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RESEARCH ARTICLE

Identification of sympatric bat species by the echolocation calls

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Abstract One hundred and thirty-eight echolocation calls of 63 free-flying individuals of five bat species (*Rhinolophus ferrumequinum*, *Myotis formosus*, *Myotis ikonnikovi*, *Myotis daubentoni* and *Murina leucogaster*) were recorded (by ultrasonic bat detector (D980)) in Zhi'an village of Jilin Province, China. According to the frequency-time spectra, these calls were categorized into two types: FM/CF (constant frequency) / FM (*R. ferrumequinum*) and FM (frequency modulated) (*M. formosus*, *M. ikonnikovi*, *M. daubentoni* and *M. leucogaster*). Sonograms of the calls of *R. ferrumequinum* could easily be distinguished from those of the other four species. For the calls of the remaining four species, six echolocation call parameters, including starting frequency, ending frequency, peak frequency duration, longest inter-pulse interval and shortest inter-pulse interval, were examined by stepwise discriminant analysis. The results show that 84.1% of calls were correctly classified, which indicates that these parameters of echolocation calls play an important role in identifying bat species. These parameters can be used to test the accuracy of general predictions based on bats' morphology in the same forest and can provide essential information for assessing patterns of bat habitat use.

Keywords bats, echolocation call, identification of species, discriminant analysis

1 Introduction

The bat (Chiroptera, Mammalia) is one of the mammals with a very wide distribution and has evolved successfully with about 18 families, 201 genera and 1107 species, worldwide. In China, there are 123 species in 30 genera and 7 families occupying 11.1% of the species of bats in the world (Zhang et al., 2005). In recent years, disturbances in the foraging habitats may have seriously affected the populations of the insectivorous bats and have led to the gradual decrease in bat number (Vaughan et al., 1996; Racey and Entwistle, 2003). The selection of bats for foraging habitats may vary not only among geographical areas but also among species (Swift and Racey, 1983). Therefore, we needed to investigate and understand the foraging habitats of the different bat species. This is important as it can help us protect them more effectively. However, due to the unique behaviors of bats, it is difficult to capture them and identify their species. At present, identification of species is always subject to the external characteristics and its accuracy was dependent on the experience and ability of the observer (Parsons and Jones, 2000).

Bats (Microchiroptera) mainly use echolocation calls to locate and hunt prey (Altringham, 1996; Simmons et al., 1979). Fenton (1997) noted that effective monitoring of echolocation calls was important in the studies on the ecology and conservation of bats. However, the structure of echolocation calls varies because of the influences of morphology, age, geographical variation, foraging habitat and foraging mode (Barclay et al., 1999). It varies not only between species, but also within species. The variation of echolocation calls can cause the researcher to incorrectly identify the species of bats according to calls. Moreover the calls which are recorded in one region or in a habitat may not be applicable to other regions, or to other habitats (Fukui et al., 2004). Thus species identification should be in the same area.

Recently, ultrasonic bat detectors have been used increasingly to study bat behaviors (Feng et al., 2000; Zhang et al., 2000). Moreover, more and more researchers

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are using bat detectors to record echolocation calls and to classify bats based on known calls (Fukui et al., 2004; Rydell et al., 2002; Vaughan et al., 1997b). Fukui et al. (2004) used the parameters of echolocation calls of the bats to distinguish eight bat species, inhabiting the forests of Southern Hokkaido in Japan, to study the patterns of habitat use and foraging behaviors and to predict the patterns of habitat use within a community. It provided essential information on the conservation and management of the habitats of bats.

In this study, we recorded the echolocation calls of five species of bats inhabiting the same area in Ji'an city, China and analyzed the characteristic parameters of these calls. We identified the species of bats by using a one-way ANOVA and discriminant function analysis (DFA). The patterns of the bats' habitat-use in this forest were then predicted. DFA is useful to resolve some practical questions, so it is broadly used in many fields (Zhang and Fang, 1982). For bats, this method is usually used to identify the species (Fukui et al., 2004; Rydell et al., 2002; Vaughan et al., 1997b). At present, stepwise discriminant analysis is the most common method in DFA, but there is no study on bats using this method in China.

