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Brian K. Hall
Department of Biology
Dalhousie University
Halifax, Nova Scotia
Canada

Adaptation of Bone Growth to Miniaturization of Body Size

JAMES HANKEN

Department of Environmental, Population, and Organismic Biology
University of Colorado
Boulder, Colorado

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Introduction

One of the most evolutionarily plastic vertebrate features is adult body size. A predominant trend towards size increase is seen in so many lineages that it has been embodied as an evolutionary “law” — Cope’s rule (Cope, 1885; Stanley, 1973). However, the opposite trend — evolutionary decrease in body size — is also widely known, and in certain respects has been of greater evolutionary significance than has size increase (Gould, 1977). Indeed, extreme body size decrease, or miniaturization, is regarded as a key factor in the evolution of the derived bauplans that define several major taxa, including the earliest reptiles (Carroll, 1969, 1970), as well as snakes (Rieppel, 1988), lizards (Carroll, 1970, 1977), and recent amphibians (Bolt, 1977, 1979; Carroll and Holmes, 1980; Milner, 1988). Moreover, miniaturization is extremely common. Among South American freshwater fishes, for example, miniaturization has independently evolved at least 34 times and is represented by no less than 85 species of adult standard length less than 26 mm (Weitzman and Vari, 1988).



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As is typical of size change generally (Schmidt-Nielsen, 1984), miniaturization has structural and functional consequences for a variety of organ systems (e.g., vascular system — Villalobos *et al.*, 1988; brain and visual system — Roth *et al.*, 1988, 1990). Nowhere, however, are the consequences more pervasive and profound than in the skeleton. This chapter addresses these consequences for the skeleton and especially for bone because of its predominance in the adult. In doing so, I hope to provide insights into how and to what extent patterns and processes of bone growth are modified in the evolution of body size decrease.

The chapter is organized into three parts. First, I briefly review models for evolutionary decrease in body size. These models depict the range of potential effects on the skeleton and how patterns of bone growth may be perturbed. They also reveal the difficulty in inferring the developmental mechanisms underlying changes in body size and adult skeletal morphology solely from adult stages. Second, I illustrate the consequences for the vertebrate skeleton that typically accompany size change and discuss them in terms of patterns and processes of bone growth. These consequences encompass four broad categories: reduction, hyperossification, increased variability, and morphological novelty. I conclude with a brief discussion of the evolutionary implications of altered patterns of bone growth and associated effects on the adult skeleton.

In this review, I draw examples exclusively from fishes, amphibians, and reptiles. This bias reflects in part my personal greater familiarity with these taxa, especially amphibians. However, it is also justified by the simple fact that, in absolute terms, miniaturization of body size is more extreme in representatives of each of these taxa than in birds and mammals. This no doubt is related to the endothermic metabolism of the latter two groups, which, as a consequence of surface area-volume relations, dictates a larger minimum adult body size for them. Thus, fishes, amphibians, and reptiles may provide more appropriate and effective models than do birds and mammals for illustrating both the consequences of miniaturization for the skeleton and the ways in which patterns and processes of bone growth are altered to effect these changes. This is not to say, however, that evolutionary decrease in body size for birds and mammals is of little consequence for the skeleton or of little interest from the standpoint of skeletal growth, as illustrated by recent studies by Bertram (1989) and Biewener (1982, 1989a, b). (See also Chapter 1.)

Mechanisms of Body Size Decrease

Understanding the morphological and functional consequences of miniaturization for the skeleton is facilitated by an appreciation of the various

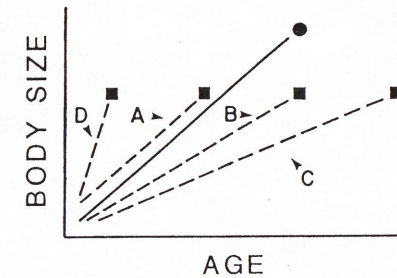


Fig. 1. Hypothetical mechanisms for evolutionary decrease in adult body size. The solid line depicts the ancestral ontogenetic trajectory relating body size to age. Reduced adult size in a descendant may result from (A) precocious cessation of growth (i.e., decrease in offset age) or (B) decrease in overall growth rate. Extending the growth period (i.e., increase in offset age) may also lead to size decrease if it is accompanied by a sufficiently low growth rate (C). Similarly, even an increase in growth rate may lead to size decrease if growth is terminated early enough (D). ●, adult ancestor; ■, adult descendant.

developmental pathways by which evolutionary decrease in body size may occur. The model of Alberch *et al.*, (1979), which relates ontogenetic and phylogenetic changes in organismal size and shape to alterations in the relative rate or timing of discrete developmental events, i.e., to heterochrony, is particularly helpful (see also Fink, 1982). In light of the model, miniaturization emerges as only one of a number of potential evolutionary results of heterochrony, but one that may follow from any of a series of distinct developmental perturbations.

Miniaturization typically involves changes in two distinct developmental parameters: the age at which overall growth stops, or offset age, and growth rate. These parameters may change in a variety of ways, either alone or in combination, to effect size decrease (Fig. 1). If the ancestral rates of organ and tissue differentiation, morphogenesis, and growth with respect to body size are retained in the smaller descendant, the effect will be to produce a pedomorphic adult morphology which resembles juvenile stages of the ancestor more closely than it does the adult (Fig. 2A). If, however, the rates of differentiation, morphogenesis, and growth relative to body size also change, the adult morphology of the descendant could, depending on the nature of the change, resemble the adult ancestor, albeit at a smaller size ("proportional dwarfism", Gould, 1971; Fig. 2B), or could even exceed that of the ancestor ("peramorphosis", Alberch *et al.*, 1979; Fig. 2C).

