EVOLUTIONARY DEVELOPMENTAL BIOLOGY —
PROSPECTS FOR AN EVOLUTIONARY SYNTHESIS
AT THE DEVELOPMENTAL LEVEL

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The relationship of development to evolution has engaged the interest of biologists for well over a century, but the subject has been something of a "fringe" issue for both fields. The famous "three-fold parallelism" between comparative anatomy, embryology, and paleontology predates darwinism, and the issue seems to re-emerge with each scientific generation. Although de Beer (1930) produced a thoughtful book on evolution and development at the beginning of the period of the evolutionary synthesis, it was not seen as being progressive by later workers. Rather, he is credited with having led the rejection of a causal relation between ontogeny and phylogeny, and the neoLamarckian ideas prevalent in the 1930s, evident in the work of MacBride and his students (Churchill, 1980). Embryology has been dismissed as being of only passing interest in the development of the evolutionary synthesis (Mayr and Provine, 1980); Hamburger (1980) refers to the absence of embryology in the synthesis as "the missing chapter," and suggests that only Schmalhausen, whose work did not become available to western scientists until 1949, could have provided it. Dobzhansky, too, felt that Schmalhausen's work had the potential of completing the evolutionary synthesis (Gilbert, 1994), but such was not to be the case, for Schmalhausen was silenced by the era of Lysenkoism in the Soviet Union and his work has not received the recognition it deserves until recently (Wake, 1986). While Huxley and de Beer, and later, Waddington, were concerned with the development-evolution relationship and made important contributions, the promise of a synthesis has yet to be attained. My thesis in this essay is that technical and conceptual advances have at last made such a synthesis an achievable goal.

Despite much excellent work by earlier workers, I believe that it was the publication of Gould's (1977) *Ontogeny and Phylogeny*, which appeared during a period of renewed interest in macroevolutionary phenomena, that stimulated development of an on-going research program into the relationship of development to evolution. His was an historical summary of the idea of recapitulation and of attempts to use heterochrony as a general explanation for departures from recapitulation. In it Gould presented his own effort to model heterochrony (the "clock-face model") and analyze it in evolutionary, ecological and paleontological perspective. In short order, his model was reformulated in a manner that made heterochrony accessible as an area of research (Alberch et

al., 1979), and Alberch (1980) threw down the gauntlet in challenging evolutionary biologists to be more conscious of new findings in developmental biology. A Dahlem Conference soon followed (Bonner 1982), and a textbook was published (Raff and Kaufman 1983); the next decade witnessed the publication of many research papers, at levels of organization ranging from molecules to whole organisms. At the end of the decade a period of consolidation occurred, with several more conferences taking place (Müller et al., 1989; Geobios Mem. Spec., 1989; Wake et al., 1991) and three major textbooks appearing (John and Miklos, 1988; McKinney and McNamara, 1991; Hall, 1992). The topic of development and evolution had become a standard expectation of conferences, and summaries of research activity dealing with specific groups are appearing (e.g., Raff, 1992a).

There was a solid intellectual foundation, reviewed by Gould (1977), on which to develop the new research initiatives. Following de Beer's (1930) reanalysis of the biogenetic law of Haeckel, there were several attempts to bring the ontogeny/phylogeny relationship into evolutionary biology. While conducting my doctoral studies on salamander osteology (Wake, 1966) I became intrigued by the possibility that some morphological features of systematic significance might have a relatively simple developmental basis (a fine example is the demonstration by Alberch and Gale [1985] that whether frogs or salamanders have four or five toes can be determined mainly by the number of cells and rate of cell proliferation). Students of salamanders always have before them the specter of the axolotl, and so learn early that to ignore the possibility of paedomorphosis is to imperil one's interpretations. Accordingly, many of us focused attention on heterochrony, which had in the past played such an important role in evolutionary narratives (e.g., origins of vertebrates). Comparative analysis of ontogenies of particular features within the context of the whole organism and its ecology and biogeography (as in my studies of the premaxillary bone, Wake, 1966, 1991) gave promise for understanding how morphology evolves.

The demonstration of the importance of hierarchical interactions in ecology, development and evolution (e.g., community dynamics giving rise to selection on growth rate or adult body size; genome size at the cell level impacting on growth and differentiation rates; Wake, 1991) has many implications. These have been studied from such diverse per-

spectives as quantitative genetics (Atchley and Hall, 1991), genome size-cell size-histogenesis (Roth et al., 1990; Roth et al., 1994), genes in relation to morphology (Nijhout, 1990), and molecular and developmental genetics in relation to ground plans (Slack, et al., 1993; Garcia-Fernández and Holland, 1994). Hierarchical analysis has been important in my own work (Wake, 1991; Roth et al., 1994), and I believe the approach is fruitful when exploring the interaction of developmental and evolutionary processes that lead to phylogenetic patterns.

Rather than attempting to summarize an active and dynamic area of research that is changing quickly, I will discuss prospects by focusing on some recent research trends. I first identify some central themes and then present a selection of topics that appear to have promise.

