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Model Systems versus Outgroups: Alternative Approaches to the Study of Head Development and Evolution[1]

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SYNOPSIS. There is widespread recognition of a recent coming together of developmental and evolutionary biology in the study of problems of mutual interest. Contemporary studies into the development and evolution of the head largely comprise two parallel approaches, or research strategies: the model systems approach and the comparative approach. The two strategies share the same general goal—greater understanding of cranial development and evolution—but typically emphasize different problems, ask different questions, and employ different methods, reflecting the contrasting backgrounds and biases of each group of investigators; there has been relatively little true synthesis. Each strategy is making important and valid contributions, but both have limitations. Resolution of many fundamental and long-standing problems in cranial development and evolution will require a combined approach that incorporates the technical and conceptual strengths of each discipline.

INTRODUCTION

The last several years have witnessed a coming together of developmental and evolutionary biology in the study of problems of mutual interest (Wake *et al.*, 1991; Hall, 1992). Among the most prominent of these problems is the development and evolution of the vertebrate head (Langille and Hall, 1989; Gans, 1989; Northcutt, 1990). Yet, the coming together of developmental and evolutionary biology, at least as applied to the head, is true only in a general sense; it is far from a total intellectual merger. Rather, current research largely comprises two independent, albeit parallel approaches: a model systems approach, which is characteristic of most contemporary studies in developmental biology; and a comparative approach, which underlies studies of evolutionary biology. Reflecting their formal training, which is typically in one or the other of these two disciplines, investigators bring to their studies the techniques and concepts unique to each. Not surprisingly, while these two research strategies share the same general goal—greater understanding of cranial development and evolution—they nevertheless emphasize different problems, ask different questions, and employ different methods; there has been relatively little true synthesis. Without question, each approach makes important and valid contributions; but, each has limitations.

In this paper, I briefly evaluate these two approaches with respect to their potential to contribute to our understanding of head development and evolution. My main conclusion is that resolution of many fundamental and long-standing problems in this field will require a combined approach that incorporates the technical and conceptual strengths of each discipline.

MODEL SYSTEMS APPROACH

The model systems approach is characteristic of most contemporary studies in developmental biology, which focus on a handful of taxa that serve as "models" for the examination of basic developmental mechanisms and processes (*e.g.*, Dawid and Sargent, 1988). For vertebrates, these taxa typically include one or two species from a

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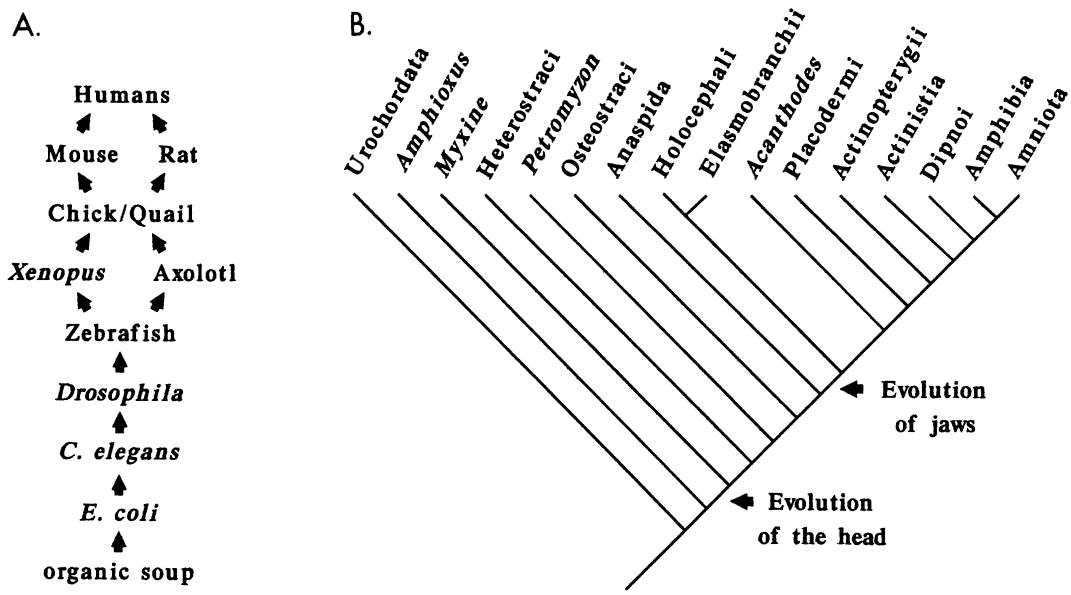

