

# Plasticity in echolocation calls of *Myotis macrodactylus* (Chiroptera: Vespertilionidae): implications for acoustic identification

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**Abstract** Poor knowledge of the intraspecific variability in echolocation calls is recognized as an important limiting factor for the accurate acoustic identification of bats. We studied the echolocation behaviors of an ecologically poorly known bat species, *Myotis macrodactylus*, while they were commuting in three types of habitats differing significantly in the amount of background clutter, as well as searching for prey above the water surface in a river. Results showed that *M. macrodactylus* altered their echolocation call structure in the same way during commuting as foraging bats do in relation to the changing level of clutter. With increasing level of clutter, *M. macrodactylus* generally produced echolocation calls with higher start, end, and peak frequencies; wider bandwidth; and shorter pulse duration. Compared to commuting, bats emitted significantly lower frequency calls with narrower bandwidth while searching for prey. Discriminant function analysis indicated that 79.8% of the calls from the three commuting habitats were correctly grouped, and 87% of the calls were correctly classified to the commuting and foraging contexts. Our finding has implications for those who would identify species by their calls.

**Keywords** Echolocation call · Commuting · Acoustic identification · DFA

## Introduction

Accurate species identification is often necessary for biodiversity management and conservation (Fenton 1997). Morphological characteristics of species are widely used in taxonomy. Nevertheless, new techniques such as DNA sequencing, for example, are increasingly applied to species identification (Jones and Teeling 2009). For bats, acoustic identification is considered a valuable approach because sophisticated echolocation (i.e., laryngeal echolocation) is highly linked to all Microchiroptera bats (Jones and Holderied 2007), and species-specific echolocation characteristics exist in many species (Fenton and Bell 1981; Parsons and Jones 2000; Adams et al. 2010). Moreover, despite biases and limitations, acoustic identification possesses several obvious advantages. First, acoustic identification could be more economical than approaches such as morphological or genetic determination which need direct species capture and greater labor costs (Preatoni et al. 2005; Parsons and Szewczak 2009). Second, acquisition of acoustic data can be achieved without a break and in a space where direct bat capture is not easy or impossible. For example, acoustic monitoring of bats at the rotor height of modern utility-scale wind turbines is more advantageous than mist netting (Kunz et al. 2007). Third, acoustic identification is an invaluable approach in identifying cryptic species in the field (Jones et al. 2000). As a result, acoustic identification has been continually applied to field surveys of bat activities for many years (Fenton 1970; Fenton and Bell 1981; O'Farrell et al. 1999; Parsons and

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Jones 2000; Rydell et al. 2002; Preatoni et al. 2005; Papadatou et al. 2008; Adams et al. 2010).

However, acoustic identification of bats may lose its power because of the intraspecific variation in echolocation calls and the absence of a “vocal signature” among species (Barclay 1999). Particularly, bats may modify echolocation pulses according to their age, sex, body size, social group, geographic location, and the specific task in hand (e.g., flying in open versus cluttered space) (Jones and Siemers 2010). It is well known that bats usually produce shorter calls with higher frequency in situations with an increasing level of clutter. In contrast, longer and lower frequency calls are generally related to situations with a reduced level of clutter. However, this phenomenon is mainly based on data from foraging habitats (but see Schaub and Schnitzler 2007b).

Commuting, the process of animals' traveling between the roosts and foraging areas, is one of the most common daily activities of bats. During commuting, echolocation calls are mainly used to avoid unexpected obstacles and to pursue routes (i.e., for spatial orientation), which often differ significantly with the tasks faced by foraging bats that have additional echolocation tasks to detect, localize, and classify potential prey (Schaub and Schnitzler 2007a). Moreover, there is evidence that bats alter their echolocation call structures according to their behavioral contexts (commuting versus foraging) (Schumm et al. 1991; Britton et al. 1997; Berger-Tal et al. 2008). If this is ubiquitous, behavioral contexts in which the bats were recorded should be taken into account for the acoustic identification of bats in the field.

