

The Ossification Sequence of *Aneides lugubris*, with Comments on Heterochrony

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ABSTRACT.—Post-hatching ontogeny is described for the skeleton of a large, direct-developing, terrestrial plethodontid salamander, *Aneides lugubris*. This species appears to have the most prolonged ontogeny of the plethodontids. Until the end of the second year of life, osteological ontogeny is similar to that of other species, but beyond that point major changes occur. Ossification of the long bones continues throughout life and is never completed. Elements such as the pelvic plate ossify continuously, but never completely. Mesopodial elements remain cartilaginous throughout life. The most dramatic changes relate to the feeding system. The anterior part of the skull becomes grotesquely enlarged in the largest specimens, with extensive coossification. Maxillary and dentary teeth decrease in number but increase in size and complexity. Features identified as "supportive adaptations" appear during ontogeny, more so in the feeding than in the locomotory mechanism. Data are interpreted in the framework of recent studies of heterochrony.

Salamanders of the family Plethodontidae present an unusually good opportunity for investigating the role of developmental modes of evolution in the production of phylogenetic patterns. The family includes a wide array of adaptive morphologies. Several species (in the genera *Gyrinophilus*, *Haideotriton*, *Eurycea*, and *Typhlomolge*) are permanently aquatic and never metamorphose from their essentially larval morphology (Dunn, 1926; Wake, 1966). In contrast, over two-thirds have abandoned the aquatic larval stage, have direct terrestrial development, and occupy a vast array of terrestrial habitats, from temperate deciduous forests to desert canyons and tropical rain forests. The terrestrial species range from surface-dwelling salamanders to fossorial and arboreal forms. Such developmental modes of evolution as heterochrony have been very important, as is evident from the neoteny of the permanently larval species. However, heterochrony is also of major significance in the direct develop-

ing species, in which its pervasive role has been documented for several lineages (Wake, 1966; Alberch and Alberch, 1981). Surprisingly, not one of the several normal tables of development available for salamanders deals with a plethodontid, despite the fact that nearly two-thirds of all living species of salamanders belong to this family. Only *Eurycea bislineata*, an eastern hemidactyline plethodontid with an aquatic larval stage, has had a thorough description of development (Wilder, 1925), and this description is not as detailed as, for example, that of Erdmann (1933) for *Triturus vulgaris*.

The present study is an attempt to provide a reasonably detailed account of post-embryonic development for a direct developing species, *Aneides lugubris*. This species was selected because, of all the plethodontids, it seems to progress the furthest in development. We believe that all plethodontids are paedomorphic relative to ancient amphibians, and that the paedomorphic process has reversed to some degree in *A. lugubris*. Accordingly, we are likely to have a more complete postembryonic development displayed in this species than in any other known to

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us, and the species is thus an appropriate one for the establishment of a baseline to serve in future comparative studies.

Aneides lugubris is a large, scansorial and arboreal species from oakwoodland habitats in California and northern Baja California. The natural history and adult morphology of the species is relatively well known (see a literature review by Lynch and Wake, 1974). The species displays morphological and behavioral adaptations for arboreality and feeding that are of special interest to us. These adaptations appear to have their foundation in two "key innovations" (identified by Wake, 1963, 1966) that distinguish the genus *Aneides* from *Plethodon* and *Ensatina* (see discussion by Larson et al., 1981). In addition, a number of morphological trends in these genera reach their culmination in *A. lugubris*. Thus, these key innovations and apparent trends will also be a major focus of this paper.

We will concentrate on developmental osteology. Aspects of osteology of *A. lugubris* have been described by many workers, and these observations were summarized and expanded by Wake (1963). Most previous work has dealt only with fully adult conditions. Here we will concentrate on production of adult conditions starting with relatively generalized hatchlings.

MATERIALS AND METHODS

An ontogenetic series of 19 *A. lugubris* were prepared for clearing and staining for cartilage (alcian blue) and bone (alizarin red S) according to the methods of Hanken and Wassersug (1981). Hatchlings through large adults of both sexes were included (snout-vent lengths [SVL] were 18.5, 20.0, 20.2, 21.5, 27.0 (2), 31.5, 34.1, 40.0, 43.9, 47.6, 48.0, 50.0, 58.0, 68.2, 74.0, 79.6, 85.0, and 88.0). No sexual dimorphism has been observed in the traits under consideration. Specimens were collected in Berkeley, Alameda County, California; and Point Reyes Station, Marin County, California. Measurements were taken with a dial calipers under a dissect-

ing microscope. Illustrations were prepared by projecting the specimens with a microprojector and outlining the projected images.

RESULTS

We describe below the state of ossification of our *least* developed specimen and then compare sequentially more advanced specimens to it in order to assess ontogenetic change in ossification.

18.5 and 20.2 mm SVL.—Skull: All bones present in adults are already ossified in this specimen. However, the premaxillae are fused only by a thin bridge on the dentigerous components, and the frontal processes are thin, straight splints projecting postero-dorsally that do not meet. The maxilla lacks the posterior enlargement typical of adults. The maxilla and prefrontal do not meet. There is no coossification of skin to skull elements. There is an enormous dorsal fontanelle between the pairs of frontals and parietals. The articular component of the lower jaw and the quadrate are cartilaginous. Tooth numbers on the dentary and maxilla are significantly higher than in adults (Fig. 1ab), but not significantly different on premaxilla and vomer (all loci were counted) (Fig. 1cd). Otic crests are absent. The parasphenoid tooth patch is not ossified to the bone. The tectum synoticum of the dorsal occipito-otic complex is cartilaginous. The urohyal is cartilaginous.

