Isolation Calls of the Bat *Saccopteryx bilineata* Encode Multiple Messages

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Abstract - The information that can possibly be encoded in a given vocalization is limited by the available acoustic space. Vocalizations composed of several elements have the potential to distribute information among distinct elements and thus encode various layers of information simultaneously. Correspondingly, the multiple messages hypothesis states that different elements of a vocalization convey different information, for example, about identity, signallers' quality or external events, which is directed to one or several receivers. The isolation call of the greater sac-winged bat, *Saccopteryx bilineata*, is a complex vocalization composed of different syllable types (variable, composite and stereotyped syllables). Pups produce isolation calls to elicit maternal care. In contrast to most bats, isolation calls of *S. bilineata* remain part of the adult vocal repertoire. In this study, we investigated the acoustic trajectories of isolation calls during ontogeny. Our analyses demonstrated that ontogenetic changes of acoustic parameters were most pronounced in stereotyped syllables. Throughout ontogeny, stereotyped syllables became shorter in duration and higher in frequency and bandwidth, thus effectively encoding information about age. Additionally, former studies revealed that composite syllables encode both an individual and a group signature. In our study, we also found an individual signature, thus corroborating the previous study. We conclude that isolation calls of *S. bilineata* encode multiple messages which are temporally segregated. The composite syllables conveyed information about individual identity and social group affiliation, whereas the stereotyped syllables encoded information about age. The temporal segregation allows the separation of information conveyed by distinct elements, thus enhancing signal reliability.

Keywords – Multiple messages hypothesis, Age signature, Individual signature, Temporal segregation, Vocal communication, Bats

Communicative signals are used to transfer information which elicits a behavioral response in the receiver (Bradbury & Vehrencamp, 2011; Simmons, 2003). Animals' signals are often highly complex, incorporating several sensory modalities (Bradbury & Vehrencamp, 2011; Hebets & Papaj, 2004). Complex signals may arise as a result of different selective pressures acting on signals. Selection pressures may affect the content of a signal (referred to as content-based selection) or the reception and correct decoding of the message by the receiver (referred to as efficacy-based selection; Guilford & Dawkins, 1991; Hebets & Papaj, 2004). Signal complexity can be achieved by producing signals which are composed of multiple messages (Candolin, 2003; Hebets & Papaj, 2004) or repetitive signal parts providing the same information (redundant signals), which is used to increase attention, reception and accurate receiver response (Hebets & Papaj, 2004). Signals can be transmitted and received via different

sensory modalities, allowing communication through visual, olfactory, tactile and/or acoustic signals (Bradbury & Vehrencamp, 2011). Acoustic signals can encode different types of information such as signallers' quality in terms of size or social rank, individual, group or species identity, sex or age, and, sometimes, different messages are directed to different receivers (e.g., Blumstein & Armitage, 1997; Charlton, Zhihe, & Snyder, 2009; Erb, Hodges, & Hammerschmidt, 2013; Ey, Hammerschmidt, Seyfarth, & Fischer, 2007; Fischer, Kitchen, Seyfarth, & Cheney, 2004; Pfefferle & Fischer, 2006; Riesch, Ford, & Thomsen, 2006; Townsend, Hollén, & Manser, 2010).

According to the multiple message hypothesis (*sensu* Hebets & Papaj, 2004), signals encoding multiple layers of information can be composed of a single element, which, in turn, is composed of distinct acoustic units (Fischer et al., 2004; Furrer & Manser, 2009; Jansen, Cant, & Manser, 2012) or of different elements emitted in a sequence (Jahelková, Horáček, & Bartonička, 2008; Koren & Geffen, 2009; Nelson & Poesel, 2007; Ryan, 1983; Templeton, Greene, & Davis, 2005). Therefore, information can be encoded in distinct acoustic units of a single element, in different elements, or in both.

When information is encoded in different elements or acoustic units of a single element, temporal segregation of information is achieved (Jahelková et al., 2008; Nelson & Poesel, 2007; Templeton et al., 2005). Temporal segregation is beneficial because it deals with problems arising from multiple message encoding. Elements have a defined acoustic "space" which can only encode a limited size of information (in bit; e.g., Beecher, 1989). Consequently, when several messages are conveyed within the same element, reliability of one or several messages diminishes. However, when multiple messages are encoded in different elements (or acoustic units of a single element) of a vocalization, each acoustic "space" can be fully exploited to encode a single message, thus enhancing signal reliability (Jahelková et al., 2008; Koren & Geffen, 2009; Nelson & Poesel, 2007). Furthermore, temporal segregation of information may allow a more precise decoding process of the receiver, therefore facilitating the behavioral response (Nelson & Poesel, 2007; Templeton et al., 2005).

Temporal segregation of information can be achieved by segmentation of a vocalization into different acoustic elements. In the black-capped chickadee, temporal segregation of a multisyllabic call is required to encode one of several messages (Templeton et al., 2005). The entire call conveys information about a predation event (e.g., stationary predator), whereas the number of "dee" elements specifies the size of the predator. A playback experiment revealed that chickadees respond accordingly to the conveyed information about predator size (Templeton et al., 2005). Temporal segregation can also be obtained by segmentation of a single element into distinct acoustic units, as for example in the contact call of banded mongooses, *Mungos mungo* (Jansen et al., 2012). This call is composed of two distinct acoustic units, a noisy and a harmonic part (Jansen et al., 2012). The noisy part is always present and encodes individual and group identity (Müller & Manser, 2008), whereas the different behavioral contexts are encoded in the duration of the harmonic part (Jansen et al., 2012).

Bats are a highly promising taxon for studying the temporal segregation of information in social vocalizations because many elements in their vocal repertoire consist of distinct acoustic units (i.e., a noisy and a tonal part) and vocalizations containing several different elements have been described for many species (e.g., Behr & von Helversen, 2004; Chaverri, Gillam, & Kunz, 2012; Chaverri, Gillam, & Vonhof, 2010; Davidson & Wilkinson, 2002; Leippert, 1994; Leippert, Goymann, Hofer, Marimuthu, & Balasingh, 2000; Pfalzer & Kusch, 2003). However, investigations on whether distinct elements in vocalizations convey different messages have only been conducted in few bat species (e.g., Bohn, Schmidt-French, Schwartz, Smotherman, & Pollak, 2009; Jahelková et al., 2008).

