Isolation call ontogeny in bat pups
(*Glossophaga soricina*)

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Abstract

Bat pups produce isolation calls to solicit maternal care. During maturation, pup isolation calls may gradually develop into echolocation calls or exist in parallel to them, depending on the species involved. We studied the ontogeny of isolation calls in nectivorous bats, *Glossophaga soricina*. Isolation calls of *G. soricina* pups were frequency modulated calls uttered in bouts of varying length. Newborn pups already produced both isolation calls and echolocation call precursors (which developed into 'normal' echolocation calls), indicating that isolation calls of *G. soricina* pups occur independently and exist in parallel to echolocation calls during ontogeny. We found strong statistical evidence for an individual signature encoded in isolation calls. Moreover, we provide evidence for considerable changes in isolation call parameters over a short ontogenetic time span. Throughout ontogeny, the call interval decreased significantly whereas most frequency parameters increased significantly and call entropy rose (i.e., isolation calls became less tonal but noisier).

Keywords

vocal ontogeny, mother–offspring recognition, individual signature, ontogenetic development, acoustic communication, Chiroptera.

1. Introduction

In vertebrates, correct offspring recognition is important in the light of parental investment and care (Ridley, 1978; Halliday, 1983). Parental care

is defined as any behaviour which increases the survival of the offspring and, therefore, raises the fitness of the parents and their young (Hamilton, 1964; Clutton-Brock, 1991). In mammals, lactation is a crucial aspect of parental care (Lee, 1997). Since lactation is energetically costly (König et al., 1988) and thus can be detrimental to maternal survival (Clutton-Brock et al., 1989), mothers should rely on a precise recognition process for identifying their offspring (Packer et al., 1992). In general, different sensory cues such as visual (e.g., Parr & de Waal, 1999), olfactory (e.g., Porter, 1998), or acoustical characteristics (Smolker et al., 1993) can be used to fulfil this task.

In bats, allonursing is very rare (e.g., Wilkinson, 1992). Thus, female bats should be able to recognise their own offspring to avoid investing resources in foreign pups (Clutton-Brock & Godfray, 1991). Mother–offspring recognition in bats is heavily biased towards olfactory and acoustical cues (reviewed in Fenton, 1985; Kunz & Hood, 2000; Wilkinson, 2003). Bat pups produce isolation calls when they are separated from their mothers to elicit maternal care. Isolation calls are distinguishable by comprising individual signatures and are thus suitable for mother–offspring recognition (e.g., Balcombe, 1990; De Fanis & Jones, 1996; Bohn et al., 2007; Knörnschild et al., 2007, 2013). Despite the fact that the general structure of isolation calls is innate (i.e., fully formed isolation calls are produced within hours after birth; reviewed in Fenton, 1985; Kunz & Hood, 2000), they can be modified over time based on social influences, i.e., social learning (Esser & Schmidt, 1989; Knörnschild et al., 2012).

Even without social learning being involved, isolation call parameters can change severely throughout ontogeny (e.g., Gelfand & McCracken, 1986; Jones et al., 1991). In most bat species studied to date, the ontogenetic change of isolation calls characteristics can be explained by maturation effects, e.g., the growth and modification of the pups' vocal tract during development (but see Esser & Schmidt, 1989; Knörnschild et al., 2012). In several bat species, pup isolation calls are precursors of echolocation calls and gradually develop into them (e.g., *Plecotus auritus*: De Fanis & Jones, 1995; *Carollia perspicillata*: Sterbing, 2002; *Myotis macrodactylus*: Wang et al., 2014). However, in the majority of bats studied to date, not only isolation calls but also echolocation calls (or echolocation call precursors) are already produced by young pups (e.g., *Noctilio albiventris*: Brown et al., 1983; *Pteronotus parnellii*: Vater et al., 2003; *Nyctalus noctula*: Knörnschild et al., 2007; *Eptesicus fuscus*: Mayberry & Faure, 2015; *Hipposideros pomona*: Jin et al., 2011). In the

latter scenario, isolation calls either drop out of the vocal repertoire when pups are weaned or mature into adult social calls, e.g., directive/contact calls (*Antrozous pallidus*: Brown, 1976; *Megaderma lyra*: Goymann et al., 1999; *Phyllostomus discolor*: Esser & Schmidt, 1989) or appeasement calls (*Saccopteryx bilineata*: Knörnschild et al., 2012).

In this study, we investigated the ontogeny of pup isolation calls in a Neotropical nectarivore, the Pallas' Long-Tongued Bat *Glossophaga soricina* (Phyllostomidae: Glossophaginae). Few phyllostomid bats have been studied with regard to their isolation calls so far (e.g., Bohn et al., 2007; Knörnschild et al., 2013). Since we had behavioural evidence that *G. soricina* mothers selectively nurse only their own offspring (pers. observations), we hypothesised that pup isolation calls encode an individual signature with sufficient inter-individual variation to facilitate maternal offspring recognition. Moreover, we tested whether sex-specific differences in isolation calls occur. We also tested whether we could observe ontogenetic changes in isolation call parameters over a relatively short time span (less than 1 month).