Building a comprehensive library of high-quality calls from known species is an essential task for acoustic identification of bats (Parsons and Szewczak 2009). Specifically, poor knowledge of the intraspecific variability in echolocation calls is recognized as a critical limiting factor in using this approach (Adams et al. 2010). In this study, we recorded and compared echolocation calls from an ecologically poorly known bat species, big-footed myotis (*Myotis macrodactylus*), while they were commuting in three types of habitats differing significantly in the amount of clutter, as well as while they were searching for prey above the water surface of a river. Most studies on *M. macrodactylus* were done in the laboratory, covering topics from structures of chromosome, caryon and mitochondrial DNA to physiological processes, and to morphological traits (Obara et al. 1976; Harada and Yosida 1978; Findley 1972; Jiang et al. 2008; Liu et al. 2009a; Hwang and Lee 2007; Lee and Mori 2004; Lee 2003; Kruskop and Tsytsulina 2001; Son et al. 1995; Sakai et al. 2003; Kawai et al. 2003). In spite of the descriptions of their echolocation calls (Fukui et al. 2004; Luo et al. 2009), to our knowledge, none were from free-flying bats in the field.

The objectives of this study were: (1) to test the general hypothesis that is whether commuting *M. macrodactylus* alter their echolocation calls in the same way as foraging bats usually do in relation to the changing level of clutter. That is, bats would produce shorter calls with higher frequencies in situations with increasing level of clutter; (2) to test whether the behavioral context (commuting versus foraging) in which the bats were recorded should be taken into account for acoustic identification of bats in the field. In supporting this hypothesis, there should be considerable variation in echolocation call characteristics between commuting and foraging contexts when bats are facing a similar level of background clutter.

## Materials and methods

### Study area

The study was conducted from July 2 to July 22, 2009, around the Dalazi Cave (125°50'9.8" E, 41°3'55.8" N) in Ji'an, Jilin Province, China, before the independent flight of the infants (Liu et al. 2009b). In front of the cave, there was a secondary forest with mostly *Quercus mongolica* and *Acanthopanax senticosus*, beside which was a large cornfield (length, > 250 m; width, c. 50 m; the corn crops were about to mature and averaged 2 m in height). Next to this cornfield, there was a river (width, 4–30 m) with middle-aged trees on the bank(s) in some sections. The cave was shared mainly by *M. macrodactylus* and *Rhinolophus ferrumequinum*, which were also the most common bat species in our study area, emitting distinctive echolocation calls (*M. macrodactylus*, FM calls; *R. ferrumequinum*, FM-CF-FM calls).

Based on observation and acoustic monitoring in the study area over the past 3 years, we found that *M. macrodactylus* typically emerged from their day roost about 15 min after sunset in July. After emergence, some individuals traveled c. 50 m in the forest, flew across a 50-m-wide cornfield to reach the river, and then they commuted down (or up) along the river. To address our first objective, we recorded the orientation calls (commuting calls) of *M. macrodactylus* at three sites that differed significantly in the amount of background clutter. They were site CF (commuting site in the forest, referred to as “highly cluttered space”) situated in front of the cave entrance and c. 40 m from it; site CC (commuting site in the cropland, referred to as “uncluttered space”), c. 25 m from both the forest edge and the river bank; and site CR (commuting site in the river, referred to as “background-cluttered space”), c. 250 m from the day roost. Similar to Britton et al. (1997), we defined commuting habitats as where *M. macrodactylus* flew in a single direction towards

