Reconstructing the Tree of Life
Taxonomy and Systematics of Species Rich Taxa
Edited by Trevor R. Hodkinson and John A.N. Parnel (2006)
CRC Press 351 pp, ISBN 0-8493-9579-8

12 Reconstructing Animal Phylogeny in the Light of Evolutionary Developmental Biology

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ABSTRACT

The relevance of evolutionary developmental biology (evo-devo) in our effort to reconstruct the tree of life has until recently been very poorly explored. However, the contribution of an evo-devo approach to the main steps of phylogenetic analysis, such as evaluation of homology, selection of characters and assessment of character polarity can be critically important, especially in species rich groups.

As independence of traits is a prerequisite for the use of coded information in the reconstruction of phylogeny, the identification of developmentally independent units is one of the areas where evo-devo may offer an especially important contribution. The way in which characters originate and change in evolution has fundamental consequences on the patterns ofevolutionary change we can reconstruct from character distribution. The remoulding of pre-existing features, genetic networks or developmental trajectories, can operate at any level of biological organisation. Comparative developmental biology supports a view that homology cannot be a relationship of the all-or-nothing kind.

12.1 DEVELOPMENT, PHYLOGENY AND THE HISTORICAL ROOTS OF EVO-DEVO

Early in the nineteenth century, the importance of developmental information in assessing relationships among different kinds of animals was already clear to some of the brightest zoologists of the time. For example, Geoffroy Saint-Hilaire[1] used congruence in ossification centres as a criterion to assess what we now call the homology between the bones of different vertebrate species, while von Baer's law[2] encapsulated the principle according to which traits diagnostic for classes appear first during development, followed by those diagnostic for orders, families, genera and species. Later criticisms to the suggestions of these pioneers, such as de Beer's magisterial analysis of heterochrony[3], did not subtract from the increasing faith in the relevance of developmental evidence in the assessment of phylogenetic relationships, but the field became increasingly complex and in need of in-depth analysis and reformulation.

Assessing homologies, however, is not the only step in phylogenetic reconstruction where a sensible evaluation of developmental evidence may be of value. As we will briefly illustrate in this article, information about development can also be critically important in evaluating the phylogenetic signal we may expect to retrieve from a character, in assessing the degree of independence between traits, and in formulating hypotheses of character polarity. Use of developmental information, however, is still uncommon in systematic literature, despite recent advances in evo-devo, a newly emerging field in which the previously independent research traditions in evolutionary biology and developmental biology are merging around a broad and still imperfectly circumscribed array of problems[4,5].

During the last two decades or so of evo-devo, the most productive field of inquiry has been the search for genes involved in body patterning and the comparative analysis of their spatial and temporal expression patterns in selected developmental stages of different organisms; for example, during segmentation or the specification of anteroposterior (AP) body patterning in metazoan embryos, or in the specification of the identity of the individual floral whorls during early lowering stages of angiosperms. The comparative framework, progressively broadening around the handful of model species to which experimental research in this field was initially confined, has revealed many important and often puzzling patterns to be interpreted in terms of phylogenetic relationships. In several instances, the result of comparative developmental genetics suggested abandoning well entrenched and long cherished phylogenetic hypotheses, in favour of new ones, or of old alternatives that had been put aside.

12.2 MORPHOLOGY TO MOLECULES TO MORPHOLOGY

Comparative developmental genetics offers an opportunity for reducing (if not bridging) the gap between molecular and morphological evidence. Molecular phylogenetics relies mainly on comparisons of nucleotide sequences which are not necessarily identical with protein coding segments, but also include blocks such as ITSs (internal transcribed spacers) and shorter sequences such as SINEs (short interspersed nuclear elements). Correspondingly, nucleotide sequences relevant in development extend well beyond the protein coding units, by including cis- and/or trans- regulatory regions. Thus, investigating the complex network of control relationships among genes involved in establishing the main features of an animal's organisation involves detailed knowledge of the whole genetic machinery, at the level of nucleotide sequences, as well as from the point of view

