Correlation between echolocation calls and morphological features among three kinds of bats*

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Abstract　　The correlation between echolocation sound and morphological features of 18 species of bats, which are subordinated to 3 families including rhinolophidae, hipposideridae and vespertilionidae is studied. Pearson's correlation and regression analysis are adopted to analyze the correlations between noseleaf width (NW) and forearm length (FAL), NW and dominant frequency (DF), FAL and DF. The results show a positive correlation between NW and FAL and a negative correlation between NW and DF in Rhinolophidae. In each family, there is a negative correlation between FAL and DF. Some species have a higher or lower frequency than predicted one according to their body size. There are significant differences of the regression equation of FAL and DF among the three families. For the bats with the same body size, the order in frequency is: hipposiderid bats > rhinolophid bats > vespertilionid bats. The mechanism of interrelationship between FAL and DF of bats, the reason why these deviations occur in correlativity of the three families and the mechanism of the interaction between morphological, physiological and ecological characteristics are discussed.

Keywords: Rhinolophidae, Hipposideridae, Vespertilionidae, echolocation calls, morphology, correlation.

In recent years, much work on the difference in the structures of echolocation calls among different species of bats has been carried out[1~9]. Many factors can affect the echolocation sound of bats including ecological, morphological and physiological factors. For example, the foraging habitats can influence DF and the number of harmonics, and so on[1]; and in different foraging phases (search phase, approach phase and capture phase), the pulse duration and FM (frequency-modulated) bandwidth are significantly different[3]. In morphology, the noseleaf width (NW) of rhinolophid bats and hipposiderid bats[4], the cochlear width and skull width of male *Rhinolophus creaghi*[5] are negatively correlated with DF, and the body sizes of bats and their preys are negatively correlated with DF and positively correlated with pulse duration[6,7]. In physiology, *Myotis daubentonii* in their first year emits echolocation calls slightly (but difference is significant) lower in frequency than those older ones; female *Rhinolophus hipposideros* emits higher frequency calls than males[8,9] etc.

In rhinolophidae and hipposideridae, more than 60 species have been recognized[10]. They are particularly amenable to a comparative study of morphological features with echolocation behavior because they have relatively stable echolocation sound[5] and highly specialized morphological features such as elaborate noseleaf, enlarged nasal cavity, large pinna, and cochlea associated with both emission and reception of echolocation calls. In Chiroptera, Vespertilionidae includes the largest number of species of bats (42 genera, 355 species), widely distributing in the world[11]. These species have a variety of echolocation calls and morphological features, which are useful to investigate echolocation behavior in bats. Our present study investigates the correlation between DF and forearm length (FAL) in 18 species of bats of 3 families for understanding how the echolocation behavior of bat functions in their communicating, foraging and their differentiation in ecological niche and other living properties.

1　Materials and methods

1.1　Bats

This study has determined the parameters of echolocation calls (at fly state) and morphological features in 8 species of rhinolophid bats (*Rhinolophus cornutus*, *R. affinis*, *R. thomasi*, *R. rouxi*, *R. blythi*, *R. ferrumequinum*, *R. pearsoni*, *R.

rex), six species of vespertilionid bats (*Myotis frater*, *Ia Io*, *M. myotis luctuosus*, *M. daubentoni*, *M. fimbriatus*, *Murina leucogaster*), and 4 species of hipposiderid bats (*Hipposideros pratti*, *Aselliscus wheeleeri*, *H. armiger*, *H. larvatus*). The correlation between echolocation sound and morphological characteristics has been analyzed. Species in the families of Rhinolophidae and Hipposideridae have relatively stable echolocation calls that are characterized by a strong constant-frequency (CF) component called DF. The CF component contains most of the energy of the sound. There exists interspecific difference in DF. Species in Vespertilionidae have variable echolocation calls that are characterized by a frequency-modulated (FM) component with one or more harmonics. It is difficult to measure their representative frequency accurately because the energy of the sound is in a wide range of frequency and the characteristics of recorded sound frequency depends on the relative angles of bat to microphone[8]. The echolocation calls in Vespertilionedae are easily influenced by the recording angle, and the frequency will change with different directionalities[12]. The power spectra of the calls often show multiple DF when a bat does not directly fly to the microphone. However, if the angles of sound recording are controlled strictly, DF is still relatively stable, and can represent the characteristic frequency of species. NW and FAL are representatives of morphological features. The correlations between FAL and DF, NW and DF (in Rhinolophidae) as well as NW and FAL (in Rhinolophidae) are analyzed, and the interactions among physiological processes, morphological features and ecological processes are discussed.

