

Developmental Evolution as a Mechanistic Science: The Inference from Developmental Mechanisms to Evolutionary Processes¹

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SYNOPSIS. Developmental Evolution (DE) contributes to various research programs in biology, such as the assessment of homology and the determination of the genetic architecture underlying species differences. The most distinctive contribution offered by DE to evolutionary biology, however, is the elucidation of the role of developmental mechanisms in the origin of evolutionary innovations. To date, explanations of evolutionary innovations have remained beyond the reach of classical evolutionary genetics, because such explanations require detailed information on the function of genes and the emergent developmental dynamics of their interactions with other genetic factors. We argue that this area has the potential to become the core of DE's disciplinary identity. The main challenge in developing a research program for DE along these lines, however, is to provide a methodological framework that accounts for the fact that developmental mechanisms continue to evolve after a character has originated. Developmental mechanisms elucidated in a derived species may therefore not provide insights into the evolutionary origin of the character in question. To meet this challenge, we propose a set of questions that may guide us in our search for valid inferences on the role of developmental mechanisms in the explanation of evolutionary innovations.

INTRODUCTION

In an influential paper from 1968, Gunter Stent characterized three clearly recognizable phases in the history of molecular biology: the so-called romantic, dogmatic and academic phases (Stent, 1968). We propose that developmental evolution (DE) is going through the same three phases, except that we prefer to call the second phase “enthusiastic” rather than dogmatic³. Today workers are still enthralled by their enthusiasm for a new enterprise; hence, DE has not yet fully entered the most mature academic phase of its history. The romantic phase of DE culminated in the Field Museum conference on Macroevolution in 1980 and the Dahlem Conference on Development and Evolution in 1981 (Bonner, 1982). At that

time DE was focused on staking out its conceptual territory and defending it against established disciplines. Formative for the romantic phase were Stephen Gould's *Ontogeny and Phylogeny* (Gould, 1977), Rupert Riedl's *Order in Living Organisms* (Riedl, 1978) (first German edition) and Rudolf Raff and Thomas Kaufman's *Embryos, Genes and Evolution* (Raff and Kaufman, 1983). There was a sense of great promise shared among the workers, but the actual accumulation of knowledge was relatively slow. This picture changed radically with the breakthroughs in *Drosophila* developmental genetics and the discovery of homologous developmental genes in other animals. We think that this led to the enthusiastic phase of DE. More and more startling discoveries were and continue to be made. While many discoveries were overinterpreted (we decline to cite examples) in the first wave of enthusiasm, general progress in DE, however, became irreversible. Examples for this progress are the discovery of the genetic basis for the evolution of butterfly wing patterns (Brakefield *et al.*,

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³ For a discussion of the prehistory of DE please refer to the papers by Hall (2000), Gilbert (2000), Laubichler (2000) and Dietrich (2000).

TABLE 1. *Research agenda of developmental evolution.*

Application of DE	Remarks
Evolution of Development	DE describes new types of characters (expression patterns and gene networks, etc.) which invites the comparative study of these characters.
Homology Assessment	Gene expression patterns can help resolve difficult problems in homology assessment.
Genotype-Phenotype Map	DE contributes to the population genetic theory of adaptation by revealing the developmental architecture of species differences.
Patterns of Phenotypic Evolution	Developmental mechanisms can either constrain or facilitate evolutionary change, i.e. developmental constraints and evolvability.
Evolutionary Innovations	DE describes the developmental mechanisms responsible for the origin of new body parts and characters.

1996; Keys *et al.*, 1999; Nijhout, 1991) and for the evolution of arthropod body regions (Averof and Akam, 1993, 1995; Carroll, 1995; Damen and Tautz, 1999). These and other cases are a clear indication that DE is maturing into a productive research enterprise. But there are still some features missing in DE that one would like to see in a mature science. One of them is an agreement about the goals of DE. We will argue in the next section that DE contributes to a set of heterogeneous research programs, but that it also makes a unique contribution, which has not been anticipated by any established research program. Furthermore we will argue that it is important for a mature DE to establish rigorous standards of evidence. It is, for example, not clear what kinds of data justify the conclusion that a particular gene is instrumental in the origin of a character, such as insect wings. Recent advances in developmental biology clearly validate the expectation that we will be able to answer such questions in the near future. But it is also clear that there are many methodological pitfalls along the way. Towards the end of this paper we will propose a set of methodological criteria (a sort of checklist or a list of "commandments," depending on one's temperament) for establishing a causal link between molecular developmental evolution and phenotypic evolution.

WHAT IS THE AGENDA OF DEVELOPMENTAL EVOLUTION?