Postcranial skeleton: The neural arch of the atlas is completely cartilaginous and consists mainly of the neural pedicel rudiment. The neural arches of all other vertebrae also consist primarily of the cartilaginous pedicels, with some ossification both anterior and posterior from the pedicel. Transverse processes are cartilaginous, and there is little ossification of the centrum. Tail vertebrae are cartilaginous. Only the centra of the vertebrae of the 18.5 mm specimen are ossified. The ribs are completely cartilaginous. The procoracoid and scapula, even in the region of the glenoid fossa, are cartilaginous. The components of the pelvic plate are weakly

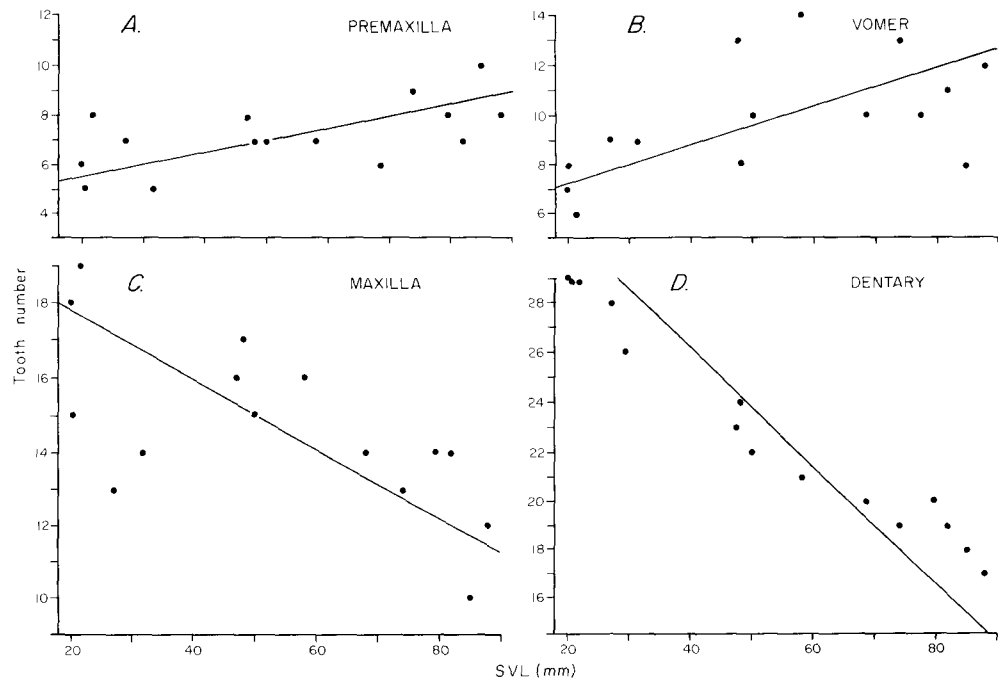


FIG. 1. Numbers of teeth at different body size in *Aneides lugubris*. A. Premaxillary tooth number vs. snout-vent length. $Y = 5.46 + 0.03X$; $r = 0.60$. B. Vomerine tooth number vs. snout-vent length. $Y = 7.15 + 0.05X$; $r = 0.54$. C. Maxillary tooth number vs. snout-vent length. $Y = 17.91 + 0.06X$; $r = 0.66$. D. Dentary tooth number vs. snout-vent length. $Y = 31.89 - 0.17X$; $r = -0.98$. Note that premaxillary and vomerine tooth numbers increase slightly with increase in body size (\approx age); maxillary and especially dentary numbers decrease significantly with increased size.

fused and entirely cartilaginous. Carpal and tarsal elements are cartilaginous, though adult in number and approximate configuration.

Central shafts of limb bones are ossified, but large proximal and distal components remain cartilaginous. Proximal phalanges are cartilaginous; distalmost are ossified but the tips are curved or irregular, rather than flattened and bifid as in other specimens.

20.0 mm.—This specimen, though slightly smaller than the larger described above, is more advanced osteologically. The skull is in a similar state, with a somewhat greater number of maxillary and premaxillary teeth and the same number of dentaries. The dorsal fontanelle is smaller, but still very large, extending both laterally and longitudinally.

Postcranial skeleton: Ribs are ossified, except for the bicapital heads and the terminal one-third. Vertebrae are more ossified, though dia- and parapophyses, posterior part of the neural arch, and the articulating facets of pre- and postzygapophyses are cartilaginous (the latter throughout life). The neural arch of the atlas is more fully ossified, though the mid-roof region and the articulating facets are cartilage. In the pectoral girdle, the components of the glenoid fossa are ossified, though distally the components are cartilaginous. Limb bones are more extensively ossified. The distal end of the femoral trochanter is cartilaginous. The phalanges are ossified and terminal ones are flattened and bifid; a ventral process for tendon insertion is present.

21.5 and 27.0 mm.—Specimens are much like those described above, but with a

decrease in dentary and maxillary tooth number and slightly more ossification of ribs, vertebrae, glenoid region, pelvic plate (62%, calculated by dividing length of ossification by mid-ventral length of the pelvic plate; Fig. 2), and long bones. In the skull, the quadrate is ossified, but the articular element of the lower jaw is not. The prefrontal and maxilla are expanded, but do not meet. The dorsal fontanelle remains prominent.

