

Evolutionary patterns in ontogenetic transformation: from laws to regularities

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ABSTRACT The concept of heterochrony derives from classical approaches to the study of ontogeny and phylogeny. Under the influence of landmark books by deBeer (1930) and Gould (1977), the traditional theories have been revised to fit into the conceptual framework of modern genetics and evolutionary theory. The current scheme, however, suffers from a problem of lack of precise definitions. The term heterochrony is now used to refer to a developmental process as well as to an evolutionary pattern. That is, it refers to a microevolutionary process of adaptation, operating in local populations under selection and to a macroevolutionary pattern based on undefined internal laws of form. Such conceptually contradictory frameworks are a source of confusion and of empirical misuse of concepts. We propose to reduce the dependence of current thinking about heterochrony on the concept of "timing" and instead focus on the organization of sequences of developmental events in ontogeny. Although Haeckelian views have been rejected, most experts would agree that some subtle parallelism between ontogeny and phylogeny does occur. This relationship deserves renewed attention and urodeles are particularly suited to study it due to their variable patterns of ontogeny and complex life cycles. Current reductionist attempts to apply the morphological terminology and postulates of classical heterochrony concepts to cellular and molecular (genetic) aspects of morphogenesis are problematic. Molecular heterochrony requires a linear or strictly hierarchical structure of gene regulation of development. In addition, isomorphism between genetic mutations and morphological changes would be required for the existing terminology to apply. Finally, we caution against a broad interpretation of heterochronic processes at the molecular level, since the approach may end up permitting the meaningless interpretation of any developmental change as heterochrony.

KEY WORDS: *phylogeny, ontogenetic sequences, heterochrony, systematics*

"The invalidity of (Haeckel's) law has been demonstrated, so often, and so conclusively, that it is easy to fall into the opposite extreme and ignore the fact that many organisms that are highly dissimilar as adults go through similar larval or embryonic stages".

Ernst Mayr in "Animal Species and Evolution" (1963, p. 606).

"It is generally recognized that, although ontogeny does not recapitulate phylogeny, sensu stricto, there is nonetheless likely to be a connection between certain phylogenetic events and certain of the ontogenetic events that have been preserved for posterity in embryology. In such cases, embryology provides phylogenetic insight of crucial significance and is indeed evolution reenacted."

Pinckney J. Harman, "Paleoneurologic, Neoneurologic and Ontogenetic Aspects of Brain Evolution" (James Arthur Lecture, 1956, Am. Mus. Nat. History, p.2-3).

Introduction

Urodeles could be rightfully considered the "*Drosophila*" of heterochrony, given their dominant role in the one hundred plus years of research that have marked this interdisciplinary subject with an old and illustrious tradition. The modern interpretation of heterochrony is a direct descendant of Haeckel's classical laws on ontogeny and phylogeny and centers on the study of evolution as a consequence of regulation in timing and rates of development

within a structurally conserved ontogeny (McKinney and McNamara, 1991). Heterochrony is currently an active and promising research subject that has been a catalyst in the explosive revival of the emerging multidisciplinary approach to evolution and development (e.g., Wake *et al.*, 1991; Hall, 1992). It derives from the most classical tradition of the study of ontogeny and phylogeny, although the original meaning of heterochrony was much narrower (Gould, 1977). The term heterochrony, like other very well-known terms such as ecology and phylogeny, was

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coined by Haeckel to define changes in the expression of specific events within the ontogeny of an organism relative to its ancestor's ontogenetic sequence. Such rearrangements ("dissociations") of an ontogeny would result in a new sequence of ontogenetic transformations that would violate Haeckel's biogenetic law, better known by the popular phrase: "ontogeny recapitulates phylogeny."

In 1867, Dumeril reported the metamorphosis of some gilled, larva-like, sexually mature axolotls into terrestrial salamanders with a generalized morphology. Ever since, the axolotl and other larval-breeding species of urodeles have become paradigmatic exceptions to the theory of recapitulation. As such singular cases of vertebrate development, they were highly appreciated by the opponents of Haeckel. Early this century, the noted zoologist and anti-Haeckelian champion, W. Garstang, felt compelled to pay poetic homage to these "reluctant developers" that had provided him with numerous empirical counter-examples to Haeckel's postulated evolution by addition of terminal stages to the ancestral ontogeny:

*"These axolotls having gills, pursue a life aquatic
But, when they should transform to newts, are naughty
and erratic"*

as well as to its more committed, mandatory non-metamorphosing relatives,

*"And newts Perennibranchiate have gone from bad to worse:
They think aquatic is bliss, terrestrial a curse.
They do not even contemplate a change to suit the weather,
But live as tadpoles, breed as tadpoles, tadpoles
altogether!"*

W. Garstang in Gould (1977, p. 178)

The phenomenon of pre-metamorphic reproduction, as expressed by the axolotl, is known as paedomorphosis¹ ("child-like morphology"). This change in ontogeny that results in a new adult morphology that looks like a juvenile or embryonic ancestral form is a special case of heterochrony. Hence, the new use of heterochrony provides a much broader meaning, which reflects a generalization of Haeckel's initial biogenetic law (see Gould, 1977, 1992; Alberch, 1985). It relaxes Haeckel's requirement of "terminal addition" as the predominant evolutionary trend and includes any change in timing and rates within a *conserved* ontogeny. This paper will review this conceptual transition and explore the implications of the new postulate that centers around the concept of regulation within a conserved pattern of development.

Heterochrony is thus a direct product of Haeckelian ideology, with its typological and internalist implications. Often Haeckel's biogenetic law is confused with Von Baer's laws. We summarize and contrast the two concepts in Figure 1 to clarify an issue often quite confusing to the outsider. We follow Richards (1992), who recently reviewed Gould's (1977) extensive historical interpretation of the views of Von Baer and Haeckel, correcting some lingering anti-Haeckelian prejudices in Gould's overall superb vindication

and rigorous study of these two central figures in the genesis of modern embryology. In the tradition of the time, the comparative study of ontogeny attempted to extract from the enormous complexity of developmental observation empirical "regularities" or rules. Such general patterns of invariance in ontogenetic organization across taxonomic groups gave rise to some fundamental postulates. Among them, Haeckel's "biogenetic law" was the better known. It states that evolution proceeds by the addition of new stages to the end of the ancestral ontogeny. As a result of this "principle of terminal addition," plus the concomitant postulate of "acceleration" in the unfolding of the ontogenetic series, an organism would go through an *abbreviated* series of ancestral adult morphologies (Fig. 1).

After a long period of prominence, Haeckel's theory fell into disrepute around the turn of the century. Several reasons for the rejection of Haeckel's views have been proposed. Curiously, most of the reasons were not of an empirical nature, with the exception of paedomorphosis, which, after all, had been known previously. Gould (1977) chronicled a sudden rise early in the twentieth century in the popularity of paedomorphosis as a phenomenon often involved in many major evolutionary transitions, such as the origin of vertebrates. Even humans were claimed to be paedomorphic apes. Among those who published during that period, most of them critical of the biogenetic law, Garstang (1922) is often noted for his influential work reviewing the accumulated empirical evidence that suggested that paedomorphosis (implying "terminal truncation," see footnote 1) was at least as common as the "terminal addition" required by the biogenetic law.

The downfall of ontogenetic approaches to evolution, however, was due to epistemological trends and changes in research methodology. Among the latter, the advent of experimental embryology excluded history from the search for immediate causal mechanisms of development. More critical, it was difficult to integrate the Haeckelian postulates with the Mendelian laws of inheritance and with "the new genetics" emphasis on the concept of mutation as the source of variability. Morgan (1903) is representative of a trend that culminated with deBeer (1930). "Recapitulation" was demolished on the grounds that it was incompatible with the prevalent explanation of new variation in terms of the newly discovered mechanisms of genetics. In addition to these two classes of arguments against recapitulation, well reviewed in Gould (1977), a more general epistemological reason for the increasing disfavor of Haeckel's views is the progressive consolidation of a new evolutionary paradigm based upon the theory of natural selection acting on populational gene frequencies. This approach led to the neodarwinian synthesis of the 1940's.

Haeckel's enthusiastic support of Darwin's theory as well as of Darwin's use of Haeckel's views as reflected in his concept of "unity of type" as fundamental evidence for descent with modification, and its corollary historical definition of homology (see Richards, 1992), may be misleading since Haeckel's biogenetic

¹Heterochrony was redefined by Alberch *et al.* (1979) into its currently accepted scheme, which centers around a key division of pattern from process. **Morphological outcomes**, which can result from a variety of underlying mechanisms, can be reduced to two *descriptive categories*: (1) **peramorphosis**, a morphology that results from *terminal addition* of stages to the ancestral ontogeny; thus a new adult morphology is generated; and (2) **paedomorphosis**, a morphology reminiscent of an embryonic or juvenile ancestral form due to the terminal deletion (truncation) of the ancestral ontogenetic sequence. On the other hand, **heterochronic processes** involve increases or decreases in the relative timing of expression, or in the rates of growth or morphogenesis, of a developmental process. For example, the earlier termination (*progenesis*) or a slow down in rate (*neoteny*) result in a "paedomorphic" phenotype; conversely a "peramorphic" form can be generated by a combination of delayed termination (*hypermorphosis*) or an *acceleration* of the ontogeny, thus allowing the process to "overdevelop" relative to its ancestor.