Our survey of the work published on developmental evolution indicates that there

are at least five partially overlapping research goals (Table 1).

1) The most obvious can be characterized as research in the *Evolution of Development*. Increasingly detailed knowledge about the mechanisms of development as a newly recognized level of biological organization invites the comparative study of these mechanisms. Perhaps the most important conclusion reached by this type of inquiry is the insight that developmental cascades constitute a level of organization and have their own evolutionary history, which is to some degree independent of the evolutionary history of the characters for which they are responsible (Abouheif, 1999; Gerhart and Kirschner, 1997; Shubin *et al.*, 1997). Increased knowledge of the phylogenetic history of developmental mechanisms also raises the question of what evolutionary forces shape development. What causes the evolution of direct and indirect modes of development? Why are imaginal discs present in some insects and not in others? These are just a few examples of important biological questions that fit into the growing set of evolutionary research programs such as molecular, phenotypic, and behavioral evolution that focus on a specific character type.

2) Another exciting implication of DE is that in some cases gene expression patterns may help to resolve longstanding questions in *assessing homologies*. Initially there was a tendency to assume that expression data can overrule any other kind of data relevant to homology assessment, but recently a

more balanced view has emerged (Bolker and Raff, 1996; Dickinson, 1995; Galis, 1999; Müller and Wagner, 1996; Wray, 1999). Nevertheless, detailed developmental studies are an invaluable source of additional information when assessing the homology of problematic characters. One can therefore expect that DE will continue to contribute to the agenda of comparative anatomy, originally set out by Owen, Gegenbauer and others for the identification of corresponding body parts in divergent animal body plans.

3) DE also promises to fill a gap in the current account of the adaptationist program in illuminating *the structure of the genotype-phenotype map* (Mayr, 1983). While it is clear that adaptations result from natural selection on spontaneous heritable variation, the genetic and developmental architecture of this adaptive variation is largely unknown. To understand the dynamics of adaptation, it is necessary to know how complex an adaptive change is at the genetic level. Recent progress in evolutionary genetics demonstrates that the genetic basis of species differences is now within the reach of experimental research. For example, it has been shown that the genetic basis of bristle pattern differences on the third leg of *Drosophila* can be traced to variation in the *cis*-regulatory sequences of *Ubx* (Stern, 1998). Similarly, variation in the regions of the axial skeleton of amniotes seems to be caused by changes in Hox gene expression domains (Belting *et al.*, 1998; Burke *et al.*, 1995).

4) From the beginning a goal of DE research has been to determine if *developmental constraints* influence patterns of evolutionary diversification (Alberch, 1983; Gould, 1977; Riedl, 1978; Wake and Larson, 1987). The *locus classicus* of this research are the papers by Alberch and Gale (Alberch and Gale, 1983, 1985) that demonstrated a causal link between patterns of digit reduction and the mode of digit development.

More recently, developmental and cell biological mechanisms have also been invoked to explain the *evolvability* of complex biological traits (Gerhart and Kirschner, 1997). Here, developmental regula-

tion may prevent unconditionally deleterious effects of mutations (Gerhart and Kirschner, 1997), which, in turn, can facilitate the evolvability of complex characters (Wagner and Altenberg, 1996). An example is the compensatory capacity of signal transduction networks characterized by weak interactions. Genes that play a central role in signal transduction, such as *ras* and *fos* have been identified, but surprisingly, in spite of the central function of these genes in the transduction cascade, deletions of these genes have only mild effects. This is because other genes can compensate for their function. This type of compensatory interaction thus provides physiological robustness and the potential for variation, *i.e.*, evolvability (Gerhart and Kirschner, 1997).

5) Finally, DE may lead to a mechanistic explanation of the origin of *evolutionary innovations* and the *origin of body plans* (Müller and Wagner, 1991). Evolutionary innovations and the evolution of body plans are hard to understand in population genetic terms since they involve radical changes in the genetic/developmental architecture of the phenotype. A good example of the power of DE in this context is the origin of butterfly eye spots (Keys *et al.*, 1999). Since this innovation has produced a qualitatively new phenotypic state, a quantitative genetic account is inherently uninformative. Indeed, knowledge on the functional interactions among the participating genes and gene products is necessary for understanding the processes that led to these new characters.