A total of 169 specimens of bats were included in the study (Table 1), which were captured from the caves in five regions (Guiyang, Anlong, Xingyi, Anshun and Zhenning) in Guizhou Province, in July and August 1999 and June 2000. The species were identified based on the descriptions in *The Beasts In Guizhou* written by Luo et al.[13].

Table 1. The species of bats

Family name	Species name	Number of bats
Rhinolophidae	*Rhinolophus cornutus*	5
	R. affinis	6
	R. thomasi	5
	R. rouxi	4
	R. blythi	16
	R. ferrumequinum	6
	R. pearsoni	7
	R. rex	8

To be Continued

Continued

Family name	Species name	Number of bats
Vespertilionidae	*Myotis frater*	5
	Ia Io	18
	M. myotis luctuosus	5
	M. daubentoni	11
	M. fimbriatus	30
	Murina leucogaster	6
Hipposideridae	*Hipposideros pratti*	3
	H. armiger	5
	H. larvatus	15
	Aselliscus wheeleeri	14

1.2 Recording of echolocation calls

The bats were captured at the entrance to the caves at about 8 pm (the time when bats usually fly away from caves), and then were put into a recording room nearby the capture locations to allow them flying free. About 2 hours later, the recording was carried out by an ultra sound detector (U30, Ultra Sound Advice) which pointed to the head of the bats when they flied to the microphone directly and recorded the echolocation calls when the bats were about 1 m away from the microphone. The duration for recording was 1.1 s with the sampling frequency of 44.1 kHz, and one bat needed to be recorded 5 times, and then the signals were fed into a portable ultra -sound processor (PUSP, Ultra Sound Advice, UK). By replaying the recorded tapes the best-quality recordings (the loudest and clearest with the least background noise) were replayed at 1/10 speed and recorded onto a digital sound recorder (Sony, MZ-R55, frequency response range: 30~20000 Hz).

1.3 Processing and analyzing of echolocation calls

The recorded echolocation signals were analyzed with a sound-processing software, Cool Edit 2000, developed by American Syntrillium Software Company on a computer. The ultra sound analysis referred to the sound spectrograms (frequency-time graph), and the Hanning window was used to obtain an analytic precision of 256 Hz. The analysis attenuation was 60 dB. The DF, pulse duration and interpulse interval of the echolocation calls were recorded and the duty-cycle which stands for the percentage of the pulse duration in the summation of the pulse duration and the interpulse interval was calculated. The data were presented as $\bar{X} \pm SD$.

1.4 Morphological feature evaluation

The morphological features (including FAL and NW) were measured using a vernier caliper, and the

measurements were presented as $\overline{X} \pm SD$. In addition, the body mass, wing length, wing width and wing area of the four species of hipposiderid bats were measured, and the wingload (body mass/ wing area) and the ratio (wing length/ wing) width were calculated.

Statistical analysis, including correlation analysis, regression analysis and significance test were performed using a software SPSS 10.0.

2   Results

2.1   Patterns of echolocation calls

When flying, the patterns of echolocation calls of 18 species of bats were recorded. As shown in Fig. 1 and Table 2, 8 rhinolophid bats emit (FM)-CF-FM echolocation calls with $1 \sim 2$ harmonics (mostly it is 1) (Fig. 1 a~h). Their pulse duration is long (>10 ms)and duty cycle is high ($>40\%$). The DF concentrates on CF component ranging from 25 kHz to 120 kHz. There are 4 hipposiderid bats which emit (FM-) CF-FM echolocation calls with 1 harmonic on average (Fig. 1, i~l), and their pulse duration is not as long as that of rhinolophid bats (<11 ms), the interpulse interval is long (>26 ms), and the duty cycle is lower than that of rhinolophid bats ($<22\%$). The DF in hipposiderid bats is higher than that in

rhinolophid bats, mostly concentrating on CF component. There are 6 vespertilionid bats which emit FM echolocation calls with $2 \sim 6$ harmonics (Fig. 1, m~r), their pulse duration is short (<5 ms) and the duty cycle is very low ($<15\%$). They have a wide modulated frequency band (>55 kHz) with DF ranging from 29.7 to 83.0 kHz.