2. Material and methods

2.1. Husbandry

We caught ten *G. soricina* mothers and their current single offspring with mist nets and hand nets near their day-roosting areas in the Santa Rosa National Park in Costa Rica (10°50′N, 86°22′W). Species identification was confirmed with a taxonomic key of Costa Rican bats (Timm & La Val, 1998). Pups were sexed, their forearm size was measured with a regular calliper and their weight was taken (*Pesola* spring scale). We marked mothers with custom-made neck collars carrying individually-coloured plastic rings (size XCS; AC Hughes, Hampton Hill, UK). All pups were pre-volant (approximately 1–5 days of age; based on census data from day-roosts and the presence/absence of an umbilical cord) when captured. Mother-pup pairs were housed together in two large outdoor flight cages (5 pairs per flight cage; Eureka: Hexagon Screen House; 3.6 × 4.2 × 2.3 m). In addition to the mother–pup pairs, one male was housed with each group to provide a natural level of social heterogeneity in the groups (*G. soricina* forms single-male-multi-female groups; Pink, 1996). The flight cages contained a custom-made day-roost mounted on the ceiling. Adults were provided ad libitum with a daily prepared nectar substitute (Nektar plus (Nekton, Pforzheim, Germany)

solution: 10 g Nektar plus in 50 ml water). Free flying moths and butter-flies were available as well. Every fifth day we measured the pups' forearm length and body weight to evaluate their physical condition and growth. We checked regularly that the neck collars fitted properly but minimised entering the flight cage to avoid unnecessary stress for the bats. After the end of our data acquisition, all bats were released at the site of capture.

2.2. Sound recordings

We used a high-quality recording set-up (500 kHz sampling rate and 12 bit depth resolution) that included an ultrasonic microphone (Avisoft USG 116Hme with condenser microphone CM16; frequency range 1–200 kHz) connected to a transportable laptop computer (JVC, MP-XP741DE). The computer contained the software Avisoft-Recorder v.4.2 (R. Specht, Avisoft Bioacoustics, Germany). To record isolation calls, each pup was separated from its mother and carefully held in both hands. After touching it gently, the pup produced isolation calls (and echolocation calls) which could be recorded with an excellent signal-to-noise ratio and without the risk of confusing them with vocalisations from nearby conspecifics. However, this approach only allowed us to record provoked isolation calls, and not spontaneously produced isolation calls (sensu Mayberry & Faure, 2015). Individual recording sessions never lasted longer than 15 minutes in total. Focal pups were recorded separately and recording sessions were conducted in a flight cage not housing any bats. Theoretically, a foraging conspecific could have passed the flight cage while we recorded the pups but this was not problematic for two reasons: First, the low microphone gain and high directionality of *G. soricina*'s echolocation calls prevented us from confusing recordings of our focal pups with those of passing conspecifics. Second, the distortion induced by the flight cage membrane would have provided us with a powerful way of discriminating between focal and unwanted recordings (but this never occurred). Immediately after the recording session, pup and mother were reunited and released together into the flight cage. The first recording session was conducted directly after mother–pup pairs were captured from their natural day-roost, while subsequent recording sessions were conducted every five days (see Table A1 in the Appendix for details).

2.3. Acoustic analyses

We used Avisoft SASLab Pro software (v.5.2.09; R. Specht, Avisoft Bioacoustics, Glienicke, Germany) for our acoustic analyses. Spectrograms were

created using a Hamming window with 1024-point fast Fourier transform and 96.87% overlap (frequency resolution 488 Hz; time resolution 0.064 ms). Start and end of isolation calls were determined automatically (-20 dB relative to the peak frequency of the signal). Even though isolation calls were multiharmonic, we used only the fundamental frequency (first harmonic) for measurements because it contained most of the sound energy. We used automated parameter measurements provided by Avisoft SASLab Pro to analyse calls. We measured three temporal parameters (duration, time to maximum amplitude, interval) and five spectral parameters (peak frequency, minimum and maximum frequency, bandwidth, entropy) averaged over the fundamental frequency of the entire isolation call. Time to maximum amplitude refers to the distance in time from the start of the call to the location of the maximum amplitude (i.e., the loudest part of the call), indicating which part of the call is emphasised. Entropy is a measure of the width and uniformity of the power spectrum (on a scale of 0–1, white noise has an entropy value of 1 and a pure tone has an entropy value of 0); it assesses whether a call can be perceived as predominantly tonal or noisy. We also determined the peak frequency at the start, centre and end of each isolation call. Moreover, we measured the above mentioned five spectral parameters (peak frequency, minimum and maximum frequency, bandwidth, entropy) at 10 different locations distributed equally over the fundamental frequency of the isolation call to estimate the frequency and entropy curvature of the call. These derived curvature parameters combined various frequency (or entropy) measurements, thus reducing multicollinearity between original acoustic parameters considerably. This was achieved by performing principal component analyses (PCAs) with varimax rotation on the above mentioned parameters (one PCA on all 40 frequency parameters and another PCA on all 10 entropy parameters). For the frequency curvature, we extracted four principal components (with eigenvalues > 1) which explained 93.3% of the total variance. For the entropy curvature, we extracted one principal component (with an eigenvalue > 1) which explained 100% of the total variance. Both PCAs 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 PCAs. In total, we obtained 11 original acoustic parameters and five derived acoustic parameters (four parameters describing frequency curvature and one

parameter describing entropy curvature) per isolation call. Parameters of isolation calls belonging to the same bout were averaged to minimise temporal dependence among calls produced in direct succession. Overall, we analysed 241 isolation call bouts from 10 different pups.