Our list is certainly not comprehensive; for example it does not include the influence of developmental evolution on the study of molecular evolution (Purugganan, 1998; Sidow, 1992; Zardoya *et al.*, 1996). We believe, however, that our list does capture the main contributions of research in DE. This list can be divided into two categories. On the one hand are those cases in which DE contributes new facts to existing research programs. These are the contributions of DE to homology assessment, the evolution of development (which can be seen as another set of biological characters that evolve) or to the explanation of the developmental architecture of adaptations. In

each case the agenda is set by an established research program and DE makes ancillary, though important, contributions. The only two dimensions of DE, which are outside the scope of existing research programs, are the idea of developmental constraints and the explanation of innovations. The concept of developmental constraints expanded the scope of the neo-Darwinian theory of evolution. It was also the first context in which developmental mechanisms acquired an *explanatory role* in evolutionary biology (Sterelny, 2000). Similarly, evolutionary innovations are outside the scope of any current research program. Through its contribution to the solution of that question, DE genuinely expands the explanatory range of evolutionary theory. We think that this is the one area where DE will have its most lasting impact on evolutionary theory and biology in general. This assessment does not mean that we downplay the contributions of DE to other research programs, but it means that we see in the problem of innovation and the evolution of body plans a unique opportunity for DE to develop its own independent identity as a research program. In the rest of the paper we will focus on the methodological challenges of explaining evolutionary innovations through developmental mechanisms. Many of the things we say will in principle apply to any attempt to link genetic events to phenotypic evolution. However, the difficulties are more pronounced in the case of innovations because many of these events happened a long time ago.

PROBLEMS IN ESTABLISHING A CAUSAL LINK BETWEEN GENETIC CHANGE AND A PHENOTYPIC INNOVATION

The question of identifying what exactly is an innovation, is beyond the scope of this paper (Müller and Wagner, 1991). Examples of innovations are the origin of new body parts, or any significant change in the identity of a character, such as the transformation of nodular bones into long-bones (Blanco *et al.*, 1998). A precise explication of innovation thus requires a theory of biological characters, which is still an unresolved issue (Wagner, 2000). Furthermore,

any change in the level of selection also qualifies as an innovation, such as the origin of multi-cellular organisms or the origin of eukaryotic cells (Buss, 1987; Maynard-Smith and Szathmáry, 1995). Here we will primarily discuss the origin of new body parts and changes in the identity of body parts.

Molecular research into the origin of a specific character is usually triggered by the discovery of certain genes that are essential for the development of that character in some model species. The discovery of sufficient and/or necessary genetic factors for a phenotypic character naturally raises the question of whether the same genes were also involved in the evolutionary origin of that character. Usually this leads to the hypothesis that the developmental causes of a character may also be part of the evolutionary cause for the origin of the character. One could call this the

Hypothesis of congruence between developmental and evolutionary causes: a gene that is sufficient for the development of a derived character state may also have been the cause of the evolutionary origin of the character state.

This is the null-hypothesis for DE research. There are at least four specific methodical and biological problems that make the assessment of this hypothesis non-trivial. Below we will discuss these problems and will propose recommendations of how to deal with them.

Of course the hypothesis of congruence between developmental and evolutionary causes requires a few qualifications. The causes of evolutionary processes, such as selection and drift are, in part, the population genetic processes that lead to the fixation of genetic variation. In that sense there cannot be any congruence between developmental mechanisms and evolutionary processes, since developmental mechanisms act at the level of individual organisms while evolutionary mechanisms act at the level of populations. However, in the case of evolutionary innovations, the specific developmental functions of the genes involved are an important part of the explanatory narrative. To state that a genetic

mutation led to a favored character, which, in turn, was selected is utterly uninformative in explaining innovation. Such an account is only sufficient for quantitative changes, identified and characterized by means of quantitative genetic techniques, where it can be shown that contributions of individual genes are not that important to the phenotypic result. In contrast, the emergence of morphological innovations depends to a large extent on the epigenetic dynamics of the involved developmental pathways (Newman and Müller, 2000). Therefore in explaining the origin of a new character, the developmental function of genes is of greater importance than in quantitative genetics. To speak of a congruence between developmental mechanism and evolutionary process means that *the developmental function of a gene in the derived species was also the developmental function for which the gene was selected when the new character arose.*

Character definition

To be successful, a research program of elucidating the genetic basis of phenotypic innovations demands a rigorous definition of the derived phenotypic character (-state). In other words, it is difficult to reach a consensus view of the genetic basis of a morphological innovation, if the precise definition of the new derived character remains unclear. An instructive example of this problem was reported in an important paper on the evolution of insect wings (Warren *et al.*, 1994). It is well known that a loss of function mutation of the *Ubx* gene in *Drosophila* transforms a haltere into a wing, which suggests that *Ubx* controls "wing number" in *Drosophila*. To further test this possibility, Carroll and collaborators (Warren *et al.*, 1994) hypothesized that *Ubx* may be responsible for the difference between four- and two-winged insects. In testing this, however, they found that in four-winged insects the expression of *Ubx* is identical to that of *Drosophila*, *i.e.*, to a two-winged insect. This finding was surprising in light of the supposed role of *Ubx* in establishing wing number. It turns out, as shown by Carroll and collaborators, that the differences between four and two-winged

