

# Miniaturization and its Effects on Cranial Morphology in Plethodontid Salamanders, Genus *Thorius* (Amphibia, Plethodontidae): II. The Fate of the Brain and Sense Organs and Their Role in Skull Morphogenesis and Evolution

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**ABSTRACT** Relative size and arrangement of the brain and paired sense organs are examined in three species of *Thorius*, a genus of minute, terrestrial salamanders that are among the smallest extant tailed tetrapods. Analogous measurements of representative species of three related genera of larger tropical (*Pseudoeurycea*, *Chiropterotriton*) and temperate (*Plethodon*) salamanders are used to identify changes in gross morphology of the brain and sense organs that have accompanied the evolution of decreased head size in *Thorius* and their relation to associated changes in skull morphology.

In adult *Thorius*, relative size (area measured in frontal plane, and length) of the eyes, otic capsules, and brain each is greater than in adults of all of the larger genera; relative size of the nasal capsules is unchanged or slightly smaller. Interspecific scaling phenomena—negative allometry of otic capsule, eye and brain size, isometry or slight positive allometry of nasal capsule size, all with respect to skull length—also are characteristic of intraspecific (ontogenetic) comparisons in both *T. narisovalis* and *Pseudoeurycea goebeli*.

Predominance of the brain and eyes in *Thorius* results in greater contact and overlap among these structures and the nasal capsules in the anterior portion of the head. This is associated with anterior displacement of both the eyes and nasal capsules, which now protrude anterior to the skull proper; a change in eye shape; and medial deformation of anterior braincase walls. Posteriorly, predominance of the otic capsules has effected a reorientation of the jaw suspensorium to a fully vertical position that is correlated with the novel presence of a posteriorly directed squamosal process and shift in origin of the quadropectoralis muscle.

Many of these changes in cranial morphology may be explained simply as results of mechanical (physical) interactions among the skeletal, nervous, and sensory components during head development at reduced size. This provides further evidence of the role of nervous, sensory, and other "soft" tissues in cranial skeletal morphogenesis, and reinforces the need to consider these tissues in analyses of skull evolution.

"In vertebrates, a quite simple change in epigenetic mechanism may have a profound and extensively different result. Moreover, the result is an integrated organism" (Davis, '64, p. 5).

"Many conspicuous features in the skeleton depend only on the capacity of bone to respond to extrinsic factors" (Davis, '64, p. 12).

A well-established principle of vertebrate development is the great degree to which nervous and sensory components may promote, or even direct, skeletal differentiation

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and morphogenesis. This is perhaps nowhere better seen than in the skull. Here, the influence of the brain and sense organs ranges from purely mechanical effects, which in large part may determine both the shape and differentiation of individual skull elements (Bassett, '72; Hall, '81; Moss, '61; Vilmann and Moss, '79), to more subtle (presumably chemical) inductive effects that influence the timing and orientation of bone formation (Schowing, '68a; Simons and Van Limborgh, '79). Earlier views of skull development (see reviews by Goss, '72, '80; Hoyte, '66; Van Limborgh, '72) held that cranial skeletal morphology was determined intrinsically, i.e., within the developing skeletal tissues, and was relatively unaffected by surrounding tissues. However, the prevailing view of skull development (often subsumed under the headings "functional craniology" or "functional cranial analysis"; see Dullemeijer, '68, '72, '74; Van der Klaauw, '46; Moss, '68a,b, '72a,b; Moss and Young, '60) stresses the critical role of neighboring tissues. These include sensory, nervous, circulatory, connective, and muscular tissues that interact to determine the ultimate size and shape of many skull elements, the intrinsic growth of which is labile and relatively indeterminate.

The predominant role of nervous and sensory components in skull development has been confirmed in numerous experimental studies which include a wide variety of vertebrates, including fishes (Pinganaud-Perrin, '73), chicks (Coulombre and Crelin, '58; Schowing, '68b,c; Silver, '62; Simons, '79; Simons and Van Limborgh, '79), amphibians (Burr, '16; Corson, '66; Leibel, '76; Richardson, '32; Twitty, '32; Washburn and Detwiler, '43), and mammals (Moss, '61; Sarnat, '82; Young, '59). These studies share a primary goal: identification of basic processes and mechanisms of vertebrate head morphogenesis. In vertebrate paleontology and comparative morphology, appreciation of the "interactive" nature of head development has provided a very effective framework for analysis of skull evolution. For example, often drastic skull rearrangements that characterize phyletic evolution in many mammalian lineages have been interpreted as direct consequences of alteration in the shape, size, or orientation of the brain or sense organs (DuBrul, '50; Van der Klaauw, '52; Radinsky, '68). Similarly, significant modifications in skull architecture that typify both the amphibian-reptilian transition

and the evolution of urodeles from early amphibians have been attributed to changes in the relative size and position of the brain, otic capsules, eyes, and nasal capsules (Carroll, '70; Carroll and Holmes, '80).

In this study I examine the changes in gross morphology, relative size, and geometrical "packaging" of the brain and sense organs that have accompanied extreme size reduction, or miniaturization, in an amphibian lineage, and the relationship of these changes to the associated modifications in skull architecture. My analysis focusses on salamanders of the plethodontid genus *Thorius*, a group comprising at least 15 terrestrial or arboreal salamanders that are among the smallest extant tailed tetrapods. Data comprise a series of measurements of gross dimensions (length, area) and arrangement of the brain and the three paired primary sense organs—otic capsules, eyes, and nasal capsules—relative to the surrounding skull in *Thorius* and selected genera of larger salamanders. Earlier (Hanken, '83), I presented an analysis of the patterns of osteological variation in the cranium of *Thorius*, stressing particularly those features that characterized the evolution of decreased head size. Three general characteristics were apparent: reduced or limited development of many individual elements; increased variability; and morphological novelty, particularly involving the jaw suspension and braincase. The first two characteristics were considered as either direct or indirect consequences of truncated development or paedomorphosis in *Thorius* relative to larger generalized salamanders; little more will be said about them here. However, I will stress the relationship among altered proportions and distribution of the brain and sense organs and the novel aspects of cranial morphology. Three primary questions will be addressed: 1) What is the relative size of the brain and sense organs in *Thorius* compared with those of larger salamanders? 2) How is packaging of these structures accommodated in a skull of drastically reduced size? 3) Do modifications in the shape, size, and/or position of the brain and sense organs impose or effect any structural rearrangements of the surrounding skull?

Two implicit assumptions, both supported by earlier studies (see above), underlie this analysis. First, many prominent features of adult skull morphology are a direct result of physical (mechanical) interactions with the

brain and sense organs. Second, many phylogenetic changes in skull morphology represent secondary responses of skeletal elements to modifications which primarily involve changes in the relative size and/or position of nervous and sensory components. A later paper will present detailed aspects of brain and sense organ structure and function (Grunwald, Hanken, and Roth, unpublished observation).

