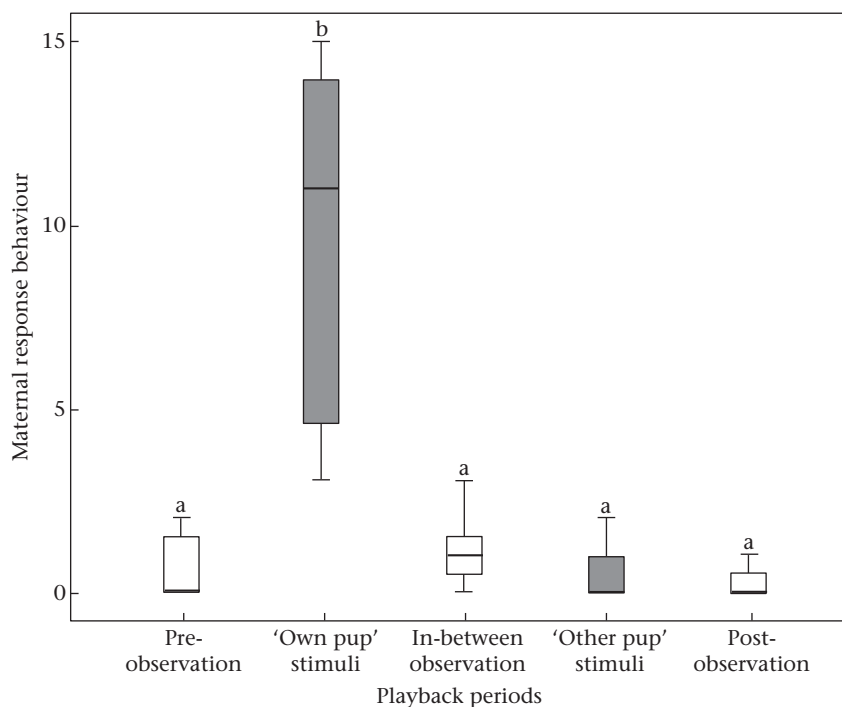


Figure 3. Maternal response behaviour (i.e. cumulative approach behaviour towards the speaker broadcasting pup isolation calls) during the 15 min playback experiment. Boxes show medians and interquartile ranges with minimum and maximum values as whiskers. Extreme values are not shown. Different superscript letters depict a significant difference ($P < 0.001$).

The extent of maternal approach behaviour towards the speaker broadcasting the isolation calls of the mothers' own pup increased significantly with increasing maternal experience (linear regression: $F_{1,6} = 8.595$, $\beta = 0.795$, $r^2 = 0.632$, $P = 0.033$). All females performed passes and search flights, but only the most experienced mothers performed additional hover flights.

Lack of Paternal Care in Reaction to Pup Isolation Calls

Harem males were never observed approaching the speaker or prompting focal females to retrieve their calling pups (mimicked by the speaker broadcasting isolation calls) during playback trials. However, harem males performed courtship behaviour towards six of the seven females tested in the playback experiment. The frequency of occurrence of male courtship behaviour towards focal females did not differ significantly between the five playback phases (Friedman test: $\chi^2_4 = 2.417$, $N = 7$, exact $P = 0.687$, power = 0.773). Correspondingly, the duration of male courtship behaviour towards focal females did not differ significantly between the five playback phases (Friedman test: $\chi^2_4 = 2.245$, $N = 7$, exact $P = 0.720$, power = 0.773). Harem males readily courted other females present in the night-roost during playback trials as well. Again, neither the frequency of occurrence nor the duration of male courtship behaviour towards other females differed significantly between the five playback phases (Friedman tests: frequency of occurrence: $\chi^2_4 = 7.282$, $N = 7$, exact $P = 0.112$, power = 0.773; duration: $\chi^2_4 = 8.350$, $N = 7$, exact $P = 0.063$, power = 0.773).

DISCUSSION

The results of our playback experiment clearly demonstrate that female *C. perspicillata* are capable of discriminating between isolation calls of own pups and age-matched pups from other females within their colony. Correspondingly, we found evidence for a strong individual signature encoded in pup isolation calls. Thus,

our results are in line with other studies reporting both statistical and experimental evidence for individual signatures in bat pup isolation calls (Mexican free-tailed bat, *Tadarida brasiliensis*: Balcombe 1990; greater sac-winged bat, *Saccopteryx bilineata*: Knörnschild & von Helversen 2008; greater spear-nosed bat, *Phyllostomus hastatus*: Bohn et al. 2007). Moreover, our results correspond to earlier work on *C. perspicillata* (Porter 1979b), which suggested that mothers might be capable of recognizing their own pups based on isolation calls alone. The experimental design used in the latter study involved the simultaneous removal of all pups from focal mothers and the subsequent presentation of isolation calls from individual pups. Only in 6 out of 16 trials did the correct mother react to broadcasts of her pup's isolation calls (Porter 1979b), providing tentative evidence for individual pup recognition at best. Yet, our results provide unequivocal evidence that maternal pup recognition (true individual recognition sensu Tibbetts & Dale 2007; Tibbetts et al. 2008) is possible based on pup isolation calls alone in *C. perspicillata*.

