



Heterochronic shifts in the ossification sequences of surface- and subsurface-dwelling skinks are correlated with the degree of limb reduction

Jasmina Hugi^{a,*}, Mark N. Hutchinson^b, D. Koyabu^a, Marcelo R. Sánchez-Villagra^a

^a Palaeontological Institute and Museum, University of Zurich, Karl Schmid-Straße 4, CH-8006 Zurich, Switzerland

^b South Australian Museum, North Terrace, Adelaide, SA 5000, Australia

ARTICLE INFO

Article history:

Received 24 June 2011

Received in revised form

26 September 2011

Accepted 13 October 2011

Keywords:

Bone formation

Fossoriality

Limb reduction

Scincid lizards

Squamates

ABSTRACT

Scincid lizards exhibit a variety of limb anatomies which reflect the functional requirements of different modes of life. Besides surface dwellers which show neither body elongation nor limb reduction, there are numerous examples that can be arranged as increasingly serpentine taxa moving in sand, humus or leaf litter. We explored the question of whether limb reduction and body elongation in skinks are linked to heterochronic shifts in the ossification sequences. The study material comprises skinks showing four different morphotypes: *Liopholis whitii*, *Lerista bougainvillii*, *Hemiergus peronii* and *Saiphos equalis*. Results showed that (i) scincid lizards with limb reductions exhibit an earlier onset of ossification in the cervical vertebrae, and (ii) ossification starts earlier in the pectoral girdle (scapula and coracoid) and pelvic girdle (ilium, ischium and pubis) relative to the timing of the onset in elements of the forelimbs and hind limbs. Furthermore, they show (iii) an earlier strengthening of the premaxilla, which first completes the anterior part of the dorsal cranial roof, and (iv) an earlier onset of ossification in the forelimb elements than in the equivalent elements of the hind limbs. The species showing the least limb reduction (*L. bougainvillii*) had the greatest developmental similarity to the normally proportioned surface-dwelling species (*L. whitii*). *S. equalis*, as the morphotype with the greatest deviation from the normally proportioned, pentadactyle form, varies the most from *L. whitii*. The heterochronic shifts in the ossification sequences are linked to a shift in the emphasis from limbed locomotion to trunk locomotion in the species with body elongation and/or limb reduction.

© 2012 Elsevier GmbH. All rights reserved.

1. Introduction

The phenomenon of limb reduction and its underlying developmental processes is a classic topic in evolutionary biology and tetrapod morphology. The squamates have undergone numerous limb reduction events, sometimes with multiple events occurring within single evolutionary lineages. In some cases, sets of morphological character states can be arranged as an evolutionary series of morphotypes in related taxa (e.g., Siler and Brown, 2011). Such lineages therefore include a variety of morphotypes, ranging from some that show neither body elongation nor limb reduction to forms with a moderately to strongly serpentine body shape (Greer, 1991; Caputo et al., 1995; Wiens et al., 2006; Skinner et al., 2008; Brandley et al., 2008).

Scincid lizards are a diverse group of squamates which are widely distributed in all continents except Antarctica (e.g., Mattison, 1989; Hutchinson, 1993). Various different morphotypes are recognised, each broadly related to their ecology, including

surface dwellers, “sand swimmers” and forms that move in humus or leaf litter. The two latter groups show body elongation and/or limb reduction, but use their limbs to a varying degree when on the surface and shift to lateral undulation with adpressed limbs when moving below the surface (e.g., Choquenot and Greer, 1989; Caputo et al., 1995). Limb reduction in skinks can vary from reduction in relative limb size to the loss of single phalangeal elements, entire digits and ultimately the entire limb. Elements are always lost along the long axis, i.e., digit IV, or the distal expanses of the foot, i.e., digit I and V (Greer, 1987, 1990, 1991; Wiens et al., 2006; Wiens, 2009). If there were any functional reasons to reduce the region of the foot along either of these major axes, i.e., to shorten the reach of the foot or to narrow its span, the digits most likely involved would be the ones which are actually affected. Skeletal elements that get lost entirely are among the last to condense and chondrify in the developmental sequence in many cases (e.g., Shapiro et al., 2007).