2 Materials and methods

2.1 Study area

The study area (125°32′–125°50′ E, 41°3′–41°28′ N) is located in the west of Zhi'an village of Ji'an city, China. This area covers 1300 ha and consists of deciduous forest, secondary deciduous forest and coniferous forest. Dominant species includes *Tilia amurensis*, *Quercus mongolica*, *Quercus liaotungensis*, *Juglans mandshurica* Maxim, *Castanea mollissima* and *Pinus tabrlaeformis*. Shrubs and herbs are abundant. There are three caves in the area that the bats inhabit. These are the Dalazi cave (125°50′ E, 41°3′ N), the Wuming cave (125°42′ E, 41°7′ N) and the Laoliujiafangshen cave (125°38′ E, 41°18′ N).

2.2 Samples collection and calls recording

Five species of bats were captured by mist nets in the study area in June and July, 2003, and in June, July and August, 2004. All bats were identified based on three books: *Mammal Species of the World* (Wilson and Reeder, 1993), *Chiroptera Key* (Wang S, personal communication) and *Wild Animal Illustrated Handbook of Jilin Province* (1988). Based on the external characteristics of the bats, *R. ferrumequinum*, *M. formosus*, *M. ikonnikovi*, *M. daubentoni* and *M. leucogaster* were identified. Among the five species, *M. formosus* was just recently documented to be in Jilin Province, China (Liu et al., 2005). The bats were taken to a temporary laboratory (9 m × 4 m × 4 m, length × width × height) to record the echolocation calls. Recordings were made by a Pettersson D980 Bat Detector (Pettersson Electronik AB, Uppsala, Sweden; frequency response ± 3.5 dB between 8 and 160 kHz), with time expanded by 10 times, to a laptop running Batsound Pro software (Pettersson). To reduce the noise disturbance, we recorded the calls when only one bat was flying freely in the lab. We marked the sexes of all the bats and body mass by using a ruler, a vernier caliper and a table balance, respectively, and released all of the captured bats after measuring the external characteristics and recording their calls.

2.3 Analysis of echolocation calls

The call structures were analyzed with Batsound Pro Version 3.10 (Pettersson Elektronik AB, Sweden) from sonogram (frequency-time graph), oscillogram (power-time graph) and power spectra (power-frequency graph), respectively. FFT (fast Fourier transform) size for calls of *R. ferrumequinum* was 1024 while those of the other species were 512. The analysis precision of the sonogram was 256 Hz with a Hanning window and the analysis attenuation was 60 dB. The following parameters from each pulse were measured: duration time (DT), starting frequency (SF), ending frequency (EF) and peak frequency (PF). The longest inter-pulse interval (IPI-L) and the shortest inter-pulse interval (IPI-S) in the sequence of echolocation calls were also measured.

2.4 Statistical analysis

Among the five species, *R. ferrumequinum* gave out typical FM/CF/FM calls while all other species produced FM calls. The sonograms of the calls of *R. ferrumequinum* could be easily distinguished from others. One-way ANOVA was applied to analyze the significant differences among other four species with Scheffe's test. The stepwise discriminate analysis was used to classify bats and the Wilks' λ value was used to examine the statistical significance of this model. All analysis was performed with SPSS 12.0, and the data was presented as mean ± SD.

3 Results

3.1 Echolocation calls

In total, 138 echolocation calls were recorded from 63 individuals of the five species in flight.

3.1.1 FM/CF/FM type

R. ferrumequinum was the only species which produced the typical FM/CF/FM echolocation calls in this area. Its phase calls began with a rise in frequency followed by a constant frequency (CF) portion and a terminal drop in frequency (Fig. 1). The peak frequency in the calls of this species was 69.6 kHz.