Several important rules follow from this model. First, the consequences of body size reduction for adult morphology may be quite variable, according to how size decrease has been achieved. Moreover, it is impossible to predict the consequences of size reduction for adult morphology from knowledge of

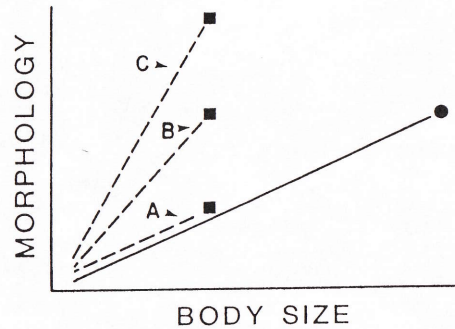


Fig. 2. Potential effects of adult body size decrease on organ and tissue morphology. If ancestral ontogenetic rates of organ and tissue differentiation, morphogenesis, and growth with respect to body size (solid line) are retained, the adult morphology of the descendant will be pedomorphic (A). If, however, differentiation, morphogenesis, and growth occur at a faster rate in the descendant, the ancestral adult morphology could be retained, albeit at a smaller body size ("proportional dwarfism"; Gould, 1971) (B). If the rates of differentiation, morphogenesis, and growth are elevated even more, then the ontogenetic trajectory may go beyond that of the ancestor ("peramorphosis"; Alberch *et al.*, 1979) (C). ●, adult ancestor; ■, adult descendant.

a single developmental parameter. Indeed, the same change in one parameter (e.g., offset age, growth rate) may produce a different adult size or morphology, depending on whether, and in what ways, other parameters are affected. Second, and conversely, similar changes in adult morphology may be brought about by fundamentally dissimilar developmental pathways. For example, evolution of pedomorphosis, as described above, may involve shortening, prolongation, or no change in the growth *period*; similarly, it may involve an increase, a decrease, or no change in the growth *rate* (Fig. 1). Finally, and following from rules 1 and 2, it is impossible to reliably infer the specific developmental changes that led either to decreased body size or to a derived adult morphology from adult stages alone. Addressing this problem requires knowledge of rates of differentiation, morphogenesis, and growth throughout ontogeny in both the descendant and putative ancestral forms — information that is rarely available in most real situations (Fig. 3). Consequently, much more is known about the effects of miniaturization on adult morphology than about the developmental mechanisms underlying them.

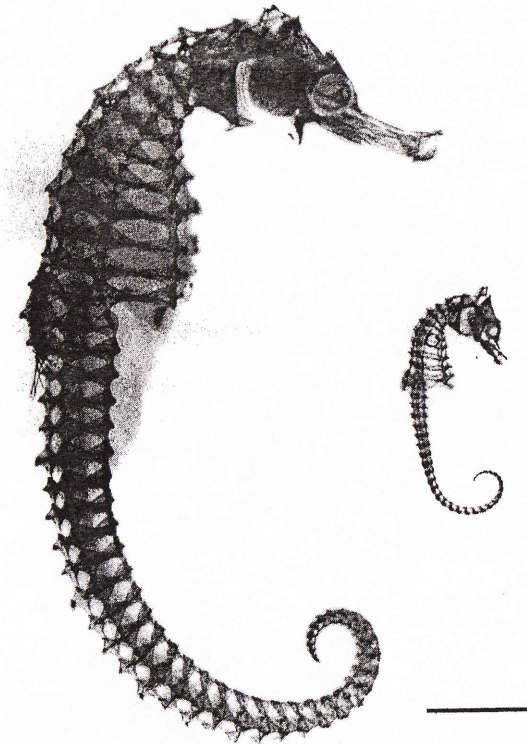


Fig. 3. Adult skeletons of the dwarf seahorse, *Hippocampus zosterae* (right) and the golden seahorse, *H. kuda*. Resolution of mechanisms underlying miniaturization and its consequences for bone growth requires knowledge of rates of differentiation, morphogenesis, and growth throughout ontogeny in both the dwarfed and putative ancestral taxa, in addition to adult morphology. Such information is largely unavailable for these fishes. Recently, Azzarello (1989) described ossification of the pterygoid series in the skull of *H. zosterae*. Scale bar equals 1 cm.

Consequences for the Skeleton

Reduction

As described above, the adult skeletal morphology of a miniaturized taxon may in theory be equally, more, or less developed than that of the ancestor. The most prevalent outcome of size decrease is reduced ossification (e.g., Fink, 1981) (Table 1). A wide range of skeletal components may be affected, including the skull, vertebrae, girdles, limbs, and/or fins. Reduced ossification may take many forms, ranging from subtle changes in the degree of

Table 1. Incidence of Four Derived Skeletal Features (reduced ossification, R; hyperossification, H; increased variability, V; and morphological novelty, N) in a Variety of Miniaturized Taxa

Taxon	Adult Size ^a	Features	Structures Affected	Citation ^b
Osteichthyes				
<i>Elachcharax miltopterus</i>	12-14	R	Skull, vertebrae, scales	1
<i>Hypheosobrycon elachys</i>	14-20	R	Skull	2
<i>Nannostomus anduzei</i>	11-16	R	Laterosensory canals, pelvic and caudal fins	3
<i>Parachanna</i> spp.	17-22	R	Skull, laterosensory canals, pectoral girdle	4, 5
<i>Priocharax arlei</i> , <i>P. pygmaeus</i>	11-17	R	Skull, pectoral girdle, and fins	6
<i>Seophaecharax</i> spp., <i>Tytocharax</i> spp.	12-25	N	Caudal fin	7
<i>Xenobrycon macropus</i>	12-20	H	Caudal fin	7
<i>X. polyancistrus</i>	11-14	R	Anal fin	8
<i>Xenobryconin</i> spp.	12-25	R, N	Laterosensory canals, scales, pectoral and pelvic girdles, fins	7
<i>Ceratostethus bicornis</i>	17-27	R, N	Skull, priaprium	9
<i>Manacopus falciifer</i>	17-27	R, N	Pelvic girdle and fins, priaprium	10
<i>Neostethus stamensis</i>	<40	N	Lower jaw	9, 11
<i>Phallostethus dunckeri</i> , <i>Phenacostethus</i> spp.	17	R, N	Skull, pectoral girdle, pelvic girdle and fins, priaprium	12, 13
<i>Oryzias latipes</i>	16-25	R, N	Skull	14
Sundacalanx praecox , <i>S. microps</i>				
	15-18	R, N	Skull and hyobranchial skeleton, pectoral and pelvic girdles and fins, scales	15
Danionella translucida				
	10-12	R, N	Skull and hyobranchial skeleton, Weberian apparatus, pelvic girdle, pectoral, pelvic and caudal fins, scales	16
Tyson belos				
	19	R	Skull, pectoral girdle, pectoral and caudal fins, scales	17
Schindleria praematura , <i>S. pietschmanni</i>				
	15-20	R	Pelvic fins	18
Amphibia				
<i>Idioceranium rusei</i>	74-113 (total)	R, H, N	Skull, vertebrae	19
<i>Lineatriton lineola</i>	40-55	H	Hyolaryngeal skeleton, mesopodials	20-23
<i>Parnimoige townsendi</i>	23	H	Hyolaryngeal skeleton, mesopodials	20-22, 24
<i>Thorius</i> spp.	14-31	R, H, V, N	Skull and hyolaryngeal skeleton, limbs (epiphyses, phalanges, mesopodials), vertebrae	21, 22, 24-30
Atiophrynus syntomopus				
	19	R	Skull, sternum, pectoral girdle, limbs (digits, phalanges)	31
Brachycephalus ephippium , <i>B. nototerega</i> , <i>Psyllophryne didactyla</i>				
	9-16	R, H, N	Skull, digits	32, 33