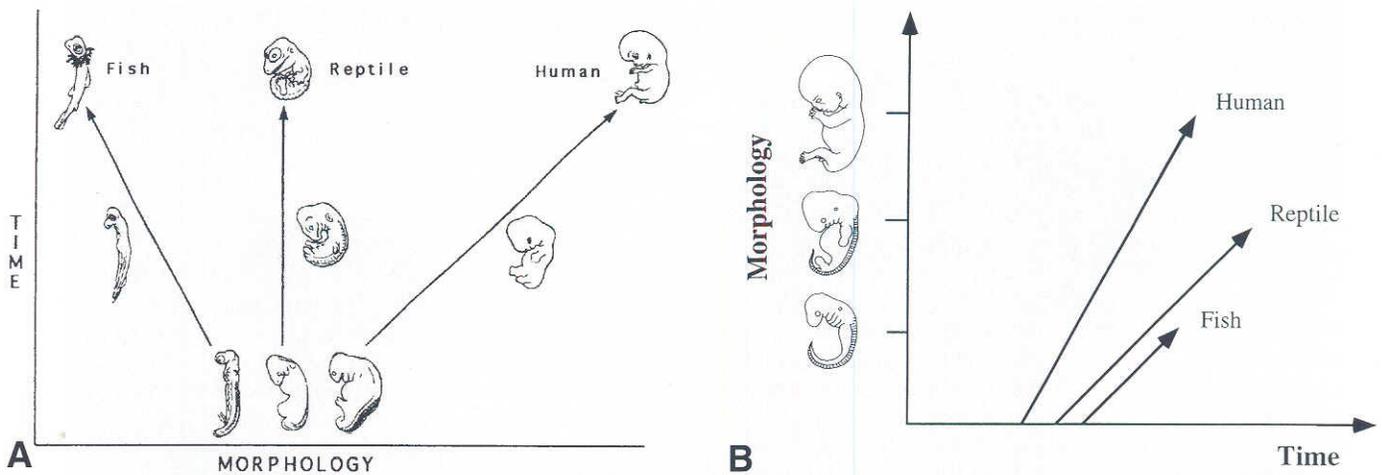


Fig. 1. Ontogenetic laws of Von Baer (A) and of Haeckel (B). (A) Von Baer's law assumes that all organisms can be classified into a small number of basic body plans, that are discrete and independent from each other. This pre-evolutionary concept is similar to Cuvier's general classes, R. Owen's archetypes or similar metaphysical concepts associated with the morphology of the times. Given this precondition, Von Baer law can be summarized as "the embryo develops from a more generalized condition of its type to the more particularized features of its species." The result is a process of progressive differentiation with a concomitant divergence in morphological similarity as the embryos develop. (B) Haeckel spoused the view that both an individual's ontogeny and a species phylogeny were analogous processes of morphogenesis through different temporal scales. Evolution proceeded by addition of new stages to the end of the ancestral ontogenetic sequence. To prevent ontogenies from becoming inordinately long in time as well as in stages, he had to add a second principle, besides terminal addition, which claimed that as an embryo develops it recapitulates an accelerated and a much abbreviated sequence of ancestral stages. Haeckel's biogenetic law requires that a homology be established between an embryonic stage of a descendant and an adult ancestral stage. Whether embryos recapitulated "adult" stages was a major issue at the time. Note, however, that if a person believes in Von Baer's laws and, unlike the nineteenth century embryologist, also accepts biological evolution, some sort of recapitulation would be observed during phylogeny.

law was deeply inconsistent with Darwin's theory of natural selection. Order in nature was interpreted by Haeckel and Darwin in radically different ways. To Darwin "ordered patterns of diversity" resulted from natural selection based on the ecological opportunities determined by environmental change. To Haeckel novelty was generated in an ordered manner by means of an internal process that effected a terminal addition. Historically, Haeckel's views have been more in line with subsequent theoretical alternatives to Darwinian natural selection. Examples range from turn-of-the-century theories of orthogenesis (e.g., Berg's "nomogenesis") to early macromutational proposals, to contemporary heterodox approaches (e.g., those proposed by Riedl, 1978 and Løvtrup, 1978). The dichotomy between internalist ("structuralist") and externalist ("functionalist") philosophies has been a recurring theme in evolutionary biology (Alberch, 1989 and references therein; as well as a recent insightful commentary by the philosopher Amudson, 1994).

In summary, the biogenetic law was essentially abandoned, and it went out of favor on the basis of theoretical, rather than empirical, arguments. Nevertheless, the existence of an empirical pattern of morphological parallelisms between ontogeny and phylogeny is undeniable. Hence, it is not surprising that efforts to develop modified versions of the biogenetic law have continued to until the present.

Many of the recurrent mistaken attributions of Haeckelian ideas to Von Baer are rooted in attempts by comparative embryologists, morphologists, and paleontologists to deal with the widespread parallelism between ontogeny and phylogeny without invoking Haeckel's much maligned influence. For example,

Morgan (1903, p. 82) already questions this strategy in his critique of the revisionist attempts of the renowned invertebrate embryologist, Hertwig: "Thus Hertwig adopts here a little from a doctrine and there a little from another, and between his attempt to reinstate the old biogenetic law of Haeckel, and to adopt a more modern point of view, he brings together a rather curious collection of statements which are not any too well coordinated." Since that time, severe criticism has ensued (for a recent example, the third edition of the popular textbook, *Developmental Biology*, by Gilbert (1991) refers to "Haeckel's disastrous union of embryology and evolutionary biology"). It resulted in an apologetic attitude by authors dealing with the subject, who felt compelled to disallow any connections with Haeckelian ideas. But we hope to demonstrate that Haeckelian concepts have survived, usually misattributed to Von Baer, in the disciplines using a comparative approach to the study of morphological pattern, such as comparative anatomy, paleontology, and classical comparative embryology.

These are areas where the neodarwinian "populational" approach has not influenced much the classical methodology. For example, Haeckelian concepts underlie most ontogenetic arguments utilized in the determination of homologies in comparative morphology (e.g., Patterson, 1983 and Nelson, 1978 [who wrongly uses Von Baer as a source, see Alberch, 1985]). Similarly, the pervasive nature of these concepts is evident from such statements as: "the occurrence of pectoral before pelvic appendages could be interpreted as phylogenetic recapitulation" (Coates, 1993; see also our introductory quote from Harman).



Fig. 2. Skulls from two young specimens of the European fire-salamander, *Salamandra salamandra*, cleared and stained with Alcian Blue (cartilage) and Alizarin Red (calcified bone). The large pre-metamorphic specimen spent two years as an aquatic larva in a mountain lake and has extensively calcified dermal bones in his fully functional skull used for feeding, breathing, etc. In contrast, the small head is from a conspecific, viviparous population from Northern Spain. It metamorphosed in the mother's reproductive tract. The larval period in this reproductive mode is greatly shortened since the fully transformed, tiny adult-like terrestrial morph was born after less than a year of gestation. The head exhibits lots of cartilage indicating very little ossification, despite the fact that it is more advanced in development than the large larva. Nevertheless, our observations clearly show that in both populations the sequence of dermal bone ossification is identical (from Dopazo and Alberch, 1994; and unpublished observations).

Heterochrony today: conflict between macro- and micro-evolutionary approaches to the concept

The publication of *Ontogeny and Phylogeny* by Gould (1977) is a landmark in the resurgence of heterochrony, a subject essentially ignored in the previous decade. Gould's masterful historical review resulted in a much simpler and more precisely defined conceptual scheme. It was followed by an elaboration of a comparative formalism that illustrated the fundamental processes and outcomes associated with heterochrony (Alberch *et al.*, 1979). The second part of Gould's book outlined a new approach to heterochrony. The emerging picture, however, introduced a new conceptual element difficult to reconcile with the historical trends just described. A perspective according to which heterochrony was not only a *developmental process*, under the hypothetical control of regulatory genes (a follow-up from deBeer's mechanistic treatment of the phenomenon), but had also been transformed from a phylogenetic (macroevolutionary) pattern into a microevolutionary process. As such, heterochronic processes became amenable to integration within a populational

context with orthodox neodarwinian concepts such as contemporary models of the evolution of life history strategies based upon adaptationist paradigms.

Heterochrony as a microevolutionary process: intrapopulational diversification of ontogeny under selective pressures controlled by ecology

Gould's thesis made good on Van Valen's famous remark that "evolution is development controlled by ecology" (emblematically quoted by Gould in his opening statement). In accord with neodarwinian postulates, ontogeny would be shaped by natural selection acting on intrapopulational variation in the basic heterochronic parameters (*sensu* Alberch *et al.*, 1979). Therefore, even if heterochrony generates macroevolutionary patterns, it can be studied at the microevolutionary level. We consider this mechanistic continuity to be a misleading image given the fundamental differences between the two approaches to heterochrony: the new approach is framed within the neodarwinian paradigm, while the traditional approach, as explained above, is rooted in an incompatible internalist

perspective.

Gould did make extensive use of the large literature on urodeles as empirical examples dealing with the evolution of ontogeny within an ecological context. In particular, the new heterochronic formalism was amenable to integration with the existing models of ecological control of metamorphosis in urodeles (Wilbur and Collins, 1973). In fact, the best examples of selection on populations of ontogenies apply to variability in the expression of metamorphosis, an event closely associated with the attainment of sexual maturity and having the timing of its expression under hormonal control. Such a phenomenon is a particular aspect of development difficult to generalize to developmental processes beyond the ones usually related to life history fitness parameters, such as length of the gestation period, size at birth, overall rate of embryonic development, age at first maturation, etc.

The last eighteen years of research on heterochrony have uncovered the operational limitations of the "new ecological approach." Given the secondary relevance of ecological arguments to the general subject of this volume, we simply outline the main conclusions from this facet of heterochronic methodology. The usefulness of heterochrony in an ecological context is operationally restricted to problems where the chronological "time" of developmental events becomes, in itself, a selective factor, such as the previously mentioned variables involved in adaptationist models of the evolution of reproductive strategies (e.g. Iwasa and Levin, 1995). Urodeles are widely used in this active area of research in evolutionary ecology. Some examples include the role of heterochrony in the diversification of metamorphic patterns and the evolution of optional larval reproduction (e.g., Whiteman, 1994) and heterochrony in sexual dimorphism or intraspecific polymorphisms (e.g., Skulason and Smith, 1995), in the genesis of the cannibalistic morphs in *Ambystoma* (Collins and Cheek, 1983; Lanoo *et al.*, 1989, 1990), or in the origin of new reproductive modes, such as in the evolution of viviparity as a result of acceleration of development associated with the presence of intrauterine sibling cannibalism in some populations of the European salamander, *Salamandra salamandra* (Dopazo and Alberch, 1994). In many other cases, besides timing itself, body size is the adaptive feature. In those cases, heterochrony transforms into its subdiscipline, allometry, and its associated aspects of functional scaling and adaptation (Gould, 1966; and M.J. Reiss, 1989 or Nicklas, 1994 for recent general reviews).

