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Echolocation call divergence in bats: a comparative analysis

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Abstract

Animal vocalizations experience pressures from ecological conditions, but their diversification may be constrained by morphology and evolutionary history. To date, the relative contribution of these factors to acoustic diversity is unclear in most vertebrate groups. Bats constitute one of the most speciose and diverse mammal groups, and most bat species rely on vocalizations for orientation, foraging, and communication. Here, we examine echolocation calls of 207 bat species across 17 families to weigh the relative role of phylogenetic inertia, natural selection, and morphological constraints in shaping echolocation call diversity in bats. Using the large dataset, we confirm that foraging guilds, phylogenetic relationships, and forearm length account for the majority of the variation in call parameters among bats. Foraging guilds play a major role in influencing call parameters in low duty cycle bats. At the family level, the variation in call parameters is primarily explained by differences in body size and phylogenetic relationships. Path analyses indicate that phylogeny determines call output not only by their direct effect on call parameters but also by having an indirect effect via foraging guilds and body size. These results demonstrate that natural selection, phylogenetic constraint, and morphological constraint shape echolocation call divergence in bats. Our findings underscore the importance of both adaptive and non-adaptive mechanisms underlying the evolution of echolocation calls in bats.

Significance statement

Ecology, morphology, and evolutionary history are tightly coupled; therefore, disentangling the relative strength of these components underlying acoustic diversity is a big challenge. Using a large dataset of bats, we assess the influence of phylogeny, ecology, and body size on echolocation call parameters. We conclude that ecological selection, phylogenetic constraint, and morphological constraint play a crucial role in shaping echolocation call divergence among bats. This study expands our knowledge of the relative contribution of adaptive and maladaptive mechanisms to echolocation call diversity at different taxonomic levels in bats.

Keywords Chiroptera · Acoustic signal · Ecology · Evolution · Phylogeny

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Introduction

Acoustic signals mediate many biological tasks, including mate selection, resource use, and social recognition in animals (Bradbury and Vehrencamp 2011). As part of a crucial sensory modality associated with survival and reproduction, hearing and vocalizations are complexly intertwined with the ecology, morphology, and evolutionary history of species (Endler 1992; Ryan and Kime 2003). The sensory drive theory proposes that animal acoustic signals are adapted to maximize information transmission and reception in their particular environment (Endler 1992). However, the available acoustic space is constrained by the sound production apparatus and phylogenetic history in a variety of species (Ryan and Kime 2003). The relative importance of phylogenetic history, ecological factors, and morphology underlying animal acoustic diversity remains poorly investigated.

Bats are the second most species-rich group of mammals with great morphological and ecological diversity (Simmons et al. 2005; Luo et al. 2019). The majority of bats have mastered the use of echolocation for spatial orientation and foraging (Schnitzler et al. 2003). Since the information contained in an echo is directly determined by the characteristics of the emitted sounds, bat echolocation calls are expected to evolve in response to environmental challenges and to optimize performance during foraging tasks (Schnitzler et al. 2003; Jones and Holderied 2007; Jakobsen et al. 2013). In particular, the emitted signal and clutter echoes produce masking effects if they overlap with prey echo, which prevents or reduces the possibility of detecting prey (Kalko and Schnitzler 1998). To mitigate auditory masking, some low duty cycle (LDC) bats utilize short frequency-modulated (FM) calls to avoid overlap of prey echo with interfering signals (Kalko and Schnitzler 1993). High duty cycle (HDC) bats emit long constant frequency (CF) calls that exploit Doppler shift compensation to maintain echo frequency within the sensitive frequency range of the auditory fovea (Schnitzler et al. 2003; Schnitzler and Denzinger 2011). Previous studies have shown that call duration, sound intensity, and frequency parameters act together to determine the effective range of bats' echolocation (Waters et al. 1995; Houston et al. 2004). Behavioral experiments on some vespertilionid bats have established the role of call bandwidth in prey detection performance (Siemers and Schnitzler 2004; Schmieder et al. 2012). Combined, these findings indicate bat echolocation calls present an excellent system to study the adaptive evolution of acoustic signals in animals.