Table 1 (continued).
Incidence of Four Derived Skeletal Features (reduced ossification, R; hyperossification, H; increased variability, V; and morphological novelty, N) in a Variety of Miniaturized Taxa

Taxon	Adult Size ^a	Features	Structures Affected	Citation ^b
<i>Didymnaphus sjoestedti</i>	19	R, N	Skull, sternum, pectoral girdle, limbs (digits, phalanges), vertebrae	34
<i>Mertensophryne micranotis</i>	24	R	Phalanges	34
<i>Phyllonastes heyeri</i> , <i>Phrynosopus bagreccio</i>	13–19	R, N	Skull, sternum, pectoral girdle	35
<i>Pseudhymenochirus merlini</i>	40	R, H, N	Skull, hyolaryngeal skeleton, sternum, pectoral girdle, phalanges	36
<i>Sminthillus limbatus</i>	8	N	Pectoral girdle	37
<i>Spea bombifrons</i>	38–64	R, H	Skull, vertebrae	38, 39
<i>Uperoleia</i> spp.	16–35	R, H, V	Skull, sternum, pectoral girdle, sacrum, vertebrae	40–49
Reptilia				
<i>Aconitias</i> spp., <i>Acontophiops lineatus</i> , <i>Anniella pulchra</i> , <i>Aprasia repens</i> , <i>Dibamus novaequinae</i> , <i>Pletholax</i> spp., <i>Typhlosaurus</i> spp.	4–15 (skull)	R, H, N	Skull	50–54
<i>Anolis bimaculatus</i>	110	R	Skull, limbs, pectoral and pelvic girdles	55
<i>Chamaetlinorops barbouri</i>	41	H, N	Vertebrae	56

^a Sizes are only approximate: some denote sample averages, others are maximum recorded values. All represent standard (snout-vent) length, in millimeters, except as otherwise indicated.

^b Citations: 1 — Weitzman, 1986; 2 — Weitzman, 1985; 3 — Fernandez and Weitzman, 1987; 4 — Weitzman and Fink, 1983; 5 — Weitzman and Vari, 1988; 6 — Weitzman and Vari, 1987; 7 — Weitzman and Fink, 1985; 8 — Weitzman, 1987; 9 — Roberts, 1971; 10 — Parenti, 1986b; 11 — Parenti, 1986a; 12 — Parenti, 1984; 13 — Parenti 1986c; 14 — Parenti, 1987; 15 — Roberts, 1981; 16 — Roberts, 1986; 17 — Springer, 1983; 18 — Watson *et al.*, 1984; 19 — Wake, 1986; 20 — Rabb, 1955; 21 — Uzzell, 1961; 22 — Wake and Elias, 1983; 23 — Wake and Lynch, 1976; 24 — Wake, 1966; 25 — Hanken, 1982; 26 — Hanken, 1983a; 27 — Hanken, 1984; 28 — Hanken, 1985; 29 — Lombard and Wake, 1977; 30 — Wake, 1970; 31 — Lynch and Ruiz-Carranza, 1982; 32 — Trueb and Alberch, 1984; 33 — Trueb and Alberch, 1985; 34 — Grandison, 1981; 35 — Lynch, 1986; 36 — Cannatella and Trueb, 1988; 37 — Griffiths, 1959; 38 — Wiens, 1989; 39 — Stebbins, 1966; 40 — Davies, 1989; 41 — Davies and Littlejohn, 1986; 42 — Davies and McDonald, 1985; 43 — Davies *et al.*, 1986; 44 — Davies *et al.*, 1987; 45 — Stephenson, 1965; 46 — Tyler and Davies, 1984; 47 — Tyler *et al.*, 1980; 48 — Tyler *et al.*, 1981a; 49 — Tyler *et al.*, 1981b; 50 — Rieppel, 1981; 51 — Rieppel, 1982; 52 — Rieppel, 1984a; 53 — Rieppel, 1984b; 54 — Rieppel, 1984c; 55 — Pregill, 1986; 56 — Forsgaard, 1983.

mineralization to the outright absence of one or more bones due to the failure of a cartilaginous precursor to ossify. Typically, it is interpreted as the result of precocious truncation of the ancestral pattern of skeletal ontogeny, i.e., pedomorphosis, because the adult skeletal morphology often resembles, in overall degree of ossification as well as gross size and shape, juvenile stages of a presumed ancestral ontogeny (e.g., Hanken, 1984; Rieppel, 1984a; Wake, 1986).

While pedomorphosis may be the most parsimonious interpretation of skeletal reduction in adults, it must be emphasized that such an interpretation remains only a hypothesis in the absence of corroborating ontogenetic data; as discussed above, developmental mechanisms responsible for skeletal reduction cannot be inferred solely from adult morphology. This is underscored by documented instances in which reduced adult ossification results not simply from precocious cessation of skeletal growth, but from secondary reduction, and sometimes complete loss, of structures that are well developed in juvenile stages. For example, in females of the unusual Philippine phallostethid fish *Manacopus falcifer*, the entire pelvic girdle and several fin rays, which are well developed in subadults, are absent in adults (Parenti, 1986b). Consequently, in instances of the limited development or absence of a bone from an adult stage, it simply is not possible, in the absence of ontogenetic data, to resolve whether this has resulted from precocious cessation of an ancestral developmental program (i.e., decrease in offset age), from change in rates of bone differentiation, morphogenesis, and growth, or even from secondary reduction from a more developed juvenile condition.