foraging sites soon after emergence. To select the above commuting habitats, not only do they have to be representative, but more importantly, the feeding opportunity of *M. macrodactylus* in these sites must be minimized or even excluded. Comparatively, there could be more controversy when defining a site from riparian areas as a commuting site for these trawling bats. However, we ensured site CR to be a qualified commuting habitat, because all bats in this site flew in the same single direction in the dusk and disappeared quickly without patrolling behaviors observed throughout our study. Moreover, no feeding activity was found at this site or at the other two commuting sites basing on the subsequent analysis of echolocation calls (i.e., no feeding buzz was observed). Site CR was not used to feed by *M. macrodactylus* in our research period probably due to the fact that there was no appropriate water surface at this site. The water surface there was heavily fragmented, and a number of stones stood out irregularly. Complete and smooth water bodies were demonstrated to be crucial to the foraging activities of trawling bats (Boonman et al. 1998). At site CR, the river was c. 10 m wide. Trees on both sides were planted at an interval of 5 m, and they formed a closed canopy above the river. The resulting vegetation tunnel was c. 8 m wide and c. 6 m high, extending c. 50 m along the river.

To address our second objective, echolocation calls from the typical foraging habitats (site FR) of *M. macrodactylus* were recorded. Site FR (foraging site in the river, referred to as “background-cluttered space”) was c. 500 m from the roost. At site FR, there was a complete body of water which covered an area of more than 30 m<sup>2</sup>, with trees along one side of the banks. Site FR was defined as foraging habitat because individuals would persist around this area, flying low over the water surface, occasionally dipping or climbing to catch insects. More importantly, feeding buzzes were recorded each night.

#### Call recording and analyzing

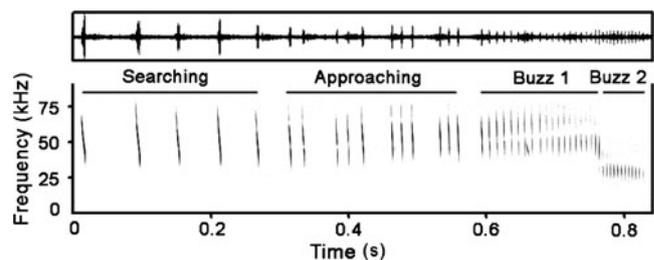
Echolocation calls were recorded using Avisoft UltraSoundGate 116 with a condenser ultrasound microphone (Avisoft Bioacoustics CM16/CPMA) connected to a laptop computer. This set of recording equipment performs well in the high-humidity environments of our study sites (<http://www.avisoft.com/usg/microphones.htm>). For commuting calls, recordings were made at the time of peak emergence of *M. macrodactylus* that traveled from the roost to their foraging areas (19:20–20:20). By contrast, foraging calls were recorded later than the bats' peak emergence time (20:00–21:30). The sampling frequency was 441 kHz, with 16-bit resolution. All sites were sampled for multiple nights (site CF, 3; site CR, 3; site CC, 3; site FR, 5), and the sampling order of these sites was randomly determined

before the onset of call recording. At each site, the microphone was held at a height of c. 1.6 m by a person standing on the ground. Throughout our study, no recording was made in nights with bad weather conditions (rainy or windy nights). To avoid the particularity of echolocation calls just after emergence (Berger-Tal et al. 2008), we chose the recording site in the forest (site CF) as far as possible, which was at least 40 m from the roost. To determine whether sexual dimorphism in echolocation calls of *M. macrodactylus* exists, which may bring some noise to our results (Kazial and Masters 2004), we captured 15 individuals (9 ♀, 6 ♂) with a mist net at the roost during the emergence time of bats a few days before our recording. Ten active individuals (5 ♀, 5 ♂) were selected for call recording in an empty room (5×4×4 m). After recording, all bats were released at the capture site before sunrise the next morning.

Echolocation calls from the search phase (Fig. 1) and commuting were automatically analyzed using Avisoft-SASLab Pro software (Avisoft Bioacoustics, Berlin, Germany). In total, calls from 154 *M. macrodactylus* individuals including 63 from site CF, 25 from site CC, 21 from site CR, and 45 from site FR were analyzed. A Fast Fourier Transformation (512 points, Hamming window) delivered spectra, which were displayed as color sonogram with temporal resolution overlap of 87.5%. We selected high-quality search phase calls for analysis according to the following criteria: (1) recordings had a high signal-to-noise ratio, and all weak signals with peak amplitudes lower than -25 dB relative to the maximal recording level were excluded; (2) selected calls were not saturated or did not overlap with echoes; (3) only one individual was present in the recording area. Only the first dominant harmonic of each call was analyzed, and in total, six parameters have been determined, including peak frequency, start frequency, end frequency, pulse duration, pulse interval, and bandwidth.