The key limiting factor of the microevolutionary approach centers on the problems with *quantification* of the ontogenetic trajectories. Quantitative values for the timing of onset and termination of developmental events, as well as of rates of growth and morphogenesis, are required to study the effects of selection acting on differential fitness within a population of ontogenetic trajectories. Severe limitations to this approach are encountered. Some are operational. For example, it is usually difficult to gather longitudinal measurements of individual ontogenetic transformations. Instead cross-sectional data from different individuals are used, which eliminates the possibility of assessing the intrapopulation variation upon which selection operates. Similarly, most field studies lack adequate "age" values for the ontogenetic samples. Consequently, we add a second tier of procedural problems to the previous limitations. For example, even if age were available, the use of chronological time is often

inappropriate in interspecific comparisons. Developmental time is a relative concept, a function of both environmental (e.g., developmental rates may vary as a function of temperature) and taxon-specific differences in physiological rates (J.O. Reiss, 1989). To overcome this problem, most heterochronic studies have substituted body size for a measure of "age." But the use of body size as the independent variable in ontogenetic plots often generates flawed inferences of heterochronic processes, as discussed by Godfrey and Sutherland (1995).

Many authors, however, are unwilling to accept the technical difficulties of devising a methodology that would permit the identification and exploration of the effects of the *processes* of heterochrony. This attitude results in the generation of an increasingly complex nomenclature describing heterochronic processes of dubious biological validity or even methodological usefulness (e.g., the subdivision of rate parameters into global and local). Such a Procrustean approach is in danger of degenerating into artificial schemes and an increasingly complex terminology. Paradoxically, the complexity of an increasingly artificial nomenclature was one of the main criticisms leveled against classical Haeckelian recapitulation.

Heterochrony as macroevolutionary pattern: phylogenetic trends, evolutionary parallelism, and the origination of new body plans

An alternative to the microevolutionary level of analysis of heterochrony is encountered in its classical tradition, based on a typological, qualitative view of ontogeny as the unfolding of a sequence of temporal events. In fact, some areas neglected by Gould (1977) in his conceptual review have been among the most active in research on the nature of ontogenetic transformations and heterochrony. Most important among these areas is the line of research, derived from cladistic systematics, on the use of ontogeny to study character transformation (e.g., Nelson, 1978; Kluge, 1985, or Wheeler, 1990; again some of these authors refer incorrectly to a variety of authors, such as Von Baer and Garstang, as the historical precedent for their ideas).

Besides its usage in systematics, heterochrony has been invoked to explain a wide range of macroevolutionary phenomena. Most intriguing is the recurrent evidence for heterochronic changes associated with the globally coordinated morphological reorganization involved in the evolutionary genesis of new body plans. Paedomorphosis is implicated in the origin of vertebrates (e.g., Garstang, 1922). Peramorphosis is invoked to explain the origin of tetrapods (Long, 1990), and there is extensive evidence supporting a paedomorphic origin of the lissamphibia, a taxonomic grouping that includes urodeles (Bolt, 1969, 1979). In addition to these specific studies of historical events in evolution, there are numerous comparative studies on heterochrony in the evolutionary history of a specific organ or functional system, for instance the brain and associated sensory systems and the CNS (e.g., Northcutt, 1990).

Similarly, heterochrony has been used to explain recurrent parallelisms in phylogeny. The independent evolution of identical features in various lineages is difficult to attribute to a probabilistic process such as selection acting on random mutations, but it is expected from heterochrony: a **system of regulation within a conserved ontogeny** (Sluys, 1989). Urodeles are characterized by the ubiquity of varying degrees of paedomorphosis throughout

**ORDERED MORPHOSPACE:
Patterns of variation in distal tarsal**

Neotropical salamanders genus Bolitoglossa

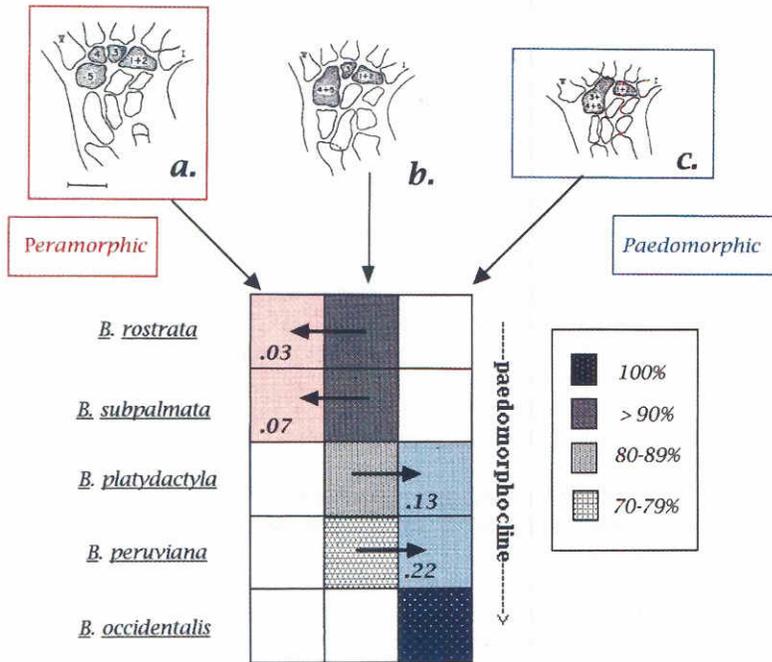


Fig. 3. The patterns of diversity in distal carpal morphology in the neotropical salamanders of the genus *Bolitoglossa* can be explained using heterochrony. A trend toward digital reduction and increases in interdigital webbing, among other highly derived features, accompanies the ecological radiation of the lowland tropical species into the arboreal habitat. Based on previous work (see citations by Alberch, Wake and colleagues in the text), we constructed an Index of Paedomorphosis to arrange the selected species into a morphocline of increasingly paedomorphic features (i.e., from less paedomorphic, highland, moderately webbed species, *B. rostrata* and *B. subpalmata*, to the extremely paedomorphic, *B. occidentalis*, an arboreal species from the tropical lowlands. Data from Alberch (1983) shows that three types of distal tarsal arrangements have been found in *Bolitoglossa*. (a) A 4-element distal tarsus that is only found in the genus as a rare atavism, although the condition is found in less paedomorphic relatives of *Bolitoglossa*; (b) the generalized condition in the genus consists of the presence of 3 distal elements; (c) a derived paedomorphic morphology, found in the most paedomorphic members of the genus, where only two elements are found. The relative peramorphic (a) and paedomorphic (c) forms are indicated. Notice that the patterns of intraspecific variability display an ordered pattern congruent with the heterochronic hypothesis. Thus, the less paedomorphic species, when a variant morphology is encountered, is always atavistic as in (a). The arrows indicate the nature of the variant (peramorphic or paedomorphic), and the numbers indicate the frequency with which it is encountered (in this case very low). (a) is the only variation found in the two species *rostrata* and *subpalmata*. The more paedomorphic forms exhibit higher levels of variation, but the

deviant morphologies always correspond to the more paedomorphic type (c). The most paedomorphic species, *B. occidentalis* displays very low variation. In fact, no variation was encountered. Our prediction is that if a variant morph is found it would correspond to (b) Data from Alberch (1993).

their phylogeny, a situation that fosters the generation of extensive parallelism among distantly related lineages (e.g., Wake, 1966, 1989; Alberch, 1981). The independent recurrence of correlated sets of paedomorphic features poses methodological difficulties in phylogenetic reconstruction, as discussed by Hecht and Edwards (1977) in relation to Perennibranchiate urodeles. In paleontology there is a long tradition of heterochronic analysis of morphological trends in the fossil record (e.g., McNamara, 1988), for example the generation of heterochronic trends in morphological transformation associated with giantism or dwarfism (e.g., Laurin and Garcia-Joral, 1990; or Roth, 1993).

From laws to regularities: evolutionary reexamination of structural patterns of heterochronic change in ontogenetic sequences

The classical postulates of Haeckel and Von Baer which attempted to identify general characteristics of ontogenetic organization and evolution were based on the comparative study of *ontogeny as a sequence of morphologies*. Heterochrony has its historical origins in this tradition. Comparative studies of ontogenetic diversity and the reexamination of morphological evolutionary events in terms of heterochrony are only meaningful if they are preceded by the demonstration of explicit regularities in specific patterns of ontogenetic transformation in evolution. Such rules of transformation must apply within a sufficiently high

taxonomic level of generality. These rules of heterochronic invariance must be demonstrated empirically or through detailed experimental analysis of cellular and molecular developmental mechanisms because, unlike erroneous implications recurrently encountered in the specialized literature, heterochrony is not a mechanism that causally constrains ontogenetic transformation. To the contrary, heterochronic analysis is only illuminating and useful because ontogenetic sequences are constrained. The origin of such rules must be sought in the underlying dynamics of development, not in heterochronic correlations that are themselves dependent upon those dynamics.

The recurrence of the subject of recapitulation in comparative morphology can be explained by Mayr's introductory quote in which his statement, "quite often it occurs" implies that there is a "regularity" or pattern according to which ontogeny often generates ancestral-like morphologies. There is, however, no estimate of the frequency of such events nor is there an explicit approach to seek that information. Traditionally, researchers have focused on exceptions, without taking into consideration that we are dealing with a statistical variable, rather than an absolute law. There may be some generic aspects to the structural organization of ontogenetic patterns that contain some useful insights on the nature of evolutionary processes.