#### MATERIALS AND METHODS

Relative size and geometrical arrangement of the brain and each pair of sense organs were quantified in each of five adult females of three species of *Thorius* (mean snout-vent length, SVL, in mm, measured to the posterior end of vent, is in parentheses): *T. pennatulus* (19.2), *T. minutissimus* (24.6), and *T. narisovalis* (28.7). These species collectively span the range of mean adult body size found in the genus, and thus would be expected to demonstrate any size-related interspecific trends in head packaging. For uniformity, only females were included. However, there are no apparent sex-related differences in head packaging other than those directly related to body size differences; in a given species of *Thorius*, mean SVL of adult females typically is slightly larger than that of adult males. Five adult females each of three larger plethodontid species, *Chiropterotriton priscus* (45.7), *Plethodon vehiculum* (49.0), and *Pseudoeurycea goebeli* (54.4), were used as reference material for evaluating head packaging in *Thorius*. These species possess a relatively generalized plethodontid morphology that may be similar to that of the ancestral form from which *Thorius* was derived (Wake, '66; Wake and Elias, '83). Ontogenetic comparisons in these species were based on examination of five juvenile *T. narisovalis* (SVL = 12.1–13.4,  $\bar{x}$  = 12.6) and five juvenile *P. goebeli* (SVL = 15.1–48.6,  $\bar{x}$  = 32.8). Spec-

imens examined are listed in Hanken ('80) and are deposited in the Museum of Vertebrate Zoology, University of California, Berkeley.

Specimens were cleared and differentially stained for bone and cartilage using the Alcian Blue-Alizarin Red S procedure (Dingerkus and Uhler, '77; Wassersug, '76) as modified by Hanken and Wassersug ('81). Skulls were photographed in dorsal view using a dissecting microscope fitted with a photo tube (Wild M8S Zoom Stereomicroscope). Specimens were immersed in glycerin and covered with a cover slip to stabilize them. All skull photographs were printed to approximately the same size on 20 × 25 cm photographic paper from which measurements of head proportions were made.

Relative sizes of the nasal capsules, eyes, otic capsules, and brain were measured by first drawing the outline of each structure (in frontal plane) as it appeared on the photograph (Fig. 1, left). The nasal capsule outline included all associated nasal cartilage visible dorsally. Otic capsule outline was defined by the clearly visible semicircular canals. Braincase dimensions were substituted for those of the brain itself. Operationally, the braincase outline was defined as a six-sided polygon symmetric about the longitudinal skull axis with the following three, paired, corner reference points: 1) dorsal articulation between the orbitosphenoid bone and posterior nasal cartilage, 2) anterior articulation of the occipito-otic and parietal bones, and 3) posterior articulation of the occipito-otic and supraoccipital bones at the level of the synotic tectum. Overall head outline was defined by the perimeter contributed in turn by the premaxilla, nasal capsule, eye, mandible, quadrate and squamosal (when visible), occipito-otic and occipital condyle. The area (cm<sup>2</sup>) of each structure then was measured from the photographs with a Dietzgen Plan-

#### Abbreviations

a,	Anterior edge of nasal capsule	NC,	Nasal capsule
a',	Posterior edge of nasal capsule	Oc,	Occipital condyle
b,	Anterior edge of eye	OC,	Otic capsule
b',	Posterior edge of eye	Pl,	<i>Plethodon vehiculum</i>
BC,	Braincase	Pm,	Premaxilla
c,	Anterior edge of braincase	Ps,	<i>Pseudoeurycea goebeli</i>
c',	Posterior edge of braincase	Q,	Quadrate
C,	<i>Chiropterotriton priscus</i>	SL,	Skull length
d,	Anterior edge of otic capsule	Sq,	Squamosal
d',	Posterior edge of otic capsule	T.m.,	<i>Thorius macdougalli</i>
E,	Eye	T.n.,	<i>Thorius narisovalis</i>
M,	Mandible	T.p.,	<i>Thorius pennatulus</i>

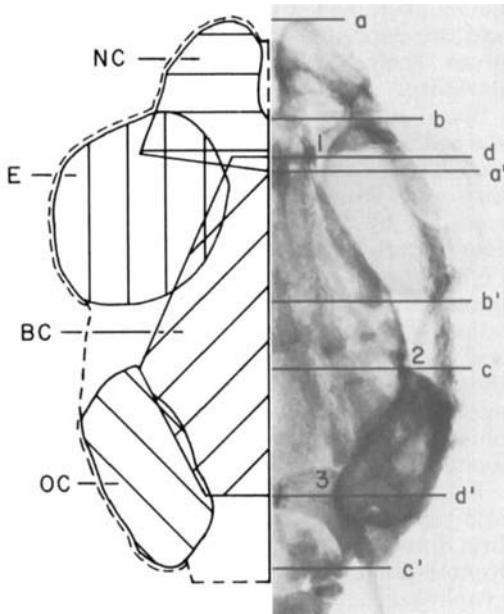


Fig. 1. Measurement techniques. Left: Outline of braincase and sense organs used to measure the proportion of head area occupied by each. Dashed line denotes head outline used to obtain overall head area. Values were calculated for one side (usually left) and doubled to give total estimate in each skull. Right: Photograph of stained skull indicating the respective anterior and posterior edges of the braincase and sense organs extrapolated to the median longitudinal skull axis. Length and position of each structure were standardized to a skull length of 100 units from the posterior edge of the occipital condyle (0) to the anterior edge of the premaxilla (100). Numbers identify the paired reference points used to delineate braincase outline (see text).

imeter no. 132. Proportion (%) of head area occupied by the paired sense organs and brain was calculated by dividing the photograph area occupied by each of these structures by the corresponding estimate of head area, multiplied by 100. Absolute area ( $\text{mm}^2$ ) of the brain and sense organs was derived using the formula,  $\text{AREA} = (\text{SL}/\text{sl})^2 \times \text{area}_p$ , in which SL = skull length (anterior edge of premaxilla to posterior edge of occipital condyle) measured directly on the specimen, sl = skull length measured on the photograph, and  $\text{area}_p$  = area measured on the photograph. To the extent that the shapes of the brain and sense organs, in transverse plane, are similar in *Thorius* and the larger genera, proportional differences in relative head area occupied by these structures will reflect corresponding differences in relative head vol-

ume. This condition is met for the nasal capsule, eye, and otic capsule, but brain shape differs markedly between *Thorius* and larger plethodontid genera such as *Pseudoeurycea* (G. Roth, personal communication) and *Hydromantes* (Grunwald, '81). However, the shape differences, particularly the increase in relative brain height in *Thorius*, are such that differences in relative brain area underestimate proportional differences in brain volume (Grunwald, Hanken, and Roth, unpublished observation).

Linear distribution and overlap of the three sense organs and brain were quantified by drawing the median longitudinal skull axis on each photograph and extrapolating a line perpendicular to this axis from the anterior and posterior edges of the nasal capsules, eyes, otic capsules, and braincase (Fig. 1, right). Proportion (%) of skull length occupied by each structure was defined operationally as the length of the skull axis segment delimited by its anterior and posterior edges, divided by skull length (sl), multiplied by 100. Occipital condyle shape and position relative to the otic capsules are similar in all species considered here, thus providing a standardized landmark for the rear skull margin in all comparisons.

## RESULTS

Relative size and distribution of the brain and sense organs generally are the same in comparisons among the three, larger reference species; interspecific variation is slight relative to the differences between each of these species and any of the three species of *Thorius* (Tables 1–3). For this reason, I will discuss in detail a comparison between a single reference species, *Pseudoeurycea goebeli*, and *Thorius*. All generalizations presented are equally true for the remaining comparisons of both *Plethodon* and *Chiropterotriton* to *Thorius*.