FIG. 1. A. "Phylogeny" of the vertebrates according to model systems. While this evolutionary scheme has never been seriously proposed, it almost seems to be implied by many of the comparisons made among these taxa. B. A more accurate depiction of vertebrate relationships is obtained when all the major groups, living and extinct, are included, as in the recent phylogenetic hypothesis of Maisey (1988). Seven of the eight vertebrate species in (A) belong to the two most derived groups, Amphibia and Amniota; the eighth (zebrafish) is in the Actinopterygii. (B modified with permission from Maisey, 1988.)

given class (but mostly tetrapods), such as the laboratory rat or mouse (mammals), the domestic chicken or Japanese quail (birds), *Xenopus* or the axolotl (amphibians), and the zebrafish (bony fish). The model systems approach is readily justified both in a biomedical context and as basic research. It is an appropriate and effective strategy for gaining a detailed understanding of many basic, and often complex, developmental mechanisms and processes, especially cellular and molecular aspects. Additional strengths include the large amount of information that is amassed concerning each model organism, and the sophisticated analytical tools and techniques that are developed to study particular problems, and which frequently are appropriate for only a single species.

There is, however, a cost associated with restricting attention to basic features of cranial development and organization in such a limited array of species, and the cost is nowhere greater than when data from model taxa are applied to problems in evolution (Fig. 1A). Basic developmental features common to these taxa are likely to have evolved (except in presumably rare cases of convergence) very early in vertebrate evolution, well before the species last shared a common ancestor (Fig. 1B). Information concerning these shared primitive features (or *symplesiomorphies,* in the jargon of systematics) provides little insight into the developmental bases of later evolutionary changes (*apomorphies*), and thus is not sufficient, or even appropriate, to address them except in the most general way. Unfortunately, these later changes include most of the evolutionary events that underlie adaptation and diversification within and among individual lineages—events that frequently motivate interest in the evolution and development of the head in the first place. Even using model taxa to investigate the origin of common, basic features can be problematic. This stems from the fact that most data have been amassed on mammals and birds, the two most recently evolved classes (and perhaps the most anatomically specialized). Extrapolating primarily from these taxa to important early events in ver-

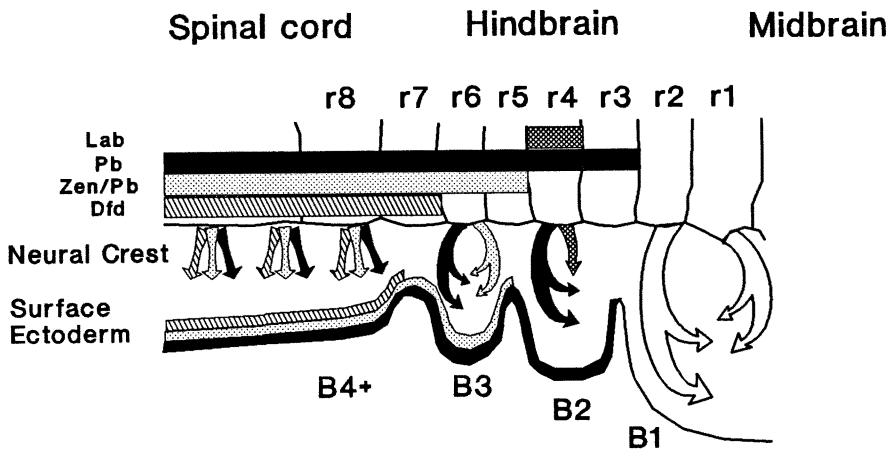

FIG. 2. The branchial *Hox* code in the mouse embryo. Horizontal bars denote the anterior limits of expression of four different gene subfamilies (Lab, Pb, Zen/Pb, and Dfd) in successive rhombomeres (r1, r2, etc.) of the developing hindbrain and spinal cord (lateral view, anterior is to the right). Unique combinations of gene expression at different axial levels (the "combinatorial *Hox* code") are largely shared by columns of neural crest (arrows) that migrate into adjacent branchial arches (B1, B2, etc.) and, later, by adjacent surface ectoderm. Redrawn with permission from Hunt *et al.* (1991*b*).

tebrate evolution (origin of the head, evolution of jaws), offers at best poor resolution of evolutionary sequences and their functional correlates (Fig. 1B).