31.5 mm.—The specimen is notable for having the urohyal ossified, as it is in all larger specimens, a further reduced dentary tooth number, and slightly more ossification of the pelvic plate, long bones, and ribs.

34.1–43.9 mm.—Skull: The frontal processes of the premaxilla are slightly to moderately dilated postero-dorsally. They do not fuse, although they approach contact posteriorly, behind the internasal gland, in the 43.9 mm specimen. There is no coossification of skin to the anterior elements of the skull, although faint ridges that may presage coossification appear on the prefrontal processes of the 43.9 mm specimen. The prefrontal lacks contact with the maxilla on the left side in the 34.1 mm specimen (and is absent on the right), is in contact on both sides in the 40.0 mm specimen, and shows some overlap in the 43.9 mm specimen. The dorsal fontanelle is greatly reduced in the larger animals, and is represented only by a straight-edged separation medially. All three specimens show a very slight ridge medially on the otic capsules that foreshadows the otic crests of larger animals. The quadrate is almost fully ossified in the three specimens, but the articular is largely cartilaginous, with the greatest amount of ossification found in the 43.9 mm specimen.

Postcranial skeleton: The neural arch of the atlas is not completely ossified, although the cartilage is nearly completely eroded in the 43.9 mm specimen. Neural arches of other vertebrae are cartilaginous posteriorly. Rib heads are ossified except for the proximal tips in the 34.1 mm specimen. The trochanter of the

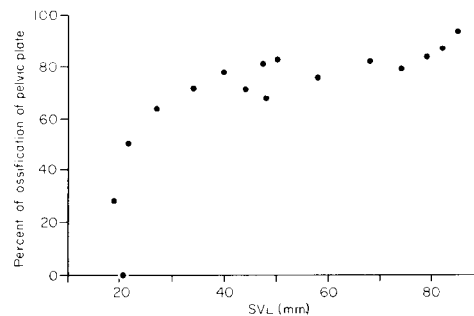


FIG. 2. Percent ossification of the pelvic plate at different sizes in *Aneides lugubris*. The pelvic plate is cartilaginous at hatching, but well ossified in the second year of age (40–50 mm SVL). Percent of ossification is calculated by dividing the length of the ossified component of the pelvic plate by the length of the plate at its ventral midline.

femur is ossified except for its distal tip. The pelvic plate shows increased ossification (69–77%; Fig. 2) as does the femur (78–85%, calculated by dividing length of ossified shaft by total length of femur; Figs. 3 and 4).

47.6–50.0 mm.—Skull: The frontal processes of the premaxilla are dilated posteriorly and in contact in the two larger specimens. Coossification is present on the premaxilla, and indicated on prefrontals and nasals. The otic crests are more pronounced. The prefrontals and maxillae show contact to slight overlap of their margins (50 mm specimen). The dorsal fontanelle has disappeared. The quadrate is well ossified, and the articular is ossified but for the synovial cap. Dentary and maxillary teeth numbers are further decreased (Fig. 1ab). The parasphenoid tooth patches are ossified to the bones, as in larger specimens.

Postcranial skeleton: The neural arch of the atlas is completely ossified (in 47.6 and 50.0 mm specimens but not in 48.0); rib heads, except for the articular facets, and dia- and parapophyses are ossified (although the posterior part of the neural arches of trunk vertebrae are still cartilaginous). Ossification occupies 67–82% of the length of the pelvic plate (Fig. 2), with pubic and ischial tips cartilaginous as are

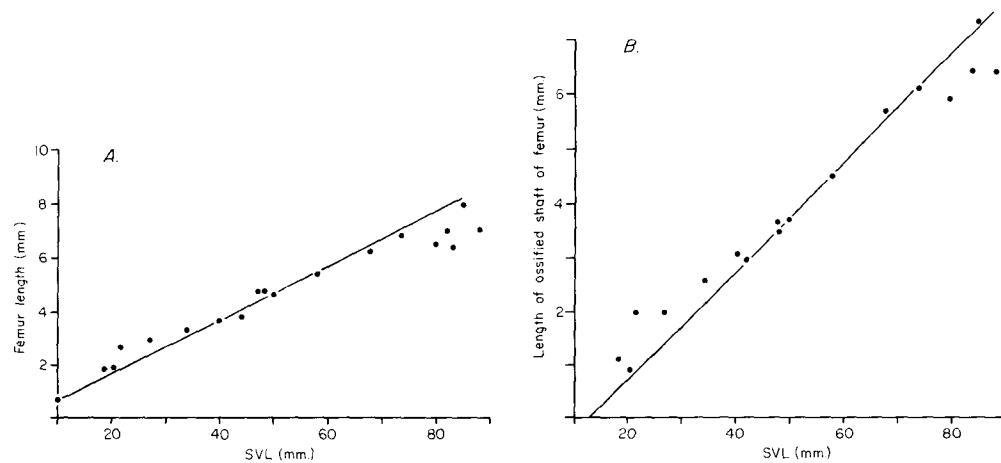


FIG. 3. Changes in the femur during growth of *Aneides lugubris*. A. Femur length vs. snout-vent length. $Y = 0.64 + 0.08X$; $r = 0.98$. Femur length is strongly correlated with body length; leg length therefore does not display important allometry. B. Length of the ossified shaft of the femur vs. snout-vent length. $Y = 0.32 + 0.08X$; $r = 0.99$.

iliac extensions. Femoral ossification is similar to that of the previously described group.