Saccopteryx bilineata is a Neotropical insectivorous bat species living in year-round stable colonies which can consist of up to 60 individuals (Bradbury & Vehrencamp, 1976; Tannenbaum, 1975). Colonies contain smaller social groups called harems, which are composed of a harem male guarding a territory of up to $2m^2$ vertical surface area where females can choose to roost during the day (Bradbury & Vehrencamp, 1976). Additionally, nonharem males which are queuing for harem access in their natal colony are also often present (Nagy, Knörnschild, Voigt, & Mayer, 2012; Voigt & Streich, 2003). Males are not able to control female movements within the colony, which leads to intense courtship efforts throughout the whole year (reviewed in Nagy & Knörnschild, 2016; Voigt et al., 2008). Females disperse

after weaning (at 12 – 14 weeks of age) to avoid inbreeding with older male relatives (Nagy, Heckel, Voigt, & Mayer, 2007). Pup births are highly synchronized, with females giving birth to one pup per year in May (Bradbury & Vehrencamp, 1976; Tannenbaum, 1975). New-born pups are already fully furred and have their eyes open (Tannenbaum, 1975). Throughout ontogeny, *S. bilineata* pups of both sexes engage in a conspicuous vocal behavior termed babbling (Knörnschild, Behr, & von Helversen, 2006). Babbling pups emit long vocal sequences containing different syllable types (the term "syllable" is used synonymously to "element") of the adult vocal repertoire as well as interspersed isolation calls and echolocation pulses (Knörnschild et al., 2006). Babbling behavior probably allows pups to acquire the highly diverse adult vocal repertoire (Behr & von Helversen, 2004) by repeatedly practicing the production of different syllable types and whole vocalization types (Knörnschild, 2014; Knörnschild, Nagy, Metz, Mayer, & von Helversen, 2010). Pups utter isolation calls to elicit maternal care (Knörnschild & von Helversen, 2008). In contrast to isolation calls of other bat species, isolation calls of *S. bilineata* are comparatively long in duration (> 1 s), and composed of three different syllable types, namely variable syllables (vs), and composite (cs) and stereotyped (ss) end syllables (Knörnschild & von Helversen, 2008). Isolation calls encode an individual and a group signature located in the composite end syllables (Knörnschild, Nagy, Metz, Mayer, & von Helversen, 2012; Knörnschild & von Helversen, 2008). Female *S. bilineata* are capable of differentiating own versus alien pups based on isolation calls alone (Knörnschild & von Helversen, 2008), which is in line with other bat species (e.g., Balcombe, 1990; Bohn, Wilkinson, & Moss, 2007; Fanis & Jones, 1995; Knörnschild, Feifel, & Kalko, 2013). However, unlike isolation calls in other bats (e.g., Engler, Rose, & Knörnschild, 2017; Esser & Schmidt, 1989; Knörnschild, von Helversen, & Mayer, 2007; Scherrer & Wilkinson, 1993; Sterbing, 2002), isolation calls of *S. bilineata* remain part of the adult vocal repertoire. They are not only emitted during mother-pup interactions and in pups' babbling trains but also during agonistic male-male interactions and male courtship (Knörnschild et al., 2012).

The aim of this study was to investigate the ontogenetic change of pup isolation calls in *S. bilineata*. We hypothesized that the majority of acoustic parameters would not change considerably during ontogeny since isolation calls remain in the adult vocal repertoire. Nevertheless, we expected to find fine-scale acoustic differences which can be used as a reliable indicator for assessing pup age. Therefore, we hypothesized that isolation calls encode different layers of information — i.e., individual identity, group affiliation and age — in different syllable types (or in different acoustic units of syllables), according to the multiple message hypothesis.

Method

Study Site and Animals

We conducted sound recordings at Barro Colorado Island, a field station of the Smithsonian Tropical Research Institute. Barro Colorado Island is located in the artificial Gatun lake (9° 9' 0''N, 79° 51' 0''W) in Panamá. We recorded vocalizations of 14 bat pups from six colonies throughout their ontogeny (from mid-May to end of August 2015, see Table 1). Colonies were roosting on the outside walls of buildings belonging to the field station and contained up to four social groups (i.e., harems) each. Each social group consisted of the harem male, several females with their respective pups and, in two colonies, nonharem males in the periphery (see Table 1 for details on group and colony composition). Since these colonies are part of a long-term project, our focal bats were well habituated to human presence and not noticeably disturbed by behavioral observations or sound recordings. Adult bats were individually identified by colored plastic bands on their forearms (A.C. Hughes Ltd. UK; size XCL; one band per forearm). All young pups were individually identified via their banded mothers, since mothers only nurse their own pups and behave aggressively towards alien pups. At approximately 60 days of age, when they had almost adult mass and size, pups were caught with mist nets (Ultrathin Mist Nets M-14; Ecotone, Gdynia, Poland) in the vicinity of their day-roosts, sexed and equipped with individually distinct colored bands. Immediately after the procedure, pups were released at the site of capture. The banding

method is well established and did not cause any noticeable negative effect on the bats' behavior or welfare. It was not possible to catch all pups because we did not want to disturb the bats by repeated capture attempts; therefore, five of 14 pups remained unsexed and unbanded (but, nevertheless, individual identification was possible via their banded mothers).

Table 1
Colony and Social Group Composition of Pups

Pup ID	Colony	Colony composition				Social group composition		Pup sex
		Harem males	*Non-harem males*	*Females*	*Pups*	*Females*	*Pups*	
1	C1	1	-	4	4 (3)	4	4 (3)	Unknown
2	E1	1	2	2	2	2	2	Unknown
3	E1	1	2	2	2	2	2	Male
4	E3	3	1	9 (8)	9 (8)	3	3	Female
5	E3	3	1	9 (8)	9 (8)	2	2	Male
6	F1	4	-	8	7	3	3	Unknown
7	F1	4	-	8	7	3	3	Female
8	SL1	1	-	2 (1)	2 (1)	2 (1)	2 (1)	Male
9	SL2	2	-	5 (4)	5 (3)	4 (3)	5 (3)	Unknown
10	SL2	2	-	5 (4)	5 (3)	4 (3)	5 (3)	Unknown
11	E3	3	1	9 (8)	9 (8)	3	3	Male
12	E3	3	1	9 (8)	9 (8)	3	3	Male
13	E3	3	1	9 (8)	9 (8)	2	2	Female
14	SL2	1	-	5 (4)	5 (3)	1	1	Female

Note. Representation of the colony and social group composition for each pup ($N = 14$). Numbers in parentheses depict changes in colony or social group composition during the field season as females and their respective pups sometime switched to a different social group or disappeared.

Sound Recordings

Sound recordings and accompanying behavioral observations of pups in the day-roost were performed during their whole ontogeny. During the first two weeks after pups were born, we restricted recordings and observations to the main activity period of bats, one hour after dawn and before dusk, respectively. With increasing age pups started to be more active throughout the entire day. Consequently, we then conducted sound recordings and behavioral observations throughout the entire day (*ad libitum* sampling *sensu* Altmann, 1974). We recorded each colony twice a week, once from dawn to midday and once from midday to dusk. We used high-quality ultrasonic recording equipment (500 kHz sampling rate, 16-bit depth resolution) to record vocalizations of individual pups. The set-up consisted of a microphone (Avisoft UltraSoundGate 116Hm, with condenser microphone CM16, frequency range 1-200 kHz ± 3 dB, connected to a laptop computer Lenovo S21e) running the software Avisoft-Recorder (v4.2.05 R. Specht, Avisoft Bioacoustics, Glienicke, Germany). Due to the excellent visibility in the day-roosts and the short recording distance (2-4 m on average), we could reliably associate isolation calls to individual pups based on mouth movements and the accompanying behavior (i.e., approaching the mother to solicit maternal care).