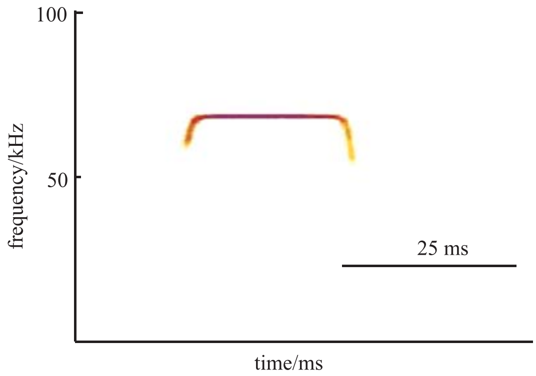

Fig. 1 Sonogram of echolocation calls of *R. ferrumequinum*

3.1.2 FM type

M. formosus, *M. ikonnikovi*, *M. daubentoni* and *M. leucogaster* used FM calls (Fig. 2). The peak frequency of *M. leucogaster* was the highest of the four species (64.3 kHz; Scheffe's test, $P < 0.05$, Table 1). The peak frequencies from high to low of other three species were 56.3 kHz (*M. formosus*), 52.0 kHz (*M. daubentoni*) and 45.3 kHz (*M. ikonnikovi*), respectively. The differences among peak frequencies of the three species were also significant (Scheffe's test, $P < 0.05$). Duration of calls of the three species was longer than that of *M. leucogaster*, but the difference was not significant (Scheffe's test, $P > 0.05$). *M. leucogaster* had a broader bandwidth than the other three species and had the greatest difference from *M. daubentoni* (Scheffe's test, $P < 0.05$). Pulse interval varied greatly between the four species (Table 1). There were significant differences between *M. daubentoni* and *M. ikonnikovi*, *M. daubentoni* and *M. leucogaster* (Scheffe's test of IPI-S and IPI-L, $P < 0.05$).

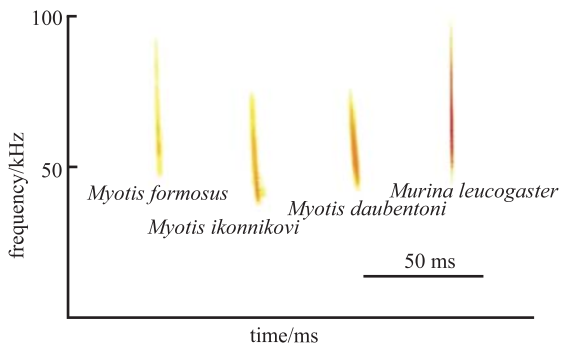

Fig. 2 Sonogram of echolocation calls of FM type of 4 bat species

3.2 Discriminant function analysis

Six parameters of echolocation calls from four bat species (*M. formosus*, *M. ikonnikovi*, *M. daubentoni* and *M. leucogaster*) were selected to classify species of bats using Wilks' λ method in a stepwise discriminate function analysis. The results of test of equality of the group means indicated that all of the six parameters were effective variances and could easily reflect classification characters (Table 2). The discrimination results were significant ($P < 0.01$) and 84.1% of 63 echolocation calls were correctly classified (Table 3). Classification rates in four species ranged from 75.0% (*M. formosus*) to 100% (*M. leucogaster*). In the ten cases of misclassification, five were misclassified as *M. formosus*, two as *M. ikonnikovi*, and two as *M. leucogaster* (Table 3).