The hypothesis that bat echolocation calls evolved adaptively to match ecological demands is supported by evidence of convergent evolution in echolocation call characteristics within guilds (Simmons et al. 1979; Neuweiler 1984; Kingston et al. 2000; Schnitzler and Kalko 2001; Schnitzler et al. 2003; Denzinger and Schnitzler 2013). In general, openspace foragers emit long narrowband calls of low frequency, which is beneficial for long-range detection of larger prey due to less attenuation and increased signal energy (Denzinger and Schnitzler 2013). However, echolocation calls of bat species that forage in edge habitats are of short duration, resulting in a marked decline in temporal overlap between prev echo and interfering signals (Kalko and Schnitzler 1998). Gleaning foragers use broadband calls to pinpoint prey in extreme clutter conditions (Denzinger and Schnitzler 2013). Broadband calls elicit many neuronal filters, and therefore improve the accuracy of range and angle determination (Kalko and Schnitzler 1998). Broadband calls covering a wide range of wavelengths could ensonify many reflecting surfaces, allowing gleaning bats to extract detailed information from echoes about prey and cluttered background (Siemers and Schnitzler 2004). Echolocation calls of flutter-detecting foragers (also termed HDC echolocators) consist of a long CF component followed by a downward broadband sweep (Jones and Teeling 2006). Such combinations enhance the performance of target detection and localization in highly cluttered conditions (Kalko and Schnitzler 1998). Moreover, increasing evidence has shown that temperature and humidity may drive the evolution of bat echolocation calls through their effects on sound attenuation (Snell-Rood 2012; Luo et al. 2014; Jacobs et al. 2017).

In addition to ecological selection, morphological and phylogenetic constraints also shape the evolution of echolocation calls among bats. There is a close association between body size and spectro-temporal parameters of echolocation calls in many LDC and HDC bats (Bogdanowicz et al. 1999; Jones 1999). Despite some exceptions, larger species tend to emit long and low frequency sounds when compared with smaller echolocators (Jones 1999; Jacobs et al. 2007). From an anatomical perspective, this can be attributed to greater lung volumes and thicker vocal chords, imposing the constraint on the production of sounds (Fitch and Hauser 2003; Luo et al. 2017). Several studies have also demonstrated that species' phylogenetic history can predict some of the diversity in echolocation calls in bats (Jones and Teeling 2006; Collen 2012; Jung et al. 2014). For instance, echolocation calls exhibit greater degree of similarity in structure between closely related species (Kingston et al. 2000; Jung et al. 2007, 2014). Call duration and frequency parameters exhibit strong phylogenetic signal in most bat lineages (Collen 2012).

Ecology, morphology, and phylogenetic history are closely coupled (Fenton and Bogdanowicz 2002; Lim and Dunlop 2008; Collen 2012), implying that these factors may simultaneously affect echolocation call diversity in bats through direct and indirect effects. Because many echolocating bats modify their calls according to ecological conditions, it is often assumed that these vocalizations are shaped primarily by ecological conditions rather than by phylogenetic history and body size of the species (Jones and Teeling 2006; Jones and Holderied 2007). Collen (2012) detected considerable phylogenetic but no spatial signal in echolocation call parameters among bats. This highlights that phylogenetic history exerts strong influence on bat echolocation call diversity. However, spatial signal is an indicator of spatial dependence, which largely reflects the effects of climate and geology on trait variation cross species (Freckleton and Jetz 2009). Low spatial signal is not equivalent to a weak contribution of foraging ecology to the evolution of call features (Freckleton and Jetz 2009). To date, it is still unknown whether the role of phylogenetic history in shaping echolocation call diversity would remain robust when incorporating information about foraging guilds, climatic factors, and body size.

The aim of this study is to disentangle the relative contribution of phylogeny, ecology, and morphology to the diversity in echolocation calls in bats. We test 3 non-mutually exclusive hypotheses concerning echolocation call divergence across bat species, i.e., ecological selection hypothesis, morphological constraint hypothesis, and phylogenetic constraint hypothesis. We integrate a large dataset spanning 207 species of bats from 17 families, which includes search-phase echolocation call parameters, body size, diets, foraging guilds, climatic factors, and phylogenetic relationships. If ecological selection is the major driver of echolocation call design, spectro-temporal parameters should be largely predicted by foraging guilds, diets, and climatic conditions. If morphological constraint determines interspecific variation in echolocation calls, bat body size should be negatively associated with frequency parameters but positively associated with call duration. Finally, according to the phylogenetic constraint hypothesis, we predict that phylogenetic components would account for interspecific variation in echolocation call parameters.

