Appendicular Skeletal Morphology in Minute Salamanders, Genus *Thorius* (Amphibia: Plethodontidae): Growth Regulation, Adult Size Determination, and Natural Variation

JAMES HANKEN Museum of Vertebrate Zoology and Department of Zoology, University of California, Berkeley, California 94720

ABSTRACT Patterns of growth and variation of the appendicular skeleton were examined in *Thorius*, a speciose genus of minute terrestrial plethodontid salamanders from southern Mexico. Observations were based primarily on ontogenetic series of each of five species that collectively span the range of adult body size in the genus; samples of adults of each of seven additional species provided supplemental estimates of the full range of variation of limb skeletal morphology.

Limbs are generally reduced, i.e., pedomorphic, in both overall size and development, and they are characterized by a pattern of extreme variation in the composition of the limb skeleton, especially mesopodial elements, both within and between species. Fifteen different combinations of fused carpal or tarsal elements are variably present in the genus, producing at least 18 different overall carpal or tarsal arrangements, many of which occur in no other plethodontid genus. As many as four carpal or tarsal arrangements were observed in single population samples of each of several species; five tarsal arrangements were observed in one population of *T. minutissimus*. Left-right asymmetry of mesopodial arrangement in a given specimen is also common.

In contrast, several unique, nonpedomorphic features of the limb skeleton, including ossification of the typically cartilaginous adult mesopodial elements and ontogenetic increase in the degree of ossification of long bones, are characteristic of all species and distinguish *Thorius* from most related genera. They form part of a mechanism of determinate skeletal growth that restricts skeletal growth after sexual maturity. Interspecific differences in the timing of the processes of appendicular skeletal maturation relative to body size are well correlated with interspecific differences in mean adult size and size at sexual maturity, suggesting that shifts in the timing of skeletal maturation provide a mechanism of achieving adult size differentiation among species.

Processes of skeletal maturation that confer determinate skeletal growth in *Thorius* are analogous to those typical of most amniotes – both groups exhibit ontogenetic reduction and eventual disappearance of the complex of stratified layers of proliferating and maturing cartilage in long bone epiphyses – but, unlike most amniotes, *Thorius* lacks secondary ossification centers. Thus, the presence of secondary ossification centers cannot be used as a criterion for establishing determinate skeletal growth in all vertebrates.

Phyletic size change is a conspicuous feature of evolution, and the phenomenon of size increase or decrease is well documented for many taxa (Stanley, '80). Less is known of the physiological mechanisms of growth regulation by which size alterations are achieved. In tetrapod vertebrates, patterns of growth and development of the skeleton – particularly the appendicular skeleton – have figured prominently in attempts to explain growth regulation overall. Early development of the appendicular skeleton is remarkably similar in all Recent

Dr. Hanken's current address is Department of Biology, Dalhousie University, Halifax B3H 4J1,Nova Scotia, Canada.

tetrapods; many differences among groups become apparent only at later stages (Hinchliffe and Johnson, '80). A typical long bone initially forms as a mesodermal condensation that subsequently differentiates into a cartilaginous model, or precursor, of the later bony element. All tetrapods, as well as bony fishes, develop a single ossification center, or diaphysis, in the midsection (shaft) of each long bone between the two cartilaginous ends, or epiphyses (Haines, '42). This single, or primary, ossification center is typical of amphibians, turtles, and crocodilians, whereas mammals, birds, and the remaining Recent, limbed reptiles - lizards and Sphenodon (the tuatara) - frequently form additional, secondary ossification centers in the cartilaginous epiphyses after the appearance of the primary center. During ontogeny in these latter groups, primary and secondary ossification centers increase in size relative to the overall length of the bone and gradually erode the intervening cartilaginous plates that separate them. Because this cartilage is the only tissue capable of longitudinal expansion (via cell proliferation and interstitial growth), longitudinal bone growth is no longer possible when primary and secondary centers meet and fuse. Consequently, secondary ossification centers are often presumed to be related intimately to a mechanism of growth regulation that confers a pattern of "determinate" growth to those amniotes that possess them (Haines, '38, '41, '42, '69; Romer, '56, '70). Tetrapods that lack secondary centers are considered to have "indeterminate" growth (Goin and Goin, '71; Goss, '74, '80; Romer, '66).

The dichotomy between determinate and indeterminate growth is well entrenched in the literature on skeletal development and has played a central role in arguments concerning the origin of particular vertebrate groups, such as lizards (Carroll, '77). In many instances, the presence or absence of secondary ossification centers is used as a sufficient criterion for identifying which mode of growth prevails in a particular taxon (for example, see Andrew and Hickman, '74). Yet thorough studies of patterns of skeletal growth on which the dichotomy is based include surprisingly few taxa; furthermore, empirical data gathered from longitudinal studies of growth of many natural populations of fish and amphibians suggest that growth patterns in these groups may be quite "determinate," regardless of the absence of secondary ossification centers. Thus, much remains to be learned concerning the mechanisms of growth and size regulation in vertebrates, the variation and distribution of these mechanisms among different taxa, and the consequences of size change in particular groups.

The genus Thorius (Amphibia: Plethodontidae) comprises a complex of at least 15 species of terrestrial lungless salamanders that live in the montane forests of southern Mexico. Representing a specialized component of the extensive radiation of plethodontid salamanders in the New World tropics, Thorius is highly derived in many respects, particularly in morphology, compared to related genera (Wake, '66). The most conspicuous attribute of Thorius is small size – as adults, body size ranges from as little as 14 mm SVL (snout to posterior end of vent) in the smallest species, to just over 30 mm SVL in the largest species. Associated with reduced size are several extreme modifications of the appendicular skeleton. The limbs are relatively small and the digits (especially the terminal phalanges) are poorly developed; in these aspects, skeletal development is truncated precociously relative to that of more generalized plethodontid salamanders, conforming to an overall pattern of pedomorphosis exhibited by much of the skeleton, including the skull. In contrast, the appendicular skeleton possesses several novel features whose presence cannot be attributed simply to truncated development. Mesopodial elements, which typically are cartilaginous throughout life in plethodontid salamanders, are ossified in adult Thorius. Furthermore, long bones, which retain relatively large and well-developed cartilaginous epiphyses in adults of most other plethodontid genera, show increased ossification in *Thorius*. Yet despite the identification of these "derived" features of the appendicular skeleton more than a century ago (Cope, 1869a), their role in limb function and skeletal maturation has remained unclear. In this paper, I present the results of an examination of skeletal growth and variation in Thorius that was conducted as part of a larger study of the mechanisms and morphological consequences of extreme size reduction in salamanders (Hanken, '80). This allows consideration of both the mechanism by which size reduction was achieved during the evolution of Thorius from larger ancestors and the means by which size differentiation is effected among species within the genus.

Analysis of size and skeletal growth regulation was based primarily on observations of five species that collectively span the range of adult body size found in *Thorius*. Supplemen-

tary observations of seven additional species provided estimates of the range of skeletal variation in the genus. I focused particularly on the appendicular skeleton for two reasons: 1) the presence of several unique, or derived, features not seen in larger plethodontid salamanders, with the presumption that the occurrence of these features is related to the process of size and growth regulation; and 2) the prominent role attributed to appendicular skeletal growth processes in the mechanism of overall growth regulation in most amniotes (see above). Specifically, I examined the following aspects of limb skeletal morphology and development: 1) variation in the appearance and number of mesopodial elements, both within and between species; 2) ossification patterns of mesopodial elements and long bones, including comparisons of species differing in adult body size; and 3) histological modifications of skeletal tissues during ontogeny, and the implications of these modifications for adult skeletal growth and maturation.

MATERIALS AND METHODS Taxonomy of Thorius

To date, nine species of *Thorius* have been formally described (Wake and Lynch, '76). Recently, I completed a taxonomic survey of the genus based on both electrophoretic and morphological analyses in which I identified the following seven additional (undescribed) species: *T. sp. A* (Puerto del Aire, Veracruz), *T. sp. B* (Volcan Orizaba and Las Vigas, Veracruz), *T. sp. B* (Volcan Orizaba and Las Vigas, Veracruz), *T. sp. C* (Cerro Pelon, Oaxaca), *T. sp. D* (Cerro Pelon, Oaxaca), *T. sp. E* (Cerro Pelon, Oaxaca), *T. sp. G* (northern Oaxaca) (Hanken, '80). Formal descriptions will be presented elsewhere. These species will be referred to below by letter designation.

