Acoustic Species Identification of Korean *Myotis* Bats (Chiroptera: Vespertilionidae)

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Abstract

We investigated structure and intensity of 267 echolocation calls that were collected from the five Korean *Myotis* species (*M. nettereri*, *M. petax*, *M. ikonnikovi*, *M. macrodactylus* and *M. formosus*). All the *Myotis* species produced typical FM call pattern with similar echolocation call shapes and outer shapes, producing steep, downward frequency-modulated calls. A pulse has two harmonies, which consist of the first harmony with wider bandwidth and the second harmony with narrower bandwidth. The PF of the first harmony is higher than that of the second harmony. The typical FM call structure, with two harmonies and wide bandwidth, might be highly related to fast flying and wide screening in the dense forests. In classification of the echolocation calls by DFA, most of calls from the five species could be well correctly classified. All calls of *M. nettereri* (100% of 17 calls), *M. formosus* (95.5% of 22 calls) and *M. ikonnikovi* (85.7% of 70 calls) could be well discriminated from those of the other species, whereas calls of *M. petax* and *M. macrodactylus* could be discriminated by 70.4% of 98 calls and 76.7% of 60 calls, respectively. Our results indicate that the five Korean *Myotis* species can be well identified by the echolocation calls with high correct classification by DFA.

Key Words: *Myotis*, echolocation call, vesperilionidae, bat, FM call

Introduction

Many bat species use echolocation call to orientate in their natural environment. In case of most insectivorous bats, they mainly depend on echolocation call for orient and forage for flying insects in the dark and also for social communication (Russo and Jones 2001). The structure and shape of echolocation calls could be influenced by various environmental factors such as habitat structures, flight height, geographical variation, age, sex, body size, social group, foraging mode and flying in open versus cluttered space (Aldridge and Rautenbach 1987; Norberg and Ray- ner 1987; Thomas et al. 1987; Fenton 1990; Barclay and Brigham 1991; Obrist 1995; Barclay et al. 1999; Russo and Jones 2003; Estrada et al. 2004; Fukui et al. 2004; Jones and simmers 2010). In many cases bat calls are species specific calls that can be used to easily identify a species and vary generally between species. Thus, most species could be discriminated by analysis of echolocation call structures. There are also the overlap between species and plasticity of echolocation calls within species, causing difficulty in identification of species using recorded echolocation calls. In some bats foraging in similar forests, the structure of echolocation calls tend to be a little similar between interspecies,
though they are different species (Kalko and Schnitzler 1993; Parsons and Jones 2000).

To date, various bat detectors like heterodyne bat detectors (Parsons et al. 2000) and time expansion detectors (Pettersson 1999; Parsons et al. 2000) have been applied to identify bat species in flight. In addition, discriminant function analysis (DFA) has been applied successfully to identify bats by the echolocation calls in Europe (Zingg 1990; Vaughan et al. 1997; Parsons and Jones 2000) and North America (Krusic and Neefus 1996; Murray et al. 1999).

There are 21 bat species described in South Korea, and 19 species of them belong to the family Vespertilionidae. In case of bats of the genus *Myotis*, seven species were described in South Korea. The *Myotis* species are similar looking and sounding bats, typically producing a frequency modulated call dominated by sweeps from high to low frequencies (Landy et al. 2011). Thus, it is hard to discriminate each of *Myotis* species with morphological characters and appearance.

The present study aimed at comprehending the respective characteristics of echolocation patterns by analyzing the echolocation calls of Korean *Myotis* bats. In addition, the study was conducted with the purpose of building a system capable of identifying species by classifying and identifying nonspecific echolocation calls. The ultrasonic calls of bats differ in the signal shape that occurs according to environment, behavior and species, and as a result, the echolocation call of bats can provide information on the identification of bat species and is known to be the most accurate and trustworthy index for identification. In this study, we collected echolocation calls of the five *Myotis* species (*M. nattereri M. petax*, *M. ikonnikovi*, *M. macrodactylus*, and *M. formosus*) that are found in similar forests in South Korea with bat detectors, investigated the characteristics and shapes of their call structures, and then examined the feasibility of identifying *Myotis* species from their echolocation calls using DFA.

