

## Ontogeny of an interactive call-and-response system in Spix's disc-winged bats

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We investigated the ontogenetic changes of two call types, the inquiry call and the response call, which comprise an interactive communication system in Spix's disc-winged bats, *Thyroptera tricolor*. We documented structural changes on both inquiry and response calls during ontogeny by recording single individual vocalizations in a flight cage and triggering response calls with playbacks of adult inquiry calls. Most ontogenetic changes in response calls were sex dependent. In addition, individuality was high for both call types across age categories. Despite the observed changes during development, both call types resembled the structure of adult calls from early ontogenetic stages, suggesting little influence of social learning in call development. Finally, we found sex dependent ontogenetic changes and high levels of individuality of calls early in life, which may indicate additional functionality in sex and pup recognition. Overall, the findings bring novel insight into the complex developmental process of the call types comprising the communication systems of Spix's disc-winged bats, and contribute to a better understanding of signal development in complex social communication systems.

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The ability to communicate effectively with conspecifics is of critical importance to social organisms, yet it often does not fully develop until adulthood. After birth, individuals begin to develop the skills to understand others, and to produce signals that convey clear messages in the correct context (Baugh, Hoke, & Ryan, 2012; Seyfarth & Cheney, 2010). In acoustic communication, for example, as individuals mature they slowly acquire their entire vocal repertoire (Conner & Whitworth, 1985), the ability to produce vocalizations with individual or group signatures (Boughman & Moss, 2003), and also learn to recognize affiliates based on characteristics of their signals (Holmes & Sherman, 1982; Sharp, McGowan, Wood, & Hatchwell, 2005). Throughout development, individuals may also learn to take turns during vocal exchanges, for example during antiphonal calling (Chaiken, 1990). Another ability that is acquired through ontogeny is functionally semantic communication; that is, to adequately associate signals with context, and to respond accordingly. Several species of mammals, for instance, learn to associate different types of conspecific alarm signals with

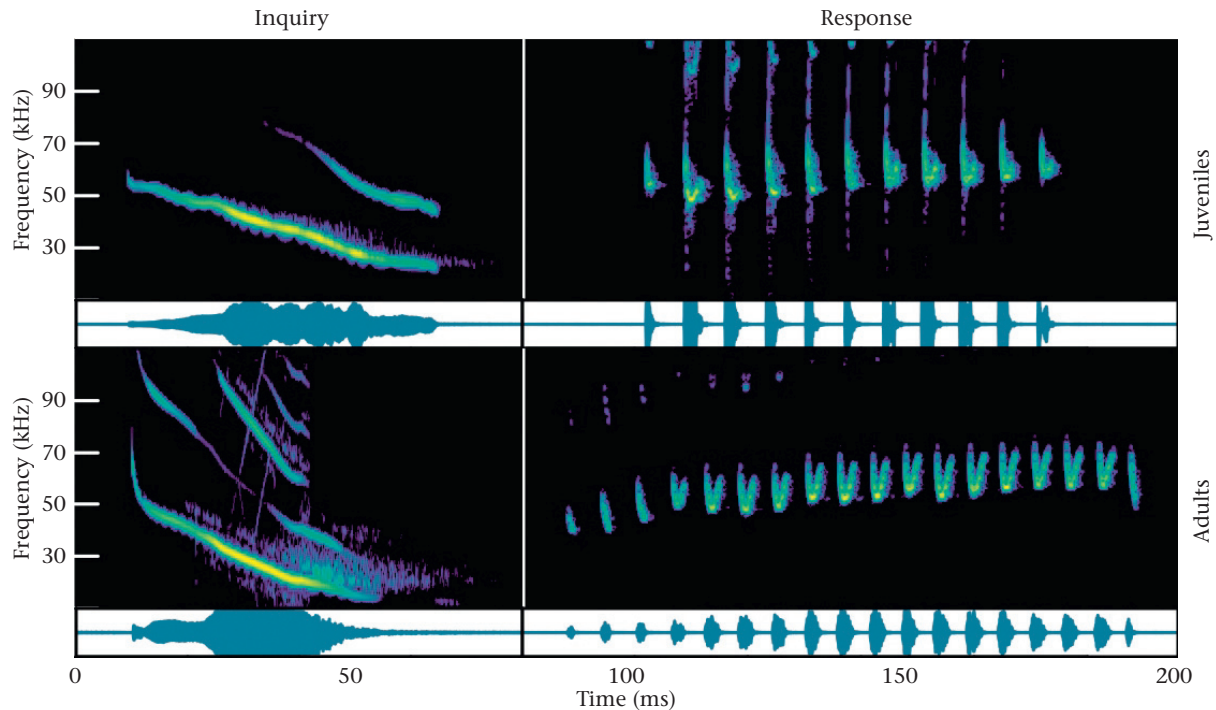
specific predators, and to adaptively respond to these signals (Fichtel, 2008; Seyfarth & Cheney, 2010).

One of the earliest call types to appear during ontogeny in many taxa are contact calls, because mother–offspring recognition is critical for offspring survival and development (Beecher, 1991). Contact calls are signals used to mediate many types of social interactions, but their main role is to allow conspecifics with some form of social affinity to locate each other (Boughman & Moss, 2003; Kondo & Watanabe, 2009). Because of their role in mediating social interactions, particularly in species with some form of parental care or while coordinating group movements, they usually emerge earlier than other types of calls and are constantly produced during daily activities and throughout an individual's life (Kondo & Watanabe, 2009). Hence, understanding the ontogeny of these signals is critical for elucidating the development and maintenance of social interactions themselves.

Spix's disc-winged bats, *Thyroptera tricolor*, use two distinct acoustic signals during the location of group members: inquiry calls and response calls (Fig. 1; Chaverri, Ancillotto, & Russo, 2018; Chaverri, Gillam, & Vohnhof, 2010); these signals are important for maintaining group cohesion, particularly since this species must locate new roost sites on a daily basis (Vohnhof & Fenton, 2004).

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**Figure 1.** Spectrograms of inquiry and response calls of juvenile and adult Spix's disc-winged bats. The estimated ages of juveniles when recorded were 42 days and 23 days for inquiry and response calls, respectively. Adults were at least 2 years old. Spectrograms were generated using customized versions of functions from the R package 'seewave' (Sueur, Aubin, & Simonis, 2008) with a hamming window function, 99% window overlap and 300 point fast Fourier transformation.

Inquiry calls are frequently emitted to maintain contact with group members during flight, even in situations of relative captivity (i.e. inside a flight cage). When an individual finds and enters a roost, it produces a response call in reply to inquiry calls from flying group and nongroup members (Chaverri & Gillam, 2013); in fact, response calls have not been recorded in any other context (Montero & Gillam, 2015), only as a result of the detection of an inquiry call, which may be easily prompted through playback. This call-and-response system resembles an antiphonal calling system but with the difference that the two signals are significantly different.

In this study, we evaluate the ontogeny of acoustic structure and individuality for inquiry and response calls in the Spix's disc-winged bat. The ontogenetic process is expected to resemble processes described in other highly vocal taxa, including bats. Juvenile social calls are typically of higher frequency than adult calls (Berg, Beissinger, & Bradbury, 2013; Brittan-Powell, Dooling, & Farabaugh, 1997; Lapshina et al., 2012), they also have higher entropy (Berg, Delgado, Cortopassi, Beissinger, & Bradbury, 2012; Liu, Wada, Jarvis, & Nottebohm, 2013) and, as individuals age, their calls can experience greater frequency modulations and thus typically have larger bandwidths (Esser & Schmidt, 1989). Older individuals with larger body size tend to produce lower-frequency signals, as low frequencies are usually constrained by the size of the vocal tract and resonance cavities, which usually covary with body size (Bradbury & Vehrencamp, 2011). For multisyllabic signals, as individuals age there may also be an increase in the number of syllables per call (Knörnschild, Nagy, Metz, Mayer, & von Helversen, 2010). In terms of the ontogeny of call individuality, previous studies have shown that this feature is not acquired until the later stages of vocal ontogeny (Brittan-Powell et al., 1997; Lapshina et al., 2012), although isolation calls in dependent young can also encode individual signatures to allow their mothers to accurately locate them (Engler, Rose, & Knörnschild, 2017).

We tested the hypothesis that the acoustic structure of the two social calls of Spix's disc-winged bats, inquiry and response, will follow similar ontogenetic paths as those experienced by other species. Specifically, we predicted that juvenile calls would have higher frequency, higher entropy, lower frequency modulation, lower bandwidth and fewer syllables in the multisyllabic (response) calls. For individuality, we expected that, if contact calls are initially used during ontogeny as isolation signals, then they should encode individual identity early during development.