One of the important reasons for distinguishing among these alternate routes to skeletal reduction concerns their implications for bone growth. In cases of pedomorphosis, the ancestral patterns and processes of bone growth seemingly are largely conserved, notwithstanding changes in timing and rate (but see discussion below). In cases of secondary reduction, however, ancestral patterns of bone growth have undergone more fundamental alterations, primarily with respect to greater emphasis on processes of bone resorption.

The variability in reduction that frequently accompanies miniaturization, as well as the difficulties in interpreting the reduction from the standpoint of mechanisms of bone growth, are well illustrated by some especially speciose dwarfed taxa. Here, closely related members of a monophyletic group frequently display a gradient of reduction, of all the skeleton or of individual bones, from modest to extreme. It is tempting to envision that such a "morphocline" represents the actual phylogenetic sequence by which size decrease and skeletal reduction occurred, via a series of truncation events at smaller and smaller sizes. This interpretation, however, is not always supported by additional evidence. For example, body size and degree of

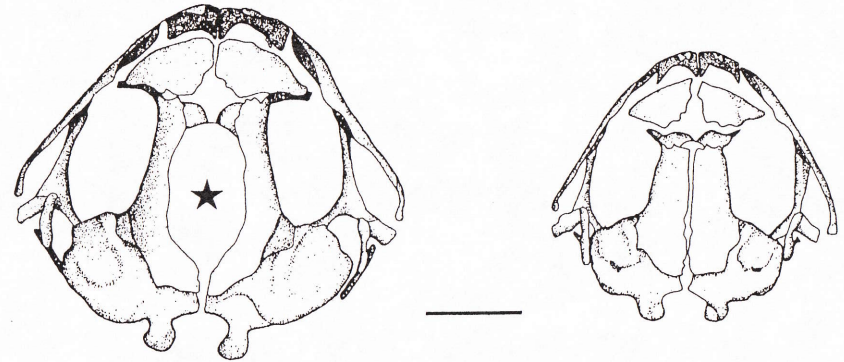


Fig. 4. Adult skulls of the miniaturized Australian frogs of the genus *Uperoleia* (dorsal views). Ossification is more complete in *U. minima* (right) than in the much larger *U. russelli* (left). Note, for example, the extensive frontoparietal fontanelle in *U. russelli* (star); these bones nearly meet in *U. minima*. Reproduced with permission from Tyler *et al.* (1981). Scale bar equals 2 mm.

skeletal reduction may not be well correlated. (If the graded array of reduced morphologies depicts the phylogenetic sequence, reduction should be minimal in the largest species and more pronounced with each successive decrease in adult body size.) Consequently, it often is impossible to resolve whether the existing array of forms represents a single graded phylogenetic sequence, or a series of reduced morphologies that independently evolved from a fully developed ancestral form. Moreover, in the absence of ontogenetic data, it is not even appropriate to accept the hypothesis of pedomorphosis as an explanation of reduced adult ossification, as opposed to secondary reduction (see earlier discussion).

An excellent example is *Uperoleia*, a genus of at least 23 species of tiny frogs endemic to Australia and New Guinea, whose osteology has been extensively studied by Davies and colleagues (Davies, 1989; Davies and Littlejohn, 1986; Davies and McDonald, 1985; Davies *et al.*, 1986, 1987; Tyler and Davies, 1984; Tyler *et al.*, 1980, 1981a, b). Adult body size in the genus ranges from 16 mm standard length (snout to vent; SL) in *U. minima* to 35 mm SL in *U. russelli*. The skeleton, especially the skull and vertebrae, is generally reduced relative to that of larger frogs. However, there is extensive interspecific variation in the degree of reduction, ranging from moderate to extreme. This is well illustrated by the frontoparietals, paired bones of the cranial roof (Fig. 4). In some species, the frontoparietals are well developed, abutting or even fusing in the dorsal midline and enclosing at most a small opening, or fontanelle, between them. In other species, they are little more than longitudinal splints of bone confined to the lateral margins of the skull roof and enclosing a broad, gaping fontanelle.

Table 2.
Cranial Variation in Frogs of the Australian Genus *Uperoleia*

Species	Exposure of Frontoparietal Fontanelle	Maxillary Teeth	Degree of Ossification	Size Class
<i>U. variegata</i>	Minimal or none	Absent	Well	Small
<i>U. minima</i>	Minimal or none	Absent	Moderate	Very small
<i>U. rugosa</i>	Minimal or none	Absent	Well	Small
<i>U. aspera</i>	Minimal or none	Absent	Moderate	Moderate
<i>U. martini</i>	Minimal or none	Present	Moderately well	Large
<i>U. fusca</i>	Minimal or none	Present	Well	Moderate
<i>U. lithomoda</i>	Slight	Absent	Moderate	Small
<i>U. micromeles</i>	Slight	Vestigial	Well	Large
<i>U. crassa</i>	Moderate	Absent	Moderate	Large
<i>U. russelli</i>	Extensive	Absent	Well	Large
<i>U. arenicola</i>	Extensive	Absent	Poor	Small
<i>U. borealis</i>	Extensive	Absent	Poor	Moderate
<i>U. talpa</i>	Extensive	Absent	Very poor	Large

Thirteen of the 23 known species are ordered according to relative exposure of the frontoparietal fontanelle in adults, which is a measure of the degree of reduced ossification of these bones. "Degree of ossification" applies to the skull overall. The four size classes encompass the full range of adult body size in these frogs, from the smallest species, *U. minima* (16 mm SL) to the largest, *U. russelli* (35 mm SL).

Data are taken from Davies, 1989; Davies and Littlejohn, 1986; Davies and McDonald, 1985; Davies *et al.*, 1986, 1987; Tyler and Davies, 1984; Tyler *et al.*, 1980, 1981a.

Other species show intermediate configurations. Together, all the species define a nearly continuous gradient in adult frontoparietal morphology (Table 2).