The early development of skeletal elements comprises the mesenchymal condensation and chondrification events that follow two conserved pathways in tetrapods: anurans and amniotes show a proximodistal direction across the limb compartments (after Rieppel, 1993a) with a postaxial dominance in the autopodium

* Corresponding author. Tel.: +41 44 634 23 47; fax: +41 44 634 49 23.
E-mail address: jasmina.hugi@gmail.com (J. Hugi).

(e.g., Shubin and Alberch, 1986). Urodeles, in contrast, show a preaxial dominance in both the zeugopodial and the autopodial region with a proximodistal direction across the entire limb (e.g., Erdmann, 1933; Shubin and Alberch, 1986; Nye et al., 2003). The subsequent ossification sequences are often not simple recapitulations of the preceding developmental programme of condensation and chondrification (e.g., Rieppel, 1992a, 1994a,b) and show plasticities in the timing, which are influenced by functional requirements in both amniotes and ‘anamniotes’. The terrestrial surface-dwelling lacertilian squamates with non-reduced limbs and no body elongation form one group within the Amniota that shows rather conserved ossification sequences. For them, only minor heterochronic shifts regarding, for example, the position of the first digit that starts ossification at the postaxial side of the autopodial region have been reported (Rieppel, 1993a; Mohammed et al., 1995; Federico and Lobo, 2006; Fröbisch, 2008; Hugi et al., 2010). There is less information available for elongate, limb-reduced species, although the studies of Shapiro (2002) and Shapiro et al. (2003), who detected an adaptive significance in limb development in a series of scincid morphoclines, have been an important contribution to this area of research. Shapiro concluded that the skeletal development of the limb cannot be explained by a simple truncation of a putative ancestral developmental programme, but rather by substrate conditions and burrowing behaviour.

Here we describe the ossification sequences of the entire skeleton and parts of the chondrification in a set of four scincid lizard species that show a trend towards increasing limb reduction and elongation, from fully surface-dwelling and pentadactyle species to elongate, fossorial and tridactyle forms. The phylogenetic breadth and the methods employed address the question: how conserved are ossification sequences among lizards with different lifestyles? Event-pairing analysis is used as a comparative approach to study the extent of possible heterochronic shifts in the ossification data (Velhagen, 1997).

2. Materials and methods

The present study is based on the ontogenetic series of four scincid lizard species from Australia. *Liopholis whitii* is a surface-active, pentadactyle species with robust limbs and the plesiomorphic presacral vertebral count of 26. *Lerista bougainvillii* is a semifossorial species with an elongate body (35–38 presacral vertebrae) but it retains a pentadactyle forelimb and hind limb. *Hemiergis peronii* and *Saiphos equalis* are both markedly elongate and fossorial in lifestyle (according to Greer, 1987, 1989 the presacral vertebral counts range between 34–38 and 38–40, respectively) and show pronounced reduction in limb size accompanied by loss of phalanges (digital formula 4-4 in *H. peronii*, 3-3 in *S. equalis*).

Embryos of *L. whitii* and *H. peronii* were obtained from specimens in the collection of the South Australian Museum, Adelaide, embryos of *S. equalis* from material collected by Prof. M.B. Thompson, University of Sydney, and embryos of *L. bougainvillii* from females collected near Burra, South Australia, during November 2009. Specimens examined were as follows: 9 *L. whitii* (snout-vent length 13.4–31.4 mm; Naturkundemuseum Berlin; Hugi et al., 2010), 8 *H. peronii* (SVL 3.4–26.5 mm), 9 *S. equalis* (SVL 6.1–30.0 mm) and 10 *L. bougainvillii* (SVL 6.0–30.3 mm). The latter three series are deposited in the collection of the Paleontological Institute and Museum of the University of Zurich (PIMUZ).

