Prenatal postcranial development in two species of sympatric Japanese wood mice (*Apodemus argenteus* and *A. speciosus*): a comparison of arboreal versus terrestrial congeners

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ABSTRACT. Habitats of two closely related Japanese field mice, *Apodemus argenteus* and *A. speciosus*, broadly overlap in many Japanese forests. *A. argenteus* being more arboreal and *A. speciosus* being more terrestrial, it is thought that such ecological segregation allows their sympatric distribution. Comparing these two congeners, whether ecological difference is reflected in postcranial development was examined. Although overall ossification sequences were virtually identical, development of the caudal vertebrae was remarkably earlier in *A. argenteus*. One of the clearest morphological differences between the two species is the relative length of the tail, which is arguably related to the degree of arboreality. I suggest that accelerated ossification of the caudal vertebrae found in *A. argenteus* is related to its elongation of the tail.

KEY WORDS: adaptation, anatomy, evodevo, prenatal, rodents

Skeletons of the mammalian body generally develop during prenatal period, and when birth takes place, most of the skeletons are already present in newborns [9]. The exception is the marsupials in which ossification of the vertebrae, pelvic girdle and hindlimb occurs after birth [20]. Ossification of their forelimb and shoulder girdle develops much earlier than the hindlimb and pelvic girdle [20]. It is argued that the earlier development of the forelimbs facilitates the "climbing" behavior of newborns [20]. Since marsupials can only have a limited time period *in utero* compared to placentals due to their primitive womb, marsupials invest on developing the forelimb earlier than the hind limb [20]. Recently, it was found that a clear developmental difference exists between bats and other boreoeutherians [8]. Bats are characterized by earlier ossification of the foot. Bats are born with nearly complete foot size, and hindlimbs are used to continuously attach to the mother. Since the forelimb is not mature enough for flight at birth and requires extended postnatal duration to be large enough to be fully functional, it was suggested that earlier developmental investment on the hindlimb is necessary in bats.

Studies on the sequence of postcranial development in monotremes [25], atlantogenatans [4] and laurasiatherians [8, 24] have revealed how ossification timing is diverse among mammals. Examples of marsupials and bats partly provide explanations for such diversity, but biological significance of ossification timing is still unknown for most mammalian taxa. In order to fill the gap in our knowledge, it is necessary to further investigate the development of ecologically well-studied species. Here, I present the postcranial developmental sequences in two closely related Japanese field mice (*Apodemus argenteus* and *A. speciosus*). The two species are both distributed widely in Japanese archipelago and often found sympatrically in forest habitat. Based on molecular evidence, the divergence time is estimated around five or six million years ago [18, 22, 23]. The large Japanese wood mouse, *A. speciosus*, is found throughout the four main islands and their peripheral islands, including Sado Island, the Izu Islands and the Satsunan Islands [23]. The small wood mouse, *A. argenteus*, has a distribution range similar to that of *A. speciosus*, including remote islands, such as Sado Island and Yakushima Island [23]. Although these two species are found mostly sympatric, it is known that their spatial usage differs considerably. *A. argenteus* is more arboreal, whereas *A. speciosus* is more terrestrial [17]. By comparing these two closely related sympatric rodents, whether ecological difference is reflected in ossification sequence was examined.

Appearance of ossification centers was observed for prenatal liquid specimens deposited at Hokkaido University Natural History Museum (Botanic Garden), Kyoto University Museum and National Museum of Science and Technology, Tokyo. I employed a high-resolution tomography (μCT) technique for detecting ossification as done in Koyabu *et al.* [9]. Shadow images were taken