Osteological analysis

The bulk of the osteological analysis was based on observations of ontogenetic series of approximately 30 specimens (10 adult males, 10 adult females, and 10 juveniles, when available) of each of the following five species: *T. pennatulus*, *T. macdougalli*, *T. minutissimus*, *T. schmidti*, and *T. narisovalis*, (Fig. 1, Table 1). Samples of approximately 20 specimens (10 adult males and 10 adult females) of each of seven additional species were used to identify mesopodial fusion combinations that are variably present in the genus: *T. troglodytes*, *T. pulmonaris*, *T. sp. B*, *T. sp. C*, *T. sp. D*, *T. sp. F*, and *T. sp. G.* Sexually mature males were idenAbbreviations

b,	compact bone	fi,	fibula
с,	centrale	i,	intermedium
c1,	centrale 1	hc,	hypertrophied cartilage
d,	diaphysis	m,	marrow cavity
d1-2,	distal carpal 1-2	mc,	mature cartilage
d3,	distal carpal 3 carpus	mt,	metatarsal 3
d4,	distal carpal 4	pc,	proliferating cartilage
d1-2,	distal tarsal 1-2	r,	radiale
d3,	distal tarsal 3 tarsus	rc,	resting cartilage
d4-5,	distal tarsal 4-5	t,	tibiale
е,	epiphysis	ti,	tibia
f,	fibulare	u,	ulnare
fe,	femur	ul,	ulna
в (
С	- 64] '}	(9)
D	= - - ⊄2	 (8)	(10)
E		 	(10)
Г 15	20 2 SVL	1 25 (mm	30 35

Fig. 1. Snout-vent length (SVL) of adult males (below) and females (above) of *T. pennatulus* (A); *T. macdougalli* (B); *T. minutissimus* (C); *T. schmidti* (D); and *T. narisovalis* (E). Mean, vertical bar; range, horizontal bar; 95% confidence interval, rectangle. Sample size is in parentheses.

 TABLE 1. Mean snout-vent length of adult males (M) and females (F) of five species of Thorius'
 Image: Comparison of Comparis

Species	Sex	SVL	SE	N
T. pennatulus	М	18.9	0.3	10
•	F	18.9	0.3	10
T. macdougalli	Μ	19.5	0.7	10
	F	22.3	0.7	10
T. minutissimus	М	22.6	0.3	9
	F	24.1	0.4	9
T. schmidti	М	23.5	0.2	8
	F	25.0	0.4	10
T. narisovalis	М	25.4	0.5	10
	F	27.8	0.4	10

'Measurements are in millimeters; SE, standard error; N, sample size.

tified by the presence of enlarged testes and/or convoluted vasa deferentia; adult females were identified by the presence of maturing follicles or swollen and/or convoluted oviducts. Detailed information about specimens examined (e.g., localities) is available in Hanken ('80) and upon request. All specimens are deposited in the Museum of Vertebrate Zoology of The University of California at Berkeley.

Specimens were preserved initially in 10% neutral-buffered formalin. External measurements of body size (snout to posterior end of vent: SVL) were taken from the formalinpreserved material. Following external measurement, all specimens were differentially stained for bone and cartilage by an alizarin red-alcian blue procedure slightly modified from that of Wassersug ('76) and Dingerkus and Uhler ('77). Linear skeletal dimensions were measured with a binocular microscope fitted with an ocular micrometer.

All specimens were scored for the presence of each of 15 potential combinations of fused mesopodial elements and 18 different overall carpal or tarsal arrangements that were identified in a preliminary survey of 12 species. In the vast majority of specimens, adjacent mesopodial elements were clearly either fused or separate. However, in a few cases adjacent elements appeared fused yet retained a conspicuous suture between them. For the purposes of this study, these instances are scored as fused. Right and left sides were scored separately.

Each mesopodial element was scored for the presence of ossification (an element was considered ossified if it showed any alizarinstained deposit). For each carpus and tarsus, an ossification index then was calculated that equaled the percentage of total elements ossified (i.e., the number of ossified elements, divided by the total number of elements, multiplied by 100). Finally, separately calculated values for right and left sides were averaged to yield one carpus and one tarsus ossification index value for each animal.

A second type of ossification index, calculated separately for both the right femur and the right third metatarsal, was used to represent the degree of ossification of long bones. For each element, the length of the ossified diaphysis (shaft) was expressed as a percentage of the total length of the element (diaphysis plus both cartilaginous epiphyses). The border between adjacent regions of cartilage and bone usually was well defined. However, in large femurs showing the maximal amount of ossification, epiphyseal cartilage at each end was reduced to a thin cap, or shell, surrounding a bony core. This maximum condition was operationally defined as 99% ossified.

One hindlimb from both juvenile (SVL = 12.5 mm) and adult (SVL = 30.9 mm) specimens of *T. sp. F* were examined in $10\text{-}\mu\text{m}$ serial sections to identify the histological modifica-

tions accompanying increased mesopodial and long bone ossification. Preservation, decalcification, paraffin sectioning, and subsequent staining in hematoxylin and eosin followed standard histological procedures (Humason, '79).

RESULTS Appearance and number of mesopodial elements

Carpus. Primitively, the carpus of plethodontid salamanders consists of eight distinct cartilages: three proximal elements (ulnare, intermedium, and radiale); two centrally located centralia (centrale and centrale 1); and three distal elements (distal carpals 1-2, 3, and 4) (Wake, '63, '66). This pattern never is seen in Thorius. Instead, many of these cartilages fuse in at least nine different combinations: 1) ulnare and intermedium; 2) ulnare, intermedium, and centrale; 3) distal carpal 4 and centrale; 4) distal carpal 3, distal carpal 4, and centrale; 5) distal carpal 1-2, distal carpal 3, distal carpal 4, and centrale; 6) distal carpal 1-2 and centrale; 7) radiale and centrale 1; 8) distal carpals 1-2 and 3; 9) distal carpals 3 and 4. (See Fig. 2 and Table 2.)

Few of these fusion combinations are common; most occur only at low frequencies but may be found in several species (Table 2). The ulnare and intermedium are fused in all specimens of all species. The centrale usually is fused with distal carpal 4, but combinations between the centrale and either ulnare-intermedium or distal carpal 1-2 appear in low frequency in many species. The fused distal carpal 4-centrale rarely joins distal carpal 3; it was fused with both distal carpals 1-2 and 3 in one immature specimen of T. schmidti. Fusion of the radiale and centrale 1 was seen in only one specimen each of T. narisovalis and T. sp. D; the latter specimen also demonstrated the only instance of fusion of distal carpals 3 and 4. The frequency of carpal fusion combination 8-distal carpals 1-2 and 3-varies greatly among species. For example, although this combination was seen in only 5-10% of the carpi of T. narisovalis, it characterized approximately half of the T. pennatulus examined.

Variable occurrence of these nine fusion combinations, both within and among species, yields nine different overall carpal arrangements in which the actual number of separate carpal units ranges from four to seven (Fig. 2). As many as four different arrangements may be present in a single sample of 20 or fewer conspecific individuals from a single locality (Table 3). The most common pattern in all species is I: ulnare-intermedium, radiale, centrale 1,



Fig. 2. Alternate carpal arrangements in *Thorius* (dorsal view). All arrangements are drawn as right carpi, although some were observed in the left carpus only (see below). Pattern I predominates in all species. The remaining patterns are rare, except for pattern II, which occurs in low to moderate frequency in most species (Table 4). Arabic numerals identify the carpal fusion combination(s) (described in text) involved in transition from one arrangements are an arrangements of the second seco

ment to another. Cartilage is stippled, except in mesopodials, which have been left unshaded. Arrangements were drawn from the following specimens (asterisk denotes left carpus): I) *T. narisovalis*, M 4456; II) *T. narisovalis*, M 4518; III) *T. narisovalis*, M 4527; IV) *T. sp. F*, M 6058; V) *T. schmidti*, M 3055; VI) *T. narisovalis*, M 4523; VII*) *T. sp. D*, HBS 2203; VIII*) *T. sp. G*, M 3808; IX) *T. troglodytes*, M 5515.