insects are caused by genetic factors downstream of *Ubx*. Is there a systematic reason for their findings? A possible answer to this question can be found when one reconsiders the character definition: instead of assuming that wing number is a unitary character, Carroll and collaborators suggested that the haltere is a character state of the hind-wing (see Warren *et al.*, 1994). In other words, fore- and hind-wings are two characters whereby the haltere is a character state of the hind-wing. Diptera, including *Drosophila*, have four "wings," but the function of its hind-wing is not to create uplift. One can see the haltere as a character state of the hind wing, rather than something completely different. *Ubx* determines hind wing identity, regardless of whether the adult character is a functional wing or a sense organ (a haltere). Therefore the differences between the Diptera and the four-winged insect are caused by genes downstream of *Ubx*.

Another example of how the definition of a character can influence a research program involves investigations into the genetic basis of the fin-limb transition. There is agreement that the main difference between the fins of the sarcopterygians (lobe-finned fishes) and the limbs in the earliest tetrapods is the distal most part, the autopodium (*i.e.*, hand and foot) (Ahlberg and Milner, 1994; Coates, 1991; Shubin, 1995; Sordino and Duboule, 1996; Laurin *et al.*, 2000). But what precisely is an autopodium? There are many morphological and developmental differences between the autopodium and the proximal parts of the limb. Some authors define the autopodium through the presence of digits (Coates, 1994; Daeschler and Shubin, 1998). So defined, the fin-limb transition has to be explained in terms of the evolution of the digital arch and the derivation of digits from radials in fins (Shubin and Alberch, 1986). Alternatively the autopodium can be defined as consisting of a segment of small nodular elements (carpals and tarsals), the so called mesopodium and distal to it a set of digits, *i.e.*, metapodials and phalanges, the so called acropodium (Fig. 1). So defined, the question is how was the developmental boundary between the lower limb

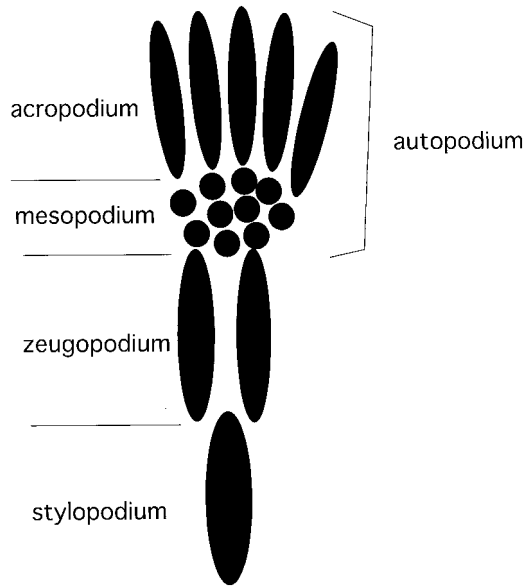


FIG. 1. The main parts of the tetrapod limb. From proximal to distal the three main segments are the stylopodium, the zeugopodium, and the autopodium, which correspond to the upper limb, lower limb, and hand/foot, respectively. The autopodium consists of two parts, the mesopodium, which corresponds to the carpus and the tarsus and primarily consists of small nodular bones in most tetrapods, and the acropodium, which consists of the metacarpals and metatarsals as well as the phalanges of digits. The existence of a mesopodial segment that separates the digits from the zeugopodium is the only consistent difference between any sarcopterygian fin and any tetrapod limb.

and the mesopodium established in evolution? In this paper we do not discuss evidence in favor of the one or the other hypotheses (for a more extensive discussion see Wagner and Chiu, 2000), but use it as an illustration that further demonstrates how the definition of a character strongly influences molecular studies aimed at elucidating its origin.

“Recency bias” in cladistic character reconstruction

Another methodological challenge to DE comes from the fact that DE data have to be obtained from extant species. Under favorable circumstances and based on a well-established phylogeny, the ancestral developmental and morphological phenotype can be reconstructed (Maddison and Maddison, 1992). The problem for understanding evo-