### Head area

The eyes, otic capsules, and brain are predominant in *Thorius* (Table 1; Figs. 2A,B, 3). In dorsal view, the eyes occupy 30–50% and the otic capsules 20–50% more head area than in *Pseudoeurycea*. The brain, which is approximately 60% larger in *Thorius*, shows the largest relative size increase. In contrast, the nasal capsules are either the same relative size, or smaller, in *Thorius*. Figure 4 depicts interspecific scaling of the brain and sense organ area in *Thorius* and larger sala-

TABLE 1. Proportion (%) of head area occupied by the nasal capsules, eyes, otic capsules, and brain in *Thorius* and three other plethodontid genera<sup>1</sup>

Species	Nasal c.	Eyes	Otic c.	Brain
<i>T. pennatulus</i>	14.0 (.3)	26.5 (.6)	17.0 (.8)	36.9 (.5)
<i>T. minutissimus</i>	15.8 (.4)	23.4 (.6)	13.5 (.6)	36.5 (.2)
<i>T. narisovalis</i> (ad.)	16.7 (.7)	22.8 (.6)	13.6 (.4)	37.4 (.4)
<i>T. narisovalis</i> (juv.)	14.5 (.5)	26.7 (.4)	15.7 (.7)	42.0 (.5)
<i>Pseudoeurycea goebeli</i>	17.1 (.5)	17.5 (.7)	11.1 (.3)	23.1 (.7)
<i>Chiropetrotriton priscus</i>	15.9 (.6)	17.7 (.7)	11.1 (.4)	26.3 (.5)
<i>Plethodon vehiculum</i>	15.2 (.7)	15.9 (.4)	11.9 (.5)	27.4 (.4)

<sup>1</sup>N = 5 for all samples; standard error is in parentheses.

TABLE 2. Proportion (%) of skull length occupied by the nasal capsules, eyes, otic capsules, and brain in *Thorius* and other plethodontid genera<sup>1</sup>

Species	SL	Nasal c.	Eyes	Otic c.	Brain
<i>T. pennatulus</i>	3.31 (.04)	27.5 (.4)	33.5 (.6)	36.5 (.8)	64.4 (.9)
<i>T. minutissimus</i>	3.93 (.05)	28.9 (1.0)	30.7 (.3)	33.8 (.4)	63.6 (.7)
<i>T. narisovalis</i> (ad.)	4.25 (.07)	28.9 (.7)	31.9 (.7)	34.7 (.9)	63.2 (.4)
<i>T. narisovalis</i> (juv.)	2.53 (.06)	28.2 (.8)	35.8 (.5)	35.5 (1.0)	67.6 (.7)
<i>Pseudoeurycea goebeli</i>	10.58 (.16)	30.6 (.8)	26.7 (.6)	26.7 (.6)	59.8 (.4)
<i>Chiropetrotriton priscus</i>	7.84 (.27)	27.8 (.7)	27.3 (.4)	27.5 (.4)	61.6 (.6)
<i>Plethodon vehiculum</i>	7.53 (.14)	28.5 (.1)	25.7 (.3)	28.0 (.4)	59.4 (.4)

<sup>1</sup>N = 5 for all samples; standard error is in parentheses. SL, skull length in mm.

TABLE 3. Relative linear position<sup>1</sup> of the anterior and posterior edges of the nasal capsules (a, a'), eyes (b, b'), otic capsules (c, c'), and brain (d, d') in *Thorius* and other plethodontid genera

Species	Nasal capsules		Eyes		Otic capsules		Brain	
	a	a'	b	b'	c	c'	d	d'
<i>T. pennatulus</i>	103.8 (.3)	76.3 (.3)	84.6 (.5)	51.1 (.2)	38.7 (.4)	2.3 (.4)	79.0 (.4)	14.2 (.4)
<i>T. minutissimus</i>	104.3 (.5)	75.4 (1.3)	83.7 (.7)	53.0 (.6)	37.3 (.2)	3.5 (.4)	77.2 (.9)	13.6 (1.0)
<i>T. narisovalis</i> (ad.)	104.1 (1.1)	75.2 (1.2)	81.9 (.9)	50.0 (.8)	37.8 (.7)	3.1 (.4)	79.2 (.5)	16.0 (.5)
<i>T. narisovalis</i> (juv.)	103.5 (.9)	75.3 (.9)	83.4 (.9)	47.6 (.4)	40.0 (1.2)	4.5 (.4)	82.0 (.7)	14.4 (1.0)
<i>Pseudoeurycea goebeli</i>	101.3 (.2)	70.6 (.6)	72.4 (.6)	45.7 (.7)	28.6 (.6)	1.9 (1.1)	72.8 (.6)	13.0 (.7)
<i>Chiropetrotriton priscus</i>	102.3 (.3)	74.5 (.9)	78.4 (.9)	51.1 (.6)	30.5 (.7)	2.9 (.3)	75.8 (1.1)	14.2 (.8)
<i>Plethodon vehiculum</i>	101.3 (.4)	72.9 (.4)	77.3 (.7)	51.6 (.4)	30.7 (.5)	2.7 (1.1)	73.6 (.7)	14.2 (.9)

<sup>1</sup>In each specimen, linear position was standardized to a skull length of 100 units from the posterior edge of the occipital condyle (0) to the anterior edge of the premaxilla (100). N = 5 for all samples; standard error is in parentheses. Proportion (%) of skull length occupied by each structure (Table 2) equals a-a', b-b', etc.

manders. Slopes of the least squares regression lines calculated from mean values for adults of each species equal 1.05 (nasal capsule), 0.81 (eye), 0.85 (otic capsule), and 0.79 (brain); *r* for each comparison exceeds 0.99. Thus, eye, otic capsule, and brain size each scale with negative allometry with respect to skull length, whereas nasal capsule size scales isometrically or possibly with slight positive allometry.

Accompanying increased relative size of the brain, eye, and otic capsule in *Thorius* is a change of shape of each organ in dorsal view (Fig. 2A,C). The eye, which is more or less circular in *Pseudoeurycea*, is ovoid, with the long axis perpendicular to the optical axis. Otic capsules are more elongate in the direc-

tion parallel to the posterior braincase walls. Brain shape change includes substantial increase in width beginning at the level of the eyes and continuing posteriorly to a maximum at the anterior articulation with the otic capsule.

Interspecific comparisons among adult *Thorius* demonstrate the same trends that characterize the comparison of *Thorius* and larger genera: negatively allometric scaling of eye, otic capsule, and brain size; isometric scaling of nasal capsule size.

