advantage if the multiplicity of infection is high enough, and there is a replicative advantage to the segments over the standard. Lin Chao (University of Maryland, College Park, USA) argued[13] that it is not enough to consider segments as the sole target of selection, since replication efficiencies must depend on the composition of the coinfection groups. Szathmáry demonstrated[14] that many models can be reformulated as well as constructed *de novo* in terms of a structured deme[15] model, where virus particles infecting the same cell correspond to one 'trait group' of Wilson[15]. Many, largely analytic, results have been derived, showing for example that there is a threshold multiplicity of infection, below which DI viruses are selected against, despite their replicative advantage.

A related question is the replicative advantage of sex in the case of segmented viruses, whose genome consists of a few, physically unlinked sequences. Setting aside the controversial multiparticle case (where each segment is encapsulated separately and the coinfection group is an entirely transient entity)[13], Chao showed convincingly that resistance against Muller's ratchet is a good candidate for the advantage of sex in the experimental system of the bacteriophage $\phi 6$[16].

The meeting was exceptional in that it brought together biochemists, virologists, molecular phylogeneticists, physicians and modellers, and it confirmed the view that viruses are excellent paradigm objects for molecular biology, epidemiology, parasite research and evolutionary biology. Their great advantage is their relative simplicity and tractability to experiment and theory; we can be sure of some rapid and interesting developments.

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Evolution and Development of the Vertebrate Skull: The Role of Pattern Formation

James Hanken and Peter Thorogood

The vertebrate skull is anatomically complex and phylogenetically diverse; it presents unique opportunities to examine the role of developmental processes in evolutionary change. Previous studies have largely examined phylogenetic trends in tissue composition or change in the timing of developmental events (heterochrony). Additional important insights may be gained if skull evolution and development are viewed from the standpoint of pattern formation. Contemporary models of pattern formation offer the possibility of linking developmental mechanisms of cranial morphogenesis from the level of genes, through cell biology, to adult form.

The skull has been a focal point for studies in vertebrate biology for more than a century. Recent studies have emphasized the prominent role played by developmental processes in mediating the evolutionary origin of the cranium, as well as its subsequent diversification[1,2]. In this article, we review several aspects of cranial development that are relevant, if not pivotal, to the study of skull evolution, but which remain largely unappreciated by comparative and evolutionary biologists. Many aspects, especially those from molecular biology, have been revealed only in the last few years; others build on the solid body of knowledge accumulated as a result of classical embryological studies earlier this century.

We concentrate on mechanisms of pattern formation – the events and processes by which cells are organized into predictable spatial arrangements (morphologies) of tissues in their proper locations – as opposed to differentiation, which is the development of phenotypically distinct, stable cell types[3,4]. (Our use of 'pattern' as a developmental concept is distinct from its use by evolutionary biologists in the context of reconstruction of phylogeny[5].) Much of the research on cranial pattern formation has been done in a noncomparative, biomedical context, but the results, as well as a number of analytical and experimental strategies, may be readily incorporated into the study of evolution of the skull and the vertebrate head in general.

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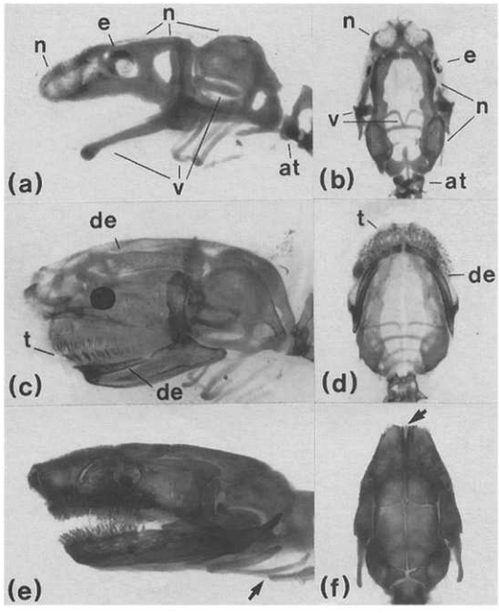

Fig. 1. Embryonic skulls of the Guatemalan caecilian *Dermophis mexicanus* (a limbless amphibian) in lateral (a, c, e), dorsal (b, f) and ventral (d) views, showing the three main skull units. (a, b) The early skull, comprising both neurocranium (n) and viscerocranium (v), is made up entirely of cartilage; at, atlas vertebra; e, eye. (c, d) Later, bone formation occurs within these units and marks the initial appearance of the dermatocranium (de); note also teeth (t). (e, f) At birth, the skull is almost entirely bony (predominantly dermatocranium), although cartilage is retained in a few places (arrows).

