Acoustic Identification of Eight Species of Bat (Mammalia: Chiroptera) Inhabiting Forests of Southern Hokkaido, Japan: Potential for Conservation Monitoring

Authors: Fukui, Dai, Agetsuma, Naoki, and Hill, David A.

Acoustic Identification of Eight Species of Bat (Mammalia: Chiroptera) Inhabiting Forests of Southern Hokkaido, Japan: Potential for Conservation Monitoring

Dai Fukui[1][*], Naoki Agetsuma[1] and David A Hill[2]

[1]*Tomakomai Experimental Forest, Hokkaido University, Takaoka, Tomakomai, Hokkaido 053-0035, Japan*
[2]*School of Life Sciences, University of Sussex, Falmer, Brighton, BN1 9QG, U.K.*

ABSTRACT—Assessing the impact of forest management on bat communities requires a reliable method for measuring patterns of habitat use by individual species. A measure of activity can be obtained by monitoring echolocation calls, but identification of species is not always straightforward. We assess the feasibility of using analysis of time-expanded echolocation calls to identify free-flying bats in the Tomakomai Experimental Forest of Hokkaido University, Hokkaido, northern Japan. Echolocation calls of eight bat species were recorded in one or more of three conditions: from hand-released individuals, from bats flying in a confined space and from bats emerging from their roost. Sonograms of 171 calls from 8 bat species were analyzed. These calls could be categorized into 3 types according to their structure: FM/CF/FM type (*Rhinolophus ferrumequinum*), FM types (*Murina leucogaster, Murina ussuriensis, Myotis macrodactylus* and *Myotis ikonnikovi*) and FM/QCF types (*Eptesicus nilssonii, Vespertilio superans* and *Nyctalus aviator*). Sonograms of the calls of *R. ferrumequinum* could easily be distinguished from those of all other species by eye. For the remaining calls, seven parameters (measures of frequency, duration and inter-call interval) were examined using discriminant function analysis, and 92% of calls were correctly classified to species. For each species, at least 80% of calls were correctly classified. We conclude that analysis of echolocation calls is a viable method for distinguishing between species of bats in the Tomakomai Experimental Forest, and that this approach could be applied to examine species differences in patterns of habitat-use within the forest.

Key words: bats, echolocation call, discriminant function analysis, TOEF

INTRODUCTION

Disturbances of foraging habitats may have serious effects on populations of insectivorous bats (e.g. Vaughan *et al.*, 1996; Law *et al.*, 1999; Racey and Entwistle, 2003). Bats use a variety of habitats for foraging (e.g. open space, forest edges, cluttered space, above water, Schnitzler and Kalko, 2001; Schnitzler *et al.*, 2003), and their requirements may vary, not only between species (Swift and Racey, 1983; Arlettaz, 1999; Vaughan *et al.*, 1997a; Russo and Jones, 2003), but also between seasons, and between geographical areas (Racey, 1998). For conservation planning to be effective, it is essential to clarify the type, or types, of habitat that each bat species typically forages in over the course of a year, so that provision can be made to protect them.

Methods that have been used to study the distribution and patterns of habitat use by bats include mist-netting (e.g. Kuenzi and Morrison, 2003; Ciechanowski, 2002), radio-tracking (e.g. Arlettaz, 1999) and observation of individuals fitted with reflective tape (e.g. Swift and Racey, 1983). All of these methods involve capturing bats, however, which will inevitably disturb their activity. Moreover, the height at which bats typically forage varies between species (Aldridge and Rautenbach, 1987; Norberg and Rayner, 1987; Neuweiler, 1989; Fenton, 1990; Schnitzler and Kalko, 1998; Schnitzler and Kalko, 2001; Schnitzler *et al.*, 2003), and capture of bats that typically fly at high altitude is very difficult. Consequently, there have been very few examples of studies in which bats were captured at heights of more than a few metres above ground level, such as in or above the forest canopy (Kalko and Handley, 2001).

Recently, the use of ultrasonic bat detectors has become increasingly common in studies of bat activity (e.g. Krusic *et al.*, 1996; Ciechanowski, 2002). Using bat detectors it is possible to monitor the activity of species that normally fly above the canopy, as well as those below it, pro-

* Corresponding author: Tel. +81-144-33-2171;
FAX. +81-144-33-2173
E-mail: daif@exfor.agr.hokudai.ac.jp

vided that the calls they give are loud enough. If we can distinguish between species using ultrasonic detectors, it should be possible to assess species differences in patterns of habitat use. Subjective identification of species from their calls can be extremely difficult and its accuracy is highly dependent on the experience and ability of the observer (see Parsons *et al.*, 2000). An alternative way of identifying calls of free-flying bats is to record them using time-expansion bat detectors, measure various call parameters from sonograms, and then categorize the calls on the basis of those parameters (e.g. Vaughan *et al.*, 1997b; Parsons, 2001; Rydell *et al.*, 2002). In order to do this, it is necessary to have a reference collection of calls of individuals of known species. These can then be analysed statistically to establish whether combinations of call parameters follow patterns that are species-specific. Methods that have been used to do this include discriminant function analysis (DFA), which has been used to identify bats in several studies in Europe and North America (Zingg, 1990; Obrist, 1995; Krusic and Neefus, 1996; Vaughan *et al.*, 1997b; Murray *et al.*, 1999; Parsons and Jones, 2000; Russo and Jones, 2002; Rydell *et al.*, 2002), and synergetic pattern recognition algorithms performed by artificial neural networks (Parsons, 2001; Parsons and Jones, 2000).