2.4. Statistical analyses

All statistical tests were conducted in SPSS (v.20; IBM SPSS Statistics, Chicago, IL, USA). To test for an individual signature in isolation calls, we performed a discriminant function analysis (DFA) with 7 pups for which we analysed at least 15 isolation call bouts (15–46 bouts per pup, 213 bouts in total). We selected 14 acoustic parameters for the DFA, namely duration, time to maximum amplitude, peak frequency (start), peak frequency (end), peak frequency (centre), peak frequency (mean), minimum frequency (mean), maximum frequency (mean), entropy (mean), frequency curvature 1–4 and entropy curvature 1. All parameters were checked for multicollinearity and included simultaneously into the DFA. We used a cross-validation procedure to estimate the correct classification success, which classified each bout based on discriminant functions established with all bouts except the bout being classified ($n - 1$ cross-validation procedure). The DFA was adjusted to the unequal number of analysed bouts per individual by computing group sizes based on prior probabilities. We performed a binomial test to check whether the obtained classification success was better than a random classification.

To investigate whether there were sex-specific differences in isolation call parameters, we used unpaired t-tests. All ten pups were included in these tests. In addition to the above mentioned 14 parameters in the DFA, we used call interval and mean bandwidth (averaged over the fundamental frequency of the entire call) as well.

For the estimation of the ontogenetic trajectory of isolation call parameters, we performed separate Linear Mixed Models (LMMs) for each call parameter (with age in 5-day steps as covariate, sex as fixed factor and pup ID as random factor) to estimate the respective slopes of the linear regression lines. Slopes were used as a basic proxy for ontogenetic development (i.e., a positive slope value indicated that a certain parameter increased during ontogeny). Seven pups, each with at least three subsequent recording sessions, were included in the analyses. Slopes were estimated for 16 acoustic parameters (see list above).

3. Results

During our recording sessions, pups emitted isolation call bouts consisting of frequency modulated, monosyllabic calls produced in rapid succession. Isolation calls, measured at the fundamental frequency, had a mean peak frequency of 50.2 ± 6.9 kHz (start of the call 66.7 ± 7.0 kHz, centre 47.2 ± 7.0 kHz, end 38.8 ± 7.6 kHz) and a mean duration of 10.3 ± 6.6 ms. The call interval was 42.0 ± 11.3 ms and the time to maximum amplitude was 4.6 ± 3.7 ms. Detailed measurements of isolation calls can be found in Table A2 in the Appendix. All pups produced both isolation calls and echolocation call precursors (i.e., calls clearly recognisable as echolocation calls but with reduced bandwidth compared to 'normal' echolocation calls) during the first recording session when pups were between 1–5 days old (Figure 1). We distinguished between echolocation call precursors and isolation calls mainly based on duration; all isolation calls were considerably longer than echolocation call precursors (see Table A2 for details; echolocation call precursors always had a duration of less than 2 ms).

3.1. Individual signature in pup isolation calls

Individual *G. soricina* pups could be distinguished based on acoustic parameters of their isolation call bouts (Figure 2, Table 1). The acoustic parameters entropy curvature, duration and call curvature 2 contributed mainly to the discriminant function 1, while peak end frequency, call curvature 1, mean minimum frequency and mean maximum frequency contributed mainly to the discriminant function 2 (Table 2). Those parameters were thus most important for distinguishing between individual pups based on their isolation call bouts. A DFA with 213 isolation call bouts of 7 pups classified 70.0% of all bouts to the correct individual (Figure 3), which was significantly higher than expected by chance (14.3%; binomial test, $p < 0.0001$).

We found no evidence for a sex-specific signature. None of the 16 analysed acoustic parameters showed significant differences between male and female pups (unpaired t-tests; $p > 0.324$ in all cases; see Table A3 in the Appendix for details), suggesting that isolation call bouts do not encode information on pups' sex.