Basic features

The adult skull comprises three basic units – the neurocranium, viscerocranium and dermatocranium – which are distinguished most readily by their location within the head and by the nature of their component skeletal tissues.

The neurocranium lies deep within the head, where it underlies the brain and partly or completely surrounds and protects the three paired special sense organs – the eye, olfactory organ and inner ear (Figs 1, 2). It first forms as cartilage, which later is replaced by a second skeletal tissue, bone.

The viscerocranium is the skeleton of the paired visceral, or branchial, arches, which constitute the walls of the primitive pharynx; it lies on either side of the head ventral and lateral to the neurocranium (Figs 1, 3a). Early in vertebrate history, the development of an anterior arch was modified to form the jaws; additional arch-derived structures include the gill skeleton in fishes and larval amphibians, and the middle ear ossicles in tetrapods. Like the neurocranium, the viscerocranium is first formed in cartilage but typically is replaced by bone.

The dermatocranium, or dermal skeleton, is exclusively bony and forms a largely superficial covering that partly or completely invests the other two units, as well as many adjacent soft tissues (Figs 1, 3b).

This ground plan generally applies to most vertebrates, but it is modified to varying extents in different taxa. For example, in sharks the neurocranium and viscerocranium don't ossify and the dermatocranium never forms. Consequently, cartilage is retained as the predominant tissue in the adult selachian skull. Because models of cranial pattern formation primarily address the development of the early, cartilaginous skull which forms in the embryo, this review will focus on the neurocranium and viscerocranium. Their form largely determines at least the initial configuration of much of the dermatocranium, which predominates in most adults.

Cranial bone and cartilage differentiate from mesenchyme (embryonic connective tissue) derived from two sources – neural crest and paraxial mesoderm[6–8]. The neural crest derives its name from its origination within paired, longitudinal ridges – the neural folds – which fuse along their medial edges to form the neural tube, the primordium of the brain and spinal cord (Fig. 4). From here, neural crest cells migrate extensively within the head to sites where they will contribute to the skull as well as to a wide range of other components, including cranial nerves, skin pigmentation, heart and teeth. The paraxial mesoderm originates at gastrulation and comes to lie on either side of the midline, or axial structures, such as the neural tube and the notochord. It makes a much smaller, but nevertheless significant, contribution to the skull. It also is the source of myogenic cells that form the cranial musculature, including the extrinsic eye muscles[4,9]; interestingly, these muscles are entirely patterned by neural crest-derived connective tissue.

Both neural crest and paraxial mesoderm contribute to all three skull units, although many individual cartilages or bones, or even entire regions (e.g. snout, face), are largely or exclusively derived from one or the other source (Fig. 3). For example, the viscerocranium is almost entirely derived from neural crest, whereas a substantial part of the cartilaginous neurocranium comes from paraxial mesoderm. Some cartilages and bones, however, receive substantial contributions from *both* neural crest and paraxial mesoderm, e.g. the frontal bone in the chicken (Frn in Fig. 3b). Moreover, the limited

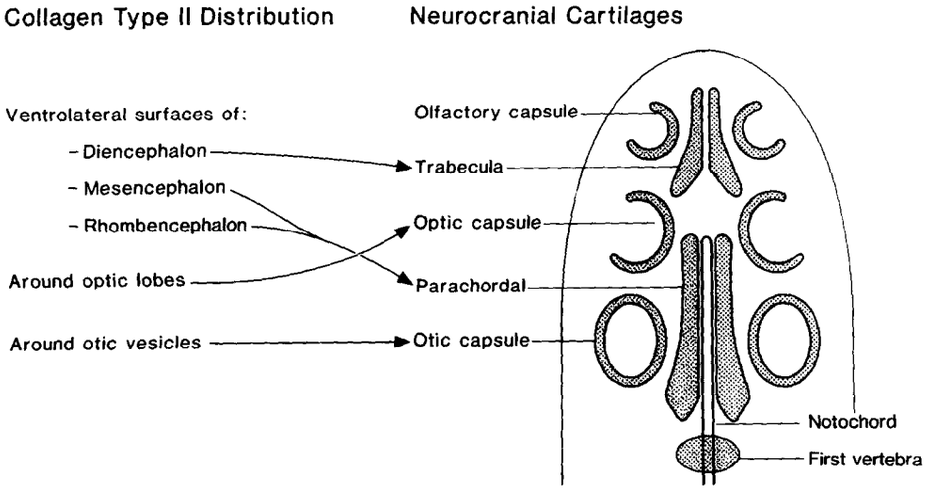

Fig. 2. Close correspondence between the early distribution of type II collagen and the initial configuration of the neurocranium in the head of the chicken embryo. Di-, mes- and rhombencephalon are regions of the brain (not illustrated), which lie medial to the three, paired sensory capsules and dorsal to the trabecular and parachordal cartilages; optic lobes and otic vesicles are primordia of the eyes and inner ears, respectively. Later expression of collagen in the olfactory (nasal) placodes, but prior to cartilage formation[17], is not indicated here.