Materials and methods

Acoustic data collection

We obtained echolocation call parameters of bats from published sources, including call duration, peak frequency (F_{peak} ; frequency of maximum energy), start frequency (F_{start} ; frequency at the start of the call), and end frequency (F_{end} ; frequency at the end of the call). These parameters are widely used to characterize echolocation vocalizations in previous studies (Table S1). Given the lack of consistency in the literature, call bandwidth in LDC bats was calculated by subtracting end frequency from start frequency. Acoustic data were chosen based upon five criteria: (1) calls were recorded during emergence from roosts, in search flight in the field, after release at the capture site, or a combination of these conditions; (2) acoustic analyses were restricted to the harmonic with the highest energy; (3) acoustic characteristics of the high-frequency call (call 1, which had the highest peak frequency) and low-frequency call (call 2, which had the lowest peak frequency) were used if data differed in geographic locations, degree of clutter, or published sources; (4) phylogenetic information for the species was available on the timecalibrated mammal supertree (Faurby and Svenning 2015), which incorporated 1146 species within the order Chiroptera; and (5) Vespertilio superans, Pipistrellus nanus, Hypsugo alaschanicus, Hypsugo savii, Chalinolobus variegatus, Myotis ricketti, Tadarida nigeriae, Mormoops blainvillii, and Dermanura cinerea were regarded as synonyms of Vespertilio sinensis, Neoromicia nana, Pipistrellus alaschanicus, Pipistrellus savii, Glauconycteris variegata, Myotis pilosus, Chaerephon nigeriae, Mormoops blainvillei, and Artibeus cinereus (IUCN 2018), respectively. Combined, we retained data from 207 bat species in 17 families (Table S1), namely Rhinopomatidae (N = 2), Megadermatidae (N = 4), Craseonycteridae (N = 1), Rhinolophidae (N = 20), Hipposideridae (N = 10), Rhinonycteridae (N = 1), Mystacinidae (N = 1), Phyllostomidae (N = 33), Mormoopidae (N = 6), Noctilionidae (N = 2), Fruipteridae (N = 1), Nycteridae (N = 1)3), Emballonuridae (N = 19), Natalidae (N = 1), Molossidae (N = 18), Miniopteridae (N = 6), and Vespertilionidae (N = 6)79).

Predictor factors

We compiled data on bat forearm length, diet, guild membership, climate, and phylogenetic components from the literature (Table S1). Forearm length was used as an estimate of body size and was sourced either from the PanTHERIA database (Jones et al. 2009) or from other published sources. Since the forearm length of adult bats does not change with seasons and food intake, it is a better indicator of body size than body mass (Bogdanowicz et al. 1999). Diets were defined using the database EltonTraits (Wilman et al. 2014) as invertebrateeating ($\geq 60\%$ diet composed of invertebrates), carnivore (\geq 60% of diet composed of vertebrates), plant-eating (\geq 60% of diet composed of fruit or nectar), and omnivore (< 60% of diet composed by one particular food). Foraging guilds were separated into five categories following previous studies (Schnitzler et al. 2003; Denzinger and Schnitzler 2013), i.e., open-space aerial forager (N = 40), edge-space aerial forager (N = 63), edge-space trawling forager (N = 11), narrow-space gleaning forager (N = 61), and narrow-space flutter-detecting forager (N = 32). Some bat species exhibit flexibility in the use of feeding space and mode, making it difficult to assign one particular guild. In such cases, we assigned the species to the most complex guild according to the degree of habitat clutter, given that they face the most difficult sensorial challenge of acoustic masking effects in cluttered environments. For climatic conditions, we obtained the annual average temperature and precipitation within species' geographic distribution by overlaying their range maps (IUCN 2018) and 2.5 arc-min bioclimatic rasters (Hijmans et al. 2005), using the package

raster version 2.1-49 (Hijmans and Van Etten 2013). To characterize phylogenetic components, we extracted the first three phylogenetic eigenvectors from a pruned supertree (Faurby and Svenning 2015) via principal coordinate analysis (PCOA) in the package ape 5.3 (Paradis et al. 2004). Phylogenetic eigenvectors allowed us to quantify the relative contributions of phylogenetic history and other predictor variables to call variation across species. PCOA generated the eigenvectors from a phylogenetic distance matrix, reflecting phylogenetic relationship between species (Paradis et al. 2004; Luo et al. 2017). The first eigenvector with higher relative eigenvalues represented the largest phylogenetic distances, and other eigenvectors with smaller relative eigenvalues reflected smaller phylogenetic distances. The three phylogenetic eigenvectors explained 51.53% of the variation in the phylogenetic distances. The other phylogenetic eigenvectors were excluded from the analysis due to the low relative eigenvalues (0-0.05).

Data analysis

Call parameters and forearm length were log10-transformed to reach normal distribution. We employed the general linear model (GLM) and post hoc Tukey's test to determine whether call parameters differed between families and between guilds. The general linear mixed model (GLMM), together with hierarchical partitioning, was applied to quantify the relative importance of predictor variables in explaining call variation using the packages lmerTest 2.0-29 (Kuznetsova et al. 2015) and hier.part 1.0-4 (Walsh and MacNally 2013). Each call parameter was repeatedly assigned as the dependent variable. Forearm length, dietary type, foraging guilds, climatic factors, phylogenetic components, and their interaction were assigned as fixed variables. The type of recording was included as a random variable. We chose the optimized GLMM based on Akaike's information criterion using the package MuMIn 1.15.6 (Barton 2016). To identify direct and indirect effects of predictor variables on call parameters among families, we carried out a path analysis using maximum likelihood in SPSS Amos 22. Path analysis involves a structural equation modeling, allowing us to test the causal relationship between exogenous and endogenous variables based on a priori hypothesis (Mitchell 1992). The coefficients of direct path represent the extent of direct effect of one variable on another, and coefficients of indirect paths indicate the indirect effects. Call parameters and their predictor variables (i.e., forearm length, foraging guilds, and phylogenetic components; Table S2) included in the optimized GLMM were used to construct path diagrams. Each call parameter, forearm length, and foraging guilds were entered into path diagrams as the endogenous variables. Phylogenetic components were entered into path diagrams as the exogenous variable. The optimized path model was also established according to Akaike's information criterion (Table S3). Because the multiple variables were not normally distributed, we corrected the *P*-value of each path using the Bollen-Stine bootstrap (Bollen and Stine 1992).