3.2. Ontogenetic trajectories of pup isolation calls

We used slope estimates of linear regressions (based on separate LMMs for each acoustic parameter) to assess the ontogenetic trajectory of isolation call parameters (Table 3). As pups matured, the call interval decreased
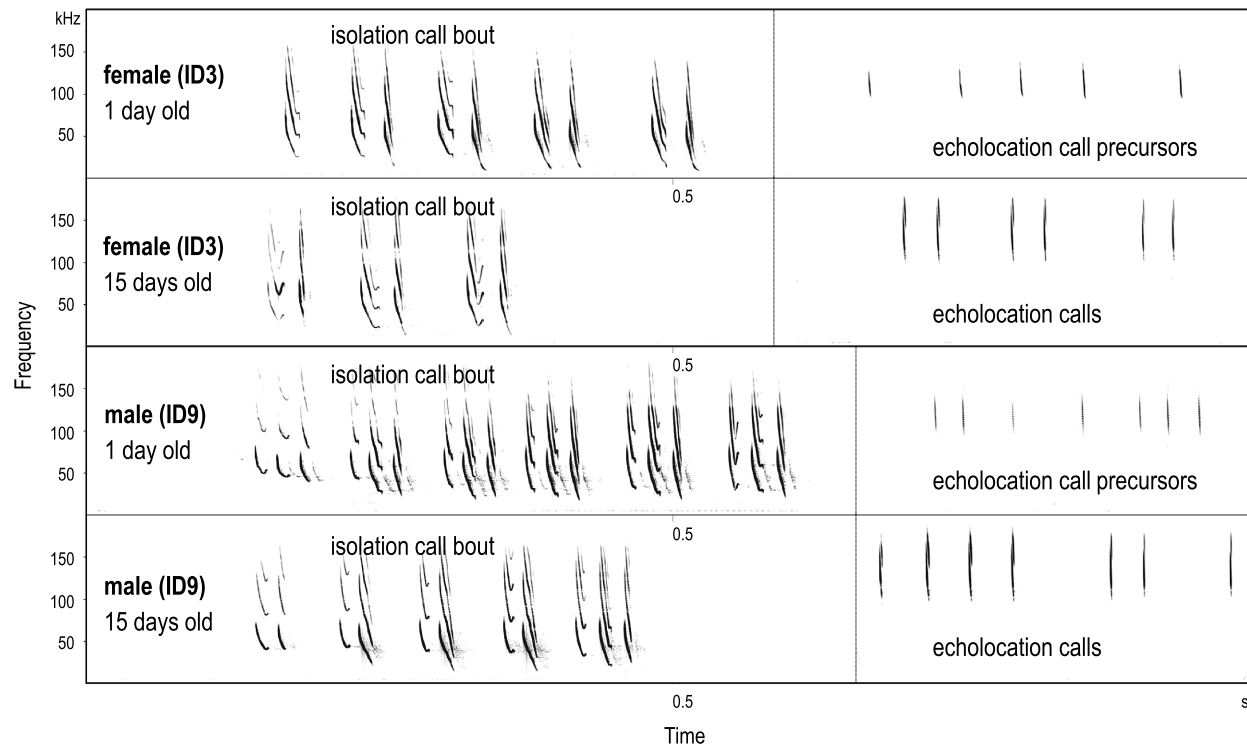
Figure 1. Isolation call bouts and echolocation call precursors (at day 1) and adult-like echolocation calls (at day 15) of two *G. soricina* pups (one female, one male), recorded at day 1 and day 15 of age. Spectrograms (frequency over time) were generated using a 1024-point fast Fourier transform, a frame size of 100% and a Hamming window with 93.75% overlap.
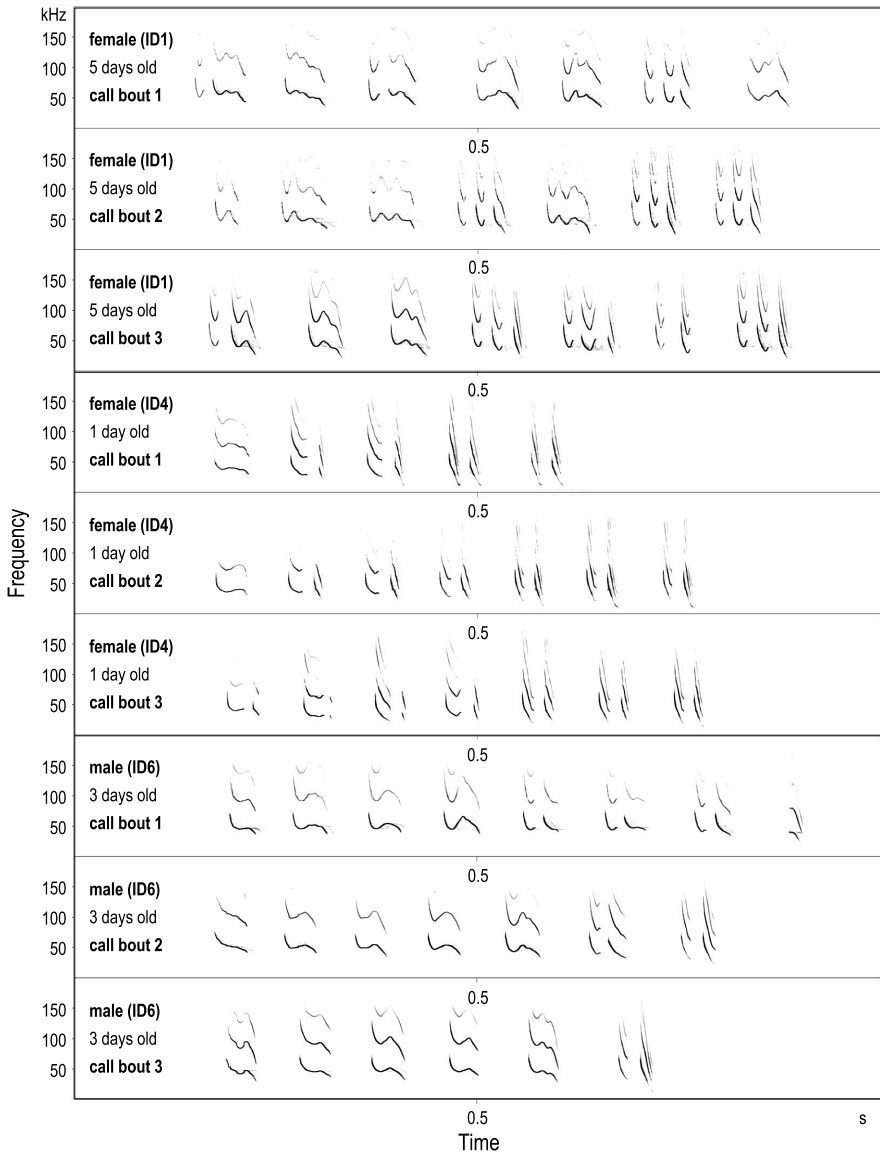
Figure 2. Three isolation call bouts from three different *G. soricina* pups (two females, one male), illustrating the individual vocal signature encoded in acoustic parameters of isolation calls. Isolation call bouts of each pup were recorded on the same day, but not in direct succession. Spectrograms were generated using a 1024-point fast Fourier transform, a frame size of 100% and a Hamming window with 93.75% overlap.

