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Postnatal development of morphological features and vocalization in the pomona leaf-nosed bat *Hipposideros pomona*

Long-ru Jin · Ai-qing Lin · Ke-ping Sun · Ying Liu · Jiang Feng

Abstract Based on mark-recapture data, we studied the postnatal development of morphological features and vocalization of the pomona leaf-nosed bat (*Hipposideros pomona*). Morphological changes indicated that body mass and length of forearm followed a linear pattern of growth until 13 days of age at mean growth rates of 0.14 g/day and 1.08 mm/day, respectively, and thereafter, growth rates slowed. The length of the total epiphyseal gap of the fourth metacarpal–phalangeal joint showed a linear increase for up to 10 days, followed by a linear decrease until day 40 at a mean rate of 0.09 mm/day. Together, two equations permitted estimation of the age of *H. pomona* pups between 1 and 40 days. The logistic equation provided the best fit to the empirical curves for body mass and length of forearm. Studies of vocal development showed that the precursors of echolocation calls were not emitted until day 7 after birth. As the pups grew, the dominant frequency (DF) of isolation calls increased and number of harmonics (NH) decreased, whereas the duration remained relatively stable. The DF and BFM_2 (the bandwidth of the terminal frequency-modulated sweep from the second harmonic) of the early echolocation calls increased; however, the NH and duration decreased.

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Introduction

Most insectivorous bats are altricial, and their young experience a postnatal growth period in which they develop appropriate sensory and locomotor skills to become independent from their mothers (Baptista et al. 2000). Many researchers have studied this period with respect to changes in behavior, physiology, and ecology to investigate life history stages and to estimate maternal investment (Kunz et al. 2009). There have been numerous studies on postnatal development of bats under both natural (e.g., Gould 1971; Kunz and Robson 1995; Hoying and Kunz 1998; Chaverri and Kunz 2006; Liu et al. 2009) and captive conditions (e.g., Rajan and Marimuthu 1999; Elangovan et al. 2003; Vater et al. 2003; Zhang et al. 2005; reviewed in Kunz et al. 2009). These studies mainly focused on two aspects: morphological feature changes and vocal development.

Studies on morphological development have shown that body mass, length of forearm, and the length of the total epiphyseal gap of the fourth metacarpal–phalangeal joint as well as changes in tooth development can be used to estimate the age of young bats (Anthony 1988; Brunet-Rossinni and Wilkinson 2009). Accurate age determination is important for behavioral, physiological, and ecological studies. In the absence of age estimate, it is impossible to determine growth rates, the timing of sexual maturity, or the development of various behavioral repertoires of an animal (Elangovan et al. 2003; Frick et al. 2010). However, compared with length of forearm and the length of the total

epiphyseal gap, body mass is not a good character for estimating age because of its sensitivity to nutritional intake, energy expenditure, and water flux (Kurta and Kunz 1987; Hoying and Kunz 1998; Stern and Kunz 1998; Kunz et al. 2009), but it is ideal for interspecific comparisons of growth rates throughout the postnatal growth period among species from different regions, although comparative studies should be based on the same model (Zullinger et al. 1984).

Studies focusing on the ontogeny of vocalizations in bats have shown that, shortly after birth, the vocal repertoire of an infant bat differs from that of an adult (Brown et al. 1983; Jones et al. 1991; Moss et al. 1997; Vater et al. 2003; Zhang et al. 2005). Generally, the vocal repertoire of an infant can be divided into two types of calls: isolation calls (mainly for communication) and early echolocation calls (mainly for echolocation). Both consist of multiple harmonics (Moss et al. 1997; Vater et al. 2003). Isolation calls normally concentrate the maximum energy on the first harmonic (Gould et al. 1973; Brown and Grinnell 1980; Knörnschild et al. 2007). However, early echolocation calls always contain the maximum energy in the same harmonic as echolocation calls of adults (Vater et al. 2003). In some species, early isolation calls of young bats may be precursors to echolocation calls (e.g., *Myotis lucifugus*-Moss et al. 1997; *Carollia perspicillata*-Sterbing 2002). However, in other species, echolocation and isolation calls appear to have separate origins (*Noctilio albiventris*-Brown et al. 1983; *Pteronotus parnellii*-Vater et al. 2003). Additionally, as the infants grow, there is a general trend for isolation calls and early echolocation calls to rise in frequency, decrease in duration and number of harmonics, and become stereotyped (Jones et al. 1991; De Fanis and Jones 1995; Moss et al. 1997).

