Vocalization development of greater horseshoe bat, *Rhinolophus ferrumequinum* (Rhinolophidae, Chiroptera)

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**Abstract.** From June to August in 2004 and 2005, we conducted the studies on spontaneous vocalization development of greater horseshoe bat, *Rhinolophus ferrumequinum*, in Zhi’an Village (Jilin province, Northeast China). In contrast to adult bats, infant bats of the greater horseshoe bat emitted calls characterized by multiharmonics and variable harmonic patterns. With the physical growth of infants, the dominant frequency, pulse duration and frequency of each harmonic of spontaneous calls increased, the number of harmonics decreased from 5–8 to 1–2 and dominant harmonic switched from first to the second with peak frequency increasing. Vocalizations of infant bats of the greater horseshoe bat could be categorized to those serving as precursors of echolocation sounds and those serving as isolation calls used to attract their mothers. According to observation on mother-infant reunion, the female adult bats only suckled their own babies, but not other pups in the same colony. And the mother recognized their own infants through both odor and vocal cues indicating that the isolation calls emitted by infant bats played an important role in mother-infant communication.

**Key words:** greater horseshoe bat, vocalization development, communication, isolation calls

**Introduction**

Insectivorous bats depend heavily on echolocation for orientation and predation in dark environments. Production of vocalizations is very important to the survival of an echolocating bat, and vocalizations during early postnatal development may lay the foundation for biosonar behaviour in the adult (Zhang et al. 2005). Simultaneously, most bats recognize and suckle their own infants (de Fanis & Jones 1995, Bohn et al. 2007), the vocalizations of infant bats are also used as important cues for mother-infant communication and recognition (Schnitzler & Henson 1980, Thomson et al. 1985, Bohn et al. 2007). Several authors have conducted studies on the ontogeny of vocalizations in microchiropteran bats (Gould 1971, Konstantinov 1973, Matsumura 1979, Habersetzer & Marimuthu 1986, Rubsammen 1987, Moss 1988, de Fanis & Jones 1995). They considered that the temporal and spectral characteristics of vocalizations of an infant bat shortly after birth varied with its physical and physiological development. There was a general trend for the development of vocalizations, which was the vocalizations will rise in frequency, decrease in duration and become increasingly stereotyped as they grow.

However, it is difficult to identify if the infant bats emit two kinds of calls serving as precursors to echolocation calls and communication isolation calls, respectively, or they just emit one kind of calls playing both two roles (Zhang et al. 2005). Bradbury (1977) and Gould (1977) suggested that early communication calls produced by some species of

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bats (e.g., some vespertilionids and phyllostomids) may be precursors to echolocation calls. However, some other authors considered that vocalizations of some species of infant bats (Moss 1988 [Eptesicus fuscus], Jones et al. 1991 [Pipistrellus pipistrellus] and Zhang et al. 2005 [Tylonycteris pachypus and T. robustula]) could be categorized as those serving as precursors of echolocation calls and those serving as communication isolation calls.

The greater horseshoe bat, *Rhinolophus ferrumequinum* (Rhinolophidae, Chiroptera) is an endangered species in Europe (Rossiter et al. 2000) and has been listed as an LR/nt species into IUCN Red List of Threatened Species (Bailie et al. 2004). Some studies have been conducted on isolation calls of the infant greater horseshoe bat. Konstantinov (1973) divided the calls of infants of the greater horseshoe bat into 3 types: 1) audible attractive calls emitted through the mouth; 2) pure-tone nasal signals emitted through the nostrils; 3) mixed signals with the first part through the mouth and the second part through the nostrils, or vice versa. Matsushita (1979) analyzed infant bats of the greater horseshoe bat by separating mother-infant pairs of various ages and indicated that infants could emit oral and nasal calls, and vocal development was low to higher frequencies and noisy to pure tones. Jones & Ransome (1993) described the various echolocation calls of adult greater horseshoe bats at different age. However, most of the studies on vocalization of infant bats of the greater horseshoe bat focused on the attractive isolation calls serving for mother-infant communication and recognition. There was no analysis on vocalizations serving as precursors of echolocation calls. Matsushita (1981) suggested early communication calls may be precursors to echolocation calls. However, when we recorded calls of infant bats in the field, we found there was significant difference between their communication isolation calls recorded with mother together and spontaneous calls recorded with mother in absence. So in this paper, we focused on documenting the spontaneous vocalization development of infant bats of the greater horseshoe bat recorded in absence of mother bats and testing if the spontaneous vocalizations of infants served as precursors of echolocation calls. In addition, the attractive isolation calls and communication behaviours of infant bats when they searched for their mother were addressed on the base of our direct observation.