of control cascades and spatiotemporal patterns of expression. In this way, as we will show through some examples, we are helped in developing comparisons among very divergent and thus hardly comparable body plans, without the need to totally ignore morphology and rely on molecular evidence only. This is critically important at high taxonomic levels, where the heuristic power of comparative morphology has been exploited since Cuvier's pioneering articulation of the animal kingdom into four 'embranchements' (the vertebrates, the articulates, the molluscs and the radiates)[6], in setting up a classification where species are ultimately grouped into phyla, that is, higher rank taxa separated by so deep differences in overall organization, as to cause major problems of comparison at morphological level[7]. On the other hand, molecular phylogenetics has provided tools for assessing phylogenetic relationships among remotely related taxa, thus overcoming the limitation of morphology, and often suggesting unconventional affinities, such as between arthropods and nematodes (in a group named Ecdysozoa[8]) or between hermit crabs and king crabs[9], affinities that are less than obvious at the level of morphology. However, this distance between morphological and molecular evidence may be shortened if we get comparative information about the identity, sequence and expression of genes which are more or less specifically involved in the generation of animal (or plant) form.

Indeed, this approach has been the source of unexpected discoveries, such as the fundamental identity of the genetic control of dorsoventral (DV) patterning in animals that are as different from each other as arthropods and vertebrates. Zoologists are familiar with a fundamental difference between them, as representatives of gastroneuralians (animals with ventral nervous cord) and notoneuralians (animals with dorsal nervous cord) respectively. In his idiosyncratic belief in a unity of plan of all animals, Geoffroy Saint-Hilaire proposed that arthropods might be equated to vertebrates, provided that we regard the dorsal aspect of the former as equivalent to the ventral aspect of the latter, and vice versa. At the time no factual argument could be advanced to support such an unconventional thesis, and the comparison was universally disregarded as a fanciful speculative exercise. However, comparative developmental genetics was eventually to rescue his idea from oblivion, by demonstrating that in *Drosophila* DV patterning is controlled by two genes (*short gastrulation* and *decapentaplegic*) that are homologous to two genes (*chordin* and *bone morphogenetic protein-4*) which perform the same job in vertebrates, but at opposite DV sides[10,11].

In the meantime, a group of genes involved in the AP patterning of the main body axis of all bilaterian metazoans had been discovered[12], thus nourishing hopes that the main traits of the hypothetical ancestor of all bilaterians, the so called Urbilateria, could eventually be inferred from comparative developmental genetic evidence. The list of traits that have been progressively added to this idealised ancestor include AP polarity and DV patterning[13], heart[13], cephalisation[14], brain and brain areas[15], a primitive photoreceptor[16], a skeleton[17], a 'humble appendage or antenna-like outgrowth'[13], hemocoel[18] and even segmentation[19]. Unfortunately, no element in this reconstruction has been evaluated using the tools of phylogenetic systematics. Indeed, doubts have often been raised as to the uniquely derived nature of most of these traits[20,21]. But the main message we want to bring forward here is not about the cavalier methods by which these phylogenetic inferences have been produced, but about the increasing availability of comparative data that can be applied to the evaluation of phylogenetic relationships among higher groups. These data are not limited to pure sequence information about genes and their products, but open vistas into an understanding of the origin and evolution of form[22–24].

12.2.1 Phylogenetic Inference from Sequence Data of Developmental Genes

The crudest level at which we can exploit comparative developmental genetics in reconstructing phylogeny is by focusing molecular phylogenetics on sequence data of selected classes of genes which play a key role in establishing the main features of animal body plans during early embryonic development.

Let us consider, for example, those classes of molecules, such as cell adhesion molecules with intracellular signal transduction pathways and gradient-forming morphogens or growth factors[25], that probably played a critical role in the very origin of metazoans from their unicellular ancestors.