## METHODS

### *Capturing and Processing Bats*

Field work was carried out at Barú Biological Station in southwestern Costa Rica during 2018 and 2019. To locate bats, we searched *Heliconia* spp., *Calathea* spp. and *Musa* spp. furled leaves, which are commonly used by Spix's disc-winged bats as roosting sites (Chaverri & Kunz, 2011; Vonhof & Fenton, 2004). Once we located a roost, we captured all group members and placed them inside a cloth holding bag. All individuals were sexed, and they were classified as dependent young if they were still suckling (i.e. attached to the female's nipple), or as independent juveniles if they were weaned but cartilaginous epiphyseal plates in metacarpals and phalanges were still present (i.e. a translucent space is seen in the metacarpal-phalange joint of the third finger upon transilluminating the bat's wing; Anthony, 1988). Individuals were classified as subadults if their plates had ossified but there were no signs of reproductive activity. If there was evidence of current reproductive activity (i.e. enlarged testes, pregnancy or lactation) or previous reproductive activity (i.e. testicular descent, keratinized nipples), individuals were classified as adults (Racey, 2009). The age (in days) of dependent young was estimated based on the length of

the forearm, an accurate ageing proxy for the Spix's disc-winged bat up to 106 days and 135 days of age for females and males, respectively, which is when young are finally capable of sustained flight (Chaverri & Vonhof, 2011).

All individuals older than 2 months were marked with transponders (Mini HPT8 Transponder; Biomark Inc., ID, U.S.A.) to individually identify callers upon recapture. We injected transponders subcutaneously in the mid-dorsal area to 23 adult bats (12 females, 11 males) after cleaning the needle, the transponder and the injection area and its surroundings with ethanol-based sanitizing gel. These transponders are  $1 \times 8$  mm and weigh approximately 0.03 g. Thus, they represent 0.67–1.07% of the bat's body mass. No anaesthesia was necessary for this procedure as the needle was not inserted but was simply used to create a very small incision through which the transponder could fit. Transponders were later scanned using Biomark's HPR Lite reader. Juveniles were accepted by their mothers after manipulation.

### Sound Recordings

We recorded 548 response calls from 21 juveniles, 50 inquiry calls from 5 juveniles and 286 inquiry calls from 23 adults. In addition, to compare response calls between age categories (non-volant juveniles, juveniles and adults), we used previous recordings from 99 calls from 13 adults, 106 calls from 8 nonvolant juveniles and 81 calls from 10 volant juveniles.

### Adults

We compared recordings of adult calls to those of juveniles to examine whether the observed ontogenetic process produces adult-like calls or whether further changes occur during maturation.

We recorded inquiry and response calls from adults in two separate ways. For inquiry calls, we released adults inside a large flight cage ( $9 \times 4 \times 3$  m) and allowed individuals to fly for a maximum of 5 min. We recorded their inquiry calls using a condenser microphone (CM16, Avisoft Bioacoustics, Glienike/Nordbahn, Germany) through an Avisoft UltraSoundGate 116Hm connected to a laptop computer running Avisoft-Recorder software (sampling rate of 375, 400, or 500 kHz, 16-bit amplitude resolution).

For recording response calls, we placed a bat inside a small transparent plastic cup, resembling the space within a tubular leaf, and inserted a circular piece of mesh at the entrance to prevent the bat's escape. Because bats only produce response calls after an inquiry call has been emitted (Chaverri et al., 2010), we broadcast prerecorded inquiry calls for 1–5 min through an UltrasoundGate Player to a broadband loudspeaker (Ultrasonic Omnidirectional Dynamic Speaker Vifa, Avisoft Bioacoustics) placed near the plastic cup. These inquiry calls were collected from five individuals from a nontest group flying within the large flight cage. A total of 67 inquiry calls were identified in the 1 min recording and they were not manipulated in any way. Spix's disc-winged bats respond indiscriminately to group and nongroup inquiry calls (Chaverri, Gillam, & Kunz, 2013), and our playback was effective at prompting response calling from roosting bats.

### Juveniles

Inquiry calls have only been recorded in Spix's disc-winged bats during flight (Chaverri et al., 2010), but individuals do not become partially volant until approximately 40 days of age (Chaverri & Vonhof, 2011). Therefore, to record inquiry calls from dependent juveniles, we wanted to mimic flight conditions as close as possible.

To prompt production of inquiry calls as early during development as possible, we gently detached the juvenile from the mother's nipple and quickly transported it to a small flight cage ( $3 \times 3 \times 2$  m). Juveniles were readily accepted by their mothers in all cases upon completion of the experiment. For young with a forearm length  $>25$  mm, we first held the uropatagium gently to determine whether the individual was flapping or already volant. Wing flapping in Spix's disc-winged bats occurs when young are approximately 25 days old, and they fly short distances at around 40 days of age (Chaverri & Vonhof, 2011). If individuals were not yet flapping, we held them on our hand and allowed them to vocalize for approximately 2 min. For juveniles with forearm length  $>25$  mm that were not yet volant, we prompted wing flapping while recording sounds emitted. Volant individuals were allowed to fly consecutively for a maximum of 1 min while recording any sounds emitted.

To record juveniles' response calls, we followed the same procedure used for adults, placing individuals inside a plastic cup and broadcasting previously recorded inquiry calls from nongroup members; however, playback was not extended beyond 2 min and we only broadcast one call every 10 s from seven different individuals. Unlike trials to record inquiry calls, response calling was triggered even for very young animals (approximately 4 days old) and was repeated when individuals were recaptured up to seven times. These data allowed us to measure changes in call parameters as individuals aged.

### Acoustic Analysis

We selected the two response calls that were produced right after a playback of an inquiry call, as those were more likely to be produced as a response to the inquiry call. Playbacks contained seven inquiry calls and most were re-recorded in all response call recordings (similar stimuli for eliciting response calls). The location of response call syllables in the frequency/time space was determined by automatic detection using the functions 'autodetec' (time detection, range 1–200 ms) and 'freq\_range' (frequency detection; 35–150 kHz) from the R package 'warbleR' v.1.1.16 (Araya-Salas & Smith-Vidaurre, 2017; R Core Team, 2019). Spectrograms of automatically detected syllables were visually inspected, and boxes showing the location of syllables were manually adjusted when necessary using Raven Pro 1.5 (Cornell Lab of Ornithology, Ithaca, NY, U.S.A.). We measured four spectrographic parameters on response call syllables: mean frequency (mean frequency of the spectrum), entropy (product of the entropies of the amplitude envelope and frequency spectrum; 0 = pure tone; 1 = noisy), skewness (asymmetry of the spectrum) and duration. Syllable level parameters were calculated as the average of all syllables within a call. We also measured two parameters at the call level: number of syllables and syllable rate (number of syllables/s). To quantify the consistency of response calls, we measured the coefficient of variation (CV, ratio of standard deviation to the mean) of the four parameters measured at the syllable level plus the CV of gap duration (silence between syllables). The two call level parameters were not included due to small sample sizes. CVs were summarized using principal component analysis and the first principal component was used to represent response call consistency. Summarizing variability in a single parameter allowed us to analyse the data using mixed-effect models (detailed below), which provide a useful statistical framework for evaluating changes in longitudinal data with complex hierarchical structure. The first principal component explained 33% of the variation, and four of the six CVs loaded negatively on this component. Hence, high PC1 values represented low acoustic variability (i.e. high acoustic consistency). Parameters were selected based on previous evidence of ontogenetic changes

in bats (Brown & Grinnell, 1980; Engler et al., 2017; Vater et al., 2003). Skewness was chosen as it represents the variation in syllable structure determined by visual inspection of spectrograms (U-shaped/symmetric syllables versus L-shaped/skewed syllables; Fig. 1). CVs were used to represent consistency as they produce relative variability measures that are comparable across parameters with different units and orders of magnitude.

The time and frequency range of inquiry calls was manually located on spectrograms using Raven Pro. For inquiry calls, we measured six acoustic parameters: duration, mean frequency, entropy, dominant frequency range, modulation index (cumulative absolute difference between adjacent measurements in a dominant frequency contour divided by the dominant frequency range) and frequency slope (difference between the dominant frequency at the start and end of the call divided by call duration). Consistency was not assessed on inquiry calls due to limited sample sizes. Modulation index, frequency slope and frequency range were chosen as they describe the most salient feature of inquiry calls: the downward frequency modulation (Fig. 1).

We also measured Mel frequency cepstral coefficients (MFCC) on both inquiry and response calls. These parameters quantify signals along a logarithmic scale that resembles pitch perception in mammals, including bats (Grinnell, 1995). MFCC have been successfully used to quantify variation in acoustic signal structure in bats (Mirzaei et al., 2012; Prat, Taub, & Yovel, 2016) and several other mammals (Clemins & Johnson, 2013; Fedurek, Zuberbühler, & Dahl, 2016; Mouy, Leary, Martin, & Laurinolli, 2008; Reby, André-Obrecht, Galinier, Farinas, & Cargnelutti, 2006; Roch, Soldevilla, Burtenshaw, Henderson, & Hildebrand, 2007). Note that these parameters have been tuned for human perception and might not accurately represent auditory perception in bats (Lyon & Ordubadi, 1982). We measured 25 cepstra on 10 warped spectral bands. The minimum, maximum, mean, median, skewness, kurtosis and variance of each MFCC and the mean and variance for the first and second derivatives were used for statistical analysis. These statistical descriptors of MFCC are commonly used in acoustic signal processing and detection (e.g. Salamon, Jacoby, & Bello, 2014). Statistical descriptors of MFCC were used for evaluating call individuality.