Embryos were dissected out of female specimens (*L. whitii*, *H. peronii*, most embryos of *S. equalis*) or from shelled eggs (all *L. bougainvillii* and two *S. equalis*) and subsequently fixed in 4% neutral buffered formaldehyde for the first 24 h and then placed into 70% ethanol. This is the standard fixation procedure for the storage in collections. The biases that fixation and other procedures

may introduce in the staining do not invalidate the results, as these effects if present would be equal across the specimens examined (Mitgutsch et al., 2011). Morphological description is based on observations with a Leica MZ-16 dissection microscope (Leica Microsystems, Wetzlar, Germany); pictures were taken with a DFC 420C digital camera (Leica Microsystems) and the accompanying IM 50 software; the drawings were processed with Adobe Illustrator CS3 (Adobe Systems, San Jose, CA, USA). The embryos were arranged based on body length, which was measured to the nearest 0.1 mm using digital callipers. Body length is defined as the distance between the tip of the snout and the end of the cloaca, a standard measurement in squamate research (Maisano, 2001, 2002a,b).

Specimens were subjected to a clearing and double-staining procedure (Dingerkus and Uhler, 1977), with modifications according to Hiroshi Nagashima, Kobe (pers. comm. 2007). This procedure visualises the onset of chondrogenesis and ossification. The chondrogenic sequences are only preserved in the autopodial regions of both the forelimbs and the hind limbs of all four skinks. No distinction is made between onset of calcification and onset of ossification as both are part of a continuous process. The first visible uptake of Alizarin red, i.e., as red-stained areas in the bones, is interpreted as the onset of ossification (Table 1). The data on the timing of the onset of ossification relative to the size of the embryo is complemented with data on the first appearance of red staining relative to its first appearance in all of the other elements (Table 1: numbers in brackets). To further visualise heterochronic shifts in the ossification sequences of the four scincid lizard species, data of four surface-dwelling lizard species with non-reduced limbs were included for event-pairing (Velhagen, 1997; see Table 2). Some elements of Table 2 are treated as compartments of skeletal groups as defined in terms of function and/or topographical region and/or members of a metameric series (Hugi et al., 2010). The first structure that ossifies within each compartment is taken to represent the onset of ossification of the group as a whole (e.g., jugal in the dermatocranium). Some skeletal elements are neither included in groups nor listed separately in Table 2 (e.g., pisiform, radiale, phalanges) due to an invariable timing of onset of ossification (postnatal ossification/never ossified) or a lack in resolution in all examined squamates (e.g., no further division within the phalanges). The 35 characters of the eight lizard species included in the present study result in 595 event-pairs. Three character states reflect the timing of one event relative to another (Table 3): before (0), simultaneously (1), or after (2) (Velhagen, 1997). Simultaneous events, which are ties, probably result from a lack in resolution, since it is unlikely that ossification of two or several bones occurs at exactly the same time. We further analysed the ossification heterochrony by the continuous analysis approach introduced by Germain and Laurin (2009). This method applies squared-change parsimony (Maddison 1991) and Felsenstein's (1985) independent contrasts to assess sequence heterochrony by providing confidence intervals on nodal values. All branch lengths are set as equal to 1 (Wiens and Slingluff, 2001) as this information is not available for all studied species. The analysis was conducted with the PDAP module of Mesquite (Maddison and Maddison, 2002; Midford et al., 2003).

Maisano (2001, 2002a,b) provided a standardised usage of skeletal terms in earlier studies, which is followed here. Symphyses in the pectoral girdle, as well as symphyses and processes in the pelvic girdle (iliac process, epi- and prepubis, ischial symphysis and metatarsal process) only ossify within secondary ossification centres in neonates (e.g., Rieppel, 1992b; Maisano, 2001, 2002a,b; Jerez and Tarazona, 2008). Processes of the pectoral girdle (suprascapula and epicoracoid), as well as the sternum calcify but never ossify (Mohammed et al., 1995). Furthermore, the elements in the skull neither fuse, nor, in some cases, do they completely ossify in embryos (e.g., there is still a parietal fontanelle in the largest

Download English Version:

<https://daneshyari.com/en/article/2791169>

Download Persian Version:

<https://daneshyari.com/article/2791169>

[Daneshyari.com](https://daneshyari.com)