The structure of echolocation calls varies, not only between species, but also within species. A variety of factors have been shown to influence call structure, including geographical variation, foraging habitat and foraging mode (Aldridge and Rautenbach, 1987; Norberg and Rayner, 1987; Thomas *et al.*, 1987; Fenton, 1990; Barclay and Brigham, 1991; Obrist, 1995; Barclay *et al.*, 1999). This means that reference calls recorded in a particular region, or in a particular habitat type, may not be applicable to other regions, or to other habitat types. Therefore, as far as possible, species identification methods should be developed in the region and habitat type where they are to be used.

The aim of this study was to develop a method for assessing interspecific variation in patterns of habitat-use by bats in the Tomakomai Experimental Forest, Hokkaido, Japan. In this paper, we examine characteristics of echolocation call structure for eight species that are found in forests in the region. We then examine the feasibility of identifying these species from their echolocation calls using discriminant function analysis. This is the first study to present an analysis of interspecific differences in echolocation calls in a community of Japanese bats.

METHODS

Study area

The main study area was Tomakomai Experimental Forest (TOEF: 42°43´N, 141°36´E), a research facility of Hokkaido University located near Tomakomai City in Hokkaido Prefecture, Japan. TOEF covers 2715 ha and consists of mature deciduous forest, secondary deciduous forest, and coniferous plantations, each of which occupies about 1/3 of the total area. Dominant tree species in the natural forests are *Quercus crispula*, *Kalopanax pictus*, *Frax-*

inus mandshurica, *Acer spp.*, and *Betula spp*. The coniferous plantations consist mainly of *Picea jezoensis* and *Abies sachalinensis*.

Five species of bat have been caught in previous surveys in the forest: *Rhinolophus ferrumequinum*, *Myotis macrodactylus*, *Myotis ikonnikovi*, *Murina leucogaster* and *Murina ussuriensis* (Fukui *et al.*, 2001). In addition, *Vespertilio superans*, *Nyctalus aviator* and *Eptesicus nilssonii* are suspected be present, although they have not been caught. These species tend to fly above the forest canopy (Abe *et al.*, 1994), and so are difficult to catch in nets set from ground level. However, all 3 species are common in Hokkaido, and one carcass of each of *Vespertilio superans* and *Nyctalus aviator* has been collected in the study area (Fukui, unpublished data). Thus, we assumed that these three species are also likely to be part of the bat community at TOEF. To obtain calls for these species, we visited roosts at other sites in the same region. Calls of *V. superans* and *N. aviator* were recorded at Centennial Woods Park in Kutchan Town (42°54´N, 140°46´E), where several roosts of each species are known (Bat Research Group of Centennial Woods Fan Club, Kutchan, 2002). The park is approximately 70 km from TOEF and consists of 27 ha of mixed woodland, largely *Populus maximowiczii* and *Alnus japonica*, surrounded by agricultural land. The calls of *E. nilssonii* were recorded at a roost in Kyogoku Town (42°49´N, 140°53´E), about 60 km from TOEF, where the roost is in the wall of a potato storehouse surrounded by fields.

Recording methods

We captured *Rhinolophus ferrumequinum, Myotis macrodactylus, Myotis ikonnikovi, Murina leucogaster* and *Murina ussuriensis* in TOEF using mist nets and harp traps from 2001 to 2003 (Capture permit number from Ministry of Environment of Japan, 2001: 2-3~5, 2002: 2-3~6, 2003: 2-39~42). We checked their sex, maturity and reproductive condition, and measured their forearm length using calipers and body weight using a balance. To avoid recording the call of the same individual twice, we tagged them with numbered aluminium bands (2.9 mm, Lambournes Ltd., Leominster, England) on their forearms.

Calls were recorded using a Pettersson D240 bat detector (Pettersson Elektronik AB, Uppsala, Sweden) linked to a digital audio tape recorder (TCD-D100, SONY, Tokyo, Japan). The D240 was set to time-expansion mode, in which it records 1.75 s of ultrasound, slows the signal down ten times to bring it into the audible range, and plays it back. This technique preserves all characteristics of the original sound and allows accurate measurement of acoustic parameters. Captured bats were initially released inside a mosquito net (8 m^3) set on the forest road, and their echolocation calls were recorded as they flew inside the net. For *Rhinolophus ferrumequinum*, calls were also recorded when the bats hung from the roof of the mosquito net. There were two reasons for recording bats in the mosquito net. First, calls of both *Murina* species are very weak, which sometimes meant that we were unable to record any calls when they were released from the hand. Second, for some species the structure of echolocation calls given in open spaces is quite different from those given in cluttered areas. The confined space of the mosquito net was intended to simulate proximity to clutter. Once bats had been recorded in the mosquito net, they were released by hand, and the calls produced on release were also recorded. All bats were released on the forest road (width > 5 m) near the point of capture. Recordings were made with the detector held at the same height, and about 2 m from the released bat.

Calls of *V. superans, N. aviator* and *E. nilssonii* were recorded from June to August 2003. Recordings were made as the bats left their roosts using the same equipment as in TOEF. The bats usually emitted loud, broadband calls immediately after emergence, which could be recorded from bats that were 20–50 m away from the roost exit.

Analysis of echolocation calls

The structure of recorded calls was analyzed using Bat Sound 3.1 software (Pettersson Elektronik AB, Uppsala, Sweden) with a sampling rate of 44.1 kHz and a Hanning window. FFT size was 512 for sonograms and 4096 for power spectra. One pulse of an echolocation call was selected at random from each bat individual in each recording situation (in the mosquito net, on hand released and during roost emergence) for analysis. We measured the following parameters from each pulse: duration (D), start frequency (SF, frequency at the start of the pulse), end frequency (EF, frequency at the end of the pulse), peak frequency (PF, frequency of maximum energy of the pulse) and center frequency (MF, frequency of highest energy taken at half pulse duration). Interpulse interval was also measured from the sequence of pulses for each individual, in each recording situation. Because the bats did not usually emit pulses at even intervals, we measured the longest interpulse interval (IPI-L) and shortest interpulse interval (IPI-S) in the sequence of echolocation calls. D, IPI-L and IPI-S were measured from oscillograms, PF from power spectra, and all other parameters from spectrograms.