Central Themes

Researchers in the fields of evolutionary and developmental biology both deal with pattern and process, but terms have been made to serve too many masters; pattern and process become conflated, and muddle follows. Process as perceived by evolutionary biologists starts with natural selection, but increasingly is seen as extending to the formation of new evolutionary units (lineage origination, establishment of species), whereas pattern emerges from cladogenesis, extinction, and events related to earth history (mass extinctions, plate tectonics, etc.). In development, process includes a wide spectrum of phenomena, from genetic signaling and autonomous cell activities (e.g., assembly of cytoskeleton, mitosis) to integration (e.g., contact inhibition, adhesion) (Wessells, 1982). As far as developmental pattern is concerned, pattern formation, morphogenesis, and even ground plans are much discussed in developmental biology. There is, however, something of a cultural and sociological gap between primary researchers in evolutionary, as contrasted with developmental, biology (Raff, 1992b). Evolutionists interested in development, especially systematists and phylogeneticists, focus on evolutionary patterns, while developmentalists are more concerned with processes and mechanisms. The mechanisms in evolutionary biology relate to changes in gene frequency (population genetics) and to the effects of many genes on overall morphology (quantitative genetics), while those in developmental biology relate to the genetic basis of patterning in early development, to genetic signaling and cell-cell interactions, etc. Accordingly, there is neither a common theme nor a common vocabulary. Furthermore, while terms are important to evolutionary biologists, developmental biologists are more pragmatic and are unlikely to debate what is meant by the term "gastrulation," for example; they look instead for common themes in gastrulation. Evolutionary biologists, in contrast, disagree on even the most fundamental concepts (e.g., homology, species), which dooms the search for simple mechanisms and makes it difficult to find common themes.

On the other hand, developmental biologists interested in evolution often accept a rather simplistic notion of what an evolutionary term (e.g., Bauplan) is, and as a result the application of their sophisticated laboratory work to questions in evolution or phylogenetics appears to fall into the realm of essentialism (e.g., Slack et al., 1993; Patel et al., 1994). Developmental biologists seek commonality — the basis of ground plans, for example; evolutionists celebrate variation and diversity and are wary of generalizations beyond those of formal mathematically based models (e.g., as in population and quantitative genetics). For example, an area of continuing controversy in evolutionary biology has to do with whether there are macroevolutionary rules and principles, or if patterns at high taxonomic levels are just the result of microevolutionary processes being played out over time in an ever-changing world.

Development meets evolution most directly in discussions of transformation of body form. At one time, developmental biologists believed strongly in genetic invention, and some still do (Løvtrup, 1987). Currently there is an increased awareness of hierarchical interactions and of generic, often physically based, factors that regulate developmental processes (Newman and Comper, 1990; Newman, 1993, 1994), and regulation is becoming a theme in the work of some molecular geneticists as well (Carroll, 1994). Evolutionists have been wary of evolutionary interpretations that relied on genes of major effect (e.g., macromutations) since the early 1930s, but they also have been slow to adopt hierarchical thinking, which is essential if modern developmental genetics is to be understood in evolutionary and phylogenetic perspective. Times are changing, and the stage is being set for mutual understanding. It may seem, with the ever-growing literature and enthusiasm generated by those studying Hox genes and related signaling systems, that we have entered a new era of focus on genetic invention, but careful attention to this large body of work reveals hierarchical perspectives dealing with levels of organization, interaction, and regulation that offer a means of connecting developmental and evolutionary approaches (see, for example, Carroll, 1994).

There is a renewed interest in ground plans, bauplans, and similar concepts, generated by new knowledge of the stability of developmental systems and the generality of underlying genetic mechanisms and signaling systems (Slack et al., 1993, Patel, 1994). Such interpretations are only possible within a phylogenetic perspective. Similarly, there is renewed interest in what might be termed the limits and conceptualization of "sameness," and this has revived interest in the ancient debate over homology (Hall, 1994). This debate involves central issues in modern developmental genetics (Wake, 1994), for which phylogenetic perspectives are crucial.

The relation of ontogeny and phylogeny — sorting pattern from process

Recapitulation will not die as an evolutionary concept, and as a grand generalization it has its ardent supporters (e.g., Nelson 1978). Recapitulation occurs when two taxa being compared share all of their ontogeny (i.e., embryos proceed through time along a definite and knowable pathway, characteristic of the lineage, termed an ontogenetic trajectory),

except for the terminal stages. In a recent re-evaluation of the idea, Mayr (1994:231) states clearly, "The observation that the embryo in the development of its organs goes, seemingly unnecessarily, through certain embryonic stages found also in its remote ancestors is an undeniable fact, and must be explained in terms that are neither metaphysical nor purely proximate, but through a conceivable evolutionary scenario." The explanation, Mayr argues, lies in the facts of development — the inducing capacities of surrounding embryonic tissues for a somatic program. The somatic program, in combination with additional nuclear genes, directs the development of organisms. The example given is the gill arch system of amniotes, which is functionless as a respiratory system but of critical importance in the development of the head and neck. In his view, recapitulation is irregular, and the explanation is that parts not being used in some manner are quickly lost. This is a strict functionalist, adaptationist explanation fully within the neodarwinian tradition.