These molecules are today inextricably involved in building and patterning the supracellular architecture of the animal body. But homologues of the corresponding genes were probably also present along the stem lineage of living metazoans; their occurrence is likely in the living representatives of the metazoans' sister group, even if the function of these genes is different from the function that evolved in the metazoan branch of the phylogenetic tree. This is indeed a pathway of inquiry where a detailed knowledge of developmental genetics suggests a class of genes on which to focus, as a source of information for assessing a charismatic node of the tree of life, namely, the splitting of metazoans from their sister group. Indeed, King and colleagues[26] have found that choanoflagellates, the most likely candidates to be the closest relatives of metazoans among the living unicells, express representatives of many cell signalling and adhesion protein families. These include cadherins, C-type lectins, tyrosine kinases and components of the tyrosine kinase signalling pathway.

Other phylogenetic studies focusing on developmentally important genes have used the *Hox* family. The reconstructed duplication event by which a proto*Hox* gene cluster would have given rise to the *Hox* genes sensu stricto and to the paralogous set of the *ParaHox* genes has been suggested as relevant to the Cambrian explosion of animal body plans[27]. Another major event in animal evolution, the origin of the vertebrate lineage, is often thought to have been accompanied by a quadruplication of the *Hox* gene cluster[28]. Other phylogenetic analyses of *Hox* genes give support to the hypothesis that insects and crustaceans form a clade to the exclusion of myriapods[29] and demonstrate the lophotrochozoan affinities of bryozoans[30].

The role of these genes in controlling basic features of the animal body has probably been overemphasised. Indeed, criticisms of the mainstream gene-centred view of development are increasingly frequent[20,31–34], but in addition to these theoretical perspectives there is also experimental evidence pointing in the same direction. For example, the deletion of an entire vertebrate *Hox* cluster may have little effect on the development of the animal's main body axis3[5–37], much less indeed than the deletion of any individual *Hox* gene in the same model animal. This asks for a serious rethink of our understanding of the role of these genes in patterning the animal body. At any rate, comparing sequences of developmentally important genes does not represent a major improvement in respect to current practice in molecular phylogenetics. We argue here instead that much more substantial progress is obtained if the basic steps in phylogenetic reconstruction are approached from an explicit evo-devo perspective.

12.2.2 Evo-Devo, Devo-Evo

Researchers involved in the evolutionary or the developmental components of evo-devo may exchange roles and provide each other with the evidence to be explained, or the framework within which to look for explanation. One may even claim that we should distinguish between an evo-devo and a devo-evo biology[38]. Irrespective of the labels we eventually apply to these efforts, such a two way perspective also applies in the area where evolutionary developmental biology meets with phylogenetics. One way is easier, but we are not really concerned with it here. This is the use of independent phylogenetic reconstructions as scenarios against which to evaluate the evolution of developmental processes. Recent examples include the evolution of segmentation in mecistocephalid centipedes[39], of dentition in basal vertebrate clades[40], of cell lineage in gastropods[41] and of metamorphosis in nemerteans[42]. The other way is using evidence from comparative developmental biology to get a sounder foundation of our assessments of homology of traits and processes, and also to discover new useful levels of comparison.

12.3 EVO-DEVO INSIGHTS INTO EVOLUTIONARY CHANGE

Independence of traits is, in principle, a prerequisite for their use in coded form in the reconstruction of phylogeny. Operationally, independence can be read as a sufficiently low level of covariation[43,44]. In biological terms, one may distinguish between developmental independence and functional

independence. The latter is often easier to ascertain, in so far as we are able to single out individual features or structural complexes performing largely distinct functions, thus behaving as minimally overlapping units from the point of view of the selective pressure acting upon the organism. Much easier, but not less important, is the identification of developmentally independent units, due especially to the pervasive pleiotropic effects of the underlying genetic networks. But this is certainly one of the areas where evo-devo may offer an especially important contribution.