Results

Echolocation call diversity

We found a wide variation in search-phase echolocation calls among families (GLM: call 1: duration: $F_{16,183} = 11.90, P <$ $0.0001; F_{\text{peak}}: F_{16,183} = 2.98, P < 0.0001; F_{\text{start}}: F_{11,92} = 1.70,$ P = 0.085; F_{end} : $F_{11.92} = 2.79$, P = 0.004; bandwidth: $F_{9.75} =$ 7.18, P < 0.0001; call 2: duration: $F_{16,183} = 12.11$, P < 0.0001; F_{peak} : $F_{16,183} = 3.29$, P < 0.0001; F_{start} : $F_{11,92} = 2.11$, P =0.027; F_{end} : $F_{11,92} = 3.29$, P = 0.001; bandwidth: $F_{9,75} =$ 5.16, P < 0.0001) and guilds (GLM: call 1: duration: $F_{4,183}$ = 6.74, P < 0.0001; F_{peak} : $F_{4,183}$ = 9.27, P < 0.0001; F_{start} : $F_{4,92} = 6.70, P < 0.0001; F_{end}: F_{4,92} = 3.50, P = 0.011;$ bandwidth: $F_{3,75} = 6.86$, P < 0.0001; call 2: duration: $F_{4,183} = 6.26$, $P < 0.0001; F_{\text{peak}}: F_{4.183} = 9.88, P < 0.0001; F_{\text{start}}: F_{4.92} =$ 7.84, P < 0.0001; F_{end} : $F_{4,92} = 3.91$, P = 0.006; bandwidth: $F_{3.75} = 8.19, P < 0.0001$), without an interaction of families and guilds (GLM: call 1: duration: $F_{3,183} = 0.71$, P = 0.55; F_{peak} : $F_{3,183} = 0.097$, P = 0.96; F_{start} : $F_{1,92} = 0.93$, P = 0.34; F_{end} : $F_{1,92} = 0.72$, P = 0.40; bandwidth: $F_{1,75} = 1.75$, P = 0.19; call 2: duration: $F_{3,183} = 0.043$, P = 0.98; F_{peak} : $F_{3,183} = 0.15$, P = 0.93; F_{start} : $F_{1.92} = 0.75$, P = 0.39; F_{end} : $F_{1.92} = 0.60$, P = 0.600.44; bandwidth: $F_{1.75} = 0.56$, P = 0.46).

Relative importance of predictor variables

Dietary type, annual average temperature, and annual average precipitation were not retained in the optimized GLMM because of weak effects on call parameters (Table S2). However, forearm length, foraging guilds, and phylogenetic components were significant predictors of call parameters (Tables 1 and 2). Forearm length correlated positively with call duration (GLMM: all P < 0.05, except for the families Emballonuridae and Molossidae) but negatively with frequency parameters (GLMM: all P < 0.05, with the exception of Emballonuridae; Tables 1 and 2), and explained 2.28–56.48% of the observed variation (Figs. 1a-d, 2a-d, and 3). Forearm length contributed only 2.44-5.73% of the variance in call bandwidth among LDC bats (Fig. 3). Foraging guilds were significantly related to call characteristics among LDC bats and all species studied, accounting for 4.37-42.02% of the variation (GLMM: all P < 0.01; Tables 1 and 2; Fig. 3). Open-space aerial foragers and narrow-space flutter-detecting foragers emitted calls of longer duration than the other guilds (Figs. 1e and 2e). In addition, open-space aerial foragers uttered calls of lower frequency and narrower bandwidth when compared with species that forage in edge and narrow

Table 1 Effects of predictor factors on acoustic parameters of the high-frequency calls