Table 1.

Assessment of model fit of the discriminant function analysis.

Function	Eigenvalue	% of variance	Test of function	Wilkins λ	χ^2	df	p
1	4.743	66.0	1 to 6	0.028	719.642	84	<0.0001
2	1.212	16.9	2 to 6	0.161	367.437	65	<0.0001
3	0.662	9.2	3 to 6	0.357	207.488	48	<0.0001
4	0.240	3.3	4 to 6	0.593	105.160	33	<0.0001
5	0.197	2.7	5 to 6	0.736	61.863	20	<0.0001
6	0.136	1.9	6	0.880	25.667	9	0.002

significantly. In addition, several frequency parameters, namely the peak frequency at the start, centre and end of each call as well as the mean minimum frequency (calculated over the entire fundamental frequency of the call) increased significantly. Moreover, the mean entropy showed a trend to increase (isolation calls became noisier and less tonal). However, it is important to note here that our assessment of ontogenetic trajectories in pup isolation calls was limited by the small sample size available ($N = 7$). Pup sex never had a significant effect in any of the conducted LMMs (all $p \geqslant 0.507$).

Table 2.

Correlations between acoustic parameters and standardised canonical discriminant functions (DF1–6), indicating the contribution of different acoustic parameters to the discriminant functions.

Parameter	DF1	DF2	DF3	DF4	DF5	DF6
Entropy curvature	−0.666*	0.352	0.230	0.394	−0.044	0.070
Duration	0.621*	−0.124	0.421	−0.097	0.281	0.080
Frequency curvature 2	−0.590*	0.061	0.332	0.300	−0.193	0.421
Peak frequency (end)	−0.032	0.665*	0.221	0.150	−0.212	0.166
Frequency curvature 1	0.091	0.643*	0.374	0.028	−0.195	0.056
Min frequency (mean)	−0.042	0.530*	0.466	0.207	−0.063	0.276
Max frequency (mean)	−0.174	0.376*	0.247	0.122	−0.220	0.322
Frequency curvature 4	−0.084	0.312	−0.656*	0.327	0.269	0.079
Peak frequency (centre)	−0.099	0.425	0.547*	0.259	−0.286	0.326
Time to max. amplitude	0.463	−0.253	0.494*	−0.010	0.458	0.015
Entropy (mean)	−0.139	−0.071	−0.052	0.406*	−0.017	−0.070
Frequency curvature 3	0.043	−0.086	0.202	0.282*	−0.054	0.280
Peak frequency (mean)	−0.146	0.390	0.404	−0.021	−0.152	0.428*
Peak frequency (start)	−0.005	0.314	0.249	0.379	−0.122	0.411*

* Highest absolute correlation between every parameter and a DF.
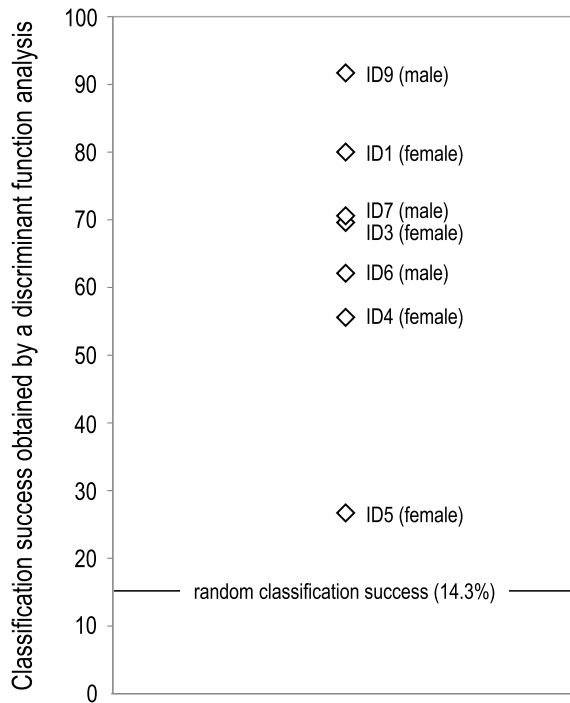
Figure 3. Classification success for seven *G. soricina* pups obtained by a DFA with $n - 1$ cross-validation (random classification success: 14.3%). ID and sex of the pups are given in the figure.