		TABLE 2. Fi	requency (%)	of nine carpa	l fusion comb	inations in a	dult Thorius ^{1,2}			
	(2)		(3)		(4)		(1)		(8)	
	Ulnare-inte + cent	srmedium srale	Distal ca + cent	arpal 4 :rale	Distal car d4-cen	pal 3+ trale	Radial	e + le 1	Distal car + distal c	rpal 1-2 carpal 3
Species	Unfused	Fused	Unfused	Fused	Unfused	Fused	Unfused	Fused	Unfused	Fused
T. pennatulus $(N = 19,20)^3$	100	I	I	100	97.4	2.7	100	I	48.8	51.2
T. macdougalli (N = 20)	100	I	I	100	100	I	100	I	85.0	15.0
T. minutissimus (N = 18)	100	I	I	100	91.7	8.4	100	I	83.4	16.7
T. schmidti (N = 18)	100	ł	I	100	94.4	5.6	100	I	88.9	1.11
T. narisovalis $(N = 20, 19)^3$	100	ł	I	100	97.5	2.5	97.5	2.5	92.3	7.8
T. troglodytes (N = 19)	100	I	2.7	97.4	97.4	2.7	100	ł	97.4	2.7
T. pulmonaris (N = 11)	100	J	I	100	100	L	100	I	91.4	8.6
$T. sp. B (N = 18, 19)^3$	100	I	I	100	100	I	100	I	51.2	48.9
T. sp. C $(N = 20)$	100	I	I	100	97.5	2.5	100	I	0.06	10.0
T. sp. D (N = 19,20) ³	100	I	2.5	97.5	92.3	7.8	97.5	2.5	87.1	12.9
T. sp. F (N = 17,18) ³	97.1	3.0	3.0	97.1	100	ł	100	I	83.0	17.0
T. sp. G $(N = 18)$	97.2	2.8	2.8	97.2	100	a.	100	I	94.5	5.6
¹ In each species, frequencie ² Combination 1, ulnare and (both carpi). Combinations d "Sample sizes for the right a	s were first calc intermedium, is 6, distal carpal 1 and left sides, re	ulated separately found in all speci 1-2 and centrale, sspectively.	y for right and mens. Combination and 9, distal ca	left sides and th tion 5, distal cau rpal 3 and dist	hen averaged to rpal 1-2, distal c al carpal 4, wen	yield a single arpal 3, and dis e seen each in	value. tal carpal 4-centrale only one of twenty s	e, was seen in only specimens of <i>T. s</i> ŗ	one juvenile T . s . D (left carpus	schmidti).

62

J. HANKEN

distal carpal 4-centrale, distal carpal 1-2, and distal carpal 3. Fusion of distal carpals 1-2 and 3 produces a second pattern (II) that occurs in low to moderate frequency in most species. Pattern III, which differs from the most common pattern in the fusion of distal carpal 3 with distal carpal 4-centrale, is low in frequency (5-10%) in at least seven species: *T. minutissimus*, *T. schmidti*, *T. pennatulus*, *T. narisovalis*, *T. troglodytes*, *T. sp. C*, and *T. sp. D*. The remaining six patterns are rare; each occurs in only one species.

The primitive arrangement of the Tarsus. tarsus in plethodontid salamanders comprises nine separate cartilages: three proximal elements (fibulare, intermedium, and tibiale); two centralia (centrale and centrale 1); and four distal elements (distal tarsals 1-2, 3, 4, and 5) (Wake, '63, '66). As in the carpus, fusions of these elements reduce the actual number of separate tarsal units present in a given specimen of *Thorius* to five to eight. The following six tarsal fusion combinations were observed (Table 4): 1) intermedium and fibulare; 2) intermedium and centrale; 3) distal tarsals 4 and 5; 4) centrale and distal tarsal 4-5; 5) centrale, distal tarsal 3, and distal tarsal 4-5; 6) distal tarsals 1-2 and 3 (Fig. 3).

Distal tarsals 4 and 5 are always fused. They fuse with the centrale in all *T. pennatulus*, but this combination appears in only low to moderate frequency, if at all, in other species. Distal tarsal 3 is fused with distal tarsal 1-2 at low to moderate frequencies in many species, especially *T. macdougalli*. The typically separate intermedium and centrale are fused only in the right tarsus of one of 17 specimens of *T. minutissimus*.

Fusion of the intermedium and fibulare exhibits the most striking pattern of variation. Here, species may be arranged in a cline based on the frequency of intermedium-fibulare fusion, from species in which the elements are nearly always separate (T. minutissimus, T. narisovalis), to species of intermediate frequency of fusion (T. macdougalli, T. sp. F), to species in which the two elements are always or nearly always fused (T. schmidti, T. pennatulus). Fusion frequency bears no obvious relationship to body size; two species of similar adult size may exhibit greatly different fusion frequencies (e.g., T. pennatulus and T. macdougalli), whereas a given fusion frequency may be shared by two species differing in mean adult size (e.g., T. narisovalis and T. minutissimus).

I observed nine different overall tarsal arrangements that, depending on which of the

six potential fusion combinations were present, had five to eight separate tarsal units (Fig. 3). In contrast to the carpus, in which the same carpal arrangement predominates in nearly all species (Table 3), each of four tarsal arrangements predominate in at least one, and often several, species (Table 5). Pattern I differs from the primitive plethodontid arrangement only in the fusion of distal tarsals 4 and 5; presumably it represents the primitive condition in Thorius. It characterizes most T. minutissimus, T. macdougalli, T. narisovalis, T. troglodytes, T. sp. C, and T. sp. D, and many T. schmidti. Pattern V has an additional fusion of the intermedium and fibulare; it appears in most T. pulmonaris and T. sp. G, and in many T. schmidti, T. sp. B, and T. sp. F. Pattern VII has an additional fusion of the centrale and distal tarsal 4-5, whereas pattern VIII has yet an additional fusion of distal tarsals 1-2 and 3; together these patterns characterize nearly all T. pennatulus. The remaining five tarsal arrangements (II-IV, VI, IX) may be derived from these four patterns by additional fusions. Each occurs infrequently in a given species, but the same pattern may appear in many different species. As many as five different tarsal arrangements may be present in a sample of 20 or fewer individuals from a single locality (e.g., T. minutissimus; Table 5).

Finally, variation in mesopodial number includes a high frequency of right-left asymmetry in mesopodial fusion and, therefore, overall carpal or tarsal arrangement (Table 6). For example, approximately one-fourth of the sample of each of several species were asymmetric for carpal fusion combination (8), distal carpal 1-2, and distal carpal 3. Combining the data concerning all 11 different mesopodial fusion combinations observed in the five species of *Thorius* examined in detail, between 20% and 70% of the specimens of a given sample were asymmetric for at least one mesopodial fusion combination and, therefore, carpal or tarsal arrangement.