**Methods**

**Bat collection and measurement**

We undertook fieldwork at Zhi’an village of Ji’an city in Jilin Province, China from June to August in 2004 and 2005. There was a large maternity colony roosting in a cave (called Dalazi Cave by local people, 125°50'9.8”E, 41°3'55.8”N, altitude 325 m, 80% humidity) every summer. The cave was surrounded by dense vegetation including arbors and shrubs (such as *Quercus mongolica* and *Acanthopanax senticosus*) and corn farmlands.

Based on our prior observation, we knew the females of greater horseshoe bats gave birth in early June in the cave. We daily checked the cave about ten days before the females’ parturition for collecting the mothers and her new pups. Ten adult bats (male: female = 3:7) and five mother-infant pairs (each mother with single pup, male: female of infants = 2:3) were captured by mist-net at the entrance of the cave after sunset in the two years. All of the bats were taken to our temporary study room (about 200m away from the cave). And the 10 adults were captured in a big cage, but each mother-infant pair was housed in a separated cage after being marked with innoxious luminous paint on fur. The bats were presented with fresh mealworms and fresh water daily.
The forearm lengths were measured (the infants were measured by hands with gloves in order to keep their odor no change) with vernier lalipers (accurate to 0.1mm). Although we did not know the exact birth date of these captive infant bats because they were born before our collection, alternatively, we estimated the age by measuring their forearm lengths (FAL), which provided a reliable age-related indicator during the lactation of several bat species (Matsunura 1979, Kunz & Anthony 1982, Rubsam 1987, Vater et al. 2003). Based on our weekly observation and measurement in the dense cluster of infants in the cave, the infant bats with the umbilical cord were assumed about one day old; the infant bats at age of one week had FAL from 25 mm to 35 mm; the infant bats at age of two weeks had FAL from 36 mm to 45 mm; the bats at age of three weeks had FAL from 46 mm to 55 mm; and bats at age of four weeks had FAL >55 mm. The FAL developments of those housed infants were consistent with the infants in the cave according our measurement. So, the age estimate in our study was acceptable and we regarded the captive bats had the same growing rate as the pups in cave.

Recording and analysis of calls

Recordings were made using a Pettersson D980 Bat Detector (Pettersson Electronik AB, Uppsala, Sweden; frequency response ± 3.5 dB between 8 and 160 kHz) with time-expanded 10 times to a laptop running Batsoundpro software (Pettersson). We put pre-aviated infant in a small cage individually (started to record after it calmed down) and the volant bats in a tent made by nylon mesh (9m×4m×4m) for acoustic recording. The communication calls were recorded from the infant and the mother respectively just after their separation and the reunion calls were picked up when they reunited. In addition, we also observed the communication behaviors between mothers and infants.

We recorded the calls from the 5 housed pups and additional 31 pups (natural growing bats) from the cave, including 9 individuals at age of one week (male: female = 2:4), 8 individuals at age of two weeks (male: female = 4:4), 9 individuals at age of three weeks (male: female = 3:6) and 8 individuals at age of four weeks (male: female = 5:3). After recording, the infants were released back to the cave where we captured them.

The calls were analyzed with BatSound Pro Version 3.10 (Pettersson) using a sampling frequency of 44.1 kHz, with 16 bit precision. The following four parameters were selected to

![Fig. 1. Typical echolocation calls of an adult greater horseshoe bat, Rhinolophus ferrumequinum. A: Sonogram, showing calls composed of long constant-frequency (CF) component with brief frequency-modulated (FM) sweeps at the beginning and the end (FM-CF-FM); B: Power spectrogram, showing dominant frequency of the call.](image)
present the features of calls: dominant frequency (DF), the frequency of each harmonic (HF), pulse duration (D) and number of harmonics. DF and HF were measured from power spectra, D from oscillograms, and the syllable type and number of harmonics from sonograms (FFT size = 1024 points). At least 30 phases of calls from each individual were analyzed. The results were analyzed by using commercial statistics software (SPSS 13.0).

Results

We measured the morphological parameters and recorded echolocation calls from 10 adult bats of the greater horseshoe bat (Table 1). The stereotyped echolocation call was shown in Fig. 1. Comparing the calls emitted by infants to those of adults, we derived following results.