4 Discussions

4.1 Characteristics of echolocation calls

The call structure of *R. ferrumequinum* in the study was a typical FM/CF/FM type which is similar to those in published literatures (Feng et al., 2000, 2002; Zhao et al., 2003). The call structure of this species in flight was complicated and was useful for obtaining more detailed information regarding the surroundings (Feng et al., 2000). The echolocation calls of *R. ferrumequinum* were influenced by flying states and foraging habitats (Schnizler, 1968) and varied in different ages and seasons (Jones and Ransome, 1993). In different regions, the peak frequency of this species was variable, e.g. 82.08 kHz in England (Parsons et al., 2000), 81.3 kHz in Italy (Russo and Jones, 2002), 65.0 kHz in Japan (Fukui et al., 2004)

Table 1 The characters of echolocation calls of five species

species	call structure	SF/kHz	EF/kHz	PF/kHz	DT/ms	IPI-S/ms	IPI-L/ms
Rhinolophus ferrumequinum (13)	FM/CF/FM	61.2 ± 4.3	57.4 ± 3.5	69.6 ± 8.3	31.7 ± 9.4	8.2 ± 1.8	57.3 ± 18.7
Myotis formosus (16)	FM	94.8 ± 5.0	44.4 ± 3.7	56.3 ± 5.0	2.1 ± 0.5	27.5 ± 11.6	61.8 ± 15.4
Myotis ikonnikovi (9)	FM	79.0 ± 13.6	38.0 ± 2.8	45.3 ± 3.6	2.3 ± 0.4	43.8 ± 15.4	84.4 ± 44.7
Myotis daubentoni (31)	FM	76.3 ± 9.9	40.0 ± 2.7	52.0 ± 3.8	2.6 ± 0.6	23.0 ± 8.9	56.1 ± 12.7
Murina leucogaster (7)	FM	101.3 ± 13.7	46.3 ± 3.1	64.3 ± 3.1	2.1 ± 0.4	41.8 ± 15.7	88.1 ± 42.7

Note: the number in the bracket after the name of the bat species is the number of specimens of the bat species.

Table 2 Results of the test of equality of group means among echolocation calls by stepwise discriminant function analysis

variables	wilk' λ	*F*	*P*
SF	0.567	15.048	0.000
EF	0.531	17.375	0.000
PF	0.376	32.617	0.000
D	0.860	3.211	0.029
IPI-L	0.647	10.749	0.000
IPI-S	0.784	5.428	0.002

and 69.6 kHz in China (this study, Jilin). Heller and Helversen (1989) considered that there seemed to be a cline for *R. ferrumequinum* in Europe. Along the cline, call frequencies decreased continuously from North West to South East. Therefore, the specific peak frequency of this species in China, which is in latitude lower than those found in Europe, might be due to this reason. The call parameters of the same bat differ from area to area. Thus, when we distinguish bat species by their echolocation calls, the controls should be known from specific individuals from the same area.

In this study, the species of the genera *Myotis* and *Murina* also used FM calls (Fig. 2). The pulse intervals of three species of *Myotis* were longer than that of *M. leucogaster* and the bandwidths were narrower than that of *M. leucogaster* which were consistent with the results of Fukui et al. (2004). Jones (1995) noted that FM calls provide detailed information on the bat's surroundings. Vaughan et al. (1997b) suggested that FM calls were suitable for bats to aerially detect, trawl and catch prey in narrow or edge spaces (Schnitzler et al., 2003). *M. leucogaster* is widely distributed in East Asia (Koopman, 1993) and emits short, broad-band and low intensity calls (Fig. 2, Table 1) which is similar to those in previous studies (Fukui et al., 2004; Kingston et al., 1999). Some authors indicated that FM calls with a short duration, a broad-band and a low intensity were useful for detecting arthropod prey in cluttered spaces (Simmons et al., 1979; Schnizler et al., 2003). Kingston et al. (1999) suggested that the calls of *Murina* species facilitated highly accurate target localization.