Does the gradient in frontoparietal morphology in *Uperoleia* correspond either with evolutionary decrease in body size or with the phylogenetic sequence of skeletal reduction? In both cases, the answer is no (Table 2). First, adult frontoparietal morphology (or inversely, size of the fontanelle) and body size are poorly correlated. Species showing minimal exposure of the fontanelle include the smallest known species in the genus, *U. minima*, as well as both moderately sized (*U. aspera*) and large species (*U. martini*). Conversely, extensive exposure of the fontanelle is found in small (*U. arenicola*), moderate (*U. borealis*), and large (*U. russelli*) species. Second, based on comparisons to putative sister taxa (*Pseudophryne*) and other outgroups (*Crinia*), in which the frontoparietals are extremely reduced (and the fontanelle correspondingly large), the more extensive development of the frontoparietal in many species of *Uperoleia* represents an *increase* in ossification over the ancestral condition for the genus. In other words, whereas extreme reduction apparently is the ancestral character state of the frontoparietal in *Uperoleia*, many species evince a reversal of this character state toward the more complete ossification characteristic of larger, more typical frogs.

Variable development of the frontoparietal in *Uperoleia* can also be used to test the hypothesis of simple truncation of the ancestral program of bone growth as a mechanism of achieving reduced ossification in adults. If the hypothesis were true, the degree of frontoparietal development should closely correspond with the degree of ossification elsewhere in the skeleton, especially in the skull. If the hypothesis were false, there likely would be little correspondence between the degree of ossification of the frontoparietals and other bones. The latter alternative prevails (Table 2). While in some species (e.g., *U. variegata*) well-developed frontoparietals are associated with a high degree of cranial ossification overall; in other species (e.g., *U. minima*) well-developed frontoparietals are found in skulls that are otherwise only moderately ossified. Similarly, species with extremely reduced frontoparietals include those with very poorly (*U. talpa*), poorly (*U. arenicola*), or well (*U. russelli*)-ossified skulls. In other words, the hypothesis of a simple truncation of the ancestral program of bone growth cannot account for the diversity in adult cranial morphology in *Uperoleia*. At the very least, there has been dissociation of the patterns of growth of individual bones, such that their development has been truncated both independently and to differing degrees.

Thus, in *Uperoleia*, the characteristically reduced ossification in adults is the result of a complex interplay between evolutionary change in body size and patterns of bone growth and morphological and developmental integration. Similar adult skeletal configurations have likely evolved repeatedly and independently, and in at least some instances represent reversals from the otherwise pervasive trend towards skeletal reduction.

Hyperossification

A second characteristic feature of the skeletons of many miniaturized taxa is hyperossification, which is bone growth or some other form of calcification (usually involving cartilage) in excess of that found in the ancestor. In terms of the developmental mechanisms and their effects on the skeleton reviewed earlier, hyperossification may be considered an example of peramorphosis (Alberch *et al.*, 1979; Fig. 2). Obviously, hyperossification stands in stark contrast to the reduced ossification that is otherwise so typical of dwarfed forms. That miniaturization can affect patterns and processes of bone growth and calcification in such a way that it may have opposite effects on the adult skeleton is remarkable. Even more surprising is the observation that hyperossification frequently occurs in groups that also show extensive skeletal reduction (e.g., *Thorius* — Hanken, 1982; *Brachycephalus* — Trueb and Alberch, 1984, 1985; Fig. 5). Extreme reduction involving one portion of the skeleton is commonly associated with excessive ossification of another

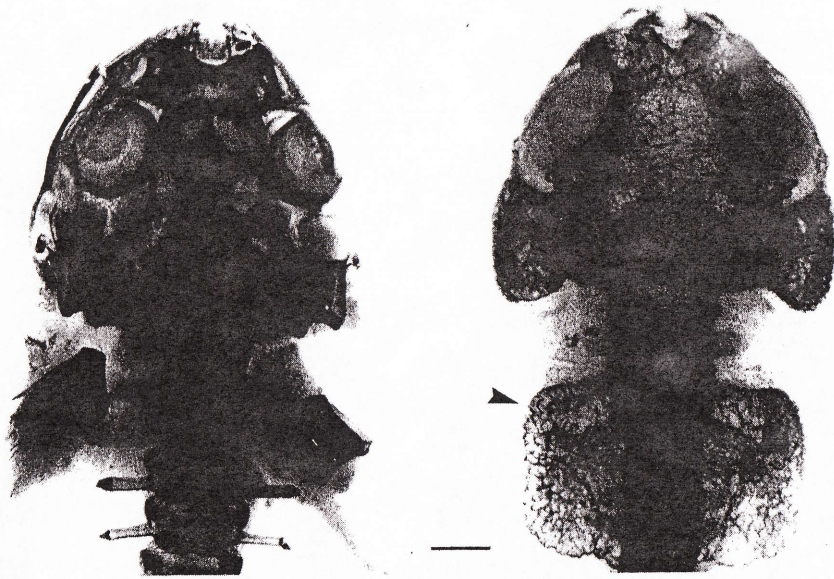


Fig. 5. Adult skulls and anterior vertebrae of two species of dwarf frogs (dorsal views). Left: *Geobatrachus walkeri* (Leptodactylidae; MCZ 20252); right: *Brachycephalus ephippium* (Brachycephalidae; MCZ 15659; pectoral girdle and forelimbs removed). *Brachycephalus* provides an outstanding example of hyperossification. Note, for example, the extensive fusion among cranial bones, which form virtually a single element dorsally; the novel dorsal shield lying above the presacral vertebrae and transverse processes (arrow); and the generally encrusted nature of the bone tissue itself. All of these features are absent in *Geobatrachus*, which displays a more generalized adult osteology for anurans, including abundant cartilage (darker areas). Scale bar equals 1 mm.

portion. Indeed, reduced ossification and hyperossification are often present within the same functional unit, such as the skull. To date, most examples of hyperossification ascribable to miniaturization come from amphibians (Table 1). It is not clear, however, if this reflects a greater liability of a derived pattern of bone growth in this class or if it is simply a consequence of the greater attention paid to this phenomenon (and miniaturization generally) in amphibians in recent years (e.g., Hanken, 1983a, 1984, 1985; Trueb and Alberch, 1985).

A number of explanations have been offered for hyperossification in miniaturized forms. These range from viewing hyperossification as a maladaptive consequence of the physiological mechanisms responsible for size decrease (e.g., "hormonal imbalance associated with dwarfing", Trueb and Alberch [1984]), to a variety of functional or adaptive explanations in which the excessive mineralization is seen to confer some physical or mechanical

advantage or benefit (e.g., Forsgaard, 1983; Rieppel, 1984a). Frequently, increased calcification is interpreted as compensating for the mechanical weakening that is presumed to result from decreased body size in general and from reduction in the number and/or size of bones in particular (e.g., Wake, 1986; Lombard and Wake, 1977; Uzzell, 1961.) In such cases, reduced ossification and hyperossification would be functionally linked and part of the same phenomenon of skeletal growth and adaptation.