Ossification pattern Mesopodial elements

General observations concerning the timing of mesopodial ossification apply equally to the carpus and tarsus. Typically, mesopodial elements are cartilaginous in both juvenile and adult plethodontid salamanders (Wake, '66). Although the elements are cartilaginous in juvenile *Thorius* (Fig. 4A), nearly all adults have some or all mesopodial elements ossified (Fig. 4B,C). In each species, intermediate values of carpal or tarsal ossification index occur in

Species	I	 II	111	IV-IX ³
$T. pennatulus (N = 19.20)^4$	46.2	51.2	2.7	_
T. macdougalli (N = 20)	85.0	15.0	_	-
T. minutissimus $(N = 18)$	75.0	16.7	8.4	-
$\begin{array}{l} T. \ schmidti\\ (N = 18) \end{array}$	83.3	11.1	5.6	-
T. narisovalis $(N = 20,19)^4$	87.3	7.8	2.5	2.5 (VI)
T. troglodytes (N = 19)	92.1	2.7	2.7	2.7 (IX)
T. pulmonaris $(N = 11)$	91.0	9.1	-	-
T. sp. B (N = 19,18)*	51.2	48.9	_	-
$\begin{array}{l} T. sp. C\\ (N=20) \end{array}$	87.5	10.0	2,5	_
T. sp. D (N = 19,20) ⁴	76.9	12.9	7.8	2.5 (VII)
T. sp. F (N = 17.18) ⁴	80.1	17.0	-	3.0 (IV)
$\begin{array}{l} T. sp. G \\ (N = 18) \end{array}$	94.5	2.8	-	2.8 (VIII)

TABLE 3. Frequency (%) of nine alternate carpal arrangements in adult Thorius^{1,2}

¹In each species, frequencies were calculated separately for the right and left sides and averaged to yield a single value. ²I: u-i, r, c1, d1·2, d3, d4-c; II: u-i, r, c1, (d1-2)-d3, d4-c; III: u-i, r, c1, d1-2, d3-(d4-c); IV: u-i-c, r, c1, d1-2, d3, d4; V: u-i, r, c1, (d1-2)-d3-d4-c; VI: u-i, r-c1, d1-2, d3, d4-c; VII: u-i, r-c1, (d1-2)-c, d3-d4; VIII: u-i-c, r, c1, (d1-2)-d3, d4; IX: u-i, r, c1, d1-2, d3, d4. ³Pattern V was seen in only a single, juvenile specimen of T. *schmidti* (both carpi). ⁴Sample sizes for the right and left sides, respectively.

	(1 Intermed fibul) dium + are	(4 Centra distal ta) ale + rsal 4-5	6) Distal tar distal ta	sal 2 + ursal 3
Species	Unfused	Fused	Unfused	Fused	Unfused	Fused
T. pennatulus $(N = 20,19)^3$	-	100	-	100	56.6	43.4
T. macdougalli $(N = 19)$	73.7	26.3	97.4	2.7	94.7	5.3
T. minutissimus $(N = 17, 18)^3$	94.3	5.8	97.2	2.8	97.2	2.8
T. schmidti (N = 18)	11.1	88.9	63.9	36.1	97.2	2.8
T. narisovalis $(N = 20)$	90.0	10.0	95.0	5.0	95.0	5.0
T. troglodytes $(N = 20,19)^3$	92.4	7.7	100	-	100	_
T. pulmonaris $(N = 11)$	18.2	81.8	100	-	100	-
T. sp. B (N = 17,19) ³	52.5	47.5	100	-	71.5	28.5
T. sp. C (N = 18,16) ³	100	-	100	-	97,2	2.8
T. sp. D (N = 18,17) ³	94.3	5.8	100	-	88.6	11.5
T. sp. F (N = 18,17) ³	48.7	51.4	97.2	2.8	100	-
T. sp. G $(N = 18)$	5.6	94.4	97.2	2.8	100	-

TABLE 4. Frequency (%) of six tarsal fusion combinations in adult Thorius^{1,2}

¹In each species, frequencies were calculated separately for the right and left sides and averaged to yield a single value. ⁴Combination 3, distal tarsals 4, and 5, was found in all specimens. Combinations 2, intermedium and centrale, and 5, centrale, distal tarsal 3, and distal tarsal 4-5, were seen only in single specimens (right tarsus) of *T. minutissimus* and *T. sp. F*, respectively. ⁴Sample sizes for the right and left sides, respectively.

64



Fig. 3. Alternate tarsal arrangements in *Thorius* (dorsal view). All arrangements are drawn as right tarsi, although some were observed in the left tarsus only (see below). Four patterns (1, V, VII, and VIII) each predominate in at least one species. The remaining patterns occur only in low frequencies, but each may be present in several species (Table 4). Arabic numerals identify the tarsal tusion combination(s) (described in text) involved in the transition

from one arrangement to another. Cartilage is stippled, except in mesopodials, which have been left unshaded. Arrangements were drawn from the following specimens (asterisk denotes left tarsus): I, III*) *T narisovalis*, M 4518; III *T narisovalis*, M 4456; IV) *T minutissimus*, M 4583; V) *T narisovalis*, M 3471; VI) *T sp. F*, MVZ 110669; VII) *T sp. F*, MVZ 110644; VIII) *T schmidt*i, M 5173; IX) *T sp. F*, M 6069.

J. HANKEN

	IIIBBB 0. I reque	ence y ()0) 01 nu	te arrennare rans	at un angemen	is in quait 1110	103	
Species	I	_11	III	v	VI	VII	V111
T. pennatulus $(N = 19,20)$	-	-	-		-	56.6	43.4
T. macdougalli (N = 20)	71.1	-	-	23.7	2.7	-	2.7
T. minutissimus (N = 18)	85.8	2.8	2.8	5.8	-	-	-
$\begin{array}{l} T. \ schmidti\\ (N = 18) \end{array}$	11.1	_	-	52.8	_	33.3	2.8
T. narisovalis $(N = 20,19)^{4}$	80.0	5.0	5.0	10.0	-	_	-
T. troglodytes $(N = 19)$	92.4	-	-	7.7	-	-	_
T. pulmonaris $(N = 11)$	18.2	-	-	81.8	-	_	-
T. sp. B (N = 19,18) ⁴	38.1	-	14.4	33.5	14.1	-	-
T. sp. C $(N = 20)$	97.1	-	2.8	_	-	-	_
T. sp. D (N = 19,20) ⁴	82.9	_	11.8	5.8	-	-	-
T. sp. F (N = 17.18) ⁴	48.7	-	-	45.8	_	2.8	-
T. sp. G (N = 18) ⁴	5.6	-	-	91.7	-	2.9	-

TABLE 5 Frequency (%) of nine alternate targal arrangements in adult Thorins^{1,2,3}

¹In each species, frequencies were calculated separately for the right and left sides and averaged to yield a single value. ²I: i, f, t, c, c1, d1-2, d3, d4-5; II: i, f, t, c1, c-(d4-5), d1-2, d3; III: i, f, t, c, c1, (d1-2)-d3, d4-5; IV: i-c, f, t, c1, d1-2, d3, d4-5; V: i-f, t, c1, c-(d4-5), d1-2, d3; d4-5; VI: i-f, t, c, c1, (d1-2)-d3, d4-5; VI: i-f, t, c1, c-(d4-5), d1-2, d3; VIII: i-f, t, c1, c-(d4-5), (d1-2)-d3; IX: i-f, t, c1, c-(d4-5). ³Patterns IV and IX were found only in single speciments (right side) of T. minutissimus and T. sp. F, respectively.

Sample sizes for the right and left sides, respectively.

	Carpal	fusion (8)	Overall o tarsal arr	carpal and angements
	Symmetric	Asymmetric	Symmetric	Asymmetric
T. pennatulus	14	5	6	13
(N = 19)	(73.7)	(26.3)	(31.6)	(68.4)
T. macdougalli	16	4	14	6
(N = 20)	(80.0)	(20.0)	(70.0)	(30.0)
T. minutissimus	13	5	11	7
(N = 18)	(72.2)	(27.8)	(61.1)	(38.9)
T. schmidti	14	4	6	12
(N = 18)	(77.8)	(22.2)	(33.3)	(66.7)
T. narisovalis	18	1	15	4
(N = 19)	(94.7)	(5.3)	(78.9)	(21.1)

TABLE 6. Frequency (%) of right-left asymmetry of carpal fusion combination (8)-distal carpal 1-2 and distal carpal 3-and overall carpal and tarsal arrangement

small mature specimens which collectively occupy a relatively narrow range of body size (Fig. 5A-E). This indicates an abrupt onset and completion of mesopodial ossification coincident with sexual maturation.

Ossification sequences of carpal and tarsal units were determined by examination of ontogenetic series of small adults. For example, in those specimens with only one ossified carpal, this unit typically was the fused ulnare-intermedium. Thus, the fused ulnare-intermedium usually is the first carpal unit to ossify. However, there is frequent variation among specimens with respect to both carpal and tarsal ossification sequences; some elements tend to ossify earlier than others, but exceptions are common. Below, I describe only the most typical patterns. There were no apparent differences in ossification sequence among species.