Acoustic Analysis

In this study, the terms "element" and "syllable" are used synonymously and are defined as an acoustic entity surrounded by silence. Syllables were classified into different categories ("syllable types") according to their acoustic properties. Furthermore, syllables were either compositional (i.e., composed of two or more acoustic units) or simple (i.e., composed of only one acoustic unit). We defined acoustic

units as separately identifiable parts with distinct acoustic features (e.g., a noisy part followed by a purely tonal part). Isolation calls of *S. bilineata* pups are multisyllabic and consist of three different syllable types, namely variable (vs), composite (cs) and stereotyped (ss) syllables (Knörnschild & von Helversen, 2008, Figure 1). Composite syllables have a noisy part ('nc') and a tonal part ('tc'), but the noisy part may be reduced or absent in some cases. Composite syllables ('nc+tc') and stereotyped syllables ('ss') are combined into so-called 'end syllables' because isolation calls always end with them. In total, we measured 356 isolation calls from 14 different pups belonging to six different social groups. To minimize temporal dependence between calls, we never measured more than two isolation calls per pup and day.
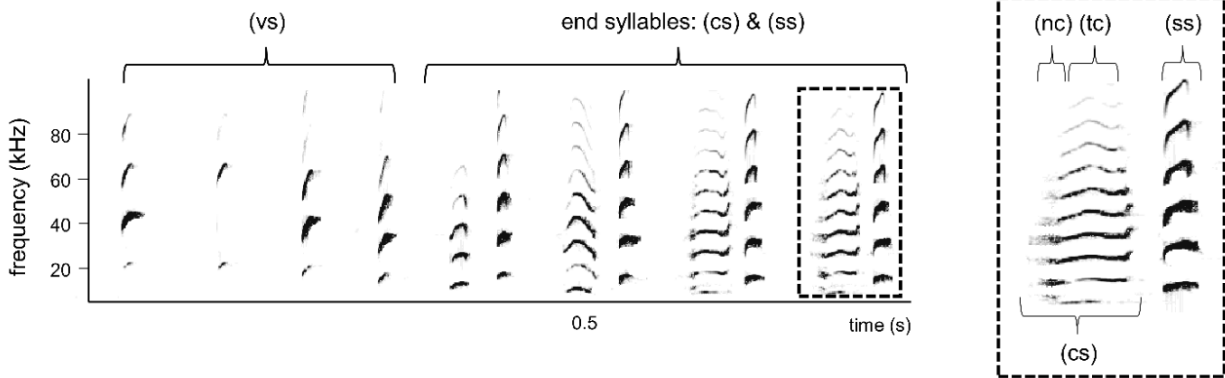
Figure 1. Entire isolation call of one pup composed of three different syllable types. **(vs)** variable simple syllables. **(tc)** tonal part of the composite syllable. **(nc)** noisy part of the composite syllable. **(cs)** composite syllable (noisy part connected to tonal part). **(ss)** stereotyped syllables. The spectrogram depicts frequency (kHz) as a function of time (s) and was generated using a 1042 point fast Fourier transform and a Hamming window with 50% overlap.

Prior to acoustic measurements, isolation calls were bandpass filtered (5-90 kHz) and normalized to 100% (Cool Edit 2000 Inc., Syntrillium Software Corporation P.O. Box 62255, Phoenix, AZ, USA). We used the software Avisoft SASLab Pro (v.5.2.09; R. Specht, Avisoft Bioacoustics, Glienicke, Germany) for our acoustic analyses. Start and end of syllables and syllable parts were determined manually based on the oscillogram and, subsequently, automatic parameter measurements provided by the software were applied. Spectrograms for measuring single syllable types were created using a Hamming window with 1024-point fast Fourier transform and 87.5% overlap (frequency resolution: 488 Hz; time resolution: 0.256 ms). Spectrograms for measuring entire call parameters were created using a Hamming window with 1024-point fast Fourier transform and 50% overlap (frequency resolution: 488 Hz; time resolution: 1.024 ms).

Some acoustic measurements were taken over entire isolation calls, while others focused on the end syllables of isolation calls because earlier work found that two vocal signatures (individual identity and social group affiliation) are encoded there (Knörnschild et al., 2012; Knörnschild & von Helversen, 2008). Isolation calls are multiharmonic and we incorporated all harmonics when measuring entire isolation calls. When measuring end syllables, however, we used only the fundamental frequency (first harmonic) because it contained most of the sound energy. For entire isolation calls, we measured duration, mean peak, minimum and maximum frequency, mean bandwidth and mean entropy. Entropy is a measure of the width and uniformity of the power spectrum and assesses how tonal or noisy a signal is (on a scale of 0-1; pure tone = 0, white noise = 1). For end syllables, different syllable types or syllable parts were measured separately (nc, tc, ss). We did not use measurements of the noisy part of composite syllables (nc) in statistical analyses because nc was not present in all end syllables (but we report all acoustic measurements, see Appendix A, Tables A1-A4). For each isolation call, we measured three end syllables and subsequently averaged measurements per syllable type and syllable part. We measured the duration, time to maximum amplitude and interval of each syllable type and syllable part. To estimate the

frequency and entropy curvature of end syllables, we measured five spectral parameters (peak frequency, minimum and maximum frequency, bandwidth, entropy) at five different locations distributed equally over the fundamental frequency of each syllable type and syllable part. Curvature parameters were obtained by performing principal component analyses (PCAs) with varimax rotation on the above mentioned parameters (one PCA on all 20 frequency parameters and another PCA on all five entropy parameters), thus reducing multicollinearity between original acoustic parameters considerably. For the frequency curvature, we extracted three principal components (with eigenvalues >1) which explained 93.4% of the total variance for tc and two principal components (with eigenvalues >1) which explained 94.6% of the total variance for ss. For the entropy curvature, we extracted one principal component for tc and ss each (with an eigenvalue >1) which explained 56.2% (tc) and 63.4% (ss) of the total variance, respectively. Both PCAs fulfilled Kaiser-Meyer-Olkin (KMO) and Bartlett's test criteria, thus assuring the appropriateness of our data for PCAs. Thus, we obtained seven derived acoustic parameters (five parameters describing the frequency curvature and two parameters describing the entropy curvature for tc and ss) per isolation call for subsequent statistical analyses.