#### Linear distribution

Measurements of relative length mirror those of relative area; eyes are 15–25%, otic capsules 27–37%, and the brain 6–8% longer

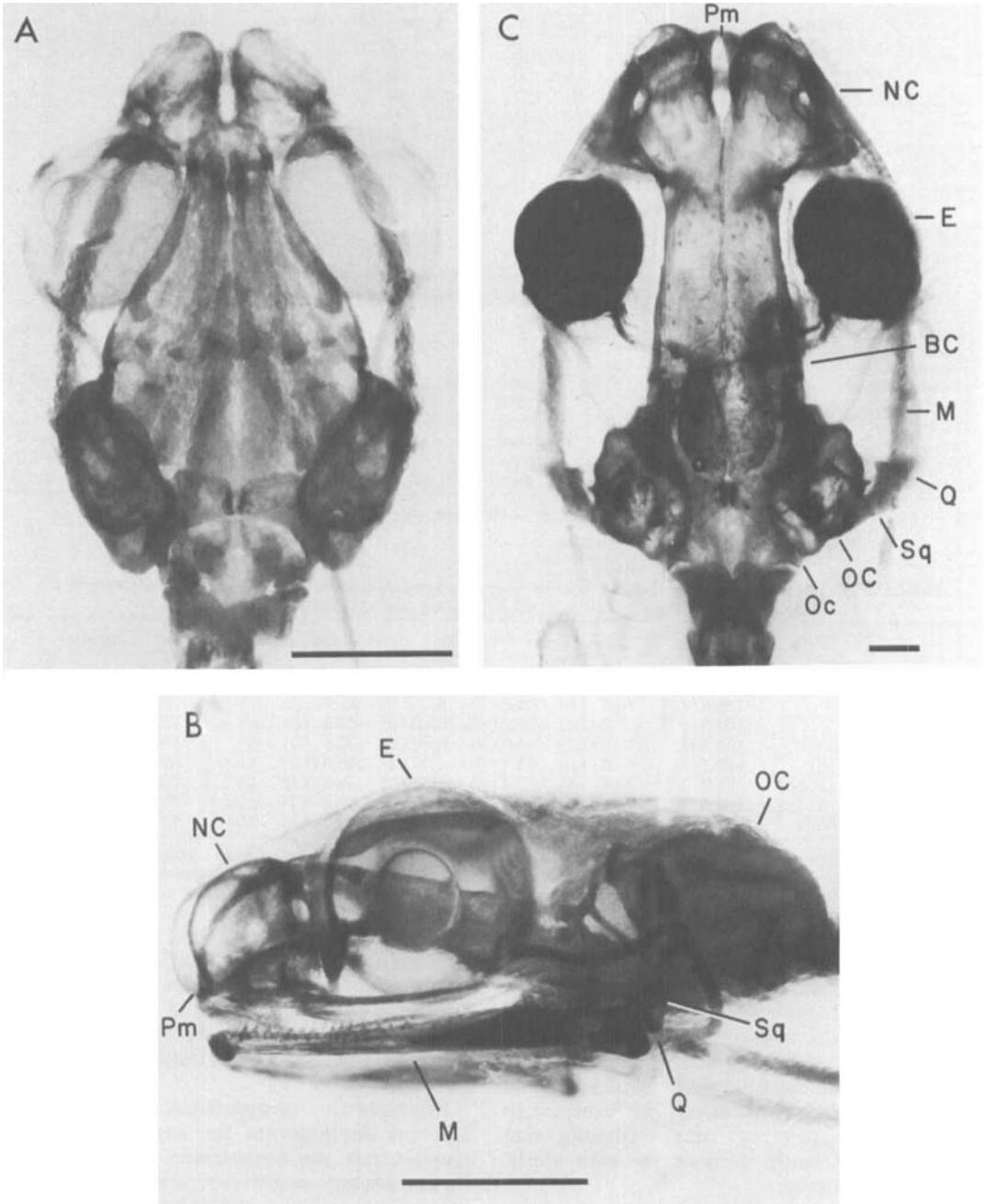


Fig. 2. Photographs of stained skulls. A, B. *Thorius pennatulus* (M 5114; SL = 19.6 mm). C. *Pseudoeurycea goebeli* (MVZ 130543; SL = 54.9 mm). A and C are dorsal views; B is a lateral view. The eyes in A and B are partially depigmented, revealing the prominent lens

within. Scale bar = 1 mm. The quadrate and squamosal, which together constitute the jaw suspensorium, are visible in dorsal view in *Pseudoeurycea* (C) but not in *Thorius* (A); they are visible in lateral view in *Thorius* (B).

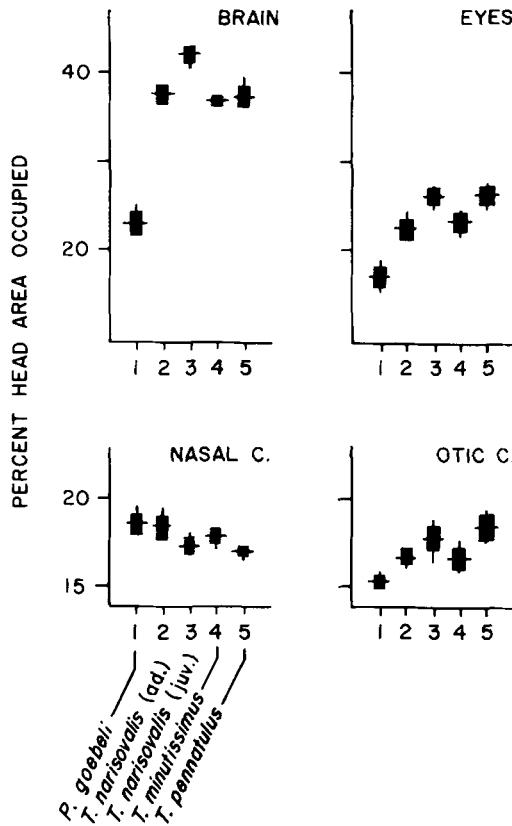


Fig. 3. Proportion (%) of head area occupied by the nasal capsules, eyes, otic capsules, and brain in *Pseudoeurycea goebeli* and three species of *Thorius*. Mean,

horizontal bar; range, vertical bar; 95% confidence interval, rectangle. N = 5 for each sample.

in *Thorius* than in *Pseudoeurycea*, whereas the nasal capsules are the same relative size or as much as 10% shorter (Table 2). Relative eye and otic capsule length are larger, and relative nasal capsule length is smaller in *T. pennatulius* compared with both *T. minutissimus* and *T. narisovalis*; differences among the latter two species are not significant ( $P > .95$ ). Relative brain length is approximately the same in all three species of *Thorius*.

Examination of the relative position of each structure reveals more subtle differences (Table 3, Fig. 5). Beginning at the rear of the skull, the substantial (30%) increase in relative length of the otic capsule in *Thorius* is achieved mostly by anterior expansion (the anterior border lies further forward). Similarly, the moderate (6%) increase in relative length of the brain is achieved primarily by

forward displacement of the anterior end of the braincase; the rear braincase margin is at approximately the same relative position in all skulls. The eye, however, both is significantly (19%) longer and lies further forward. The nasal capsules, although showing little change in relative length, also have been displaced anteriorly with respect to the rear skull margin (occipital condyles); in each comparison of *Thorius* with *Pseudoeurycea*, the posterior nasal capsule margin is shifted forward by as much as 6% of head length. The anterior margin, which typically overhangs the skull only slightly in larger salamanders, protrudes well beyond the premaxilla (cf. Fig. 2A,C).