While mechanical explanations for hyperossification may be plausible in some instances, to my knowledge none has ever been tested by functional analysis, at least not among the groups considered here; they remain speculative. Moreover, alternative and frequently less-obvious explanations for the same phenomenon are rarely considered, even though they may provide, in at least some cases, a more parsimonious and consistent explanation for the data.

An excellent example comes from Mexican salamanders of the genera *Thorius*, *Parvimolge*, and *Lineatriton*. Miniaturization has evolved independently and via different routes in these three taxa, resulting in both convergent and dissimilar morphologies (Wake, 1966; Wake and Elias, 1983). One of the features they share is calcification of mesopodial elements, which typically remain cartilaginous in adults of larger, related taxa (Hanken, 1982; Rabb, 1955; Uzzell, 1961; Wake, 1966; Wake and Elias, 1983). This phenomenon was first analyzed in a meaningful way by Uzzell, who offered a mechanical explanation in proposing that hyperossification "may compensate for the paedomorphic and presumably weak condition of the adult foot of these genera; the absence of calcified mesopodials in other weak-limbed plethodontid genera may reflect different kinds of locomotion in those forms" (1961, p. 83). This was subsequently challenged by Wake (1966), who pointed out that Uzzell's postulated distribution of mechanical forces in the limb did not agree with that inferred from patterns of mesopodial fusion and digital loss. Wake, however, did not offer an alternative explanation for the excess calcification. Later, Hanken (1982) documented that mesopodial calcification, at least in *Thorius*, was actually only one manifestation of a broader pattern of hyperossification involving the entire limb skeleton, which in turn was part of an overall mechanism of skeletal growth regulation that conferred determinate growth in these salamanders. In *Thorius*, onset of limb hyperossification is not strictly associated with a particular body size, as might be expected if it were a primarily mechanical phenomenon. Instead, it coincides with sexual maturity, which occurs at widely different body sizes in different species.

Where does this leave the mechanical explanation for limb hyperossification in the remaining two genera, *Parvimolge* and *Lineatriton*? At present, phylogenetic analyses cannot resolve whether mesopodial calcification in these genera is homologous to that in *Thorius* or if it evolved independently

(Wake and Elias, 1983). Thus, what holds for *Thorius* may not, indeed need not, apply to them. Results from *Thorius*, however, indicate that a mechanical explanation for hyperossification is not the only plausible one. To further underscore the difficulties that may accompany efforts to account for derived patterns of bone growth such as hyperossification, it should be pointed out that hyperossification may have different explanations, even within the same skeleton. Thus, whereas in *Thorius* mechanical factors do not adequately explain mesopodial calcification, they do provide a much more satisfactory and realistic explanation for calcification in the hyolaryngeal skeleton (Lombard and Wake, 1977).

Finally, it is important to remember that, while hyperossification is observed in many miniaturized taxa, it may not in every instance primarily result from phylogenetic decrease in body size. As with reduced ossification, the evolution of hyperossification must be analyzed in a rigorous phylogenetic context in order to distinguish those instances that accompanied body size decrease from those that did not. For example, in the small pipid frog *Pseudhymenochirus merlini*, some examples of cranial hyperossification (coalescence of frontoparietals, fusion of tympanic annulus) cannot be attributed to size decrease, as they also are characteristic of larger, more-primitive species (Cannatella and Trueb, 1988). However, other examples of hyperossification within the same skull (ossification of ceratohyals, prootic-squamosal fusion) are unique to *Pseudhymenochirus* and are therefore more appropriately interpreted as correlates of size decrease.

Increased Variability

The third feature associated with miniaturization is increased intraspecific variability of the adult skeleton. This includes variation among or within individuals in the size, shape, or even presence/absence of individual bones. Increased variability is the most difficult feature to document and, therefore, to defend, as characteristic of dwarfed taxa, simply because of the paucity of studies that assay skeletal variation in natural populations of vertebrates, miniaturized or not. Nevertheless, extensive skeletal variability has been observed in several dwarfed forms (Table 1), providing further insights into how ancestral patterns and processes of bone growth have been affected by size change. Marshall and Corruccini (1978) provide numerous additional examples of increased variability in tooth dimensions accompanying dwarfism in fossil mammals.

The observed variation is basically of two kinds with respect to bone growth. The first variation involves typically late-forming elements, whose development is precociously truncated as part of the common pattern of reduced ossification via pedomorphosis described above. While a common level of development may be attained by many individuals in a species,

Table 3.
Skeletal Variation in Five Species of the Diminutive Mexican Salamander Genus *Thorius*

Species	Septomaxilla			Right-Left Asymmetry	Distal Carpal 1-2 + Distal Carpal 3	
	Absent	Present, Small	Present, Large		Fused	Right-Left Asymmetry
<i>T. pennatulus</i>	62	3	35	25	51	26
<i>T. macdougalli</i>	97	3	—	5	15	20
<i>T. minutissimus</i>	86	8	6	28	17	28
<i>T. schmidti</i>	89	3	8	11	11	22
<i>T. narisovalis</i>	85	—	15	—	8	5

Values denote frequencies (%) observed in approximately 20 specimens per species, which are arranged in order of increasing adult body size. Intraspecific variation overall is much more extensive than that represented by these two characters from the skull and limb skeleton, respectively. For example, seven different carpal fusion combinations are each variable in at least one species, and right-left asymmetry of overall carpal or tarsal pattern ranges as high as 68% in some species.