Statistical Analysis

All statistical tests were conducted in SPSS (v.20; IBM SPSS Statistics Chicago, IL, USA) and R (v.3.02; R Development Core Team 2008). To estimate the ontogenetic trajectory of isolation call parameters, we performed separate General Linear Mixed Models (GLMMs) for each call parameter (with age in 10-day steps as covariate, sex as fixed factor and pup ID as random factor; Gamma distribution with log link function). In addition, we performed separate linear regressions for each pup and call parameter to obtain the respective individual slopes of the linear regressions. Slopes were used as a basic proxy for visualizing ontogenetic development (i.e., a positive slope value indicated that a certain parameter increased during ontogeny). GLMMs and linear regressions were conducted for 19 acoustic parameters, namely tc duration, tc time to maximum amplitude, tc mean peak frequency, tc mean bandwidth, tc frequency curvature 1-3, tc entropy curvature 1, ss duration, ss time to maximum amplitude, ss mean peak frequency, ss mean bandwidth, ss frequency curvature 1-2, ss entropy curvature 1, entire call duration, entire mean peak frequency, entire mean bandwidth, and entire mean entropy.

To test for the existence of an individual signature in isolation calls, we performed a discriminant function analysis (DFA) on the whole data set. To test whether the strength of the individual signature remained stable or changed during ontogeny, we performed two more DFAs on a subset of the data (only including pups for which we had a sufficient amount of recorded isolation calls to split the data into two ontogenetic phases). We adjusted the DFAs to the unequal number of analyzed calls per pup by computing group sizes based on prior probabilities (calls per pup varied within each DFA but not between DFAs). We used a cross-validation procedure to estimate the correct classification success ('n-1' cross-validation procedure), which classified each call based on discriminant functions established with all calls except the call being classified. Subsequently, we performed a Binomial test to check whether the obtained classification success was better than a random classification.

In the first DFA (testing the existence of an individual signature), we used 14 pups for which we analyzed at least 17 isolation calls each (17-38 isolation calls per pup, 356 calls in total). We selected 16 acoustic parameters, namely tc duration, tc interval, tc frequency curvature 1-3, tc entropy curvature 1, ss duration, ss interval, ss frequency curvature 1-2, ss entropy curvature 1, entire call duration, entire mean peak frequency, entire mean bandwidth, entire mean entropy, and number of variable syllables of entire call. All parameters were checked for multicollinearity and included simultaneously into the DFA.

In two subsequent DFAs (testing the strength of the individual signature during ontogeny), we used ten pups for which we analyzed at least nine isolation calls for each of two ontogenetic phases (9-15 isolation calls per ontogeny phase and pup, 220 calls in total). The number of calls per pup was equal for both ontogenetic phases to ensure a direct comparison of the classification success obtained by both DFAs. We split our data set into two ontogenetic phases based on pup age, resulting in one data set with pups younger than 34 days and a second data set with pups older than 34 days. This age corresponded to

the onset of independent foraging which inaugurates a phase of greater independence of pups, even though they are still nursed by their mothers (Knörnschild et al., 2012). We selected eight acoustic parameters, namely tc duration, tc frequency curvature 1-2, tc entropy curvature 1, ss duration, ss frequency curvature 1-2, and ss entropy curvature 1. Again, all parameters were checked for multicollinearity and included simultaneously into the DFA. The classification success of individual pups was compared between both ontogenetic phases using a Wilcoxon test. All statistical tests were conducted in SPSS and R.

Results

Ontogenetic Trajectories of Isolation Calls

We conducted separate General Linear Mixed Models (GLMMs) to assess the ontogenetic trajectory of isolation call parameters. Taken together, ontogenetic changes were more pronounced in the stereotyped syllables than in the composite syllables' tonal part (for details see Table 2). As pups matured, the mean peak frequency of the tonal component of composite syllables showed a strong trend to decrease, $t = -1.93$; $p = 0.053$. In contrast to this, the mean peak frequency and bandwidth of the stereotyped syllables increased significantly, mean peak frequency: $t = 4.94$, $p < 0.001$; bandwidth: $t = 6.51$, $p < 0.001$, (Figure 2). This also resulted in a significant increase of the entire isolation calls' peak frequency, $t = 3.47$, $p < 0.001$ because the stereotyped syllables had a higher relative amplitude than the composite syllables. The duration of entire isolation calls increased with age as well, $t = 8.72$, $p < 0.001$. Most other significant ontogenetic changes concerned the stereotyped syllables. For example, duration and the time to maximum amplitude decreased significantly during ontogeny, whereas entropy significantly increased, duration: $t = -3.37$, $p < 0.001$; time to maximum amplitude: $t = -3.07$, $p = 0.002$; entropy: $t = 2.70$, $p = 0.006$). Pup sex had a negligible effect in all of the conducted GLMMs (see Appendix B, Table B1).

Individual Signature

Most isolation calls could be correctly classified to the respective pup. A DFA with 356 isolation calls of 14 pups classified 48.9 % of all calls to the correct individual, which was significantly higher than expected by chance (7.1%; binomial test: $p = 0.0009$, $N = 14$). Differences between isolation calls of different pups were subtle (Figure 3), but a previous study showed that these differences are sufficient for correct maternal offspring recognition (Knörnschild & von Helversen, 2008). Misclassifications were significantly more likely to occur between pups from the same social group (intra-group misclassification) than between pups from different social groups (inter-group misclassifications; Wilcoxon test: $Z = -2.667$; $p = 0.008$, $N = 12$; see Table 3).

In both ontogenetic stages, pups could be statistically distinguished based on acoustical parameters of isolation calls (pups younger than 34 days: 46.4% classification success; pups older than 34 days: 56.4% classification success; $N = 10$ pups in each case). Again, the obtained classification success was significantly better than expected by chance (10%), binomial test: $p = 0.001$ for ontogeny phase 1, $p = 0.01$ for ontogeny phase 2. We found no significant difference between the classification success for individual pups from both ontogenetic phases, Wilcoxon test: $Z = -1.174$; $p = 0.24$, ns ($N = 10$; see Table 4), suggesting that the strength of the individual signature remains stable during ontogeny.