Recordings were resampled to a 375 kHz sampling rate prior to analyses. Acoustic analyses were conducted with a hamming window function, 90% window overlap and 300 (inquiry) or 600 sample (response) fast Fourier transformation, which resulted in a frequency resolution of 625 and 1250 Hz and a time resolution of 0.08 and 0.16 ms for inquiry and response calls, respectively. Both call types have harmonic structure, but only the fundamental frequency (first harmonic) was used for analysis because it contained most of the energy. All acoustic parameters were measured using the functions 'specan' (spectrographic parameters) and 'mfcc\_stats' (descriptors of cepstral coefficients) from the R package 'warbleR'. Raven Pro outputs were imported into R using the package 'Raven' v.1.0.6 (Araya-Salas, 2017).

### Statistical Analysis

#### Call ontogeny

We used Bayesian generalized linear multilevel (mixed) models in the R package 'MCMCglmm' (Hadfield, 2010) to assess common patterns of ontogenetic changes across individuals in the selected spectrographic and call level (response calls) parameters. We ran separate models for each acoustic parameter as a response, for both response and inquiry calls. Both sexes were included in all analyses. Age (as a continuous variable), sex and their interaction were included as predictors when evaluating changes in response calls in juveniles (e.g. all nonadult individuals, which include nonvolant

and volant juveniles). Age was treated as a categorical variable when comparing nonvolant juveniles, volant juveniles and adults. We did not include forearm length in the models as it was highly correlated to age ( $r = 0.79$ ). Including forearm in the models would not only add very little new information, but would also bias model estimates and inflate their precision (Bonate, 1999). Acoustic parameters from response calls of nonvolant (4.23–19.5 days old) and volant juveniles (49.9–65.2 days old) were not compared as this is implicitly included when evaluating ontogenetic changes in juveniles (e.g. when age was used as a continuous variable across early developmental stages). A similar approach was used to compare acoustic parameters of inquiry calls between juveniles and adults. Acoustic parameters were z-transformed prior to analysis to produce standardized effect sizes that would be comparable across models. Age as a continuous variable was mean-centred (by subtracting the mean age of the individual from each of its observations). This procedure does not affect the interpretation, because the variables retain their original units, but it makes effect size meaningful when including interaction terms (Schielzeth, 2010). We applied a model selection procedure based on the deviance information criterion (DIC) to determine the relative fit of competing models, including a null (intercept-only) model. The best model was defined as the one with the lowest DIC. We only evaluated the statistical significance of predictors for models that differed by more than 2 DIC units from the null model. Hence, model selection procedures in which the delta DIC of the null model was equal to or lower than 2 were considered as evidence of no significant effect(s) for the predictors involved.

We replicated all Bayesian mixed models three times using identical parameters, but randomly sampling the starting values from a Z distribution. We retained 9700 posterior samples for each model (chain length = 100 000, burn-in = 3000, thinning interval = 10). We evaluated model performance by comparing the trace and distribution of estimates among the three replicates. We also assessed the independence of successive sampled values (i.e. autocorrelation) of the MCMC chain and used the Gelman–Rubin diagnostic to check for convergence between the three runs (Hadfield, 2012). We present effect sizes as mean posterior estimates as well as the highest posterior density (HPD) interval (e.g. 95% credible interval). Effect sizes in which credible intervals did not overlap with zero were considered to have an effect on the response variable.

#### Individuality

We evaluated ontogenetic changes in call individuality (i.e. the discriminability of individuals based on call features) for both response and inquiry calls. Supervised random forest analysis (Breiman, 2001) in the R package 'ranger' (Wright & Ziegler, 2017) was used for discriminating individuals within each age category based on call structure. This method was preferred over most common alternatives (i.e. discriminant function analysis) as it can handle nonmonotonic relationships, is robust to outliers and it performs well on highly dimensional data (Breiman, 2001; Valletta et al., 2017). We used Box–Cox-transformed spectrographic, call level (for response calls) and MFCC parameters to characterize call structure. Collinear parameters ( $r > 0.9$ ) were excluded from the analyses. The out-of-bag error (the mean prediction error on each sample using only the random forest trees that did not have that sample) was used as a metric of classification performance. The same number of individuals and calls per individual were used to make individuality measures comparable across age categories. We used linear regression to assess a quadratic model in which individuality decreases during intermediate age categories. The fit of the model was compared to a null (intercept-only) model using the Akaike information criterion (AIC), and the 95% credible interval of



the quadratic term was used for assessing statistical significance. For response calls, age was categorized by splitting the age range between 1 and 70 days (the best sampled age range) into four periods of equal length, while including adults as a single category. We used the six individuals with the highest sample size within each age category, randomly sampling six calls per individual (the maximum number of calls available for some combinations of individuals and age categories). This procedure was repeated 100 times, and the mean out-of-bag error was calculated for each category. A similar approach was taken for evaluating inquiry call individuality in juveniles and adults. The five individuals with the highest sample size for each age group were used for assessing inquiry call individuality. A subsample of calls of equal size across categories (two individuals with 2 calls and three individuals with 10 calls, due to small sample size of some individuals) was randomly selected, and the procedure was repeated 100 times. The R code for the acoustic and statistical analyses is provided in the Supplementary Material.

### Ethical Note

All sampling protocols followed guidelines approved by the American Society of Mammalogists for capture, handling and care of mammals (Sikes et al., 2016) and the ASAB/ABS Guidelines for the use of animals in research. This study was conducted in accordance with the ethical standards for animal welfare of the Costa Rican Ministry of Environment and Energy, Sistema Nacional de Áreas de Conservación, permit no. SINAC-ACOPAC-RES-INV-008-2017 (Decree No. 32553-MINAE). Protocols were also approved by the University of Costa Rica's Institutional Animal Care and Use Committee (CICUA-42-2018).

In this study, we captured Spix's disc-winged bats in the wild by searching *Heliconia* spp., *Calathea* spp. and *Musa* spp. furled leaves. To avoid disturbing bats while in their roosts, we approached the leaf very quietly and searched for bats with an extendable mirror; if the presence of bats was confirmed, we identified individuals within the leaf with a passive integrated transponder (PIT) tag reader (HPR Plus, Biomark Inc., ID, U.S.A.) with a custom-made antennae ('Bat wand', Biomark) sensitive enough to identify all bats within the leaf. If the leaf was occupied by a group of interest, we placed a transparent plastic bag at the opening of the leaf and carefully pinched the leaf at the bottom, causing the bats to crawl to the opening and into the plastic bag. Once the bats were in the plastic bag, they were transferred into cloth bags for transportation; capture sites were no more than 15 min away (by foot) from the flight cage where experiments were conducted.

While performing flight cage experiments, we kept each social group together in the same bag to avoid any social disturbance; this does not result in conflicts and mimics natural social conditions that may decrease stress. Moreover, we kept bags in a ventilated area with no direct exposure to sunlight. If bats were participating in individual trials, after the trial we returned them to the same bags. We performed the flight cage experiments with minimal manipulation of bats. For this, we liberated the bats inside the flight cage directly from the cloth bag. If the bats entered a furled leaf placed inside the cage, we retrieved them following the procedure explained above. If bats failed to enter the leaf after 5 min, we captured them using a hand-net.

When recording the response calls of bats, individuals were placed singly inside a transparent plastic tube with small holes on the sides to aid in ventilation. The top of the tube was also kept well ventilated, but prevented the bat's escape by means of a circular piece of mesh placed at the entrance (see Sound Recordings above). Bats remained calm and in a natural resting position (vertical and

attached to the tube's inner wall) when they were introduced into this apparatus.

At the end of the experiments, we provided mealworms (*Tenebrio molitor*) and water to all individuals. We released the entire social group by placing all the individuals inside the same or a nearby leaf where they were found roosting earlier in the day. We have always used this technique for returning bats to their habitat, and individuals remain calm and in their natural roosting positions immediately after their return.

The procedures explained above, including manipulation, might cause some distress to some individuals. Thus, we decided not to perform individual trials on bats that were visually in distress and/or showing uncommon behaviours. However, these measures were not necessary during our experiments.