4.2 Species identification of bats

The results show that the stepwise discriminant function analysis of six parameters of echolocation calls was a feasible method for identifying and classifying bats. The overall level of accuracy of the DFA was 84.1% (Table 3) which was higher than those obtained by some other studies (Parsons and Jones, 2000; Vaughan et al., 1997b). The higher accuracy of classification may result from the relatively few species in the study including only one species of *Murina* and the use of only two types of calls in this study. In particular, only the echolocation calls in flight were analyzed. Fukui et al. (2004) suggested that some species of bats changed the structures of their echolocation calls in response to variations in the environment of the flight space. For example, *M. macrodactylus* varied the characteristics of its calls according to whether it was in open space or cluttered space (Fukui et al., 2004). Vaughan et al. (1997) noted that the echolocation calls recorded in different environments could promote the reliability of classification.

Ten individuals in 63 samples were misclassified and the misclassification rate was 15.9%. The reasons for misclassification might be that the species of *Myotis* had an adaptive radiation (Fenton and Bogdanowicz, 2002) and the structure of the echolocation calls among the sympatric species was similar. Furthermore, morphology also influenced call design and could cause convergence in call design between species of *Myotis* with similar morphs (Parsons et al., 2000). So the discrimination of species was sometimes difficult (Fukui et al., 2004).

Our study results proved that the DFA of parameters of echolocation calls is a feasible method for identifying bats in the same area. When the unknown echolocation calls were investigated and recorded, we can apply this method to identify certain species of bats. To check the accuracy of DFA, we may compare the results of classification with those of external characteristics. On the basis of the recorded data in the field, this approach can provide much more quantitative data about activity patterns and provide essential information for predicting the patterns of habitat use of bats in the same area.

Table 3 Summary of classification of four species of bats by discriminant function analysis

classification	true species			
	Myotis formosus	*Myotis ikonnikovi*	*Myotis daubentoni*	*Murina leucogaster*
Myotis formosus	12	1	4	0
Myotis ikonnikovi	1	8	1	0
Myotis daubentoni	1	0	26	0
Murina leucogaster	2	0	0	7
Number of true species	16	9	31	7
Number of correct classification	12	8	26	7
Percent of correct classification/%	75.0	88.9	83.9	100

Note: model relied on 6 parameters; overall correct classification rate was 84.1%.

With the development of analysis methods, this approach should be combined with an artificial neural network in future. At that time, more data should be obtained and echolocation calls should be analyzed more deeply. The individual bats can then be accurately identified at the species level. The patterns of habitat use of every species can be analyzed for promoting conservation of diversities of bats.

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References

Altringham J D (1996). Bats-biology and behaviour. New York: Oxford University

Barclay R M R, Fullard J H, Jacobs D S (1999). Variation in the echolocation calls of the hoary bat (*Lasiurus cinereus*): influence of body size, habitat structure, and geographic location. Canadian Journal of Zoology, 77: 530–534

Feng J, Li Z X, Chen M, Zhou J, Zhao H H, Zhang S Y (2002). The Echolocation comparison and the differentiation of ecology niche of five species bats live in one cave. Acta Ecologica Sinica, 22(2): 150–155 (in Chinese)

Feng J, Zhang S Y, Li Z X, Sheng L X, Wang L X (2000). Echolocation calls of greater horseshoe bat at different behavior. Acta Zoologica Sinica, 46(2): 230–232 (in Chinese)

Fenton M B, Bogdanowicz W (2002). Relationships between external morphology and foraging behaviour: bats in the genus *Myotis*. Canadian Journal of Zoology, 80: 1004–1013

Fenton M B (1997). Science and the conservation of bats. Journal of Mammalogy, 78: 1–14

Fukui D, Agetsuma N, Hill D A (2004). Acoustic identification of eight species of bat (Mammalia: Chiroptera) inhabiting forests of southern Hokkaido, Japan: potential for conservation monitoring. Zoological Science, 21: 947–955

Heller K G, von Helversen O (1989). Resource partitioning of sonar frequency bands in rhinolophoid bats. Oecologia, 63: 178–186

Jones G, Ransome R D (1993). Echolocation calls of bats change over a lifetime are influenced by maternal effects. Proceedings of the Royal Society, London, 252B: 125–128