Table 2
Ontogenetic Development of Acoustic Parameters

Syllable type	Dependent variable	Estimate	t	p	Trajectory
Tonal part of CS	Duration (ms)	-0.010135	-1.160	0.247	n.s.
Tonal part of CS	Time to max. amplitude (ms)	0.014780	1.002	0.316	n.s.
Tonal part of CS	Mean peak freq. (kHz)	-0.008092	-1.930	0.053	n.s.
Tonal part of CS	Mean bandwidth (kHz)	-0.002916	-0.268	0.788	n.s.
Tonal part of CS	PC1 entropy	-0.013856	-2.142	0.032	decrease*
Tonal part of CS	PC1 call curv.	-0.021493	-2.636	0.008	decrease**
Tonal part of CS	PC2 call curv.	-0.001279	-0.191	0.848	n.s.
Tonal part of CS	PC3 call curv.	0.008929	1.300	0.194	n.s.
Stereotyped	Duration (ms)	-0.018117	-3.370	< 0.001	decrease***
Stereotyped	Time to max. amplitude (ms)	-0.040350	-3.072	0.002	decrease**
Stereotyped	Mean peak freq. (kHz)	0.020765	4.940	< 0.001	increase***
Stereotyped	Mean bandwidth (kHz)	0.072170	6.510	< 0.001	increase***
Stereotyped	PC1 entropy	0.021806	2.707	0.006	increase**
Stereotyped	PC1 call curv.	0.038622	4.701	< 0.001	increase***
Stereotyped	PC2 call curv.	0.034560	5.800	< 0.001	increase***
Entire call	Duration (s)	0.085696	8.729	< 0.001	increase***
Entire call	Mean peak freq. (kHz)	0.039714	3.470	< 0.001	increase***
Entire call	Mean bandwidth (kHz)	-0.007587	-1.610	0.108	n.s.
Entire call	Mean entropy	-0.009124	-2.470	0.013	decrease*
Entire call	Harmonics to noise ratio	0.003677	0.530	0.598	n.s.

Note. GLMMs with two fixed factors, age (in 10-day blocks) and sex (female, male, unknown), and one random factor, pup ID 1-14. Results for sex are not listed in the table (but can be found in the appendix) because they were non-significant in most of the cases. $*p < 0.05$. $**p < 0.01$. $***p < 0.001$.
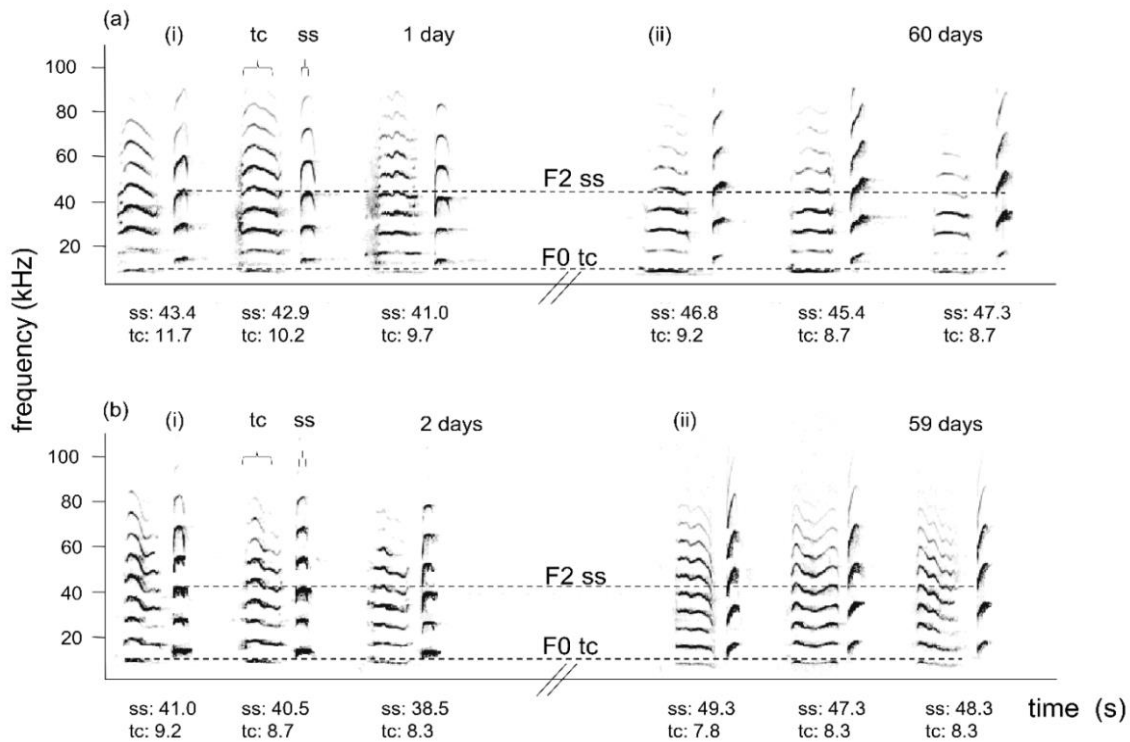
Figure 2. Isolation call end syllables of two pups at different ages. Three consecutive end syllables of one pup (unknown sex) **(a)** at one day (i) and 60 days of age (ii) and of a male pup **(b)** at two days (i) and 59 days of age (ii). Dashed lines serve as visual orientation for the ontogenetic change of acoustic parameters of tc and ss with age. F0 (fundamental frequency): depicts the decrease of mean peak frequency of the tonal part of the composite syllable. The rather subtle decrease in frequency of the tonal unit of the composite syllable with age is further illustrated by mean values of the peak frequency (kHz) of each tonal part (tc). F2 (second harmonic): depicts the increase of the mean peak frequency of the stereotyped syllable. Mean values of the peak frequency (kHz) of each stereotyped syllable (ss) are given as well. Spectrograms depict frequency (kHz) as a function of time (s) and were generated using a 1042 point fast Fourier transform and a Hamming window with 50% overlap.

Table 3

Intra- and Inter-group Misclassifications of Isolation Calls to Individual Pups (N = 14) Obtained by a DFA and the Classification Success Calculated Separately for Each Social Group (N = 4 groups)

Pup ID	Group ID	Intra-group misclassification	Inter-group misclassification	Classification success within social groups (%)
2	2	3.20	2.96	78.2%
3	2	16.70	4.86	(50% random classification success)
4	3	9.08	3.03	
5	3	4.78	3.43	58.8%
11	3	2.63	2.63	(20% random classification success)
12	3	10.83	3.40	
13	3	5.95	3.70	
6	4	16.70	5.55	70.8%
7	4	3.30	3.33	(50% random classification success)
9	6	16.70	0.75	
10	6	1.65	1.52	73.2% (33.3% random classification success)
14	6	14.70	6.42	