comparative data available regarding the relative contributions of neural crest and paraxial mesoderm to the skull in different species suggest that in such composite elements the interface between regions derived from the two different embryonic sources may vary among taxa[8,10].

Models of cranial pattern formation

At present, there are basically two different models of embryonic cranial pattern formation. The two models are complementary insofar as they generally apply to different regions of the skull; they differ with respect to the degree to which pattern is specified intrinsically within the skeletogenic (skeleton-forming) cells, as opposed to pattern being imposed by extrinsic signals from the extracellular environment. Evidence supporting each model is derived primarily from the study of a few common laboratory species, mostly amniotes such as the domestic chicken, the Japanese quail and the laboratory mouse. However, additional data from a phylogenetically more diverse array of taxa, including teleost fish and amphibians, support the view that these morphogenetic models hold for embryonic skull development in all vertebrates.

The first model generally applies to the neural crest-derived viscerocranium. It proposes that patterning information is to a significant degree intrinsic to skeletogenic neural crest cells by the time they arrive at their eventual sites of differentiation within the visceral arches, including the lower jaw. Until very recently, support for this 'intrinsic patterning' model came primarily from experiments in which small regions of premigratory cranial neural crest (along with adjacent portions of the neural fold) from a donor embryo are grafted in place of a corresponding section of neural crest in a host embryo of equivalent age[4,6,11]. Regardless of whether the manipulations involve neural crest from the same region in different species (heterospecific grafts), or crest from different regions in the same species (heterotopic grafts), the morphology of skeletal elements derived from the graft corresponds to that of the donor. In other words, the neural crest-derived visceral skeleton shows donor-specific patterning, regardless of the host environment.

Intrinsic pattern specificity, however, is not absolute; neural crest cells can and do respond to external cues. The complex balance between intrinsic and extrinsic control is shown by neural crest ablation studies. Deletion of large portions of neural crest results in the loss of skeletal elements normally derived from these cells, yet when small portions are deleted, remaining crest cells frequently 'regulate' to replace the missing structures[12,13].

Claims of intrinsic patterning of at least some skeletogenic cells based on data from experimental embryology are bolstered by recent studies addressing the role of homeobox genes in cranial development (Box 1). One group in particular, the *Antennapedia* (*Antp*)-class *Hox* genes, may provide a molecular basis for intrinsic differences in the pattern-forming ability of neural crest and other cells.

In the head, these genes are expressed initially within the developing hindbrain, where several gene subfamilies differ in their anterior limits of expression, coincident with apparent axial segment boundaries in the central nervous system[14] (Box 1). Consequently, different hindbrain regions are characterized by unique combinations of *Hox* gene expression. Moreover, the neural crest cells that migrate into the branchial arches generally retain an array of *Hox* gene expression that corresponds to the particular hindbrain region from which they arose. Thus, the mesenchyme of each arch is characterized by a different combination of *Hox* gene expression, which is secondarily acquired by adjacent cell populations such as the overlying ectoderm. This arch-specific, combinatorial pattern of homeobox gene expression, or 'branchial *Hox* code', may, along with other regulatory genes, determine the development of arch-specific morphological patterns in structures derived from these neural crest and other cells[15]. However, a definite causal link between *Hox* gene expression and cranial morphology has yet to be demonstrated unequivocally.