Statistical procedures

For the five species for which calls were recorded from captured bats, multivariate analysis of variance (MANOVA) was used to test for differences between calls recorded in the mosquito net and on hand release. Peak frequency, duration and bandwidth (SF minus EF) were compared using Scheffe's test when significant difference had been confirmed by one-way analysis of variance (ANOVA) on calls of all species, except *R. ferrumequinum*.

R. ferrumequinum emits FM/CF/FM calls typical of rhinolophids (e.g. Vaughan *et al.*, 1997b), while all other species produced FM or FM/QCF calls. As sonograms of calls of *R. ferrumequinum* could be easily distinguished from others by eye, they were omitted from the main statistical analyses. Multivariate discriminant function analysis (DFA) was applied to call parameters of the remaining seven species. Quadratic analyses were used because Box's M test showed that covariance matrices were not homogeneous ($p<0.001$). Quadratic discriminant analyses were performed using all seven parameters. Subsequently, the analyses were performed using only the five parameters (SF, EF, PF, MF and D) that could be taken from a single pulse, to assess the degree to which species discrimination would be possible in cases where only one pulse was recorded. Wilk's lambda values were obtained with a MANOVA to test for statistical significance of DFA models. MANOVA was performed with StatView 5.0 for Macintosh, and other tests were performed with SYSTAT 10 for Windows. In all tests, values of $p<0.05$ were considered statistically significant.

RESULTS

In total, 171 echolocation calls were recorded from 129 individuals of eight species. Of these, 40.4% were recorded on hand-release, 33.9% in the mosquito net, and 25.1% during roost emergence (Table 1).

Characteristics of echolocation call

Calls of each species were categorized into FM/CF/FM, FM and FM/QCF type, as defined by Schnitzler and Kalko (2001).

FM/CF/FM type

Rhinolophus ferrumequinum produced typical FM/CF/FM echolocation calls, i.e. calls with a long, strictly constant-

Table 1. Species recorded and number of bats recorded at each situation

| Species	Situations		
	Hand-released	Mosquito net	Leaving roost
Rhinolophus ferrumequinum	8	4	
Myotis ikonnikovi	27	5	–
Myotis macrodactylus	10	9	–
Murina ussuriensis	11	20	–
Murina leucogaster	13	20	–
Eptesicus nilssonii	–	–	12
Nyctalus aviator	–	–	11
Vespertilio superans	–	–	21
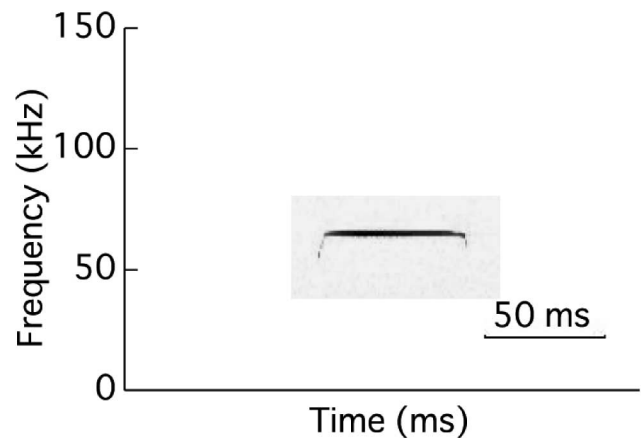
Fig. 1. Sonogram of echolocation calls of *R. ferrumequinum*.

frequency component (CF) preceded and followed by a brief, frequency-modulated (FM) sweep (Fig. 1). Peak frequency in the calls of this species was 65.0 kHz (Table 2). *R. ferrumequinum* was the only species with FM/CF/FM type calls in the TOEF.

FM type

All four species of the genera *Myotis* and *Murina* emitted FM calls (Fig. 2). The peak frequency of *Murina ussuriensis* was the highest of the four species (86.3 kHz; Table 2, Scheffe's test, $p<0.05$). There was little difference between the peak frequencies of other three species. Duration of calls of the two species of *Myotis* were longer than those of the two species of *Murina* (Scheffe's test, $p<0.05$). Both species of *Murina* had broader bandwidth than the two *Myotis* (Scheffe's test, $p<0.05$). In all species, interpulse interval was very variable (see Table 2). MANOVA showed that the only significant differences between hand-release calls and mosquito net calls were for *My. macrodactylus*, (Wilk's lambda=0.20, $F_{7,11}=6.3$, $p<0.05$). MF for this species in the mosquito net was higher than that of hand released calls (ANOVA, $F_{1,17}=6.2$, $p<0.05$), and D, IPI-S and IPI-L in mosquito net were shorter than those of hand-released bats, respectively (D: $F_{1,17}=4.5$, $p<0.05$; IPI-S: $F_{1,17}=21.2$, $p<0.05$;
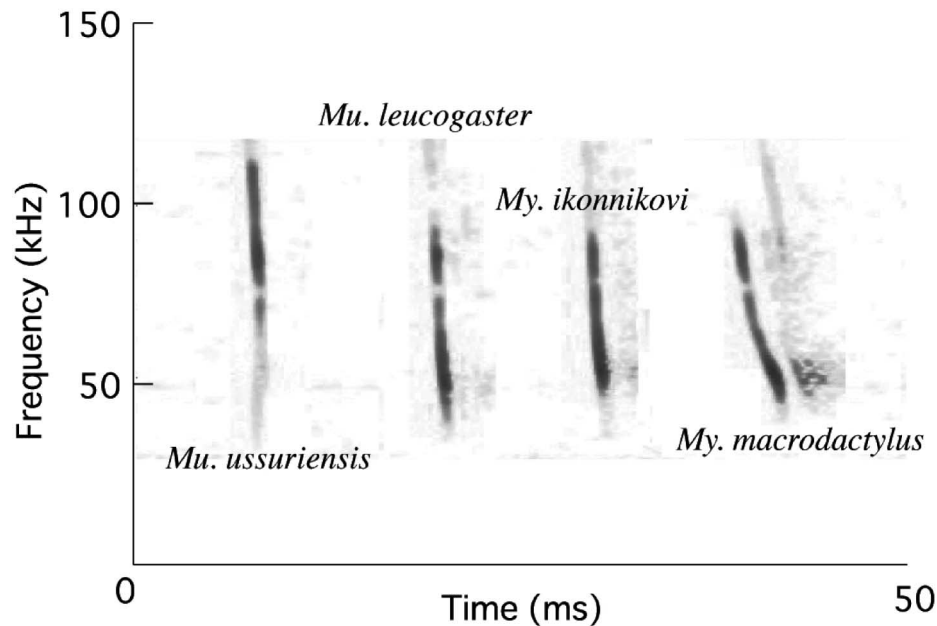
Fig. 2. Sonograms of echolocation calls of *Myotis macrodactylus, Myotis ikonnikovi, Murina leucogaster* and *Murina ussuriensis*.