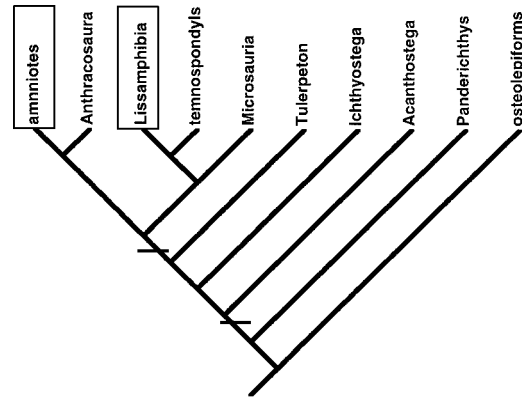


FIG. 2. Phylogeny of tetrapods according to the phylogenetic analysis of Ahlberg and Milner (1994). The two closest relatives of tetrapods are the Panderichthyd and the osteolepiforms. The oldest known tetrapods are *Acanthostega*, *Ichthyostega* and *Tulerpeton*, which are all Devonian forms. The two major groups of recent tetrapods are the amniotes and the lissamphibians, indicated by a shaded box. Note that the most recent common ancestor of extant tetrapods was more recent than any of the Devonian forms, originating most likely in the late Devonian or the early Carboniferous. This implies that the most recent common ancestor of extant tetrapods is much more recent than the epoch in which the fin-limb transition occurred.

lutionary innovation is that the reconstructed character states may not reflect the events at the origin of the character. There is a “recency bias” built into cladistic character reconstruction methods. By the very nature of the cladistic method applied to recent species, the deepest node that in principle can be reconstructed is the most recent common ancestor of the extant clade. For instance, following the phylogenetic hypotheses of Ahlberg and Milner (1994) and Laurin (1998) the most recent common ancestor of extant tetrapods is most likely from the Carboniferous age (*ca.* 340 MA) (Fig. 2) (but see [Coates, 1994] for a dissenting view). The fin-limb transition, however, happened about 30 million years earlier in the Devonian. The limb morphology of the Devonian forms is also quite different from that of recent forms and all the carboniferous forms (Coates and Clack, 1990; Coates, 1991, 1996). Hence the most recent common ancestor of extant tetrapods is not the direct product of the fin-limb transition, but rather the product of the trans-

formation of the archaic limbs into modern ones.

Temporal dissociation between origin and canalization of a character

Closely related to the methodological problem outlined in the last paragraph is a biological problem. The “recency bias” of cladistic character reconstruction would not be a problem for the reconstruction of the developmental and genetic bases of innovations, if the developmental architecture of the character remains unchanged after its origin. There can, however, be a temporal dissociation between the origin and the canalization of a character⁴. Again the difference between Devonian and recent tetrapod limbs is an excellent example.

As mentioned above, the typical limb of a recent tetrapod is pentadactyl. This limb type is fundamentally different from that of Devonian forms (Fig. 3). The Devonian tetrapods have up to eight digits and the digits can be grouped into at least two different morphological classes that differ in size and cross section (Coates, 1991; Coates and Clack, 1990). Furthermore the morphology of the mesopodium is not comparable to that of modern forms. These morphological facts show that the pentadactyl limb arose after the fin-limb transition and is the product of the canalization of the phenotype of the archaic limbs. Many developmental features that are universal (or nearly so) among recent tetrapods were not necessarily acquired during the fin-limb transition but rather by the Devonian and early Carboniferous tetrapods which evolved the pentadactyl limb. For instance almost all tetrapods examined have a digital arch from which most of the digits develop (Shubin and Alberch 1986)⁵. There are, however, other modes of digit development described for extant species (Fig. 4). Hence a possible explanation of the difference between archaic and modern tetrapod limbs is that ar-