However, other workers, while accepting the idea of complicated interactions between parts during ontogeny, focus more on phylogenetic issues. Ontogenies are perceived as being predictable and constrained within lineages, suggesting that they are not mosaic but highly integrated. Evolution occurs mainly by the addition of novel traits to the terminal stages of ontogenetic trajectories (which are repeated, or recapitulated, from ancestor to descendant, differing only with respect to the novel added feature), was formalized by Alberch el al. (1979) as a phylogenetic manifestation of peramorphosis, defined as the morphological expression of a particular cell-level process. There is a rich terminology. Gould (1977) and subsequently Alberch et al. (1979) and others redefined old terms as names for "pure" processes, such as hypermorphosis — a temporal extension of a developmental process, and acceleration — an increase in the rate at which a developmental process proceeds). Thus, process (such as cell division rate) was linked to, but discrete from pattern (the appearance of novel morphology in a descendant, relative to an ancestor). The extreme alternative is reverse recapitulation, a phylogenetic manifestation of paedomorphosis, the morphological expression of a particular process (among which are progenesis — a temporal truncation of development, and neoteny — a decrease in the rate at which a development proceeds). In these cases descendants do not show novelty, but rather re-express as adult states conditions found in the ontogeny of ancestors (in practice, phylogeneticists assess conditions in sister taxa and appropriate outgroup taxa, for ancestors can only be inferred, not known).

"Reverse recapitulation" is a rather cumbersome term, but it conveys the impression of derivative taxa "backing down," phylogenetically, an ontogenetic trajectory that is characteristic of related taxa by failing to complete it developmentally. Mayr (in litt.) objects strongly to the concept of reverse recapitulation. It was introduced in a strictly formal sense by Alberch et al. (1979), in order to idealize a symmetry between evolution by terminal addition versus elimination of terminal stages. Perhaps "incomplete recapitulation" would be a more neutral term.

Patterns of both recapitulation and reverse recapitulation are kinds of heterochrony, phylogenetic differences among taxa with respect to the timing of developmental processes. Heterochrony itself is often treated as a very general term (e.g., Raff and Wray, 1989; McKinney and McNamara, 1991). To treat heterochrony in such a manner has confused evolutionary terminology by conflating developmental process with phylogenetic pattern. For example, Raff and Wray (1989) argue that heterochrony at the level of whole organisms ("global heterochrony") is uncommon and probably not very significant, whereas heterochrony at the level of particular developmental and genic processes is common and important. I would argue that it is global heterochrony that is most likely to have phylogenetic implications, and that to describe subtle shifts in timing of gene action as heterochrony is to reduce the value and utility of the term. Heterochrony with respect to whole organisms and ontogenies has its greatest value in phylogenetics as a relatively uncommon phenomenon. I have in mind such phenomena as the overall body form of larvae versus adults, and ontogenetic trajectories of organ systems (for example, limbs), and parts (for example, presence or absence, and shape, of skull bones) in relation to the whole organism (e.g., Alberch and Alberch, 1981). McKinney and McNamara (1991) argued that recapitulation and reverse recapitulation are unnecessary, and that heterochrony is a sufficient inclusive term for peramorphosis and paedomorphosis, but I disagree. Much of their book is an attempt to make virtually everything in evolutionary biology some kind of heterochrony, thus rendering the concept nearly empty of intellectual content. Confusion is particularly bad with respect to heterochronic processes versus heterochronic patterns. This is where terms like recapitulation and reverse recapitulation are useful, for they are terms relating to phylogenetic patterns.

The word "heterochrony" serves too many masters. The term needs modification and restriction relative to its overuse by McKinney and McNamara. A start would be to expand our process terminology, first by distinguishing process heterochrony from heterotopy (differences among taxa in relative position within the embryo where developmental processes proceed, e.g., the differing results of ectodermal-mesenchymal interactions involving neural crest cells in vertebrates, see Zelditch and Fink, 1995). There is value in developing evolutionary perspectives on heteroplasy (e.g., differing rates of cell proliferation leading to patterns of intertaxon allometry) as well. Workers should be careful to differentiate between developmental mechanics and processes, and phylogenetic pattern.