4. Discussion

The high classification success of our DFA indicates that isolation call bouts of *G. soricina* pups encode an individual signature. Such a vocal signature probably facilitates mother–offspring recognition, especially when pups become increasingly mobile during ontogeny and mothers cannot rely solely on spatial memory to retrieve the correct pup (for overviews see Kunz & Hood, 2000; Wilkinson, 2003). We suggest playback experiments to clarify whether vocal mother–offspring recognition in *G. soricina* is unidirectional/nonmutual (as in, e.g., *Tadarida brasiliensis*: Balcombe, 1990; *Pipistrellus pygmaeus*: DeFanis & Jones, 1996; *Saccopteryx bilineata*: Knörnschild & von Helversen, 2008) or bidirectional/mutual (as in e.g., *Plecotus auritus*: De Fanis & Jones, 1995; *Phyllostomus discolor*: Esser, 1998). Moreover, it is unclear to date whether the strength of the individual signature remains the same during the ontogeny of *G. soricina* pups (as in e.g.,

Table 3.
Linear regression slopes as a proxy for the ontogenetic trajectory of isolation call parameters in *G. soricina* pups.

Parameter	*F*	df	*p*	Slope	Trajectory
Duration	0.072	1, 21.264	0.79	−0.07 ms	None
Interval	15.046	1, 21.540	0.001	−3.05 ms	Shorter
Time to max. amplitude	1.479	1, 21.723	0.237	0.30 ms	None
Peak frequency (start)	7.195	1, 25.931	0.013	2.1 kHz	Higher
Peak frequency (end)	5.029	1, 24.911	0.034	1.7 kHz	Higher
Peak frequency (centre)	5.222	1, 24.425	0.031	1.6 kHz	Higher
Peak frequency (mean)	3.09	1, 24.677	0.091	1.3 kHz	Higher
Min frequency (mean)	5.833	1, 24.467	0.024	1.5 kHz	Higher
Max frequency (mean)	3.351	1, 23.964	0.08	1.4 kHz	Higher
Bandwidth (mean)	0.017	1, 22.333	0.898	0.1 kHz	None
Entropy (mean)	3.036	1, 23.695	0.094	0.0052	Greater
Frequency curvature 1	5.769	1, 24.960	0.024	0.1883	More
Frequency curvature 2	8.441	1, 21.446	0.008	0.1468	More
Frequency curvature 3	1.217	1, 26.000	0.28	0.0754	None
Frequency curvature 4	2.142	1, 23.086	0.157	0.1133	None
Entropy curvature	13.738	1, 21.410	0.001	0.1809	More

Slope estimates based on LMMs with age in 5-day steps as covariate, sex as fixed factor and pup ID as random factor; sex never had a significant effect in the models.

Tadarida brasiliensis: Balcombe, 1990; *Saccopteryx bilineata*: Knörnschild et al., 2012) or whether it changes (as in e.g., *Pipistrellus pygmaeus*: Jones et al., 2001; *Nyctalus noctula*: Knörnschild et al., 2007). In addition to an individual signature, pup isolation calls can also encode group or family affiliation (Scherrer & Wilkinson, 1993; Bohn et al., 2007; Knörnschild et al., 2007, 2012). We could not test this for *G. soricina* with our current dataset, because the exact social origin of the mother–pup pairs in our study appeared unclear. Moreover, we did not detect a sex-specific vocal signature in isolation calls of *G. soricina* pups, which corroborates findings from other bat species (e.g., *Saccopteryx bilineata*: Knörnschild et al., 2012; *Carollia perspicillata*: Knörnschild et al., 2013).