Table 2.   The morphological features and parameters of echolocation calls in 18 species of bats ($\overline{X} \pm SD$)

Species (n)	FAL (mm)	NW (mm)	DF (kHz)
R. cornutus (5)	37.2±1.1	6.48±0.15	118.5±0.5
R. affinis (6)	50.0±2.0	—	93.4±0.4
R. thomasi (5)	47.4±2.0	7.80±0.14	89.7±0.7
R. rouxi (4)	45.7±2.4	9.74±3.31	87.2±0.5
R. blythi (16)	40.2±0.8	7.19±0.64	75.4±0.4
R. ferrumequinum (6)	54.9±2.9	11.35±0.53	63.5±0.2
R. pearsoni (7)	56.3±2.6	—	61.1±0.2
R. rex (8)	55.4±1.1	13.36±0.57	26.8±0.2
M. myotis luctuosus(5)	63.7±1.4	—	47.1±6.6
M. daubentoni (11)	34.9±1.2	—	83.0±4.0
M. fimbriatus (30)	40.0±4.0	—	53.0±7.8
I. Io (18)	76.7±1.8	—	29.7±2.3
M. leucogaster (6)	47.0±1.2	—	43.2±5.7
M. frater (5)	39.3±1.0	—	60.0±2.6
H. armiger (5)	90.9±1.0	—	76.2±0.8
H. pratti (3)	93.4±3.8	—	76.4±0.5
H. larvatus(15)	62.0±1.1	—	96.0±0.6
A. wheeleeri(14)	42.3±2.5	—	132.4±8.7

— Undetected
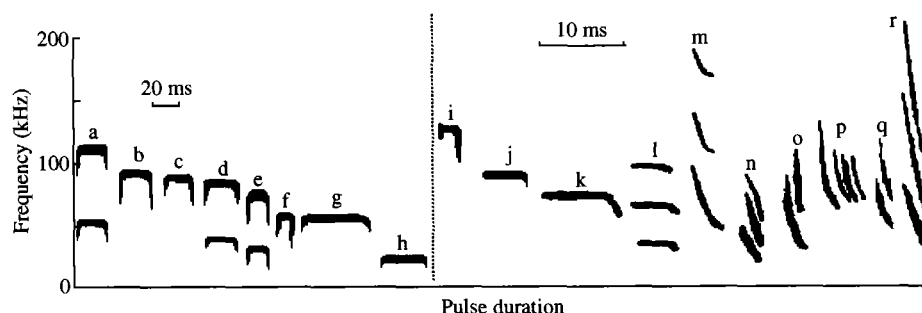

Fig. 1.   Patterns of echolocation calls of 18 species of bats.
a, R. cornutus; b, R. affinis; c, R. thomasi; d, R. rouxi; e, R. blythi; f, R. ferrumequinum; g, R. pearsoni; h, R. rex; i, A. wheeleeri; j, H. larvatus; k, H. pratti; l, H. armiger; m, M. frater; n, I. Io; o, M. myotis luctuosus; p, M. daubentoni; q, M. fimbriatus; r, M. leucogaster.

2.2   Morphological features

FAL (18 species) and NW (6 species) are given in Table 2. The wingloads of H. armiger, H. pratti, H. larvatus and A. wheeleeri are 20.27, 18.02, 9.38 and 5.73 respectively; and the wing as-

pect ratios of them are 6.98, 7.06, 5.56 and 4.64 respectively.

2.3   Correlation between the echolocation calls and morphological features

Pearson's correlation analysis was adopted to explore the correlation between the echolocation calls

and morphological features. The results are shown in Table 3.