Vocalization development with age in infant bats

In contrast to adult bats, infant bats of the greater horseshoe bat emitted calls characterized by multi-harmonics and variable harmonic patterns. The variation in syllables of one representative individual was given in Fig. 2 (The terminology of the syllable types were according to Kanwal et al. (1994) and Ma et al. (2006)). Infant bats at age of one week were rather inactive and quiet for most of the time both in the cave and in captivity. They just emitted calls as shown in Fig. 2A sometime (occupying 30% of the total calls). In the most time when we recorded, they emitted audible irregular noise burst (occupying 70% of the total calls). Infant bats at age of two weeks were active while they were hanging on the body of their mothers and were characterized by vocal activity. The bats at age of three weeks could fly a short distance and produced ultrasonic calls more like adults. The bats at age of four weeks could fly freely and emitted the same calls as adults actively.

As the infant bats growing up, the spontaneous calls with short duration and low frequency became progressively more like the constant frequency (CF) echolocation calls emitted by the adult bats. The dominant frequency, pulse duration and frequency of each harmonic of spontaneous calls all increased with age growing, as shown in Fig. 3, Fig. 4

Table 1. Variation of bodysize and call parameters of bat in four age groups.

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Forearm length (mm)</th>
<th>Dominant frequency (kHz)</th>
<th>Duration (ms)</th>
<th>Number of harmonics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 week old bats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=11, male: female = 4:7)</td>
<td>29.20±3.70</td>
<td>16.63±4.55</td>
<td>≤3 days old</td>
<td>5-8</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>3.28±0.49</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&gt;3 days old</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>13.56±5.29</td>
<td></td>
</tr>
<tr>
<td>2 weeks old bats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=13, male: female=6:7)</td>
<td>39.42±3.58</td>
<td>35.25±5.86</td>
<td></td>
<td>4-7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16.26±5.00</td>
<td></td>
</tr>
<tr>
<td>3 weeks old bats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=14, male: female=5:9)</td>
<td>52.28±2.94</td>
<td>66.57±4.87</td>
<td></td>
<td>2-6</td>
</tr>
<tr>
<td>4 weeks old bats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=13, male: female=7:6)</td>
<td>58.69±3.47</td>
<td>69.08±1.45</td>
<td></td>
<td>1-3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28.87±6.74</td>
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Explanations: n, number of infant bats which were recorded. In each group, five individuals were cultured in study site and others were captured from the cave every week. We analized at least 30 syllables from each individual.
Fig. 2. Typical vocal repertoire of an infant greater horseshoe bat, *Rhinolophus ferrumequinum*, at different age with forearm length (FAL) of 25-62mm. A: Syllables from the infant at age of 1 week. (a) and (b), very short monosyllables with multi-harmonics emitted by the infants with FAL of 25mm and 28mm at age of less than three days. (c) and (d), Quasi-constant-frequency (QCF) emitted by the infant with FAL of 31 mm and 35mm at age from 4 days to a week. B: Syllables emitted by the infant at age of 2 weeks. (a) and (b), Trapezoidal QCF (tQCF) syllables emitted by the infant with FAL of 37mm and 39mm. (c) and (d), echolocation call-like syllables emitted by the infant with FAL of 41mm and 45mm. C: Syllables emitted by the infant at age of 3 weeks. (a), (b) and (c), biosonar pulses with multiharmonics emitted by the infant with FAL of 47mm, 49mm and 55mm, respectively. D: Syllables emitted by the infant at age of 4 weeks. (a) and (b), long FM-CF-FM signals emitted by infant with FAL of 57mm and 62mm, which were similar to the signals emitted by adult bats.

Fig. 3. Changes in the dominant frequency (DF) of spontaneous calls emitted by infant bats of the greater horseshoe bat, *Rhinolophus ferrumequinum*, with forearm length (FAL) during growth (Pearson Correlation, 2-tailed test, n=36, \( r=0.805 \), \( P<0.01 \)). Means ± standard deviation (SD) of upper and lower frequencies are illustrated. Increasing of frequency and duration mainly occurred in the post-natal three weeks. In the fourth week when the pups could fly, they emitted calls that were similar to
the echolocation calls of adults, with little changes in frequency and duration. Simultaneously, as the bats growing, the most relative intense harmonic transform from the first harmonic to the second one, and the number of harmonics also decreased from 5–8 to 1–3 (Fig. 6).