Evo-devo explicitly addresses the generative mechanisms underlying the evolution of organismal form. An extreme reductionist view of the evolutionary process would argue that at the basis of evolutionary change there is nothing more than a change in the underlying regulatory networks of developmental genes[45]. Alternative views of the evolution of organismal form would maintain that generative mechanisms are not restricted to the genetic circuitry involved in individual development. These mechanisms also arise from the physical properties of biological materials, the self organisational capacities of cells and tissues, and the dynamics of epigenetic interactions among developmental modules[20,46,47]. However, although evo-devo does not coincide with developmental genetics, it must be said that our current understanding of development and the mechanisms of its evolutionary change is much more advanced at the level of the genes.

Due to their pervasive occurrence, the 'nongenetic' components of the developmental processes (physicochemical properties of living matter and epigenetic interactions) are likely to be relevant in the evaluation of character independence. However, even adopting a narrow view of evo-devo, limiting its scope to developmental genetics, leaves a lot to say about the assessment of homology, character independence and character polarity.

12.3.1 How the Genetic Network Evolves

The traditional view on how the genetic makeup of organisms changes during evolution has centred on the gene's coding sequence. Through a cascade of causal processes, first at the genetic and later at the developmental level, a new allele, or a new allele combination in a new genotype, causes a new ontogenetic trajectory, more precisely, a new reaction norm. However, more recent studies are now challenging this view[48–51]. The evolution of the genetic machinery underlying the diversity of organismal form is mainly due to differences in gene regulation. Activating or repressing the expression of a gene at a new developmental stage or at a new location in the body can produce dramatic variations in the resulting phenotype. Researchers increasingly attribute evolutionary novelties to stretches of DNA with regulatory, rather than coding functions. These sequences, called enhancers, present binding sites for factors that regulate gene transcription. Sometimes the enhancer simply contains multiple copies of the same binding site; at other times, it has sites for different transcription factors. Moreover, recent results suggest that the effect of an enhancer on a gene is determined not just by the combination of transcription factors to which it can bind, but also by the spacing between its different binding sites[48]. Thus the same subset of transcription factors can be used to regulate different genes, by simply changing the spatial configuration with which these proteins bind along the enhancer. Modifications in the order and spacing of binding sites can affect the behaviour of the same gene across different species. Evolutionary change via enhancer rearrangement seems to be simple and effective. For instance, the vertebrate gene *Hoxc8* is involved in defining the number and shape of thoracic vertebrae. There is direct evidence that the enhancer, rather than the coding sequence, plays a pivotal role in generating different axial morphologies amongst species (for example, se Wang et al.[49]). Subtle species-specific differences in the enhancers correlate with the different anterior boundary of *Hoxc8* expression within the embryo. This can contribute to explain the great diversity in the number of thoracic vertebrae among vertebrates[50]. However, there are other ways in which development can be changed through changes at the gene level. Alonso and Wilkins[51] have recently challenged the view that enhancer elements are the only, or the main, sites of gene regulation and therefore the principal players in the evolution of developmental processes. They explored the potential of many different factors involved in the regulation

of gene expression, both at the transcriptional and posttranscriptional levels. These 'alternative regulative levels', as they call them, have many of the features, like flexibility, modularity and a combinatorial nature, which make enhancers critical contributors of genetic source materials to the evolution of development. They are sites of 'initial' genetic change, that may be either strengthened or replaced by subsequent 'secondary' genetic changes at other regulatory levels, including the level of the enhancers.

Evolutionary changes at the level of the genome's regulatory elements make it possible that different parts of the same animal are built by exploiting the same gene network or the same developmental module. 'Tinkering', 'multi-functionality', 'redundancy' and 'modularity' are common at the roots of phenotypic variation, but their impact on phenotypic evolution is far from being generally acknowledged. Character independence cannot be inferred from comparative anatomy or descriptive embryology alone and is just one working hypothesis among others in phylogenetic reconstruction. By expanding on the scope of current methods for estimating robustness of phylogenetic trees, it would probably be profitable to develop methods able to cope with an unknown level of character covariation.