Table 3. Correlation coefficient and regression equation

Feature	Species	Correlation coefficient/Regression equation
NW and DF	Rhinolophidae	$r = -0.88$, $n = 6$, two-tailed t-test, $p = 0.02$
NW and FAL	Rhinolophidae	$r = 0.92$, $n = 6$, two-tailed t-test, $p < 0.01$
FAL and DF	Rhinolophidae	$r = -0.76$, $n = 8$, two-tailed t-test, $p = 0.03$
FAL and DF	Hipposideridae	$r = -0.97$, $n = 4$, two-tailed t-test, $p = 0.04$
FAL and DF	Vespertilionidae	$r = -0.81$, $n = 6$, two-tailed t-test, $p = 0.05$
NW and DF	Rhinolophidae	$DF = -10.12NW + 171.20$ ($df = 5$, $r^2 = 0.78$, F-test, $p = 0.02$)
NW and FAL	Rhinolophidae	$FAL = 2.56NW + 22.90$ ($df = 5$, $r^2 = 0.84$, F-test, $p < 0.01$)
FAL and DF	Rhinolophidae	$DF = -2.88FAL + 216.47$ ($df = 7$, $r^2 = 0.57$, F-test, $p = 0.03$)
FAL and DF	Hipposideridae	$DF = -1.04FAL + 170.49$ ($df = 3$, $r^2 = 0.93$, F-test, $p = 0.03$)
FAL and DF	Vespertilionidae	$DF = -0.89FAL + 97.33$ ($df = 5$, $r^2 = 0.66$, F-test, $p = 0.05$)

The regression equations of NW against DF, NW against FAL and FAL against DF are shown in Fig. 2 and Table 3. In Rhinolophidae, the ratio between NW and wavelength varies from 2.79 to 1.17, with a mean value of 1.66, which means 1.66 wavelengths per NW.
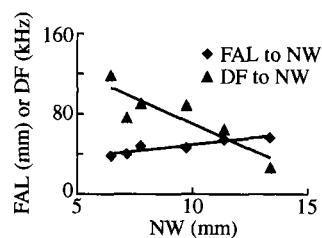
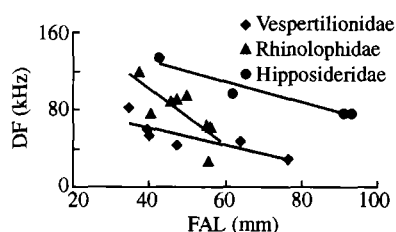

Fig. 2. Regression of NW against DF and NW against FAL.

Fig. 3. Regression of FAL against DF in three families.

3 Discussion

Robinson[4] reported that there was no correlation between FAL and NW in rhinolophid bats and hipposiderid bats after he measured NW and DF of 9 bat species (*R. luctus*, *R. coelophyllus*, *R. pear-sonii*, *R. acuminatus*, *R. pusillus*, *R. Malayanus*, *R. stheno*, *R. affinis* and *R. ferrumequinum*), while Jones[9] assumed that the larger individuals should have larger vocal tracts, therefore, there was a correlation between FAL and NW. Our study revealed a significantly positive correlation between NW and FAL in Rhinolophidae, which supported the Jones' conclusion. Our study, moreover, determined NW and DF of the other five species (*Rhinolophus cornutus*, *R. thomasi*, *R. rouxi*, *R. blythi* and *R. rex*) and also found a negative correlation between them, confirming the negative correlation between NW and DF in bats. Robinson found[4] that the ratio between NW and wavelength was 1.97 in rhinolophid bats and hipposiderid bats, while our study showed that the ratio was 1.66 in rhinolophid bats.

Heller et al.[7] and Zhang et al.[14] measured the body size and DF in rhinolophid bats and hipposiderid bats respectively and found a significantly negative correlation between DF of echolocation calls and body size. We investigated and compared those parameters for the three bat families and found a negative correlation between FAL and DF, which was consistent with the previous studies. The negative correlation found can be explained by the following facts. From physiology, large individuals have large vocal tracts, emitting low-frequency calls. From kinematics, a large-size body should fly with high speed so as to produce adequate buoyancy to support its body in the air. Because the larger size body reduces its maneuverability required to fly in the cluttered space, the large individuals can only fly in the relatively less cluttered space to detect and search for the distant targets. The high attenuation in the atmosphere of high frequency calls means that high-frequency calls are only operational within a narrow range. Therefore large species use low-frequency calls to detect distant preys. From ecology, large individuals need more preys for energy, therefore the large individuals feed on the preys with large body size. The body size of preys was thought to relate with the frequency of echolocation sound in Rhinolophidae and Hipposideridae[9]. If a spheriform diameter is shorter than the wavelength of the sound, the intensity of echo reflected from the sphere is weak[15]. Similarly, a large prey can easily be detected by the bats with a wide range of wavelength (or frequency); but a small prey can only be detected by the bats with wavelength short enough (frequency high enough) to be reflect-

ed[15]. It is inferred that the bats with high frequency can feed on both large and small preys, but the bats with low frequency can only feed on large preys. Jones et al.[9] found that the proportion of moths and beetles depended on the frequency produced by bats, and the proportion of moths was higher in the food of bats for the bats with high frequency than that for the bats with low frequency.