| Calls | Factors | All | LDC | HDC | Emba | Molo | Phyl | Rhin | Vesp |
|--------------------|-----------|-------------|--------------|-----------|---------------------|--------------------|--------------------|--------------------|--------------------|
| Dura | Fore | 0.49** | 0.54** | 0.82** | _ | 1.08 ^{NS} | 0.66** | 0.81** | 0.34 ^{NS} |
| | Guil | - 0.068** | - 0.14*** | _ | - 180.02** | _ | _ | _ | _ |
| | PVR1 | - 0.0051* | _ | - 0.22* | -55.72^{NS} | 1.11 ^{NS} | 0.95^{NS} | 0.80^{NS} | -0.020^{**} |
| | PVR2 | 0.0048*** | 0.0018** | 0.25* | 38.68** | -6.59^{NS} | - | 1.14 ^{NS} | _ |
| | PVR3 | 0.011*** | _ | - 0.41** | 3.18* | 0.75^{NS} | _ | 4.30 ^{NS} | _ |
| | Guil:PVR1 | 0.0025*** | _ | - | 58.99 ^{NS} | _ | - | _ | - |
| | Guil:PVR2 | _ | _ | _ | - 28.19** | _ | - | _ | _ |
| | Guil:PVR3 | - 0.0053*** | _ | - | - 2.27** | _ | - | _ | _ |
| F _{peak} | Fore | - 0.85*** | - 0.79*** | - 1.21*** | -445.66^{NS} | - 2.02*** | - 0.61** | - 1.41** | - 0.96*** |
| | Guil | 0.081*** | 0.076*** | _ | _ | _ | _ | _ | _ |
| | PVR1 | 0.0014*** | _ | _ | 18.45 ^{NS} | 1.43 ^{NS} | 0.16 ^{NS} | -4.88^{NS} | 3.99** |
| | PVR2 | - 0.0016*** | - 0.0022*** | _ | 95.50 ^{NS} | 3.19 ^{NS} | _ | -5.32^{NS} | 3.95** |
| | PVR3 | _ | _ | 0.044*** | 9.10 ^{NS} | _ | _ | -20.61^{NS} | 0.33*** |
| | Fore:PVR1 | _ | _ | - | -10.37^{NS} | _ | - | _ | _ |
| | Fore:PVR2 | _ | _ | - | -55.92^{NS} | _ | - | _ | _ |
| | Fore:PVR3 | _ | _ | - | -5.33^{NS} | _ | - | _ | _ |
| F _{start} | Fore | - 0.77*** | - 0.70*** | - 0.92** | _ | _ | - | _ | - 0.83*** |
| | Guil | 0.067*** | 0.084*** | - | _ | _ | - | _ | _ |
| | PVR1 | _ | _ | - | _ | _ | - | _ | 3.35** |
| | PVR2 | _ | _ | - | _ | _ | - | _ | 3.35** |
| | PVR3 | _ | _ | 0.041** | _ | _ | _ | _ | 0.29** |
| F _{end} | Fore | - 0.83*** | - 0.65*** | - 0.97** | _ | _ | - | _ | - 1.05*** |
| | Guil | 0.045** | 0.047** | - | _ | _ | - | _ | _ |
| | PVR1 | 0.0023*** | _ | - | _ | _ | - | _ | 0.017** |
| | PVR3 | _ | _ | 0.037** | _ | _ | - | _ | |
| Band | Fore | _ | -0.47^{NS} | _ | _ | _ | - | _ | - 0.48* |
| | Guil | _ | 0.16*** | _ | _ | _ | _ | _ | _ |
| | PVR1 | _ | - 0.0052** | _ | _ | _ | _ | _ | - 0.83*** |
| | PVR2 | _ | - | - | _ | _ | _ | - | - 0.73*** |

Values are coefficients of optimized linear mixed models

Dura call duration, F_{peak} peak frequency, F_{start} start frequency, F_{end} end frequency, Band bandwidth, All all bats studied, LDC low duty cycle bats, HDC bats high duty cycle bats, Emba Emballonuridae, Molo Molossidae, Phyl Phyllostomidae, Rhin Rhinolophidae, Vesp Vespertilionidae, Fore forearm length, Guil foraging guilds, PVR1, PVR2, and PVR3 phylogenetic eigenvectors extracted from phylogenetic distance matrix

NS Not significant

*P < 0.05

**P < 0.01

***P < 0.0001

Families with less than 15 species studied were excluded from the analysis in order to improve model power and reduce estimation bias

spaces (Figs. 1f–i and 2f–i). The independent effects of phylogenetic components on call parameters ranged from 1.79 to 55.37% across different bat groups. Path analysis identified that phylogenetic components exerted direct and indirect impacts on call output by acting over multiple pathways among acoustic parameters, foraging guilds, and forearm length (Fig. 4). The standardized direct effects of phylogenetic components on call parameters ranged from -0.35 to 0.68. The standardized indirect effects of phylogenetic components on call parameters varied from -0.18 to 0.18.

