SHORT COMMUNICATION

Ossification sequence in the mole *Talpa occidentalis* (Eulipotyphla, Talpidae) and comparison with other mammals

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The skeleton of mammals has been the subject of detailed anatomical and functional studies for centuries, and investigations of its ontogeny enjoy a long tradition (Starck 1995). However, knowledge of the timing of ossification is restricted to few species. Most available data of this kind pertain to species used in the lab or domesticated animals (e.g., Curgy 1965). Here we report on the ossification timing in the Iberian mole *Talpa occidentalis* (Fig. 1), based on the study of a developmental series obtained in studies of reproductive biology and growth in this species (e.g., Barrionuevo et al. 2004). This study complements those on the ossification patterns in the European mole *Talpa europaea* (Prochel 2006; Goswami and Prochel 2007) and in other eulipotyphlans (Prochel et al. 2004).

The sequence of developmental events in mammalian evolution has been subject of some attention in recent years (e.g., Bininda-Emonds et al. 2003), correlated with the development of analytical techniques to study sequence heterochrony (Richardson et al. 2001). Relatedly, studies of ossification timing have been conducted, concentrating on heterochrony (Smith 1997; Sánchez-Villagra 2002; Schoch 2006) and modularity (Goswami 2007).

Twenty-nine specimens of *T. occidentalis* (Cabrera, 1907) were cleared and double-stained for bone and cartilage (Dingerkus and Uhler 1977; Prochel 2006). The onset of ossification, shown by intake of alizarin red, was examined in 25 cranial and 24 postcranial elements and was recorded in (prenatal) stages 7–9 (Barrionuevo et al. 2004). The results were compared with those resulting from previous studies of a similar kind of 22 prenatal stages of *T. europaea* for the cranium (Goswami and Prochel 2007) and the postcranium (Prochel 2006).

Onset of ossification data of 11 selected cranial elements in eight placentals and four marsupials and of postcranial elements in 10 therian and three sauropsid species were compiled in two matrices of pairs of events (i.e., onset of ossification of each element is related to every other one), as described in Smith (1997). Sequence data for the non-talpid species, including the sauropsids used as outgroups, were taken from Sánchez-Villagra (2002 and references therein), Nunn and Smith (1998), and literature cited therein. A similar analysis was performed by Sánchez-Villagra (2002) on the same postcranial data set without the talpids, and by Prochel (2006) without *T. occidentalis*.

The cranial matrix including 12 taxa and 11 elements resulted in 55 event-pairs (characters), whereas the postcranial matrix of 14 taxa and 24 elements resulted in 276 event pairs (characters). These 'characters' were mapped on existing cladograms, which include the species examined or close relatives (Fig. 2) using Mesquite (Maddison and Maddison 2006). We coded

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Fig. 1. Lateral view of a macerated skull of an adult *Talpa occidentalis* (TO 506).

in each event-pair (= character) the relative timing of the onset of ossification of the two elements, using 0 = before, 1 = simultaneous, and 2 = after.

Cranial ossification began in stage 8 and proceeded quickly. The first specimens with ossified cranial elements display 16 cranial elements in which ossification has started (Tables 1 and 2). This lack of resolution is due to a lack of early stage 8 specimens. The basisphenoid was visible only as a small centre of ossification in the most advanced stage 8 specimen. The lacrimal was anteroposteriorly elongate in stage 8, but in later stages, it was ovoid and bore a well-defined lacrimal canal. The infraorbital canal was complete by stages 8–9. The mastoid was only weakly ossified at its dorsal boundary, near the parietal, in stages 8 and 9, but it was well ossified by stage 10. The jugal was not discernable in any specimens, potentially due to early closure of the zygomaticomaxillary suture. This suture fused soon after the first appearance of the jugal in stage 8 specimens of *T. europaea* (Goswami and Prochel 2007), and this period was poorly resolved in *T. occidentalis*, as noted above. Canines and premolars were first observed by staining of the enamel by alizarin red in specimens of stages 8–9, and incisors appeared in stage 9. The alisphenoid was visible as a small ossification centre in stages 8–9, and was well ossified in stage 10. The orbitosphenoid was visible for the first time in stage 10. The ethmoid appeared as a small ossification centre in stage 10.