Reverse recapitulation is manifest in a number of taxa of perennibranchiate but sexually mature salamanders (Siren Ambystoma mexicanum, Necturus, Typhlomolge give examples of independent derivation of the phenomenon in four distantly related families). These are dramatically clear cases, in which derived taxa resemble in their overall morphology the non-terminal ontogenetic states of ancestral or out-group taxa. The term "perennibranchiates" refers to the fact that these taxa retain larval-like gills and remain aquatic throughout life. The processes responsible for the pattern have pro-

duced ecomorphologies (phenotypes tightly connected to particular ecologies — in the present case, aquatic versus terrestrial habitats) that are profoundly distinct from those of immediate ancestors (Reilly, 1994). The genetic and developmental mechanisms underlying this evolutionary transition from terrestrial to aquatic adults are relatively simple, for they are readily attained. Within *Ambystoma* (tiger salamanders, axolotl and mole salamanders) close genealogical relatives may remain in the larval state or metamorphose into terrestrial adults, and may even show polymorphism within a species or population (Shaffer, 1993; Collins et al., 1993). In the case of perennibranchiation, the global effects of the phenomenon are superficially evident in morphology and profoundly evident in ecology, but metamorphosis in salamanders is not a dramatic event, and perennibranchiate and fully metamorphosed animals differ little with respect to most organ systems. Reilly (1994) argued that we should examine the genetic, developmental, morphological, ecological and phylogenetic aspects of heterochronic phenomena separately so that we can differentiate between phenotypic plasticity (phenotypic differences caused by environmental rather than genetic differences) and genetic fixation (at the level of species).

Perennibranchiation is dramatic in its ecological implications, but its more long-term, evolutionary and phylogenetic implications are less evident. In salamanders it leads not to radiative evolution, but seems to be a dead end. There are only two genera and a handful of species in each of the exclusively perennibranchiate families Proteidae and Sirenidae. In contrast, organismal-wide paedomorphosis in direct developing salamanders of the family Plethodontidae has had far more profound implications, judging from the combination of morphological and taxonomic diversification. Although the morphological expression of the underlying developmental processes is superficially less evident than in the perennibranchiate taxa, the morphological diversity encompasses a far greater array of morphological combinations and associations, including substantial novelty (Wake 1966, 1991). Furthermore, although direct development is found only in the Plethodontidae, itself only one of the ten families of salamanders, the direct-developing taxa constitute about two-thirds of the living species of salamanders (Wake 1966, 1987).

I first encountered paedomorphosis in direct-developing taxa when I found taxonomic characters (mainly bones and their parts) that varied among taxa with respect to the time of their appearance (Wake 1966). Those characters that appear very late in ontogeny, or that are found only in the oldest and largest members of a population or species (see also Smirnov 1994), suggest that the taxon displays organismal-wide paedomorphosis, and so the entire morphology and morphological ontogeny must be evaluated within this mental framework.

Elsewhere (Wake 1989) I presented an example from salamanders of the genus *Batrachoseps* (Slender Salamanders, family Plethodontidae) of a pattern of intertaxon heterochrony. The species of this western North American lineage all develop directly from eggs laid on land, and there is no

larval stage. One infers that the morphology is paedomorphic, in relation to out-group taxa within the plethodontid tribe Bolitoglossini, because all species (currently there are 8 species recognized, but several new species are currently being described) display adult morphologies (such as a very large cranial fontanelle, and only four toes) that represent embryonic or juvenile stages of out-groups and inferred ancestors. This is not very controversial, for the logic and data are relatively straight-forward. However, species vary with respect to other traits (all late-appearing features, such as processes of bones, secondary separation of bones, and presence or absence of bones). It is unclear whether there is a paedomorphocline, a sequence of derived taxa each more paedomorphic than the last (McNamara, 1986), with slender species being more derived and paedomorphic, or a peramorphocline, a sequence of taxa showing progressively more peramorphic characters. If the latter situation holds, relatively robust, more fully developed (with respect to the traits listed above) species are more derived. Having been derived from a paedomorphic lineage (that is, one that has shown reversed evolution), they now would be showing a second reversal in having morphologies that resemble the situation before the paedomorphic phase associated with the establishment of the lineage. Other more complex hypotheses are only slightly less parsimonious. Recent work in my lab shows that different stages of paedomorphosis are displayed within each of two major clades of *Batrachoseps*, so at least the notion of a simple paedomorphocline can be rejected. It is much more difficult to reject the hypothesis that there has been a phylogenetic reversal within the clade that includes the robust species. The very existence of heterochrony implies character instability and suggests that characters are more labile than we generally assume that they are in phylogenetic analyses. While robust phylogenetic hypotheses are essential for correct interpretation, in cases such as I have described, with organismal-wide paedomorphosis, nearly every character becomes suspect, and there is general insecurity in relation to "which end is up" (i.e., character polarity). To give one example from *Batrachoseps*, it is equally parsimonious from out-group analysis as to whether presence or absence of a prefrontal bone is ancestral. All but one of the described species lacks the bone, but a second species gains a tiny speck of bone in the correct position very late in life, following achievement of sexual maturity. Are these two species displaying an ancestral trait, or have they undergone peramorphic evolution within the framework of general paedomorphosis and restored a trait absent from their common ancestor? I know of no way to solve this problem by ontogenetic and phylogenetic analysis of living taxa; this is an instance in which a better fossil record could be decisive.

The reason that Hennig's (1966) cladistic phylogenetic procedures have been so successful among morphologists is that morphological characters often persist for long periods of time, through numerous branching events. Differential *character persistence* is universal. Some systematic characters are labile phylogenetically; others, usually termed conservative, show greater persistence. It is easy to find exam-

ples of high character persistence: spiral cleavage in several major and minor taxa, the notochord and brain stem of craniates, and the tripartite body plan of insects. I believe the terminology is appropriate at all levels of the organismal hierarchy. Thus, the *Hox* gene cluster is highly persistent. I distinguish character persistence from *stasis*, persistence of the full morphology through numerous branching events (Wake et al., 1983).