#### Ontogenetic comparisons

Examination of juvenile *T. narisovalis* and *Pseudoeurycea goebeli* provides information

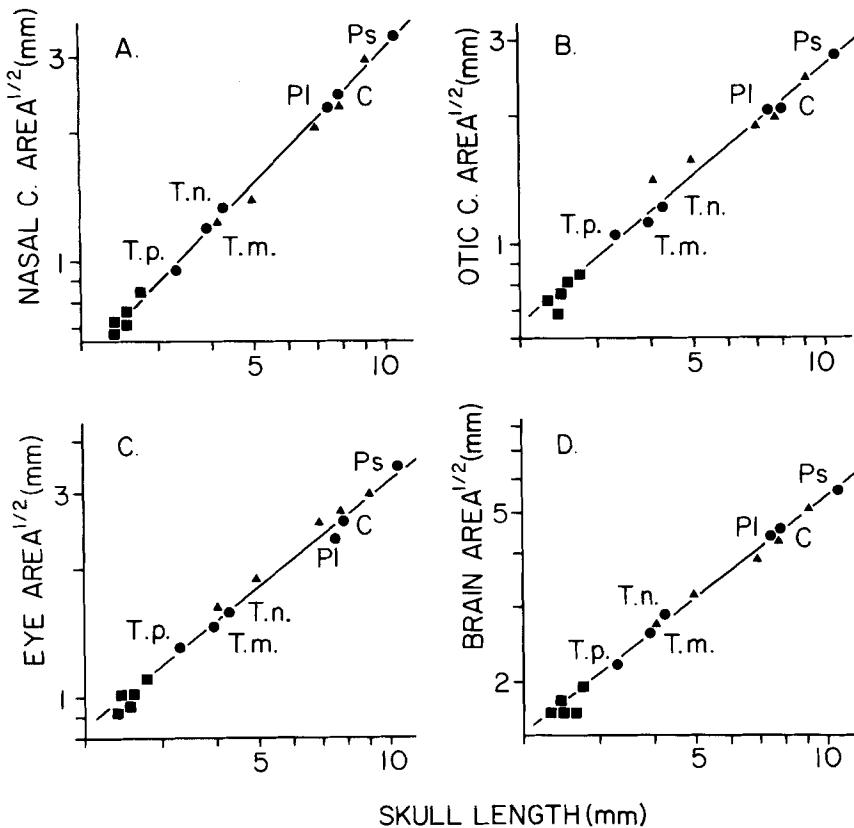


Fig. 4. Allometric scaling of (A) nasal capsule, (B) otic capsule, (C) eye, and (D) brain area relative to skull length in *Thorius* and larger salamanders. Scale is log-log. Closed circles represent the mean value of five adults of each species; these were used to compute the least

squares regression line in each graph. Individual juvenile specimens of *Pseudoeurycea goebeli* ( $\blacktriangle$ ) and *T. narisovalis* ( $\blacksquare$ ) are also indicated. Regression slopes are discussed in text.

about the ontogeny of head packaging. In both species, negative allometry of otic capsule, eye, and brain size (area) contrasts with the isometry or slight positive allometry of nasal capsule size (Tables 1, 2 Figs. 4, 5B,C). As a result, the predominance of the brain and eyes in tiny juveniles may be quite extreme: in some juvenile *Thorius*, the brain occupies nearly 43% of head area.

#### DISCUSSION Head packaging

Cranial miniaturization in *Thorius* is characterized by extreme reduction or loss of much of the ossified skeleton, especially the anterior elements, which typically is present in larger, adult salamanders (Hanken, '83). In contrast, the otic capsules, eyes, and brain,

which have not diminished in proportion to the absolute dimensions of the head, occupy a greater proportion of head area and head length in *Thorius* than in larger salamanders. In effect, the skull has shrunk around these relatively expanded nervous and sensory components (Fig. 2A,B). Furthermore, the geometrical packaging and arrangement of the brain and sense organs suggest 1) space available to contain these structures is severely limited; 2) there is "competition" for head space among these structures, especially anteriorly; and 3) the predominant brain, otic capsules, and eyes have imposed structural rearrangements on much of the skull that remains.

In the occiput, otic capsule enlargement is achieved primarily by expansion anteriorly

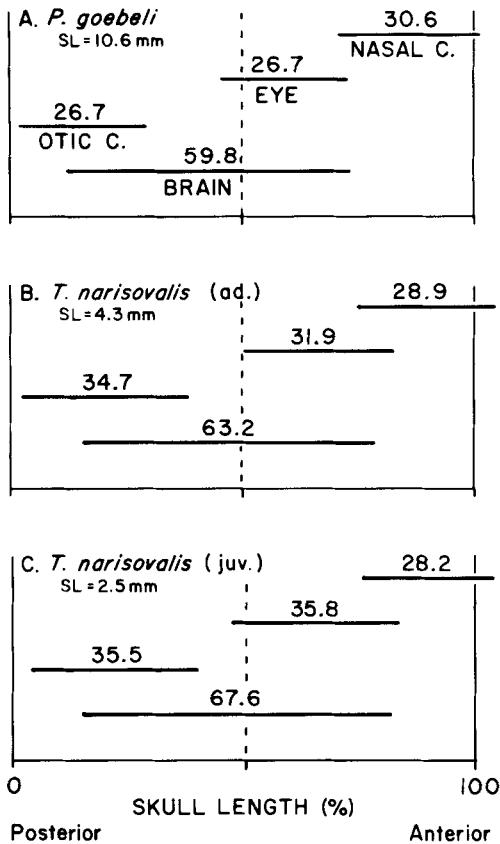


Fig. 5. Schematic representation of the relative length and position of the nasal capsule, eye, otic capsule, and brain in (A) adult *Pseudoeurycea goebeli* and (B) adult and (C) juvenile *Thorius narisovalis*. Relative length is expressed as a percentage of total skull length from the posterior edge of the occipital condyle (0) to the anterior edge of the premaxilla (100). N = 5 for each sample.

(Fig. 5). This brings the anterior margin of the otic capsule closer to the eye, but these structures remain well separated. Similarly, transverse broadening of the otic capsule is effected by lateral expansion of the capsule from its origin on the posterolateral braincase wall, but this apparently neither restricts nor is restricted by lateral expansion of the hindbrain. Transverse broadening of the hindbrain and otic capsule, however, has substantial effects on the configuration of lateral skull elements. In larger salamanders, such as *Pseudoeurycea*, the quadrate articulates on the lateral surface of the otic capsule from which it descends ventrolaterally to an articulation with the lower jaw;

together with the narrow squamosal, the quadrate thus constitutes the jaw suspensorium (Fig. 2C). In *Thorius*, the quadrate maintains its conservative articulations with both the otic capsule and lower jaw but, because of lateral expansion of the hindbrain and otic capsule relative to the remaining skull and lower jaw, it is rotated to a nearly vertical orientation beneath the otic capsule (Fig. 2A,B). This is seen clearly by comparing the two skulls illustrated in Figure 2 (A,C); the jaw suspensorium is visible from dorsal view in *Pseudoeurycea*, but is nearly hidden from dorsal view in *Thorius*. The significance of the reorientation of the jaw suspensorium elements for mechanisms of jaw opening and closure is unknown.

Rotation of the quadrate and squamosal represents only one of a suite of three features involving the jaw suspensorium which are unique to *Thorius*. The two remaining features are 1) a posteriorly directed, bony, squamosal process or spur, and 2) a shift in the origin of the quadropectoralis muscle from the quadrate—as is typical of most plethodontid salamanders—to the squamosal, where it attaches to this process (Tanner, '52). I suggest that, as with the reorientation of the jaw suspensorium, these two features represent secondary consequences of increase in relative size of the brain and otic capsule. The influence of muscle attachment in promotion of bone growth is well demonstrated (e.g., Washburn, '47); in *Thorius*, development of a bony spur may represent only a passive response of the developing squamosal to altered muscle patterning—quadrate to squamosal—effected by reorientation of the jaw suspensorium. Accordingly, the developing squamosal of other, closely related plethodontid genera should have the potential for evoking this response, and a posteriorly directed process has been described in a single *Oedipina complex* (Wake, '66) although the origin of the quadropectoralis muscle in this specimen is not known.