This study investigated in detail the morphological features, DF, foraging habitats and prey types in 4 species of hipposiderid bats, which can explain interaction among the morphological features, kinematic features and echolocation calls. *H. armiger* and *H. pratti* have a bigger body size, with higher wingload, poor flexibility and higher wing aspect ratio and a fast flying ability. These two species often detect distant preys in the open leaf canopy ($h > 6$ m) of broadleaf forest; and their main food includes more species in Coleoptera (41.61% for *H. armiger* and 43.28% for *H. pratti*) than those in Lepidoptera (22.30% for *H. armiger* and 26.15% for *H. pratti*). They have lower DF, which is adapted to their morphological and ecological features. *H. larvatus* and *A. wheeleeri* have a smaller body size, with lower wingload, good flexibility and lower wing aspect ratio, and a slow flying ability. They often detect preys nearby in highly cluttered space. *H. larvatus* forages preys in the branches and leaves (2 m ~ 6 m) of broadleaf forest, and *A. wheeleeri* forages preys in the branches and leaves (0.5 m ~ 2 m) of broadleaf forest, shrubbery and bamboo forest. The main food of *H. larvatus* is similar to that of Coleoptera (37.64%) and Lepidoptera (31.13%); the main food of *A. wheeleeri* includes more species in Lepidoptera (43.28%) than those in Colepidoptera (29.08%). The DF of the two species is higher, which is adapted to their morphological and ecological features.

The bats with the same body size in the three families have different DF (Fig. 3). In general, the frequency in hipposiderid bats is the highest, then followed by those in rhinolophid bats and vespertilionid bats. Most rhinolophid bats and hipposiderid bats emit calls with higher frequencies for their large body size than the species in Vespertilionidae, because rhinolophid bats and hipposiderid bats generally put more energy into the second, rather than the first harmonics in their calls, while vespertilionid bats put more energy into the first, rather than the second harmonics (in general, the second harmonic has high-er frequency than the first harmonic). Francis et al.[5] found that for the bats with the same body size, the echolocation frequency of hipposiderid bats averaged about 50 kHz higher than that of rhinolophid bats, but they did not discuss the reason for that. Our study showed that for a given body size, the echolocation sound frequency of hipposiderid bats was 30 ~ 70 kHz higher than that of rhinolophid bats, probably because the structure and size of noseleaf (sound emitting apparatus) and cochlea (sound receiving apparatus) in the two families might be different and their prey species and foraging strategy might be different. So far the research on physiological structure and ecological features of bats is not enough, so the different DF in Rhinolophidae and Hipposideridae should be studied further in both fieldwork and in laboratory. In addition, there is considerable deviation from the regression line (Fig. 3). The negative correlation between DF and body size is a rough trend, the bats with the same body size in the same family as well as in the different families have different DF, in addition, the previous study has suggested that the other morphological, physiological and ecological factors can also influence DF[2~9], therefore, echolocation frequency can be inferred not only from body size, but also from other factors.

In addition, because there exists summer swarming, which results in the smaller number of female bats in summer, we could not analyze the difference in echolocation calls and morphological characteristics between the males and females. The specimens recorded are all male bats in this investigation.

In conclusion, there are significantly negative correlations between body size and DF (in three families) and between NW and DF (in Rhinolophidae). Each family has a different regression equation and within each family the correlation is not strict, with many dots fluctuating around the regression lines rather than falling on the lines. All these indicate that the echolocation behavior of bats is an adaptation, influenced by morphological, physiological and ecological features simultaneously. The body size, sound emission organs and sound reception organs determine the basic structure of echolocation sound, thus determine main ecological processes indirectly (such as foraging region, diet type and foraging mode). The echolocation sound parameters (such as frequency, pulse duration, pulse repetition and FM bandwidth) may be changed to some extent to adapt to different foraging region, diet type and to make bats detect,

locate, approach and capture their preys successfully.

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