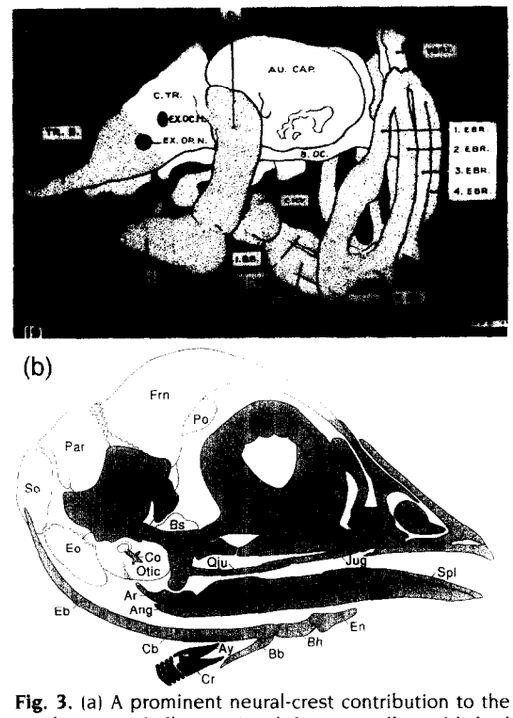

Fig. 3. (a) A prominent neural-crest contribution to the cartilaginous skull (anterior: left) was well established by the early part of this century, as seen in this larval salamander (*Ambystoma*) depicted by Stone in 1926. *From Ref. 39, with permission.* (b) The full extent of crest contribution to the bony skull in any vertebrate was not discovered until decades later, as seen in this domestic chicken skull (anterior: right). Crest-derived elements are lightly stippled; unshaded elements are derived from paraxial mesoderm. Lateral mesoderm-derived laryngeal cartilages (dark stipple) are also shown. Bones: Ang, angulat; Den, dentary; Eth, ethmoid; Frn, frontal; Jug, jugal; Max, maxilla; Nas, nasal; Pal, palatine; Par, parietal; Pfr, prefrontal; Pmx, premaxilla; Ptr, pterygoid; Qju, quadratojugal; San, supra-angular; Scl, scleral ossicles; Spl, splenial; Sqm, squamosal; Vom, vomer. Cartilages: Ar, articular; Ay, arytenoid; Bb, basibranchial (caudal); Bh, basihyoid (rostral basibranchial); Bs, basisphenoid; Cb, ceratobranchial; Co, columella; Cr, cricoid; Eb, epibranchial; En, entoglossum; Eo, exoccipital; Io, inter-orbital septum; Mc, mandibular (Meckel's) cartilage; Nc, nasal capsule; Po, postorbital; Os, orbitosphenoid; Qd, quadrate; So, supraoccipital. *Courtesy of D. Noden.*

The second model applies primarily to the neurocranium, and posits instead that gross spatial patterning of these cartilages is established by skeletogenic mesenchyme cells conforming to a molecular 'prepattern' expressed in the neural and sensory epithelia of the developing head[16]. A key molecule involved seems to be type II collagen, which is produced at this early stage of development by the epithelium of the developing brain and special sense organs and deposited at the interface with surrounding mesenchyme (Fig. 2). The spatial distribution of epithelially derived type II collagen closely resembles the neurocranium, which forms later[17–19]. Moreover, its transient expression appears to

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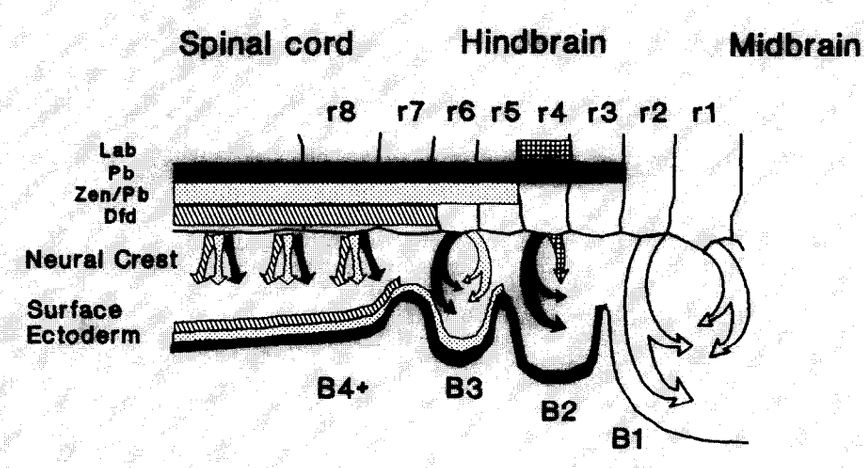
Box 1. Homeobox genes

Homeobox genes are a group of regulatory elements that play a key role in animal development. They were first discovered in the *Drosophila* embryo, where they are involved in specifying regional identity along the body axis. Their name ultimately derives from the nature of their mutant phenotypes, in which body parts are frequently transformed into parts appropriate for a different body region, or segment, a phenomenon termed 'homeosis'. Thus in the *Bithorax* gene mutation, the second thoracic segment, which normally bears a pair of balancing organs (halteres), is transformed into an extra first thoracic wing-bearing segment, producing a fly with two pairs of wings rather than one – a 'homeotic' mutation. Similarly, in *Antennapedia*, antennae are transformed into legs. All of these genes contain a region of 180 nucleotide base pairs, the 'homeobox', which encodes for a DNA-binding domain within the protein gene product[24]. These proteins bind to other genes, and in this way regulate their expression. It is because of this putative role in controlling the expression of such 'downstream' genes that homeobox genes have been attributed a regulatory role within the hierarchical operation of the genome.