Ontogenetic trajectories display high persistence. This phenomenon has been indirectly recognized by some previous workers in a curious way — they have proposed that paedomorphosis is a kind of escape from specialization for lineages (e.g., de Beer, 1930). Extreme paedomorphosis in miniaturized members of various phyla has led to the loss of traits that are considered parts of bauplans and thus deeply embedded in ontogenetic trajectories (e.g., coeloms; reviewed by Hanken and Wake, 1993).

Character persistence occurs to varying degrees across taxa of any rank. Among salamanders, all plethodontids are lungless, but only some salamandrids are. Most salamanders are five-toed, but three genera of plethodontids have independently become four-toed, and all species of *Batrachoseps* are four-toed. There is a well-justified, general assumption that five is the number of toes that became fixed early in tetrapod phylogeny, and this character has had great persistence, but with noteworthy exceptions that themselves have shown persistence at another level (e.g., the two toes of artiodactyls, limblessness in various saurian clades and in caecilians). But cladistic approaches are invalid when character persistence is low (the "flip side" of character persistence is homoplasy). For example, when dealing with mitochondrial DNA sequences it is risky to attempt to identify synapomorphic and symplesiomorphic substitutions in third positions. It is easier if one is working with a coding sequence, such as cytochrome b, which can be analyzed cladistically at the level of its encoding for amino acids, because amino acids show much greater persistence than bases. Much of the on-going argument over how to analyze adaptation is instead an argument over character persistence. Some workers (e.g., Baum and Larson, 1991) adopt an implicit premise that adaptive traits have high character persistence, while others (Reeve and Sherman, 1993; Frumhoff and Reeve, 1994) believe that such traits have low persistence. Organismal-wide (global) paedomorphosis is, in a cladistic sense, a reduction in persistence in many (terminal) characters at once, and when it is later followed by peramorphosis, confusing degrees of homoplasy are encountered. In such a situation cladistic analysis can be difficult because many equally parsimonious arrangements of taxa are possible, and the most parsimonious may be incorrect because of false information from many traits. This is a pattern encountered within the large bolitoglossine clade of plethodontid salamanders, where early in the history of the clade there apparently was general (high persistence) paedomorphosis and this was followed by peramorphic changes of low persistence (Wake, 1966, 1991; Wake and Elias, 1983). A way out of this dilemma for cladistic analysis is to be able to recognize organismal-wide paedomorphosis at a high taxonomic level, and then code indi-

vidual traits accordingly.

Failure to recognize organismal-wide paedomorphosis can have profound consequences. For generations the brains of salamanders have been accepted as simple, generalized and primitive with respect to their organization and degree of histogenesis. A recent phylogenetic analysis (as recommended above) has shown that, with a high degree of probability, the brains not only of salamanders but also of caecilians and frogs are secondarily simplified, and the nervous system is only a part of an organismal-wide paedomorphic syndrome (Roth et al. 1993). In the most extreme cases, which are highly derived phylogenetically, the brains take on an appearance similar to those of early embryos of out-groups. This is a deceptively simple interpretation, however, because the secondary simplification is founded on derived patterns of connectivity and organization, and represents a mix of embryonic (i.e., paedomorphic) and derived traits, and in some cases novel characters, not represented in out-group taxa. Somewhat surprisingly, the most simplified brains are not those of perennibranchiates such as *Ambystoma mexicanum*, but those of direct-developing species with complex behaviors such as members of the genera *Batrachoseps* and *Hydromantes*, and cladistically basal perennibranchiates such as *Necturus* (Roth et al., 1994). The mixing of cladistically derived, reversed (from paedomorphosis) traits with persistent traits can produce substantial morphological novelty. This outcome, termed *ontogenetic repatterning* (Wake and Roth, 1989), can affect many seemingly unconnected traits at once, with profound implications (as in the organization of the neural control of feeding and brainstem organization in salamanders, Wake, 1993).

The relation of ontogeny to phylogeny is no longer studied with the goal of finding phylogenetically ancestral conditions of whole organisms or even of traits, but with the awareness that it is the entire ontogeny of organisms that is subject to evolutionary change. A character cannot be separated from its ontogeny (de Queiroz, 1985), nor can a character be fully separated or isolated from its organismal milieu. Nonetheless, it is the general stability and conservatism of characters and organisms during their ontogeny and phylogeny that encourages us to believe that there are lessons to be learned for phylogeny from ontogeny.

