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The ontogeny of the baculum in *Nyctalus noctula* and *Vespertilio murinus* (Chiroptera: Vespertilionidae)

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Patterns of baculum development were studied in *Nyctalus noctula* and *Vespertilio murinus* and found to be similar in both species. A cartilaginous baculum anlage was evident at late embryonic stages. Initially, the distal part of the baculum develops during embryogenesis as a cartilage. Then the growth of the shaft and formation of the proximal portion occurs and it is shortly followed by ossification of the whole structure. The baculum continues to grows during the first months of the postnatal life and its length is correlated with that of the forearm. The baculum reaches its definitive shape and size by the age of 1.5 month in *N. noctula* and two months in *V. murinus*. Therefore, after this time period, the shape and size of the baculum cannot be used for age determination.

Key words: Chiroptera, *Nyctalus noctula*, *Vespertilio murinus*, baculum, ontogenesis

INTRODUCTION

During the last few decades, the baculum (os penis) shape has been successfully used in the taxonomy of some mammalian groups (e.g., Didier, 1954; Rabeder, 1976; Strelkov, 1989*a*; Baryshnikov and Abramov, 1997; Benda and Tsytsulina, 2000). The employment of shape features is especially effective in instances when it is difficult to differentiate species using general morphology. For example, species of *Plecotus*, *Barbastella*, and *Myotis* (*Selysius* subgenus) that are morphologically very similar can be differentiated by baculum morphology (Strelkov, 1989*a*, 1989*b*; Benda and Tsytsulina, 2000; Kruskop and Lavrenchenko, 2000; Tsytsulina, 2001). Bacula are well studied in Palaearctic bats and described in more than 160 Vespertilionidae species of the world's fauna (e.g., Thomas, 1915; Chaine, 1926; Topál, 1958; Hill and Harrison, 1987; Strelkov, 1989*a*, 1989*b*; Yoshiyuki, 1989; Smirnov, 2000).

The baculum is a heterotopic skeletal element, which is located inside a distal part of the glans penis and provides support for adjacent tissues of the copulatory organs. Opinions differ on the baculum's origin and function. According to Jellison (1945), the baculum is an additional bone of the skeleton. On the other hand, Ottow (1955) and Nickel *et al.* (1973) noted the absence of any relation between the baculum and the rest of the skeleton. They stated that the baculum is a result of calcification of the distal part of cavernous bodies. Recently it was reported that the length of the baculum is positively associated with relative testis mass and/or male body mass in Chiroptera on the species level. However, after phylogenetic testing, baculum length was not found to be significantly associated with testis and/or body mass (Hosken *et al*., 2001). Age peculiarities of baculum shape have been studied in detail in Rodentia and Carnivora (e.g., Friley, 1949; Callery, 1951; Heidt, 1970; Tarasov, 1974, 1984; Aksenova, 1980). In Chiroptera, however, they were studied only in *Nyctalus aviator* (Maeda, 1978) and in this species a correlation between increasing condylobasal length, forearm length and baculum length was shown. Yoshiyuki (1989) noted that age variation is remarkable in baculum shape and size in *Murina silvatica*, *Vespertilio superans*, *Myotis macrodactylus*, and *Rhinolophus cornutus*. However, Yoon *et al.* (1990) studied baculum development in *V. superans* and found no morphological differences between bats aged from 10 to 202 days.

For the purpose of providing basic data on the development of the baculum and to partly address paucity of information, we analysed age peculiarities of the baculum shape in *Nyctalus noctula* and *Vespertilio murinus*. We collected data on baculum shape variation in adults and during their development in these species to clarify whether the baculum's size and shape may be used for age determination and in these species.

Materials and Methods

The bacula of 20 males of *N. noctula* and 13 males of *V. murinus* of different age from the collection of Department of Zoology and Ecology of Penza State Educational University (Penza, Russia) were used for this study. The examined specimens [species – locality – age and collection number] are as follows: *N. noctula* — Russian, Penza Region – embryo (1 ♂): 128B; two hours after birth (2 ♂♂): 141, 187; 2–3 days after birth (2 ♂♂): 127A, 139; 4–7 days after birth (2 ♂♂): 131, 132; 10–16 days after birth (2 ♂♂): 126A, 134; young able to fly, 40 ± 5 days (1 ♂): 331; 4 adult ♂♂: 239, 303, 486, 487. Russia, Uljanovsk Region — young able to fly, 40 ± 5 days (1 ♂): 762. Russia, Orenburg Region — adult ♂: 514. Northern Caucasus — 3 adult ♂♂: 7X–8X. North-Western Kazakhstan — adult ♂: 427. *V. murinus* — Russia, Penza Region — new-born bat (1 ♂): 393; 7–15 days after birth (1 ♂): 395; 16–25 days after birth (1 ♂): 396; 3 adult ♂♂: 388, 389, 484; Uljanovsk Region — young able to fly, 25–35 days (1 ♂): 782; Saratov Region — 3 adult ♂♂: 773, 774, 775; Volgograd Region — adult ♂: 776; Bashkir Republic — 2 adult ♂♂: 522, 523. Specimens without wide cartilage streaks in the metacarpalia and phalanx articulations were considered adults. Overwintered bats were considered at least one-year old. In young bats (except embryos, newborn and adult bats) age was determined based on methods proposed for age groups in *N. noctula* (Heise, 1993; Ivantseva and Ivantsev, 2000) and *V. murinus* (Kozhurina, 1999).