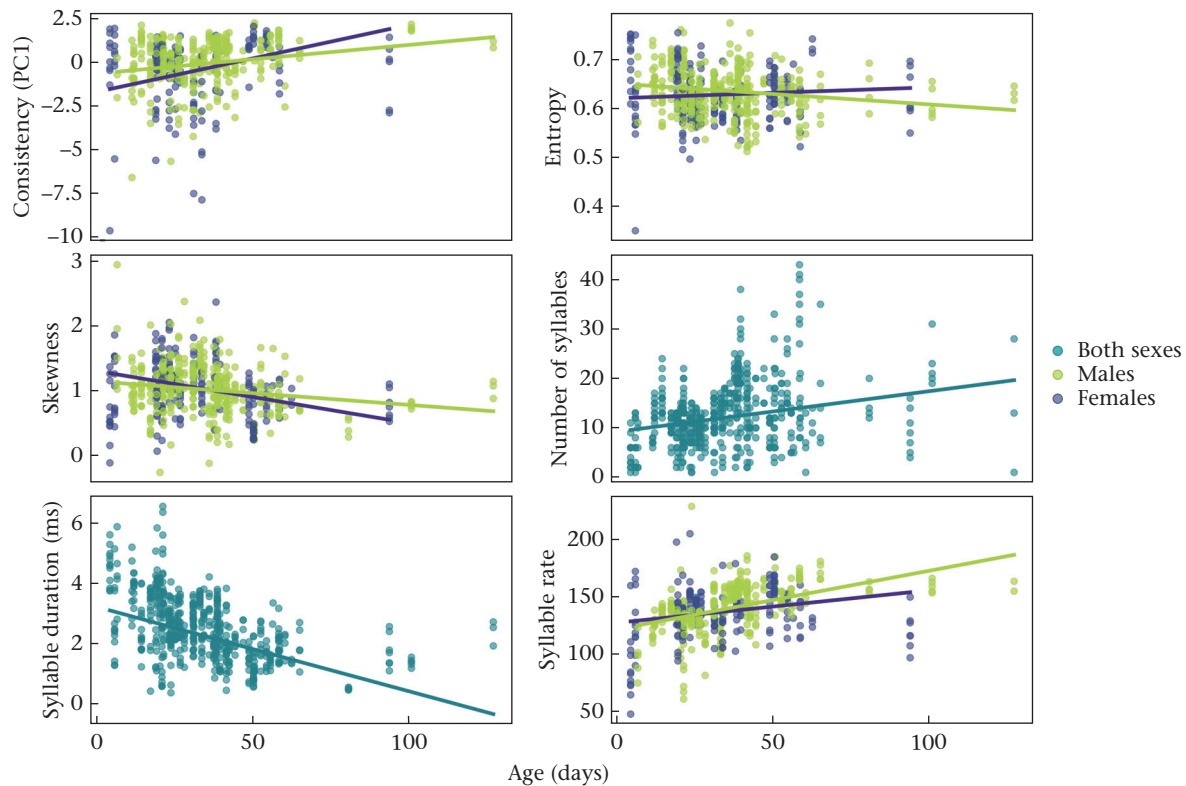
## RESULTS

### Call Ontogeny

A total of 22 young bats were used to test ontogenetic changes in response calling; all bats responded in at least one of the trials except one. The age of the youngest individual producing response calls was estimated at 6 days for males (mean: 25 days;  $N = 13$ ) and 4 days for females (mean: 26 days;  $N = 8$ ). The best statistical models for ontogenetic change in juveniles contained age as a predictor for size of the seven acoustic parameters ( $N = 21$ ; Appendix, Table A1). In five of the six acoustic parameters there was a significant effect of age: syllable rate, number of syllables and call consistency (PC1 on CVs) increased during ontogeny, while syllable duration and skewness decreased (Fig. 2, Appendix, Table A2). In addition, for four of the acoustic parameters the best model included the interaction between age and sex as predictor. This interaction was significant (i.e. the slope of ontogenetic change differed between sexes) for four of these parameters: males showed a more pronounced slope than females for syllable rate, while the slope of skewness and consistency was more pronounced in females. Differences in the ages when males and females were recorded prevented us for comparing exactly the same developmental period in both sexes. This difference could partially explain the observed pattern. However, statistical analyses excluding observations of males older than 106 days produced qualitatively equivalent results. Notice that for entropy only the interaction term was significant and only males showed a significant decrease. The statistical significance of a model with mean frequency as a response was not evaluated as the best model did not show a better fit compared to the null model (Appendix, Table A1).

We also compared call structure between adults and nonvolant and volant juveniles. In six of the seven acoustic parameters evaluated, the best model included age category as predictor ( $N = 29$ ; Appendix, Table A3). Significant differences were found between nonvolant juveniles and adults for three of these parameters: nonvolant juveniles showed lower consistency, syllable rate and number of syllables (Fig. 3, Appendix, Table A4). In addition, volant juveniles showed significantly lower syllable duration, number of syllables, syllable rate and skewness compared with adults (Fig. 3, Appendix, Table A4). A significant interaction between age and sex was detected only for mean frequency: adult males showed a higher frequency while adult females showed a lower frequency when compared to volant juveniles. We did not evaluate the statistical significance of a model with entropy as a response as the model did not show a better fit compared to the null model (Appendix, Table A3).

The age of the youngest male producing inquiry calls was estimated at 40 days ( $N = 13$ ; mean: 63 days). The age of females when the first inquiry calls were produced was not estimated due to lack



**Figure 2.** Scatterplots of acoustic parameters by age for the six parameters of response calls that had significant ontogenetic changes. Only parameters for which the statistical model had a better fit compared to the null model are shown. Lines depict the best fit line according to Bayesian linear mixed models. Data for the sexes are shown separately when models indicated a significant interaction between age and sex.

of forearm data. For inquiry calls, the best model included age category as a predictor for only three of the seven acoustic parameters: mean frequency, frequency range and call duration ( $N = 27$ ; Appendix, Table A5). The three parameters showed a significant difference: juveniles had a higher mean frequency, wider frequency range and longer call duration compared to adults (Fig. 4, Appendix, Table A6). Sex was not included as a predictor in any of the best models returned by our model selection procedure.

### Individuality

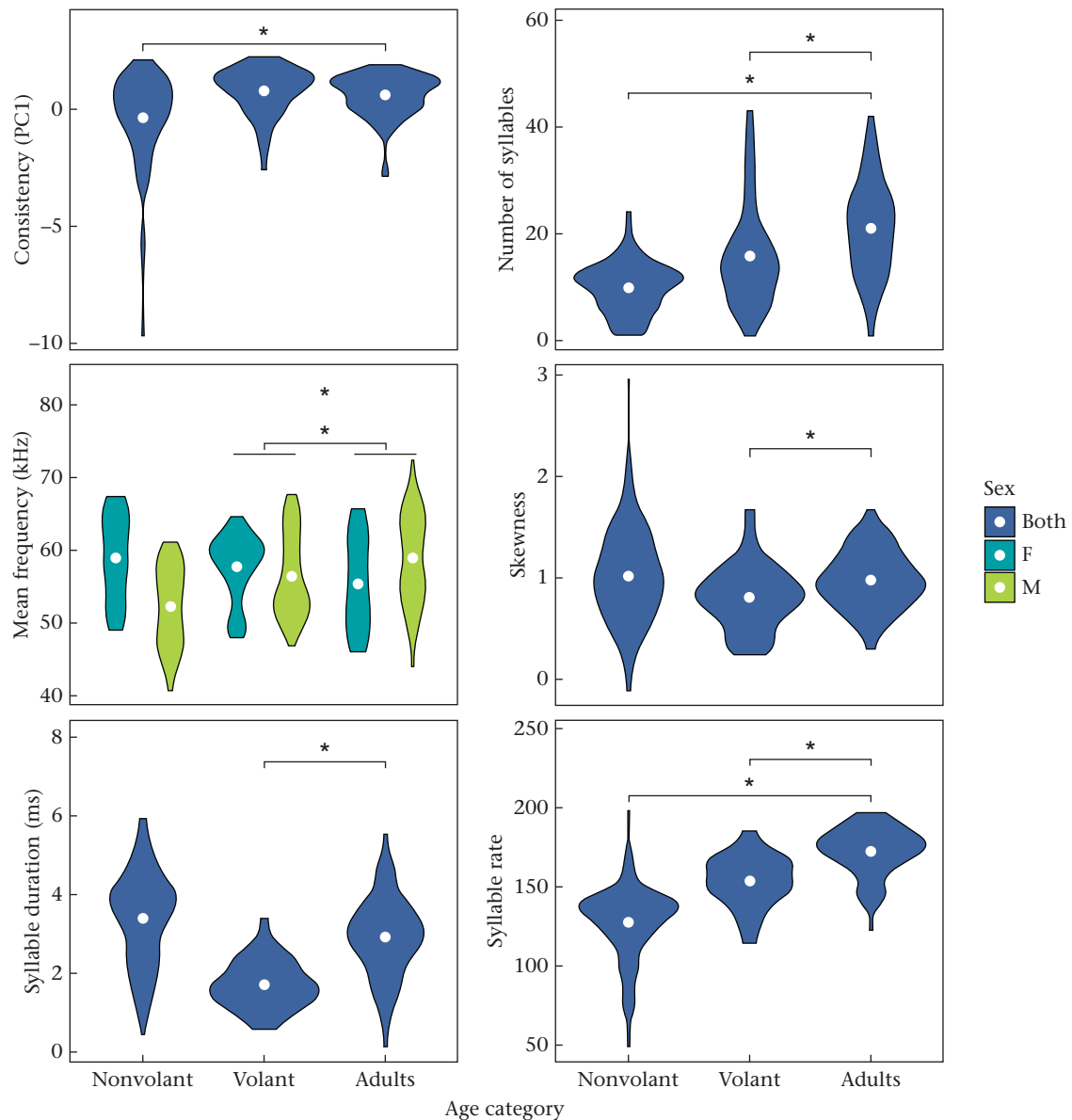
Response call individuality was high ( $> 0.5$ ) for all age categories. Furthermore, the linear model predicting a decrease in call individuality during intermediate ontogenetic stages, which included a quadratic term for age, had a lower AIC than the null model ( $-1104$  versus  $-745$ , respectively). A significant effect was found for the quadratic term (effect size: 1.30; lower CI: 1.14; upper CI: 1.46). Individuality was high early in life (4 days to ca. 20 days old; Fig. 5) but decreased later during ontogeny up to approximately 50 days of age, but then slightly increased towards adulthood. In contrast to response calls, inquiry call individuality showed no clear differences between juveniles and adults (Fig. 5); in fact, individuality was high for both age classes.