Table 2. Descriptive statistics for time and frequency parameters in echolocation calls of 8 species of bats. Table shows mean and minimum –maximum of parameters. SF, start frequency; EF, end frequency; PF, frequency of maximum energy; MF, middle frequency; D, duration; IPI-S, shorter inter pulse interval; IPI-L, longer inter pulse interval.

Species	Call structure	SF (kHz)	EF (kHz)	PF (kHz)	MF (kHz)	D (ms)	IPI-S (ms)	IPI-L (ms)
Rhinolophus ferrumequinum	FM/CF/FM	55.9	50.9	65.0	65.0	26.8	10.6	34.5
		52.4–59.1	47.3–56.9	62.8–66.6	62.8–66.6	12.0–80.0	2.0–45.0	5.8–65.0
Myotis ikonnikovi	FM	90.0	43.2	50.6	59.9	2.0	42.3	65.9
		55.2–118.1	37.8–50.1	47.3–55.9	47.6–75.2	0.9–3.4	21.0–84.0	32.0–138.0
Myotis macrodactylus	FM	82.4	39.4	49.2	56.4	3.0	41.0	65.8
		54.1–91.7	31.6–47.7	45.0–51.8	47.9–63.6	1.7–5.0	21.0–84.0	44.0–144.0
Murina ussuriensis	FM	112.6	50.7	86.3	81.5	1.7	26.1	48.4
		90.0–136.6	44.9–58.4	81.5–89.8	69.3–97.9	1.0–4.1	16.0–54.0	29.0–78.0
Murina leucogaster	FM	104.9	43.6	51.2	71.2	1.8	30.8	55.5
		82.8–140.0	35.6–50.1	47.0–56.5	59.2–90.3	1.1–2.7	20.0–69.0	36.0–118.0
Eptesicus nilssonii	FM/QCF	57.9	26.1	30.5	33.2	6.3	75.3	122.9
		51.8–64.7	24.3–27.7	28.3–31.6	31.6–35.0	4.4–8.0	50.0–92.0	95.0–185.0
Nyctalus aviator	FM/QCF	38.7	20.2	21.1	23.3	12.0	221.4	264.8
		27.1–54.1	17.6–22.6	20.2–23.3	19.8–29.9	2.2–17.8	21.4–415.0	37.3–415.0
Vespertilio superans	FM/QCF	48.1	21.8	24.2	26.7	6.2	105.0	144.5
		33.3–55.7	18.1–23.2	21.8–26.5	21.8–31.6	2.3–19.9	27.0–409.0	68.0–409.0

IPI-L: $F_{1,17}=9.1$, $p<0.05$).

FM/QCF type

Calls of *Eptesicus nilssonii, Vespertilio superans* and *Nyctalus aviator* were all defined as FM/QCF type. These pulses had two components: they began with steep frequency modulation (FM), and moved to shallow frequency modulation (quasi-constant frequency, QCF) in latter part of the pulse (Fig. 3). Calls of *E. nilssonii, V. superans* and *N.*

aviator had lower peak frequencies (30.5, 24.2 and 21.1 kHz respectively, $p<0.05$ by Scheffe's test), longer durations (6.3, 6.2 and 12.0 ms respectively, $p<0.05$) and shorter bandwidth (31.8, 26.4 and 18.6 kHz respectively, $p<0.05$) than the other four FM type species (Table 2). Among FM/QCF species, for duration and bandwidth, there were no differences between *E. nilssonii* and *V. superans* (Scheffe's test, $p=0.09$, 0.99). Peak frequencies between each species were significantly differ (Scheffe's test, $p<0.05$). Calls of *N.*
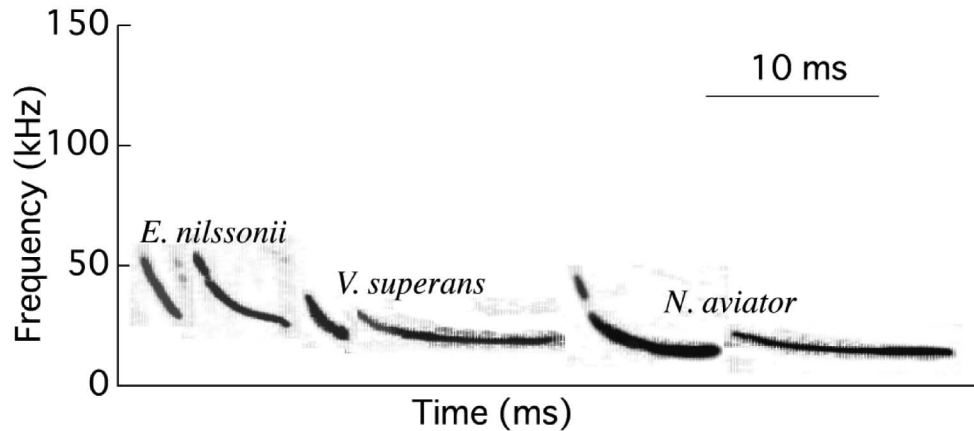
Fig. 3. Sonograms of echolocation calls of *Vespertilio superans, Nyctalus aviator* and *Eptesicus nilssonii*. Two calls of each species are included, in order to show some intraspecific variation.