Most studies on postnatal development of bats have been conducted on members of the family Vespertilionidae, with few studies focusing on Hipposideridae, especially those species located in subtropical regions. The pomona leaf-nosed bat (*Hipposideros pomona*) is a widespread species, occurring through Bangladesh and India to Burma, Thailand, Laos, Cambodia, Vietnam, West Malaysia, and South China (Simmons 2005). To our knowledge, no published data are available on its postnatal development or on its vocal repertoire. In this study, we first investigated the postnatal development of morphological features and vocalizations in *H. pomona* based on mark-recapture data. Our aims were: (l) to measure the sizes of bat pups at birth, to derive age estimation equations based on length of forearm and the length of the total epiphyseal gap, and to compare the growth patterns of body mass and length of forearm using three non-linear equations; (2) to examine the origin of early echolocation calls and to investigate how the vocalizations of the young change with age.

Materials and methods

Study area

The study was conducted in an abandoned air-raid shelter (N 22°37′, E 103°50′; ca. 250 m long, 2 m wide, and 2 m high) in Hekou County, Yunnan Province, China. This site had two entrances and housed a mixed colony of ca. 500 pomona leaf-nosed bats and 100 ashy leaf-nosed bats (*Hipposideros cineraceus*). The surrounding area was covered by dense forest of mostly rubber tree (*Hevea brasiliensis*) and banana tree (*Musa nana*).

Morphological measuring and acoustic recording

Based on prior investigation, we knew that the females of *H. pomona* gave birth in this area from mid-April to early May. Thus, from April 5, 2009, we checked the cave daily with lights for evidence of parturition. When newborn pups were observed (using morphological features to identify neonates of *H. pomona*), we captured neonates by hand following the nightly emergence of adults. We used an odorless marker to denote the locations from which we had captured neonates. Upon capture, young bats were placed in cloth holding bags and were transferred to a temporary laboratory (5 m×4 m×3 m) ca. 60 m from the roost site. After the sex of each pup was determined, a numbered alloy band (Porzana Ltd, UK) was placed on the forearm of each bat for individual identification. Neonates with an attached umbilical cord were assumed to be 1 day old (Kunz and Robson 1995). Body mass was recorded to the nearest 0.01 g using an electronic balance (ProScale LC-50, America). Length of forearm was measured to the nearest 0.01 mm with electronic vernier calipers (TESA-CAL IP67, Switzerland). The length of the total epiphyseal gap of the fourth metacarpal–phalangeal joint was measured to the nearest 0.01 mm using the calipers while the wing of the bat was spread out over a transparent solid plastic sheet illuminated from below with a strong torch to visualize the gap (Sharifi 2004a). To standardize potential error, all measurements were taken by the same person, and each measurement was made three times, from which an average was taken. After measurements were completed, calls from handheld bats were recorded with a condenser ultrasound microphone Avisoft Bioacoustics CM16 (Berlin, German) (the microphone was held 20–40 cm from each bat) and digitized through a PC laptop running Avisoft Recorder USG software (version 4.40, Avisoft Bioacoustics, Berlin, Germany). The sample rate was 500 kHz at 16 bits resolution.

To limit disturbance and possible abandonment, measurement of morphological characters and recording of pups' calls took place while adults were out foraging and

within a time frame of 1.5 h total. After measuring and recording, pups were returned to the site of original capture before females returned. The roost shelter was visited every 3 days (Table 1), and visits were made until the majority of the pups were volant and could no longer be captured, even with the aid of mist-nets and a hand-net by the end of mid-June, 2009. Additionally, we mist-netted postpartum females at the shelter entrances and measured their body mass and length of forearm, and recorded their echolocation calls in late June 2009 for reference. All field work was conducted under license from local authorities.

Data analysis

We used the professional vocalization software Avisoft SASLab Pro (version 4.40, Berlin, Germany) to manually analyze calls. We chose 30–35 calls with high signal-to-noise ratio from each bat on each visit day. For each call, we generated a spectrogram, a power spectrum, and an oscillogram. Spectrograms and power spectra were obtained by applying a fast Fourier transform (FFT) of 512 points and a Hamming window with 87.5% overlap. When the calls of the young were analyzed, a basic problem is how to define the precursors of echolocation calls (versus isolation calls). Based on some studies (Vater et al. 2003; Liu et al. 2007) and the development of vocalization in our study, the calls with constant frequency–frequency modulated (CF–FM) structure and dominant frequency (DF) locating in the second harmonic were defined as early echolocation calls (others as isolation calls). For each isolation call, we considered the following variables: the frequency of maximum energy (i.e., DF) from the fundamental component, pulse duration (DT) and number of harmonics (NH). For each echolocation call, we measured the total duration of the CF–FM component (DT), the frequency of maximum energy (DF), and the bandwidth of the terminal frequency modulated (FM) from the second harmonic (BFM$_2$). The DF was measured from power spectra and pulse duration from oscillograms. The NH and BFM$_2$ were taken from spectrograms. One-way analysis of variance was used to compare the difference of echolocation call parameters (DF, DT, and BFM$_2$) between young at 40-day age and adults.