Carpus. The carpus usually ossifies from seven separate centers. The sole exception noted was the fused distal carpal 4 and cen-



Fig. 4. Cleared and differentially stained (bone and cartilage) right hindlimbs of juvenile and adult *T. narisovalis* (dorsal view) showing ossification of tarsal elements and long bones. A. M 4443, juvenile male, SVL = 21.8 mm; B. M 4453, adult female, SVL = 26.8 mm; C. M 4456, adult female, SVL = 28.4 mm. The cartilaginous epiphyses present in the long bones of A are reduced or lacking in B

and C. Tarsals are cartilaginous in A but the intermedium and fibulare are partly ossified (appearing black in photograph) in B; all the tarsals are at least partly ossified in C. Note the fused intermedium – fibulare in A, the fused distal tarsal 4-5 and centrale in C, and the reduced phalangeal number of the second digit in C. Bar equals 0.5 mm.

trale, which, though usually ossifying as a single center, ossified from two separate centers in one carpus of one adult specimen of T. sp. F (SVL = 27.1 mm), making a total of eight centers in this carpus. The predominant ossification sequence is: ulnare-intermedium (ossifying as two centers); distal carpal 4-centrale; radiale; centrale 1; distal carpal 1-2 or distal carpal 3. This sequence differs slightly from that of Wake ('66) who, citing the observations of Uzzell ('61), reported that the radiale ossified before distal carpal 4-centrale.

Tarsus. Eight separate centers ossify in the tarsus in the following sequence: intermedium; fibulare; centrale, tibiale, centrale 1 or distal tarsal 4-5; distal tarsal 1-2; distal tarsal 3. This sequence, too, represents only some of the ossification sequences examined as there were numerous exceptions. For example, the tibiale, fibulare, centrale 1, and distal tarsal 4-5 each were ossified before the intermedium in at least one specimen.

The five *Thorius* species differ in mean adult body size (Table 1; Fig. 1). The body size at which mesopodial ossification is initiated also differs between species, corresponding to a shift in the timing of sexual maturity relative to body size among species. For example, in *T. pennatulus*, the smallest species, sexual maturity and carpal ossification commence at body sizes as small as 15 mm SVL, and carpal ossification is complete by 20 mm SVL (Fig. 5A). *Thorius macdougalli*, with a larger mean adult body size, typically initiates sexual maturity and carpal ossification at body sizes greater than 20 mm SVL, although carpal ossiJ. HANKEN



Fig. 5. Carpal mineralization index vs. body size in T. pennatulus (A); T. macdougalli (B); T. minutissimus (C); T. schmidti (D); and T. narisovalis (E). (\blacksquare) adult male, (\blacktriangle)

adult female, (\bigcirc) juvenile. Mean adult size of adult males (M) and females (F) of each species is indicated on the abscissa.

fication may be complete in animals less than 25 mm SVL (Fig. 4C). And in *T. narisovalis*, the largest species, maturity and ossification are delayed until nearly 25 mm SVL, and ossification may be incomplete in animals as large as 30 mm SVL or more (Fig. 5E).

Femur

Epiphyses of small juvenile specimens occupy as much as 30% of femur length and may include even the proximal and distal ends of the central shaft (Fig. 4A). With increasing size, the proportion of femur length ossified increases gradually until in the largest specimens each epiphysis is reduced to a thin cartilaginous cap, or shell, surrounding a bony core (Fig. 4B,C).

Plots of femur ossification index versus body size for each species are presented in Figure 6A-E. As was observed in the ossification of mesopodial elements (Fig. 5A-E), relative ossification of the femur is a function of body size. However, unlike the abrupt "steplike" appearance of carpal ossification that occurs over a relatively narrow range of body size in each species coincident with sexual maturation, ossification of the femur is a gradual process that begins well before the onset of sexual maturity. Femurs of most adults of all species are fully (i.e., 99%) ossified, but femur ossification differs greatly among juveniles, with degree of ossification proportional to body size within each species.

The pattern of increased ossification with increased body size is evident in each species. However, species differ in the degree of femur ossification over a broad range of body size. For instance, in T. pennatulus femur ossification is complete by 20 mm SVL, whereas in T. narisovalis, a much larger species, complete femur ossification typically is attained only at body sizes in excess of 25 mm SVL; juvenile T. narisovalis of a body size comparable to that of adult T. pennatulus have no more than 80% of the femur ossified (cf. Fig. 6A,E). Here, too, as was seen in the ossification of mesopodial elements (see above), differences among species in the degree of femur ossification at a given body size parallel species differences in mean adult body size at sexual maturity.

Third metatarsal

Ossification of the third metatarsal demonstrates the same general phenomenon as was observed for the femur. Ossification is least in small specimens, in which cartilage may constitute up to 40% of the length of the element, and is proportional to body size within each species (Figs. 4, 7A-E). Ossification increases gradually, beginning well before sexual maturity. Finally, species differ in the degree of metatarsal ossification over a broad range of body size, again in a regular manner correlated with species differences in mean adult body size and size at sexual maturity.

Histological modifications during ontogeny

Examination of serial sections of hindlimbs of juvenile and adult *Thorius* allowed determination of the exact nature of mesopodial and long bone mineralization, and characterization of important differences in growth parameters of the appendicular skeleton before and after sexual maturity. In describing the histology of these mineralizing tissues I employ the terminology of Andrew and Hickman ('74).

Mesopodials of juvenile specimens are entirely cartilaginous, with neither internal cavities nor mineralization (Fig. 8A). Long bones, such as the femur, of juveniles have well-developed cartilaginous epiphyses that fill the entire volume of each bulbous end and adjacent regions of the narrow shaft (Fig. 9A). A layer, or zone, of resting cartilage forms the articulating facet peripherally and extends centrally towards the shaft for approximately half the length of the epiphysis. There it grades into a layer several cells thick comprising columns of flattened, compressed, dividing cells-the socalled zone of proliferating cartilage-that gives way to a zone of hypertrophied, or maturing, cartilage near the base of the epiphysis. In the neck of the shaft a final, thin, poorly defined layer of mature cartilage cells is disrupted and replaced by a marrow cavity that fills the interior of the surrounding bony diaphysis. This complex of stratified layers of skeletogenous tissue in successive stages of differentiation and maturation, especially the proliferative zone, is characteristic of actively growing bone (Andrew and Hickman, '74; Ham and Cormack, '79).

The appearance of adult mesopodials is strikingly different from that of juvenile elements (Fig. 8B). Cartilage, which fills the mesopodial elements of juveniles, is reduced to a thin layer of resting cartilage surrounding a large internal marrow cavity in adults. This layer is thickest where it forms articulating facets with adjacent elements, but it is never more than two to four cells deep and there are few dividing cells. Peripherally, a thin layer of compact bone surrounds this cartilaginous layer, except at the articulating facets. These histological modifications of adult mesopodials are indicative of intracartilaginous, or endochondral, ossification, and not calcified cartilage as has been claimed previously by Uzzell ('61).



Fig. 6. Femur ossification index vs. body size in T. pennatulus (A); T. macdougalli (B); T. minutissimus (C); T. schmidti (D); and T. narisovalis (E). (\blacksquare) adult male, (\blacktriangle)

adult female, (\bullet) juvenile. Mean adult size of males (M) and females (F) of each species is indicated on the abscissa.



Fig. 7. Metatarsal 3 ossification index vs. body size for T. pennatulus (A); T. macdougalli (B); T. minutissimus (C); T. schmidti (D); and T. narisovalis (E). (\blacksquare) adult male, (\blacktriangle)

adult female, (${\scriptsize \textcircled{}}$) juvenile. Mean adult size of males (M) and females (F) of each species is indicated on the abscissa.