Table 3. Summary of classification of 7 species of bats by discriminant function analysis. Model relied on 7 parameters (SF, EF, PF, MF, D, IPI-S and IPI-L). Overall correct classification rate was 92%.

Classified as:	True spcies						
	My. i.	*My. m.*	*Mu. u.*	*Mu. l.*	*E. n.*	*N. a.*	*V. s.*
Myotis ikonnikovi	26	0	0	4	0	0	0
Myotis macrodactylus	0	19	0	0	0	0	0
Murina ussuriensis	0	0	31	0	0	0	0
Murina leucogaster	6	0	0	29	0	0	0
Eptesicus nilssonii	0	0	0	0	12	0	0
Nyctalus aviator	0	0	0	0	0	11	3
Vespertilio superans	0	0	0	0	0	0	18
n	32	19	31	33	12	11	21
n correct	26	19	31	29	12	11	18
% correct	81	100	100	88	100	100	86

aviator had longer duration and broader bandwidth than other two FM/QCF type species (Scheffe's test, $p<0.05$). These calls showed considerable variation in duration (D), interpulse interval (IPI), MF and SF (Table 2).

Discriminant function analysis

Quadratic discriminant function analysis using all 7 parameters resulted in 92% of 171 records of echolocation calls being correctly classified to one of the seven species (Table 3). MANOVA showed that the model was significant (Wilk's lambda=0.001, $F_{42,688}=62.0$, $p<0.05$). The first 3 discriminant functions explained 99.9% of total variation. For four species classification was 100%. Ten of the 13 cases of misclassification involved two species, *My. ikonnikovi* and *Mu. leucogaster* (Table 3). For the preceding analysis, 45 bats contributed more than one data point, as they were recorded both in the mosquito net and on hand release. When the analysis was re-run with only one call taken for each individual bat (n=126), 85% of calls were correctly classified.

In the canonical score plot, the cluster of *Mu. ussurien-*
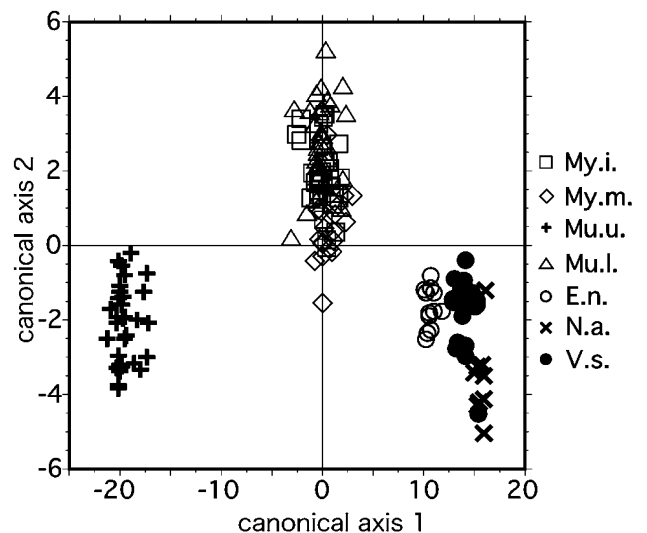
Fig. 4. Canonical score plots of each individual by DFA using 7 parameters. My. i.; *Myotis ikonnikovi,* My. m.; *Myotis macrodactylus,* Mu. u.; *Murina ussuriensis,* Mu. l.; *Murina leucogaster,* E. n.; *Eptesicus nilssonii,* N. a.; *Nyctalus aviator,* V. s.; *Vespertilio superans.*

Table 4. Summary of classification of 7 species of bats by discriminant function analysis. Model relied on 5 parameters (SF, EF, PF, MF and D). Overall correct classification rate was 87%.

Classified as:	True spcies						
	My. l	*My. m.*	*Mu. u.*	*Mu. l.*	*E. n.*	*N. a.*	*V. s.*
Myotis ikonnikovi	23	3	0	3	0	0	0
Myotis macrodactylus	3	16	0	0	0	0	0
Murina ussuriensis	0	0	31	0	0	0	0
Murina leucogaster	6	0	0	30	0	0	0
Eptesicus nilssonii	0	0	0	0	12	0	0
Nyctalus aviator	0	0	0	0	0	8	3
Vespertilio superans	0	0	0	0	0	3	18
n	32	19	31	33	12	11	21
n correct	23	16	31	30	12	8	18
% correct	72	84	100	91	100	73	86

sis was distinct from clusters of other species (Fig. 4). The other three FM type species (*My. macrodactylus, My. ikonnikovi* and *Mu. leucogaster*) showed some overlap. Clusters of 3 FM/QCF type species were contiguous, but with very little overlap (Fig. 4).