12.3.2 Tinkering at the Level of Developmental Genes

Reviewing the conceptual framework of evolutionary developmental biology, Arthur[5] lists a number of key concepts that represent the toolbox for evo-devo investigation. Amongst these is a group of patterns and processes related to the 're-use of developmental genes in evolution'. Co-option of developmental genes (or gene networks) for different functional roles, sometimes involving gene duplication, is considered a major source of evolutionary change. Taking this idea even further, co-option can cause the formation of new structures, rather than simple change in old ones.

A more extreme concept is Minelli's axis paramorphism[52]. In metazoans there seems to be a general correspondence between the organisation of the appendages and the organisation of the main body axis of the same animal. Minelli interpreted metazoan appendages (secondary axes) as the product of a duplicate expression of genes already involved in growth and patterning of the main axis, that is, as axial paramorphs of the latter. Following the paramorphism hypothesis, arthropods' potential to produce periodically arranged structures along the main axis was exploited in producing segmented appendages[53].

The hypothesis of axis paramorphism has non-negligible consequences in establishing character polarity for traits related to arthropod appendages. Consider the old question of the mutual relationships between the antenna and the conventional (locomotory) leg of arthropods. The question is whether the antenna is to be regarded as a specialised leg, or vice versa. In other terms, which is the ancestral condition of the arthropod appendage? Different opinions have been expressed recently about the plausibility of the two alternatives (antenna first, or leg first), mainly in the light of developmental genetic evidence. For example, Dong and colleagues[54] favoured the antenna-first hypothesis, whereas Casares and Mann[55] supported the 'leg-first' hypothesis, although in a later paper these two authors have adopted a less clear cut option[56]. Considering paramorphic relationships between the main body axis, already segmented and patterned in AP sequence, and its serial appendages, which to some extent would therefore be already diverse (and segmented) since their very first expression, the whole question of the primacy of the leg versus the antenna would become meaningless[53,57], and the scheme of character transition from one form to the other is no longer applicable.

The great plasticity of the gene network and the non-strictly hierarchical modes of evolutionary change are also attested to by the numerous examples of homologous characters that are formed via different developmental pathways[58]. The evolution of simultaneous body segmentation in arthropods may illustrate this point.

Body segmental units originate almost simultaneously in the *Drosophila* embryo. The so called segmentation genes, classified into gap, pair-rule and segment-polarity genes, create a hierarchical

cascade of gene activity, leading from the early gap genes to the later expressed pair-rule and segment-polarity genes. These genes encode proteins that are eventually localised in the embryo according to segmental periodicity. In other arthropods, segments originate sequentially in an AP progression from a subterminal region. Simultaneous and sequential segmentation can both occur within the same animal. In many insects with embryo intermediate between short and long germ-band type, the most anterior segments originate synchronously, whereas the remaining segments are sequentially specified from a posterior sub-terminal zone[59]. At least for a significant posterior portion of the main body axis, sequential segmentation is generally considered the primitive condition in arthropods, and mechanisms for the evolutionary change from sequential to simultaneous segmentation have been proposed. These are based on a gradual cellular-to-syncytial transition in the blastoderm where the same segment-forming gene network operates[60], or on a progressive increase (from the anterior) of the number of segmental units falling under the control of gap genes[61].

In this case history of segmentation processes, the downstream developmental processes are conserved, whereas the earliest phase of the segmentation process has changed. This is just one of the many examples that comparative developmental biology can offer in support to a view that homology cannot be a relationship of the all-or-nothing kind. Because evolutionary change is a continuous process, based on the remoulding of pre-existing features, along with the underlying genetic networks that control their development, homology can only be partial[20,62–66]. The view of a character remaining the same (homologue) throughout a number of possible states, defining as many steps in an evolutionary sequence that can be linearly polarised and coded to fill in a phylogenetic data matrix, probably rests on a misrepresentation of how organisms evolve.