Packaging of the eyes, nasal capsules, and brain in *Thorius* must be discussed as one phenomenon because of mutual interactions among these structures in the anterior portion of the head. Relative size of the eyes and brain both have increased, but most transverse expansion of the brain is achieved posteriorly, behind the eyes (Fig. 2A,C). Anteriorly, lateral brain expansion appears to be limited by the eyes which, as seen in dorsal view, overlap and intrude medially

against the braincase. In response, anterior braincase walls deflect inwardly as they conform to the margin of the eyes. (The accompanying change in eye shape suggest that the eyes actually are pressing against the braincase.) This results in a change in gross shape of the braincase from the parallel-sided structure that is typical of the skull of larger salamanders in which the eyes are well separated from the braincase (Figs. 2C, 6). Correspondingly, location of the relatively longer eyes more anteriorly may be ascribed to mechanical interactions with the anterior braincase walls which have displaced the eye forward. This, in turn, brings the eyes into contact and overlap with the nasal capsules which are likewise displaced further forward and now protrude conspicuously beyond the anterior margin of the skull (Figs. 2A,C, 5).

Numerous experimental studies have demonstrated the degree to which the jaw suspensorium, braincase, and other skull elements can be influenced by perturbations

in the mechanical environment during development (Corsin, '66; Pinganaud-Perrin, '73; Silver, '62; and above). While such manipulations have not been performed on plethodontid salamanders, the diversity of vertebrates which have been studied, combined with the general similarity of results, suggest that mechanical interactions are a conservative characteristic of vertebrate development that may be applied to analyses of cranial evolution of plethodontid salamanders. This supports the interpretation of modifications in jaw suspensorium and braincase morphology in *Thorius* presented above.

Perhaps the most pertinent experiments are two that utilized a heteroplastic grafting procedure in which sensory placodes were grafted between larvae of two species of ambystomatid salamanders, *Ambystoma maculatum* and the larger *A. tigrinum*. In such manipulations, the grafted organs typically demonstrate great fidelity to their characteristic, i.e., species specific, growth rate and attain their normal adult size regardless of their new host environment. In this manner, the response of the developing host skull to the presence of an uncharacteristically small or large sense organ may be examined. Washburn and Detwiler ('43) made reciprocal grafts of optic placodes and observed pronounced effects on the shape of the chondrocranium and the orientation of the nasal and otic capsules of the host skull. In hosts of both species, normal skull development was altered to accommodate the smaller or larger eye. Leibel ('76) transplanted otic vesicles. When grafted into the presumptive otic region of *A. maculatum*, the presumptive otic vesicle of *A. tigrinum* attained its normal, larger size and, by moving the dorsal articulation of the quadrate laterally, effected a reorientation of the jaw suspensorium from a ventrolateral to a fully vertical displacement. Thus, by simply replacing the normal otic vesicle with one of a larger growth rate and finite size, Leibel experimentally produced a series of skeletal modifications that mimic those seen in the phylogeny of plethodontid salamanders.

#### Functional considerations

In a consideration of the geometry of the vertebrate skull, Gans ('74) observed that maintenance of skull functions (e.g., protection of the brain, food acquisition, support of the sense organs) becomes more difficult as external dimensions are constrained. He suggested that different structures will respond

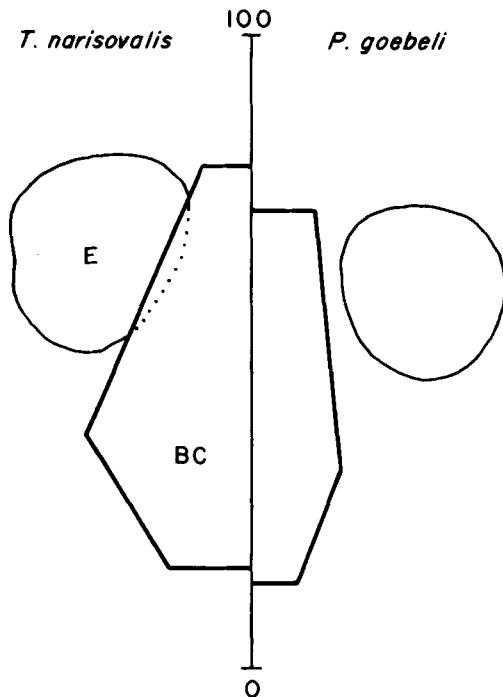


Fig. 6. Schematic representation of braincase shape (dorsal view) in adult *Thorius narisovalis* (M 3558; SL = 4.25 mm) and *Pseudoeurycea goebeli* (MVZ 130531; SL = 10.8 mm). Braincase dimensions were standardized to the same skull length to illustrate relative braincase size, shape, and position in the two genera.

differently to changes in scale in order to maintain functional efficiency. In several amphisbaenid reptiles, for example, reduction of skull diameter is associated with extreme modifications of skull architecture presumably to maintain rigidity demanded by the dominant role of the head in burrowing. The skull of another amphisbaenid, *Bipes*, is widest at the level of the otic capsules, which bulge inward against the lateral walls of the braincase. Gans believed the otic capsules to be the limiting factor in skull reduction in these animals because of the need to maintain a certain minimum internal diameter of the semicircular canals for their proper function as sensory acuity decreases as the distance between these paired structures is reduced (Gans, '60, '74; Jones and Spells, '63; see also Carroll, '70). Rieppel ('81) proposed analogous interpretations of several pronounced modifications in head morphology that have accompanied extreme size reduction in scincoïd lizards; these modifications include reduction and loss of post temporal fossae and the upper temporal arcade, and increase in the relative size of adductor musculature, braincase, and otic capsule.

Predominance of the eyes, brain, and otic capsules of *Thorius* suggest that, here too, these nervous and sensory structures are approaching their lower size limit for efficient skull function. This has been confirmed by recent detailed anatomical studies of the eye and brain in which morphological differences between *Thorius* and larger salamanders were interpreted as functional compensations for the reduced size of these structures. In the eye, altered curvature of the inner surface of the retina apparently serves to maintain a minimum "working distance" between the retina and the relatively enlarged lens which otherwise nearly completely fills the vitreous cavity (G. Roth, personal communication). Novel features of brain ultrastructure in *Thorius* include a more intricate neuronal branching pattern (increased arborization) in the optic tectum, and greater branching angle between adjacent dendrites on a given neuron (Grunwald, '81; Grunwald, Hanken and Roth, unpublished observation). The most complete study yet available, though, is that of Lombard ('77), who investigated the inner ear morphology of plethodontid salamanders. Lombard identified several modifications of the inner ear of *Thorius*, some of which have appeared independently in other small salamanders.

First, the periotic canal and periotic cistern are reduced greatly. Second the periotic sac, which normally extends from the inner ear into the braincase, is enlarged to such an extent that it actually invades the occipital condyles from within (this condition has appeared independently in *Batrachoseps*, another genus of diminutive plethodontid salamanders). Last, the internal diameter of the semicircular canals is relatively great, confirming the predicted negative allometry between canal bore and overall size based on functional analysis of inner ear design (Jones and Spells, '63).