Quadratic discriminant function analysis using 5 parameters indicated that 87% of 171 calls were classified into correct species (Table 4). MANOVA showed that the model was significant (Wilk's lambda=0.001, $F_{30,594}=98.8$, $p<0.001$). The first three discriminant functions explained 99.7% of total variation. Classification rates in each species ranged from 72% (*My. ikonnikovi*) to 100% (*E. nilssonii* and *Mu. ussuriensis*). Twenty-eight percent of *My. ikonnikovi* calls were misclassified as *Mu. leucogaster* and *My. macrodactylus*, and 27% of *N. aviator* calls as *V. superans* (Table 4). Clusters of *N. aviator* and *V. superans* overlapped extensively in the DFA score plot when 5 parameters were used

Fig. 5. Canonical score plots of each individual by DFA using 5 parameters. My. i.; *Myotis ikonnikovi,* My. m.; *Myotis macrodactylus,* Mu. u.; *Murina ussuriensis,* Mu. l.; *Murina leucogaster,* E. n.; *Eptesicus nilssonii,* N. a.; *Nyctalus aviator,* V. s.; *Vespertilio superans.*

(Fig. 5). The degree of overlap between clusters of other species was similar.

DISCUSSION

Characteristics of echolocation calls

Of the eight species found in the broad-leaved boreal forests of southern Hokkaido, *R. ferrumequinum* and *E. nilssonii* have been studied in other parts of their distribution, and parameters of their echolocation calls have been published (Vaughan *et al.*, 1997b; Parsons and Jones, 2000; Russo and Jones, 2002; Zingg, 1990). For the remaining six species (*Mu. ussuriensis, Mu. leucogaster, My. ikonnikovi, My. macrodactylus, V. superans* and *N. aviator*) this study provides the first description of their call structures.

Mu. ussuriensis is one of the smallest of the Murininae (forearm length 28.3–34.0 mm) and is distributed in eastern Russia, Korea and Japan. *Mu. leucogaster* is the largest species of the genus (forearm length 40–44 mm) and is distributed in eastern Asia (Koopman, 1993). Both *Murina* species emitted FM calls of very short duration, broad bandwidth and low intensity (Fig. 2, Table 2), which were similar to those described for three *Murina* species in Malaysia (Kingston *et al.*, 1999). Short, broad-band and low intensity FM calls are thought to be used for the detection of arthropod prey in clutter (Simmons *et al.*, 1979; Neuweiler, 1989; Schnitzler *et al.*, 2003). Kingston *et al.* (1999) suggested that calls of *Murina* species in Malaysia facilitate highly accurate target localization in terms of both range and angle estimation and can thus be interpreted as an adaptation to foraging in the highly cluttered environment of the forest understory. Therefore, echolocation calls of *Murina* in this study area would be suitable for highly cluttered forest understory.

The two *Myotis* species in this study emitted typical FM calls. The interpulse interval for these species was longer than for the *Murina ussuriensis*, and the bandwidth was narrower than *Murina* species. Although there is no information
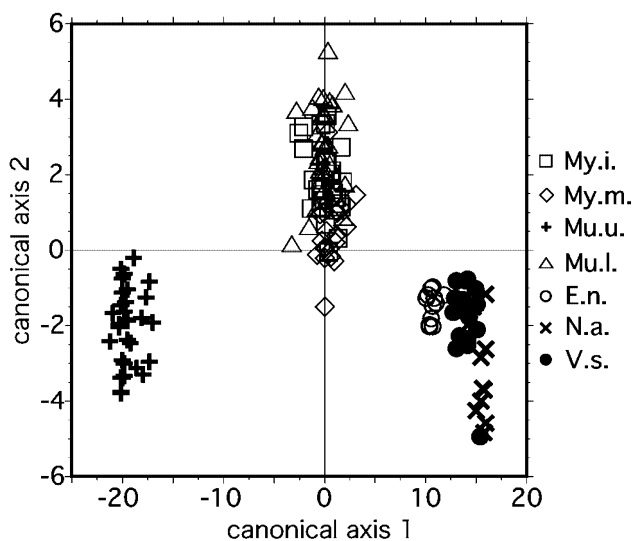
about habitat use by either *My. ikonnikovi* or *My. macrodactylus*, studies of other species suggest that this call type is characteristic of "narrow or edge space aerial/trawling forager" (Schnitzler *et al*., 2003). For *My. macrodactylus*, temporal parameters (D, IPI-S and IPI-L) of hand-released calls and calls recorded in the mosquito net were significantly different. This suggests that *My. macrodactylus* varies the characteristics of its calls according to whether it is in cluttered space or uncluttered (or backcluttered) space. For DFA, it is preferable to analyze both types of call structure together, as under natural conditions, it is possible that *My. macrodactylus* flies in both uncluttered and cluttered space.

The call structures of the three FM/QCF species presented here are similar to those of related European species described in the literature (*N. leisleri, N. noctula* and *E. serotinus*; Vaughan *et al*., 1997b). These species are expected to forage mainly in open spaces (Vaughan *et al*., 1997a), because FM/QCF calls are suitable for use in open environments with some obstacles (Simmons *et al*., 1979). In our results, the degree of frequency modulation of the QCF part was very variable (Fig. 3). Such variation has also been reported in previous studies (Vaughan *et al*., 1997b). It is known that FM/QCF bats emit relatively long, narrow-band signals in uncluttered space, but emit shorter and broader-band signals in background cluttered space such as gaps or edges (Schnitzler and Kalko, 1998, 2001). When we recorded the calls of these species the bats had just left their roost and were dispersing in various directions. Some bats were recorded flying in fairly open space, whereas others were flying near to clutter, and this may have been the source of variation in the structure of calls recorded.