Fig. 8. Hematoxylin and eosin-stained sections through carpal elements of juvenile and adult *Thorius*. A. Ulnare, intermedium, and adjacent distal portion of ulna of a juvenile specimen of *T. minutissimus* (M 4636; SVL = 12.5

Correlated with mesopodial ossification is extensive reduction of the cartilaginous epiphysis in adult long bones (Fig. 9B). In each epiphysis, cartilage is limited to a peripheral layer of articulating cartilage one to three cells thick that sheathes the joint articulation. Zones of proliferating and hypertrophied cartilage, both of which are conspicuous in juveniles, are absent, although a few cells in the articulating layer are dividing. The marrow cavity and its surrounding layer of bone - which in juveniles are restricted to the narrow shaft and barely reach the base of each expanded end – have invaded both ends and filled their entire volume below the thin layer of epiphyseal cartilage. The extreme reduction of cartilage, especially the proliferative layer, and the consequent nearly complete ossification of both ends of the femur and adjacent mesopodial elements, suggest that the adult appendicular



mm). B. Ulnare and adjacent distal portion of ulna of an adult female specimen of T. sp. F (TJP 14569; SVL = 30.9 mm).

skeleton has lost the physiological capacity for longitudinal growth and elongation that is typical of juvenile elements.

DISCUSSION

Skeletal growth pattern in Thorius

The appendicular skeleton of *Thorius*, as compared to that of most plethodontid salamanders, demonstrates conspicuous and extensive increase in the degree of ossification during ontogeny. Increased ossification of long bones occurs as a gradual process, beginning prior to sexual maturity, and is proportional to body size in each species; maximum ossification is achieved soon after sexual maturity (Fig. 6). However, unlike bone growth in most amniotes, *Thorius* lacks secondary ossification centers at all stages. Instead, increased ossification is solely the result of longitudinal expansion of the primary (diaphyseal) center of



Fig. 9. Longitudinal sections through femurs of juvenile and adult *Thorius*. A. Proximal end of femur of a juvenile specimen of *T. minutissimus* (M 4736; SVL = 12.5 mm). B.

ossification. In contrast, mesopodial ossification appears abruptly and coincidentally with sexual maturity (Fig. 5); it may be seen as merely the culmination of a general maturation process that involves the entire limb skeleton. Increased ossification involves additional qualitative, as well as quantitative, changes. Most important among these is the loss of the complex of stratified layers of skeletogenous tissue from the ends of long bones, especially the proliferative zone, and the consequent reduction in the size and development of cartilaginous epiphyses (Fig. 9). As a result, and as is the case in most amniotes, the physiological capacity for longitudinal growth of the appendicular skeleton in juveniles is absent from adults. This constitutes a mechanism of determinate growth unique to urodele amphibians and analogous to that found in most amniotes. The extensive ossification of much of the adult



Distal end of femur of an adult T. sp. F (TJP 14596; SVL = 30.9 mm).

appendicular skeleton contrasts sharply with the otherwise "reduced," pedomorphic morphology that generally characterizes the skeleton of *Thorius*, particularly the limbs and skull (Wake, '66). In view of the relation of the increased ossification to the pattern of determinate appendicular skeletal growth, it seems likely that these novel features are only part of a more fundamental mechanism of overall growth regulation that restricts adult body size in *Thorius* by precocious truncation of somatic development relative to that observed in larger, generalized plethodontid salamanders.

The novel mineralizations in the appendicular skeleton of *Thorius* were first identified by E.D. Cope more than a century ago in his original description of the genus (1869a). Cope presented the mineralizations as a taxonomic character that distinguished *Thorius* from all other plethodontid genera, and most subsequent discussions of the mineralizations have considered them only in this context (for example, see Cope, 1869b; Dunn, '26; Hall, '52; Hilton, '46, '48; Rabb, '55; Taylor, '44). Two exceptions are the works of Uzzell ('61) and Wake ('66), who offered functional (i.e., biomechanical) models to explain the increased mineralization in the appendicular skeleton. Uzzell also described variation in mesopodial ossification in a single species, *T. pulmonaris*.

Wake proposed that the increased ossification of long bones acted as "strengthening compensations for paedomorphic weakening (66: 46), an explanation similar to that claimed earlier by Uzzell for the ossified mesopodial elements. Both authors cited the independent development of one or both of these traits in other genera of small salamanders as additional evidence in support of their ideas (see Rabb, '55). However, while mechanical influences can have a dominant role in effecting both the sequence and extent of skeletal development in vertebrates generally (Abdalla, '79; Hall, '71, '75, '78; Hoyte and Enlow, '66; Murray, '36; Murray and Drachman, '69) and have been invoked to explain the calcification or ossification of hyoid cartilages in many salamanders, including Thorius (Lombard and Wake, '77; Özeti and Wake, '69; Uzzell, '61), I consider biomechanical explanations of the unique limb mineralizations of Thorius unlikely for several reasons.

First, Uzzell ('61) inferred that force transmission was greatest through postaxial mesopodial elements because they were the first to mineralize. Yet, as indicated by Wake ('66), the postaxial elements are lost or reduced most frequently in plethodontid salamanders, and thus they seem to play a minor role in force transmission through the carpus and tarsus; they would not be expected to mineralize first if mineralization occurred in response to mechanical stress. Second, my observations of the variability of the sequence of mesopodial ossification suggest that the sequence cannot reflect the pattern of force transmission unless the pattern itself is highly variable. Most significantly, if ossification occurred in response to mechanical stress we would expect ossification to appear at a more or less constant size in all animals. Instead, interspecific comparisons have revealed the onset of ossification to be correlated with the onset of sexual maturation, rather than a single body size for the genus. Therefore, it is unlikely that mechanical factors play a primary role in effecting increased ossification in the appendicular skeleton of *Thorius.* If these factors do influence ossification events in the limb, it is as a result of an interaction between these stimuli and a predisposition of these cartilages to increased ossification conferred by physiological alterations associated with sexual maturation.

Increased ossification of the appendicular skeleton in Thorius is a derived condition with respect to larger, generalized plethodontid salamanders that display the primitive condition of well-developed cartilaginous epiphyses and cartilaginous mesopodial elements in both juveniles and adults (Wake, '66). However, ossification of mesopodial elements actually represents the secondary appearance of a feature that primitively was present in urodeles but was lost by ancestors of modern plethodontid salamanders (Romer, '66). Thus, the presence of ossified mesopodials may be interpreted in at least two ways. In one, mesopodial ossifications represent an atavism – the reappearance of bones homologous to those of nonplethodontid salamanders as a result of the reactivation of a developmental "program" that is otherwise dormant in plethodontid salamanders (see Raikow ('75) for an example from birds) – whereas in the other, ossified mesopodials are a neomorph, a truly novel morphological feature with a different developmental basis from those seen in nonplethodontid salamanders. Because mesopodial ossification appears regularly as part of a coordinated mechanism of appendicular skeletal growth regulation that is unique to Thorius, and not as an independent or rare developmental anomaly, I favor the latter hypothesis.

Interspecific size differentiation, skeletal variation, and morphological novelty

While the skeletal growth pattern of Thorius differs greatly from that of other plethodontid salamanders, it is strikingly similar in all species of Thorius examined so far. In fact, the only major difference among species with respect to limb ossification phenomena involves variation in the timing of these processes relative to body size - species characterized by a relatively small mean adult body size initiate and complete ontogenetic increase in ossification at a smaller body size than do larger species (Figs. 5-7). Thus it appears that alteration in the timing, relative to body size, of processes conferring determinate skeletal growth has been employed as a means of achieving adult size differentiation within the genus. This provides further evidence of the dominant role of heterochrony (sensu Gould, '77) in effecting morphological diversification among plethodontid salamanders (Alberch, '80, '81; Alberch and Alberch, '81; Larson, '79; Wake, '66).