Preparation of the bacula followed standard procedures using dry, alcohol-fixed and fresh material (Friley, 1947; White, 1951). Soft tissues were macerated in a 6–7% solution of KOH and stained with alizarin red 'S' for 1–2 days depending on the material storage. After maceration, the remaining soft tissue was removed from the bacula under a binocular microscope (MBS-1). Figures were made with a BIOLAM R-11 microscope and Lucida camera.

In our opinion, forearm length is the most accessible and representative measurement for a growing animal. It is also useful when analyzing alcohol and dry preserved specimens. To determine the degree of contingency and character relationship between the size of a growing baculum and the forearm length, a coefficient of correlation between the baculum length and the forearm length was calculated and a regression equation was produced. To determine the significance of the regression equation the *t*-test was used (STATISTICA for Windows 5.0, StatSoft, Inc., 1995). Baculum and forearm measurements are shown in Table 1.

Results

Nyctalus noctula has one of the largest bacula among Vespertilionidae. The baculum consists of two parts: one is a long, thin rod bifurcated at its distal end and the another is an enlarged proximal part which takes up about 1/3 of the overall baculum

TABLE 1. Baculum and forearm lengths of *Nyctalus noctula* and *Vespertilio murinus* (in mm) at different age

Age	Baculum				Forearm			
	n	$\bar{x}$	SD	Range	*n*	$\bar{x}$	SD	Range
				Nyctalus noctula				
Embryo	1	1.05	–	–	1	17.90	–	–
2 hours	2	1.18	–	1.09–1.26	2	21.65	–	21.5–21.8
2–3 days	2	1.68	–	1.65–1.70	2	25.20	–	23.9–26.5
4–7 days	2	1.98	–	1.9–2.05	2	30.65	–	28.2–33.1
10–16 days	2	2.53	–	2.5–2.55	2	37.30	–	36.3–38.3
40 days	2	4.88	–	4.65–5.10	2	52.35	–	52.1–52.6
Adults	9	5.92	0.23	5.50–6.25	9	53.21	1.03	52.0–55.4
				Vespertilio murinus				
1 day	1	0.53	–	–	1	25.80	–	–
7–15 days	1	0.51	–	–	1	38.50	–	–
16–25 days	1	0.91	–	–	1	40.00	–	–
Adults	10	1.65	0.12	1.46–1.85	10	44.39	1.36	42.7–46.8

length (Fig. 1). A deep cut divides the proximal part into right and the left branches. As a rule, in adults both branches have straight medial and semicircular lateral sides. From a lateral view, the proximal part of the baculum curves down at an angle of 30–45° from the rod's main axis. There is an incavation on the dorsal side of the proximal part of the baculum. This cavity forms its cone with a basis on the upper edges and its top in the middle of the proximal part. The bifurcated apical end also has a hollow on the ventral side.

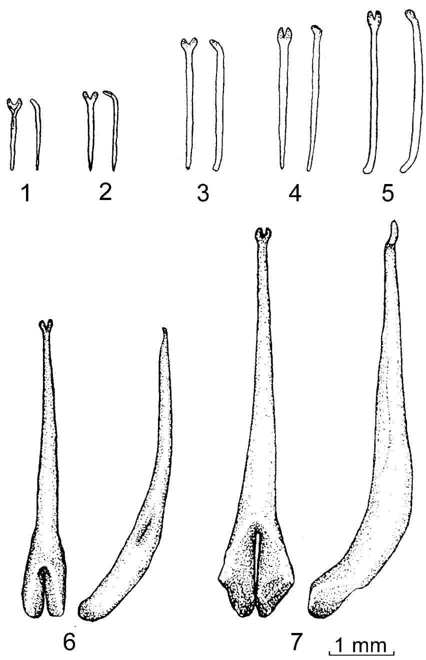

FIG. 1. Bacula of *N. noctula* at different age. 1: embryo (last days before a birth); 2: two hours after birth; 3: 2–3 days after birth; 4: 4–7 days after birth; 5: 10–16 days after birth; 6: young male able to fly (40 ± 5 days); 7: adult male. The given views are from dorsal and left lateral sides (from left to right)