The call structures of *R. ferrumequinum* (Vaughan *et al*., 1997b; Parsons and Jones, 2000; Russo and Jones, 2002) and *E. nilssonii* (Zingg, 1990) have been described previously for other regions. *R. ferrumequinum* has a very wide distribution across temperate Europe and Eurasia (Koopman, 1993). This species is typical of narrow-space CF bats, which hunt from perches for passing insects (Schnitzler and Kalko, 1998). In Europe, the peak frequency of this species is quite different from that in Japan (England: 82.08 kHz, Parsons and Jones, 2000; Italy: 81.3 kHz, Russo and Jones, 2002; Japan: 65.5 kHz, Taniguchi, 1985 and 65.0 kHz, this study). Heller and Helversen (1989) noted that for *R. ferrumequinum* in Europe there seems to be a cline along which call frequencies decrease continuously from NW to SE. This trend may possibly extend as far as the Japan, as the frequency of calls of *R. ferrumequinum* in Japan was much lower than in Europe. The distribution of *E. nilssonii* also includes parts of temperate Europe and Eurasia (Koopman, 1993). The peak frequency of this species is 28.0 kHz in Switzerland (Zingg, 1990), which is lower than in Japanese individuals (30.5 kHz, this study), although the difference is smaller than that found for *R. ferrumequinum*. The occurrence of such geographic variation in call parameters means that studies that use characteristics of echolocation calls to attempt to identify species must be sure to compare them to calls of known individuals from the same region.

Discriminant function analysis

The results show that Discriminant Function Analysis of parameters of echolocation calls is a feasible method for identifying bats in the assemblage of species found in broad-leaved boreal forests in southern Hokkaido. The overall level of accuracy of the DFA was high (92%; Table 3), and remained high even when the analysis was based on only 5 parameters obtained from a single pulse (87%; Table 4). The rates of correct classification were comparable to, or better than, those achieved by other studies that have used DFA to classify calls from individual bats of known species (Obrist, 1995; Zingg, 1990; Vaughan *et al*., 1997b; Krusic and Neefus, 1996; Murray *et al*., 1999). One reason why this study obtained higher accuracy of classification than some others may have been that it was based on relatively few species. In particular, only two species of *Myotis* inhabit the study region. In many cases the echolocation calls of sympatric *Myotis* species are similar in structure and show extensive large overlap in spectral and temporal parameters making them difficult to distinguish from one another (Krusic and Neefus, 1996; Vaughan *et al*., 1997b).

It is important to note, however, that certain species, such as *My. macrodactylus, E. nilssonii, N. aviator* and *V. superans*, changed the structure of their echolocation calls in response to changing environmental conditions of the flight space. It is therefore essential to develop a reference collection of calls that includes recordings of individuals of each species flying in a variety of environments. As far as possible, the collection should include all environmental conditions in which the bats may be encountered in the field.

Although classification by DFA was more accurate for some species than for others, accuracy was high for all species. This suggests that it should be possible to study species-specific patterns of habitat use by bats in forests in the study area using acoustic monitoring alone. In practice this may be less straightforward than it appears to be, however. Levels of activity cannot be compared directly across species, because different species give calls of different intensity (Waters and Jones, 1995), and this influences the distance that they can be detected from. It should be possible to examine the habitat requirements of each species independently by assessing relative levels of foraging activity in various environmental conditions. However, this may also be problematic for certain species. The calls of the three FM/QCF type species were very loud, and could be detected at distances of over 50 m (Fukui, personal observation), which means that they may be recorded from habitat types adjacent to those being surveyed. This problem could be overcome to some extent by ensuring that sampling of a particular habitat type was always done as far as possible from borders with other habitat types. By contrast, the calls of the two *Murina* species and *Rhinolophus ferrumequinum* were very quiet, and could only be recorded in

close proximity. Consequently, in a survey of habitat use, the calls of these species may be detected very rarely, even in areas where they are frequently active. In view of this limitation, acoustic monitoring should probably not be used in isolation, but should be supplemented with survey techniques involving capture.

Assessing species differences in habitat use

Patterns of habitat use and foraging behavior of bats have been interpreted in terms of the structure of echolocation calls (Neuweiler, 1989; Schnitzler *et al.*, 2003), as mentioned above, and aspects of wing morphology (Norberg and Rayner, 1987). In theory, it should be possible to predict the kinds of habitat that each species in a bat community is likely to be associated with on the basis of these characteristics. Such predictions could then be used to develop plans for conservation management that would enhance habitats for bats. However, it is first necessary to establish whether the predictions are valid for a broad range species by assessing patterns of habitat use in the field.

This study has shown that acoustic identification of species is a promising method for establishing patterns of habitat use by the bat community in boreal broad-leaved forests in southern Hokkaido. When calls of unknown bats are recorded in the field, they can be classified to species using the methods described here. This approach is less invasive, and can provide much more quantitative data about activity patterns, than surveys based on capturing bats. Such data can then be used both to test the accuracy of general predictions based on call structure and wing morphology, and to provide profiles of the habitat requirements of individual bat species which is essential information for promoting their conservation (see Vaughan *et al.*, 1997; Russo and Jones 2003).

ACKNOWLEDGEMENTS

REFERENCES

Abe H, Ishii N, Kaneko Y, Maeda K, Miura S, Yoneta M. (1994) A Pictorial Guide to the Mammals of Japan. Tokai University Press, Tokyo (in Japanese)

Aldridge HDJN, Rautenbach IL (1987) Morphology, echolocation and resource partitioning in insectivorous bats. J Anim Ecol 56: 763–778

Arlettaz R (1999) Habitat selection as a major resource partitioning mechanism between the two sympatric sibling bat species *Myotis myotis* and *Myotis blythii*. J Anim Ecol 68: 460–471

Barclay RMR, Brigham RM (1991) Prey detection, dietary niche breadth, and body size in bats: why are aerial insectivorous bats so small? Am Nat 137: 693–703