We outline a method to carry out a comprehensive, systematic survey within an objective, comparative approach to test hypotheses about general patterns of transformation in the

evolution of ontogeny (e.g., see Rieppel, 1988, for an outline of contemporary comparative methodology for reconstructing the history and patterns of relationship in modern systematics).

The following kinematic description of development does not make any mechanistic assumptions. Specifically, it does not presuppose recapitulation. Rather, we formalize a method to describe and compare developmental sequences in a broad search for regularities and invariance.

Deconstructing ontogeny as a sequence of "developmental events"

Let us define ontogeny as a sequence of discrete morphological transformations, referred to as "developmental events." Such "events" could be described under a wide range of metrics. For example, events can be qualitative morphological descriptions (e.g., the classical stages from "Normal Tables" of development) or value ranges determined by quantitative phenotypic measurements (e.g., volume concentrations, a transient chemical state within a chain of reactions, or any other phenotypic variable amenable to expression as part of a temporal sequence of phenotypic transformation). It is important to remember that heterochrony is a morphological-level theory. It is based on *phenotypic* comparisons among stages of ontogeny. Furthermore, as elaborated in Alberch (1985), to defend the Haeckelian roots of the method we must be able to define correspondences ("homologies") among equivalent embryological or adult stages in different ontogenies.

For illustrative purposes, we list four distinct, but evolutionarily homologous, ontogenies, A to G, characterized by the following sequences of morphological transformation:

A= 0
 B= 0→1→2
 C= 0→1→2→3→4
 D= 0→1→2'→3
 E= 0→1→2→3
 F= 0→1→2'
 G= 0→1→2

These series of transformations result in the adult morphologies, 0, 2,4,3,3,2', and 2 respectively. Hence, B and G are identical (the reason for its redundant presence will be clear later), while D and E correspond to a commonly encountered instance of a similar adult phenotype being attained through somewhat different ontogenetic pathways.

Examination of the above patterns of transformation illustrate the following properties: (1) Absence of *dissociation*, a term that in the literature on heterochrony means that the sequence remains invariant throughout phylogeny in the absence of reversals in the order of appearance of the events within the series, (e.g. 2→1 would be a case of reversal); (2) Absence of non-terminal additions (=insertions); (3) Branching development, which graphically can be expressed as:



In this example, two distinct developmental pathways lead from state A to states B and C. This type of branching is not rare in development. In fact, in the absence of branching processes in

ontogeny, the whole range of organismal diversity would consist of different morphologies corresponding to stages within a single ontogenetic sequence leading from the unicellular amoeba to the most complex multicellular terminal stage (primate) as implied in the classical notion of the "scale of beings" (e.g., see Gould, 1977, for references). Hence, diversification requires branching in developmental series, but it is important to emphasize that heterochrony depends on the minimization of such processes.

Structural stability of ontogenetic sequences

For any given ontogenetic sequence to contain information, the sequence must be ordered, even if "dynamical continuity" is *not* assumed or required. (Dynamical "continuity" in a process means that the stages of the sequence are expressions of a single underlying causal mechanism). An ordered pattern may be indicative of an underlying constraint which in turn is an emergent property of the dynamics of the developmental process. In this case, by "ordered" we mean the relative absence of dissociation, branching, and non-terminal alteration of the sequence.

Constancy in sequence allows for predictability of hypothetical transformations. For example, based on the information provided by the above illustrative sequences, we could postulate that a mutation or experimental perturbation (e.g., phenocopy) that forces or induces the "overdevelopment" of ontogeny A, morphology 1 would be the most likely to be generated. Furthermore, given the observation that 1 is present, we would predict that, should further "overdevelopment" occur, 2 will follow. This property of invariance is characteristic of a causal sequence, which requires a relationship between events such that the antecedent is a prerequisite for the expression of the subsequent event.

There are two classes of sequences: "temporal" and "causal" (Campbell and Ritchie, 1983). A "temporal" ordering of events in a sequence does not necessarily connect the expression of neighboring events. Hence, a strictly "temporal" arrangement does not preclude the possibility of inversions or point deletions occurring without generating cascading effects downstream. For "causal sequences" embryological tissue inductions constitute a typical example.

Empirical examples of ontogenetic and phylogenetic patterns of variation

Intraspecific and interspecific patterns of variability within sequences require empirical approaches in order to be recognized. We provide a couple of illustrative examples. In many ways, we are just formalizing the procedure of many studies of heterochrony. Due to limitations of "longitudinal" data, researchers end up resorting to sequence analysis and comparative analysis of size-based ontogenetic trajectories built from "cross-sectional" data (i.e., many specimens arranged by increasing size as opposed to sequential ontogenetic measurements on the same individuals, known as "longitudinal").

Intraspecific variation in ontogeny: cranial ossification in the urodele *Salamandra salamandra*

One aspect of development that is rarely examined is the presence of individual variation in ontogenetic patterns. It is usually assumed that all individuals within a species are

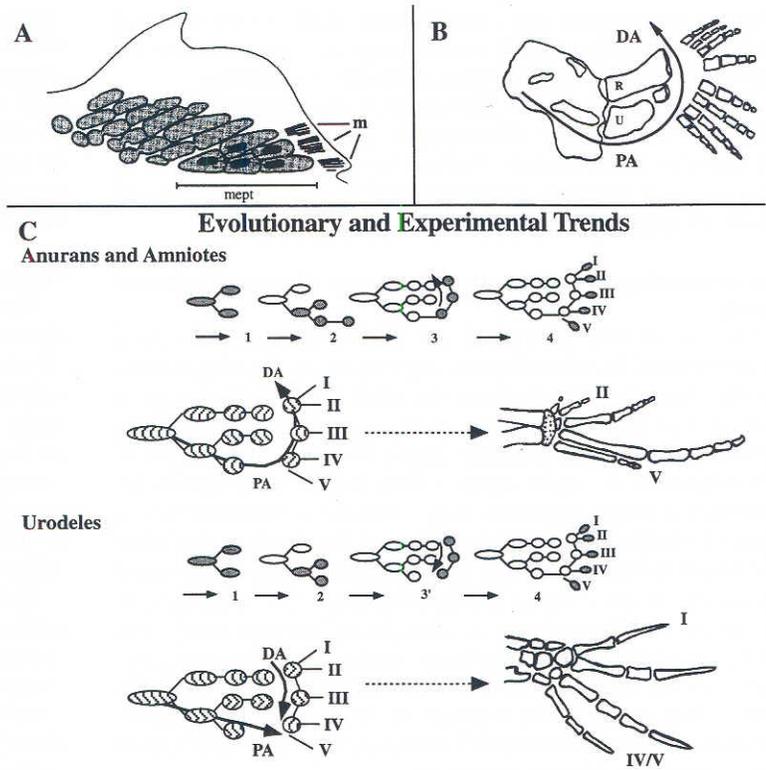


Fig. 4. Limb development and evolution. (A) Posterior branching pattern of prechondrogenic elements during pelvic fin development in the sturgeon *Acipenser ruthens* (from Coates, 1994). The branching scheme, as proposed in Shubin and Alberch (1986) is overlaid on the posterior side of the adult fin skeleton. This posterior branching is a general feature of tetrapod ontogeny. (B) Schematic restoration of the *Acanthostega* forelimb (from Coates and Clack, 1990) showing the polydactylous character of early tetrapods. Overprinted, the bend of the primary axis into the digital arch, associated with the origin of a novel structure in the tetrapod taxa: the digits. (C) Evolutionary and experimental trends. Branching and segmentation diagrams indicating the sequence of chondrogenetic condensations are shown to illustrate the unique reversal of the digital arch in urodeles (bottom). Experimental reduction in the size of the early limb bud results in different outcomes in urodeles vs anurans as the former lose posterior elements while the latter do not differentiate the anteriormost element ("the thumb"). Identical experimental perturbation generates distinct outcomes that parallel ontogenetic and phylogenetic differences. Abbreviations: m, muscular bundles; mept, metapterygium; R, radius; U, ulna; DA, digital arch; PA, primary axis; I, II, III, IV and V, digits 1, 2, 3, 4 and 5, respectively.

embryologically identical. In the case of sequences of events with unknown underlying developmental interdependence, it is critical to examine variability among individuals in character transformation.

To this end, as part of an ongoing project on the evolution of life history strategies and reproductive modes in Iberian populations of the European "fire-salamander," *Salamandra salamandra*, 229 specimens at various stages of cranial ossification — usually associated with metamorphosis — were examined. The following sequence of ossification was constructed (Dopazo, 1995 and unpubl. data):

parasphenoid → parietal → exoccipital → quadrate → otic → maxillary → prefrontal → nasal

Of the 229 specimens from six populations, only 4 individuals (all from the same population) deviated from the norm. That is, knowing the above sequence, any individual with just one ossification would have the parasphenoid bone ossified; if two events have differentiated, they would be the ossifications of the parasphenoid and the parietal, and so on. The four exceptions exhibited an identical pattern that consisted of the alteration of the terminal events into:

..... → otic → prefrontal → nasal → maxillary

It is important to emphasize that the changes occurred in the terminal region of the sequence. The invariance of the series of differentiation events — and an illustration of the relative nature of chronological age — is the fact that, among the populations sampled, we included representatives of two extreme life history patterns (Fig. 2): (1) the "viviparous reproductive mode," in which metamorphosis occurs at a relatively small size inside the

mother's reproductive tract and with scant calcification; and (2) a population composed of individuals that metamorphose up to two years after being spawned. At metamorphosis time they almost double the viviparous metamorphosing specimens in size, and with a considerable amount of skull bone calcification (for more information on *Salamandra* interpopulational variability in reproductive modes, see Joly, 1981; Dopazo and Alberch, 1994; Alcobendas *et al.*, 1996; Alcobendas, Castanet and Alberch, submitted). Both these groups displayed the identical sequence of ossification events. The four exceptions were not members from either of the two populations just discussed. They belonged to a generalized ovoviviparous population.