### DISCUSSION

Contact calls are a key feature of animal social communication. However, our knowledge of contact call development is still very limited, particularly for complex communication systems involving the interactive exchange of different call types (Rivera-Cáceres & Templeton, 2019). We investigated the ontogenetic changes of the two call types comprising the communication system involved in

the location of group members and roost sites in Spix's disc-winged bats. Our results show that both inquiry and response calls are produced early in life, and both call types go through structural changes during ontogeny. Developmental changes were detected in six acoustic parameters for response calls and in three parameters for inquiry calls. Furthermore, most ontogenetic changes were sex dependent, but specifically for response and not inquiry calls. Acoustic individuality was also high for both call types across age categories, although response call individuality seemed to be modulated throughout development whereas individuality for inquiry calls remained high and did not seem to change significantly throughout an individual's ontogeny.

Both call types, inquiry and response, were readily produced by juveniles when facing the social context in which they are typically used by adults. Response calls were produced by nonvolant juveniles that were tested during inquiry call playbacks. Similarly, inquiry calls were emitted by juveniles when held on the experimenter's hand and during their first flights in experimental cages; we could not detect inquiry call production before flight, and thus cannot conclusively confirm or rule out that bats are unable to produce them before they are volant. Nevertheless, juveniles were already able to respond accordingly by emitting response calls during very early stages of development. Furthermore, adults seem to respond to juvenile inquiry calls and to use response calls from juveniles to find the group's roost (G. Chaverri, personal observation), indicating that the signals are functional at this stage. Overall, the results suggest that the neural and morphological substrates involved in call production are sufficiently developed and integrated at early stages of life in Spix's disc-winged bats. The early onset of social calls has also been observed in other bat species (Boughman & Moss, 2003), and in a nonvocal-learning bird (Derégnaucourt, Saar, & Gahr, 2009),

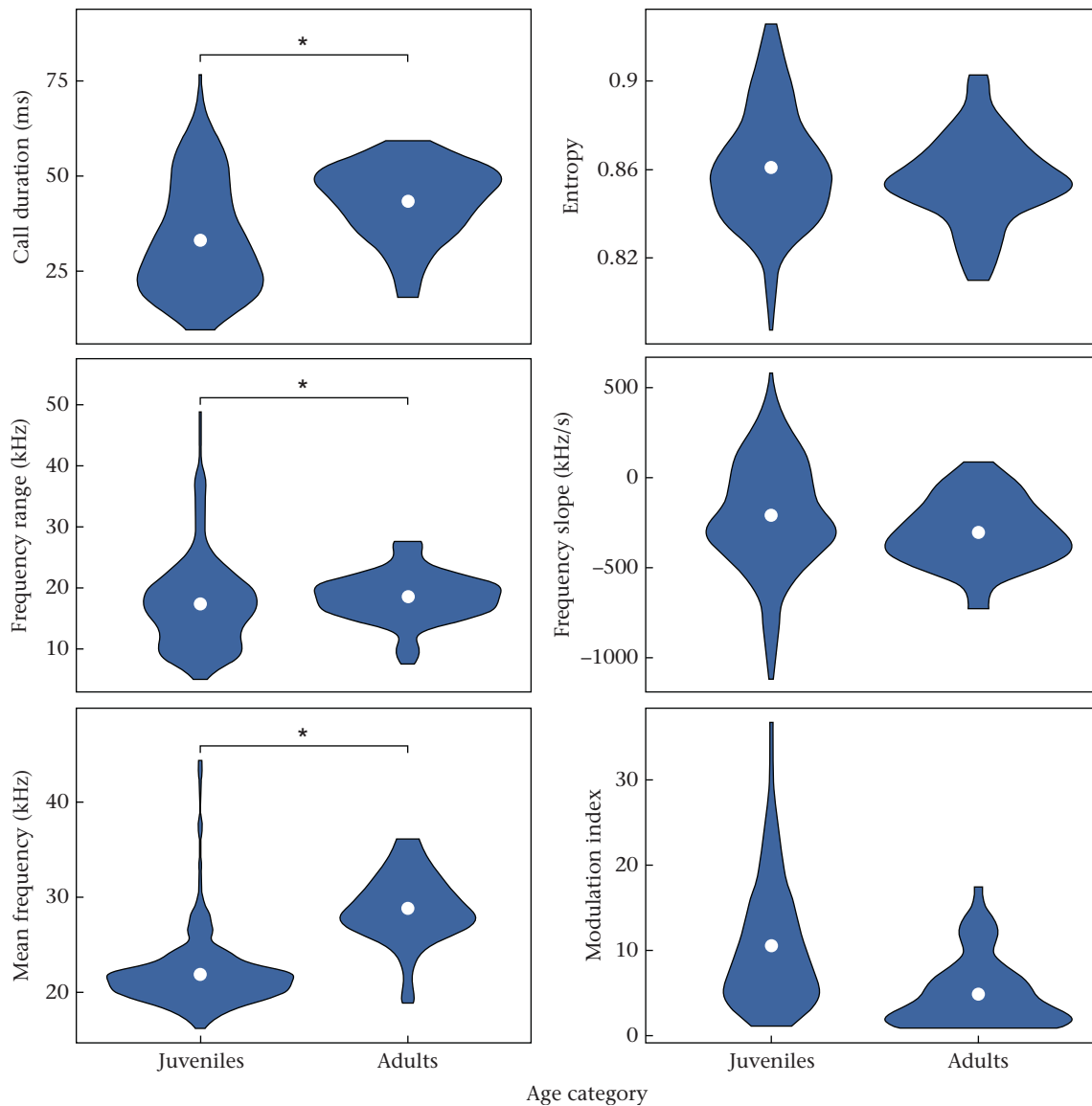


**Figure 3.** Violin plots showing the distribution of six response call acoustic parameters compared between juveniles (nonvolant and volant) and adults. Violin plots show the frequency distribution of values across the response variables. White dots show mean values. Asterisks indicate significant differences between the groups linked by brackets, except for mean frequency, in which significant differences in ontogeny between sexes were found. Only parameters for which the statistical model had a better fit compared to the null model are shown.

although it contrasts with the prolonged development of songs in vocal-learning birds (Araya-Salas & Wright, 2013; Hultsch & Todt, 2004), which may indicate that call development is, to a larger extent, innately determined in this bat species. The ability to emit social calls since early stages is likely favoured during evolution by the crucial role that these calls play during parent–offspring communication (Beecher, 1991).

Most ontogenetic changes in the social calls of Spix's disc-winged bats were predicted based on patterns of vocal development in other species. We found a decrease in entropy and syllable duration, and an increase in syllable rate, number of syllables and syllable consistency in response calls (Fig. 2). We also found a decrease in call duration and mean frequency during development in inquiry calls (Fig. 4). Indeed, similar ontogenetic paths on social calls have been previously documented in a variety of taxa (Berg et al., 2013; Brittan-Powell et al., 1997; Brown & Grinnell, 1980;

Lapshina et al., 2012; Sikes, 2016; Vater et al., 2003). Higher entropy and lower consistency during vocal development has been observed in birds (Berg et al., 2013; Liu, Feng, Jiang, Wu, & Sun, 2007) and could be the result of little laryngeal and respiratory control (Berg et al., 2013). This lack of control could also explain the lower syllable rate, shorter duration and fewer number of syllables in early ontogenetic stages. Repeated frequency-modulated sounds can require a high level of motor proficiency that can be difficult to attain during development (Podos, Peters, & Nowicki, 2004). However, our prediction of a decrease in frequency during development was just partially met as frequency only decreased in inquiry calls but not in response calls. Inquiry calls have much lower frequencies than both echolocation and response calls (~16–35 kHz, Chaverri et al., 2010). Low frequencies are usually constrained by body size (Bradbury & Vehrencamp, 2011). Hence, it seems likely that the observed ontogenetic changes in frequency



**Figure 4.** Violin plots showing the distribution of six inquiry call acoustic parameters compared between juveniles and adults. White dots show mean values. Asterisks indicate significant differences. Only parameters in which the statistical model had a better fit compared to the null model are shown.