Discussion

Our comparative analyses corroborate a predominant role of phylogenetic components in determining echolocation call duration and end frequency within the order Chiroptera, but in much greater extent than reported by previous studies (Jones and Teeling 2006; Jones and Holderied 2007; Collen 2012; Jung et al. 2014). Upon taking phylogeny into account, we find that call duration and spectral parameters are closely linked to guild membership, supporting the hypothesis of

| Calls | Factors | All | LDC | HDC | Emba | Molo | Phyl | Rhin | Vesp |
|--------------------|-----------|-------------|--------------|-----------|----------------------|-----------------------|--------------------|--------------------|---------------|
| Dura | Fore | 0.54** | 0.58*** | 0.80*** | _ | 1494.41 ^{NS} | 0.69** | 0.62** | 0.50^{*} |
| | Guil | - 0.074** | - 0.15*** | _ | - 177.75** | _ | _ | _ | _ |
| | PVR1 | - 0.0058* | _ | - 0.21* | -33.08^{NS} | 291.52 ^{NS} | 1.10 ^{NS} | 2.64 ^{NS} | -0.027^{**} |
| | PVR2 | 0.0049*** | 0.0019*** | 0.24* | 36.68* | -223.86^{NS} | _ | 0.84^{NS} | _ |
| | PVR3 | 0.011*** | _ | - 0.40** | 3.16* | -42.69^{NS} | _ | 9.22 ^{NS} | _ |
| | Guil:PVR1 | 0.0027*** | _ | - | 34.65 ^{NS} | 163.54 ^{NS} | — | - | _ |
| | Guil:PVR2 | _ | _ | - | - 25.71** | 120.55 ^{NS} | — | - | _ |
| | Guil:PVR3 | - 0.0049*** | _ | - | - 2.19* | 24.40 ^{NS} | — | - | _ |
| Fpeak | Fore | - 0.84*** | - 0.79*** | - 1.20*** | -577.20^{NS} | - 1.79*** | - 0.61** | - 1.40** | - 0.97*** |
| I ···· | Guil | 0.086*** | 0.081*** | _ | _ | _ | _ | _ | _ |
| | PVR1 | 0.0014*** | _ | _ | -8.63^{NS} | -0.84^{NS} | 0.18^{NS} | -5.33^{NS} | 4.15*** |
| | PVR2 | - 0.0016*** | - 0.0022*** | _ | 127.63 ^{NS} | -2.77^{NS} | _ | -4.41^{NS} | 4.11*** |
| | PVR3 | _ | _ | 0.044*** | 12.08 ^{NS} | _ | _ | -20.81^{NS} | 0.34*** |
| | Fore:PVR1 | _ | _ | - | 4.21 ^{NS} | _ | — | - | _ |
| | Fore:PVR2 | _ | _ | - | -74.61^{NS} | _ | — | - | _ |
| | Fore:PVR3 | _ | _ | - | -7.07^{NS} | _ | — | - | _ |
| F_{start} | Fore | - 0.77*** | - 0.67*** | - 0.92** | _ | _ | — | - | - 0.85*** |
| | Guil | 0.079*** | 0.10*** | - | _ | _ | - | - | _ |
| | PVR1 | _ | _ | _ | _ | _ | _ | _ | 3.52** |
| | PVR2 | _ | _ | _ | _ | _ | _ | _ | 3.52** |
| | PVR3 | _ | _ | 0.042** | _ | _ | — | - | 0.31** |
| Fend | Fore | -0.84*** | - 0.66*** | - 0.98** | _ | _ | _ | - | - 1.06*** |
| | Guil | 0.052*** | 0.057** | - | _ | _ | — | - | _ |
| | PVR1 | 0.0022*** | _ | - | _ | _ | _ | - | 0.019** |
| | PVR3 | _ | _ | 0.036** | _ | _ | — | - | _ |
| Bandwidth | Fore | _ | -0.35^{NS} | - | _ | _ | — | - | -0.50^{NS} |
| | Guil | _ | 0.19*** | - | _ | _ | — | - | _ |
| | PVR1 | _ | - 0.0049** | _ | - | - | _ | _ | - 0.84*** |
| | PVR2 | _ | _ | _ | _ | - | _ | _ | - 0.76*** |
| | | | | | | | | | |

Table 2 Effects of predictor factors on acoustic parameters of the low-frequency calls

Values are coefficients of optimized linear mixed models

Dura call duration, F_{peak} peak frequency, F_{start} start frequency, F_{end} end frequency, Band bandwidth, All all bats studied, LDC low duty cycle bats, HDC bats high duty cycle bats, Emba Emballonuridae, Molo Molossidae, Phyl Phyllostomidae, Rhin Rhinolophidae, Vesp Vespertilionidae, Fore forearm length, Guil foraging guilds, PVR1, PVR2, and PVR3 phylogenetic eigenvectors extracted from phylogenetic distance matrix

NS Not significant

*P < 0.05

**P < 0.01

***P < 0.0001

Families with less than 15 species studied were excluded from the analysis in order to improve model power and reduce estimation bias

adaptive evolution of call design in response to ecological pressure (Neuweiler 1984; Jones and Holderied 2007; Denzinger and Schnitzler 2013). At the family level, the variation in call parameters is largely explained by differences in body size and phylogenetic relationships. Together, this provides strong evidence supporting that the effects of phylogeny, ecology, and body size on echolocation calls diversity vary at different taxonomic levels in bats.