Note. Misclassifications were averaged per pup. Two of 14 pups are not listed here because we did not have any recordings for fellow pups from their social group.
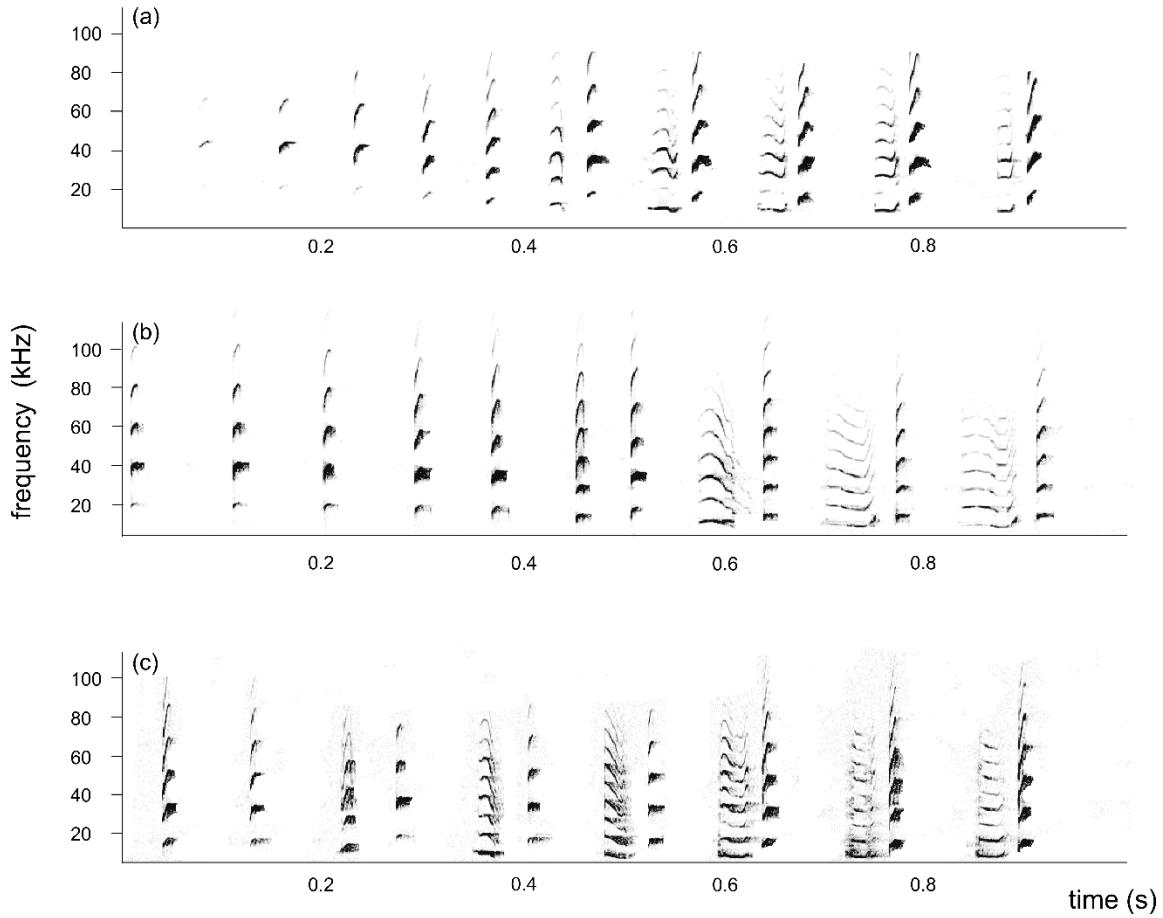
Figure 3. Spectrograms of isolation calls encoding an individual signature from three pups at similar ages. **(a)** female bat pup, 46 days of age. **(b)** unknown sex, 49 days of age. **(c)** male bat pup, 44 days of age. Pups belonged to three different colonies. Spectrograms depict frequency (kHz) as a function of time (s) and were generated using a 1042 point fast Fourier transform and a Hamming window with 50% overlap.

Table 4
Strength of Individual Signature During Ontogeny (N = 10 pups)

Pup ID	Classification success < 34 days of age (%)	Classification success > 34 days of age (%)	Relative change of classification success
2	80.0	66.7	decrease
3	30.0	10.0	decrease
4	11.1	44.4	increase
5	70.0	80.0	increase
7	46.7	80.0	increase
8	45.5	90.9	increase
9	75.0	66.7	decrease
11	20.0	30.0	increase
12	11.1	33.3	increase
13	44.4	33.3	decrease

Note. Four of 14 pups had to be excluded from the analysis because we did not have sufficient recordings throughout ontogeny for a comparison of signature strength.

Discussion

This study shows that multisyllabic isolation calls of *S. bilineata* pups encode multiple messages in different syllable types (multiple messages hypothesis *sensu* Gerhardt, 1992; Hebets & Papaj, 2004). While it was already established that the tonal part of the composite syllables in isolation calls encodes information about individual identity and group affiliation (Knörnschild et al., 2012; Knörnschild & von Helversen, 2008), this study found conclusive evidence that the stereotyped syllables convey information about age. In contrast to the stereotyped syllables, the acoustic parameters of composite syllables remained generally stable during ontogeny.

The ontogenetic change in stereotyped syllables is most probably a by-product of vocal tract maturation and not influenced by the vocal learning processes shaping the group signature encoded in the tonal part of the composite syllables (Knörnschild et al., 2012). The ontogenetic increase in body mass and the accompanying growth of the vocal folds does not contradict the frequency increase in the stereotyped syllables. Fundamental frequencies are not only influenced by the length and mass of the vocal folds but also by their tension (Bradbury & Vehrencamp, 2011; Fitch & Hauser, 2003). Tension of vocal folds is achieved through laryngeal muscle control and muscular control becomes more sophisticated during ontogeny (Bradbury & Vehrencamp, 2011). Until now, the vocal tract of *S. bilineata* has not been investigated. Nevertheless, the most parsimonious assumption is that the acoustic changes in stereotyped syllables result from maturation processes, suggesting that they are an honest signal about the signaller (Bradbury & Vehrencamp, 2011; Fitch & Hauser, 2003).

As a receiver, information about signaller age could be useful in different social interactions such as during agonistic encounters, for mate choice, or during predator encounters where signaller reliability is crucial for a receiver's response (Blumstein & Daniel, 2004; Blumstein & Munos, 2005; Charlton et al., 2009; Erb et al., 2013; Ey et al., 2007; Fischer et al., 2004; Mathevon, Koralek, Weldele, Glickman, & Theunissen, 2010; Reby & McComb, 2003). In *S. bilineata,* pup age might be useful for females to adequately allocate maternal care. However, it is unlikely that females would rely on a single signal for assessing how much maternal care should be invested. Most probably, other signals such as pup behavior (solicitation for food), foraging efficacy, and morphological cues, such as size, influence the allocation of maternal care as well. To test the mothers` ability to discriminate between pup ages based on stereotyped syllables alone, one could perform a playback experiment in which mothers had to distinguish between correct versions of their own pups` isolation calls and incorrect versions in which the stereotyped syllables have been artificially shifted upwards or downwards in frequency. If mothers evaluate age information in pup isolation calls, they should react most strongly to the correct versions.