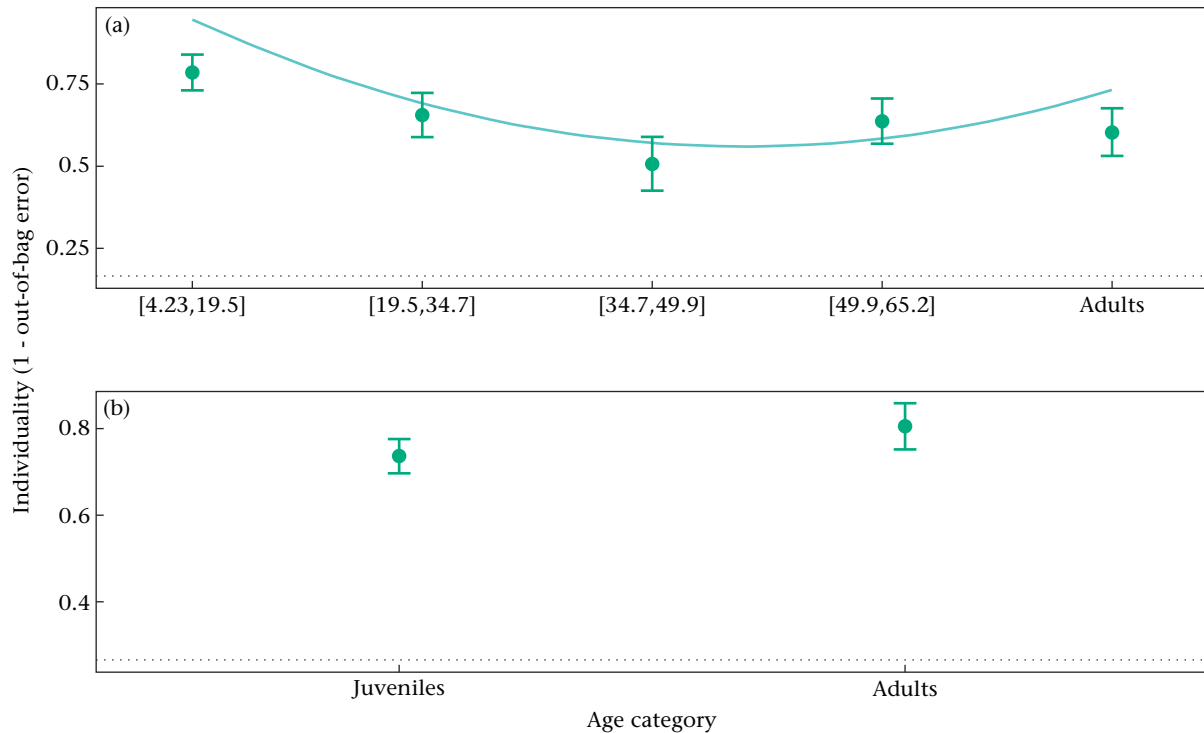
could indicate a morphological constraint only affecting inquiry call structure as body size increases during development.

Our results seem to indicate differences between the developmental processes of response and inquiry calls: six acoustic parameters changed in response calls (Fig. 2) but only three acoustic parameters changed in inquiry calls (Fig. 4). However, this variation could result from the different developmental periods in which each call type was evaluated, rather than actual ontogenetic variation between call types. Response calls were recorded as early as in the first week of life while inquiry calls were only recorded after individuals were at least 8 weeks old. Yet, when comparing response call structure between volants (49.9–65.2 days old, closer to the age range for juveniles in the inquiry call analysis) and adults (Fig. 3), five acoustic parameters remained significantly different but only one parameter showed development differences between sexes. Nevertheless, the comparison is still not ideal as the strong differences in acoustic structure between call types (Fig. 1) prevented us from measuring the same acoustic parameters. As a result, only two parameters (duration and mean frequency) were

measured on response and inquiry calls; both parameters differed during ontogeny for both call types. Overall, there is no conclusive evidence to support differential ontogeny between the two main call types in the communication system of Spix's disc-winged bats.

We observed sex differences during early ontogeny of response calls (Fig. 2). These differences might also occur in inquiry calls, but the age range in which these calls were evaluated may have precluded us for detecting a similar pattern (discussed above). Nevertheless, sex-specific ontogenetic differences were not found between volant juveniles and adults in either response or inquiry calls, suggesting that differences between sexes occur mostly during early development and that different ontogenetic trajectories may converge later in life. This ontogenetic sexual dimorphism could result from differences in body size; females tend to be larger than males in this species (Chaverri & Vonhof, 2011). However, the acoustic traits that typically covary with body size (e.g. frequency and duration) did not differ between sexes. Most varying parameters were related to syllable shape (skewness) or overall call structure (consistency, entropy, syllable rate). Sex-related





**Figure 5.** Individuality of (a) response and (b) inquiry calls. Individuality was measured as 1 - out-of-bag error from supervised random forest on spectrographic parameters and statistical descriptors of Mel cepstral coefficients. Black dotted horizontal lines indicate the error expected by chance. The curved line in the top panel shows the best fit line for the quadratic linear regression model predicting response call individuality by age. Numbers in the X axis in (a) correspond to the age range of juveniles in days for each period.

ontogenetic differences in call structure could also arise from sexual developmental dimorphism in the organs involved in vocal sound production, as has been observed in other mammals (Frey, Volodin, Volodina, Soldatova, & Juldachev, 2011). Alternatively, differences between sexes may be attributed to social learning in vocal production (Briefer & McElligott, 2011; Knörnschild, 2014; Zann, 1985). The geographical variation of both call types in Spix's disc-winged bats has been previously examined as a proxy for vocal learning (Montero, Sagot, Phillips, Baker, & Gillam, 2015). Dialect-like spatial variation at a small geographical scale typically suggests that behaviours are socially learned (Araya-Salas et al., 2019; Sewall, Young, & Wright, 2016). However, no conclusive evidence was found supporting vocal learning of social calls in this species (Montero et al., 2015). Finally, the call's function may be additionally related to sexual recognition or sexual selection, and not exclusively to maintaining contact with group members as has been presumed so far (Chaverri & Gillam, 2016), which could explain sex differences in call development. Finally, difference in age ranges between males and females might explain the observed sex-dependent ontogeny. However, age ranges between sexes largely overlap. Therefore, we find it very unlikely that this small range difference will generate significantly different ontogenetic patterns among sexes for most acoustic parameters.

We found strong individual signatures on both inquiry and response calls even during early ontogenetic stages. Indeed, high call individuality in adults has been documented in this species for both types of calls (Gillam & Chaverri, 2012). Furthermore, we were able to track the development of response call individuality and found a decrease in individuality during intermediate stages (Fig. 5a). This contradicted our prediction based on previous research, which suggested an increase in call individuality as pups develop (Brittan-Powell et al., 1997; Lapshina et al., 2012). The

observed pattern could arise if response calls are used early in life for mother-offspring recognition and only later develop into fully functional response calls. The individuality of response calls early in life could be critical in Spix's disc-winged bats if mothers depend on these vocalizations for relocating pups. If so, this would predict that pup recognition from mothers would decrease during development. Additional experiments on mother-offspring recognition would be warranted to evaluate this hypothesis.

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### Supplementary Material

Supplementary material associated with this article is available, in the online version, at <https://doi.org/10.1016/j.anbehav.2020.05.018>.