The first eight postcranial elements to start ossification in stage 8 are elements in the upper part of the body (clavicle, scapula, humerus, radius, ulna, ribs, and cervical vertebrae. in addition to calcified tendons disto-palmar to the third phalanx). Elements of the lower appendicular skeleton, including the femur, tibia, and fibula, as well as the thoracic vertebrae, are next to begin ossification, in early stages 8–9. Later in stages 8–9, the ilium begins ossification, followed closely by the lumbar vertebrae. The sacral and caudal vertebrae, sternum, pubis, and ischium begin ossification by stage 10. The distal elements of the appendicular skeleton are last to ossify, starting with the phalanges of the hand

starting later in stage 10. These are followed by the tarsals and the phalanges of the foot. In the latest stage 10 specimens, the metatarsals begin ossification. The carpals do not begin ossification in any specimens examined in this study, and are thus presumably the last postcranial elements to ossify.

The cranial ossification sequence of *T. occidentalis* broadly resembles that of *T. europaea*, with the main differences being that the lacrimal and alisphenoid ossify later and the basisphenoid ossifies earlier in *T. europaea* than in *T. occidentalis*. The delay of the alisphenoid is also evident in raw timing of ossification, as the alisphenoid is not visible in stages 8 or 9 of *T. europaea*, but appears in stages 8–9 in *T. occidentalis*. Other differences in relative timing of ossification are probably due to differences in resolution, as *T. europaea* specimens include more early and intermediate stages, whereas later stages are better represented in *T. occidentalis*.

As reported for *T. europaea* (Goswami and Prochel 2007) and shown here for *T. occidentalis*, the cranial elements in these moles appear in a very short period during prenatal development. Therefore it is a well-resolved sequence of ossification for most cranial elements difficult to obtain. However, we found that there is at least one unequivocal autapomorphy for the talpids in the onset of cranial ossification: talpids are the only mammals included in this study in which the alisphenoid appears after the basisphenoid. This character is not resolved in *Mus*, but is well resolved in all other taxa. Another potential autapomorphy is that the basisphenoid starts to ossify before the exoccipital in *T. occidentalis*. The exoccipital ossifies before the basisphenoid in all of the other mammals considered here, with the possible exception of the other talpid, *T. europaea*, in which it is unresolved. Thirteen other changes in the cranial onset of ossification sequence appear as unequivocal autapomorphies of talpids, six of which are unique for talpids. However, in all but two of these cases, the two *Talpa* species show the 'simultaneous' ossification condition. Thus, it is possible that these features reflect of lack of resolution of the data, and these autapomorphies require analysis when more detailed sequence data become available.

Several clades other than talpids show cranial sequence synapomorphies. In *Tupaia* and *Tarsius* and in *Manis* and *Felis* the jugal starts to ossify before the exoccipital, which is possibly a derived condition among the placentals. In *Manis* and *Felis* the jugal starts to ossify before the squamosal. This character is unresolved in two of the marsupials, *Dasyurus* and *Perameles* and is opposite in all other mammals. The parietal ossifying before the exoccipital appears to be a marsupial synapomorphy.

The postcranial changes in the onset of ossification that characterize the two talpids concern vertebrae, the

Fig. 2. Phylogeny of the taxa considered in the: (a) cranial and (b) postcranial event-pairing comparisons. The phylogeny in (a) is based on Amrine-Madsen et al. (2003) for placentals. Current controversies about the relationships among australidelphians marsupials are reflected in the lack of resolution in parts of the tree (Asher et al. 2004; Phillips et al. 2006). The phylogeny in (b) is taken from references in Sánchez-Villagra (2002).

scapula, and sternum versus either hind limb, pelvic portions or autopodial elements. The vertebral column has to be stable and holds the relatively large and derived humerus (Sánchez-Villagra et al. 2004) and also provides a surface of attachment for shoulder muscles in the thoracic region. As in *T. europaea* (Prochel 2006) the

first calcification seen in the hand of *T. occidentalis* are calcified tendons disto-palmar to the third phalanx.