We used an independent sample t test to compare length of forearm and body mass of males and females at birth. Linear regression equations were derived to predict age from pooled data on forearm length (1–13 days) and the length of the total epiphyseal gap (10–40 days). To derive an age-predictive equation for the measurements, the axes on the growth curve were reversed and age for the specific periods were considered as the dependent variable (Kunz and Anthony 1982). Ninety-five percent confidence and prediction intervals were plotted for the regression equations for lengths of forearm and total epiphyseal gap. In addition, growth data of body mass and length of forearm in young bats were fitted to the three models: logistic, Gompertz, and von Bertalanffy equations (Table 2). The Levenberg–Marquardt algorithm was used to derive the best fit to the three non-linear equations. Results from the three different models were compared by the goodness of fit obtained from each model (Zullinger et al. 1984).

All statistical analysis was performed in SPSS 15.0 (SPSS Inc., Chicago, IL, USA), and data were described using means±SD unless stated otherwise.

Results

Each female observed produced a single pup. For 56 newborns with umbilical cord captured, no significant difference was found between length of forearm in males (17.96±0.42 mm) and females (18.03±0.50 mm, $t=0.5$, $df=54$, $P>0.05$), although average forearm length of female was slightly longer. Similarly, body mass of male (2.31± 0.11 g) and female (2.36±0.12 g) young was not significantly different ($t=1.6$, $df=54$, $P>0.05$). Length of forearm and body mass of newborns were 44.1% and 39.7% of that of adult females (length of forearm, 40.82± 1.1 mm; body mass, 5.89±0.6 g, $n=31$), respectively.

Morphological development

Newborns were naked with a gray dorsal and a pink ventral part. They had closed eyes, folded ears, and deciduous teeth. Four days after birth, ears of the pups were raised partly with gray fur increasing in the dorsal part and gray fur becoming

Table 1 Number of individuals captured on day 1 and subsequently re-captured on other sampling occasions

Parameter	Day of sampling													
	1	4	7	10	13	16	19	22	25	28	31	34	37	40
Number of re-captured bats	56	32	26	25	29	25	28	28	18	14	12	9	8	6
Marked bats re-captured (%)	–	57	46	45	52	45	50	50	32	25	21	16	14	11

Table 2 The three growth models were fitted in this paper

Model name	Model
Logistic	$W = A(1 + \exp(-K(t - I)))^{-1}$
Gompertz	$W = A\exp(-\exp(-K(t - I)))$
von Bertalanffy	$W = A(1 - (1/3)\exp(-K(t - I)))^3$

The parameters A and K in each model were estimated for the growth of mass in the neonate population. Similar equations were used for length of forearm

A is the asymptotic value (grams), W is the body mass (grams) at age t (days), K is the growth rate constant (day^{-1}), and I is the age at the inflection point (days)

visible in the ventral part. Eye slits appeared after 1 week, and the eyes were completely opened by 13 days. After 19 days, the young could fly awkwardly for a short distance when they were dropped from hands. After 31 days, most young bats could fly and turn agilely in the recording room, by which time the mean body mass and length of forearm were 4.94± 0.25 g and 38.61±0.76 mm, and they were 83.2% and 95.9% of adult values, respectively.

Empirical growth curves from 56 young based on changes in body mass, length of forearm, and the length of the total epiphyseal gap are shown in Fig. 1. During the first 13 days, body mass (Fig. 1a) and length of forearm (Fig. 1b) increased linearly with growth rates of 0.14 g/d and 1.08 mm/d, respectively. Subsequently, the growth rates of these two characters decreased to a relatively constant value. Evaluation of coefficients of determination for the relationships between forearm length and age (R^2=0.98) and between body mass and age (R^2=0.93) indicated that length of forearm can be used to reliably estimate the age of young *H. pomona* up to 13 days of age, by which time average length of forearm was 30.6±0.76 mm. Thus, the equation for estimating age from length of forearm is valid if this dimension is≤30.6 mm. The equation is as follows (Fig. 2a):

$$\text{Age(days)} = 0.92 \, \text{Forearm length} \qquad (1)$$
$$-15.61 \left(R^2 = 0.98, df = 167, P < 0.05\right)$$

In contrast, the length of the epiphyseal gap increased linearly until day 10 and then decreased linearly at a mean rate of 0.09 mm/day (Fig. 1c). At 10 days of age, length of forearm of pups was 27.84±0.7 mm. A linear regression equation estimating the age was derived from the total length of the epiphyseal gap until day 40. The equation is as follows (Fig. 2b):

$$\text{Age(days)} = -10.87 \, \text{Length of the total epiphyseal gap} \qquad (2)$$
$$+ 53.91 \left(R^2 = 0.83, df = 201, P < 0.05\right)$$