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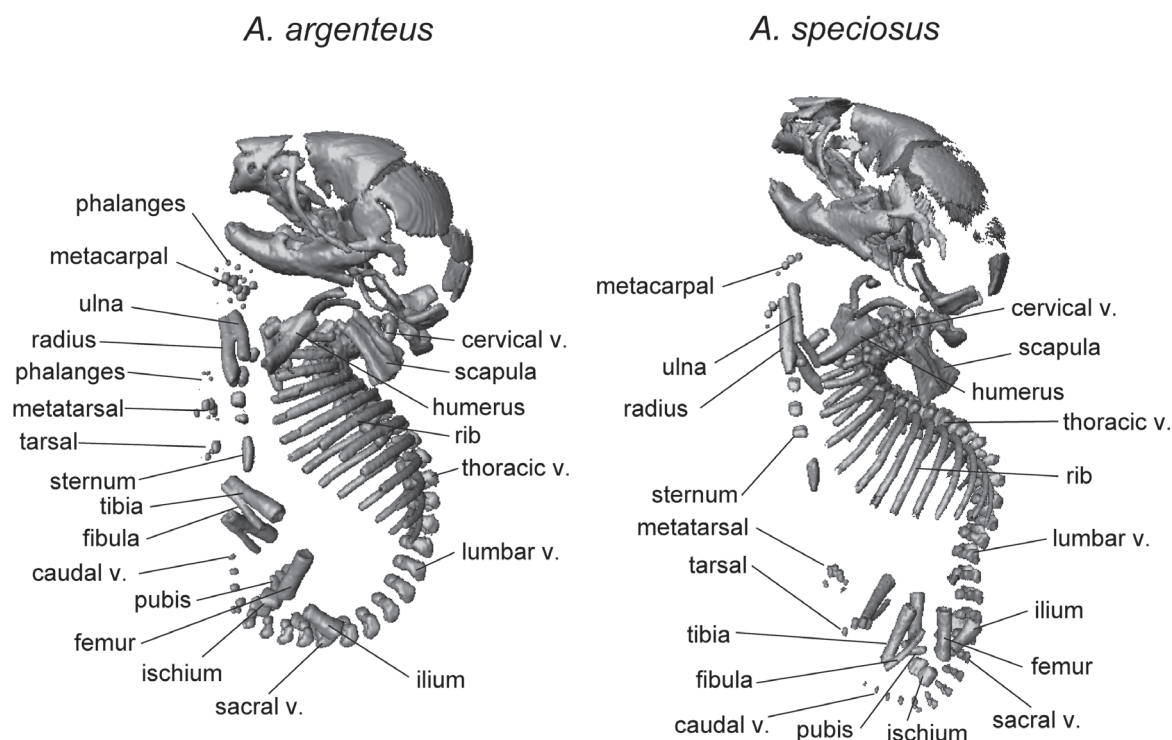
Fig. 1. Three-dimensional reconstructions of developing skeletons of fetal *A. argenteus* (crown-rump length 21.0 mm) and *A. speciosus* (crown-rump length 22.5 mm).

by μCT at the University Museum, The University of Tokyo, Japan (TXS225-ACTIS, TESCO, Tokyo, Japan). Three dimensional visualization, analysis of shadow images and crown-rump length measurements were conducted in Amira 5.3 (Visage Imaging GmbH, Berlin, Germany).

I first described the sequence of appearance of ossification centers for 24 postcranial elements (Fig. 1). Then, to examine the rank variation in sequence of a particular ossification event, the rank of each ossification event was scaled as:

$(r–1)/(rmax–1)$

in which r is the absolute rank of a given ossification event, and $rmax$ is the total number of ranks for each species [7]. By this manner, the relative ranks of each species are scaled between 0 and 1, allowing to remove the differences of maximum rank between species resulting from differing levels of sampling resolution between species. A similar approach as standardizing the absolute rank r by the maximum number of ranks ($rmax$) has been applied in previous sequence heterochrony studies [24, 28]. As the ranks are scaled between $1/rmax$ and 1, the scaled ranks of the earliest bone to ossify can vary, depending on $rmax$. However, the method used here circumvents this problem, because the scaled ranks of the earliest event will always be scaled to zero [7]. Nevertheless, some noise remains, because species with higher $rmax$ have a lower influence on the variance [7]. Similarity of ossification sequence was examined by computing the Pearson's correlation between scaled ranks.

Presence or absence of ossification centers for studied specimens is given in Table 1 (*A. argenteus*) and Table 2 (*A. speciosus*). Specimens with greater crown-rump length tended to show more bones, but some specimens did not follow this rule. In total, 10 ranks from 11 specimens for *A. argenteus* and 10 ranks from 12 specimens for *A. speciosus* (Table 3) were described.

In both species, the clavicle was the first bone to ossify. The humerus was found to be earlier than the radius, ulna, femur and fibula in both species. The ilium was always earlier than other bones associated with the pelvic girdle (sacral vertebrae, ischium and pubis). Sacral and caudal vertebrae were later than cervical, thoracic and lumbar vertebrae. The metacarpal was always later than the radius, ulna and humerus, but earlier than the metatarsal. The manual phalanges and pedal phalanges were later than the metacarpal bones and metatarsal bones. The carpal bones were the last to ossify, preceded by the tarsal bones. Similarity of scaled ranks was significantly high ($r=0.91$, $P<0.001$), suggesting that the sequence of ossification is highly comparable.