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Appendix

Table A1

Deviance information criterion (DIC), delta DIC, log likelihood (logLik) and degrees of freedom (df) for the model selection procedure on response call ontogeny in juveniles

Response	Predictors	df	logLik	DIC	Delta	Weight
<b>Mean_freq</b>	<b>Age + sex + age:sex</b>	<b>6</b>	<b>-644.0917</b>	<b>1310.701</b>	<b>0.000000</b>	<b>5.683621e-01</b>
Mean_freq	Null	3	-645.7765	1311.926	1.2245884	3.081124e-01
<b>Mean_freq</b>	<b>Age</b>	<b>4</b>	<b>-646.1141</b>	<b>1313.754</b>	<b>3.0526219</b>	<b>1.235255e-01</b>
Entropy	Age + sex + age:sex	6	-739.5998	1498.321	0.0000000	7.976226e-01
<b>Entropy</b>	<b>Null</b>	<b>3</b>	<b>-742.3224</b>	<b>1501.803</b>	<b>3.4825731</b>	<b>1.398190e-01</b>
Entropy	Age	4	-742.9530	1503.412	5.0910720	6.255834e-02
<b>Skewness</b>	<b>Age + sex + age:sex</b>	<b>6</b>	<b>-721.3082</b>	<b>1463.116</b>	<b>0.0000000</b>	<b>8.622298e-01</b>
Skewness	Age	4	-723.9567	1466.784	3.6678777	1.377696e-01
<b>Skewness</b>	<b>Null</b>	<b>3</b>	<b>-736.7806</b>	<b>1491.728</b>	<b>28.6120775</b>	<b>5.279458e-07</b>
Num_syllables	Age	4	-641.2205	1303.279	0.0000000	5.903079e-01
<b>Num_syllables</b>	<b>Age + sex + age:sex</b>	<b>6</b>	<b>-641.0507</b>	<b>1304.010</b>	<b>0.7304981</b>	<b>4.096877e-01</b>
Num_syllables	Null	3	-653.3139	1326.863	23.5837286	4.466188e-06
<b>Syll_duration</b>	<b>Age + sex + age:sex</b>	<b>6</b>	<b>-650.2093</b>	<b>1320.076</b>	<b>0.0000000</b>	<b>6.401759e-01</b>
Syll_duration	Age	4	-651.0208	1321.228	1.1522557	3.598241e-01
<b>Syll_duration</b>	<b>Null</b>	<b>3</b>	<b>-685.3835</b>	<b>1390.724</b>	<b>70.6482810</b>	<b>2.918906e-16</b>
Syll_rate	Age + sex + age:sex	6	-655.7364	1332.508	0.0000000	6.224752e-01
<b>Syll_rate</b>	<b>Age</b>	<b>4</b>	<b>-656.6321</b>	<b>1333.508</b>	<b>1.0001349</b>	<b>3.775248e-01</b>
Syll_rate	Null	3	-682.5551	1384.997	52.4890174	2.490444e-12
<b>PC1_consistency</b>	<b>Age + sex + age:sex</b>	<b>6</b>	<b>-582.3475</b>	<b>1185.576</b>	<b>0.0000000</b>	<b>9.240753e-01</b>
PC1_consistency	Age	4	-585.4975	1190.574	4.9981031	7.592469e-02
<b>PC1_consistency</b>	<b>Null</b>	<b>3</b>	<b>-600.0382</b>	<b>1218.727</b>	<b>33.1501761</b>	<b>5.851107e-08</b>

Rows containing the best models are shown in bold.

Table A2

Effect sizes for the evaluated predictors from models on response call ontogeny in juveniles

Response	Predictor	Effect_size	CI_2.5	CI_97.5	pMCMC	Intercept	N			No. of observations		
							Total	Males	Females	Total	Males	Females
Entropy	Age	0.0045	-0.0035	0.0131	0.2854	-0.0725	21	13	8	548	347	201
Entropy	Age:sexM	-0.0130	<b>-0.0233</b>	<b>-0.0021</b>	<b>0.0153</b>	-0.0725	21	13	8	548	347	201
Skewness	Age	-0.0202	<b>-0.0280</b>	<b>-0.0121</b>	<b>0.0001</b>	0.0488	21	13	8	548	347	201
Skewness	Age:sexM	0.0111	<b>0.0008</b>	<b>0.0214</b>	<b>0.0363</b>	0.0488	21	13	8	548	347	201
Num_syllables	Age	0.0126	<b>0.0081</b>	<b>0.0172</b>	<b>0.0001</b>	-0.0899	21	13	8	548	347	201
Syll_duration	Age	-0.0264	<b>-0.0334</b>	<b>-0.0193</b>	<b>0.0001</b>	-0.2382	21	13	8	548	347	201
Syll_duration	Age:sexM	0.0063	-0.0028	0.0152	0.1703	-0.2382	21	13	8	548	347	201
Syll_rate	Age	0.0132	<b>0.0059</b>	<b>0.0204</b>	<b>0.0010</b>	-0.1278	21	13	8	541	343	198
Syll_rate	Age:sexM	0.0105	<b>0.0008</b>	<b>0.0206</b>	<b>0.0365</b>	-0.1278	21	13	8	541	343	198
PC1_consistency	Age	0.0241	<b>0.0147</b>	<b>0.0327</b>	<b>0.0001</b>	-0.2001	21	13	8	466	315	151
PC1_consistency	Age:sexM	-0.0138	<b>-0.0253</b>	<b>-0.0022</b>	<b>0.0202</b>	-0.2001	21	13	8	466	315	151

Rows and 95% credible intervals (CI 2.5 and CI 97.5) for significant effects are shown in bold.

**Table A3**

Deviance information criterion (DIC), delta DIC log likelihood (logLik) and degrees of freedom (*df*) for the model selection procedure on response call ontogeny compared between juveniles (nonvolant and volant) and adults

Response	Predictors	<i>df</i>	logLik	DIC	Delta	Weight
<b>Mean_freq</b>	<b>Age + sex + age:sex</b>	<b>8</b>	<b>-267.3709</b>	<b>563.9749</b>	<b>0.000000</b>	<b>9.126064e-01</b>
Mean_freq	Age	5	-270.0399	568.6667	4.6917651	8.739360e-02
Mean_freq	Null	3	-286.7401	600.5769	36.6019431	1.028662e-08
<b>Entropy</b>	<b>Null</b>	<b>3</b>	<b>-354.3676</b>	<b>731.9012</b>	<b>0.000000</b>	<b>4.677987e-01</b>
Entropy	Age	5	-354.5167	732.6071	0.7059062	3.286801e-01
Entropy	Age + sex + age:sex	8	-354.0371	733.5658	1.6645353	2.035212e-01
<b>Skewness</b>	<b>Age + sex + age:sex</b>	<b>8</b>	<b>-358.5480</b>	<b>742.8172</b>	<b>0.000000</b>	<b>9.987351e-01</b>
Skewness	Age	5	-367.1023	756.1603	13.3430619	1.264856e-03
Skewness	Null	3	-382.5558	783.9999	41.1826377	1.139605e-09
<b>Num_syllables</b>	<b>Age</b>	<b>5</b>	<b>-328.3041</b>	<b>678.1609</b>	<b>0.000000</b>	<b>6.255427e-01</b>
Num_syllables	Age + sex + age:sex	8	-328.5402	680.1985	2.0376139	2.258368e-01
Num_syllables	Null	3	-328.3623	681.0354	2.8744474	1.486205e-01
<b>Syll_duration</b>	<b>Age + sex + age:sex</b>	<b>8</b>	<b>-286.4817</b>	<b>598.4043</b>	<b>0.000000</b>	<b>6.578099e-01</b>
Syll_duration	Age	5	-287.7615	599.7114	1.3070994	3.421901e-01
Syll_duration	Null	3	-308.4651	642.6538	44.2494783	1.619751e-10
<b>Syll_rate</b>	<b>Age</b>	<b>5</b>	<b>-239.8177</b>	<b>504.3797</b>	<b>0.000000</b>	<b>6.933191e-01</b>
Syll_rate	Age + sex + age:sex	8	-240.4084	506.0171	1.6373650	3.057622e-01
Syll_rate	Null	3	-245.0400	517.6323	13.2525543	9.187081e-04
<b>PC1_consistency</b>	<b>Age + sex + age:sex</b>	<b>8</b>	<b>-322.1095</b>	<b>662.9012</b>	<b>0.000000</b>	<b>8.928831e-01</b>
PC1_consistency	Age	5	-326.9360	667.1458	4.2445492	1.069307e-01
PC1_consistency	Null	3	-331.9215	679.8519	16.9506259	1.862143e-04

Rows containing best models are shown in bold.