**Communication behaviors and calls between mothers and infants**

We also recorded the attractive isolation calls emitted when infant bats (the five individuals from the captured mother-infant pairs) seeking for their mother by separating mother-infant pair. The infant bats produced consecutive oral sound (isolation calls) shortly after they left

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**Fig. 4.** Changes in the duration of spontaneous calls emitted by infant bats of the greater horseshoe bat, *Rhinolophus ferrumequinum*, with forearm length (FAL) during growth (Pearson Correlation, 2-tailed test, n=36, r=0.720, P<0.01). Means ± standard deviation (SD) of upper and lower duration are illustrated.

**Fig. 5.** Changes of the frequency of each harmonic of calls emitted by infant bats (the 5 infant bats housed in temporary study room) of the greater horseshoe bat, *Rhinolophus ferrumequinum*, with forearm length (FAL) (Pearson Correlation, 2-tailed test, n=5, H1: r=0.963, P<0.01; H2: r=0.920, P<0.01; H3: r=0.853, P<0.01; H4: r=-0.837, P<0.01; H5: r=0.783, P<0.01; H6: r=0.108, P=0.817; H7: r=0.998, P=0.002). H1-H8 represent the first to the eighth harmonic of calls, respectively.
from the female bat (Fig. 7), which was quite different from that emitted by the infant bats recorded with mother in absence. When the infant bats emitted isolation calls, they usually opened mouths and emitted audible “quack-quack” sounds with the movement of the head and slow crawling, seemed to look for their mothers and companions. While they produced pure ultrasound, they didn’t open mouths or move heads. Infant bats only emitted isolation calls with audible sound in the former two post-natal weeks. Furthermore, the percentage of the calls with oral component decreased as the infants grew up (Fig. 8). Infant bats at age of more than two weeks only produced pure ultrasound (Fig. 8). They didn’t produce isolation calls with oral sound even if the mother bats flew to them.

According to our observation, all the infant bats produced isolation calls together to attract the female bat when the female bat flew to them. The female bat would evade when the infant bats that were not her baby approached her. But when her own baby was close to her, the female bat held it with her arms and began to suckle, and the baby ceased calling.

Discussion

**Vocalization development of spontaneous calls with age**

Vocalizations during postnatal growth play an important role in the development of biosonar behaviour in adult echolocating bats. In this study we focused on the development of
spontaneous vocalizations of the greater horseshoe bats under the mother bats was in absence. It is extension of study on the vocalization of echolocating adult animal depending on experience acquired as a juvenile.

In our result, the syllables emitted by infant bats <3 days (Fig. 1A (a) and (b)) were very short and quite different from the isolation calls (Fig. 7). Matsuruma (1979) found that in infant greater horseshoe bats at age of one week, the unceasing attractive calls emitted by the separated infants through the opened mouth were partially audible to the human ear and sounded like an insect’s chirp. When we recorded short syllables emitted by independent infants at age of one week, infants didn’t open mouth, but closed their mouths with heads and ears shaking. Zhang et al. (2005) considered the shortest calls produced by pups of Tylonycteris pachypus or T. robustula were precursors of echolocation calls and the longest calls were i-calls. So we considered the very short calls emitted by the infants at age of one week were the precursors of echolocation calls, but not isolation calls. However, the patterns and parameters of calls emitted by infant bats at age of more than three days, especially at age of two weeks in our result were very similar to the attractive calls reported.
by Matsumura (1979, 1981). Matsumura (1981) reported the attractive calls of greater horseshoe bats at age of three week including oral syllables or oral and ultra mixed-type syllables. In our result, vocalizations of infant bats at age of more than three weeks had similar spectro-temporal features to echolocation pulses of adult bats.

Therefore, our findings consist partially with that vocalizations of infant bats could be categorized to those serving as precursors of echolocation sounds and those serving as isolation calls used to attract their mothers (Brown et al. 1983, Moss 1988 & Jones et al. 1991), though there might be overlap in these two kinds of sounds. Furthermore, our result illustrate that greater horseshoe bats emit signals which resemble adult sonar calls from the day of birth. However, further investigation should be conducted to identify discrete differences between i-calls and certain shorter calls that resembled adult echolocation calls of infant greater horseshoe bats at age of two weeks.

Matsumura (1979) concluded that in infant bats of R. ferrumequinum, Nippon the character of the pure nasal calls changes with increasing age: the fundamental frequency of the harmonics of a syllable increases from 12 kHz to 36 kHz, the second harmonic reached 68–70 kHz at age of four weeks. Some studies on the ontogeny of vocalization in other species of bats have also shown that echolocation calls develop by showing an increase in frequency during post-natal growth (Rubsam en 1987, Moss 1988, Jones et al. 1991, Moss et al. 1997). In our result, not only frequency of fundamental and second harmonic but also DF and frequency of each harmonics of spontaneous calls emitted by infant greater horseshoe bats with mother in absence increased with age. So we suggested that the frequency of spontaneous calls emitted by infant greater horseshoe bats had the similar development trend to their isolation calls.