58.0 mm.—Skull: Otic crests are pronounced. The dorsal flange of the maxilla and the prefrontal overlap slightly. The frontal processes of the premaxilla are dilated and fused at one point, forming a fontanelle. Coossification is more extensive, spreading from the premaxilla to prefrontal and nasals. The trends in dentition continue (Fig. 1).

Postcranial skeleton: Ossification of pelvic plate (76%) and femur (83%) progresses (Figs. 2-4). Ossification of the atlas is complete, as in all larger specimens.

68.2-79.6 mm.—Skull: The tectum synoticum is almost fully ossified. Maxillary-prefrontal overlap is more extensive. Fusion of frontal processes of the premaxilla is more extensive, as is coossification of skin to anterior skull elements. The frontals and parietals have grown tightly together and form a median ridge. Dentary and premaxillary, but not maxillary, tooth numbers are reduced (Fig. 1). Both quadrate and articular components of the jaw articulation are nearly fully ossified. Coossification is more extensive.

Postcranial skeleton: The ossification of

the pelvic plate (79-83%) and the femur (88-91%) continues. The neural arches are fully ossified posteriorly. The trochanter of the femur is ossified but for a cartilaginous cap. The 68.2 mm specimen has a developmental anomaly involving its anterior vertebrae and their ribs. The vertebrae are shortened, cramped together, and appear to have sites of fusion. A rib is absent from one side of one vertebra; ribs appear to have duplicated on the opposite side of the adjacent vertebra.

80.0 mm.—Skull: The trends of more extensive coossification and diminished dentary and maxillary tooth numbers are evident. Otic crests are more pronounced, and the maxilla extends farther dorsally in its prefrontal articulation. The jaw articulation is nearly fully ossified.

Postcranial skeleton: The pelvic plate is more extensively ossified (86%) and the femur is the most extensively ossified of any specimen (93%) (Figs. 2 and 4).

85.0 mm.—This specimen is the most extensively ossified of the sample. Maxillary and vomerine tooth numbers are least for the sample, dentary nearly so; however, the premaxillary number is greatest in the sample (Fig. 1). Coossification, fusion of prefrontal processes of the pre-

ERRATA: Figure 3. Regression equation for A is $Y = 1.88 + 0.26X$; for B is $Y = -0.88 + 0.27X$.

maxilla, secondary overlap of the fontanelle by bone, and overlap of maxillary and prefrontal area more extensive than in smaller specimens. The frontal bones are fused together medially, as are the parietals, and there is a prominent medial ridge.

Postcranial skeleton: Significant changes have occurred in the postcranial skeleton. All elements are more heavily ossified. The pelvic plate is nearly fully ossified (93%) and the femur is 92% ossified, there being only a thin cartilaginous cap articulating at the acetabulum and a more extensive cap at the distal end. In this ontogenetic series, the ossification of elements forming the glenoid fossa has increased only slightly but progressively with age (size). The posterior margins of the suprascapulae are ossified.

88.0 mm.—Trends in ossification of the skull continue including those of tooth number, overlap, fusion, and coossification. However, the pelvic plate is less extensively ossified (88%) than in the 85.0 mm specimen, as is the femur (91%). Also, there is no indication of bone in the suprascapulae. The mass of all ossified elements appears to be increasing in the 85.0 and 88.0 mm specimens, both in dimensions and in depth of staining.

DISCUSSION

Summary of the Ossification Sequence.—At hatching, most of the bones that compose the adult skeleton have at least begun to ossify, including those of the skull. However, several ossifications associated with feeding and locomotion are not present in hatchlings. Ossification of the jaw articulation and the frontal processes of the premaxilla, contact of prefrontal with maxilla, and development of the otic crests occur later. The glenoid fossa and the pelvic plate are cartilaginous at hatching, though ossification begins very shortly thereafter. The distal phalanges are ossified, but not the more proximal ones, and the shafts of the long bones are not well ossified. However, by the end of the first year post-hatching (~30–35 mm SVL, estimated by size *vide* Miller, 1944), the

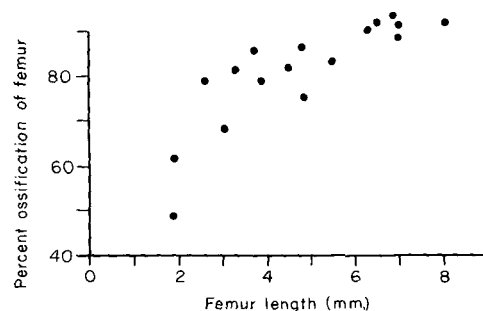


FIG. 4. Percent of ossification of the femur as a function of femur length in *Aneides lugubris*. The femurs of immature animals (<50 mm SVL; see Fig. 3) are less completely ossified than those of adults. In adults, the shaft is 80–95% ossified, but the articular facets and some of the shaft remain cartilaginous.

locomotor apparatus is well ossified. The contacts, overlap, and fusion of anterior skull elements mentioned by Wake (1963) as a “key innovation” differentiating *Aneides* from *Plethodon* have not yet occurred.