However, and in contrast to Porter's previous study (Porter 1979b), we found no evidence that male *C. perspicillata* reacted to the presentation of pup isolation calls. Porter (1979b) reported that, in 50% of trials, harem males responded to isolation call playbacks by inspecting the speaker and by prompting the respective mothers to approach the speaker (i.e. nudging them with folded wings and harassing them until the mothers approached the speaker). Porter (1979a, b) interpreted the males' behaviour as paternal care, a behaviour that has been found extremely rarely in bats (Kunz & Hood 2000). Specifically, Porter wrote: "Harem males appear to guard these separated infants by chasing away any bats who approach the infant and by approaching, vocalizing and/or chasing mothers until they reunite with their infants" (Porter 1979a, page 414). Paternal care in *C. perspicillata* would be a surprising phenomenon, since harem holders are not always the biological fathers of females' current pups (Porter & McCracken 1983), because females can switch freely between different harems (Porter 1978; Williams 1986). Porter (1979b) concluded that harem males provide care to unrelated pups to increase their reproductive fitness by retaining the pups' mothers in the harem. Porter wrote: "Since *Carollia* harem males do seem to contribute significantly to the survival of infants born in their harems by guarding them and by attempting to reunite them with their mothers, females may also be selecting mates on the basis of their competency as caretakers" (Porter 1979a, page 415). However, the postpartem oestrus of *C. perspicillata* females makes such a scenario unlikely since harem males impregnate females 3–10 days after parturition (Badwaik & Rasweiler 2000). During this time, pups remain almost constantly attached to their mothers, making paternal care as suggested by Porter (1979b) superfluous. Moreover, the results of our playback experiment indicate that harem males engage in courtship activities instead of paternal care. Courting male *C. perspicillata* exhibit stereotypic behaviours, including frequent wing poking (M. Knörnschild, personal observations; see Supplementary Video S1), which Porter presumably misinterpreted as males prompting females to respond to isolation call playbacks (Porter 1979a, b). Assuming that paternal care occurs in *C. perspicillata* and that it is possible to replicate Porter's original experiment (1979b), we would have expected differences in male behaviour during the different playback phases. However, male behaviour was not influenced by the presence or absence of playback stimuli and was not restricted to the focal female whose pup was missing, indicating that male courtship behaviour, not paternal care, is the most likely explanation for the observed behaviour. Correspondingly, no evidence for paternal care was found in free-living *C. perspicillata* colonies (Williams 1986; Fleming 1988).

Our results suggest that more experienced mothers show stronger responses to pup isolation calls than less experienced mothers. However, we only have correlative evidence and it is possible that maternal age acts as an additional factor since the experienced mothers in our study were older than the less experienced mothers. The positive relation between maternal experience and responsiveness to signalling offspring has not been demonstrated in bats until now, but it is a well-known phenomenon in other taxa (reviewed in Fleming & Li 2002; Poindron 2005). Maternal experience can have a crucial effect on the extent of maternal behaviours such as grooming, nursing and protecting offspring. Enhanced maternal responsiveness of multiparous females compared to primiparous females has been documented in rodents (e.g. laboratory rats, *Rattus norvegicus*: Fleming & Li 2002), lagomorpha (e.g. European rabbit, *Oryctolagus cuniculus*: González-Mariscal et al. 1998), artiodactyla (e.g. domestic sheep, *Ovis aries*: Dwyer & Lawrence 2000; Keller et al. 2003), perissodactyla (e.g. domestic horses, *Equus caballus*: Juarbe-Díaz et al. 1998) and primates (e.g. cynomolgus macaques, *Macaca fascicularis*: Timmermans & Vossen 1996). It is hypothesized that primiparous mothers show reduced responsiveness to signalling offspring because their inexperience causes neophobia and/or anxiety to deal with offspring's needs (reviewed in: Fleming & Li 2002; Poindron 2005). Therefore, it is possible that multiparous *C. perspicillata* mothers showed a stronger reaction towards pup isolation calls than primiparous mothers because the former already had experience with retrieving fallen pups from the ground, a potentially dangerous situation for wild bats due to the increased predation risk.

To conclude, our study demonstrates that pup isolation calls facilitate vocal mother–offspring recognition and subsequent maternal care behaviour that augments with increasing maternal experience. However, pup isolation calls did not elicit paternal care behaviour, which disproves an earlier assumption regarding the recognition of third-party social relationships in *C. perspicillata* (Porter 1979a, b). Thus, our results provide clarity about the parental care efforts and associated cognitive abilities of a bat species that is thoroughly studied in diverse research fields such as physiology (e.g. Laska 1990; Delorme & Thomas 1996), reproductive biology (e.g. Rasweiler & Badwaik 1997; Cretokos et al. 2005), neuroscience (e.g. Esser & Eiermann 1999; Scalia et al. 2013), ecology (e.g. Heithaus & Fleming 1978; Galindo-Gonzalez et al. 2000) and behaviour (e.g. Ratcliffe & ter Hofstede 2005; Ammersdörfer et al. 2012).

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Supplementary Material

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