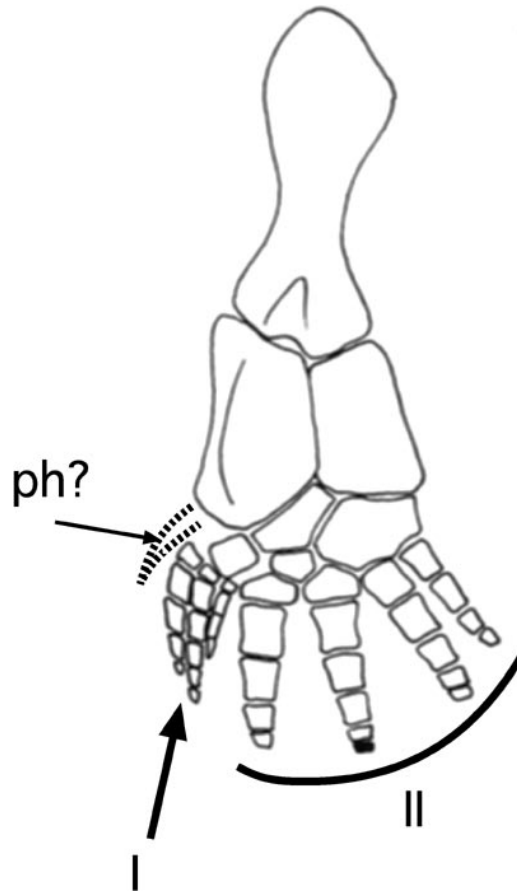


FIG. 3. Hindlimb of *Ichtyosteaga*, an archaic tetrapod (after Coates, 1991). Note that the limb has seven digits and the digits form two clearly recognizable groups (I and II) with their own size trends and shapes (not shown). The inserted element labeled “ph?” is shown in the drawing of the fossil as a poorly ossified structure but not in the reconstruction on which this drawing is based on (see Coates, 1991). Based on the location and the structure this element could be a prehallux (see Fig 4 for the development of a prehallux). The mesopodium is also different from that of extant tetrapods. (anterior is to the left and posterior to the right)

chaic limbs may have used more than one mode of digit development, leading to different kinds of digits. In contrast, digit development in modern tetrapods is almost completely monopolized by the digital arch. This switch from two or three parallel modes of digit development to one (the digital arch) may explain the canalization of the modern limb. We suggest that the mode of digit development in modern tetrapods

⁴ For a definition and discussion of the canalization concept see Gibson and Wagner (2000).

⁵ In addition the most posterior digit, DV, develops from an independent condensation. The only exception to the Shubin-Alberch model of digital arch development are the urodeles.

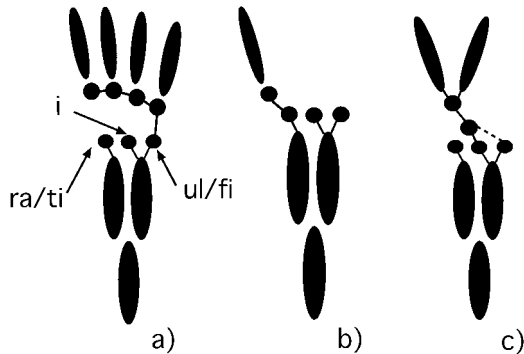


FIG. 4. Modes of digit development. The autopodium has three growth zones, emanating from the three proximal mesopodials: ra/ti = radiale or tibiale, i = intermedium, and ul/fi = ulnare or fibulare in the fore- and hindlimb respectively. a) The digital arch originates from the radiale/fibulare and is the source of digit development in most tetrapods, *i.e.*, the amniotes and the anurans. b) The radiale/tibiale can also give rise to a digit called the pre-pollex or pre-hallux in the hand or foot respectively (also called pre-digits). In extant forms a prehallux is a frequent character of anurans and pre-digits are a not uncommon natural variation in urodeles (Rienesl and Wagner, unpublished). In reptiles pre-digits are occasionally observed (Jacques Gauthier, personal communication). c) In urodeles the digits I and II develop from a mesenchymal condensation that is always derived from the intermedium (*Triturus*, [Blanco and Alberch, 1992]; *Salamandrella*, [Schmalhausen, 1910; Vorobyeva and Hinchliffe, 1996]; *Ranodon*, *Ambystoma*, [Hinchliffe and Vorobyeva, 1999; Vorobyeva *et al.*, 1997]), or from both, the intermedium and the ulnare/fibulare. We suggest that the archaic limbs of Devonian tetrapods (see Fig. 3) have digits derived from all three pathways, which would explain the morphological heterogeneity and their poly-dactyl condition. The canalization of the pentadactyl limb may have been achieved by suppression of the two digit development pathways emanating from the intermedium and the radiale/tibiale. As a result the modern limbs develop their digits primarily from the digital arch. (Anterior is to the left and posterior to the right.)

(the digital arch) may be the product of the secondary stabilization of the limb phenotype and may not have been acquired during the fin-limb transition. Indeed, only those developmental characters that explain the morphological features common to all tetrapods, including the archaic forms, are candidates for accounting for the fin limb transition. We argue elsewhere that the feature common to all tetrapods, archaic and modern, is the existence of a mesopodial-acropodial configuration (Fig. 1) and that

the genetic mechanism for the development of this feature is more likely involved in the fin-limb transition than mechanisms involved in digit development (Wagner and Chiu, 2000). This example also shows that paleontological data are an essential component of DE research.

Recruitment of genes into existing developmental pathways

Another fact that makes it problematic to draw inferences of evolutionary processes from developmental mechanisms is the finding that the developmental function of a gene can fundamentally change without affecting the morphology of phenotypic character(s). One of the most striking examples is the fact that *even-skipped*, a pair rule gene essential for the development of postcephalic segments in *Drosophila*, and most likely other higher insects, does not have this function in segment development in grasshoppers (Patel *et al.*, 1992). This shows that development and its genetic regulation is a feature with its own evolutionary history that is partially autonomous of the evolutionary history of the characters they “code for.” The genetic machinery active in the development of a derived species may not be the same as the one that was active in the ancestor that originally acquired the character. Consequently the genes that have been recruited into the development of a character after its origin have nothing to do with the genetic mechanisms responsible for the origin of a character.