Foraging guilds are the significant explanatory variable of interspecific variation in echolocation calls. This supports the sensory drive hypothesis, which states that signals, sensory systems, and signaling behaviors coevolve as a function of habitat (Endler 1992). The sensory drive framework is valuable for understanding the evolution of echolocation in bats (Jones 1999; Jones and Holderied 2007). As shown by multiple studies (Neuweiler 1984; Kingston et al. 2000; Jones and Holderied 2007; Denzinger and Schnitzler 2013; Jakobsen et al. 2013; Jung et al. 2014), echolocating bats do optimize echolocation signals to meet perceptual



Fig. 1 Relationship among body size, foraging guilds, and acoustic parameters of the high-frequency calls at the order level. **a** Call duration and forearm length. **b** Peak frequency and forearm length. **c** Start frequency and forearm length. **d** End frequency and forearm length. **e** Call duration and guilds. **f** Peak frequency and guilds. **g** Start frequency and guilds. **h** End frequency and guilds. **i** Bandwidth and guilds. Circles denote low duty cycle bats. Triangles denote high duty cycle bats. OA, open-space

challenges imposed by foraging demands, and species within the same guild exhibit convergent acoustic phenotypes. For example, the search-phase echolocation calls of open-space aerial foraging insectivorous bats are characterized by relatively long duration, low frequency, and narrow bandwidth. Such calls facilitate long-range detection of larger insects, since these sounds increase the signal energy, suffer less attenuation, and their echoes rapidly activate neuronal filters tuned to the narrowbands (Kalko and Schnitzler 1998; Schnitzler and Kalko 2001). Conversely, for foragers in moderate and extremely cluttered spaces, the echoes from vegetation and continual emissions of outgoing calls can mask prey echo (Schnitzler and Kalko 2001). To mitigate acoustic masking, some of these LDC bats must utter short broadband frequency-modulated calls to increase the width of the overlap-free window (Kalko and Schnitzler 1993). A broadband call that covers a multitude of wavelengths

aerial forager; EA, edge-space aerial forager; ET, edge-space trawling forager; NG, narrow-space gleaning forager; NF, narrow-space flutterdetecting forager. Data in box plots represent the upper and lower adjacent values (highest and lowest horizontal line, respectively), 25% and 75% quartile with median value (box) and outliers (dots). NS, not significant, **P* < 0.05, ***P* < 0.01, ****P* < 0.0001. Statistical significance is based on post hoc Tukey's test

confers advantages in prey classification and preybackground discrimination, given that sound of short wavelength reflects more efficiently from small target than that of long wavelength (Siemers and Schnitzler 2004). A broadband call also activates more neuronal filters compared with narrowband signal, enhancing the accuracy of range and angle determination (Kalko and Schnitzler 1998). One recent study showed that short, broadband echolocation calls evoked highly precise neural signals in the inferior colliculus of the big brown bats (Eptesicus fuscus), presumably owing to an increased number of active neurons and neural synchrony (Luo et al. 2018). HDC bats emit long duration, narrowband CF calls followed by a downward broadband sweep. In some HDC species, the beginning portion of the calls also contains an upward broadband sweep (Jones and Teeling 2006). In these circumstances, prey echo overlaps temporally with interfering signals. However, HDC bats avoid



Fig. 2 Relationship among body size, foraging guilds, and acoustic parameters of the low-frequency calls at the order level. **a** Call duration and forearm length. **b** Peak frequency and forearm length. **c** Start frequency and forearm length. **d** End frequency and forearm length. **e** Call duration and guilds. **f** Peak frequency and guilds. **g** Start frequency and guilds. **h** End frequency and guilds. **i** Bandwidth and guilds. Circles denote low duty cycle bats. Triangles denote high duty cycle bats. OA, open-space

acoustic masking by extracting information from the CF component of prey echo, i.e., frequency shifts caused by the relative movement between bat and fluttering insect, and acoustic glints generated by the wingbeats of fluttering insect (Fenton et al. 2012). A combination of narrowband and broadband signals are suited for detection and location of fluttering insect in highly cluttered spaces (Schnitzler and Kalko 2001; Schnitzler and Denzinger 2011). These previous findings, together with the results presented here, indicate that ecological selection acts as an important shaping force for generating echolocation call diversity in bats.