#### Statistical analysis

All statistical analyses were performed using SPSS 14.0 statistical software (SPSS Inc., Chicago, IL, USA). We first



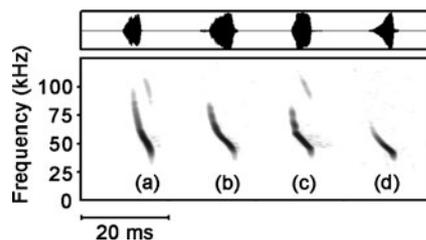
**Fig. 1** Spectrogram and oscillogram of echolocation calls of *M. macrodactylus* while searching for, approaching, and capturing prey over the water surface

performed Kruskal–Wallis test to determine the normality of the call parameters. Since all the six call parameters were normally distributed in most cases, one-way ANOVA and two-tailed *T* test were used to compare calls from different commuting habitats and for sexual dimorphism or behavioral contexts, respectively. For others, Mann–Whitney Test was used to compare calls from different behavioral contexts. To examine the percentage of calls that can be correctly assigned to their respective habitat type during commuting or to their behavioral context (commuting versus foraging), discriminant function analysis (DFA) associated with cross-validation was used. Cross-validation is a procedure that constructs a discriminant function based on all calls but one and then uses this function to classify the excluded call. We used end frequency, pulse interval, and bandwidth for DFA, because these variables were all used to distinguish bat species (Berger-Tal et al. 2008), and had low correlation with each other in our study (all within-groups correlation <0.35). To avoid pseudoreplication, only one call per bat was randomly selected for the above statistical analyses. The level of significance was set at  $P < 0.05$ .

## Results

*M. macrodactylus* produced downward frequency-modulated signals containing 1–2 harmonics, but the first harmonic was always more prominent than the second. Echolocation calls of *M. macrodactylus* were typical for this genus in structure, with an initial steep frequency-modulated sweep followed by a shallower sweep, ending with a second, rapid FM sweep (Fig. 2). No sexual dimorphism in echolocation calls of *M. macrodactylus* existed (two-tailed *T* test, start frequency:  $t = 1.08$ ,  $P = 0.31$ ; end frequency:  $t = 0.75$ ,  $P = 0.48$ ; pulse interval:  $t = -0.05$ ,  $P = 0.96$ ; pulse duration:  $t = 0.40$ ,  $P = 0.70$ ; peak frequency:  $t = -1.57$ ,  $P = 0.15$ ; bandwidth:  $t = 0.64$ ,  $P = 0.54$ ).

There were significant differences in all the six signal parameters among the three commuting habitats (one-way ANOVA, all  $P \leq 0.001$ ; Table 1). During commuting, with



**Fig. 2** Spectrogram and oscillogram of representative echolocation calls of *M. macrodactylus* while commuting and searching for prey. *a* Highly cluttered space, commuting; *b* background-cluttered space, commuting; *c* uncluttered space, commuting; *d* background-cluttered space, foraging

increasing level of clutter, *M. macrodactylus* generally produced echolocation calls with higher start, end, and peak frequencies; wider bandwidth; and shorter pulse duration. Moreover, during foraging, bats produced longer search phase calls with significant lower frequencies and narrower bandwidth than during commuting (two-tailed *T* test, start frequency:  $t = 12.70$ ,  $P < 0.001$ ; end frequency:  $t = 7.60$ ,  $P < 0.001$ ; bandwidth:  $t = 11.77$ ,  $P < 0.001$ ; Mann–Whitney test, pulse interval:  $Z = -4.98$ ;  $P < 0.001$ ; pulse duration:  $Z = -3.23$ ,  $P = 0.001$ ; peak frequency:  $Z = -6.99$ ,  $P < 0.001$ ; Table 1).