This and the previous argument about the possible secondary canalization of a character reflect the fact that the development of a character continues to evolve after the evolutionary origin of that character. The difference between the two cases is that in the first, canalization, the character itself shows obvious signs of morphological evolution from the archaic to modern types. In the second example the character, the insect segment, does not show an evolutionary trend in its morphological character identity. The main difference here is the mode of development (long germ versus short germ development) without any effect on the phenotypic character. This is the only rea-

son for distinguishing between canalization and recruitment. For the elucidation of the developmental basis of innovations these two cases have the same effect, namely that one has to examine the evolution of the developmental mechanisms in addition to the evolution of the character itself.

These examples certainly do not exhaust all the possible methodological pitfalls that one is likely to encounter in research into the origin of characters. They may, however, suffice to show that the inference from developmental mechanisms to evolutionary processes needs methodological constraints in addition to those imposed by developmental genetics. In the next section we propose a small set of “questions” that may guide the formulation of a valid argument that links a genetic to a phenotypic event in evolution.

A CHAIN OF QUESTIONS LINKING GENETIC TO PHENOTYPIC EVOLUTION

The natural starting point of any DE project on the origin of a novel character is the discovery of a genetic mechanism for the development of a derived character, say the limb or the insect wing. Therefore the answer to the first question we propose below will be the *starting point* rather than the result of a DE project. Nevertheless, the results from developmental genetics have to be scrutinized as to whether they, in fact, imply a meaningful hypothesis about the origin of a novel character.

A) *What is the developmental mechanism that accounts for the derived character (-state)?*

This question actually stands for a complex of related questions that have to do with defining the phenotypic innovation and with establishing the developmental mechanism that specifically accounts for the innovation. What exactly is the phenotypic difference between the derived and the ancestral character state? What is the taxonomic group for which this character state is synapomorphic? What are the outgroup taxa and do they represent the ancestral character state? Does the identified developmental mechanism account specifically for the derived character state or is it responsible for more fundamental and more

ancient (plesiomorphic) character states within the lineage?

An example to illustrate the last question is the function of *Shh* in limb development. Clearly *Shh*, which is released in the ZPA (zone of polarizing activity), is necessary for proper digit development (Riddle *et al.*, 1993). Its role in digit development, however, is derivative of a more general and more ancient function. The ZPA determines the anterior–posterior polarity of paired appendages in vertebrates. Fins also have an anterior–posterior polarity and it seems that *Shh* already has this function in fins, since it is expressed in a similar location in zebrafish fin buds as it is in tetrapod limb buds (Krauss *et al.*, 1993). A gene with an essential function in the development of a character thus may have this function because of a more ancient role than the one related to the development of the specific character.

B) *Does the developmental mechanism for the derived character (-state) map to the same node on the phylogeny as the derived character (-state)?*

This question is the first test of the causal efficacy of the developmental mechanism in the evolutionary process. If the developmental changes are a cause for the morphological difference then they have to be coincidental. The evolutionary change in development can neither be older nor younger than the origin of the character state it is supposed to explain. In addition, a positive answer to this question eliminates one of the problems discussed above, namely that the development of a character can continue to evolve after the character originated. Only those developmental mechanisms that were active in the ancestor can account for the evolutionary origin of the character. Our ability to answer this question is limited by the “recency bias” of character reconstruction as discussed above. There are situations where the reconstructed nodes are not a close approximation of the event that led to the origin of a character. But there are other kinds of data that could be employed to resolve the issue.

For instance one would expect that a mechanism, call it D, that depends on another mechanism, say A, will have evolved

after the mechanism A. This is not an absolute rule, because upstream mechanisms can evolve after downstream mechanisms, but in general it may be used as an indication of the possible sequence of evolutionary events. For example, there are two groups of Hox genes involved in the development of the autopodium. These are the *AbdB* homologues on Hox gene clusters A and D. Mutational analyses have shown that the developmental function of the *HoxD* genes is downstream of the *HoxA* genes (Zákány *et al.*, 1997). It also happens to be the case that the *HoxA* genes are involved in setting up the boundary between the anlage of the hand and the lower arm, while the *HoxD* genes regulate the number and morphology of the digits. From these data Duboule and collaborators have concluded that the function of *HoxA* genes may be more ancient than that of the *HoxD* genes. Note that the lack of direct descendants from the Devonian tetrapods prevents us from reconstructing the events that occurred between the origin of limbs and the origin of the penta-dactyl limb.