Echolocation call parameters are also predicted by phylogenetic components in bats, especially at the order level. This supports the argument of a previous comparative study (Collen 2012), and suggests that shared phylogenetic history affects interspecific divergence in echolocation calls. Several possible mechanisms explain

aerial forager; EA, edge-space aerial forager; ET, edge-space trawling forager; NG, narrow-space gleaning forager; NF, narrow-space flutterdetecting forager. Data in box plots represent the upper and lower adjacent values (highest and lowest horizontal line, respectively), 25% and 75% quartile with median value (box) and outliers (dots). NS, not significant, **P* < 0.05, ***P* < 0.01, ****P* < 0.0001. Statistical significance is based on post hoc Tukey's test

this pattern. First, some bat pups possess genetic signatures for precursors of echolocation sounds (Scherrer and Wilkinson 1993; Monroy et al. 2011). This predicts a genetic basis on the inheritance of call characteristics across generations, and ultimately across species through cladogenesis. Consistent with this idea, we reveal evolutionarily conserved acoustic characteristics in searchphase echolocation calls in both LDC and HDC bats. Path analysis also identifies significant direct effects of phylogenetic components on echolocation call parameters. Second, the size of the sound production apparatus (e.g., larvnx) and associated morphological traits in bats are not independent of phylogenetic history (Collen 2012), and similar call features would be found between closely related species. In this case, phylogenetic history of bats may affect echolocation call output indirectly by acting on morphological traits involved in sound production, as shown in our path diagrams (Fig. 4).



Fig. 3 Independent effects of predictor factors on echolocation call parameters. a1-e1 The duration, peak frequency, start frequency, end frequency, and bandwidth of the high-frequency calls, respectively. a2-

Third, we detect pronounced phylogenetic effects on foraging guilds that species belong to (Fig. 4). If these bats use habitatdependent echolocation signals, it is possible that echolocation calls show similar adaptations within some lineages.

As expected, call duration scales positively with forearm length and peak frequency does so negatively in most bat groups, even in a phylogenetic context. A similar relationship between echolocation call parameters and the proxy for body size has been reported in Rhinolophidae, Hipposideridae, Emballonuridae, Vespertilionidae, and Molossidae (Bogdanowicz et al. 1999; Jones 1999; Wu et al. 2015; Jacobs and Bastian 2018). These findings indicate that bat echolocation calls depend on the acoustic production apparatus, which is associated with body size. For LDC bats, use of shorter call duration might also represent an adaptation to echolocation with higher frequencies, given that a combination of both features could minimize the interference from clutter echoes (Waters et al. 1995; Schmieder et al. 2012). However, some bats have an echolocation frequency

e2 The duration, peak frequency, start frequency, end frequency, and bandwidth of the low-frequency calls, respectively. For abbreviations, see footnote in Table 1

significantly higher or lower than expected based on their body size (Jacobs et al. 2007, 2014; Wu et al. 2015). This provides additional support for the adaptive evolution of echolocation calls through ecological selection. A deviation from size-dependent relationships benefits bats in evolutionary arms races with prey, given that many eared moths are particularly sensitive to echolocation frequencies between 20 kHz and 60 kHz but less sensitive to allotonic frequencies (Bogdanowicz et al. 1999; Ter Hofstede et al. 2013). A deviation in echolocation call frequency may also be attributed to food resource partitioning between species (Kingston et al. 2000; Shi et al. 2009), selection for decreased snout length and increased bite force (Jacobs et al. 2014), and character displacement to facilitate intraspecific communication (Jacobs et al. 2007).

In summary, our phylogenetic comparative analyses demonstrate that phylogenetic components, foraging guilds, and body size each contribute to the diversity of echolocation calls in bats, supporting the importance of adaptive and nonFig. 4 Path diagram for direct and indirect effects of selected variables on echolocation call parameters at the order level. a High-frequency call parameters. b Low-frequency call parameters. Green arrows indicate negative effects, and red arrows indicate positive effects significant at P values < 0.05. Gray arrows represent nonsignificant relationships (P values > 0.05). Lines correspond to standardized partial regression coefficients on the diagram. For abbreviations, see footnote in Table 1



adaptive mechanisms in the evolution of echolocation signals in bats. We find that the role of these factors in shaping echolocation call parameters varies at different taxonomic levels. We confirm that phylogenetic components determine echolocation call output via multiple, direct and indirect pathways across acoustic parameters, guild membership and body size. This study constitutes the first attempt to disentangle direct and indirect phylogenetic effects on bat echolocation calls, which facilitates a better understanding of the nature of echolocation call diversity. Our combination of phylogenetic and ecological frameworks can be applied to explore the evolution of acoustic signals in other animal taxa. However, it should be noted that other factors such as mate choice and FOXP2 gene may also play a role in influencing echolocation call parameters in bats (Li et al. 2007; Puechmaille et al. 2014). Further research is needed to assess whether sexual selection and neutral processes (e.g., genetic drift) may mold echolocation call diversity in bats by exploring the relationships among call parameters, mating system, and functional genes involved in sound production.

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Authors' contribution BL and JF designed the study; BL, LZ, TL, and YHX collected the data; BL and YL conducted statistical analysis; BL, LLM, and SES wrote the paper.

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Data availability The datasets generated and/or analyzed during the current study are presented in electronic supplementary material.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement This article does not contain any studies with human participants or animals performed by any of the authors.

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