Role of Hox *genes in branchial arch patterning*

The attributes of the model systems approach are nicely illustrated by studies of the role of homeobox genes in cranial development. There is compelling evidence that at least one subset of these genes, the *Antennapedia* (*Antp*)-class or *Hox* genes, play a fundamental role in patterning the branchial region of the head during embryonic development, including the hindbrain, associated neural crest, branchial arches, and overlying integument (Balling *et al.*, 1989; Hunt *et al.*, 1991*a*). Of particular interest is the proposal that differential rostrocaudal expression of *Hox* genes constitutes a "combinatorial *Hox* code" that mediates branchial segment identity (Hunt *et al.*, 1991*b*; Fig. 2).

Hox genes are also being used to address a number of fundamental problems in vertebrate evolution, such as the origin of the head and other basic features of the *bauplan* (Gaunt, 1991; Hunt *et al.*, 1991*a*; Holland, 1992). Data from model systems, however, which have proven so effective in analysis of the role of *Hox* genes in cranial development *per se*, are often less effective in this evolutionary context. Because most data on patterns of cranial *Hox* gene expression in vertebrates comes from mammals (mice and humans), they provide only a limited capacity to resolve either the specific phylogenetic sequence or the function of evolutionary changes (molecular or morphological) that occurred early in vertebrate history. Evolutionary hypotheses based primarily on these data must be tested using comparable data from phylogenetically more appropriate vertebrate taxa, *e.g.*, primitive fishes, as well as putative outgroups, *e.g.*, amphioxus (Holland, 1992). Analyses based largely on mammals also frequently do not take into account their extreme cranial specialization—both anatomical and developmental—in comparison to other vertebrates (Morriss-Kay and Tuckett, 1991). This specialization may severely qualify any resulting evolutionary hypotheses regarding the origin of the head.

A potentially fascinating topic is the role of *Hox* and other putative developmental control genes in the evolutionary diversification of the head *following* its origin. What is the molecular basis, for example, of the enormous interspecific variation in the morphology of branchial arch derivatives seen in vertebrates, especially anamniotes? The significance of a combinatorial *Hox*

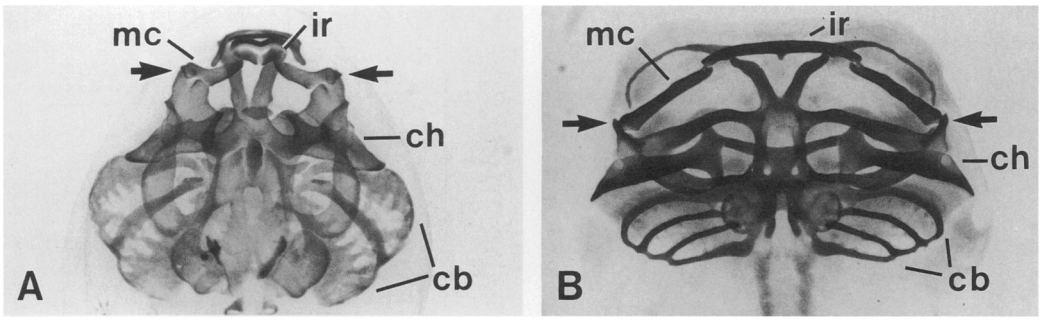

FIG. 3. Interspecific variation in branchial-arch cartilages of larval anurans. A. The Oriental fire-bellied toad, *Bombina orientalis* (Discoglossidae), displays a generalized morphology. B. In the South American frog, *Lepidobatrachus laevis* (Leptodactylidae), the morphology of many elements is grossly different, and virtually the entire branchial-arch skeleton is hypertrophied. For example, elongate Meckel's (mc) and infrarostral (ir) cartilages of the first (mandibular) arch provide an enormous gape (arrows) in this carnivorous species. Cartilage is stained with Alcian blue; both specimens are shown in ventral view. Additional abbreviations: ch, ceratohyal cartilage; cb, ceratobranchial cartilages I–IV.