Concerning the event-pairing analysis for postcranial elements, of 276 event-pairs, 46 (17%) are different between the two talpids, one species having value '0' and the other one '2'. The rest of 230 event pairs (83%) are

Table 1. Relative timing of the first appearance of ossification in selected cranial skeletal elements of *Talpa europaea* and *T. occidentalis*

	T. europaea	*T. occidentalis*
Premaxilla	1	1
Maxilla	1	1
Palatine	1	1
Dentary	1	1
Frontal	1	1
Nasal	1	1
Parietal	1	1
Squamosal	1	1
Presphenoid	1	1
Pterygoid	1	1
Basioccipital	1	1
Supraoccipital	1	1
Exoccipital	2	1
Mastoid	2	1
Basisphenoid	2	2
Jugal	3	?
Ectotympanic	3	1
Lacrimal	4	1
Canine	4	3
Premolar	4	3
Petrosal	5	5
Alisphenoid	6	4
Incisor	6	5
Ethmoid	6	6
Orbitosphenoid	6	6

The numbers represent relative timing and order of onset of ossification, starting with '1'.

Table 2. Relative timing of the first appearance of ossification in selected postcranial skeletal elements of *Talpa europaea* and *T. occidentalis*

	T. europaea	*T. occidentalis*
Clavicle	1	1
Ribs	1	1
Radius	2	1
Ulna	2	1
Tibia	2	2
Fibula	2	2
Cervical vertebra	2	1
Thoracic vertebra	2	2
Scapula	2	1
Humerus	3	1
Femur	3	2
Lumbar vertebra	3	4
Ilium	4	3
Sternum	5	5
Sacral vertebra	5	5
Caudal vertebra	6	5
Metacarpal	7	7
Phalanges pes	7	7
Metatarsal	7	8
Ischium	7	5
Pubis	7	5
Phalanges manus	7	6
Tarsals	7	7
Carpals	7	9

The numbers represent relative timing and order of onset of ossification, starting with '1'.

either identical for both talpids or at least one of the species is unresolved because of lack of resolution ('1').

Ten event pairs are unambiguous autapomorphies of mammals in the current sampling. One event-pair characterizes both talpids and otherwise only marsupials, the scapula starting ossification before the femur. There are seven unambiguous autapomorphies of both talpids involving a change in relative timing to the following conditions: scapula before femur, sternum before phalanx in pes and before metatarsal, cervical vertebrae before ilium and before femur, thoracic vertebrae before ilium and caudal vertebrae before phalanx in manus.

It is difficult to build ontogenetic series from sampling of wild populations. Great numbers of moles were available to us as a by-product of ongoing studies of the development of the sexual anatomy and reproduction in this species. However, resolution for ossification sequence is poor, as ossification happens in a very short period of time (see Goswami and Prochel 2007). As shown by Sterba (1977), in *T. europaea* the highest growing rate is between stage 8 and 9 in the last fifth of is intrauterine development. In both talpids examined here, *T. europaea* and *T. occidentalis*, this is reflected by

the fast onset of ossification and growth of several postcranial skeletal elements in these developmental stages.

There is a relatively low number of synapomorphies in the ossification sequence for the moles, but, considering the lack of resolution in the ontogenetic data, there are potentially many more. Future studies should concentrate on the short period encompassing the appearances of the skeletal elements in question, particularly within the early part of stage 8. This study demonstrates that intrageneric variation in ontogenetic series exists. However, variation at this taxonomic level is likely to be much less in mammals than in ectotherms (cf. Mabee et al. 2000).

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