12.3.3 Evolution of Ontogenies versus Evolution of Characters

The way in which characters originate and change in evolution has fundamental consequences on the patterns of evolutionary change we can reconstruct from character distribution. Organismal form is not the product of one overall hierarchy of developmental modules, either at the level of genetic network or at the level of epigenetic interactions. Some hierarchical relationships occur only locally, both in spatial and temporal senses. This affects the production of variation in evolution. For instance, phylogenetically independent structures in different organisms can exploit the same genetic toolkit, or the same physical properties of living matter, in producing new variants. The remoulding of pre-existing features, genetic networks or developmental trajectories, can operate at any level of organisation. This 'tinkering' can overcome structural and functional boundaries between subsystems within an organism, by exploiting pattern and processes of one subsystem for the use of others[53]. The non-strictly hierarchical nature of evolutionary changes of ontogeny will unavoidably cause homoplasy.

12.4 DEALING WITH CHARACTERS FROM AN EVO-DEVO PERSPECTIVE

The new approach to phylogenetic reconstruction based on evolutionary developmental biology begins with the step of articulating the phenotype into a set of manageable and meaningful traits, and proceeds until the step is reached at which a suitable coding for character states can be eventually adopted.

Let us start with the identification of characters to be used in phylogenetic reconstruction. To delimit characters is basically the same as to identify homologies[62,63]. In this respect, evo-devo has broadened the scope of the search for homology, traditionally limited to a comparison of anatomical traits, to encompass also physiological and especially developmental processes. Gilbert and Bolker[69] introduced the term 'homology of process' to describe "the relationship between patterns that are composed of homologous proteins and that are related by common ancestry". However, as one of

the present article's authors has remarked elsewhere[20], this still means reducing organ homology to gene homology, something conceptually and methodologically equivalent to reducing species phylogeny to gene phylogeny. As the latter reduction is conceptually unwarranted and must be rejected[70,71], so process homology is not to be reduced to the shared involvement of homologous genes in two developmental sequences, but must be firmly rooted in the shared origin of the developmental pattern itself. This opens several interesting questions, two of which will be dealt with here.

The first question is whether we can formulate hypotheses of homology between developmental stages, rather than between specific developmental events. We think we can, but very cautiously. Nobody will contend that to enter in a data matrix a character 'larva' with states such as 'trochophora', 'caterpillar' and 'tadpole' would be other than plain nonsense, but what about suggesting the grasshopper prelarva as homologous to the larva of beetles or flies? Evidence in favour is admittedly tenuous[72], but the hypothesis cannot be discounted hastily[73,74], and only a thorough exploration of the developmental sequences both upstream of these putatively homologous stages and during the same will hopefully clarify the issue.

Problems with the comparison of developmental stages are sometimes even more subtle. When comparing stages of two closely related insects, with a similar postembryonic developmental schedule but with different number of instars, we may consider whether there is necessarily homology between equally numbered stages of the two species. That is, we may ask whether the fifth and last larval stage of butterfly species A is homologous to the fifth but penultimate larval stage of butterfly species B. In our understanding, there is no universally valid answer to this question, but as a basic rule, we believe that individual instars in a developmentally 'smooth' sequence (that is, one along which moults are only punctuations of the animal's basically continuous growth) cannot be individually treated as homologues. The only meaningful comparison, in the example, would be one between the character state 'larval development through four instars' and 'larval development through five instars', but excluding a direct stage-to-stage comparison between the two species.

The second question is whether we can still rely on the traditional all-or-nothing notion of homology. Our answer is firmly 'no'. All developmental sequences investigated in some detail, and especially those for which a detailed analysis has been performed in terms of genetic control of developmental events, have shown that characters, morphological and developmental alike, are not produced by unique and perfectly well integrated complexes, or networks, of genes. Locally acting dynamics allow the recognition of more or less individualised developmental modules[75–78], but overlaps and cross links are such as to oppose a simply hierarchical dissection of development and, hence, a strictly hierarchical view of homology. A combinatorial approach to homology has been suggested as a viable alternative[63].