Does ontogeny recapitulate phylogeny? Sometimes, in a limited way, more in some taxa, less in others, and probably never in the extreme form envisaged by Haeckel. The degree to which it does hold is determined only with difficulty, and so the value of recapitulation as a general guide is very low, as has long been recognized (e.g., de Beer, 1930). Nonetheless, it is surprising to find how readily biologists in many fields leap to the conclusion that a trait that appears early in ontogeny and then transforms is likely to display the ancestral condition at its first appearance. Traditions die slowly. Those who would choose to use recapitulation as a premise in their work would be well advised to study such detailed analyses as that of Mabee (1993; see also Mabee, 1989), who showed that for centrarchid fishes only 52% of a large set of characters evolved by terminal addition. Hence use of an ontogenetic criterion for determining character state po-

larity has low value. The problem of "which way is up" in evolution and phylogeny will not soon disappear!

Hierarchical perspectives on development and evolution.

Development is a hierarchical phenomenon in which a complex of genetic signals, physiological signals, cell-cell interactions, generic physical factors, and self-organizational properties interact to produce an ontogeny. During the past decade there has been enormous progress in our understanding of the nature of hierarchical interactions during the development of the vertebrate head, as exemplified by the paper in this volume by Holland (p. 63). I will briefly consider this issue not from the perspective of the genetic signalling that appears to be so important in head development, but from the perspective of an evolutionist and phylogeneticist, trying to understand how heads have evolved.

That there is a relation of *Hox* genes to neuromeres seems indisputable, and many labs are actively engaged in research to pinpoint the specific mechanisms and interactions that relate genes to morphology. The most impressive evidence that the genes are specifically related to neuromere formation comes from the concordance of neuromere order, the arrangement of genes within gene families on chromosomes, and the general (there are some specific exceptions) sequence of gene expression. At points as yet undetermined, but apparently within the craniate lineage (a critical need is for more work on basal fish lineages), there has been extensive paralogous duplication, resulting in four gene families all showing the same general ordering, but having different specific patterns of expression (Garcia-Fernández and Holland, 1994). This area of research is one in which the zeal to find a common developmental genetic ground plan for the vertebrate head has proceeded with only the most general kind of comparative structure, and with little attention to variation. That is beginning to change, as can be seen from the recent paper by Gilland and Baker (1993), who show that within the general pattern of conservation there is also variation in neuromere-gene relationship. The species differences found indicate the likelihood of shifting relations between genetically determined rhombomere identity and cranial nerves. Although the generality is impressive at this early stage of comparative developmental genetics, the issues of homology and conservatism versus change are likely to loom large in the future. The need for a detailed phylogenetic analysis of the impressive new ontogenetic data, from a broader comparative base than now exists, is critical (Meyer, 1996, presents a detailed exposition of this point).

There long has been controversy concerning the pattern of segmentation of the vertebrate head, and whereas once the issue was one of how many segments were incorporated into the head (e.g., de Beer, 1937), and later the role of novel vertebrate developmental interactions (e.g., those involving the neural crest; Gans and Northcutt, 1982; Northcutt and Gans, 1982), now the issues being raised relate to differences in numbers and identity of rhombomeres and somitomeres, to the nature of differences in head versus body development

and organization, and to differences in genome and cell size.

Whereas Gans and Northcutt argued for a new perspective on the organization of the head and focused on novel features associated with interactions of neural crest cells, recent work has gone even further and has focused on major differences in head-body origination. Holland (1996:63–70) and Fernández-Garcia and Holland (1994) have shown that it may be more appropriate to view amphioxus as being mainly head rather than mainly body, in terms of the pattern of gene product distribution during development. Fritzsch and Northcutt (1993) proposed that cranial and spinal nerves of vertebrates may not be homologues, and argued that the old view that ocular motor nerves were homologues of spinal motor nerves and of the so-called somatic motor component of ventral roots of brainstem mixed nerves can no longer be strongly defended. Northcutt (1993) refutes the influential model of Goodrich (1930), based on modification of trunk-like segments in head origins, and argues against a close relationship between nerves and mesodermal derivatives. He envisions as many as four separate cranial-caudal series of special nerves having arisen in the heads of basal vertebrates. These were derived independently with relationship to each of the iterative developmental tissues of the head (neural crest, neuromeres, placodes, somitomeres), each in a different manner. Gilland and Baker (1993) have gone further, making comparisons between the cranial region of vertebrate embryos and the primary gastrula of amphioxus. In their view the vertebrate head is primary, the homologue of nearly the entire gastrula of ancestral chordates, and craniogenesis during gastrulation is the proper structural starting point for examining the critical roles of brain segmentation and of the evolution of functional roles for the neural crest in craniates. These new perspectives have turned old ideas around, and now we have the image of an ancestral vertebrate head that had to invent a body!

One would think, given these new perspectives, that there would be more attention to the region of the craniovertebral joint, but that is generally not the case. As I have pointed out elsewhere (Wake, 1993), the vagus nerve (X) is of special significance because most of the function of this nerve is associated with the body, although it is derived from one to several rhombomeres (the number is unclear and probably varies among taxa) and gives every indication of being a serial homologue of nerves V, VII and IX. Conversely, the motor nucleus of the hypoglossal nerve (XII) lies in the spinal cord of frogs and salamanders, and exits through vertebrae, although it serves head muscles (the origin of these muscles from dorsal somitic muscle that migrates ventrally was of key importance in Goodrich's model). The motor nucleus of the spinal accessory nerve (XI), only recently mapped in amphibians (Roth et al., 1984; Wake et al., 1988; Ota et al., 1987), lies in the vertebral column, outside the brainstem, yet the nerve migrates anteriorly to exit through the head together with the completely separate vagus. De Beer (1937) and other workers thought amphibians had incorporated fewer segments from the body into the head. This issue needs to be thoroughly reevaluated given new findings relating to head organization.