code lies in its potential ability to explain, at least in part, the rostro-caudal gradient in branchial arch morphology characteristic of vertebrates. Yet, in some interspecific comparisons, differences in the morphology of elements of a given arch rival, if not exceed, the differences between adjacent arches in a single species (Fig. 3). Are these differences in morphology mirrored by differences in the *Hox* code, or is the code constant across major taxa? If relatively invariant, as suggested by the limited data available from non-mammalian vertebrates (Holland, 1992), does this mean that *Hox* genes do not mediate evolutionary changes in branchial region morphology which underlie adaptive diversification at lower taxonomic levels? Or, instead, do more subtle differences in gene expression (*e.g.*, timing or level of expression within individual segments) relate to evolutionary changes? These questions, however, cannot be adequately addressed using only model systems, which in general are too different and too distantly related from one another to formulate meaningful comparisons that address the developmental mechanisms underlying morphological evolution within individual lineages.

COMPARATIVE APPROACH

The comparative approach is the fundamental mode of analysis used by evolutionary biologists (Rieppel, 1988). It seeks to document patterns of phylogenetic change and organismal adaptation (Harvey and Purvis, 1991). It evaluates how various kinds of mechanisms—of which development is only one—effect and mediate these phenomena. Moreover, its overall aim is as much to account for the origin and fate of unique and specialized characteristics, as of general features.

There are obvious weaknesses of the comparative approach, relative to the model systems approach, when applied to head development and evolution. Because so many taxa are considered, the level of background knowledge accumulated for any one species typically is far below that available for model taxa. Second, most vertebrate species simply are not suitable for intensive laboratory analysis due to practical limitations, such as their inability to be bred in captivity. Finally, evolutionary biologists generally have been reluctant, or at least slow, to incorporate into their analyses many modern analytical tools and experimental techniques that have the potential to resolve (at least in part) many long-standing problems in head evolution.

At the same time, the comparative approach has unique strengths. It offers the potential to assess phylogenetic diversity in developmental pattern, or even mechanism. It also can provide a rigorous and explicit phylogenetic context for testing hypotheses of evolutionary change and evaluating adaptation (Rieppel, 1988; Harvey and Purvis, 1991). Each kind of infor-

mation provides insights that are not otherwise obtainable, and which can be used to frame key studies; both are needed for a complete understanding of head development and evolution. Neither is provided by the model systems approach. The following two examples illustrate these points.

Alternate patterns of cranial ontogeny in anamniotes

Contemporary models of cranial pattern formation focus almost exclusively on amniotes, in which the form and structure of the adult skull are largely established during embryonic development, in addition to the initial differentiation of all primary skeletal tissues (*e.g.*, Noden, 1988; Thorogood, 1988; Wood *et al.*, 1991). This relatively simple pattern of cranial development, however, is not the only one in vertebrates, nor possibly even the predominant one. Many fishes and amphibians instead display a complex life history, involving distinct larval and adult stages separated by a discrete metamorphosis (Fig. 4A). In many taxa, this complex life history is reflected in a biphasic pattern of cranial development, which underlies the formation of highly specialized, and anatomically distinct, larval and adult skulls (Hanken and Summers, 1988; Rose and Reiss, 1993; Figs. 5, 6).

Current models of cranial development do not readily accommodate or explain this biphasic pattern, which instead poses a number of interesting questions concerning underlying developmental mechanisms, especially those mediating skull metamorphosis (Alberch, 1989). What is the embryonic derivation of the many cartilages and bones which, in at least some taxa, do not form until metamorphosis? In particular, do these structures form as a result of compartmentalization of larval *versus* adult cell lineages, akin to that seen in many invertebrates? If postmetamorphic structures are derived at least in part from the embryonic neural crest, then is their skeletal pattern specified intrinsically, as in neural crest-derived components that form during embryogenesis (Noden, 1988)? Most studies of the mechanisms underlying cranial development in fishes and amphibians, however, have focused on basic features of embryonic development shared with