The second year of life is taken to include animals from approximately 35 to 50 mm snout-vent length. These animals are also juveniles. Age class data, surprisingly, are not available for *A. lugubris*. Our estimates of the second year span and age at maturity are based on Miller (1944) and examination of data taken during preparation of osteological specimens for which SVL and gonad condition were recorded, and are similar to those of Lynch (1974). Gonads of both sexes showed inception of maturation at 48–50 mm SVL, so we estimate that age of maturity is two years. A number of osteological features correlate with this estimate. Those features associated with strengthening the feeding apparatus have appeared by the end of the second year post-hatching, and are therefore “adult” features. These include the ossification of the urohyal, to which hyoid muscles attach; development of the otic crest, to which the expanding jaw musculature attaches; dilation, contact and fusion of the frontal processes of the premaxilla; contact and overlap of the prefrontals and maxillae; coossification of skin

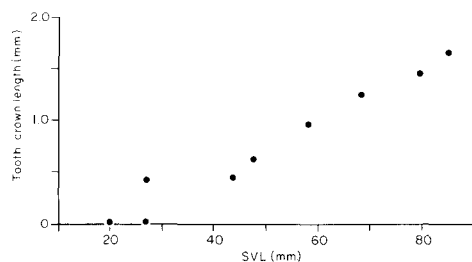


FIG. 5. Length of the crown of the longest dentary tooth as a function of body size in *Aneides lugubris*. Tooth crowns of hatchlings are 0.1 mm long; tooth crown length increases throughout life. The longest tooth crown (usually fourth from the antero-medial end) of the left jaw ramus was extracted from each specimen and mounted in glycerine, then measured with an ocular micrometer on a Wild dissecting microscope.

to bone anteriorly on the skull; ossification of the articular components for the lower jaw; fusion of the parasphenoid patch to ventral elements of the braincase; and reduction in number of maxillary and dentary teeth concomitant with their elongation, recurving, and thickening. These together are "supportive adaptations" related to one of the two "key innovations" discussed by Wake (1963, 1966) and Larson et al. (1981), specifically fusion of the dentigerous processes of the premaxillae presumed to be at the base of adaptations for feeding. The neural arch of the atlas becomes ossified, as do the transverse processes of trunk vertebrae, the neural arches of most trunk vertebrae, and the rib heads. The locomotor apparatus was well ossified at the end of the first year after hatching, but ossification of those elements increases during the second year. However, we observe an ontogenetic increase in number of caudal vertebrae. Our sample size is small (10), for several specimens have broken or regenerated tails. The trend is from 26 caudal vertebrae in a 20.2 mm animal to 32–35 in large adults. This parallels Noble's (1931) observation of post-hatching addition of caudal vertebrae in *Batrachoseps*.

Osteological change during the "adult" years involves further development of structures associated with feeding and

locomotion. Otic crests become more pronounced, prefrontal-maxillary overlap increases, fusion of frontal processes of the premaxillary increases, coossification is more extensive, and dentary and maxillary tooth numbers decrease (Fig. 1). As Wake (1963, 1966) mentioned, these fewer, larger teeth are concentrated anteriorly, especially as the maxilla develops its cleaver-like, non-dentigerous posterior flange.

One of the most dramatic ontogenetic trends in the species relates to the teeth and jaws. Young animals are very much like generalized plethodontids in having teeth that are of similar size on all tooth-bearing elements. However, in *A. lugubris* starting as early as 27 mm profound changes begin to occur. The first indication is an increase in size of the maxillary and dentary teeth (Figs. 5, 6), a trend which continues throughout life. Dorsio-ventral expansion of the posterior part of the maxilla begins at about 34 mm, and pronounced expansion is present by 40 mm. The maxilla becomes increasingly massive, and more and more tightly articulated with the prefrontal. Initiation of an interlocking articulation between the two bones is evident as early as 34 mm. By 34 mm the maxilla has grown posteriorly to assume the proportions of a generalized plethodontid. At this point the bone is somewhat less than 40% edentulous posteriorly, considerably greater than in members of the related genera *Plethodon* and *Ensatina* (Wake, 1963). As growth proceeds the maxilla becomes increasingly massive, and the posterior portion becomes greatly expanded dorso-ventrally to form a cleaver-like structure (Wake, 1963). At sizes above 80 mm the ventral portion of this expanded structure becomes broadened and very rugose, and the dorsal part becomes a stout bar, much broader than the dorsal part of the cleaver. The portion of the maxilla that is edentulous increases to about 50% by 50 mm to between 55 and 70% in animals larger than 80 mm. The teeth become increasingly long and saber-shaped (Fig. 6). Maxillary and dentary teeth are unicuspid