It is important to remember, however, that some increase in the relative size of the brain and sense organs is likely as a result of the extrapolation of conservative growth relationships to small head size. Negative allometry of brain size relative to body size is typical of vertebrates generally (Bauchot, '78); Radinsky ('81) recently documented negative allometry for otic bulla volume and orbit area in interspecific comparisons of carnivorous mammals. Similar trends are seen in ontogenetic comparisons of *Thorius* and *Pseudoeurycea* (Fig. 4). Therefore, greater relative brain, eye, and otic capsule size may be expected in *Thorius*, with respect to larger plethodontid salamanders, even in the absence of functional considerations (see Cheverud, '82a, for a discussion of the complex relationship between ontogenetic and interspecific allometry). Predominance of the brain, eyes, and otic capsules in *Thorius* likely represents both allometric effects and compensations that maintain functional efficiency at reduced head size.

#### *Integrating mechanisms of skull development and skull evolution*

The developing skull is considered most appropriately as a complex of different tissues that respond in a predictable way to the influence of neighboring nonskeletal structures; its growth should be interpreted in the context of overall head development (Moss, '68a, '72c). Of particular importance is the dynamic aspect; cranial morphogenesis comprises a sequence of interactions between tissues and structures that differ in their response capabilities, which culminates in the production of the complex adult structure (Blechs Schmidt and Gasser, '78; Horder, '81). This view of cranial morphogenesis can explain both the high degree of regularity and correlated growth (*sensu* Twitty, '32) that is typical of skull development under normal

conditions (Cheverud, '82b) and provides a mechanism for the relatively great structural alterations achieved with only slight experimental alteration (e.g., DuBrul and Laskin, '61; Leibel, '76).

But most important to the evolutionary morphologist, this view provides a mechanism to achieve the often radical, yet coordinated, changes in head morphology that characterize much of vertebrate phylogeny (Davis, '64; Frazetta, '75). Thus, a relatively minor, but genetically based, alteration in the development of a given nonskeletal head component (e.g., the intrinsic growth rate of the eye) may initiate a series of compensatory responses in the surrounding skull, thereby effecting a change in overall head morphology. A genetic change that directly affects skull development per se (e.g., rate of deposition of extracellular matrix, rate of cell division) would not be required for the appearance of a repeatable, permanent change in skull morphology. In fact, results of studies of the mechanics of bone growth and morphogenesis emphasize the degree to which adult bone form is not under direct genetic control (Grüneberg, '63; Moss, '68b; Riesenfeld, '69); instead, they emphasize the degree to which modification in bone form is usually a biomechanical consequence of genetic changes, primary targets of which are adjacent structures.

The cranial morphology of *Thorius* is a product of this system of interactions during head development at reduced size. Several unique structural modifications that have accompanied the evolution of decreased head size appear primarily to be consequences of mechanical interactions with adjacent nervous and sensory components, especially the predominant eyes, otic capsules, and brain. This, combined with the overall reduction or loss of many other skull elements as a result of paedomorphosis (Hanken, '83), can account for many of the cranial features that distinguish *Thorius* from larger plethodontid salamanders. Similar analyses, which consider the range of developmental, functional, and phylogenetic constraints to morphological structure, will likely be of value in future studies of vertebrate skull evolution.

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#### LITERATURE CITED

- Bassett, C.A.L. (1972) A biophysical approach to craniofacial morphogenesis. *Acta Morphol. Neerl.-Scand.* 10:71-86.
- Bauchot, R. (1978) Encephalization in vertebrates. *Brain Behav. Evol.* 15:1-18.
- Blechsmidt, E., and R.F. Gasser (1978) Biokinetics and Biodynamics of Human Differentiation, Principles and Applications. Springfield: Thomas.
- Burr, H.S. (1916) The effects of the removal of the nasal pits in *Amblystoma* embryos. *J. Exp. Zool.* 20:27-57.
- Carroll, R.L. (1970) Quantitative aspects of the amphibian-reptilian transition. *Forma Functio* 3:165-178.
- Carroll, R.L., and R. Holmes (1980) The skull and jaw musculature as guides to the ancestry of salamanders. *Zool. J. Linn. Soc.* 68:1-40.
- Cheverud, J.M. (1982a) Relationships among ontogenetic, static, and evolutionary allometry. *Am. J. Phys. Anthropol.* 59:139-149.
- Cheverud, J.M. (1982b) Phenotypic, genetic, and environmental morphological integration in the cranium. *Evolution* 36:499-516.
- Corsin, J. (1961) Étude de quelques corrélations morphogénétiques dans le développement du chondrocrâne de *Salmo*. *Bull. Soc. Zool. France* 86:772-785.
- Corsin, J. (1966) Quelques problèmes de morphogénèse chez les urodèles. *Coll. Int. Cent. Natl. Rech. Sci.* 163:295-300.
- Coulombre, A.J., and E.S. Crelin (1958) The role of the developing eye in the morphogenesis of the avian skull. *Am. J. Phys. Anthropol.* 16:25-37.
- Davis, D.D. (1964) The giant panda—a morphological study of evolutionary mechanisms. *Fieldiana Zool. Mem.* 3:1-339.
- 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.
- DuBrul, E.L. (1950) Posture, locomotion, and the skull in lagomorpha. *Am. J. Anat.* 87:277-313.
- DuBrul, E.L., and D.M. Laskin (1961) Preadaptive potentialities in the mammalian skull: An experiment in growth and form. *Am. J. Anat.* 109:117-132.
- Dullemeijer, P. (1968) Some methodological problems in