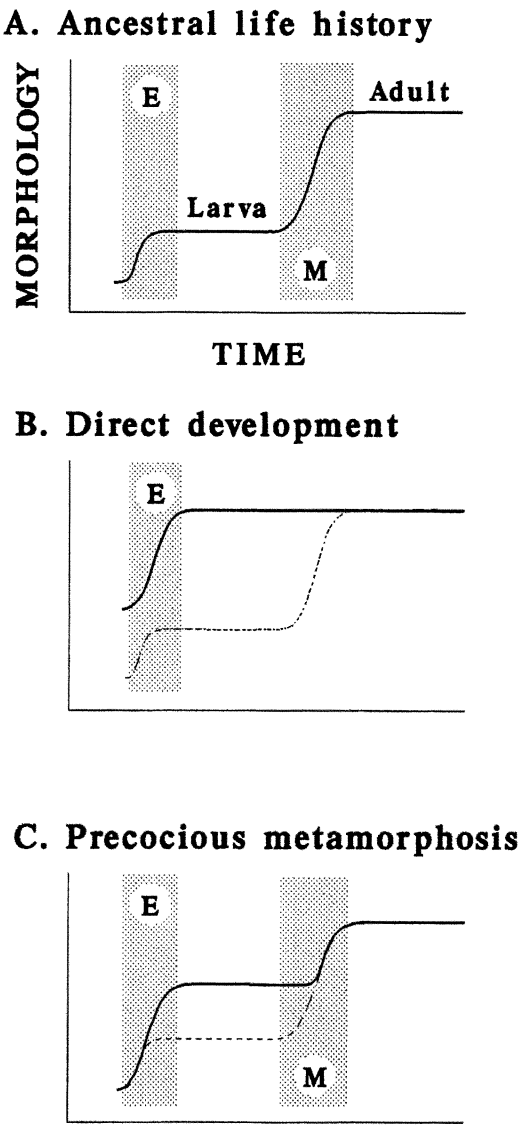

FIG. 4. Alternative life-history/developmental patterns in amphibians. The ancestral life history (A) appears as a dashed line in (B) and (C). Abbreviations: E, embryogenesis; M, metamorphosis.

amniotes (*e.g.*, tissue interactions mediating chondrogenesis, mechanisms of neural crest cell migration); they have almost completely ignored the metamorphic events that mediate the development of the definitive adult form.

Consideration of non-traditional species also provides unique and important opportunities to analyze skull evolution. Again, this is well illustrated by anamniotes, and especially amphibians. The complex life

history described above is generally regarded as the ancestral pattern among living taxa (Duellman and Trueb, 1986). In addition, many species display a wide variety of alternative life history modes, and their evolution has often had dramatic consequences for cranial ontogeny. Indeed, these organisms, and the evolutionary changes they represent, have provided some of the most compelling evidence for heterochrony, or change in the relative timing of developmental events, as a primary mechanism of cranial evolution in vertebrates (Hanken, 1989).

For example, direct development is a derived life history mode characterized by loss of the free-living, aquatic larval stage; it has evolved repeatedly within all three living amphibian orders (Duellman and Trueb, 1986; Fig. 4B). As one might expect, evolution of direct development has had contrasting effects on the ancestral, biphasic pattern of cranial development in different lineages. Some taxa largely recapitulate the ancestral ontogeny during embryogenesis, first forming larval-specific structures which then undergo a virtual metamorphosis before hatching (*e.g., Nectophrynoides tornieri*—Orton, 1949). In other taxa, much of the larval ontogeny has been abandoned (*e.g., Eleutherodactylus coqui*—Hanken *et al.,* 1992). Interestingly, while substantial alterations to early ontogeny need not have any obvious consequences for adult morphology, they may facilitate the subsequent evolution of morphological novelty (Hanken, 1992).

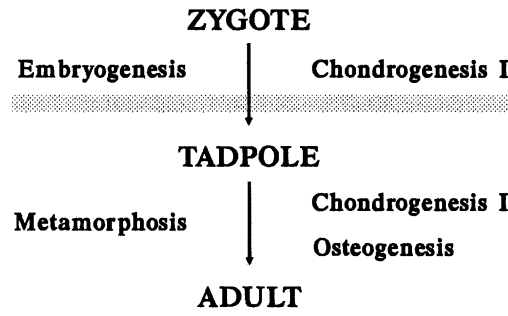

FIG. 5. Biphasic pattern of cranial development in metamorphosing anurans, which display the ancestral, complex life history (*e.g., Bombina orientalis,* Fig. 6). Horizontal, shaded bar denotes hatching.