Molecular probes constructed from sequences of the *Drosophila* homeobox-containing genes have been used to screen the genomes of other species for homologous genes, detectable by virtue of their highly conserved base sequence. Homeobox genes are now known to be present in a wide variety of invertebrate phyla, as well as vertebrates[38]. Among the most intensely studied homeobox genes are those of the *Antennapedia* (*Antp*)-class. In the few vertebrate species studied in detail, up to 38 *Antp*-class '*Hox*' genes have been identified per genome, typically comprising 13 gene subfamilies arrayed in four homologous clusters, *Hox*-1–*Hox*-4, each on a different chromosome[27]. As in *Drosophila*, vertebrate homeobox genes have been implicated in the specification of anatomical pattern[14,15,24]. The expression domains of four *Hox* gene subfamilies (Lab, Pb, Zen/Pb and Dfd – see hatched areas) in the mouse hindbrain and branchial region are shown below (lateral view, anterior is to the right). r1, r2, etc., denote hindbrain segments (rhombomeres); B1, B2, etc., denote branchial arches. *Figure redrawn from Ref. 15, with permission.*

coincide with the extracellular matrix-mediated tissue interactions that underlie the development of the neurocranial cartilages[10,16].

According to this model, development of the cartilaginous neurocranium follows an interaction between skeletogenic cells and the collagen, which effectively signals or directs their differentiative fate; the collagen may also 'entrap' these cells during earlier periods of cell migration. Indeed, it is this ability to respond to signals from the external environment (and other cells therein) which is critical. Skeletal pattern would then follow simply as a consequence of where the cartilages are induced to form, which in turn reflects the spatial distribution of collagen. Moreover, unlike the first model, which specifically addresses morphogenetic mechanisms of neural-crest-derived cells, the source of the skeletogenic mesenchyme is relatively unimportant for the second model because these cells are believed to contain relatively little intrinsic patterning information. Consequently, neurocranial cartilages of mixed composition – crest-*and* mesoderm-derived – can and do arise. Finally, while most research has focused on the morphogenetic role of type II collagen, its widespread embryonic distribution[20,21] makes it likely that either additional 'patterning' molecules are involved, or the collagen binds differentially a second class of molecules (e.g. growth factors) which themselves serve to signal selectively.

These two models of cranial pattern formation incorporate features unique to the skull, yet each exemplifies more general and widespread mechanisms of skeletal morphogenesis. The intrinsic model illustrates a common phenomenon in which initial pattern specification is confined to the tissue that will later express the pattern – in this case, the neural crest-derived mesenchyme of the branchial arches. In an equivalent fashion, the pattern of the appendicular skeleton is apparently specified in the mesenchyme of the developing limb bud. In both cases, associated epithelia may serve a secondary, almost passive role (albeit a necessary one). In the extrinsic model, pattern is imposed upon a naive but responsive mesenchyme by a second tissue – in this case, the epithelia of the brain and sense organ primordia – via a molecular prepattern. Similar examples of extrinsic patterning abound; they involve not only nervous and sensory tissues, but also muscular and other soft tissues determining skeletal, and especially cranial, form. Moreover, the influence is not always inductive (as in the early neurocranium), but may be physical as well, as in the mechanical influence of the brain and sense organs on skull form at later stages of development[22].

Evolutionary implications

The skull has played a prominent role in virtually every major adaptive transition in vertebrate history. Its diversification has involved a complex combination of modifications to embryo and adult, including change in the number and form of individual elements, in tissue composition and in functional relations among constituent parts and with adjacent soft tissues. Recent examinations of the developmental bases of these evolutionary trends have emphasized the importance of change in the relative timing of developmental events, or heterochrony[2,23]. These evolutionary modifications should also be viewed in terms of the mechanisms of spatial patterning, which provide unique insights that complement

those gained from consideration of developmental timing. Such a combined approach offers a more comprehensive and integrated understanding of skull development and evolution than either does alone. Here we highlight three of the many important evolutionary implications of the current models of cranial pattern formation. These implications are largely unexplored and represent logical topics for future research.