Discriminant function analysis showed that 79.8% of the calls from commuting habitats were correctly classified, with the highest correct classification occurred in the highly cluttered space (Table 2). Moreover, 87% of the calls were correctly classified to the commuting and foraging contexts despite the high variations of call parameters caused by different levels of background clutter of the commuting habitats (Table 2).

## Discussion

The results of this study confirmed our first hypothesis that during commuting, *M. macrodactylus* altered their echolocation call structure in the same way as foraging bats do in relation to the changing level of clutter (Kalko and Schnitzler 1993; Schnitzler and Kalko 2001; Siemers et al. 2001a; Wund 2006; Bartonicka and Rehak 2005). With an increasing level of clutter, *M. macrodactylus* generally produced echolocation calls with higher start, end, and peak frequencies; wider bandwidth; and shorter pulse duration, though the pulse interval was shorter in uncluttered space than that in background-cluttered space. While commuting in the uncluttered space, *M. macrodactylus* produced echolocation calls with the lowest peak frequency, narrowest bandwidth, and longest pulse duration, which were similar to larger-sized bat species that commute more frequently in open space (Limpens and Kapteyn 1991; Verboom and Huitema 1997). These call traits enabled bats to detect targets at a greater distance in open space (Neuweiler 1989; Kalko and Schnitzler 1993). Our data also confirmed the prediction proposed by Limpens and Kapteyn (1991) that bats may use calls with lower frequency and narrower bandwidth while commuting in open habitats than in edge habitats. In contrast, the echolocation calls were characterized by the highest peak frequency, the widest bandwidth, and the shortest pulse duration, when *M. macrodactylus* were commuting in the highly cluttered space. Higher frequency calls are more effective in detecting small targets in shorter range (Jones and Holderied 2007). An increase in bandwidth is believed to be advantageous for bats within cluttered space because of the illumination of the sonar scene with a wider range of wavelengths; thus, they may develop a detailed

**Table 1** Parameters of echolocation calls emitted by *M. macrodactylus* while commuting and searching for prey

Sites	Start frequency (kHz)	End frequency (kHz)	Peak frequency (kHz)	Bandwidth (kHz)	Duration (ms)	Interval (ms)
Site CF	81.29±6.01	43.56±1.50	52.92±2.53	37.73±5.47	5.26±0.56	55.53±7.50
Site CR	77.53±7.64	42.80±1.92	50.17±4.21	34.73±7.20	6.27±0.93	84.82±16.57
Site CC	71.54±5.97	42.04±1.96	49.36±3.78	29.50±5.33	7.11±1.22	68.47±15.78
Site FR	62.64±6.27	40.83±1.64	45.84±4.15	21.79±6.35	6.43±1.18	86.35±32.70

characterization of the background contours (Siemers and Schnitzler 2004). Moreover, reduced pulse duration is essential to avoid temporal overlap of calls and returning echoes (Holderied et al. 2006; Jones and Holderied 2007). As a result, echolocation calls could provide bats with precise spatial information which was essential for safe orientation in highly cluttered space.

During commuting, *M. macrodactylus* altered call parameters in response to habitat types; such plasticity could be important for bats to reduce the effects of isolation of fragments and then for a successful commuting between roosts and foraging areas. Some bat species relied strongly on edge habitat structures during commuting, and they would choose to make a detour to avoid crossing an open space (Krull et al. 1991; Limpens and Kapteyn 1991; Murray and Kurta 2004). Both acoustic orientation and predation risk were suggested to account for this phenomenon (Limpens and Kapteyn 1991; Ekman and Jong 1996; Verboom and Huitema 1997; Verboom et al. 1999; Schaub and Schnitzler 2007b). Limpens and Kapteyn (1991) hypothesized that the shorter detecting range of higher frequency calls of small bats was the primary reason for them to depend on edge habitats more strongly than larger bats. The same viewpoint was held by Verboom and Huitema (1997), who found that *Pipistrellus pipistrellus* was confined to edge habitat while *Eptesicus serotinus* was not. Alternatively, Ekman and Jong (1996) argued that predation risk should be largely responsible for the avoidance by smaller bats of open areas, since smaller species survive attacks from predators significantly less often (Speakman 1991).