Together, these two equations (i.e., Eqs. 1 and 2) allow us to predict the age of young *H. pomona* from 1 to 40 days after birth.
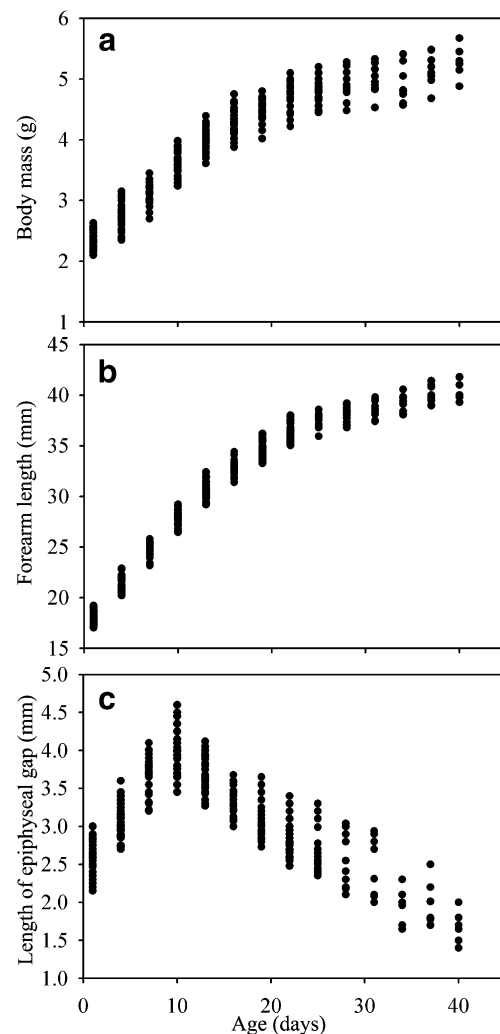

Fig. 1 Empirical growth curves for **a** body mass (*n*=316), **b** length of forearm (*n*=316), and **c** the length of the total gap of the fourth metacarpal–phalangeal joint (*n*=316) of young *H. pomona* from day 1 to 40. Some points represent more than one observation

The logistic, Gompertz and von Bertalanffy models gave a good fit to the changes in length of forearm and body mass. A summary of growth parameters derived from the three non-linear growth models is given in Table 3. Based on the sum of squares model, the logistic model appeared to provide the best fit for body mass and length of forearm (Table 3).

Vocalization development with age

Newborn *H. pomona* were usually silent and only sporadically emitted multiple harmonic calls with fundamental frequencies usually above 20 kHz. As the pups grew older, they vocalized more actively.

During the first 4 days, young bats emitted signals with various structures, including upward sweep to constant frequency (CF; 11–14 ms; Fig. 3a), noisy quasi-CF (QCF;
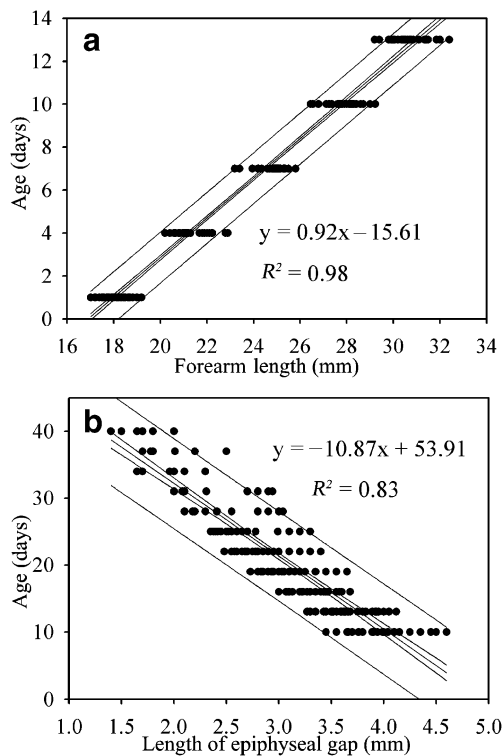
Fig. 2 Regression line estimating the age of *H. pomona* from **a** the values of length of forearm up to 13 days, and **b** the values of epiphyseal gap length at 10–40 days. Some points represent more than one observation. The predictive equation is valid for length of forearm ≤30.6 mm and >30.6 mm, respectively. *Narrow* and *wide* bands indicate 95% confidence and prediction intervals, respectively. Age (days)=0.92×length of forearm−15.61 (R^2=0.98, df=167, P<0.05) and age (days)=−10.87×length of epiphyseal gap+53.91 (R^2=0.83, df=201, P<0.05), respectively

harmonics with most energy concentrating on the fundamental frequency (Fig. 3e) and they did not develop into echolocation calls during the later postnatal growth (Fig. 4a–d, g). Thus, these calls mentioned above were all isolation calls. Based on the definition of the early echolocation calls as mentioned previously (see section "Materials and methods"), we did not distinguish the precursors of echolocation calls until the seventh day in three young bats, but they were faint in energy and were comprised of only four components (Fig. 4e–h).