**Table A4**

Effect sizes for the evaluated predictors from models comparing response call acoustic parameters between juveniles (nonvolant and volant) and adults

Response	Predictor	Effect_size	CI_2.5	CI_97.5	pMCMC	<i>N</i>	No. of observations						
							Total	Nonvolant	Volant	Adults	Total	Nonvolant	Volant
Mean_freq	Nonvolant_vs_adults	-0.0047	-1.1140	1.2172	0.9975	29	8	10	13	286	106	81	99
<b>Mean_freq</b>	<b>Volant_vs_adults</b>	<b>1.2850</b>	<b>0.1800</b>	<b>2.4832</b>	<b>0.0318</b>	<b>29</b>	<b>8</b>	<b>10</b>	<b>13</b>	<b>286</b>	<b>106</b>	<b>81</b>	<b>99</b>
Mean_freq	SexM	1.0485	-0.1006	2.1438	0.0693	29	8	10	13	286	106	81	99
Mean_freq	Nonvolant_vs_adults:sexM	-1.1278	-2.7169	0.3800	0.1489	29	8	10	13	286	106	81	99
<b>Mean_freq</b>	<b>Volant_vs_adults:sexM</b>	<b>-2.0629</b>	<b>-3.6378</b>	<b>-0.5161</b>	<b>0.0070</b>	<b>29</b>	<b>8</b>	<b>10</b>	<b>13</b>	<b>286</b>	<b>106</b>	<b>81</b>	<b>99</b>
Skewness	Nonvolant_vs_adults	0.7804	-0.2325	1.8091	0.1165	29	8	10	13	285	106	81	98
<b>Skewness</b>	<b>Volant_vs_adults</b>	<b>-1.0161</b>	<b>-1.9862</b>	<b>-0.1058</b>	<b>0.0390</b>	<b>29</b>	<b>8</b>	<b>10</b>	<b>13</b>	<b>285</b>	<b>106</b>	<b>81</b>	<b>98</b>
Skewness	SexM	-0.2368	-1.1037	0.7048	0.5779	29	8	10	13	285	106	81	98
Skewness	Nonvolant_vs_adults:sexM	-0.5713	-1.8917	0.8210	0.4062	29	8	10	13	285	106	81	98
Skewness	Volant_vs_adults:sexM	1.0721	-0.2518	2.3834	0.0922	29	8	10	13	285	106	81	98
<b>Num_syllables</b>	<b>Nonvolant_vs_adults</b>	<b>-1.0898</b>	<b>-1.5124</b>	<b>-0.6632</b>	<b>0.0001</b>	<b>29</b>	<b>8</b>	<b>10</b>	<b>13</b>	<b>286</b>	<b>106</b>	<b>81</b>	<b>99</b>
<b>Num_syllables</b>	<b>Volant_vs_adults</b>	<b>-0.6459</b>	<b>-1.0743</b>	<b>-0.2110</b>	<b>0.0043</b>	<b>29</b>	<b>8</b>	<b>10</b>	<b>13</b>	<b>286</b>	<b>106</b>	<b>81</b>	<b>99</b>
Syll_duration	Nonvolant_vs_adults	0.4424	-0.3319	1.2039	0.2518	29	8	10	13	286	106	81	99
<b>Syll_duration</b>	<b>Volant_vs_adults</b>	<b>-1.2624</b>	<b>-1.9648</b>	<b>-0.5005</b>	<b>0.0014</b>	<b>29</b>	<b>8</b>	<b>10</b>	<b>13</b>	<b>286</b>	<b>106</b>	<b>81</b>	<b>99</b>
Syll_duration	SexM	-0.3053	-0.9621	0.4220	0.3734	29	8	10	13	286	106	81	99
Syll_duration	Nonvolant_vs_adults:sexM	-0.0316	-1.0826	0.9114	0.9639	29	8	10	13	286	106	81	99
Syll_duration	Volant_vs_adults:sexM	0.6489	-0.3448	1.6004	0.1839	29	8	10	13	286	106	81	99
<b>Syll_rate</b>	<b>Nonvolant_vs_adults</b>	<b>-1.6641</b>	<b>-2.0645</b>	<b>-1.2471</b>	<b>0.0001</b>	<b>29</b>	<b>8</b>	<b>10</b>	<b>13</b>	<b>281</b>	<b>104</b>	<b>80</b>	<b>97</b>
<b>Syll_rate</b>	<b>Volant_vs_adults</b>	<b>-0.9036</b>	<b>-1.3261</b>	<b>-0.5095</b>	<b>0.0001</b>	<b>29</b>	<b>8</b>	<b>10</b>	<b>13</b>	<b>281</b>	<b>104</b>	<b>80</b>	<b>97</b>
<b>PC1_consistency</b>	<b>Nonvolant_vs_adults</b>	<b>-0.9831</b>	<b>-1.7496</b>	<b>-0.2447</b>	<b>0.0072</b>	<b>28</b>	<b>8</b>	<b>9</b>	<b>13</b>	<b>245</b>	<b>93</b>	<b>59</b>	<b>93</b>
PC1_consistency	Volant_vs_adults	0.3259	-0.3882	1.0885	0.3732	28	8	9	13	245	93	59	93
PC1_consistency	SexM	0.0777	-0.5271	0.7262	0.7946	28	8	9	13	245	93	59	93
PC1_consistency	Nonvolant_vs_adults:sexM	0.3267	-0.5831	1.2893	0.4728	28	8	9	13	245	93	59	93
PC1_consistency	Volant_vs_adults:sexM	-0.3000	-1.2801	0.6183	0.5311	28	8	9	13	245	93	59	93

Rows and 95% credible intervals (CI 2.5 and CI 97.5) for significant effects are shown in bold.



**Table A5**Deviance information criterion (DIC), delta DIC log likelihood (logLik) and degrees of freedom (*df*) for the model selection procedure on response call ontogeny in juveniles

Response	Predictors	<i>df</i>	logLik	DIC	Delta	Weight
<b>Call_duration</b>	<b>Age</b>	<b>4</b>	<b>-277.2300</b>	<b>580.1603</b>	<b>0.000000</b>	<b>0.595314098</b>
Call_duration	Age + sex + age:sex	6	-277.3837	580.9937	0.8334818	0.392426116
Call_duration	Null	3	-281.2728	587.9258	7.7655294	0.012259786
<b>Mean_freq</b>	<b>Age</b>	<b>4</b>	<b>-287.4203</b>	<b>600.6877</b>	<b>0.000000</b>	<b>0.519585208</b>
Mean_freq	Age + sex + age:sex	6	-287.3498	600.8624	0.1746947	0.476126454
Mean_freq	Null	3	-292.2883	610.2820	9.5942633	0.004288338
<b>Entropy</b>	<b>Null</b>	<b>3</b>	<b>-416.2397</b>	<b>854.0050</b>	<b>0.000000</b>	<b>0.478003406</b>
Entropy	Age	4	-416.6994	854.9629	0.9578765	0.296094785
Entropy	Age + sex + age:sex	6	-416.4688	855.5041	1.4990348	0.225901810
<b>Freq_range</b>	<b>Age + sex + age:sex</b>	<b>6</b>	<b>-402.6839</b>	<b>828.6203</b>	<b>0.000000</b>	<b>0.814258254</b>
Freq_range	Age	4	-404.9149	831.6813	3.0610390	0.176224379
Freq_range	Null	3	-408.6106	837.5186	8.8983187	0.009517367
<b>Modulation_index</b>	<b>Null</b>	<b>3</b>	<b>-387.9509</b>	<b>797.7304</b>	<b>0.000000</b>	<b>0.616802344</b>
Modulation_index	Age	4	-388.9146	799.4758	1.7454562	0.257706287
Modulation_index	Age + sex + age:sex	6	-389.1299	800.9150	3.1846233	0.125491369
<b>Freq_slope</b>	<b>Age + sex + age:sex</b>	<b>6</b>	<b>-445.0349</b>	<b>911.8661</b>	<b>0.000000</b>	<b>0.553159440</b>
Freq_slope	Age	4	-446.8132	913.4850	1.6189152	0.246210945
Freq_slope	Null	3	-447.3791	913.8945	2.0283716	0.200629615

Rows containing best models are shown in bold.

**Table A6**

Effect sizes for the evaluated predictors from models comparing inquiry call acoustic parameters between juveniles and adults

Response	Predictor	Effect_size	CI_2.5	CI_97.5	pMCMC	<i>N</i>			No. of observations		
						Total	Juvs	Adults	Total	Juvs	Adults
<b>Call_duration</b>	<b>Age.classjuveniles</b>	<b>1.0103</b>	<b>0.4315</b>	<b>1.5635</b>	<b>0.0004</b>	<b>27</b>	<b>5</b>	<b>23</b>	<b>336</b>	<b>50</b>	<b>286</b>
<b>Mean_freq</b>	<b>Age.classjuveniles</b>	<b>1.2252</b>	<b>0.6608</b>	<b>1.8182</b>	<b>0.0002</b>	<b>27</b>	<b>5</b>	<b>23</b>	<b>336</b>	<b>50</b>	<b>286</b>
<b>Freq_range</b>	<b>Age.classjuveniles</b>	<b>1.2455</b>	<b>0.3193</b>	<b>2.2092</b>	<b>0.0063</b>	<b>27</b>	<b>5</b>	<b>23</b>	<b>336</b>	<b>50</b>	<b>286</b>
Freq_range	SexM	-0.0970	-0.6740	0.5052	0.7530	27	5	23	336	50	286
Freq_range	Age.classjuveniles:sexM	-1.0141	-2.4557	0.2251	0.1172	27	5	23	336	50	286
Freq_slope	Age.classjuveniles	-0.7177	-1.6765	0.2104	0.1351	27	5	23	336	50	286
Freq_slope	SexM	0.0687	-0.4910	0.6660	0.8154	27	5	23	336	50	286
Freq_slope	Age.classjuveniles:sexM	0.6792	-0.5764	2.0186	0.2902	27	5	23	336	50	286

Rows and 95% credible intervals (CI 2.5 and CI 97.5) for significant effects are shown in bold.