(1) *Models of cranial pattern formation, and in particular recent molecular studies, offer a vital genetic perspective on the developmental basis of skull diversification. They also provide new data that may resolve longstanding problems concerning the evolutionary origin of the skull and head.* For example, contrasting patterns and sites of homeobox gene expression (especially *Antp*-class and *msh*-like genes) along the body axis have been used to argue that different segmental patterning mechanisms apply to the postcranial or trunk region, to the hindbrain and visceral arches (including skeletal components) and to more anterior regions of the head[15,24,25]. This, in turn, supports the view[26] that the vertebrate head, or at least its anterior portion, evolved as a new structure with a fundamentally different organization and developmental program from the anterior region of nonvertebrate chordate ancestors[27,28]. Until now, data in support of this view, as well as competing theories that deny a fundamental distinction between head and trunk, have come largely from descriptive embryology and gross adult morphology, which have been insufficient to resolve the debate[29]. Virtually nothing, however, is known about the role of homeobox genes in cranial evolution following the initial origin of the head[30].

(2) *The two models lead to precise, albeit fundamentally different predictions regarding the developmental locus of taxon-specific patterning of cranial tissues, and of evolutionary change in skull form.* The intrinsic model implies that the locus of evolutionary change in skull form resides in the skeletogenic cells, particularly those derived from the neural crest. The extrinsic model implies that the locus of evolutionary change in skull form resides in neural and sensory components, whose effect is mediated by molecules deposited in the extracellular matrix or by direct mechanical molding. Moreover, to the extent that the two models apply to different skull units – viscerocranium versus neurocranium – they identify region-specific differences in the developmental mechanisms mediating cranial evolution.

The remarkable degree to which taxon-specific patterning can be localized to a given embryonic tissue is demonstrated in a series of classical embryological studies involving chimaeric embryos produced by heterospecific grafting. Most of the experiments used amphibians; a wide range of structures was examined, including cartilages, bones and teeth as well as non-skeletal components such as balancers in larval urodeles and the specialized oral apparatus of larval anurans[6,31–33]. Typically, taxon-specific patterning could be localized to a single embryonic tissue, such as neural crest or ectoderm. Among the most compelling results are those of Wagner[34], who grafted neural folds (neural crest and overlying ectoderm) unilaterally from the yellow-bellied toad (*Bombina variegata*) to the Alpine newt (*Triturus alpestris*) and examined the skull, and especially the visceral skeleton, in the chimaeric larvae. Right and left sides differed dramatically in skeletal pattern as well as tissue type, in a manner corresponding precisely to the source of the neural crest – frog or salamander – giving rise to each side (Fig. 5).

These classical studies involving heterospecific chimaeras were largely done in the four decades after 1920[33], yet despite their relevance they have been largely ignored, or at least underused, by evolutionary biologists. In retrospect, it's easy to see why. These studies primarily addressed issues in developmental biology, such as the nature of embryonic induction, in which differences in intrinsic patterning were used either as a marker for cell lineage or to assess the developmental roles played by interacting tissues. Although the results were discussed with regard to their implications for homology[32], the studies were not fundamentally evolutionary in orientation. Also, because most were published in German, they have remained outside the scope of most English-speaking biologists. They become very relevant again, however, in the light of contemporary questions in both developmental and evolutionary biology. Interestingly, renewed interest in these questions has coincided with the renewed widespread use of interspecific chimaeras in developmental biology[35].