We observed a considerable change of isolation call parameters even during a relatively short ontogenetic time span of 15 days (median; range 11–26 days). Since we analysed provoked isolation calls (instead of spontaneously produced isolation calls), it is possible that we underestimated the ontogenetic change in call parameters; a recent study on *Eptesicus fuscus* pups (Mayberry & Faure, 2015) found that provoked isolation calls

resembled those of younger bats (possibly because provoked calls are likely uttered under greater distress than spontaneously produced calls and 'sounding younger' might facilitate maternal assistance). Comparable to other bat species, several frequency parameters of *G. soricina*'s isolation calls increased, while the inter-call interval decreased (as in, e.g., *Tadarida brasiliensis*: Gelfand & McCracken, 1986; *Phyllostomus discolor*: Esser & Schmidt, 1989; *Pipistrellus pygmaeus*: Jones et al., 1991; *Nycticeius humeralis*: Scherrer & Wilkinson, 1993; but see Schmidt et al., 1981; van Parijs & Corkeron, 2002). Similar ontogenetic trajectories are found for echolocation calls, no matter whether they develop from pup isolation calls (as in, e.g., *Carollia perspicillata*: Sterbing, 2000; *Myotis macrodactylus*: Wang et al., 2014) or occur independently (as in, e.g., *Eptesicus fuscus*: Moss, 1988; *Vespertilio sinensis*: Jin et al., 2012; *Artibeus jamaicensis*: Carter et al., 2014). In *G. soricina*, newborn pups produced not only isolation calls but also echolocation call precursors, indicating that isolation calls and echolocation calls occur and develop independently in our focal species, even though they show several parallel ontogenetic trajectories (except the ontogenetic increase in isolation call entropy, which is not to be expected for echolocation calls since increasing entropy indicates that calls become noisier). In some bat species, isolation calls mature into adult social calls (e.g., Brown, 1976; Esser & Schmidt, 1989; Goymann et al., 1999; Knörnschild & von Helversen, 2008). At present, we can only speculate whether this is the case in *G. soricina* or whether isolation calls are dropped from the adult vocal repertoire when pups are weaned. There is a certain resemblance between pup isolation calls (in their simple, downward modulated form; e.g., the last isolation call depicted in Figure 1) and adult contact calls (which are produced by adult males during tandem flights with females; Knörnschild et al., 2010) but such a superficial resemblance alone is not conclusive evidence for an ontogenetic development from pup isolation calls to adult contact calls. Nevertheless, it is an interesting area for future research on the vocal communication of *G. soricina*.

Our study shows that bouts of isolation calls of *G. soricina* pups encode enough inter-individual variation to facilitate maternal offspring recognition. Moreover, it adds to the growing body of evidence that communication calls and echolocation calls occur independently during ontogeny and thus follow different developmental and evolutionary pathways in most bat species studied to date. In concordance with these findings, different neural control

mechanisms are responsible for the production of communication calls and echolocation calls in bats (e.g., Fenzl & Schuller, 2005, 2007; Metzner & Schuller, 2010).

Acknowledgements

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Appendix

Table A1.
Focal *G. soricina* pups in our analyses.

Individual (ID)	Sex	Time span (days)	Date of recording	Number of analysed bouts
ID1	Female	19	14 January 2016	10
			19 January 2016	9
			24 January 2016	7
			29 January 2016	11
ID2	Female	1	7 January 2016	8
ID3	Female	21	12 January 2016	12
			17 January 2016	10
			22 January 2016	11
			27 January 2016	6
			1 February 2016	8
ID4	Female	11	4 January 2016	7
			9 January 2016	6
			14 January 2016	5
ID5	Female	16	7 January 2016	8
			12 January 2016	3
			17 January 2016	1
			22 January 2016	3
ID6	Male	11	21 January 2016	10
			26 January 2016	10
			31 January 2016	9
ID7	Male	26	4 January 2016	5
			9 January 2016	6
			14 January 2016	8
			19 January 2016	5
			24 January 2016	6
			29 January 2016	5
ID8	Male	1	19 January 2016	10
ID9	Male	16	17 January 2016	7
			22 January 2016	10
			27 January 2016	9
			1 February 2016	10
ID10	Male	6	24 January 2016	7
			29 January 2016	3

ID2, 8 and 10 were only included in the analysis of sex-specific differences in call parameters due to the low number of recording sessions per pup.

Table A2.

Measurements of isolation calls from 10 *G. soricina* pups.

Pup ID	Session	Duration (ms)	Interval (ms)	Disttomax (ms)	Peak frequency (kHz)				Mean frequency (kHz)		Bandwith (mean, kHz)	Entropy (mean)
					Start	End	Centre	Mean	Min	Max		
1	1	18.88	64.27	6.80	68.0	39.8	45.5	45.5	40.6	60.2	19.5	0.3594
	2	22.09	46.65	6.71	70.9	43.4	49.8	47.9	45.1	61.2	16.1	0.3489
	3	21.83	50.50	13.94	73.5	52.1	53.5	55.9	50.3	66.7	16.3	0.3050
	4	18.98	52.81	7.99	72.3	49.0	50.9	53.5	47.1	63.8	16.6	0.3359
2	1	8.75	38.46	2.45	61.5	31.5	40.1	47.6	35.8	61.3	25.5	0.3826
3	1	5.98	49.01	2.15	55.9	30.3	41.2	45.4	35.5	56.2	20.7	0.3701
	2	3.78	52.60	1.58	76.9	49.9	63.4	65.1	53.5	78.2	24.6	0.3574
	3	4.20	42.98	1.71	77.1	44.5	55.9	58.9	46.2	77.0	30.7	0.4188
	4	2.94	40.22	1.45	78.1	49.6	60.6	61.5	52.9	76.2	23.3	0.3879
	5	2.66	31.96	1.28	74.3	53.3	63.6	63.2	55.3	77.7	22.3	0.3872
4	1	8.81	45.76	3.24	53.8	27.4	37.1	41.5	30.5	54.0	23.5	0.3303
	2	7.88	41.90	2.49	65.2	36.7	45.1	52.1	38.5	63.1	24.6	0.3542
	3	7.46	47.27	3.39	68.2	37.4	49.7	55.7	41.3	67.9	26.5	0.3715
5	1	9.39	38.46	3.93	56.7	33.2	38.7	41.7	35.0	55.4	20.3	0.3420
	2	7.97	30.87	4.91	63.3	38.2	41.9	44.2	39.2	57.2	18.0	0.3673
	3	9.03	34.00	5.01	70.1	45.9	50.0	54.6	44.7	66.4	21.6	0.3825
	4	7.77	32.69	2.65	69.1	43.0	49.5	56.1	44.9	67.3	22.4	0.4058
6	1	22.18	74.62	11.27	63.6	34.4	45.1	48.0	39.1	56.6	17.5	0.3367
	2	19.68	49.82	11.20	65.9	31.3	43.5	44.5	37.6	55.7	18.0	0.3516
	3	28.66	62.09	16.58	66.8	29.2	46.0	44.6	39.6	53.7	14.1	0.3642