- a holistic approach to functional morphology. *Acta Biotheor.* (Leiden) 18:204-214.
- Dullemeijer, P. (1972) Methodology in craniofacial biology. *Acta Morphol. Neerl.-Scand.* 10:9-23.
- Dullemeijer, P. (1974) Concepts and Approaches in Animal Morphology. Assen, The Netherlands: Van Gorcum.
- Frazetta, T.H. (1975) Complex Adaptations In Evolving Populations. Massachusetts: Sinauer.
- Gans, C. (1960) Studies on amphibiaenids (Amphisbaenia, Reptilia). I. A taxonomic revision of the Trogonophinae, and a functional interpretation of the amphibiaenid adaptive pattern. *Bull. Am. Mus. Nat. Hist.* 119:129-204.
- Gans, C. (1974) Biomechanics. Philadelphia: Lippincott.
- Goss, R.J. (1972) Theories of growth and regulation. In R.J. Goss (ed): Regulation of Organ and Tissue Growth. New York: Academic, pp. 1-11.
- Goss, R.J. (1980) Physiology of Growth. New York: Academic.
- Grüneberg, H. (1963) The Pathology of Development. New York: John Wiley & Sons.
- Grunwald, W. (1981) Neuroanatomische Untersuchung zum Aufbau des Tectum Opticum bei Lungenlosen Salamandern (Plethodontidae). Thesis, Univ. of Bremen.
- Hall, B.K. (1981) Specificity in the differentiation and morphogenesis of neural-crest derived scleral ossicles and of epithelial scleral papillae in the eye of the embryonic chick. *J. Embryol. Exp. Morphol.* 66:175-190.
- Hanken, J. (1980) Morphological and Genetic Investigations of Miniaturization in Salamanders (Genus *Thorius*). Ph.D. thesis, Univ. of Calif., Diss. Abstr. no. 8113059.
- Hanken, J. (1983) Miniaturization and its effects on cranial morphology in plethodontid salamanders, genus *Thorius* (Amphibia: Plethodontidae). I. Osteological variation. *Biol. J. Linn. Soc.* (in press).
- Hanken, J., and R.J. Wassersug (1981) The visible skeleton. *Funct. Phot.* 16:22-26, 44.
- Horder, T.J. (1981) On not throwing the baby out with the bath water. In G.G.E. Scudder and J.L. Revelle (eds): Evolution Today. Pittsburgh: Hunt Institute, pp. 163-180.
- Hoyle, D.A.N. (1966) Experimental investigations of skull morphology and growth. *Int. Rev. Gen. Exp. Zool.* 2:345-407.
- Jones, G.M., and K.E. Spels (1963) A theoretical and comparative study of the functional dependence of the semicircular canal upon its physical dimensions. *Proc. R. Soc. Lond. [Biol.]* 157B:403-419.
- Klaauw, C.J. Van der. (1946) Cerebral skull and facial skull. *Arch. Neerl. Zool.* 7:16-37.
- Klaauw, C.J. Van der. (1952) Size and position of the functional components of the skull. A contribution to the knowledge of the architecture of the skull, based on data in the literature. *Arch. Neerl. Zool.* 9:1-599.
- Leibel, W.S. (1976) The influence of the otic capsule in ambystomid salamanders. *J. Exp. Zool.* 196:85-104.
- Limborgh, J. Van. (1972) The role of genetic and local environmental factors in the control of postnatal craniofacial morphogenesis. *Acta Morphol. Neerl.-Scand.* 10:37-47.
- Lombard, R.E. (1977) Comparative morphology of the inner ear in salamanders (Caudata: Amphibia). In M.K. Hecht and F.S. Szalay (eds): Contributions to Vertebrate Evolution, 2. Basel: Karger, pp. 1-143.
- Moss, M.L. (1961) Rotation of the otic capsule in bipedal rats. *Am. J. Phys. Anthropol.* 19:301-307.
- Moss, M.L. (1968a) A theoretical analysis of the functional matrix. *Acta Biotheor.* (Leiden) 18:195-203.
- Moss, M.L. (1968b) Functional cranial analysis of mammalian mandibular ramal morphology. *Acta Anat.* 71:423-447.
- Moss, M.L. (1972a) An introduction to the neurobiology of oro-facial growth. *Acta Biotheor.* (Leiden) 22:236-259.
- Moss, M.L. (1972b) New research objectives in craniofacial morphogenesis. *Acta Morphol. Neerl.-Scand.* 10:103-110.
- Moss, M.L. (1972c) The regulation of skeletal growth. In R. Goss (ed): Regulation of Organ and Tissue Growth. New York: Academic Press, pp. 127-142.
- Moss, M.L., and R.W. Young (1960) A functional approach to craniology. *Am. J. Phys. Anthropol.* 18:281-291.
- Pinganaud-Perrin, G. (1973) Effets de l'ablation de l'oeil sur la morphogenèse du chondrocrâne et du crâne osseux de *Salmo irideus*. *Acta Zool.* 54:209-221.
- Radinsky, L.B. (1968) A new approach to mammalian cranial analysis, illustrated by examples of prosimian primates. *J. Morphol.* 124:167-180.
- Radinsky, L.B. (1981) Evolution of skull shape in carnivores. I. Representative modern carnivores. *Biol. J. Linn. Soc.* 15:369-388.
- Rieppel, O. (1981) The skull and the jaw adductor musculature in some burrowing scincomorph lizards of the genera *Acontias*, *Typhlosaurus* and *Feylinia*. *J. Zool. Lond.* 195:493-528.
- Richardson, D. (1932) Some effects of heteroplastic transplantation of the ear vesicle in *Amblystoma*. *J. Exp. Zool.* 63:413-445.
- Riesenfeld, A. (1969) The adaptive mandible: an experimental study. *Acta Anat.* 72:246-262.
- Sarnat, B.G. (1982) Eye and orbital size in the young and adult. *Ophthalmologica* 185:74-89.
- Schowing, J. (1968a) Mise en évidence du rôle inducteur de l'encéphale dans l'ostéogénèse du crâne embryonnaire du poulet. *J. Embryol. Exp. Morphol.* 19:88-93.
- Schowing, J. (1968b) Influence inductrice de l'encephale embryonnaire sur le développement du crâne chez le poulet. I. Influence de l'excision des territoires nerveux antérieures sur le développement crânien. *J. Embryol. Exp. Morphol.* 19:9-22.
- Schowing, J. (1968c) Influence inductrice de l'encephale embryonnaire sur le développement du crâne chez le poulet. II. Influence de l'excision de la corde et des territoires encéphaliques moyen et postérieur sur le développement crânien. *J. Embryol. Exp. Morphol.* 19:23-32.
- Silver, P.H.S. (1962) In ovo experiments concerning the eye, the orbit, and certain juxta-orbital structures, in the chick embryo. *J. Embryol. Exp. Morphol.* 10:423-450.
- Simons, E.V. (1979) Control Mechanisms Of Skull Morphogenesis. A Study On Unilaterally Anotic Chick Embryos. Lisse: Swets & Zeitlinger.
- Simons, E.V., and J. Van Limborgh (1979) The effects of experimental unilateral anotia on skull development in the chick embryo. VI. Discussion of the results in relation to the problem of the control of skull morphogenesis. *Acta Morphol. Neerl.-Scand.* 17:81-103.
- Tanner, W.W. (1952) A comparative study of the throat musculature on the plethodontidae of Mexico and Central America. *Univ. Kansas Sci. Bull.* 34, pt. 2: 583-677.
- Twitty, V.C. (1932) Influence of the eye on growth of its associated structures, studied by means of heteroplas-

- tic transplantation. *J. Exp. Zool.* 61:333-374.
- Vilmann, H., and M. Moss (1979) Spatial position of the lateral semicircular canal in 14-60-day-old rat heads. *Scand. J. Dent. Res.* 87:171-177.
- Wake, D.B. (1966) Comparative osteology and evolution of the lungless salamanders, family Plethodontidae. *Mem. South. Calif. Acad. Sci.* 4:1-111.
- Wake, D.B., and P. Elias (1983) New genera and a new species of Central American salamanders, with a review of the tropical genera (Amphibia, Plethodontidae). *Nat. Hist. Mus. Los Angeles Co., Contrib. Sci.* (in press)
- Washburn, S.L. (1947) The relation of the temporal muscle to the form of the skull. *Anat. Rec.* 99:239-248.
- Washburn, S.L., and S.R. Detwiler (1943) An experiment bearing on the problems of physical anthropology. *Am. J. Phys. Anthropol.* 1:171-190.
- Wassersug, R.J. (1976) A procedure for differential staining of cartilage and bone in whole formalin fixed vertebrates. *Stain Technol.* 51:131-134.
- Young, R.W. (1959) The influence of cranial contents on postnatal growth of the skull in the rat. *Am. J. Anat.* 105:383-415.