Data are from Hanken (1982, 1984).

remaining individuals develop to substantially greater or lesser degrees. In other words, variation in the stage at which the ancestral pattern of bone growth is truncated leads directly to variation in adult morphology. The magnitude of variation is larger than in nonminiaturized species, in which individuals typically complete the ontogenetic sequence, resulting in the bone(s) in question being fully formed in most individuals. Variation of this kind is most conspicuous in cases in which truncation of the ancestral program of skeletal ontogeny leads to the failure of a bone to form in some but not all individuals. Even when present, the bone may be very poorly developed (e.g., septomaxilla in *Thorius* — Table 3). If this involves paired bones, there may even be right-left asymmetry in terms of presence or absence. In such cases, the mean extent to which the species completes the ancestral ontogeny can be thought of as lying precisely atop the developmental threshold (*sensu* Falconer, 1981) for the bone in question. Individuals for which the combination of prevailing genetic and environmental factors fails to exceed the threshold will lack the bone; those in which the combination exceeds the threshold will have it.

An interesting consequence of this kind of variation for phylogenetic analysis is seen in speciose taxa. Because the skeleton often is a primary source of morphological characters for taxonomic and phylogenetic studies, extensive intraspecific variation may obscure interspecific differences in adult morphology, even when such differences exist. Consequently, the systematics of many groups of miniaturized vertebrates has been difficult to resolve

using morphology alone, and researchers often have to resort to nonskeletal features or molecular techniques to discern phylogenetic relationships (e.g., *Thorius* — Hanken, 1983b, 1984).

The second kind of variation involves features that form early in development and whose variability reflects a fundamental alteration of skeletal patterning. Hence, unlike the first kind of variation, it cannot be readily linked to alterations in the patterns or processes of bone growth at later stages. One example is the extensive variability in mesopodial arrangement and phalangeal number in *Thorius* (Hanken, 1982, 1985; Table 3). The primary reason for excluding bone growth as a factor in accounting for this variation is very simply that mesopodial and phalangeal patterns are established during early stages of chondrogenesis, well before bone has formed. Moreover, there is no evidence that the observed skeletal patterns change once ossification begins (Hanken, unpublished data). This does not mean, however, that the variation is unrelated to evolutionary change in body size. Indeed, smaller body size, acting through decreased cell number or absolute size of limb primordia during embryonic development, may well underlie many of the documented evolutionary changes in skeletal patterning (Alberch and Gale, 1983, 1985; Hanken, 1985; Wake and Larson, 1987). Alternatively, increased skeletal variability in dwarfed taxa may simply reflect relaxed selection pressures on the morphology of certain structures when built at a much smaller size (Gould, 1977; Hanken, 1984). In other words, excessive variation may follow as a by-product of size decrease and have nothing directly to do with the particular developmental mechanism by which miniaturization is achieved.

Morphological Novelty

The final feature prevalent in the skeletons of miniaturized vertebrates is morphological novelty. Novel morphologies are observed in virtually all regions of the skeleton, although the skull, distal portions of the limbs, and the limb girdles are affected most frequently (Table 1). There are three categories of novelties that differ in the extent to which the novel features can be regarded as specific adaptations that permit or allow size decrease.

The first category includes novel skeletal arrangements that represent virtually inevitable consequences of adult body size decrease. Unlike features that apparently solve problems posed by body size reduction (see below), these novel arrangements seem to represent little more than side effects of miniaturization. Such changes frequently occur, for example, when conservative allometric growth relations are extrapolated to tiny adult body sizes, resulting in substantial change in the relative proportions among skeletal and nonskeletal components. In such cases, a novel shape or disposition of

a cartilage or bone may simply represent a mechanical consequence of the altered physical positioning of these components brought on by the change in relative sizes — a packaging effect. While novelties of this kind may appropriately be viewed as by-products of changes primarily affecting other tissues, they nevertheless often entail a substantial and fundamental change in structure and/or function. In this way, they may represent an important source of morphological variation for subsequent adaptation and diversification (Hanken, 1985).

In salamanders of the genus *Thorius*, for example, extrapolation of the negatively allometric relationship between brain, eye, and inner ear size relative to body size (a characteristic of vertebrates generally) to their tiny adult head size has resulted in predominance of these organs and a substantial shift in their relative positions (Hanken, 1983a). This, in turn, has effected a dramatic change in braincase shape and a reorientation of the jaw suspension in the tiny skull, which barely exceeds 3 mm in total length in some species. If such changes in cranial morphology are a consequence of size decrease acting in concert with evolutionarily conservative allometric growth patterns, then similar scaling effects dictated by the brain and sense organs should accompany cranial miniaturization in other groups. Indeed, this is the case, in groups as divergent as Recent amphibians (Carroll and Holmes, 1980; Milner, 1988) and squamate reptiles (Carroll, 1969, 1970; Rieppel, 1981, 1984c). Interestingly, while morphological novelty is observed in each of these cases, the particular novel structure or arrangement often differs, reflecting fundamental differences in the “initial conditions” of cranial structure among these groups before miniaturization.

The second category of morphological novelty comprises changes in the skeleton that may be interpreted as specific adaptations that permit size decrease: in other words, structures that maintain or confer skeletal function at reduced body size. In some cases this involves little more than enhanced deposition of bone, which may compensate for the mechanical weakening that presumably follows as a result of the reduction or even loss of other portions of the skeleton (see above section on reduced ossification). In the miniaturized caecilian *Idiocranium russeli*, for example, extensive ossification of the mesethmoid region of the skull may compensate for reduction of other cranial bones, and thereby maintain rigidity and strength necessary for use of the head in burrowing by this fossorial species (Wake, 1986).

In other instances, the anatomical changes are more extensive, involving functional systems that comprise both skeletal and nonskeletal components. For example, novel configurations of the braincase (closure) and upper temporal arcade (reduction or loss) have evolved repeatedly in the skulls of miniaturized fossorial lizards (Rieppel, 1981, 1984a, b, c; Table 1). In the extremely derived skull of *Dibamus novaeguineae*, there is in addition a novel dorsal enlargement of the coronoid process on the lower jaw (Fig. 6). These