Although the overall ossification sequences were virtually similar between the two species, I identified a noteworthy difference between the two: the onset timing of the caudal vertebrae. The sequence of ossification center appearance is highly conservative among the two *Apodemus*, but the timing of the appearance of the caudal vertebrae seems to be accelerated in *A. argenteus*. While their ossification timing is later than the manual phalanges and pedal phalanges in *A. speciosus*, they appear earlier than the phalanges in *A. argenteus*. The author was only able to observe the presence of the caudal vertebrae in two very late fetuses in *A. speciosus*, whereas they were visible in six fetuses in *A. argenteus*, possibly reflecting the early onset of ossification of the caudal vertebrae.

Table 1. Presence/absence of 24 postcranial bones in sampled fetuses of *A. argenteus*

Crown-rump length (mm)	8.1	11.4	12.0	13.7	19.7	20.1	20.6	18.1	20.3	20.8	21.0
Clavicle	2	2	2	2	2	2	2	2	2	2	2
Humerus		2	2	2	2	2	2	2	2	2	2
Ribs		2	2	2	2	2	2	2	2	2	2
Femur				2	2	2	2	2	2	2	2
Radius		2	2	2	2	2	2	2	2	2	2
Ulna		2	2	2	2	2	2	2	2	2	2
Scapula		2	2	2	2	2	2	2	2	2	2
Cervical vertebrae		2	2	2	2	2	2	2	2	2	2
Thoracic vertebrae			1	2	2	2	2	2	2	2	2
Tibia				2	2	2	2	2	2	2	2
Fibula				2	2	2	2	2	2	2	2
Lumbar vertebrae				2	2	2	2	2	2	2	2
Sacral vertebrae					2	2	2	2	2	2	2
Caudal vertebrae						2	2	2	2	2	2
Ilium				2	2	2	2	2	2	2	2
Manual phalanges								2	2	2	2
Pedal phalanges									2	2	2
Ischium					2	2	2	2	2	2	2
Pubis					2	2	2	2	2	2	2
Metacarpal					2	2	2	2	2	2	2
Metatarsal						2	2	2	2	2	2
Tarsals											1
Carpals											
Sternum					2	2	2	2	2	2	2

2: present, 1: slightly present.

Table 2. Presence/absence of 24 postcranial bones in sampled fetuses of *A. speciosus*

Crown-rump length (mm)	8.3	8.5	14.8	18.0	21.6	18.5	19.0	21.6	23.8	21.1	22.5	22.6
Clavicle	2	2	2	2	2	2	2	2	2	2	2	2
Humerus			2	2	2	2	2	2	2	2	2	2
Ribs			2	2	2	2	2	2	2	2	2	2
Femur			2	2	2	2	2	2	2	2	2	2
Radius			2	2	2	2	2	2	2	2	2	2
Ulna			2	2	2	2	2	2	2	2	2	2
Scapula			2	2	2	2	2	2	2	2	2	2
Cervical vertebrae			2	2	2	2	2	2	2	2	2	2
Thoracic vertebrae			2	2	2	2	2	2	2	2	2	2
Tibia			2	2	2	2	2	2	2	2	2	2
Fibula			2	2	2	2	2	2	2	2	2	2
Lumbar vertebrae			2	2	2	2	2	2	2	2	2	2
Sacral vertebrae				2	2	2	2	2	2	2	2	2
Caudal vertebrae											2	2
Ilium			2	2	2	2	2	2	2	2	2	2
Manual phalanges										2	2	2
Pedal phalanges										2	2	2
Ischium				1	2	2	2	2	2	2	2	2
Pubis					2	2	2	2	2	2	2	2
Metacarpal				2	2	2	2	2	2	2	2	2
Metatarsal						2	2	2	2	2	2	2
Tarsals											1	1
Carpals												
Sternum					2	2	2	2	2	2	2	2

2: present, 1: slightly present.