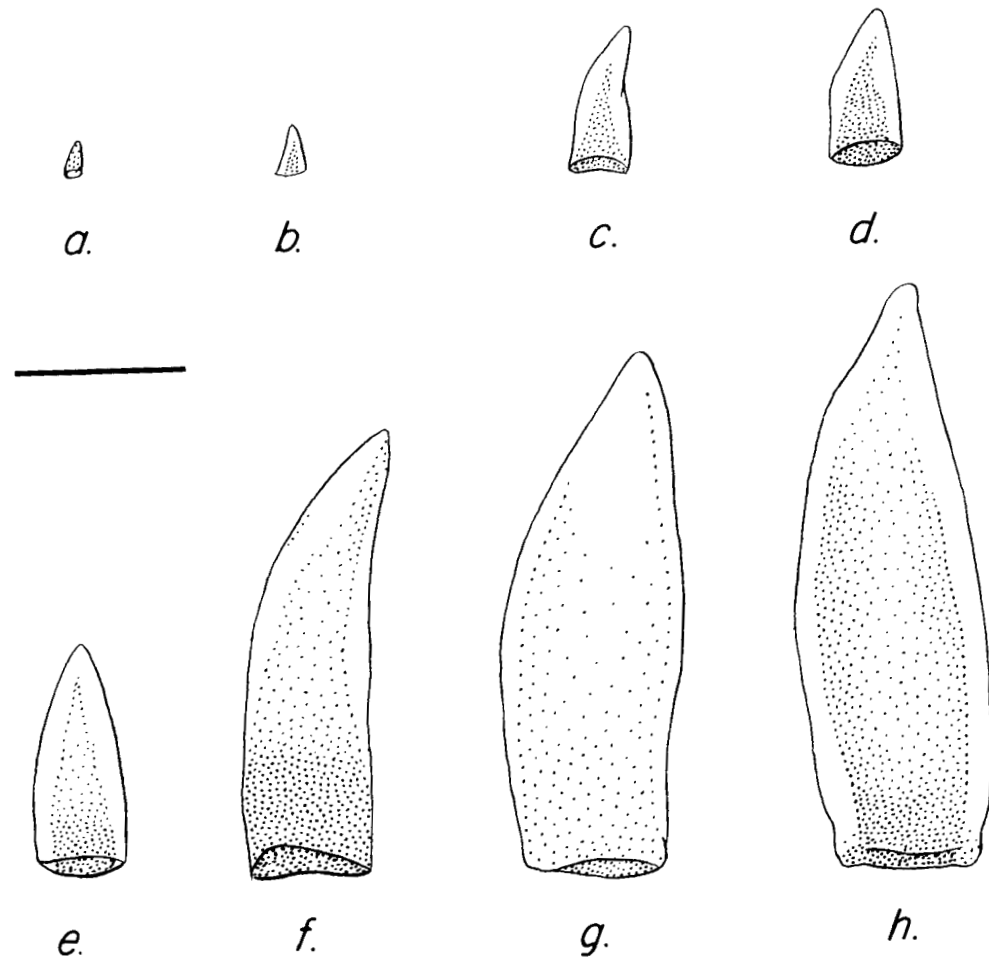


FIG. 6. Tooth crown ontogeny in *Aneides lugubris*. A. Specimen 20 mm SVL; B. 27 mm SVL; C. 27 mm SVL (more osteologically advanced specimen than in B); D. 43.9 mm SVL; E. 47.6 mm SVL; F. 68.2 mm SVL; G. 79.6 mm SVL; H. 85 mm SVL. Camera lucida drawings of glycerine mounted tooth crowns. Note that crowns assume a flattened, recurved, blade-like form with age. Bar = 0.5 mm.

throughout development. The teeth become increasingly flattened, being conical in the smallest animals and extremely flattened, with sharp cutting edges, at sizes greater than about 60 mm. The basal diameter to length ratio for the largest maxillary teeth changes from between .5 to .8 in animals below 30 mm to between .4 and .5 in animals between 30 and 50 mm, to less than .2 in animals greater than 80 mm. While great changes are taking place in the maxillary and mandibular regions, the vomer remains small, and in

a relative sense it is poorly developed. The vomerine teeth in adults are no larger in absolute size than are those in animals in the 20 to 30 mm range.

Other changes in "adulthood" include strengthened ossification of vertebrae and ribs, and the glenoid fossa and pelvic plate elements, the long bones, and the phalanges are increasingly ossified (Figs. 7 and 8). In the adult specimen with the most extensive ossification (85 mm SVL), the tectum synoticum has almost completely ossified and only articular caps of most

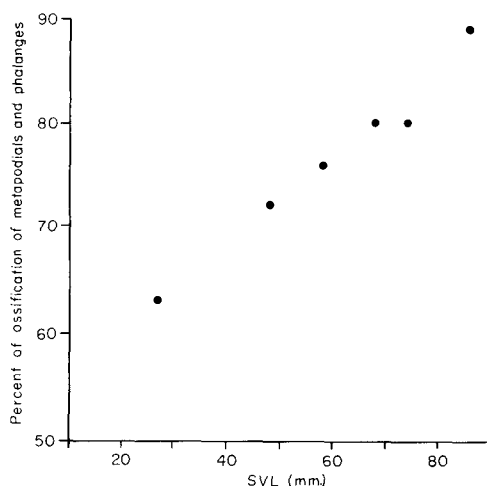


FIG. 7. Percent ossification of metapodials and phalanges vs. snout-vent length in *Aneides lugubris*. Ossification increases throughout life, but is not complete even in large, old specimens. The length of the ossified component and the total length of each of sixteen metapodial and phalangeal elements of the right hind foot were measured. The sum of ossified lengths was divided by the sum of total lengths of the elements to obtain the percentage of ossification of each foot.

joint-forming elements are cartilaginous (ribs, pre- and postzygapophyses, articular of lower jaw, caps of long bones and phalanges, etc.). The hyoid skeleton (except for the urohyal), the carpals, and the tarsals remain cartilaginous throughout life. The pelvic plate is nearly fully ossified as are the femurs. In the pectoral girdle, the glenoid fossa is well ossified, but the distal aspects of the elements forming it are cartilaginous. However, the posterior margins of the suprascapulae of the 85 mm specimen include large crescents of ossification contrary to Wake's (1963, 1966) report that the elements are cartilaginous throughout life. We conclude that while *Aneides lugubris* has the most extensively ossified skeleton found in plethodontids (but see Hanken, 1982, for a discussion of increased limb ossification associated with dwarfing in *Thorius*), some potential for further trends in the direction of increased ossification remains.