Nevertheless, some small species necessarily commute across open spaces since bats cannot always keep themselves in sheltered edge habitats (Verboom and Huitema 1997; Nicholls and Racey 2006; Russell et al. 2009). For example, *M. macrodactylus* commutes regularly across a 50-m-wide cornfield in our study area. We argue that plasticity in echolocation calls may be valuable to these smaller species in commuting across open spaces as it allows bats to produce calls that help to detect objects at a greater distance, which may profit them to use further landscape elements for orientation and (or) may be a prerequisite for bats to achieve higher flight speeds to reduce predation risk (Britton et al. 1997).

Our second hypothesis was supported by the fact that bats emitted significantly lower frequency calls with narrower bandwidth while searching for prey than commuting. This was in accordance with the results from another trawling bat *Myotis dusycnerne* which was observed to produce calls with lower maximum and minimum frequencies as well as narrower bandwidth while foraging above an open lake than commuting at a canal (Britton et al. 1997). However, for other bat species, there was no significant difference of echolocation call frequencies as well as bandwidth between commuting and foraging (e.g., *Pipistrellus kuhli*, Berger-Tal et al. 2008; *Vespertilio murinus*, Schaub and Schnitzler 2007a). Thus, we speculate that lower frequency calls with narrower bandwidth were probably advantageous for trawling bat species while searching for prey, but not for the other two species above. This kind of echolocation strategy was perhaps an active adaptation to the high moisty aquatic

**Table 2** Classification results of the DFA among commuting habitats and between behavioral contexts

Three commuting habitats <sup>a</sup>	Correct (%)	Site CF	Site CR	Site CC
Site CF	87.3	55	2	6
Site CR	72.0	5	18	2
Site CC	66.7	4	3	14
Commuting and foraging contexts <sup>b</sup>	Correct (%)	Commuting	Foraging	
Commuting	85.3	93	16	
Foraging	91.1	4	41	

The italicized number indicates the correctly classified calls for each case

<sup>a</sup> 79.8% of cases correctly classified

<sup>b</sup> 87.0% of cases correctly classified

environment where higher frequency calls would suffer stronger atmospheric attenuation, which may shorten the detection range of these trawling bats. It may also allow the trawling bats to detect prey at longer distances if the increased humidity from foraging sites does not affect the echolocation range greatly. Meanwhile, the reduced frequencies and bandwidth may not cause problems for bats while searching for prey above the water surface, as it could be easier for bats to perceive prey on the water surface than on vegetation or on the ground (Siemers et al. 2001b).

In our study, 79.8% of the calls were correctly classified to the commuting habitats. This indicated that clutter conditions alone had strong effects on the echolocation behavior of bats during commuting. It has been proposed that it is important to develop clutter-specific call libraries to improve species classification accuracy using echolocation calls (Broders et al. 2004). Our results support this idea because call parameters from different commuting habitats differed significantly. However, it is still not enough to build unambiguous patterns that can help identify the commuting habitats by the calls' characteristics. It seems that the misclassification of calls would increase with decreasing level of background clutter (i.e., uncluttered space), which in turn challenges the idea above.

Moreover, 87% of the calls were correctly classified to the commuting and foraging contexts despite the high variations of call parameters caused by different levels of background clutter of the commuting habitats. This demonstrated that behavioral contexts indeed impose much pressure on the echolocation behavior of bats. This finding has implications for those who would identify species by their calls.

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