The regularity and precise timing of limb ossification phenomena in Thorius are superimposed on a pattern of extreme variation in the actual composition of the limb skeleton, as for example with the variation in mesopodial fusion combinations and overall carpal and tarsal arrangement (Tables 2-5). Both the carpus and tarsus manifest trends toward reduction in the number of separate elements, as cartilaginous units that are separate in the respective presumed ancestral arrangements coalesce to give as few as four elements in the carpus (Fig. 2) and five units in the tarsus (Fig. 3). Moreover, decrease in mesopodial number has evolved independently several times, as revealed by the different carpal or tarsal arrangements that contain the identical (reduced) number of separate elements (e.g., carpal patterns II, III, VI, VIII). Six carpal arrangements (III-VIII) and at least four tarsal arrangements (II-IV, VI) are not found in other plethodontid salamanders (Wake, '66); thus miniaturization of the appendicular skeleton in Thorius has been accompanied by the appearance of novel morphological arrangements not found in ancestors. However, variation in the number of limb elements is only part of a general pattern of extreme skeletal variation associated with the trend towards skeletal reduction in the genus (Wake, '66). Interestingly, a similar pattern of extreme variation and novelty in the composition of limb skeletal elements accompanying decreased relative limb size is common in many vertebrate lineages, such as the lizard genus *Bachia* (Lande, '78) and the plethodontid salamander genus Oedipina (Wake, '66), suggesting that increased skeletal variation, and the appearance of novel morphological arrangements, may represent an inevitable "by-product" of reduction via developmental truncation (see also Marshall and Corruccini, '78).

The magnitude of carpal and tarsal variation in *Thorius* is exceptional among amphibians. Intraspecific and, more importantly, intrapopulational variation in carpal and tarsal arrangement is rare in most plethodontid salamanders, although mesopodial fusion combinations distinguish many plethodontid genera and, in some cases, individual species from congeners (Alberch, '80, '81; Larson et al., '81; Wake, '66). Furthermore, the appearance of novel mesopodial arrangements may have significant evolutionary consequences. For example, Larson et al. ('81) identified the appearance of novel carpal and tarsal arrangements as one of two "key innovations" that permitted the adoption of an arboreal existence by the ancestors of the salamander genus Aneides. They also cited the absence of intermediate (i.e., transitional) arrangements as evidence that the unique arrangements in Aneides arose in "single steps" from the presumed ancestral arrangements seen in the closely related genus Plethodon. In Thorius, certain carpal and tarsal arrangements do distinguish some species. But several other arrangements, which are as different as those that distinguish species of Thorius from each other (and as different as those that distinguish Aneides from Plethodon), are present together within restricted local populations of each of many species of Thorius. In some populations, mesopodial polymorphism involves as many as four different carpal or five different tarsal arrangements and may include a high frequency of right-left asymmetry in single specimens. In addition, stages intermediate between fused and unfused states were observed occasionally in many combinations, as has been observed in detailed analyses of the development and genetic basis of mesopodial fusion in other vertebrates (Grüneberg, '63; Grüneberg and Hus-'65; Wright, '34). In Thorius, the ton, establishment of novel morphological features of the limb skeleton (often comparable in magnitude to those distinguishing species, or even genera) may require no mechanism other than the fixation of a particular trait that occurs variably within a single population.

Skeletal growth regulation in tetrapods

Mammals and birds demonstrate a pattern of determinate growth in which maximum adult body size is attained relatively early in life, and it is clear that many skeletal features peculiar to these groups, particularly the presence of secondary (epiphyseal) ossification centers, are related to this mechanism of overall growth regulation. However, many authors have used the association between secondary ossification centers and determinate skeletal growth in these groups to claim the absence of determinate skeletal growth in tetrapods that lack secondary centers (e.g., Andrew and Hickman, '74; Carroll, '77; Goss, '74, '80; Haines, '38, '42, '69). This claim is unjustified for at least two reasons. First, fusion of primary and secondary ossification centers in many mammals often is correlated poorly with attainment of maximum adult size. Specifically, in rodents

(Dawson, '29, '34a, b, '35; Strong, '26), guinea pigs (Zuck, '38) and opossums (Washburn, '46) fusion may be delayed until late in life, years after the cessation of longitudinal growth. Furthermore, a given specimen may demonstrate great regional variation in the timing of epiphyseal fusion within the body; in some bones, epiphyseal fusion may never occur (Washburn, '46). Second, most birds have only a single epiphyseal ossification center that lies at the head of the tibiotarsus (Bellairs and Jenkin, '60; Hinchliffe and Johnson, '80); regulation of growth of the remaining elements is achieved without secondary centers. Thus, although secondary (epiphyseal) ossification centers are components of the pattern of determinate skeletal growth that is seen in many amniotes, they are not a requisite feature of the mechanism conferring precise growth and size regulation in the entire skeleton in these groups.

Amphibians typically lack secondary ossifications in long bones, although calcified cartilage has been described in the epiphyses of several frogs (Haines, '42). Among plethodontid salamanders, adult Thorius are unique in the extent of appendicular ossification, including long bones and mesopodial elements, although they too lack secondary centers. This "excessive" ossification is part of a mechanism of determinate skeletal growth that confers 1) size reduction relative to larger, generalized plethodontid salamanders, and 2) adult size differences among species within the genus. However, empirical estimates of growth in natural populations of other plethodontid salamanders suggest that skeletal growth may be quite determinate in these groups as well; in many taxa, such as Batrachoseps attenuatus (Hendrickson, '54), Bolitoglossa rostrata (Houck, '77), and Desmognathus ochrophaeus (Tilley, '77, '80), little or no overall size increase is observed following the attainment of sexual maturity. The mechanism of skeletal growth regulation and adult size differentiation in these groups (and, presumably, other amphibians) does not involve the conspicuous modifications of the appendicular skeleton that are characteristic of Thorius. These results suggest the existence of a plurality of mechanisms of growth regulation in tetrapods, involving a variety of skeletal modifications. Knowledge of the full extent of this diversity awaits analysis of the mode and mechanism of skeletal growth, and growth regulation, in other groups.

ACKNOWLEDGMENTS

This study was undertaken while I was a graduate student in zoology at the University

of California at Berkeley, during which time many people provided aid and encouragement. Doug Eakins, Tom Hetherington, and H. Bradley Shaffer accompanied me on field trips to Mexico and (except in rare instances) enthusiastically collected Thorius. John Cadle, Carlos Ceron, Julie Feder, Martin Feder, Jim Lynch, Ted Papenfuss, Bob Seib, David Wake, Marvalee Wake, and Tom Wake provided additional material. Richard Wassersug helped me adapt the double-stain procedure for *Thorius*; Marvalee Wake allowed access to her histology lab. James Patton, Richard Wassersug, Brian Hall, Marvalee Wake, Sally Susnowitz, and two reviewers provided helpful comments and suggestions on various drafts of this paper. Mary Primrose, Terry Collins, and Phyllis Thompson helped prepare the figures. David Wake made the original suggestion that I study Thorius, and contributed enthusiasm, logistic support, and valuable intellectual discussion throughout my stay at Berkeley. This research was partially supported at the University of California, by a Louise M. Kellogg Grant-in-Aid and an Annie Alexander Memorial Fellowship from the Museum of Vertebrate Zoology, a Center for Latin American Studies Grantin-Aid, the Department of Zoology, and National Science Fondation grant DEB-7803008 to D.B. Wake; it was completed while I was a Killam Postdoctoral Fellow at Dalhousie University.

LITERATURE CITED

- Abdalla, O. (1979) Ossification and mineralization in the tendons of the chicken (Gallus domesticus). J. Anat. 129:351– 359.
- Alberch, P. (1980) Ontogenesis and morphological diversification. Am. Zool. 20:653-667.
- Alberch, P. (1981) Convergence and parallelism in foot morphology in the neotropical salamander genus *Bolito*glossa. I. Function. Evolution 35:84-100.
- Alberch, P., and J. Alberch (1981) Heterochronic mechanisms of morphological diversification and evolutionary change in the neotropical salamander, *Bolitoglossa occidentalis* (Amphibia: Plethodontidae). J. Morphol. 167: 249-264.
- Andrew, W., and C.P. Hickman (1974) Histology of the Vertebrates. St. Louis: Mosby.
- Bellairs, A.D.A., and C.R. Jenkin (1960) The skeleton of birds. In A.J. Marshall (ed.): Biology and Comparative Physiology of Birds. New York: Academic, pp. 241-300.
- Carroll, R.L. (1977) The origin of lizards. In S.M. Andrews, R.S. Miles, and A.D. Walker (eds.): Problems in Vertebrate Evolution. New York: Academic, pp. 359-396.
- Cope, E.D. (1869a) A review of the species of the Plethodontidae and Desmognathidae. Proc. Acad. Nat. Sci. Philadelphia. 1869:93-118.
- Cope, E.D. (1869b) Natural history miscellany . . . new salamander. Am. Nat. 3:222.
- Dawson, A.B. (1929) A histological study of the persisting cartilage plates in retarded or lapsed epiphyseal union in the albino rat. Anat. Rec. 43:109–129.
- Dawson, A.B. (1934a) Further studies on epiphyseal union in the skeleton of the rat. Anat. Rec. 60:83-86.