Isolation calls and early echolocation calls changed in their frequency/time structure with the age of the growing bat (Figs. 5 and 6). Specifically, isolation calls were usually multi-harmonic. Typically, the first harmonic was the most intense and other harmonic energy decreased accordingly. The number of harmonics decreased (Fig. 4a–d) and DF increased (Fig. 5). However, the duration of isolation calls remained relatively stable (Fig. 6). Interestingly, during 19–28 days after birth, except for some short isolation calls, some distinct longer isolation calls (the maximum duration up to 75 ms) were recorded when the young were handheld, mainly including W-shaped FM (Fig. 7a), QCF-FM (Fig. 7b), QCF (Fig. 7c), and downward FM (Fig. 7d). After 28 days of age, young bats that were handheld seldom emitted isolation calls, although they occasionally produced some calls like distress calls of adults emitted from the mouth. However, in the roost shelter, young bats still produced isolation calls when female adults approached them even after 4 weeks.

Early echolocation calls typically contained four harmonics, and the second harmonic was usually the most intense. With the development of young bats, the first and fourth harmonics disappeared, but the second and third harmonics remained (Fig. 4e–h). Additionally, the DF of echolocation calls increased quickly during the second week and thereafter increased slowly (Fig. 5). From 7 days

9–12 ms; Fig. 3b), downward FM sweeps (7–10 ms; Fig. 3c), short (7–10 ms) and long (13–18 ms) QCF (Fig. 3d), CF–FM (7–11 ms; Fig. 3e) and short CF (4–9 ms; Fig. 3f). Those CF–FM calls contained up to seven

Table 3 Growth parameters in *H. pomona*, derived from the logistic, Gompertz, and von Bertalanffy growth models

Growth model	Parameter	Body mass versus age				Length of forearm versus age			
		Estimate	SE	CV (%)	MSS	Estimate	SE	CV (%)	MSS
Logistic	A	5.17	0.041	0.79	13.22	40.68	0.153	0.38	156.32
	K	0.12	0.004	3.42		0.11	0.002	1.82	
	I	2.88	0.174	6.04		3.09	0.084	2.72	
Gompertz	A	5.31	0.053	1.00	13.62	41.81	0.197	0.47	159.14
	K	0.09	0.003	3.41		0.08	0.001	1.20	
	I	−0.96	0.159	−16.65		−0.94	0.073	−7.76	
von Bertalanffy	A	5.37	0.059	1.10	13.84	42.34	0.223	0.53	164.62
	K	0.08	0.003	3.85		0.07	0.001	1.35	
	I	−2.75	0.182	−6.61		−2.84	0.082	−2.89	

Note: *A* asymptotic size of forearm length (centimeters) or body mass (grams), *K* growth rate constant, *I* inflection point, *SE* is standard error, *CV* is coefficient of variation, *MSS* is model sum of squares
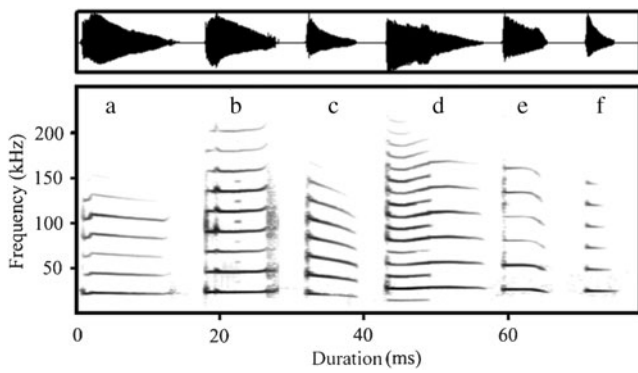
Fig. 3 Vocal repertoire of infant *H. pomona* during the first 4 days. *Top* of each graph presents oscillogram and *bottom* presents spectrogram of respective calls. **a** upward sweep to CF, **b** noisy QCF, **c** downward FM, **d** short and long QCF, **e** CF-FM, and **f** short CF. All spectrograms were produced using a 512-point FFT and a Hamming window with 87.5% overlap
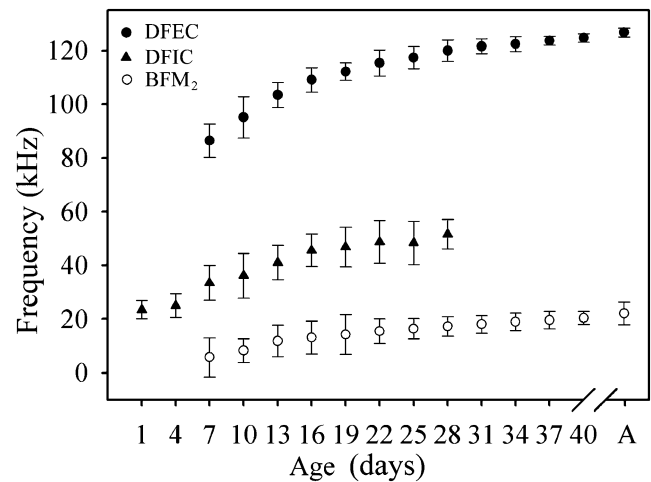