Table A2.

(Continued.)

Pup ID	Session	Duration (ms)	Interval (ms)	Disttomax (ms)	Peak frequency (kHz)				Mean frequency (kHz)		Bandwith (mean, kHz)	Entropy (mean)
					Start	End	Centre	Mean	Min	Max		
7	1	6.02	39.33	1.73	63.5	40.6	47.0	51.4	42.9	62.8	19.9	0.3254
	2	6.04	39.54	1.62	69.0	40.5	48.3	56.1	44.4	70.5	26.0	0.3898
	3	7.12	39.16	2.71	72.2	43.0	48.2	52.6	43.2	67.1	23.8	0.3872
	4	5.33	28.40	2.97	80.3	49.0	55.4	56.8	48.2	72.7	24.4	0.3922
	5	7.65	29.89	3.07	61.6	34.6	42.1	44.5	36.1	56.7	20.6	0.3577
	6	4.62	52.78	1.65	64.7	33.4	44.7	51.1	40.4	65.5	25.1	0.3895
8	1	10.74	40.08	5.07	52.1	32.6	38.8	39.9	33.0	50.3	17.2	0.3748
9	1	7.99	30.06	2.41	57.7	26.8	39.3	44.7	33.1	55.6	22.4	0.3721
	2	7.65	32.97	3.57	65.6	32.0	42.4	42.3	34.7	58.6	23.8	0.3983
	3	8.01	33.50	3.83	63.6	31.7	40.4	41.0	33.2	55.3	22.1	0.3956
	4	8.95	29.85	4.12	63.9	31.2	40.2	42.0	33.7	57.5	23.7	0.3776
10	1	7.56	40.92	3.36	73.8	41.4	49.2	52.5	43.0	69.0	25.9	0.3978
	2	13.08	40.92	5.61	64.2	42.2	53.3	54.7	48.6	64.0	15.4	0.3157

Values are averaged per individual, bout and recording session.

Table A3.
Results of the *t*-tests that were used for evaluating possible sex differences between isolation calls.

Parameter	*t*-test for mean equality			Mean difference	Standard error of difference	95% confidence interval of difference	
	t	df	*p*			Lower	Higher
Duration (ms)	−0.388	8	0.708	−1.589	4.089	−11.019	7.842
Interval (ms)	0.019	8	0.985	0.140	7.427	−16.986	17.266
Time to max. amplitude (ms)	−0.769	8	0.464	−1.727	2.245	−6.904	3.450
Peak frequency (start) (kHz)	0.311	8	0.764	1.252	4.030	−8.041	10.545
Peak frequency (end) (kHz)	0.782	8	0.457	2.850	3.642	−5.550	11.249
Peak frequency (centre) (kHz)	0.464	8	0.655	1.639	3.529	−6.499	9.776
Peak frequency (mean) (kHz)	1.048	8	0.325	3.469	3.309	−4.162	11.100
Min frequency (mean) (kHz)	0.608	8	0.56	2.024	3.327	−5.649	9.697
Max frequency (mean) (kHz)	0.987	8	0.352	3.922	3.972	−5.238	13.082
Bandwidth (mean) (kHz)	0.863	8	0.413	1.895	2.196	−3.168	6.958
Entropy (mean)	−0.793	8	0.451	−0.008	0.010	−0.032	0.016
Frequency curvature 1	0.684	8	0.514	0.270	0.395	−0.641	1.182
Frequency curvature 2	0.249	8	0.810	0.110	0.443	−0.910	1.130
Frequency curvature 3	−0.17	8	0.869	−0.058	0.343	−0.849	0.732
Frequency curvature 4	0.083	8	0.936	0.030	0.365	−0.812	0.873
Entropy curvature 1	0.277	8	0.789	0.128	0.462	−0.938	1.193