**Materials and Methods**

**Sample collection**

We collected each individual of *Myotis petax*, *M. ikonnikovi*, *M. macrodactylus*, *M. nattereri* and *M. formosus* from various forests in South Korea using mist-net (3.5x9 m;
Echolocation Call

Avinet, USA) (Table 1). Echolocation calls from each species were collected using the hand-releasing method (Russo and Jones 2002) right after bat capture at each collection site, and then the individual was released back to its natural habitats.

**Echolocation call analysis**

Total 267 echolocation calls were recorded from the hand-released bats after the capturing using ultrasound bat detector (Batbox griffin, England). The ultrasound bat detector was set to time-expansion mode of 1.5s in time length to slow the echolocation sound signals down ten times, which will be able to bring them into the audible range. The structure of echolocation calls was analyzed using Raven pro version 1.5 software (Cornell Laboratory of Ornithology Natick, NY, USA). From each call structure, we measured the following seven parameters according to Fukui et al. (2004) (Fig. 1): (1) start frequency (SF; frequency at the start of the pulse), (2) end frequency (EF; frequency at the end of the pulse), (3) peak frequency (PF; frequency of maximum energy of pulse), (4) bandwidth (BW; difference between maximal and minimal frequencies), (5) duration (D; time between start and end of a pulse), (6) interpulse interval (IPI; beginning of a call to the start of next call) and (7) the number of harmony (NH).

**Data analysis**

Echolocation calls of *M. petax*, *M. ikonnikovi*, *M. macrodactylus*, *M. nattereri* and *M. formosus* were analyzed using DFA implemented in SPSS statistics ver. 20. The five parameters of SF, EF, PF, D and PI were used for the DFA analysis. Ahead of the DFA analysis, MANOVA test and Box’s M analysis were conducted to test multivariate normal distribution and the verification of the covariance matrix, respectively.

**Results and Discussion**

**Characteristics of echolocation call structure**

All of 267 echolocation calls of the five *Myotis* species, which were recorded from the hand-releasing method, showed highly similar call structure and could be categorized into FM types. The FM type of the five *Myotis* species were typical call pattern of the genus *Myotis*, producing steep, downward frequency-modulated calls (Fig. 2).

The calls of all the five *Myotis* species have two harmonies of the first and second harmony. The first harmony of *M. formosus* call is basically similar shape to that of *M. petax*, *M. ikonnikovi*, *M. macrodactylus*, and *M. formosus* calls, though it is a little longer in frequency and thicker in pulse interval than that of the four species. In case of *M. nattereri*, both of the first and second harmonies are relatively weaker than those of the other species. The second harmony of *M. macrodactylus* has higher frequency and wider pulse interval than that of the other species (Fig. 2).

Descriptive values of the six parameters of echolocation calls are shown in Table 2. Average SF value was highest in
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Fig. 3. Canonical score plots of echolocation calls of the five Korean Myotis species by DFA using five parameters (SF, start frequency; EF, end frequency; PF, peak frequency; D, duration; and IPI, interpulse interval).

Discriminant function analysis of echolocation call

We selected the five factors (SF, EF, PF, D and IPI) from structure of 267 echolocation calls from the five Korean Myotis species, and then the factors were used as parameters for DFA to estimate whether the five Korean