The multiple messages conveyed in *S. bilineata's* isolation calls (individual ID, group affiliation, age) are encoded in different syllable types and thus temporally segregated. This segregation has the advantage that information can be encoded without a reduction in signal reliability or receivers' decoding accuracy (Hebets & Papaj, 2004; Jahelková et al., 2008; Koren & Geffen, 2009; Nelson & Poesel, 2007; Ryan, 1983). A former study (Knörnschild & von Helversen, 2008) showed that the individual signature, encoded in the tonal part of composite syllables, facilitated maternal offspring recognition based on isolation calls alone. The strengths of the individual signature in both the latter and our present study are comparable and surprisingly low for a signal under strong natural selection pressure (allonursing does not occur in *S. bilineata*; Knörnschild & von Helversen, 2008). The comparatively low strength of the individual signature may be caused by the fact that a second signature is encoded in the tonal part of composite syllables as well, suggesting that the individual signature is, to a certain degree, masked by the group signature (Knörnschild et al., 2012). This signature, conveying information about social group affiliation, increases in strength during ontogeny, while the individual signature strength seems to remain stable as pups mature (Knörnschild et al., 2012, and this study). Since information about age is temporally segregated from information about individual identity and group affiliation (age information is encoded in a different syllable type, the stereotyped syllables), it might be easier for conspecifics to decode the information content of pup isolation calls.

Our findings correspond to other incidents of temporal segregation of information in various taxa. In white-crowned sparrows, for example, males produce a multisyllabic song encoding multiple messages, namely identity and local dialect, which receivers perceive accordingly (Nelson & Poesel, 2007). In the rock hyrax (*Procavia capensis*), males sing complex multisyllabic songs which are presumably used for mate attraction and rival deterrence (Koren & Geffen, 2009). Hyrax song encodes information about body weight, size, body condition, hormonal levels and social status in different song elements (Koren & Geffen, 2009). The above mentioned studies and others (e.g., Bohn et al., 2009; Jahelková et al., 2008; Müller & Manser, 2008; Templeton et al., 2005) demonstrate that the temporal segregation of information occurs in vocalizations under both natural selection and sexual selection pressures.

To conclude, our study shows that multisyllabic isolation calls of *S. bilineata* pups convey different layers of information encoded in two different syllable types. This finding highlights the possibility that more animal vocalizations than previously anticipated encode multiple messages. Future work will benefit especially from focussing on multisyllabic vocalizations which are emitted in different social contexts and/or directed towards different receivers.

Acknowledgements

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Appendix A

Table A1
Acoustic Parameters: Noisy Part of Composite Syllables

Pup ID	Duration (ms)	Time to max. amplitude (ms)	Mean peak freq. (kHz)	Mean bandwidth (kHz)	PC1 entropy	PC1 call curv.	PC2 call curv.	PC3 call curv.
1 (16)	2.99	2.33	27.6	5.6	-0.413	-0.103	-0.320	-0.033
2 (21)	2.54	2.01	33.7	4.9	-0.376	0.606	-0.344	-0.231
3 (14)	4.66	4.20	29.6	5.7	-0.031	0.187	-0.235	-0.131
4 (7)	3.43	2.81	26.1	4.0	-1.164	-0.218	-1.001	-0.215
5 (8)	3.45	2.57	22.9	6.5	-0.019	-0.679	0.144	-0.088
6 (14)	4.10	3.42	22.8	6.1	0.156	-0.595	0.187	-0.224
7 (24)	7.06	6.36	27.2	5.6	-0.192	-0.117	-0.410	0.109
8 (31)	9.01	7.37	29.5	7.0	0.610	0.085	0.672	-0.457
9 (5)	3.45	3.17	26.5	4.6	-1.018	-0.164	-0.690	-0.137
10 (15)	3.43	2.70	26.4	6.9	0.755	-0.267	0.477	-0.153
11 (27)	4.61	3.59	32.9	6.3	-0.045	0.361	0.237	0.733
12 (15)	14.38	13.11	28.5	7.4	0.660	-0.081	0.396	0.735
13 (7)	4.78	4.08	30.9	5.7	-0.120	0.293	-0.154	-0.268
14 (10)	5.45	3.73	24.9	4.2	-0.731	-0.396	-0.709	-0.178

Note. Raw data of the different parameters are presented as mean ($\bar{x}$) of all measurements per pup. Note that the noisy part was not present in every call and thus the mean presented here is an average of only those calls which had a noisy part. Call number per pup is given in parentheses after the pup ID.

Table A2
Acoustic Parameters: Tonal Part of Composite Syllables

Pup ID	Duration (ms)	Time to max. amplitude (ms)	Mean peak freq. (kHz)	Mean bandwidth (kHz)	PC1 entropy	PC1 call curv.	PC2 call curv.	PC3 call curv.
1 (19)	23.84	8.92	8.9	1.8	-0.305	-0.134	0.100	-0.097
2 (32)	22.59	9.1	8.8	1.8	0.331	-0.206	-0.211	0.061
3 (24)	23.38	8.66	8.8	1.8	-0.029	0.049	-0.347	-0.254
4 (22)	26.09	14.42	8.9	1.4	-0.828	0.044	0.001	-0.229
5 (26)	32.57	16.45	9.2	2.2	0.207	0.090	-0.015	0.341
6 (18)	24.82	9.91	9.2	1.9	-0.355	0.058	0.054	0.355
7 (30)	27.46	12.77	9.2	1.4	-0.645	-0.088	0.470	-0.237
8 (33)	26.33	18.13	10.6	3.3	1.111	-0.622	1.695	0.291
9 (24)	18.94	5.55	9.2	2.4	-0.065	0.411	-0.669	0.113
10 (30)	16.86	6.78	9.1	2.5	0.739	0.367	-0.825	0.079
11 (38)	17.55	8.05	8.6	1.3	-0.206	-0.178	-0.274	-0.315
12 (23)	20.45	7.47	10.3	2.2	0.264	0.844	0.082	0.107
13 (21)	25.83	14.28	8.4	1.6	-0.490	-0.312	-0.256	-0.147
14 (17)	18.93	9.43	8.9	1.7	-0.633	-0.014	-0.158	0.042

Note. Raw data of the different parameters are presented as mean ($\bar{x}$) of all measurements per pup. Note that the total call numbers differ between individual pups. Call number per pup is given in parentheses after the pup ID.