It was ascertained that the *N. noctula* baculum could be distinguished in the late stages of the embryogenesis, when it is a cartilage anlage above the urethra. A newly born noctule has a cartilage anlage, which is quite thin and small (about 1 mm long) and has almost equal width along its full length (Fig. 1). Later this anlage develops into the distal part of the baculum. By this time, a bifurcation on the apical pole is distinct. The baculum remains cartilaginous up to second or third day after birth. The cartilaginous anlage ossifies completely by about the seventh day after birth. The ossification starts in the central axis of the distal part of the anlage and spreads over its periphery. Starting from the same day seven, baculum length increases gradually. The proximal part forms before 15 to 20 days after the commencement of solo flight, i.e., from 15 to 40 days. At the age of one month young bats are able to fly and their forearm length is about 90–96% of that of adults. At this time, the proximal part has formed completely and the baculum has its definitive shape with a well defined urethral gutter. At this age the baculum has smooth and even surface. It reaches a definitive size (with the length of up to 6 mm) and relief at the age of about one year. Changes in the baculum occur mainly during the intensive growth period of the animal. The length of the growing baculum is correlated with the forearm length (see Table 1). The regression equation is expressed in the following form: $b = -2.07 + 0.15R$, where b is the baculum length and R is the forearm length ($r = 0.97$, $n = 20$, $P < 0.001$).

Vespertilio murinus adults have a massive baculum, which differs considerably in shape from those of all other bat species. The two parts of the baculum are separated from each other by a deep constriction (Fig. 2). The distal part has a round-like shape. There is a well-expressed jag with a front spike on the ventral side of the distal part. In some cases the jag was absent or broken most likely because of a heavy load on the penis during copulation. A small hollow is usually present on the front edge of the baculum's distal part. Older specimens may have such hollows on the lateral edges of the baculum. The proximal part of the baculum has the same length or is slightly longer. A well-expressed wedge-shaped groove bifurcates the hind section of the proximal part. The dorsal side of the proximal part is flat, while on the ventral side there is an urethral gutter, which goes from the top of the proximal groove almost to the base of the jag. There are combs on each side of the gutter, and these two may split at the ends of branches of the proximal part and thus form a hollow less-pronounced than the urethral one. The baculum may become thinner in some older males.

Baculum development in *V. murinus* is similar to that of *N. noctula*. The first cartilaginous element is evident in the late embryonic stages and is represented by an anlage of the distal part of the baculum (Fig. 2). During the first postnatal month this structure grows intensively, and the rate of growth slows down abruptly by the end of the second month. On days 7 to 15, when the forearm length is about 26.0–38.5 mm, the baculum ossifies and a jag which is typical of this species appears as a small protuberance. At this time, the baculum is

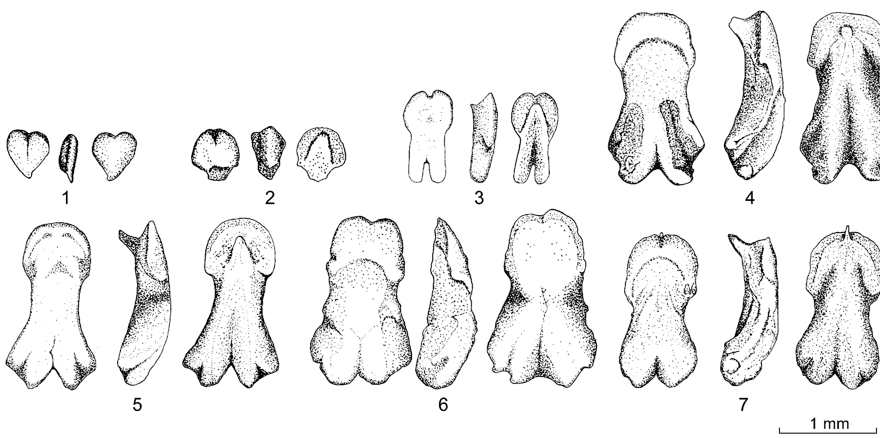

FIG. 2. Bacula of *V. murinus* at different age. 1: new-born bat; 2: 7–15 days after birth; 3: 16–25 days after birth; 4 – young male able to fly (25–35 days); 5–7: adult male. The given views are from dorsal, lateral and ventral sides (from left to right)

very thin from a lateral view and heart-shaped when viewed from above. Further changes in baculum structure pertain mainly to growth of its proximal part.