Fig. 5 Changes of the mean values of dominant frequency of echolocation calls (DFEC) and isolation calls (DFIC), and bandwidth of the terminal FM₂ (BFM₂) with age during the growth of young in *H. pomona*. *A* represents adults and *error bar* represents 2SD

to 40 days, the BFM₂ increased (Fig. 5) and the duration decreased (Fig. 6). By day 40, there was significant difference in DF ($F_{1, 35}$=33.83, $P<0.05$) between the young and adult calls (DF, 126.6±0.68 kHz). However, there was no significant difference in BFM₂ (adult, 21.33± 1.76 kHz, $F_{1, 35}$=3.81, $P>0.05$) and duration (adult, 5.78± 0.52 ms; $F_{1, 35}$=2.59, $P>0.05$).

Discussion

For newborn *H. pomona* in the present study, we found no significant differences between males and females both in body mass and length of forearm, which was consistent with studies on *Hipposideros terasensis* (Cheng et al. 2002) and *Tadarida brasiliensis mexicana* (Kunz and Robson 1995). However, a significant difference in body mass existed among that of *Artibeus watsoni* (Chaverri and Kunz 2006). Additionally, body mass of neonates are 11–43% of

that of adults (Altringham 1996; Kunz et al. 2009). In this study, the mean mass at birth of neonates of *H. pomona* was relatively large (39.7% of that of adults), which is generally associated with an advanced stage of development and is believed to aid the conservation of heat generated to maintain constant body temperature when the mother leaves the roost to forage (Reiter 2004). Carrying a heavier fetus during pregnancy and the associated increase in wing loading, however, can reduce flight maneuverability of pregnant females, which may, in turn, have increased predation risks from raptorial birds (McLean and Speakman 2000). Whereas, interestingly, after 2 weeks, some *H. pomona* females occasionally carried their young during foraging bouts even when they were large in size (body mass of young bats were ca. 65% of that of adults by that time) in our study (Long-ru Jin, personal observation), which suggested that *H. pomona* has a good flight ability
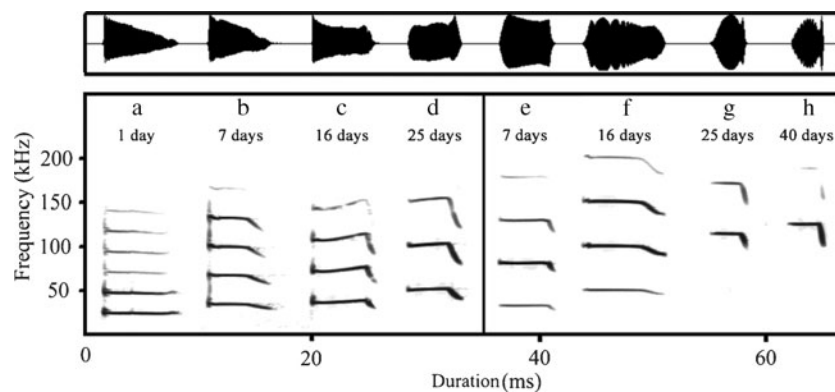