The intraspecific invariance just described cannot be extended to apply to either an interspecific or intergeneric characteristic, as was illustrated in Alberch *et al.* (1979) for *Ambystoma* species. There is a considerable amount of variability among urodele taxa in sequence of ossification, a feature that calls for a more detailed treatment of the sequences prior to their use in heterochronic comparisons (K. Dunlap, pers. comm.).

Heterochrony and the interpretation of patterns of intraspecific and intrageneric variability in the neotropical salamanders of the genus *Bolitoglossa*

The salamanders of the genus *Bolitoglossa* exhibit an extraordinary range of morphological variability associated with the evolutionary radiation of this genus into the arboreal niches of the tropical lowlands (see Wake and Lynch, 1976, for an introduction to this speciose genus of neotropical urodeles). Alberch (1983) analyzed the morphological variability found

among several species of *Bolitoglossa*. The sample included generalized, highland species such as *B. rostrata* and *B. subpalmata*, already used as a basis for comparison in Alberch and Alberch (1981), and two distinct types of lowland, highly derived, fully webbed, arboreal species: (1) large body size forms, such as *B. platydactyla*, or even extremely large, e.g., *B. doffeini*; and (2) dwarf arboreal convergent forms, such as the Mexican species, *B. occidentalis* and the Amazonian lowland species, *B. peruviana* (see Alberch, 1981 for further discussion on their convergent morphology).

There are two basic points that we wish to illustrate with the example. First, knowledge of the ontogenetic sequence can be used to measure the incidence of paedomorphosis in this group. A series of traits from Alberch and Alberch (1981) were used to derive a "paedomorphic index." A plot of the patterns of intraspecific variability against the degree of paedomorphosis shows that the least variable forms are one or the other extreme in the continuum of degree of paedomorphosis. The species that exhibit intermediate paedomorphic features are the most variable at the intraspecific level. Second, the expression of atavisms is also non-random and can be explained as a function of the paedomorphic degree of the species (Fig. 3). In conclusion, heterochrony is not only useful for interpreting the morphologies expressed, but also the relative degree of populational variability.

Searching for regularities: a hypothesis of evolution by terminal modification of ontogenetic sequences

The previous examples demonstrate that the analysis of ontogenetic sequences can provide insights into the nature of the processes of morphological evolution. Most important from a methodological perspective, the information obtained from developmental sequences could not have been derived from any alternative analytical procedure at other levels of biological organization (i.e., genetic, populational,...). This is not an attack on the adaptationist program, rather it purposes to make explicit that our proposal, within current developmentalist approaches to evolution, is a source of knowledge qualitatively different and methodologically autonomous from classical neo-Darwinian adaptationism.

In summary the fundamental requirements for an insightful, and locally predictive, heterochronic analysis are listed below:

- i **Dissociation events**, i.e., changes in the order of expression of ontogenetic events, must be rare.
- ii **Non-terminal conservancy**: addition or deletion of the events in the sequence, or any insertion within the ancestral sequence (the latter is equivalent to Haeckelian caenogenesis), must also be rare.
- iii **Branching transitions in ontogenetic transformations** must be rare: a process of morphogenesis or pattern formation that cannot be described as a linear expression of events is beyond the scope of the methodology.

Terminal modification: a hypothesis

Assuming the low frequency of the above three phenomena as a precondition, the following corollary can be formally stated as a working hypothesis: "the evolutionary mode of diversification of a

morphological feature, or the structural physical arrangement characteristic of an integrated functional system must exhibit the following two patterns of organization in the apportionment of ontogenetic and phylogenetic diversity: (1) adult polymorphisms must share a common underlying pattern of ontogeny. That is, despite varying lengths, (i.e., the number of developmental events expressed) characteristic of the ontogeny leading to each phenotype, a stable sequence of developmental events is maintained; (2) evolution must have occurred by modification of the number of developmental events within a conserved sequence of transformational events. Furthermore, conservancy of a generic sequence of ontogeny means that the genesis of derived characters must occur by "terminal modification"² of the events at the end of the species-specific sequence of ontogeny.

The logic of the above statement makes it clear that the central issue is not the phenomenon of "terminal modification," since given the previous three postulates, the only possible way for evolutionary changes to occur without violating the preconditions is by acting on the last events in the ontogenetic sequence. Instead, terminal modification may not be due to the action of any specific process, such as the "regulatory genes" invoked by Gould (1977) or the rate genes that, according to the heterodox evolutionist Goldschmidt (1940), controlled macroevolution. The greater lability of the terminal stages of ontogeny is a property of the inherent conservancy of sequences of development. Hence, it is clear that a broad, comprehensive empirical study is required to objectively test the stability of sequence. Since the results of such a study would depend on the choice of developmental system as well as taxonomic group, our point is not to argue for the validity of the above assertion, but instead to formulate a hypothesis. *It would be a contradiction to argue for a flexible interpretation of developmental pattern and, at the same time, to assert that development can be a useful analytic tool in evolutionary studies.* Any author who embarks on such an approach is not being logically coherent, and the research program would lead to the reconstruction of *ad hoc* scenarios of anecdotal interest, without any conceptual theoretical value. The arguments for these assertions are similar to the ones used against "scenarios" in the adaptationist program (Gould and Lewontin, 1979; Eldredge, 1993).

Phylogenetic analysis of ontogenetic sequences.

Mostly inspired by Nelson (1973 and 1978), systematists have been researching the issue of patterning in ontogeny and phylogeny from the perspective of sequence analysis. Fink (1982) revised the terminology proposed by Alberch *et al.* (1979), suggesting the use of outgroups to establish polarity in ontogenetic sequences. Other treatments of the subject can be found in Kluge and Strauss (1985), Kluge (1985), Humphries (1988), Wheeler (1990) and Northcutt (1990), among others. We exclude references on how to use ontogenetic transformations as characters in phylogenetic analysis and its methodological approaches (e.g., de Queiroz, 1985; although some of the previous references also touched upon the issue). Our concern is limited to the phenomenon of evolutionary patterns in ontogenetic transformations. In this respect, most of the previously quoted authors have defended variations of the terminal modification

²Terminal modification results in the genesis of two classes of morphological outcomes: terminal addition (**peramorphosis**) and terminal deletion (**paedomorphosis**).

hypothesis (Northcutt, 1990). There is a fair amount of consensus that the emphasis placed on timing by de Beer (1930) and Gould (1977) and its methodological revision in Alberch *et al.* (1979) detracted from the macroevolutionary analysis of ontogeny. Furthermore, to varying degrees the majority of authors consider ontogenetic sequences to be a valid tool for comparative evolutionary analysis as well as a source of phylogenetic information.

The hypothesis of "terminal modification" is non-committal regarding the relative manifestation of terminal addition (peramorphosis) vs. terminal deletion (paedomorphosis) throughout evolution. This is an empirical issue that needs to be tested in a system where an adequate previous knowledge of phylogenetic relationships already exists. Phylogenetic analysis based on molecular characters would be particularly appropriate since it is necessary to base heterochronic conclusions on a phylogeny constructed with characters independent of the effects of heterochrony (see Wake, 1989; Wray and Bely, 1994 for a discussion of this topic).

The empirical results of Mabee (1993) and Krauss (1988) are to our knowledge the best comprehensive studies that survey a wide range of traits to test hypotheses on the nature of ontogenetic transformations during evolution. Both studies empirically support evolution by terminal modification in an extraordinarily high frequency (up to 70-80%) of cases (Krauss, 1988; Mabee, 1993). Mabee's (1993) paper is a model on how to carry out this type of global survey on the evolution of the structure of ontogenetic sequences. Her use of alternative phylogenetic schemes is thorough and objective. Thus, her arguments against an unqualified acceptance of terminal modification as suggested by Krauss (1988), without a careful assessment of potential artifactual errors due to the method used in character scoring, are warranted; see also Mabee (1989). Therefore, a cautious conclusion would be that the results are interesting enough to justify more comprehensive studies on the issue. The results seem to be more convincing that it is not possible to state that peramorphosis is more common than paedomorphosis, although Krauss (1988) implies the former to be more common. Furthermore, the incidence of any of the above two heterochronic outcomes maybe taxon dependent. For example, urodeles seem to be a group with a major incidence of paedomorphosis throughout the evolution of most of their lineages (Wake and Larson, 1987). Nevertheless, the validity of the proposed heterochrony methodology is not dependent on the relative frequencies of peramorphosis or paedomorphosis; terminal modification (and, more explicitly, sequence stability) is the key requirement.

Reductionism: molecular and cellular approaches to heterochrony

It must be emphasized that heterochrony historically has been a theory of *morphological transformation*. Hence, any reductionist application of the theory is limited by a terminology grounded not only on the process-related variable of timing, but also on morphological appearance. One of the first extensions to the gene level was the proposal of so-called, "heterochronic" genes of the Lin system in *C. elegans* (Ambros, 1988 for review). Such genes appeared to control the timing of cell division and differentiation. Given the highly mosaic pattern of development in this nematode,

there is a close relationship between mutation and phenotypic effect. That property makes this usage of heterochrony a special case. Given the lack of space and the focus of this volume on urodeles, we will not discuss the heterochronic studies at this level carried out in invertebrates.

One general epistemological problem with reductionist approaches to heterochrony is the need to be cautious in extrapolation. The broad approach of McKinney and McNamara (1991), who treat heterochrony, almost without distinction, as both a pattern and a developmental process under hormonal and genetic control, leads to equating heterochrony with development. Since the latter can be abstracted as a temporal process of gene expression and since any morphological change throughout evolution must involve an underlying change in development, it would follow that almost any developmental change implies some kind of alteration in the temporal pattern of gene expression. Hence, taken to its logical conclusion, any instance of morphological evolution can be ultimately treated as a heterochronic or heterotopic alteration in genetic expression. Such an all-encompassing view loses its analytic usefulness.