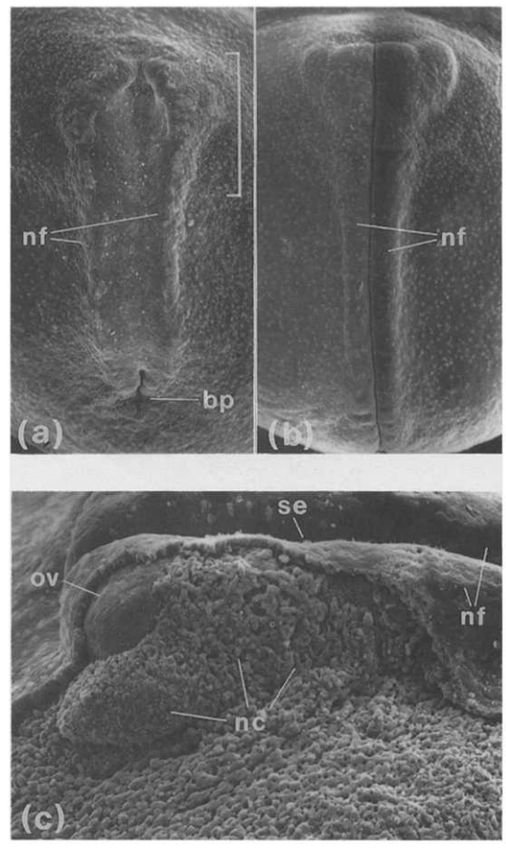


Fig. 4. Embryos of the Puerto Rican direct-developing frog *Eleutherodactylus coqui* at the time of neural crest cell formation and migration, seen in dorsal (a, b) and lateral (c) views. (a) Paired neural folds (nf) initially are widely separated on either side of the midline. The bracket delineates the head region; bp, blastopore. (b, c) Later, neural folds come into contact medially. In (c), surface ectoderm (se) has been partly removed to reveal the prominent optic vesicle (ov), which will form the eye, and a mass of neural crest cells (nc) migrating into the future jaw region. *Scanning electron micrographs courtesy of D. Moury.*

(3) *Both models of pattern formation provide a developmental basis for the coordinated evolution of highly integrated functional systems, such as the vertebrate head.* Any viable theory of the evolution of complex structures must be able to account for correlated change in constituent parts that is necessary to maintain the functional integrity

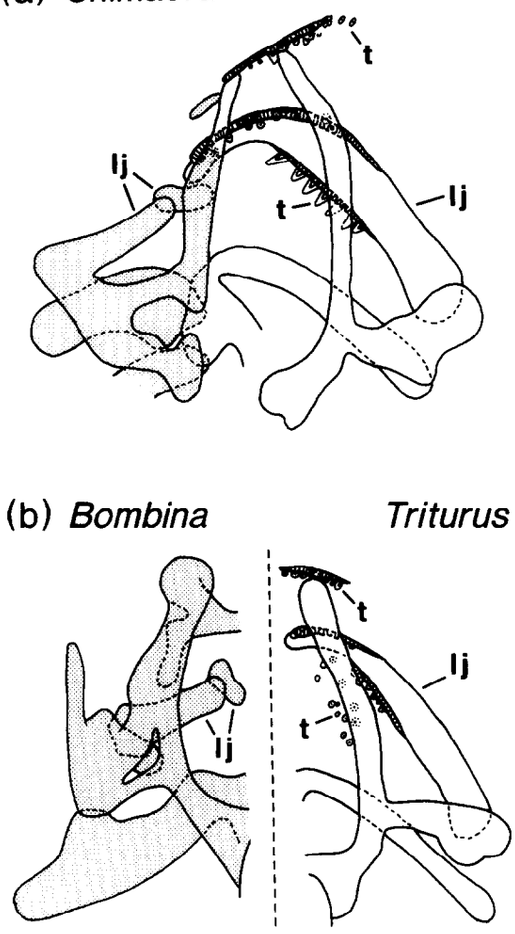


Fig. 5. Skull patterning in a chimaeric amphibian produced by replacing cranial neural crest from the left side of a salamander embryo (*Triturus*) with crest from a frog embryo (*Bombina*). The morphology of each side of the chimaeric skull (a) corresponds closely with the normal pattern characteristic of each species (b); all are dorsal views. Thus, the left side of the chimaeric skull is *Bombina*, or donor, in type, whereas the right side is *Triturus*, or host, in type. Note, for example, the bipartite lower jaw (lj) which is characteristic of larval frogs but not urodeles, and the bones (cross-hatched) and teeth (t) present in larval urodeles but not anurans. *Modified from Ref. 34, with permission.*

critical for survival[36,37]. This challenge is especially acute for the skull and other cranial components, in which individual parts are highly distinct anatomically and developmentally, yet functionally interdependent. Notwithstanding the obvious differences between the two models of pattern formation discussed above, here we stress their fundamental similarity: both portray the developing head as a highly interactive system of precisely coordinated differentiation, morphogenesis and growth. Both models also attribute a predominant role to the brain and sense organs in cranial patterning, either directly (via a molecular prepattern) or indirectly (via a *Hox* code conferred upon the emigrating neural crest). Indeed, comprehensive understanding of skull evolution can only come when cranial ontogeny is viewed in the broader context of overall cephalic, and especially neural, development.

This developmental integration is well illustrated by the neural crest, which mediates embryonic patterning not only of the visceral skeleton but also several non-skeletal tissues, such as cranial muscles and some integumental derivatives[4,8,11]. To date, the significance of this patterning role has been perceived largely in terms of its providing a means of integration among distinct components in a single organism. Yet, of equal significance is its probable role in maintaining anatomical and functional integration during morphological transformation in an evolutionary series of organisms, i.e. during vertebrate phylogeny.