C) What are the developmental changes that occurred at the origin of the derived character (-state)?

The answer to this question requires the reconstruction of the developmental mechanism in the ancestor of the derived clade. This will only be possible if appropriate out-group species are available for investigation. This information is also necessary to constrain the possible candidates for the allelic substitutions (or other genetic changes) that caused the morphological difference. An example of such an hypothesis is the suggestion that the origin of the autopodium (hand and foot) may have been caused by the evolution of a non-overlapping expression domain of the genes *Hoxa-11* and *Hoxa-13* (Fig. 5). In tetrapods the expression domains for these two genes are non-overlapping when the autopodium develops (Davis *et al.*, 1995; Haack and Gruss, 1993; Yokouchi *et al.*, 1991, 1993), while they are overlapping in zebrafish (Sordino *et al.*, 1995). This scenario predicts that the origin of the autopodium is caused by changes in the regulation of these two genes, either by upstream regulators,

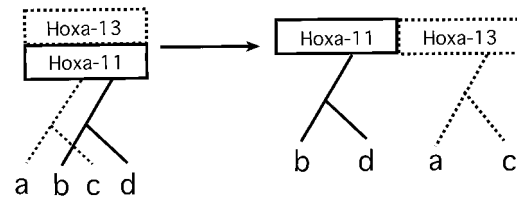


FIG. 5. A hypothesis for a developmental transition that may explain the origin of the autopodium. In almost all extant forms the genes *Hoxa-11* and *Hoxa-13* are expressed in locally exclusive domains, *Hoxa-11* in the prospective zeugopodium and *Hoxa-13* in the prospective autopodium, causing the differentiation of the auto- from the zeugopodium. We suggest that the ancestral state was one of overlapping expression domains, as seen in zebrafish (Sordino and Duboule, 1995) and that the fin limb transition was associated with the evolution of the non-overlapping expression domains. (proximal is to the left, distal to the right)

mutations in *cis*-regulatory sequences or in the activator and repressor domains of the protein. Preliminary results show that the evolution of the derived expression pattern is coincidental with the acquisition of new putative repressor domains in the N-terminal region of the *Hoxa-11* encoded protein (Chiu *et al.*, 2000). In general the study of the sequence evolution of developmental regulatory genes is a powerful tool for detecting candidate mutations that may be responsible for developmental and phenotypic transformations (Purugganan, 1998).

D) Are the genetic differences sufficient to cause the derived character (-state)?

This is experimentally the most difficult question to answer. It requires the identification of genetic differences that may account for the developmental differences and the ability to introduce genetic elements into in-group as well as out-group species. Advances in transgenic technology, however, make the thought of performing these experiments less crazy than they were a few years ago. For instance there is increasing evidence that specific changes in the *cis*-regulatory region of Hox genes may account for differences in the size of body regions of vertebrates (Belting *et al.*, 1998; Burke *et al.*, 1995). Ideally one would like to cause a transformation towards the derived state in an outgroup species by introducing a genetic element from a derived species and an atavistic character state by

introducing a genetic element from an out-group species into a derived species. We are not aware of a case where both experiments have been performed.

To summarize, if all the answers to these questions support a hypothesis about the developmental mechanism for the origin of a novel phenotypic character, then the conclusion is all but unavoidable that this mechanism in fact was instrumental in causing the origin of the derived character (-state).

CONCLUSIONS

Developmental Evolution makes important contributions to the agenda of established research programs, such as the assessment of homology and the evolution of adaptations. In addition, DE opens up new areas of research that have not been part of any of the established research programs, especially in elucidating the genetic factors that are responsible for the origin of evolutionary innovations, and, in particular, the origin of new characters. We think that it is this area in which DE will eventually make its most distinctive contribution to evolutionary biology and where it may find its own disciplinary identity. The main methodological challenge of this research program is to link developmental mechanisms to evolutionary processes. We have exemplified the problems with a number of examples and suggested a "check list" for constructing a valid chain of inferences between developmental mechanisms and evolutionary innovation. From this list we conclude that a successful DE project needs essential input from at least five biological disciplines: 1) developmental biology, which provides insights into the proximate mechanisms of character development; 2) evolutionary genetics, which provides insights into the forces acting on the developmental genes; 3) systematics, which provides us with the comparative methods for testing the evolutionary assertions; 4) comparative anatomy, which helps us to coherently define the characters and the character states we seek to understand; and 5) paleontology, which provides us with information about the sequence of phenotypic transformations that led to the character

states found in extant forms. All of these contributions are essential for DE.

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