Patterns of Morphological Change in Plethodontids.—Few ontogenetic data are

available for other plethodontid species. Wilder's (1925) study of *Eurycea bislineata* provides the most information on developmental osteology, but she concentrated on the events of metamorphosis in that semi-aquatic species. Since *Eurycea* lack the specializations for feeding and locomotion of *Aneides*, and retain an aquatic larval stage, comparisons of *E. bislineata* and *A. lugubris* are not really fruitful beyond those of Wake (1966). Scattered allusions to "larval" or various individual ontogenetic stages (but rarely series) exist, but do not facilitate comparison of ossification sequence. However, Hanken (1982) has recently presented a detailed account of ontogenesis of the appendicular skeleton in *Thorius*, a miniature salamander that has direct development. *Thorius* is at the opposite end of the size and development spectrum from *Aneides*, and in *Thorius* increased ossification is a proximate means of achieving adult size. In addition, a few studies have focused on the ontogeny of particular elements, such as Uzzell (1961) and Wake (1980) on carpals and tarsals, and Wake and Lawson (1973) and Wake (1970) on vertebrae. Lynch's (1981) study of ontogenetic variation in *A. flavipunctatus* utilizes only externally observable characters, and so provided no osteological evidence of use for our study. Further, the work of Larson (1980) and Larson et al. (1981) utilized morphological data from Wake (1960, 1963, 1966) and Lynch (1974) for comparison with molecular data, rather than adding new information on ontogenesis relative to considerations of pedomorphosis and origin of morphological novelties. We therefore regard this as a baseline study, with comparable data for related species still to be gathered. However, our data do allow some consideration of patterns of morphological evolution.

We are particularly interested in the ontogenetic features of the two "key innovations" identified by Wake (1963)—changes for scansorial locomotion and strengthening the jaws for feeding. As Larson et al. (1981) note, it appears that each "key innovation" is the result of a

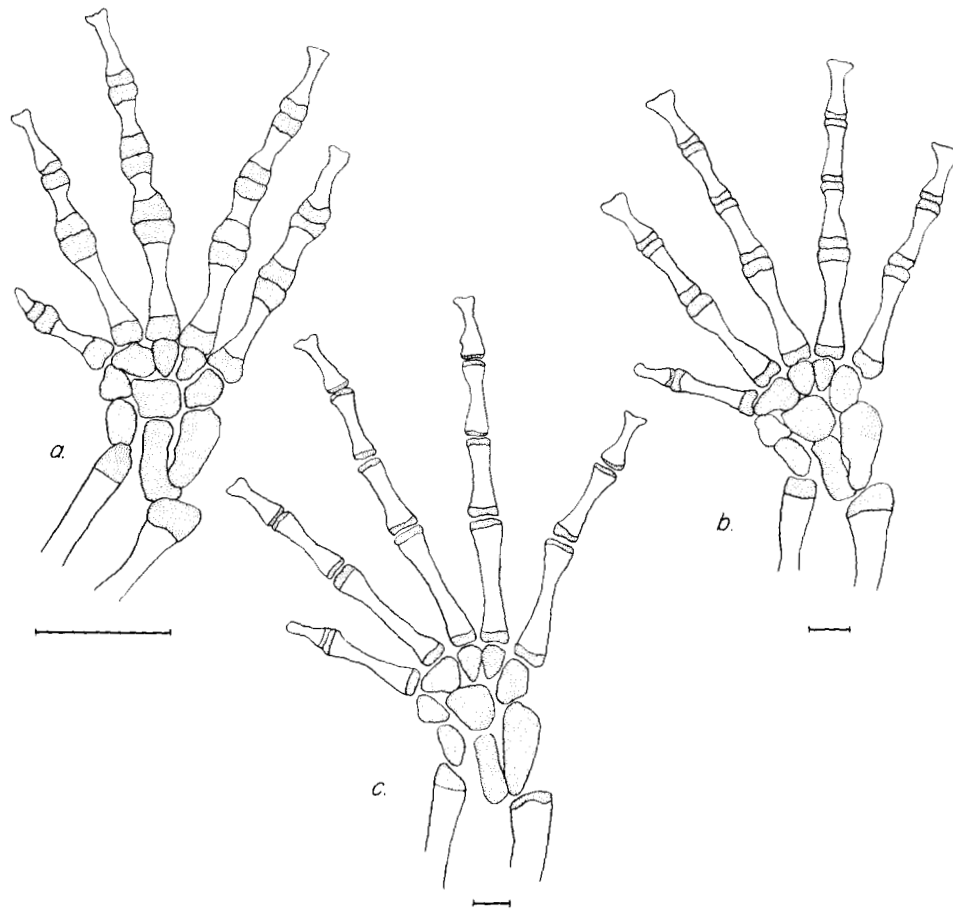


FIG. 8. Ontogeny of the skeleton of the right hind foot in *Aneides lugubris*. A. 27.0 mm SVL specimen; B. 68.2 mm SVL specimen; C. 85.0 mm SVL specimen. Note the increase in ossification of the shafts of metapodials, phalanges, radius and ulna and concomitant decrease in cartilage. Mesopodials remain cartilaginous throughout life. The first digit appears to lengthen relative to other digits with age. Bar = 1.0 mm. Cartilage stippled.

particular morphological change that happened over a short period of (evolutionary) time. Larson et al. (1981) determined through analysis of morphoclines in the five species of *Aneides* (largely adult material) that though the "key innovations" may have been produced by single developmental alterations, associated morphological modifications ("supportive adaptations") occurred gradually by accumulation of several independent genetic changes.