Evolution of cranial ontogeny in ceratophryine frogs

Lepidobatrachus is a genus of very unusual South American frogs. Adults are aquatic and have large heads and jaws; they are aggressive predators (Ruibal and Thomas, 1988). Perhaps most unusual, however, are the tadpoles. They show several specialized features of cranial anatomy not seen in generalized larval anurans, including grossly hypertrophied jaws and hyobranchial cartilages, which give the larva an enormous head (Ruibal and Thomas, 1988; Hanken, 1992; Fig. 3B). These specializations constitute an adaptation for obligate carnivory, involving consumption of large, live prey which are swallowed whole, unlike typical tadpoles which are microphagous herbivores.

These cranial specializations make *Lepidobatrachus* a potentially excellent species for analyzing developmental phenomena seen in many other vertebrates, *e.g.,* macrocephaly (Elinson, 1991). Our understanding of head development in these frogs is enhanced, however, when these features are analyzed in a rigorous historical context that also considers closely related taxa. Such an

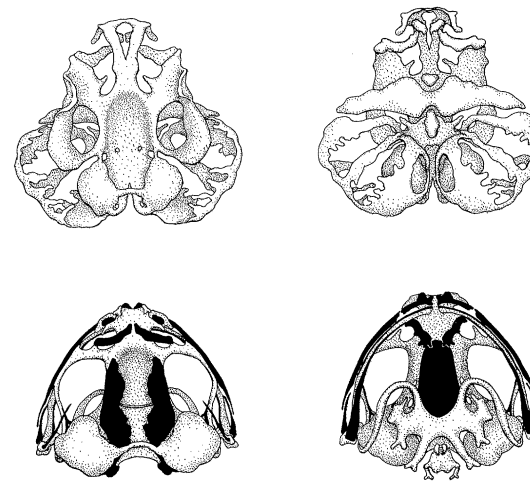

FIG. 6. Larval (top) and early postmetamorphic (bottom) skulls of *Bombina orientalis* (left, dorsal view; right, ventral view). The skull of larval anurans is highly specialized, including several functionally significant cartilages not found in other vertebrates. Metamorphosis involves the resorption or extensive remodeling of many of these and other cartilages, as well as the formation of additional cartilages and bone. Cartilage is stippled; bone is solid black.