A recent example of this usage of the concept of heterochrony in a molecular survey, including urodeles, was reported by Collazo (1994). He studied the variability in the relative timing of fibronectin expression during gastrulation. The report highlights once more the singularity of urodeles in terms of development. The result is an interesting insight into this developmental feature, and it should reinforce the appeal to be cautious in extrapolations from urodele development, given their uniqueness relative to other tetrapods. To describe it as a heterochronic shift, however, has the pernicious effect of equating most comparative studies of development with heterochronic surveys.

Heterochrony as an evolutionary subject must be viewed as a search for global patterns in structural organization of ontogeny, a methodology that leads to the prediction of regularities in the transformation and expression of specific morphologies.

Limb development and evolution as a test case: heterochrony and the expression of Hox genes

The hypothetical developmental sequences listed in "Deconstructing ontogeny as a sequence of 'developmental events'" correspond to a selection of patterns of development in some selected species, as well as to experimentally generated ontogenies, chosen to illustrate the ontogenetic transformations associated with the fin to limb transition (Fig. 4). Limb development is illustrated as a proximo-distal temporal sequence of branching and segmentation spatial patterns in early chondrogenetic foci (Shubin and Alberch, 1986). The proto-ancestral fish fin, corresponding to the species *Accipenser*, is from Coates (1994), and the representative of the most primitive tetrapods, *Acanthostega*, is based on Coates and Clack (1990). The rest are based on previous research on the development and evolution of the tetrapod limb (Burke and Alberch, 1985; Shubin and Alberch, 1986; Muller and Alberch, 1990; Blanco and Alberch, 1992).

The analysis of the ontogenetic sequence of *Accipenser* suggests that postaxial dominance (*sensu* Shubin and Alberch, 1986) was already present in the pre-tetrapod limb. Such posterior branching activity was developmentally extended to generate the digital arch in the early polydactylous tetrapods (Fig. 4A). Hence,

the tetrapod limb originated by peramorphosis (see Long, 1990 for an elaboration on this thesis).

If polydactyly is the ancestral condition (Fig. 4B), it follows that the pentadactylous condition must be considered a paedomorphic form. This conclusion illustrates both the hierarchical nature of the definition of heterochronic outcomes as well as the importance that determinations be based on broad phylogenetic surveys of morphological variability (Coates, 1993). From an ahistorical and structuralist perspective, however, it is more relevant to highlight the fact that, regardless of polarity, limbs evolved by terminal modification and that most morphologies can be shown to be variations within a conserved theme.

Finally, we illustrate the striking parallel between the four-toed species of salamanders and anurans. Both adult morphologies have evolved repeatedly in each amphibian order, an example of the widespread parallelism associated with heterochrony. In salamanders as well as in frogs, the four-toed phenotype is the result of a paedomorphic truncation of their respective five-toed generalized ontogenies (Alberch and Gale, 1985). The digits, however, have been lost differently in each group: frogs have lost the anteriormost (the thumb), while the urodeles have lost the posterior digit. Such a difference corresponds to the opposite polarity of development of their respective digital arches. Furthermore, the experimental morphologies generated by reducing the number of cells in the early limb bud resulted in the expression of teratological four-toed limbs, identical in each case to the corresponding evolutionarily derived species (Alberch and Gale, 1983). The different inter-ordinal responses to the same experimental perturbation — a reflection of the reversed polarity of the Urodelan limb ontogeny — and the morphological identity between the experimental limb and the species-characteristic four-toed morphology emphasizes the constrained nature of morphological variability (Alberch, 1989).

The reversed polarity between the urodele and anuran limb ontogeny is an example of a "branching" ontogeny (see "Deconstructing ontogeny as a sequence of developmental events" and Fig. 4), which is one of the classes of ontogenetic patterns that preclude a heterochronic interpretation. Hence, the four-toed anurans and urodeles are *paedomorphic* in relation to their primitive morphologies, but *the unique developmental pattern of urodeles, relative to anurans and other tetrapods, cannot be interpreted from a heterochronic perspective.*

Recently, there have been attempts to resort to the terminology of heterochrony to refer to experimental results obtained in developmental genetics. In particular, Dollé *et al.* (1993) have generated truncated ontogenies, morphologically similar to the Alberch and Gale (1983) forms illustrated in Figure 4, by altering the expression of *Hoxd* genes during the development of the mouse limb. Their reference to "neoteny" implies that a paedomorphic morphology has been generated by a specific process of slowing down the rate of development in accord with their observation of temporal and spatial reduction of rates of cell proliferation. As was discussed in Alberch (1985), a slow down in the rate of cell proliferation does not precisely match a slow down in rate of morphological shape change as implied by the formalism (Alberch *et al.*, 1979). In fact, the evolutionary results in Alberch and Gale (1985) suggest that the Dollé *et al.* (1993) forms, a result of the reduced rate of cell proliferation, tend to be associated with reductions in overall size, which traditionally is a feature of

progenetic forms. Hence, their assessment of neoteny is not conclusively warranted by their evidence. This lack of correspondence poses methodological problems that need to be more carefully examined, since using an old term with a new meaning is likely to end up being more confusing than helpful. Nevertheless, it is easy to avoid this problem, by using the process-free term of paedomorphosis to describe their results.

The interpretation of experimentally induced arrest of development in terms of heterochrony is part of a larger theoretical scheme outlined by Duboule (1994). The temporal pattern of the proximo-distal expression of the *Hox* genes along the body axis correlated with their spatial colinearity leads him to view the *Hox* complex as a central regulator of fundamental morphological pattern (phylotypic model), where heterochrony is invoked within a conceptual context congruent with the one espoused in this article.

Duboule (1994) appears to imply that such patterns of expression hold for the ontogenetic and phylogenetic patterns of *Hox* genes in vertebrate evolution. He explicitly postulates a morphological theory wherein *Hox* genes play a role in the determination of the vertebrate body plan: "*the phylotypic stage is not a point, nor a stage, but rather a succession of stages, and [I] propose that the concomitant activation of the gene is neither a coincidence, nor a consequence of this event, but instead is the cause of the apparent invariance of this developmental event*" (Duboule, 1994, p. 139). We had not seen this paragraph when we wrote the outline for an explicit conceptual scheme of heterochrony and its correlated requirements, so we were struck by the precise concordance of Duboule's statement: that is, "temporal sequence," causality, and morphological pattern (the emphasis on "succession" and "cause" is from the original). The hypothesis is extended to apply to the observed pattern of higher lability of terminal events in limb patterning ("this could account for the 'distal variability, proximal stability observed amongst tetrapod limbs'" (Duboule, 1994, p. 140, quoting Hinchliffe, 1991, on the apportionment of variability).

The hypothesis is further reinforced by the suggestion of Yokouchi *et al.* (1991) of a correlation between the anterior-posterior sequence of expression of *Hox* and the morphological sequence, Figure 4, of branching and segmentation of cartilaginous elements described in Shubin and Alberch (1986). Similarly, Sordino *et al.* (1995) suggested that tetrapod digits, the most distal and consequently terminal stage in limb morphogenesis, are also recent evolutionary traits and not homologous to any fin structures (Shubin and Alberch, 1986 and Coates, 1995). Furthermore they are controlled in their expression by *Hoxd-13*, which, in turn, is also the last gene to be expressed in the sequence. This gene is not expressed in the latest phase of development of the teleost fin.

Molecular heterochrony: a conceptual outline and explicit requirements for an evolutionary approach at the molecular level

A priori, it is possible to extend the definition of heterochrony to the genetic level, but to avoid truisms derived from all-encompassing approaches, we reiterate the requirements that apply: (1) evidence for a "causal" sequence of gene expression along a temporal axis; (2) invariance in the ordinal arrangement of the sequence events, plus a minimum of insertions and deletions

throughout evolution; (3) most evolutionary modifications must be restricted to alterations in the genes expressed in the terminal portion of the sequence; (4) furthermore, given the fact that heterochrony is a theory that describes morphological outcomes, a rigid isomorphism between gene sequence of expression and morphological pattern of transformation in ontogeny needs to be postulated.

The last requirement is more formal than restrictive. If evolution proceeds by terminal modification of conserved linear arrays of gene expression, molecular heterochrony can provide major insights into the nature of evolution of ontogeny. It would, however, require a new terminology to describe the outcome, if gene-to-phenotype isomorphism is not present. As their Greek roots indicate, the terms paedomorphosis and peramorphosis refer to morphology. Hence, they ought to be restricted to that level of analysis.

Linear patterns of temporal gene expression, isomorphic with morphological change, will generate a pattern according to Duboule's hypothesis. In spite of Duboule's remarks, the issue of reductionism in heterochrony is still not resolved. It will eventually depend on the structure of gene control systems during development. As was argued previously for phenotypic-level sequences of development, the fundamental issue is the stability of the sequence. At the morphological level, the stable ordering along a sequence may reflect an emerging property, rather than an expression of mechanistic causality. But at the gene level, a more direct causal interaction among the members of a sequence is required. More specifically, we contend that only linear, or strictly tier-hierarchical, systems of gene control could produce the required invariant sequence.