Fig. 4 Modification of the isolation call (left, **a–d**) and echolocation call (right, **e–h**) with age. *Top* of each graph presents oscillogram and *bottom* presents spectrogram of respective calls. Oscillograms and sonograms of the isolation calls produced by the same infant of *H.* *pomona* aged 1 (**a**), 7 (**b**), 16 (**c**), and 25 (**d**) days; oscillograms and sonograms of the echolocation calls produced by the same infant of *H. pomona* aged 7 (**e**), 16 (**f**), 25 (**g**), and 40 (**d**) days
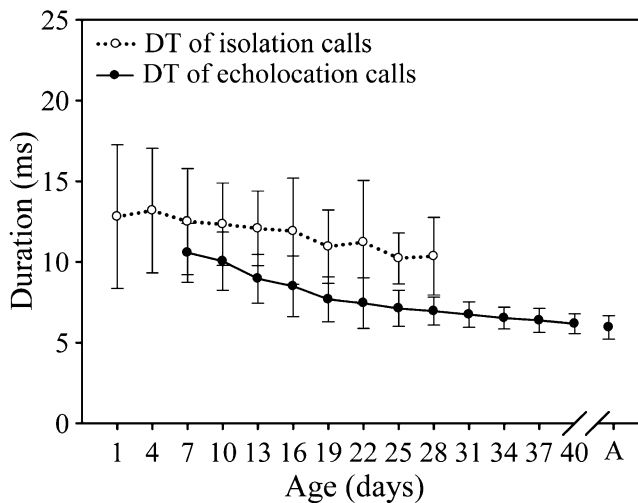
Fig. 6 Changes of the mean values of pulse duration of isolation calls and echolocation calls with age during growth of young in *H. pomona*. *A* represents adults and *error bar* represents 1SD

and that their forage area is close to their roost site, and it might indicate that their predators are scarce in this area.

Morphological development

The characteristics of neonates in *H. pomona*, including closed eyes, folded ears, and deciduous teeth, were similar to those of most other bat species. However, the neonates have gray dorsal part, and their eyes fully opened late, which is different from those of most other species (e.g., *Pipistrellus mimus*-Isaac and Marimuthu 1996; *Myotis blythii*-Sharifi 2004b) except *H. terasensis* (Cheng et al. 2002), and *Hipposideros sperois* (Habersetzer and Marimuthu 1986). Additionally, we also observed that *H. cineraceus* and *Hipposideros larvatus* pups had characteristics in common with *H. pomona*. Thus, the possibility that this is a phenomenon common to the Hipposideridae bats invites further investigation.

Sustained flight of young *H. pomona* occurred in a 31-day-old, by which time they had achieved 83.2% of adult body mass and 95.9% of adult forearm proportions. This is similar to juveniles of other microchiropterans, which typically begin to fly when they attain 70% of adult body

mass and 90% of adult forearm length (Kunz and Robson 1995; Kunz and Hood 2000; Kunz et al. 2009). Having a small body size and a larger skeletal size at the onset of flight would result in a lower wing loading and, in turn, would contribute to increased maneuverability and decreased the cost of flight, which is important for the survival of young bat (Hughes et al. 1995). Additionally, in some bat species, the young would experience a loss of body mass during the early flight and weaning period and such reduction in body mass may reflect the poor foraging ability (Sharifi 2004a). However, the phenomenon of temporary reduction in body mass was not reported in development of other bat species (e.g., *M. blythii*-Sharifi 2004b; *Myotis formosus*-Shen and Lee 2000). In our study, the changes of body mass were similar to the latter phenomenon.

Although body mass also increased linearly in the early postnatal growth, the coefficient was lower than that between length of forearm and age. Thus, age estimation of young bats should be based on length of forearm and the length of the total epiphyseal gap, which is consistent with other studies (Hood et al. 2002; Sharifi 2004a; Kunz et al. 2009). However, on the other hand, postnatal growth rates in bats are influenced by climate, food supply, roost temperature, latitude, maternal factors, colony size, and anthropogenic factors (Allen et al. 2009; Kunz et al. 2009), which might limit the application of growth equations to their general areas of origin. Thus, as suggested by Kunz et al. (2009), we encourage researchers to compare the changes in different years and to generate geographically specific equations whenever possible.

Of the three non-linear growth models, the logistic model best described growth rates for length of forearm and body mass in free-ranging *H. pomona*. Similar conclusions were made for *Pipistrellus pipistrellus* (Hughes et al. 1995), *Plecotus auritus* (De Fanis and Jones 1995), and *T. brasiliensis mexicana* (Kunz and Robson 1995). However, in other studies (e.g., Kunz 1974), the von Bertalanffy model gave the best fit for body mass. Given the relatively few studies that have compared different models, the general significance of these differences remains unclear. In addition, on the basis of reviews by
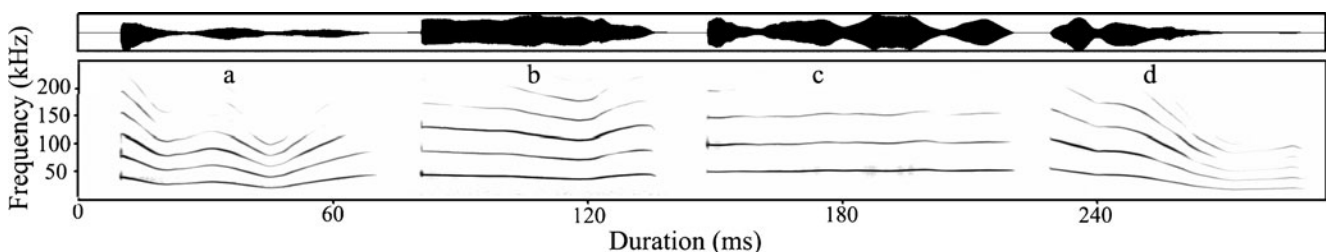