Some species of bats were studied and a common conclusion was drawn that duration of calls in infant bats decreased with the age or FAL growing, including bats of Hipposideros speoris (Habersetzer & Marimuthu 1986), Rhinolophus rouxi (Rubsam en 1987), Eptesicus fuscus (Moss 1988) and Pipistrellus pipistrellus (Jones et al. 1991). However, duration of calls in infant bats of R. ferrumequinum didn’t shorten but increased with age in our results. It is a little unexpected for us. But Vater et al. (2003) found that there was a large increase in CF$_2$ duration of spontaneous vocalizations with age in infant bats of Pteronotus parrnellii. He considered that long duration of calls emitted by adult bats were relevant to the flight and prey detection, adult like acoustics of the sonar calls were achieved just before the time when young bats began to fly and forage (Vater et al. 2003). The infant bats may learn to exploit the information contained in the CF component by lengthening the CF duration of spontaneous echolocation during their development (Vater et al. 2003). In our study, we recorded calls emitted by infant bats without mothers or other induced behaviour, just like the spontaneous calls of Pteronotus parrnellii (Vater et al. 2003), thus our result may reflect the spontaneous development of infant greater horseshoe bats without being disturbed by mothers. Camclang et al. (2006) found that in juvenile big brown bats, Eptesicus fuscus, the isolation call duration increased with increasing body temperature. Young pups are unable to thermoregulate and experience a wide range of body temperatures, thus body temperature may potentially affect their ability to produce consistent vocalizations in our study. But this should be identified in the further study.

The development of vocalization of the infants is influenced by many factors, such as the production system (Suthers & Fattu 1973, de Fanis & Jones 1995),
survival habitats (Powers et al. 1991, Rubsam en 1987, Jones & Ransome 1993), acoustic and auditory controlling mechanisms of vocal apparatus (Fenton 1985, Moss 1988, Moss et al. 1997). Variation of spontaneous calls in infant bats in our study may be caused by the difference in timing between the contractions of immature cricothyroid muscles and opening of immature glottal gate. Pye (1980) indicated that different types of echolocation calls were generated by opening and closing the glottal gate at different phases of the contraction/relaxation cycle of the cricothyroid muscles that modulate the tension of the vocal folds. Kingston & Rossiter (2004) suspected that a change in cochlear dimensions initiated the switch because the functional onset and tuning of the cochlear filter are innate processes arising from postnatal maturation of the cochlea, which the vocalization system tracks by auditory feedback. Therefore, as the development of cricothyroid muscles and glottal gate, the difference in timing would be smaller and smaller, consequently, the pattern of calls in young bats would be more and more stereotyped.

Roles of behavior and calls of infant bats between mother–infant communications

Although adult bats are known to exhibit complex social organization, the mother-infant pair is undoubtedly one of the most fundamental social units. According to our observation, when infant bats at age of one week searching for their mother, they produced attractive calls with audible components. When they vocalized, they performed oriented ear and head movements, and seemed to be able to passively localize a sound source. The oriented calls could be found in other species of bats, such as Eptesicus fuscus (Moss et al. 1997) and P. parnelli (Vater et al. 2003).

Based on the observation, we suggested that the infant bats with FAL<40mm produced isolation calls with oral components to attract their mothers, and female bats recognized their babies by these calls, which supported the conclusion of Matsumura (1979). We also found that the female bat would refuse her own baby whose odor has changed (after being measured with naked hands). Therefore female bats of R. ferrumequinum may recognize their babies not only through the attractive calls but also through the odors. Furthermore, our result proved that female bats of R. ferrumequinum only suckle their own babies. They didn’t nurse babies for others within the same species colony, unlike the bats of Nycticeius humeralis which would nurse relative infants in the same colony (Scher rer & Wilkinson 1993).

Matsumura (1979) proposed that infant bats of R. ferrumequinum nippon at the age of more than four weeks were able to fly a short distance and mother-infant bonds became looser. From our observation, infant bats of R. ferrumequinum at the age of three weeks were able to fly a short distance and mother-infant bonds became looser, i.e. the infant bats observed by us developed faster. The possible cause may be that the habitats where the bats in our study lived had suitable temperature, humidity and abundant nutrition sources. However, the further possible cause should be explored in our future study.

Acknowledgments

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**LITERATURE**