There is little empirical evidence on the topological structure of gene control networks in eukaryotes that it is relevant to the evolutionary question of heterochrony. As an exception, we can cite the preparation of maps that orient the sequences of cellular cytodifferentiation during development from a global perspective that focuses on potential pathways and structural organization (e.g., Slack, 1985; Brady *et al.*, 1995). Conversely, if genetic control of morphology is the result of complex network of multi-level interactions (e.g., recent models by Burstein, 1995, and Loomis and Sternberg, 1995), the hypothesis will not be valid. Similarly, the hypothesis of the evolutionary origination of *Hox* clusters through gene duplication would result in redundancies within the regulatory systems. In addition, Wagner's (1994) model proposing that *Hox* patterns of activity have combinatorial properties would not support Duboule's linearly temporal causality in expression and regulatory function. Finally, the lack of one-to-one gene mutation-to-morphological effect causality in homeotic mutants (Carroll, 1995) does not appear encouraging to a heterochronic translation to the gene level. In conclusion, it is too early to make a judgment due to the lack of specifically focused studies. For example, most research has focused on the combined effects of genes on phenotype (e.g., Condie and Capocchi, 1994; Davis *et al.*, 1995), but to invoke heterochrony in *Hox* genes, it would be useful to know the effects of altering the temporal order in gene expression. More specifically, to know the effects of one gene's misexpression on subsequent events within a specific ontogenetic sequence (for example, to study the effects of experimental misexpression of *Hoxd-11* on the activity of *Hoxd-12* and *Hoxd-13*).

Conclusion

Does evolution proceed mostly by changes in the terminal stages of ontogeny? How much information about the history of an organism is encoded in its sequence of developmental transformations? More than a century since Haeckel proposed that ontogeny recapitulates phylogeny, most experts cannot provide definitive answers to these questions. Haeckel has been dismissed, his theories rejected, but, as any comparative study of embryology would illustrate, some subtle parallel between ontogeny and phylogeny exists. We do not argue for a nostalgic revival of Romantic morphology. But, we believe it is time to reexamine the empirical phenomenon afresh. Previous studies were tarnished by their objective to debunk Haeckel's maximalist opinions. As a result, authors have tended to focus on exceptions to his law, rather than approaching the issue from a comprehensive perspective. There are many valid reasons for the downfall of Haeckel's views, but some relate to a generic prejudice of the neodarwinian synthesis against internalist approaches to evolution.

The phenomenology of evolution by terminal modification as a common theme is a reasonable hypothesis based on existing empirical data. Nor are we arguing that any ontogenetic sequence is invariant and relevant. To the contrary, our postulate is based on previous empirical knowledge. That is, *given* an invariant sequence, it will contain useful evolutionary information. A mechanistic explanation, however, is not necessarily implied. One could speculate that, in agreement with the high level of complexity that characterizes development, the global transformational regularities in morphological development and evolution are an emergent property without a single underlying mechanistic cause. Rather they represent a sort of statistical average of the millions of interactions that characterize the developmental process. Nevertheless, the rescue of the comparative method and the incorporation of a historical perspective to developmental biology will be a positive catalyst.

The framework proposed here suggests that reductionist research programs must be combined with comparative searches for global regularities in developmental patterns. This is clearly the case for heterochrony, where a systematic characterization is required before it is possible to explore *local* causality. Approaches such as the genetic analysis of *Drosophila* mutants by DeSalle and Grimaldi (1993) give insights into the old question of the parallelisms between ontogeny and phylogeny, which have never been satisfactorily studied. Scientists keep focusing on the variability of early stages of development, such as gastrulation, to dismiss the topic, rather than focusing on, say, overall distribution of the "timing of phenotypic effect" of all known developmental genes. We are dealing with a statistical property, rather than a physics-like law. Renewed concepts, such as the bauplan and zootype (Slack *et al.*, 1993), derived from the classic theoretical morphology of the 18th century are being pushed forward by molecular and developmental biologists. Such an unprejudiced attitude towards the comparative aspects of evolutionary development will be very positive for the establishment and consolidation of an alternative internalist approach within a more comprehensive evolutionary theory.

References

- ALBERCH, P. (1981). Convergence and parallelism in foot morphology in the neotropical salamander genus *Bolitoglossa*. 1. Function. *Evolution* 35: 84-100.
- ALBERCH, P. (1983). Morphological variation in the neotropical salamander genus *Bolitoglossa*. *Evolution* 37: 906-919.
- ALBERCH, P. (1985). Problems with the interpretation of developmental sequences. *Syst. Zool.* 34: 46-58.
- ALBERCH, P. (1989). The logic of monsters: evidence for internal constraint in development and evolution. *Geobios* 19: 21-57.
- ALBERCH, P. and ALBERCH, J. (1981). Heterochronic mechanisms of morphological diversification and evolutionary change in the neotropical salamander, *Bolitoglossa occidentalis* (Amphibia: Plethodontidae). *J. Morphol.* 167: 249-264.
- ALBERCH, P. and GALE, E. (1983). Size dependence during the development of the amphibian foot. Colchicine induced digital loss and reduction. *J. Embryol. Exp. Morphol.* 76: 177-197.
- ALBERCH, P. and GALE, E. (1985). A developmental analysis of an evolutionary trend. Digital reduction in amphibians. *Evolution* 39: 8-23.
- ALBERCH, P., GOULD, S.J., OSTER, G.F. and WAKE, D.B. (1979). Size and shape in ontogeny and phylogeny. *Paleobiology* 5: 296-317.
- ALCOBENDAS, M., DOPAZO, H. and ALBERCH, P. (1996). Geographic variation in allozymes of populations of *Salamandra salamandra* (Amphibia, Urodela) exhibiting distinct reproductive modes. *J. Evol. Biol.* 9: 83-102.
- AMBROS, V. (1988). Genetic basis for heterochronic variation. In *Heterochrony in Evolution: A Multidisciplinary Approach* (Ed. M.L. McKinney). Plenum Press, New York.
- AMUNDSON, R. (1994). Two concepts of constraint: adaptationism and the challenge from developmental biology. *J. Philos. Sci.* 61: 556-578.
- BLANCO, M.J. and ALBERCH, P. (1992). Caenogenesis, developmental variability, and evolution in the carpus and tarsus of the marbled newt *Triturus marmoratus*. *Evolution* 46: 677-687.
- BOLT, J.R. (1969). Lissamphibian origins: possible protolissamphibian from the lower Permian of Oklahoma. *Science* 166: 888-891.
- BOLT, J.R. (1979). *Amphibamus grandiceps* as a juvenile dissorophid: evidence and implications. In *Mazon Creek Fossils* (Ed. M.H. Nitecki). Academic Press, New York, pp. 529-563.
- BRADY, G., BILLIA, F. and ISCOVE, N. (1995). Analysis of gene expression in a complex differentiation hierarchy by global amplification of cDNA from single cells. *Curr. Biol.* 5: 909-922.
- BURKE, A.C. and ALBERCH, P. (1985). The development and homologies of the chelonian carpus and tarsus. *J. Morphol.* 186: 119-131.
- BURSTEIN, Z. (1995). A network of developmental gene hierarchies. *J. Theor. Biol.* 174: 1-11.
- CAMPBELL, R.L. and RICHIE, D.M. (1983). Problems in the theory of developmental sequences: Prerequisites and precursors. *Hum. Dev.* 26: 156-172.
- CARROLL, S.B. (1995). Homeotic genes and the evolution of arthropods and chordates. *Nature* 376: 479-485.
- COATES, M.I. (1993). Hox genes, fin folds and symmetry. *Nature* 364: 195-196.
- COATES, M.I. (1994). The origin of the vertebrate limbs. *Development (Suppl.)*: 169-180.
- COATES, M.I. (1995). Fish fins or tetrapod limbs - a simple twist of fate? *Curr. Biol.* 5: 844-848.
- COATES, M.I. and CLACK, J.A. (1990). Polydactyly in the earliest tetrapod limbs. *Nature* 347: 66-69.
- COLLAZO, A. (1994). Molecular heterochrony in the pattern of fibronectin expression during gastrulation in amphibians. *Evolution* 48: 2037-2045.
- COLLINS, J.P. and CHEEK, J.E. (1983). Effect of food and density on development of typical and Cannibalistic Salamander Larvae in *Ambystoma tigrinum nebulosum*. *Am. Zool.* 3: 77-84.
- CONDIE, B.G. and CAPECCHI, M.R. (1994). Mice with targeted disruptions in the paralogous genes *hoxa-3* and *hoxd-3* reveal synergistic interactions. *Nature* 370: 304-307.
- DAVIS, A.P., WITTE, D.P., HSIEH-LI, H.M., POTTER, S.S. and CAPECCHI, M.R. (1995). Absence of radius and ulna in mice lacking *hoxa-11* and *hoxd-11*. *Nature* 375: 791-795.
- DE BEER, G.R. (1930). *Embryology and Evolution*. Oxford University Press, Clarendon, Oxford (UK).
- DE QUEIROZ, K. (1985). The ontogenetic method for determining character polarity and its relevance to phylogenetic systematics. *Syst. Zool.* 34: 280-299.
- DESALLE, R. and GRIMALDI, D. (1993). Phylogenetic pattern and developmental process in *Drosophila*. *Syst. Biol.* 42: 458-475.
- DOLLÉ, P., DIERICH, A., LEMEURE, M., SCHIMMANG, T., SCHUHBAUR, B., CHAMBON, P. and DUBOULE, D. (1993). Disruption of the *Hoxd-13* gene induces localized heterochrony leading to mice with neotenic limbs. *Cell* 75: 431-441.
- DOPAZO, H. (1995). Evolución intraespecífica de una característica macroevolutiva: viviparismo en *Salamandra salamandra* (Amphibia: Salamandridae). Ph.D. Dissertation, Universidad Autónoma de Madrid.
- DOPAZO, H. and ALBERCH, P. (1994). Preliminary results on optional viviparity and intrauterine siblicide in *Salamandra salamandra* populations from Northern Spain. *Mertensiella* 4: 139-160.
- DUBOULE, D. (1994). Temporal colinearity and the phylotypic progression: a basis for the stability of a vertebrate Bauplan and the evolution of morphologies through heterochrony. *Development (Suppl.)*: 135-142.
- DUMERIL, A. (1867). Métamorphoses des batraciens urodèles à branchies extérieures de Mexique dits axolotls, observées à la Ménagerie des Reptiles du Muséum d'Histoire Naturelle. *Ann. Sci. Nat. Zool.* 7: 229-254.
- ELDREDGE, N. (1993). History, function and evolutionary biology. *Evol. Biol.* 27: 33-50.
- FINK, W.L. (1982). The conceptual relationship between ontogeny and phylogeny. *Paleobiology* 8: 254-264.
- GARSTANG, W. (1922). The theory of recapitulation: a critical restatement of the biogenetic law. *J. Linn. Soc. Zool.* 35: 81-101.
- GILBERT, S.F. (1991). *Developmental Biology*. Sinauer Associates, Inc., Sunderland, Massachusetts.
- GODFREY, L.R. and SUTHERLAND, M.R. (1995). Flawed inference: why size based tests of heterochronic processes do not work. *J. Theor. Biol.* 172: 43-61.
- GOLDSCHMIDT, R. (1940). *The Material Basis of Evolution*. Yale University Press, Hanover, Massachusetts.
- GOULD, S.J. (1966). Allometry and size in ontogeny and phylogeny. *Biol. Rev.* 41: 587-640.
- GOULD, S.J. (1977). *Ontogeny and Phylogeny*. The Belknap Press of Harvard University Press, Cambridge, (MA).
- GOULD, S.J. (1992). Ontogeny and Phylogeny Revisited and Reunited. *BioEssays* 14: 274-279.
- GOULD, S.J. and LEWONTIN, R.C. (1979). The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist program. *Proc. R. Soc. Lond. Biol.* 205: 581-598.
- HALL, B.K. (1992). *Evolutionary Developmental Biology*. Chapman & Hall, London.
- HECHT, M.K. and EDWARDS, J.L. (1976). The determination of parallel or monophyletic relationships: the proteid salamanders — A test case. *Am. Nat.* 110: 653-670.
- HINCHLIFFE, J.R. (1991). Developmental approaches to the problem of limb structure in evolution. In *Developmental Patterning of the Vertebrate Limb* (Eds. J.R. Hinchliffe, J.M. Hurlé and D. Summerbell). Plenum Publishing Corp., New York, London, pp. 313-324.
- HUMPHRIES, C.J. (1988). *Ontogeny and Systematics*. Columbia University Press, New York.
- IWASA, Y. and LEVIN, S.A. (1995). The timing of life history events. *J. Theor. Biol.* 172: 33-42.
- JOLY, J. (1981). Données écologiques sur la salamandre tachetée *Salamandra salamandra*. *Ann. Sci. Nat. Zool.* 10: 301-366.
- KEIL, F.C. (1981). Constraint on knowledge and cognitive development. *Psychol. Rev.* 88: 197-227.
- KLUGE, A.G. (1985). Ontogeny and the phylogenetic systematics. *Cladistics* 1: 13-27.
- KLUGE, A.G. and STRAUSS, R.E. (1985). Ontogeny and systematics. *Annu. Rev.*