The two "key innovations" and their supporting morphological changes appear

to be products of distinctly different "programs" for developmental time in *A. lugubris*. The morphology presumed correlated with arboreal/scansorial locomotion, rearrangement of tarsals and carpals, is already established at hatching. The carpal and tarsal arrangements are organized for the specialized transmission of forces (Wake, 1980). The limbs grow with a constant relationship to body length (Fig. 3), so they are proportionately longer throughout life than in non-arboreal species, rather than products of allometric growth. The feet also grow in an essen-

tially isometric manner (Fig. 7). Further, distal phalanges are ossified, and become curved and bifid very shortly after hatching; proximal phalanges ossify just after hatching as well. The pelvis and shoulder girdles are cartilaginous at hatching, but ossification begins just after hatching and is well advanced by the end of the first year of life.

In contrast to the early inception of locomotor adaptations is the developmental timing of those features associated with the strengthening of the feeding apparatus. Almost all of these structural changes take place late in the second year of life, as sexual maturity is achieved. Development of otic crests and ossification of the urohyal for jaw and hyoid muscle attachments, strengthening the premaxilla by fusion of the frontal processes, the correlated inception of coossification and the associated secondary bony overlap, jaw and dentition changes, and the complex articulation of the frontals and maxillae all occur at maturity, and slowly increase throughout life. The skull at the end of the second year is similar in morphology to that of an adult *Plethodon*, but much growth remains to occur. If the modifications of the skull are indeed specializations for feeding in arboreal situations, and given that juveniles are indeed scansorial, questions arise about feeding mechanics of juveniles. We do not know whether feeding mechanics of juvenile and adult *A. lugubris* differ. However, *A. lugubris* of all sizes take prey of a range of sizes, though large adult *A. lugubris* can subsist on very few, very large prey items (Maiorana, 1978; Lynch, 1974; Zweifel, 1949; pers. obs.), so it is possible that the morphological adaptations of the feeding apparatus in adults facilitate taking a greater range of prey types and sizes, particularly very large items, and aid in efficient acquisition of them.

Heterochrony, or selective modulations of developmental pattern, has been considered a major feature influencing the diversification of the plethodontid salamanders (Wake, 1966; Alberch et al., 1979; Larson et al., 1981). Paedomorphosis, or

retention of juvenile morphological characters of an ancestor by adults of descendants, has been cited by Wake (1966) as the primary heterochronic pattern involved in plethodontids. We do not have ontogenetic data for other species of *Aneides*, so we cannot directly evaluate patterns of heterochrony. Yet, we can compare our ontogenetic information for *A. lugubris* with the morphocline analysis of the several species of *Aneides* by Larson et al. (1981) and with the comparative study by Wake (1963) in order to gain further insight into patterns of heterochrony. The morphology of the premaxilla has been extensively analyzed by Wake (1963, 1966) and Larson et al. (1981) as a case in point. We will use the concepts presented in Alberch et al. (1979) to evaluate the developmental patterns of features associated with the "supportive adaptations" in *Aneides*, especially in *lugubris*. Within *lugubris* it appears that the ontogenetic trajectories for the suites of characters would differ. The onset of growth appears to be earlier (pre-hatching) for the locomotor adaptations than for the feeding specializations (two years and sexual maturity). Concomitantly, we extrapolate that the growth rate is less for the locomotor relative to the feeding specializations. The offset signal provides a more difficult concept. In a general sense, it may simply be a limiting size for both sets of adaptations. However, it can vary among elements within a complex. For example, the offset signals for shape and arrangement of carpal and tarsal elements could be interpreted as occurring very early, but the offset signal for the size of these components would be met very late in life in these indeterminately growing forms. In other words, there may be separate offset signals for size, arrangement, and shape. Likewise, we cannot determine from our data and those in the literature which of the modes of heterochrony of Alberch et al. (1979) are the "best fit" to the observed ontogeny. For example, the complex prefrontal-maxillary joint, the fusion of frontal processes of the premaxilla, and the extensive coossifica-

tion all appear to be aspects in which the phenotype of the descendant (*A. lubugris*) transcends that of the ancestor (whether a more primitive species of *Aneides*, or a *Plethodon*-like ancestor). This can occur by positively perturbing the shape growth rate (acceleration) or by positively perturbing the offset signal (hypermorphosis). The fusion of the premaxillae may be either a progenetic (a decrement in the offset signal) paedomorphic event (the bones are fused in all larval and embryonic plethodontids; Wake, 1963), or a secondary peramorphic event (as considered, in a sense, by Wake, 1963, 1966, and Larson et al., 1981). The change in carpal-tarsal structure may involve a pre-displacement phenomenon, with growth forces operable earlier producing more complex shapes at earlier stages (Wake, 1980, and in preparation). We present these conjectures, and unanswered questions, to indicate the degree to which our data are useful in this context, and the greater degree to which more comparative morphological, developmental, and genetic data are needed. We believe that our data provide new information on developmental timing of the morphology associated with a set of macroevolutionary events, and concur with Larson et al. (1981) that these multiple-character changes may be the result of gradually accumulating genetic changes. A single developmental origin need not be suggested, though intermediate states in phenotypic expression are not apparent, and an "adaptive shift" with major phyletic implications results.

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