Table 3. Postcranial events ranked according to relative timing of onset of ossification and scaled ranks of *Apodemus argenteus* and *A. speciosus*

A. argenteus			A. speciosus		
rank		scaled rank	rank		scaled rank
1	Clavicle	0.00	1	Clavicle	0.00
2	Humerus	0.11	2	Humerus	0.11
2	Ribs	0.11	2	Ribs	0.11
2	Radius	0.11	2	Radius	0.11
2	Ulna	0.11	2	Ulna	0.11
2	Scapula	0.11	2	Scapula	0.11
2	Cervical vertebrae	0.11	2	Cervical vertebrae	0.11
3	Thoracic vertebrae	0.22	2	Thoracic vertebrae	0.11
4	Femur	0.33	2	Femur	0.11
4	Tibia	0.33	2	Tibia	0.11
4	Fibula	0.33	2	Fibula	0.11
4	Lumbar vertebrae	0.33	2	Lumbar vertebrae	0.11
4	Ilium	0.33	2	Ilium	0.11
5	Sacral vertebrae	0.44	3	Sacral vertebrae	0.22
5	Metacarpal	0.44	3	Metacarpal	0.22
5	Ischium	0.44	4	Ischium	0.33
5	Pubis	0.44	5	Pubis	0.44
5	Sternum	0.44	5	Sternum	0.44
6	Caudal vertebrae	0.56	6	Metatarsal	0.56
6	Metatarsal	0.56	7	Manual phalanges	0.67
7	Manual phalanges	0.67	7	Pedal phalanges	0.67
8	Pedal phalanges	0.78	8	Caudal vertebrae	0.78
9	Tarsals	0.89	9	Tarsals	0.89
10	Carpals	1.00	10	Carpals	1.00

It is evident that the early onset of the caudal vertebrae is a species-specific developmental pattern of the *A. argenteus* and that a heterochronic shift has occurred at some point in the lineage of *Apodemus*. It is known that habitats of these two congeneric rodents broadly overlap in many forests [17]. Horizontal habitat segregation occurs between the two species in sympatric environments [17, 19]. In addition, *A. argenteus* exploits arboreal habitats in summer and autumn, and *A. speciosus* explicitly utilizes terrestrial habitats over the year [17], resulting in vertical habitat segregation. It is now widely accepted that such ecological partitioning facilitates interspecific competition and thus allows sympatric distribution of the two [15, 17, 19]. Identification of the two species by outer morphology is often difficult, but it is known that the relative length of the tail is clearly different [1]. The tail length is generally longer than the crown-rump length in *A. argenteus,* whereas it is equivalent to or shorter than the crown-rump length in *A. speciosus* [1]. It is reported that despite its significantly smaller body size, *A. argenteus* possesses more number of vertebrae [11], presumably reflecting more number of caudal vertebrae and longer tail in this species. This long tail is routinely employed for arboreal locomotion and facilitates improved balancing on tree substrates [5].

I suggest that the earlier appearance of the caudal vertebrae in *A. argenteus* is related to its long tail, which is essential for its arboreal locomotion. I argue that earlier initiation of vertebral development is linked to its lengthening of the tail compared to *A. speciosus*. Previously, the author and colleagues discovered that bats are characterized by earlier ossification of the foot and the thumb of the manus [8]. It is known that the foot length of bat neonates is almost equal to the feet of adults [6, 13, 29], while neonatal foot length falls between 30 to 50% in other mammalian taxa [3, 10, 16]. Bats' thumbs are also well developed already at birth and similar in size to those of adults [6]. It is known that their feet and hand thumbs are utilized to tightly grab the mother or cling to the wall of the nursery areas [2, 12, 13].

The case reported here suggests that developmental changes can lead to changes in adult morphology. Nonetheless, the evolutionary impact or consequence of heterochronic shifts in ossification sequence is still disputed [14, 21, 26, 27]. Most of the studies examining whether earlier appearance of characters is correlated to larger prominence in adults have often compared distantly related species. However, such hypothesis can only be tested within a phylogenetically independent context or by comparisons of closely related taxa as done in this preliminary report. It must be noted that not all detected changes in onset of ossification are exclusively related to changes in adult morphology. This is because changes in adult morphology can be achieved by multiple pathways, i.e. shifting the timing, tempo and mode of development. Besides, even if the onset of ossification is accelerated, there may be cases that the developmental speed is even decreased, resulting in little change of adult morphology. In studies of ossification heterochrony [7–9, 14, 21, 26, 27], often the tempo and mode of development are not treated. In order to test whether earlier appearance of characters is correlated to larger prominence in adults, one should compare closely related species and examine not only the onset timing but also tempo and mode of development.

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