- Ecol. Syst.* 16: 247-68.
- KRAUSS, F. (1988). An empirical evaluation of the use of ontogeny polarization criterion in phylogenetic inference. *Syst. Zool.* 37: 106-141.
- LANOO, M.J., LOWCOCK, L. and BOGART, J.P. (1989). Sibling cannibalism in noncannibal morph *Ambystoma tigrinum* larvae and its correlation with high growth rates and early metamorphosis. *Can. J. Zool.* 67: 1911-1914.
- LANOO, M., SWEET, M., LADEHOFF, N., FANGMAN, E. and COLLINS, W. (1990). Time to metamorphosis as a function of larval size in a population of *Ambystoma tigrinum* salamanders consisting of cannibal and typical morph phenotypes. *J. Iowa Acad. Sci.* 97 (4): 121-126.
- LAURIN, B. and GARCIA-JORAL, F. (1990). Miniaturization and heterochrony in *Homoeorhynchia meridionalis* and *H. cynocephala* (Brachiopoda, Rhynchonellidae) from the Jurassic of the Iberian Range, Spain. *Paleobiology* 16: 62-76.
- LONG, J.A. (1990). Heterochrony and the origin of tetrapods. *Lethaia* 23: 157-166.
- LOOMIS, W.F. and STERNBERG, P.W. (1995). Genetic networks. *Science* 269: 649-656.
- LÖVTRUP, S. (1978). On Von Baerian and Haeckelian recapitulation. *Syst. Zool.* 27: 348-352.
- MABEE, P.M. (1989). An empirical rejection of the ontogenetic polarity criterion. *Cladistics* 5: 409-416.
- MABEE, P.M. (1993). Phylogenetic interpretation of ontogenetic change: sorting out the actual and artefactual in an empirical case study of centrarchid fishes. *Zool. J. Linn. Soc.* 107: 175-291.
- MCKINNEY, M.L. and MCNAMARA, K.J. (1991). *Heterochrony. The Evolution of Ontogeny*. Plenum Press, New York.
- MCNAMARA, K.J. (1988). Patterns of heterochrony in the fossil record. *Tree* 3: 176-180.
- MORGAN, T.H. (1903). *Evolution and Adaptation*. McMillan and Co., London, New York.
- MULLER, G.B. and ALBERCH, P. (1990). Ontogeny of the limb skeleton in *Alligator mississippiensis*: developmental invariance and change in the evolution of archosaur limbs. *J. Morphol.* 203: 151-164.
- NELSON, G. (1973). Negative gains and positive losses: a reply to J.G. Lundberg. *Syst. Zool.* 22: 230.
- NELSON, G. (1978). Ontogeny, phylogeny, paleontology, and the biogenetic law. *Syst. Zool.* 27: 324-345.
- NIKLAS, K.J. (1994). *Plant allometry. The Scaling of Form and Process*. Chicago University Press, Chicago.
- NORTHCUTT, G. (1990). Ontogeny and phylogeny: a re-evaluation of conceptual relationships and some applications. *Brain Behav. Evol.* 36: 116-140.
- PATTERSON, C. (1983). How does phylogeny differ from ontogeny? In *Development and Evolution* (Eds. B. Goodwin, N. Holder and C. Wylie). Cambridge University Press, Cambridge, pp. 1-31.
- REISS, J.O. (1989). The meaning of developmental time: Attempt at a metric for comparative embryology. *Am. Nat.* 134: 170-189.
- REISS, M.J. (1989). *The Allometry of Growth and Reproduction*. Cambridge University Press, Cambridge.
- RICHARDS, R.J. (1992). *The Meaning of Evolution*. Chicago University Press, Chicago.
- RIEDL, R. (1978). *Order in Living Organisms: A System Analysis of Evolution*. Wiley Press, New York.
- RIEPEL, O. (1988). *Fundamentals of Comparative Biology*. Birkhäuser, Basel.
- ROTH, V.L. (1993). Dwarfism and variability in the Santa Rosa Island Mammoth (*Mammuthus exilis*): an interspecific comparison of limb-bone sizes and shapes in elephants. In *Third California Islands Symposium* (Ed. F.G. Hochberg). Santa Barbara Museum of Natural History, Santa Barbara (CA), pp. 433-442.
- SHUBIN, N.H. and ALBERCH, P. (1986). A morphogenetic approach to the origin and basic organization of the tetrapod limb. *Evol. Biol.* 20: 319-387.
- SKULASON, S. and SMITH, T.B. (1995). Resource polymorphisms in vertebrates. *Tree* 10: 366-370.
- SLACK, J. (1985). Homoeotic transformations in man: implications for the mechanism of embryonic development and for the organization of epithelia. *J. Theor. Biol.* 114: 463-490.
- SLACK, J.M.W., HOLLAND, P.W.H. and GRAHAM, C.F. (1993). The zootype and the phylotypic stage. *Nature* 361: 490-492.
- SLUYS, R. (1989). Rampant parallelism: an appraisal of the use of nonuniversal derived character states in phylogenetic reconstruction. *Syst. Zool.* 38: 350-370.
- SORDINO, P., VAN DER HOEVEN, F. and DUBOULE, D. (1995). Hox gene expression in teleost fins and the origin of vertebrate digits. *Nature* 375: 678-681.
- WAGNER, A. (1994). Evolution of gene networks by gene duplications: a mathematical model and its implications on genome organization. *Proc. Natl. Acad. Sci. USA* 91: 4387-4391.
- WAKE, D.B. (1966). Comparative osteology and evolution of the lungless salamanders, family *Plethodontidae*. *Memoirs of the Southern California Acad. Sci.* 4: 111.
- WAKE, D.B. (1989). Phylogenetic implications of ontogenetic data. *Géobios Mem. Spec.* 12: 369-378.
- WAKE, D.B. and LARSON, A. (1987). Multidimensional analysis of an evolving lineage. *Science* 238: 42-48.
- WAKE, D.B. and LYNCH, J.F. (1976). The distribution, ecology, and evolutionary history of plethodontid salamanders in tropical America. *Bull. Nat. Hist. Mus. Los Angeles Co.* 25: 1-65.
- WAKE, D.B., MABEE, P., HANKEN, J. and WAGNER, G. (1991). Development and evolution: the emergence of a new field. In *The Unity of Evolutionary Biology*, Vol. 1 (Ed. E.C. Dudley). Dioscorides Press, Portland (OR), pp. 582-588.
- WHEELER, Q. (1990). Ontogeny and character phylogeny. *Cladistics* 6: 225-268.
- WHITEMAN, H.H. (1994). Evolution of facultative paedomorphosis in salamanders. *Q. Rev. Biol.* 69: 205-221.
- WILBUR, H.M. and COLLINS, J.P. (1973). Ecological aspects of amphibian metamorphosis. *Science* 182: 1305-1314.
- WRAY, G.A. and BELY, A.E. (1994). The evolution of echinoderm development is driven by several distinct factors. *Development (Suppl.)*: 97-106.
- YOKOUCHI, Y., SASAKI, H. and KUROIWA, A. (1991). Homeobox gene expression correlated with the bifurcation process of limb cartilage development. *Nature* 353: 443-445.