Table 2. Descriptive statistics for six parameters in echolocation calls of the five Korean Myotis species

| Species  | SF (kHz) | EF (kHz) | PF (kHz) | D (ms) | IPI (ms) | NH |
|----------|----------|----------|----------|--------|----------|----|
| M. petax | Mean±SD  | 109.2±7.6 | 38.2±2.8 | 79.9±16.2 | 2.3±0.4 | 59.7±18.1 | 2 |
|          | (Range)  | (90.3~127.4) | (29.5~43.3) | (51.3~96.5) | (1.2~3.4) | (23.8~128.0) |
| M. ikonnikovi | Mean±SD | 115.0±5.7 | 39.5±3.9 | 60.4±9.3 | 2.6±0.6 | 67.5±16.1 | 2 |
|          | (Range)  | (95.4~127.0) | (31.6~45.4) | (36.4~85.4) | (1.6~3.7) | (45.2~126.3) |
| M. macrodactylus | Mean±SD | 104.4±3.5 | 26.8±1.6 | 62.0±20.5 | 2.7±0.4 | 53.5±21.6 | 2 |
|          | (Range)  | (94.9~111.5) | (23.3~30.4) | (41.3~99.2) | (1.9~3.6) | (14.7~96.2) |
| M. nattereri | Mean±SD | 78.0±3.0 | 13.4±0.7 | 43.9±14.6 | 3.8±2.7 | 81.5±62.2 | 2 |
|          | (Range)  | (72.2~82.0) | (12.5~15.8) | (20.7~55.8) | (1.4~8.0) | (29.2~168.9) |
| M. formosus | Mean±SD | 106.5±5.0 | 29.0±2.7 | 71.2±14.5 | 2.0±1.5 | 60.3±12.8 | 2 |
|          | (Range)  | (94.1~112.6) | (23.5~33.2) | (49.6~93.7) | (1.5~2.5) | (31.1~91.9) |

M. petax (109.2±7.6), whereas it was lowest in M. nattereri (78.0±3.0). Average EF value was highest in M. ikonnikovi (39.5±3.9), whereas it was lowest in M. nattereri (13.4±0.7). Average PF value was highest in M. petax (79.9±16.2), whereas it was lowest in M. nattereri (43.9±14.6). Average D value was highest in M. nattereri (3.8±2.7), whereas it was lowest in M. formosus (2.0±1.5). Average IPI value was highest in M. nattereri (81.5±62.2), whereas it was lowest in M. macrodactylus (53.5±21.6). As shown in morphological shape of echolocation calls (Fig. 2), interestingly, the echolocation call of M. nattereri is discriminated from the calls of the other species by the highest average values in D and IPI and the lowest average values in SF, EF and PF.

Table 2. Descriptive statistics for six parameters in echolocation calls of the five Korean Myotis species
Myotis species could be discriminated by the echolocation calls. According to canonical score plots derived by DFA (Fig. 3), most of 267 calls from the five Myotis species were well separated among the species, with the exception of some overlapping between calls from *M. petax* and *M. ikonnikovi*. Calls of *M. nettereri* were well separated from those of the other species.

The DFA on the echolocation calls of five species of genus *Myotis*, with similar outer shapes and echolocation call shapes, resulted in 79.8% out of 267 echolocation calls being correctly classified (Table 3). Out of the five species, *M. nettereri* was 100% classified, while the remaining four species were respectively classified as follows: *M. formosus* 70.4%, *M. ikonnikovi* 85.7%, *M. macrodactylus* 76.7% and *M. petax* 70.4%.

In *M. petax* bats, 98 echolocation calls were analyzed by DFA. Of them, 69 calls were correctly classified as *M. petax* call, and the others were classified to calls of *M. ikonnikovi* (22 calls), *M. formosus* (four calls), *M. macrodactylus* (two calls) and *M. nettereri* (one call). In *M. ikonnikovi* bats, 70 echolocation calls were analyzed by DFA. Of them, 60 calls were correctly classified as *M. konnikovi* call, and the others were classified to calls of *M. petax* (seven calls), *M. macrodactylus* (two calls) and *M. formosus* (one call). In *M. macrodactylus* bats, 60 echolocation calls were analyzed by DFA. Of them, 40 calls were correctly classified as *M. macrodactylus* call, and the others were classified to *M. formosus* calls. In *M. nettereri* bats, all of 17 echolocation calls, which were analyzed by DFA, were correctly classified to *M. nettereri* call, as also shown in the canonical score plots (Fig. 3). Finally, 21 of the 22 *M. formosus* calls were correctly classified and one call was classified to *M. petax* call.