Future research

Far more is known about the basic mechanisms of vertebrate cranial development than how these mechanisms mediate the evolution of the skull. Future research should proceed along several lines. For example, both current models of cranial pattern formation primarily address embryonic development. While this may be an appropriate focus in amniotes (reptiles, birds and mammals), in which many aspects of adult form are established in the embryo, neither model readily accommodates the more complex modes of development seen in many fishes and amphibians[30]. In these taxa, metamorphosis represents a second, post-hatching period of morphogenesis that in many species effects profound changes in cranial organization and architecture. The nature of the developmental processes that underlie such instances of post-metamorphic pattern specification are virtually unknown, as are their implications for cranial evolution in the taxa involved.

Indeed, a fundamental topic remaining to be adequately addressed in all vertebrates is the relation of initial embryonic patterning to adult skull morphology, and especially to the dermatocranium, whose form is not specifically addressed by either model of pattern formation discussed above. To what extent can initial patterning be modified by adaptive, functionally mandated changes later in ontogeny? To what extent are these adaptive responses required for the development of the normal adult morphology, and what is their role in skull evolution?

A third, related area concerns the level of taxonomic resolution conferred by the two different morphogenetic mechanisms. Do they confer species-level differences in skeletal pattern, as opposed to more general features distinguishing higher taxonomic levels, or are species-specific features determined primarily by additional processes that underlie subsequent growth and remodeling, both before and after birth? Most existing studies of cranial pattern formation can't be used to address this question, simply because the taxa considered are too distantly related. Studies are urgently needed that evaluate the evolution of cranial patterns – and underlying patterning mechanisms – among closely related species and in the context of a well defined phylogeny.

By following these and other complementary approaches, the enormous potential contribution of models of cranial morphogenesis to our understanding of skull evolution may finally be realized, and a meaningful integration of evolutionary and developmental approaches to skull form be achieved.

Acknowledgements

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The Evolutionary History of the Seed Plant Male Gametophyte

William E. Friedman

The role of the male gametophyte in the early history of seed plants remains an underappreciated but critical part of the evolution of a suite of characters that ultimately came to define seed plants. Recent paleobotanical discoveries and studies of extant primitive seed plant male gametophytes, when coupled with phylogenetic analyses of seed plants, provide insight into many key events that occurred during the early evolution of seed plants. These discoveries are changing our ideas concerning the multiple origins of the sulcus (pollen grain germinal aperture) and pollen tube, the structural and physiological relationships of the male gametophyte with the host sporophyte tissues in primitive seed plants, and the evolution of siphonogamy (conduction of non-motile sperm via a pollen tube) from a zooidogamous (swimming sperm) condition.

Eight years before Darwin's initial publication in 1859 on the process of evolution, Wilhelm Hofmeister[1] first established the existence of two alternate generations within the life cycle of land plants, the gametophyte (gamete-producing organism) and the sporophyte (spore-producing organism). Hofmeister was further able to demonstrate that seed plant species comprise three distinct classes of organisms: the sporophyte, male gametophyte (sperm-producing organism), and female gametophyte (egg-producing organism). When placed within the context of evolutionary theory, it is clear that each of these organismal generations maintains a distinctive ontogeny and undergoes complex interactions with its environment, each is a focal point for selection and the consequent potential for evolutionary change, and each possesses a unique evolutionary history.

Traditionally, most investigations of seed plant structure and evolution have focused on the sporophyte generation of the life cycle. There have been relatively few studies of the structural and functional diversity of the male gametophytes of extant seed plants, particularly among nonflowering seed plants[2]. Even less information exists concerning the developmental and reproductive biology of the male gametophytes of various early (Paleozoic) groups of seed plants[3,4]. In essence, the seed plant male gametophyte is, as Heslop-Harrison once wrote, the 'forgotten generation'[5]. This is unfortunate because the early evolution and diversification of seed plants may have been closely tied to the biology of the male gametophyte[6–8]. Fortunately, analysis of the reproductive biology of primitive extant and extinct groups of seed plants, coupled with an understanding of phylogeny (Fig. 1), can provide a framework for examining the evolutionary history of the seed plant male gametophyte.

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Life histories of male gametophytes among extant seed plants

The ontogeny of the male gametophyte among extant seed plants (Cycadales, Coniferales, *Ginkgo biloba*, Gnetales and angiosperms) begins with a single-celled haploid microspore (a product of meiosis) that forms within the microsporangium of the sporophyte. Male gametophytes of