At days 15 to 25, when bats have a forearm length about 89% of that of adults and are still incapable of solo flight, the proximal part of the baculum is presented by a small ossified wishbone with a well expressed urethral gutter on its ventral side. Later, the basis of the wishbone elongates further and becomes thicker. In *V. murinus* the baculum forms completely by maturation. The regression equation takes the following form: $b = -1.18 + 0.06R$, where b is baculum length and R is forearm length ($r = 0.98$, $n = 13$, $P < 0.001$; see also Table 1).

Discussion

In both species, the baculum develops in the following way: a cartilaginous distal part forms first**,** this formation is then followed by the development of the rod and proximal end of the baculum, and at the same time, the baculum ossifies. This process starts from the baculum's central axis and spreads to its periphery. Differences between baculum development in *N. noctula* and *V. murinus* are minimal and are limited to the time of the baculum first appearance and to the intensity of ossification. In both *N. noctula* and *V. murinus*, the baculum of young bats, born in the current summer prior to the start of hibernation, is easily distinguished from that of adults by its size and shape. In the spring, when bats return from hibernacula to summer roosts and their age is at least one year, all males have bacula of similar size and shape.

Baculum development in *Nyctalus aviator*, as described by Maeda (1978), seems to be similar to that of *N. noctula*. For example, in new born bats of both species there is no ossification of the baculum. Ossification starts in the distal part and is evident in 10-day old bats. The distal part forms first and the proximal part becomes evident on day 35. The baculum gradually increases in length until the age of 3 to 9 months. At this same time the baculum forms completely in its shape. Thus, similar to *N. noctula* the baculum in *N. aviator* forms at the age of the beginning of solo flight. Maeda (1978) showed that the total length of the baculum becomes significantly greater with increasing condylobasal length and forearm length ($r = 0.80$, $P < 0.01$ and $r = 0.65$, $P < 0.01$, respectively; $n = 16$). In the case of *N. noctula* correlation with forearm length was even higher ($r = 0.97$, $P < 0.001$, $n = 20$).

Yoon *et al.* (1990) studied the development of pseudobaculum in *V. superans* and made some comments on baculum development. They found no differences in baculum shape between 10 day, 42–86 day and 92–202 day old bats, but the last ones slightly differ in size. This seems to be incorrect because all other bones increase in size and change shape during an animal's life (Romer and Parsons, 1992). Baculum development in *N. noctula* and *V. murinus* is similar to some other mammals. For example, in hamsters, initial stages of ossification were recorded at day 3 or 4 (Callery, 1951). In voles, *Microtus arvalis* and *M. subarvalis*, ossification is initiated at day five to ten and further ossification as well as increasing of baculum length and widening of its proximal part, takes place by day 15 to 25 (Aksenova, 1980). These similarities suggest an ancient origin of this structure. In Carnivora, the duration of baculum formation is extended by up to several months (Tarasov, 1984). For example, in *Mustela vison*, ossification begins on the first month after birth. By the age of two months the bone increases in size and an evident urethral gutter appears on the ventral side of the baculum. During the following 3–4 months the baculum continues to increase in size. The apical end of the baculum ossifies first and only afterwards the proximal part ossifies. The baculum reaches its final size and shape by the age of 6–7 months. In the family Canidae, the process of baculum formation lasts longer and continues for one-two years after the birth (Tarasov, 1984).

In all of the above mentioned mammal species, the baculum reaches its definitive size by the maturation. In bat species, the examined baculum reaches its definitive size and shape by the beginning of active flight. At this time the baculum of young bats is not different from that of adults. Therefore, it appears that bacular development is completed by sexual maturity. This conjecture is supported by the enlargement of the testis and epididymides, which occurs in young bat males during the first autumn (Sosnovtzeva, 1974; Entwistle *et al*., 1998; D. G. Smirnov, unpubl. data). Whether young males participate in copulation during the first autumn (and their success) still remains unknown. However, based on histological evidence of genitalia in *N. noctula* and *V. murinus,* it was shown that males mature by their first autumn after birth but do not participate in the autumn matings (Stukanova, 1977).

In summary, since in *N. noctula* and *V. murinus* the baculum grows and changes its shape up to the age of either 1.5 month (*N. noctula*) or two months (*V. murinus*), determination of the bat's age based on the baculum size and shape, is possible before this age. Later the baculum practically does not change and thus becomes unsuitable for age determination. Nevertheless, the absence of further ontogenetic changes allows the baculum to be used as a reliable taxonomical character for species determination even in young flying bats.

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