- Dawson, A.B. (1934b) Additional evidence of the failure of epiphyseal union in the skeleton of the rat. Studies on wild and captive gray Norway rats. Anat. Rec. 60:501-511.
- Dawson, A.B. (1935) The sequence of epiphyseal union in the skeleton of the mouse with special reference to the phenomenon of "lapsed" union. Anat. Rec. 63:93-99.
- Dingerkus, G., and L.D. Uhler (1977) Enzyme clearing of alcian blue stained whole small vertebrates for demonstration of cartilage. Stain Technol. 52:229-232.
- Dunn, E.R. (1926) The Salamanders of the Family Plethodontidae. Northampton, Mass: Smith College.
- Goin, C.J., and O.B. Goin (1971) Introduction to Herpetology. 2nd Ed. San Francisco: Freeman.
- Goss, R.J. (1974) Aging versus growth, Perspect. Biol. Med. 17:485-494.
- Goss, R.J. (1980) Physiology of Growth. New York: Academic.
- Gould, S.J. (1977) Ontogeny and Phylogeny. Cambridge: Harvard University.
- Grüneberg, H. (1963) The Pathology of Development. New York: Wiley.
- Grüneberg, H., and K. Huston (1965) The development of bovine syndactylism. J. Embryol. Exp. Morphol. 19:251-259.
- Haines, R.W. (1938) The primitive form of epiphysis in the long bones of tetrapods. J. Anat. Long. 72:323-343.
- Haines, R.W. (1941) Epiphyseal structure in lizards and marsupials. J. Anat. 75:282-294.
- Haines, R.W. (1942) The evolution of epiphyses and of endochondral bone. Biol. Rev. 17:267-292.
- Haines, R.W. (1969) Epiphyses and sesamoids. In C. Gans (ed.): Biology of the Reptilia. New York: Academic, Vol. 1, pp. 81-115.
- Hall, B.K. (1971) Histogenesis and morphogenesis of bone. Clin. Orthop. 74:249-267.
- Hall, B.K. (1975) Differentiation and maintenance of articular (secondary) cartilage on avian membrane bones. Ann. Rheum. Dis. 34(Suppl.):145.
- Hall, B.K. (1978) Developmental and Cellular Skeletal Biology. New York: Academic.
- Hall, C.W. (1952) Comparative osteology of salamanders of the genus *Thorius*. Master's thesis, University of Kansas.
- Ham, A.W., and D.H. Cormack (1979) Histophysiology of Cartilage, Bone, and Joints. Philadelphia: Lippincott.
- Hanken, J. (1980) Morphological and genetic investigations of miniaturization in salamanders (genus *Thorius*). Doctoral thesis, University of California.
- Hendrickson, J.R. (1954) Ecology and systematics of salamanders of the genus *Batrachoseps*. Univ. Calif. Publ. Zool. 54:1-46.
- Hilton, W.A. (1946) Skeletons of Mexican and Central American salamanders of the family Plethodontidae. J. Entomol. Zool. 38:1-8.
- Hilton, W.A. (1948) The carpus and tarsus of salamanders. J. Entomol. Zool. 40:1-13.
- Hinchliffe, J.R., and D.R. Johnson (1980) The Development of the Vertebrate Limb. Oxford: Clarendon.
- Houck, L.D. (1977) Life history patterns and reproductive biology of neotropical salamanders. In D.H. Taylor and S.I. Guttman (eds.): The Reproductive Biology of Amphibians. New York: Plenum, pp. 43-72.
- Hoyte, D.A.N., and D.H. Enlow (1966) Wolff's law and the problem of muscle attachment on resorptive surfaces of bone. Am. J. Phys. Anthropol. 24:205-214.
- Humason, G.L. (1979) Animal Tissue Techniques. San Francisco: Freeman.
- Lande, R. (1978) Evolutionary mechanisms of limb loss in tetrapods. Evolution 32:73-92.
- Larson, A. (1979) Paedomorphosis in relation to rates of morphological and molecular evolution in the salamander

Aneides flavipunctatus (Amphibia, Plethodontidae). Evolution 34:1-17.

- Larson, A., D.B. Wake, L.R. Maxson, and R. Highton (1981) A molecular phylogenetic perspective on the origins of morphological novelties in salamanders of the Tribe Plethodontini (Amphibia, Plethodontidae). Evolution 35: 405-422.
- Lombard, R.E., and D.B. Wake (1977) Tongue evolution in the lungless salamanders, family Plethodontidae. II. Function and evolutionary diversity. J. Morphol. 153: 39-80.
- Marshall, L.G., and R.S. Corruccini (1978) Variability, evolutionary rates, and allometry in dwarfing lineages. Paleobiology 4:101-119.
- Murray, P.D.F. (1936) Bones: A Study of the Development and Structure of the Vertebrate Skeleton. Cambridge: Cambridge University Press.
- Murray, P.D.F., and D.B. Drachman (1969) The role of movement in the development of joints and related structures: The head and neck in the chick embryo. J. Embryol. Exp. Morphol. 22:349-371.
- Özeti, N., and D.B. Wake (1969) The morphology and evolution of the tongue and associated structures in salamanders and newts (Family Salamandridae). Copeia 1969: 91-123.
- Rabb, G.B. (1955) A new salamander of the genus Parvimolge of Mexico. Breviora 42:1-9.
- Raikow, R.J. (1975) The evolutionary reappearance of ancestral muscles as developmental anomalies in two species of birds. Condor 77:514–517.
- Romer, A.S. (1956) Osteology of the Reptiles. Chicago: University of Chicago Press.
- Romer, A.S. (1966) Vertebrate Paleontology (3rd ed). Chicago: University of Chicago Press.
- Romer, A.S. (1970) The Vertebrate Body (4th ed). Philadelphia: Saunders.
- Stanley, S.M. (1980) Macroevolution. San Francisco: Freeman.
- Strong, R.M. (1926) The order, time, and rate of ossification of the albino rat (*Mus norvegicus albinus*) skeleton. Am. J. Anat. 36:313–355.
- Taylor, E.H. (1944) The genera of plethodont salamanders in Mexico, Part 1. Univ. Kansas Sci. Bull. 30(Pt. 1):189-232.
- Tilley, S.G. (1977) Studies of life histories and reproduction in North American plethodontid salamanders. In D.H. Taylor and S.I. Guttman (eds.): The Reproductive Biology of Amphibians. New York: Plenum, pp. 1-41.
- Tilley, S.G. (1980) Life histories and comparative demography of two salamander populations. Copeia 1980:806-821.
- Uzzell, T.M., Jr. (1961) Calcified hyoid and mesopodial elements of plethodontid salamanders. Copeia 1961:78-86.
- Wake, D.B. (1963) Comparative osteology of the plethodontid salamander genus Aneides. J. Morphol. 113:77-118.
- Wake, D.B. (1966) Comparative osteology and evolution of the lungless salamanders, family Plethodontidae. Mem. So. Calif. Acad. Sci. 4:1-111.
- Wake, D.B., and J.F. Lynch (1976) The distribution, ecology, and evolutionary history of plethodontid salamanders in tropical America. Nat. Hist. Mus. Los Angeles Co. Bull. 25:1-65.
- Washburn, S.L. (1946) The sequence of epiphyseal union in the opossum. Anat. Rec. 95:253-363.
- Wassersug, R.D. (1976) A procedure for differential staining of cartilage and bone in whole formalin-fixed vertebrates. Stain Technol. 51:131-134.
- Wright, S. (1934) An analysis of variability in number of digits in an inbred strain of guinea pigs. Genetics 19:506–536.
- Zuck, T.T. (1938) The age order of epiphyseal union in the guinea pig. Anat. Rec. 70:389-399.