It may be that part of the problem with relation to amphibian head organization arises from the fact that there are fewer somitomeres in amphibian heads (as in elasmobranchs) as compared with teleosts and amniotes (Jacobson, 1993). It is equally parsimonious, with the data at hand, to argue either: 1) that low numbers were ancestral and high numbers have been gained independently in teleosts and amniotes, or 2) that high numbers were ancestral and that low numbers have been evolved independently in elasmobranchs and amphibians, or 3) that low numbers were ancestral and have been retained in elasmobranchs, but were evolved independently again in amphibians. I favor the latter hypothesis, on the grounds that various kinds of somitic tissue are present in very low quantities in amphibians (e.g., sclerotome; Wake, 1970; Wake and Lawson, 1973), that amphibians show secondary simplification of the entire nervous system (Roth et al., 1993), and that modern amphibians have larger than average to enormous genome and cell sizes and they probably arose from ancestors which had large genomes and cells. The connection of this last point is not immediately obvious. However, somitomeres are expansion figures in early embryogenesis that accumulate cells as they expand (Jacobson, 1993), and rhombomeres, like segments, are condensations of cells that require certain numbers of cells before they self-organize. Furthermore, rhombomeres first are laid out segmentally and then they subdivide. I suggest that a major factor in amphibian development (and especially salamanders) has been large cell size, which has led to the reduction and probable loss of resegmentation (often considered to be a universal feature of vertebral formation in tetrapods), in the trunk, reduction through failure of units to subdivide in the posterior head, and possibly with new combinations of the remaining iterative cell masses in the head. Thus the number of segments in the head of salamanders and frogs as compared with amniotes and some fishes could be the absence not of primary segmentation but of secondary segmentation, plus some amalgamation.

There is a strong positive correlation between genome size and cell size in vertebrates, and in large-genomed taxa there are important implications for rate of cell division, morphogenesis and adult morphology (Sessions and Larson, 1987; Roth et al. 1994). This is especially true for relatively small organisms. Miniaturization often leads to disruption of ground plans, and in such taxa as those constituting the marine interstitial fauna, secondary (in a phylogenetic sense) simplification of adult morphology is nearly an expectation (reviewed by Hanken and Wake, 1993). However, it has not been generally appreciated that there is a difference between physical size, in which organisms are compared by mass or linear dimensions, and biological size, in which genome and cell size in relation to physical size, within a phylogenetic framework (in order to determine the direction of character state change), are the important parameters. Using such criteria, the physically large lungfishes (which have the largest genomes, and cells, among vertebrates) are biologically small, but the physically small salamanders (and some frogs) are in effect biological miniatures. In such organisms we should expect, and we do find, paedomorphic morphologies

that are secondarily simplified, but are in fact only partially recapitulatory. Thus, the optic tectum and the tegmentum of relatively large genomed salamanders, frogs, and lungfishes are apparently embryonic in histological and some aspects of neuronal structure and organization, but they have fully adult physiological organization and neurological connectivity, comparable to less simplified out groups. Physically small mammals such as shrews are biologically large, in comparison to other small but metabolically less active mammals, but all small mammals are biologically large in comparison with the large-genomed, metabolically slow, developmentally retarded salamanders and lungfishes, some of which are much larger than shrews in physical dimensions. Genome size variation is not great in amniote vertebrates, but in most organisms it is a factor that should not be overlooked.

Increasingly in developmental biology there is an appreciation of the importance of cell number at critical stages of morphogenesis, such as in the organization of early condensations. Busturia and Lawrence (1994) used genetic manipulations to produce *Drosophila* embryos with reduced numbers of abdominal primordial cells; such embryos were unable to produce morphological patterns normally seen in development, but denticle bands were fused to those in adjacent segments and some rows were missing, bristles normally present were absent, and a pigment band was reduced. In another example, molecular-level factors involved in skeletal morphogenesis in mice were examined with respect to the role of the gene superfamily known as transforming growth factor beta (Storm et al., 1994). Mutations known as *brachypodism* result in marked shortening of the limb skeleton. Tickle (1994) observed that the number of founder cells for each limb element might be reduced in mutants, which translates into insufficient growth that leads to digital defects. Alberch and Gale (1985) showed that reductions in cell division rate alone can lead to the reduction in the numbers of digits in both frogs and salamanders.