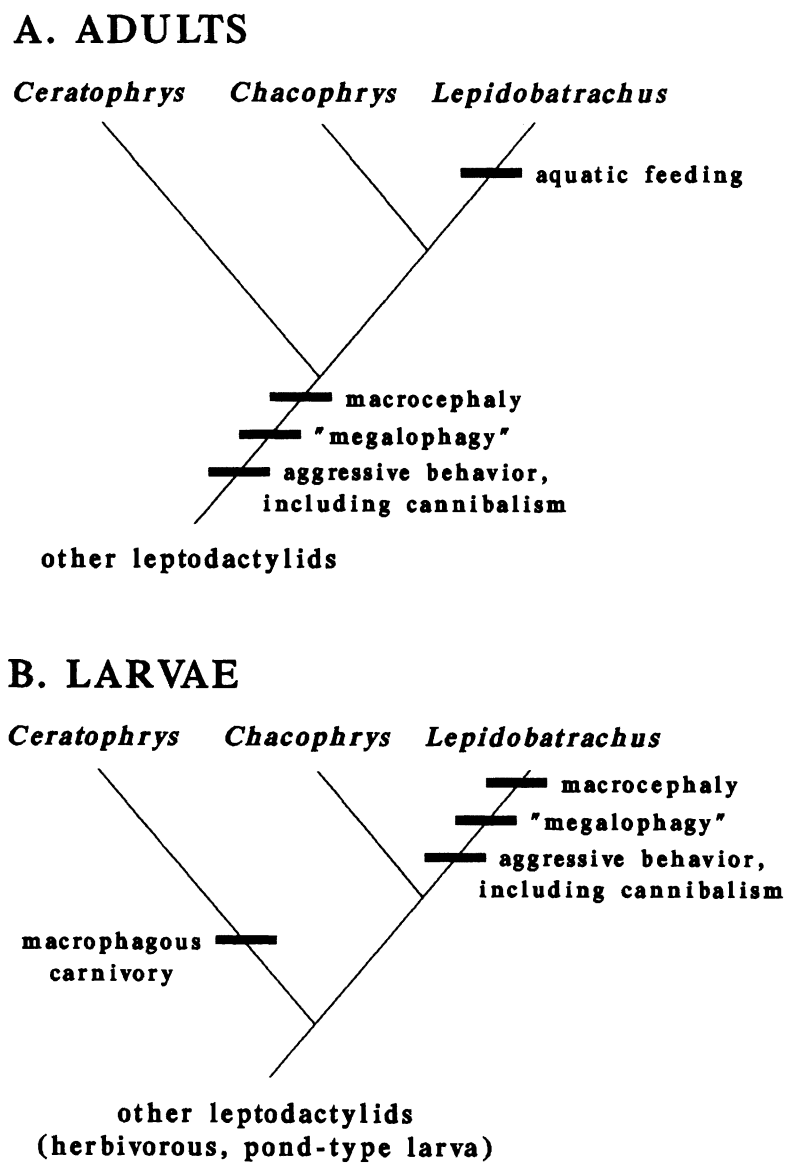

FIG. 7. A. Scheme of phylogenetic relationships among the three genera of ceratophryine frogs inferred from molecular data (Maxson and Ruibal, 1988), on which is superimposed the distribution of adult morphological and behavioral characters. B. Same phylogeny as in (A), showing the distribution of larval characters.

analysis allows one to explicitly define the likely sequence by which these features evolved, and provides additional insights into patterns of development in this and related species.

For example, *Lepidobatrachus* is one of three genera in the leptodactylid subfamily Ceratophryinae, whose phylogenetic relationships have been defined by molecular comparisons (Maxson and Ruibal, 1988). Because adults of all three genera have enlarged heads and jaws, it is most parsimonious to assume that these characteristic, derived (with respect to other leptodactylid frogs) features arose once in their immediate common ancestor and have been retained in each genus (Fig. 7A). A similar comparison for larvae shows that many of the fea-

tures unique to *Lepidobatrachus* in fact are identical to those that evolved earlier in the adult, and which typically, in the two related genera, form at metamorphosis (Fig. 7B). Thus, in the evolution of *Lepidobatrachus,* the metamorphic development of derived features characteristic of the skull of adult ceratophryine frogs appears to have been advanced into the embryonic period (Fig. 4C). This hypothesis can be tested because of the many predictions it makes about larval cranial morphology and embryonic development in *Lepidobatrachus.* One would expect, for example, the larval jaw morphology in *Lepidobatrachus* to resemble a metamorphic stage of the presumed ancestral pattern development seen in related genera, and this is indeed the case (Jennings *et al.,* 1991; Fig. 3B).

CONCLUSIONS

A comprehensive understanding of cranial development and evolution requires consideration of the basic features of the head in all vertebrates, as well as the specialized, unique features that characterize individual lineages and which have played an important role in their adaptation and diversification. At present, most studies utilize either one of two parallel approaches—the model systems approach, or the comparative approach. Solution of many outstanding problems, however, will require a combined approach that incorporates the technical and conceptual strengths of each.

Developmental biologists trained to work on at most a few model organisms must apply the same rigor to their analyses of evolutionary pattern and process that they routinely do to their analyses of developmental mechanism. This means choosing the phylogenetically most appropriate taxa for the problem under investigation, as well as relevant outgroups. Standard model species that are excellent for studying basic developmental processes need not be—and very likely are not—the most appropriate species for examining fundamental questions in head evolution. To the extent that the most appropriate groups are not available or otherwise feasible for investigation, then inferences about evolutionary events must be qualified accordingly.

Comparative biologists must make greater use of the extensive variety of laboratory tools and techniques now available for studying developmental processes, as well as experimental methods in general. They also need to become more familiar with the impressive body of knowledge concerning developmental mechanisms that has been amassed in the study of model taxa, and which is directly relevant—indeed indispensable—for an understanding of head evolution in individual lineages.

If such a synthesis can be achieved, then there is the real possibility of finding answers to many of the fundamental questions in cranial biology and evolution, which have long remained elusive.

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