Fig. 7 Vocal repertoire with distinct characteristics produced by different young *H. pomona* bats between 19–28 days. **a** 19 day old, **b** 22 day old, **c** 25 day old, and **d** 28 day old

Kunz and Hood (2000), growth constant rates of body mass of all tropical microchiropterans were 0.04–0.11 (n=11), and the rates of most temperate microchiropteran species were 0.12–0.25 (n=13). In our study, the constant rate of body mass was 0.12, which embodied the regional features (i.e., a transition zone between subtropical and tropical environments).

Vocal development of young bats

During the first 4 days, the pups of *H. pomona* emitted various structure signals, which were also seen in the studies of *Rhinolophus ferrumequinum* (Liu et al. 2007) and *P. parnellii* (Vater et al. 2003). On the basis of call characteristics, they were identified as isolation calls. During ontogeny in *H. pomona*, early echolocation calls increased in DF and decreased in number of harmonics until they resembled adult's echolocation calls. Thus, we inferred that early echolocation calls in *H. pomona* did not originate from isolation calls, but developed independently, which is consistent with the studies on *N. albiventris* (Brown et al. 1983), *P. pipistrellus* (Jones et al. 1991), *P. parnellii* (Vater et al. 2003), and *R. ferrumequinum* (Liu et al. 2007). However, in other studies (e.g., *P. auritus*-De Fanis and Jones 1995; *M. lucifugus*-Moss et al. 1997; *C. perspicillata*-Sterbing 2002), echolocation calls develop from communication calls by a continuous change in signal parameters, such as duration, frequency content, modulation rate, and harmonic content.

Newborn *H. pomona* always emitted isolation calls of the same relatively high fundamental frequency (>20 kHz) with the maximum energy as *Hipposideros speoris* (Habersetzer and Marimuthu 1986), but neonates of larger-sized rhinolophids use lower frequencies (<20 kHz; e.g., *R. ferrumequinum*-Matsumura 1979; *Rhinolophus rouxi*-Rübsamen 1987). In previous studies, the dominant frequency of echolocation calls is was negatively correlated with body size (Francis and Habersetzer 1998). Considering both echolocation and isolation calls emitted through vocal tract, we inferred that those differences among fundamental frequencies might result from differences in body size.

Previously studies have shown that the developmental trend of isolation calls in bats generally falls into two categories: (1) increasing in DF, decreasing in number of harmonics and duration (e.g., Jones et al. 1991; Zhang et al. 2005); or (2) remaining stable in structure throughout the lactation period (e.g., Parijs and Corkeron 2002). Our results for *H. pomona* were generally similar to the former. Additionally, the duration of isolation calls remained relatively stable. This was unexpected and awaits further investigation. During 19–28 days, some individuals emitted long duration isolation calls (Fig. 7). Given low sample size, these long calls were excluded from our statistical analysis.

For early echolocation calls, the DF and BFM_2 of echolocation calls both increased, which may involve maturation at the effector (laryngeal muscles, cartilage stiffness, tendons) and at the level of central neuronal control (Vater et al. 2003). For CF–FM echolocating bats, the CF portion is used to evaluate relative motion, whereas the terminal downward FM is used to determine target distance. Thus, a higher CF may help detect moving targets and a wider bandwidth may convey more target-specific and higher resolution information, which is advantageous for spatial orientation during hunting and target capture by young bats. The duration of echolocation calls in juvenile *H. pomona* decreased until it resembled that of adults, which is similar to *H. speoris* (Habersetzer and Marimuthu 1986). However, in the studies on *R. ferrumequinum* (Liu et al. 2007) and *P. parnellii* (Vater et al. 2003), the duration of echolocation calls increased as juveniles matured.

When young bats began to forage, there was a significant difference in DF of echolocation calls compared with those of adults. This difference is also found in other studies (Buchler 1980; Jones and Ransome 1993; Chen et al. 2009). Wund (2005) suggested that young bats may learn to modify their echolocation calls to enhance performance once they have begun to forage. Thus, we inferred that young *H. pomona* would increase the DF of echolocation calls when they begin to forage and gain experience in a new habitat.

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