Prospects for a merger of development and evolution

At present the fields of development and evolution are mainly separate, but there are prospects for an integration. I have given some examples of areas of opportunity. Two book-length treatments have appeared (Raff and Kaufman, 1983; Hall, 1992), but the first predated much of the recent excitement in molecular genetics and the second was written by a scientist whose work has dealt mainly with morphogenesis and not with the molecular biology of development. Those who focus on molecular developmental genetics are mainly focused on genetic invention and novelty (genes of large effect) (e.g., Tabin, 1993; Tabin and Laufer, 1993), whereas developmentalists such as Hall recognize the complexities and hierarchical nature of development, and have established working relationships with quantitative geneticists (Atchley and Hall, 1992), who characteristically focus on many genes, each of minor effect. I believe that the resolution of arguments concerning the merits of these contrasting approaches will come from comparisons of relatively closely related taxa, for most of the research (with a few

notable exceptions, e.g., Raff et al., 1991 on sea urchin morphogenesis, and Nijhout, 1990, on butterfly wing patterns), has dealt with too few and too distantly related taxa. There has been a great deal of highly sophisticated developmental genetic work in the past several years, but mainly the focus has been on conserved systems (highly persistent ones, using my suggested terminology). For example the discovery of an apparent common dorsal-ventral patterning signal in insects and vertebrates has renewed speculation that there was a reversal (note the recurrent theme of genetic invention) of the dorsal-ventral axis in some common ancestor (Holley, et al., 1995). However, other workers are focused more acutely on evolutionary issues. The recent work of Carroll (summarized in Carroll, 1994) on the developmental-genetic basis for differences in arthropod body plans stimulated interest in the possibility of analyzing the influence of developmental regulatory mechanisms underlying morphological transitions. Such work may point the way for new investigations into the genetic and developmental foundations of morphological diversity within an appropriate evolutionary and phylogenetic framework.

Evolutionary developmental biology is a field of great promise. Barriers that formerly separated the disciplines of development and evolution are being broken down, and new research questions and programs are being formulated. As the new field develops it will be increasingly necessary to maintain communication with the core disciplines, and this will require that practitioners understand and appreciate the philosophical and conceptual issues in evolution (such as arguments over homology, and methods of phylogenetic analysis) and the methodological and strategic issues in development (such as the hierarchical nature of the interaction of molecular and cytological factors in morphogenesis), so that the empirical core of the fields can be made relevant to both. The success of the new field will depend critically on bringing developmental approaches to the appropriate taxonomic level, such as the populational and interpopulational levels for evolutionary analysis, and the interspecific (i.e., intrageneric or intrafamilial) levels for phylogenetic analysis. There are hurdles, even barriers, to be crossed if a development-evolution synthesis is to be attained. Evolutionists must be convinced that development has something to offer them, and vice-versa. Amundson (1994:576) has offered a penetrating analysis of this issue, and has made clear what the task of students of comparative ontogenies will be "to demonstrate that a knowledge of the processes of ontogenetic development is essential for the explanation of evolutionary phenomena." It will not be easy, as witness the recent attack of Reeve and Sherman (1993) on the concept of developmental constraint. In questioning whether my structuralist (Wake 1991) explanation for why small frogs and salamanders often lose one toe, but a different one in the two taxa (five in salamanders; one in frogs, following Alberch and Gale, 1985), Reeve and Sherman argue that the functionalist (adaptationist) approach must always be conducted first in order to determine if any other kind of explanation is necessary. Amundson (1994) has made a useful comparison be-

tween constraint on form and constraint on adaptation that has relevance here. In essence, Reeve and Sherman have made a category mistake. The argument is not about constraint on adaptation but about constraint on form generation; knowledge of the developmental pathways in frogs and salamanders enables predictions (e.g., concerning unknown or unstudied taxa of frogs or salamanders) to be made. This framework leads to the recognition of constraints, not on adaptation but on form. We who are interested in a synthesis of development and evolution must make clear that our goals are not to replace neodarwinism, but to expand it by focusing on form and its causes, the central problem in development. Similarly, we must make clear to developmentalists that study of variation and its genetic basis, and careful phylogenetic analysis, central issues in evolution, have relevance in developmental biology as well.

I have argued elsewhere (Wake, 1991; Wake and Larson, 1987; Wake and Roth, 1989) that a synthesis and integration of three perspectives on the evolution of form are required for a full picture of the question: how do organisms evolve? One of these perspectives is neodarwinian functionalism — the heart of evolutionary biology. A second is biological structuralism — the rules of form generation and transformation, deeply embedded but not totally subservient to developmental biology. The third is history — both the contingencies of history and knowledge of the genealogical relationships of lineages. I believe that we are not far from a time when these three approaches, appropriately integrated, will form the heart of a modern science of evolutionary developmental biology.

Conclusion

We are on the threshold of a new venture in evolutionary biology, the long-awaited merger of studies on the ontogenetic production and phylogenetic transformation of organismal form. This new field is demanding, for it requires understanding of mechanisms of development and of evolutionary change, and perspectives on current dynamics and on history, both the history of ideas and concepts, and the one true but only partially known history of life on this planet. I predict that the years ahead will at last witness a fruitful synthesis that will bring new excitement to developmental evolutionary biology.

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