Calls of *M. nettereri* and *M. formosus* showed high correct classification rates by DFA. The echolocation call of *M. nettereri* resulted in 17 pulses being classified into a single group during DFA (Table 3, Fig. 3). Calls of *M. formosus* and *M. macrodactylus* were overlapped with those of only one species (*M. petax* call and *M. formosus*, respectively) (Table 3).

According to Parsons and Jones (2000), five *Myotis* species (*M. bechsteinii*, *M. brandti*, *M. daubentonii*, *M. mystacinus*, and *M. nettereri*) were classified with average correct classification rates of 72% to 91% by DFA. In another previous study (Mathieu et al. 2011), higher overall correct classification rate of 88.9% was achieved by DFA. Correct classification of 100% of both *M. daubentonii* and *M. mystacinus* was achieved by both typical call outlines. For *M. nettereri*, 79.6% of calls were correctly classified by call morphology, but the addition of maximum frequency improved this to 96.3%. In this study, the echolocation calls of the five Korean *Myotis* species were successfully classified by DFA. Overall correct classification of 79.8%, ranging from 70.4% (*M. petax*) to 100% (*M. nettereri*), was achieved by DFA. This correct classification rates are similar or better in comparison with the results of the previous studies (Parsons and Jones 2000; Mathieu et al. 2011).

Identification of a number of bat species, especially in bats of the genus *Myotis*, using recorded echolocation calls can be difficult, due mainly to the overlap between species and plasticity of echolocation calls within species (Kalko and Schnitzler 1993; Parsons and Jones 2000). Multivariate analyses of call parameters (e.g. DFA) have often been used to identify unknown echolocation calls (Zingg 1990; Russo and Jones 2002; Papadatou et al. 2008). The DFA have been shown to achieve high rates of correct species identification (Zingg 1990; Obrist 1995; Vaughan et al. 1997; Jones et al. 2000; Parsons and Jones 2000; Russo and Jones 2002; Papadatou et al. 2008). In this study to examine the feasibility of identifying Korean *Myotis* species from DFA-based echolocation classification, FM calls of the five Korean *Myotis* species could be successfully classified with

### Table 3. Summary of classification of echolocation calls of the five Korean Myotis species by DFA based on the five parameters (SF; start frequency, EF; end frequency, PF; peak frequency, D; duration, and IPI; interpulse interval)

| Species* | True species |
|----------|--------------|
|          | MP | MI | MM | MN | MF |
| **MP**   | 69 | 7  | 0  | 0  | 1  |
| **MI**   | 22 | 60 | 0  | 0  | 0  |
| **MM**   | 2  | 2  | 46 | 0  | 0  |
| **MN**   | 1  | 0  | 0  | 17 | 0  |
| **MF**   | 4  | 1  | 14 | 0  | 21 |
| n         | 98 | 70 | 60 | 17 | 22 |
| n correct | 69 | 60 | 46 | 17 | 21 |
| % correct | 70.4 | 85.7 | 76.7 | 100.0 | 95.5 |

*MP, Myotis petax; MI, Myotis ikonnikovi; MM, Myotis macrodactylus; MN, Myotis nattereri; MF, Myotis formosus.*
relatively high correct classification rate. Thus, DFA-based call classification will provide a quick and easy method of distinguishing the Korean Myotis species for bat monitoring in field conditions and could be extended to include other species of bats that share conventional acoustic parameters.

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Conflict of interest

The authors declare that they have no competing interests.

Ethics approval

All animal experiments throughout the study were conducted in accordance with guidelines of Kangwon National University for the care and use of animals.

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