Jones G (1995). Flight performance, echolocation and foraging behavior in noctule bats *Nyctalus noctula*. Journal of Zoology, 237: 303–312

Kingston T, Jones G, Akbar Z, Kunz T H (1999). Echolocation signal design in Kerivoulinae and Murininae (Chiroptera: Vespertilionidae) from Malaysia. Journal of Zoology, 249: 359–374

Koopman K F (1993). Order Chiroptera. In: mammal species of the world: a taxonmic and geographic reference. Washington DC: Smithsonian Institution Press, 137–241

Liu Y, Lun X W, Li Z X, Jin L R, Zhang X C, Feng J (2005). A new record of Vespertilionidae in Jilin province. Chinese Journal of Zoology, 40(1): 101–103 (in Chinese)

Parsons S, Boonman A M, Obrist M K (2000). Advantages and disadvantages of techniques for transforming and analyzing Chiropteran echolocation calls. Journal of Mammalogy, 81: 927–938

Parsons S, Jones G (2000). Acoustic identification of twelve species of echolocating bat by discrimimant function analysis and artificial neural networks. Journal of Experimental Biology, 203: 2641–2656

Racey P A, Entwistle A C (2003). Conservation ecology of bats. In: Bat Ecology. Kunz T H, Fenton M B, Chicago: The University of Chicago Press, 680–745

Russo D, Jones G (2002). Identification of twenty-two bat species (Mammalia: Chiroptera) from Italy by analysis of time-expanded recordings of echolocation calls. Journal of Zoology, 258: 91–103

Rydell J, Arita H T, Santos M, Granados J (2002). Acoustic identification of insectivorous bats (order Chiroptera) of Yucatan, Mexico. Journal of Zoology, 257: 27–36

Schnizler H U, Moss C F, Denzinger A (2003). From spatial orientation to prey acquisition in echolocating bats. Trends in Ecology & Evolution, 18: 386–394

Schnizler H U (1968). Ultrasonic sounds of horseshoe bats (Chiroptera-Rhinolophidae) in different orientation situations. Vergleichende Physiologie, 57: 376–408

Simmons J A, Fenton M B, O'Farrell M J (1979). Echolocation and the pursuit of prey by bats. Science, 203: 16–21

Swift S M, Racey P A (1983). Resource partitioning in two species of vespertilionid bats (Chiroptera) occupying the same roost. Journal of Zoology, 200: 249–259

Vaughan N, Jones G, Harris S (1996). Effects of sewage effluent on the activity of bats (Chiroptera: Vespertilionidae) foraging along rivers. Biological Conservation, 78: 337–343

Vaughan N, Jones G, Harris S (1997b). Identification of British bat species by multivariate analysis of echolocation call parameters. Bioacoustics, 7: 189–207

Wild Animal Conservation Society of JiLin Province, 1988. Wild animal illustrated handbook of Jilin province (amphibia, reptilia, mammalian), Jilin Science Press, 41–45 (in Chinese)

Wilson D E, Reeder D M, eds (1993). Mammal species of the world. Smithsonian Institution Press

Zhang J S, Zhang J P, Liang B, Zhang S Y (2005). The latest report of status and species of bats (Chiroptera) in the world. Chinese Journal of Zoology, 40(2): 79 (in Chinese)

Zhang S Y, Zhao H H, Feng J, Sheng L X, Wang H, Wang L X (2000). Relationship between echolocation frequency and body size in two species of *Hipposideros*. Chinese Science Bulletin, 45(7):740–743 (in Chinese)

Zhang Y T, Fang K T (1982). Introduction to multivariate statistical analysis. Beijing: Science Press, 393–401 (in Chinese)

Zhao H H, Zuo M X, Liang B (2003).Correlation between ear length and call frequency in *Rhinolophus*. Acta Zoologica Sinica, 49(1): 128–133 (in Chinese)