Table A3
Acoustic Parameters: Stereotyped Syllables

Pup ID	Duration (ms)	Time to max. amplitude (ms)	Mean peak freq. (kHz)	Mean bandwidth (kHz)	PC1 entropy	PC1 call curv.	PC2 call curv.
1 (19)	6.96	2.30	16.5	2.9	0.724	0.724	-0.195
2 (32)	7.22	2.73	15.2	2.2	0.771	0.771	-0.281
3 (24)	6.42	2.09	14.9	2.7	0.445	0.445	0.018
4 (22)	5.36	1.86	13.9	1.7	-0.351	-0.351	-0.253
5 (26)	6.61	2.29	16.0	1.7	-0.048	-0.048	-0.184
6 (18)	7.21	2.43	14.6	2.6	0.392	0.392	0.341
7 (30)	7.58	2.37	15.2	3.0	0.162	0.162	0.510
8 (33)	7.18	2.85	14.6	2.1	0.013	0.013	-0.186
9 (24)	6.98	2.19	12.3	2.8	-0.630	-0.630	0.518
10 (30)	6.87	2.41	14.5	3.6	0.133	0.133	0.704
11 (38)	5.48	2.21	12.9	1.6	-0.392	-0.392	-0.464
12 (23)	6.11	2.15	14.1	1.7	-0.434	-0.434	-0.473
13 (21)	5.66	2.52	14.6	2.3	-0.179	-0.179	0.171
14 (17)	6.73	2.36	13.34	1.9	-0.749	-0.749	-0.076

Note. Raw data of the different parameters are presented as mean ($\bar{x}$) of all measurements per pup. Note that the total call numbers differ between individual pups. Call number per pup is given in parentheses after the pup ID.

Table A4
Acoustic Parameters: Entire Call

Pup ID	Duration (s)	Mean peak freq. (kHz)	Mean bandwidth (kHz)	Mean entropy	Harmonics to noise ratio
1 (19)	0.91	31.8	52.9	0.362	21.61
2 (32)	1.08	28.1	52.7	0.353	20.54
3 (24)	1.54	26.6	56.1	0.385	18.25
4 (22)	1.50	32.3	50.7	0.342	21.95
5 (26)	1.50	31.2	49.9	0.313	22.69
6 (18)	1.67	28.0	52.6	0.345	23.56
7 (30)	1.20	31.6	51.5	0.349	20.54
8 (33)	1.01	30.1	53.6	0.369	21.18
9 (24)	1.41	26.2	43.7	0.317	23.16
10 (30)	1.74	30.5	44.4	0.330	22.62
11 (38)	1.41	30.2	48.8	0.334	20.26
12 (23)	1.29	25.9	49.7	0.351	18.98
13 (21)	1.14	28.2	47.5	0.347	19.40
14 (17)	1.15	31.2	46.9	0.325	20.84

Note. Raw data of the different parameters are presented as mean ($\bar{x}$) of all measurements per pup. Note that the total call numbers differ between individual pups. Call number per pup is given in parentheses after the pup ID.

Appendix B

Table B1

Ontogenetic Development of Acoustic Parameters in Female and Male Pups

Syllable type	Dependent variable	Sex	Estimate	t	p	Trajectory
Tonal part of CS	Duration (ms)	Female	0.127925	1.46	0.146	n.s.
Tonal part of CS	Duration (ms)	Male	0.107603	1.32	0.187	n.s.
Tonal part of CS	Distance to max. amplitude (ms)	Female	0.47125	3.166	0.001	Increase **
Tonal part of CS	Distance to max. amplitude (ms)	Male	0.31888	2.308	0.021	Increase *
Tonal part of CS	Mean peak freq (kHz)	Female	-0.018560	-0.51	0.607	n.s.
Tonal part of CS	Mean peak freq (kHz)	Male	0.051538	1.55	0.121	n.s.
Tonal part of CS	Mean bandwidth (kHz)	Female	-0.257784	-2.287	0.022	Decrease *
Tonal part of CS	Mean bandwidth (kHz)	Male	0.047766	0.456	0.648	n.s.
Tonal part of CS	PC1 Entropy	Female	-0.237559	-3.802	0.0001	Decrease ***
Tonal part of CS	PC1 Entropy	Male	0.091533	1.581	0.113	n.s.
Tonal part of CS	PC1 call curv.	Female	-0.065979	-0.916	0.359	n.s.
Tonal part of CS	PC1 call curv.	Male	-0.020092	-0.302	0.762	n.s.
Tonal part of CS	PC2 call curv.	Female	0.101359	1.363	0.173	n.s.
Tonal part of CS	PC2 call curv.	Male	0.150005	2.167	0.030	Increase *
Tonal part of CS	PC3 call curv.	Female	-0.056057	-1.064	0.287	n.s.
Tonal part of CS	PC3 call curv.	Male	-0.003933	-0.081	0.935	n.s.
Stereotyped	Duration (ms)	Female	-0.125893	-2.58	0.010	Decrease *
Stereotyped	Duration (ms)	Male	-0.096351	-2.13	0.033	Decrease *
Stereotyped	Distance to max. amplitude (ms)	Female	-0.076780	-0.96	0.336	n.s.
Stereotyped	Distance to max. amplitude (ms)	Male	-0.031500	-0.44	0.661	n.s.
Stereotyped	Mean peak freq (kHz)	Female	-0.022286	-0.51	0.613	n.s.
Stereotyped	Mean peak freq (kHz)	Male	-0.011453	-0.28	0.780	n.s.
Stereotyped	Mean bandwidth (kHz)	Female	-0.212450	-1.87	0.061	n.s.
Stereotyped	Mean bandwidth (kHz)	Male	-0.388830	-3.69	0.0002	Decrease ***
Stereotyped	PC1 entropy	Female	-0.176338	-2.24	0.024	Decrease *
Stereotyped	PC1 entropy	Male	-0.111754	-1.53	0.125	n.s.
Stereotyped	PC1 call curv.	Female	-0.011949	-0.14	0.892	n.s.
Stereotyped	PC1 call curv.	Male	-0.003267	-0.04	0.968	n.s.
Stereotyped	PC2 call curvature	Female	-0.013777	-0.23	0.819	n.s.
Stereotyped	PC2 call curv.	Male	-0.141397	-2.53	0.011	Decrease *
Entire call	Duration (s)	Female	-0.032539	-0.34	0.733	n.s.
Entire call	Duration (s)	Male	-0.011165	-0.13	0.900	n.s.
Entire call	Mean peak freq (kHz)	Female	0.087851	1.42	0.156	n.s.
Entire call	Mean peak freq (kHz)	Male	0.003581	0.06	0.948	n.s.
Entire call	Mean bandwidth (kHz)	Female	0.000422	0.01	0.991	n.s.
Entire call	Mean bandwidth (kHz)	Male	0.054037	1.59	0.112	n.s.
Entire call	Mean entropy	Female	-0.004142	-0.13	0.893	n.s.
Entire call	Mean entropy	Male	0.028272	1.00	0.319	n.s.
Entire call	Harmonics to noise ratio	Female	-0.072516	-1.52	0.129	n.s.
Entire call	Harmonics to noise ratio	Male	-0.095553	-2.20	0.028	Increase *

Note. GLMMs with two fixed factors, age (in 10-day blocks) and sex (female, male, unknown), and one random factor, pup ID 1-14. *$p < 0.05$. **$p < 0.01$. ***$p < 0.001$.