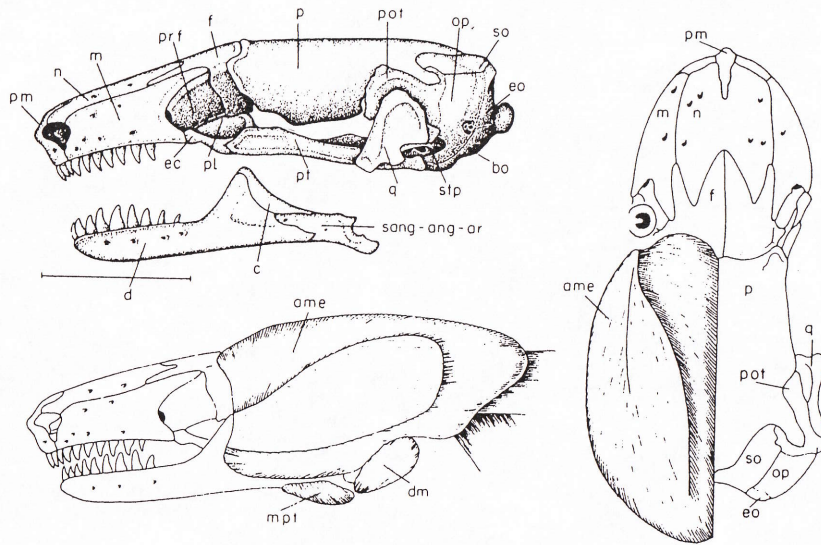


Fig. 6. Skull and superficial jaw adductor musculature of the miniaturized, fossorial lizard, *Dibamus novaeguineae* (Dibamidae) in lateral (left) and dorsal (right) views. Novel features include a closed lateral braincase wall formed by a descending process of the parietal (p) and an anterior process of the prootic (pot); absence of the postorbitofrontal and squamosal, i.e., the upper temporal arcade; and a dorsally expanded coronoid process (c) on the lower jaw. Additional abbreviations: ame, m. adductor mandibulae externus; ang, angular; ar, articular; bo, basioccipital; d, dentary; dm, depressor mandibulae; ec, ectopterygoid; eo, exoccipital; f, frontal; m, maxilla; mpt, m. pterygoideus; n, nasal; op, opisthotic; pl, palatine; pm, premaxilla; prf, prefrontal; pt, pterygoid; q, quadrate; sang, surangular; so, supraoccipital; stp, stapes. Reproduced with permission from Rieppel (1984c). Scale bar equals 2 mm.

changes are associated with — and, indeed, permit — posterodorsal expansion of the jaw adductor musculature that maintains an effective fiber length, which is important in assuring an adequate gape and enhancing mechanical advantage for feeding. Without these modifications, it is unlikely that miniaturization could have proceeded to the tiny adult skull sizes attained in some genera. Interestingly, however, not all small lizards show these modifications — only those that are also fossorial. Thus, evolutionary and developmental responses by the skeleton to size decrease have been mediated by skull function as well as habitus and life history.

The third category of morphological novelty contains a series of functionally diverse, unique specializations that are the most difficult to explain in the context of miniaturization. Unlike the examples discussed above, they are not readily interpreted either as primary, functionally mandated changes that facilitate or permit size decrease or as incidental, yet sometimes inevitable, consequences of miniaturization. Instead, if their evolution is causally

linked to size decrease, it most likely is a result of their relation to other features of the organism that are more directly affected by size change, such as reproductive mode or clutch size (Miller, 1979). A bizarre yet wonderful example is the priaprium, or subcephalic copulatory organ, of male fishes in the teleost family Phallostethidae (Parenti, 1984, 1986c; Roberts, 1971). Males use this structure to hold onto the female during head-to-head courtship, which leads to internal fertilization of her eggs. The skeletal morphology of the priaprium, which is unique to these tiny fishes among all teleosts, is so odd that resolving homologies between it and skeletal structures in other fishes — indeed, whether it is homologous to skeletal structures in other fishes — has been a vexing problem since it was first described more than 75 years ago (Parenti, 1986b). In fact, this question was only adequately resolved recently following careful comparative and ontogenetic analyses by Parenti (1986b), who demonstrated that the priaprium is derived from pelvic fin structures, particularly the rays and girdle.

Each of these examples of morphological novelty involves modification of the ancestral patterns of bone growth. In some instances, the modifications may represent little more than a labile morphogenetic response of osteogenic tissues to changes primarily involving adjacent, nonskeletal tissues, especially nervous and sensory components and musculature, which are known to exert a predominant influence on skeletal pattern formation and growth generally (reviewed by Hanken, 1983a; Moss, 1968, 1972). Such an explanation likely applies to novel arrangements that seemingly are incidental consequences of size decrease (first category, above) as well as those that primarily comprise increased mineralization in areas of high mechanical stress (e.g., the hyolaryngeal skeleton of small bolitoglossine salamanders [Lombard and Wake, 1977]). Other instances, however, such as the novel configuration of the braincase in miniaturized burrowing reptiles (Rieppel, 1981, 1984a, b, c) and the priaprium of phallostethid fishes (Parenti, 1984, 1986c; Roberts, 1971), are not readily explained by this model. Instead, they likely denote a more fundamental alteration to the developmental processes underlying skeletal pattern formation and growth. Thus, just as the novel skeletal configurations in miniaturized vertebrates are diverse, so too are the ways in which bone growth has been modified to effect these evolutionary changes. Together, these examples document the significant potential for evolutionary change that resides within general patterns and processes of bone growth and which can be evoked with relatively little perturbation.

Concluding Remarks

Four distinct features of skeletal morphology are associated with miniaturization of adult body size in vertebrates: reduced ossification, hyperossification, increased variability, and morphological novelty. None of these features is an inevitable consequence of size decrease, nor is size reduction a prerequisite for the evolutionary appearance of any one of them. Nevertheless, the high frequency with which dwarfed taxa display them (Table 1) is evidence of the significant effect that miniaturization may have on skeletal development, and especially bone growth. It is thus surprising how frequently body size *per se* is ignored in considerations of the derived features of the skeleton in dwarfed forms. One likely explanation is the frequent difficulty of evaluating a specific instance of reduced ossification, morphological novelty, etc., in the context of size decrease vs. plausible alternatives such as retention of an ancestral trait.

The adult skeleton of dwarfed forms is the product of both phylogenetic and ontogenetic constraints and functional modification mediated by natural selection (Hanken, 1983a, 1984; Trueb and Alberch, 1985). The main difficulty, of course, is to partition the effects of these often-conflicting influences. This can be especially difficult when the consequences of small size for the skeleton are not especially important in terms of natural selection, whose primary target may lie elsewhere. As the evolutionary changes that accompany size decrease are mediated in large part by patterns and processes of bone growth, greater knowledge of these phenomena likely will aid our understanding of how miniaturization itself is achieved.

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