Barclay RMR, Fullard JH, Jacobs DS (1999) Variation in the echolocation calls of the hoary bat (*Lasiurus cinereus*): influence of body size, habitat structure, and geographic location. Can J Zool 77: 530–534

Bat Research Group of Centennial Woods Fan Club, Kutchan (2001) Bats in Mt. Yotei and Niseko Range, Hokkaido, Japan, No. 1. –Report on 1997~2000 Faunal Survey–. Bull Otaru Mus 14: 127–132

Ciechanowski M (2002) Community structure and activity of bats (Chiroptera) over different water bodies. Mammal Biol 67: 276–285

Fenton MB (1990) The foraging behaviour and ecology of animal-eating bats. Can J Zool 68: 411–422

Fukui D, Ishii T, Agetsuma N, Aoi T (2001) Efficiency of harp trap for capturing bats in boreal broad-leaved forest in Japan. Eurasian J For Res 3: 23–26

Heller K-G, von Helversen O (1989) Resource partitioning of sonar frequency bands in rhinolophoid bats. Oecologia 80: 178–186

Kalko EKV, Handley CO Jr (2001) Neotropical bats in the canopy: diversity, community structure, and implications for conservation. Plant Ecol 153: 319–333

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

Koopman KF (1993) Order Chiroptera. In "Mammal Species of the World: a Taxonomic and Geographic Reference" Ed by DE Wilson, DM Reeder, Smithonian Institution Press, Washington DC, pp 137–241

Krusic RA, Neefus CD (1996) Habitat associations of bat species in the White Mountain National Forest. In "Bats and Forests Symposium" Ed by RMR Barclay, RM Brigham, Ministry of Forests, Victoria, pp 185–198

Krusic RA, Yamasaki M, Neefus CD, Pekins P (1996) Bat habitat use in White Mountain National Forest. J Wildl Manage 60: 625–631

Kuenzi AL, Morrison ML (2003) Temporal patterns of bat activity in southern Arizona. J Wildl Manage 67: 52–64

Law BS, Anderson J, Chidel M (1999) Bat communities in a fragmented forest landscape on the south-west slopes of New South Wales, Australia. Biol Conserv 88: 333–345

Ministry of the Environment (2002) Threatened Wildlife of Japan: Red Data Book 2nd ed – Vol. 1, Mammalia. Japan Wildlife Research Center, Tokyo

Murray KL, Britzke ER, Hadley BM, Robbins LW (1999) Surveying bat communities: a comparison between mist nets and the Anabat II bat detector system. Acta Chiropt 1: 105–112

Neuweiler G (1989) Foraging ecology and audition in echolocating bats. Trends Ecol Evol 4: 160–166

Norberg UM, Rayner JM (1987) Ecological morphology and flight in bats (Mammalia; Chiroptera): wing adaptations, flight performance, foraging strategy and echolocation. Phil Trans R Soc Lond B 316: 335–427

Obrist MK (1995) Flexible bat echolocation – the influence of individual, habitat and conspecifics on sonar signal design. Behav Ecol Sociobiol 36: 207–219

Parsons S (2001) Identification of New Zealand bats (*Chalinolobus tuberculatus* and *Mystacina tuberculata*) in flight from analysis of echolocation calls by artificial neural networks. J Zool 253: 447–456

Parsons S, Boonman AM, Obrist MK (2000) Advantages and disadvantages of techniques for transforming and analyzing Chiropteran echolocation calls. J Mammal 81: 927–938

Parsons S, Jones G (2000) Acoustic identification of twelve species of echolocating bat by discriminant function analysis and artificial neural networks. J Exp Biol 203: 2641–2656

Racey PA (1998) The importance of the riparian environment as a habitat for British bats. In "Behaviour and Ecology of Riparian Mammals" ED by N Dunstone, ML Gorman, Symp Zool Soc

Lond 71: 69–91

Racey PA, Entwistle AC (2003) Conservation ecology of bats. In "Bat Ecology" Ed by TH Kunz, MB Fenton, The University of Chicago Press, Chicago, pp 680–743

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

Russo D, Jones G (2003) Use of foraging habitats by bats in a Mediterranean area determined by acoustic surveys: conservation implications. Ecography 26: 197–209

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

Schnitzler H-U, Kalko EKV (1998) How echolocating bats search and find food. In "Bat Biology and Conservation" Ed by TH Kunz, PA Racey, Smithsonian Institution Press, Washington DC, pp 183–196

Schnitzler H-U, Kalko EKV (2001) Echolocation by insect-eating bats. BioScience 51: 557–569

Schnitzler H-U, Moss CF, Denzinger A (2003) From spatial orientation to food acquisition in echolocating bats. Trends Ecol Evol 18: 386–394

Simmons JA, Fenton MB, O'Farrell MJ (1979) Echolocation and pursuit of prey by bats. Science 203: 16–21

Swift SM, Racey PA (1983) Resource partitioning in two species of vespertilionid bats (Chiroptera) occupying the same roost. J Zool 200: 249–259

Taniguchi I (1985) Echolocation sounds and hearing of the Greater Japanese Horseshoe Bat (*Rhinolophus ferrumequinum nippon*). J Comp Physiol A 156: 185–188

Thomas DW, Bell GP, Fenton MB (1987) Variation in echolocation call frequencies recorded from North American vespertilionid bats: a cautionary note. J Mammal 68: 842–847

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

Vaughan N, Jones G, Harris S (1997a) Habitat use by bats (Chiroptera) assessed by means of a broad-band acoustic method. J Appl Ecol 34: 716–730

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

Waters DA, Jones G (1995) Echolocation call structure and intensity in five species of insectivorous bats. J Exp Biol 198: 475–489

Zingg PE (1990) Acoustic species identification of bats (Mammalia: Chiroptera) in Switzerland. Rev Suisse Zool 97: 263–294