12.4.1 Segmentation

One of the grand traits of animal organisation on which evolutionary developmental biology offers a renewed perspective is segmentation, a key trait in the taxonomy of some species rich groups, such as the mecistocephalid centipedes[39]. Body segmentation has long been regarded as a character useful in recognising affinities at very high taxonomic levels, for example, as an argument through which zoologists have supported, until recently, Cuvier's[6] pre-evolutionary concept of a taxon Articulata that should include the two major groups of segmented invertebrates, annelids and arthropods. Modern insights into segmentation mechanisms have cast increasing doubt as to the equivalence of segmentation mechanisms in the two groups[79] and the origin of segmentation is now generally regarded as either very deep in animal phylogeny (via a segmented Urbilateria[13,19,80,81]) or, as we prefer to believe, as having evolved in annelids and arthropods convergently[82]. Adopting a broader evo-devo perspective helps with the interpretation of segmented features of animal body architecture in a much more articulated way[83,84]. In this way, we realise that phylogenetically closely related species may differ in their segmentation mechanism to a considerable extent, whereas

unexpected similarities in these mechanisms may occur between much more distant lineages. Comparative evidence suggests that the concept of segmentation applies to organs rather than to whole organisms, overall segmentation resulting when independently segmented structures eventually develop to share period and phase of their repetitive patterns. One may even argue that segmentation is a 'generic' property of bilaterians[85], that is, that it depends on basic physicochemical properties of living matter more than on the specific expression patterns of a restricted set of genes. In this perspective, it seems that the traditional distinction between the 'true' segmentation of annelids, arthropods and vertebrates, and the 'pseudosegmentation' of animals such as tapeworms and kinorhynchs should be abandoned. This is obviously important, given the use we may want to make of segmentation in phylogenetic reconstruction.

12.4.2 Larval Legs versus 'True' Legs

Atavisms or 'evolutionary Lazarus features'[20] can strongly perturb the process of reconstructing phylogeny, but combining insights from developmental genetics with those from comparative morphology may help avoid pitfalls. One may wonder whether the larval legs of caterpillars are homologous to the other paired appendages of the trunk, a question traditionally studied only in respect to the generalised lack of nongenital abdominal appendages in pterygote insects and the presence of short appendages in the abdomen of more distantly related hexapods. In this case, in addition to providing one more argument in favour of adopting a combinatorial view of homology, developmental genetics has demonstrated that changing from presence to absence of a major anatomical trait may be obtained at relatively low cost. It has been shown, in fact, that the presence of larval legs in the abdomen of lepidopteran larvae is due to the expression of the *distal-less* gene in the abdominal segments, a pattern of expression which is otherwise suppressed in most insect orders, thus determining the absence of limbs on the corresponding segments. A conspicuous morphological difference between lepidopteran larvae and other larval or adult insects is thus basically explained by the de-repression of the expression of this gene[86]. Caterpillar prolegs should be therefore examined more closely for their specific morphology rather than evaluated in plain terms of presence in lepidopterans versus absence in other insect orders.

12.4.3 Character States: Discontinuous Variation

One of the largest benefits of the ongoing dialogue between developmental biologists and evolutionary biologists is a better understanding of the causes of discontinuous variation in circumstances where an explanation in terms of natural selection would be at loss. An example is variation in the number of leg-bearing segments in centipedes. This number is always odd in adult centipedes: 15 in Scutigeromorpha, Lithobiomorpha and Craterostigmomorpha, 21 or 23 in Scolopendromorpha and 27 to 191 in Geophilomorpha. Intraspecific variation in segment number is known for one scolopendromorph only (*Scolopendropsis bahiensis* (Brandt))[87] but is widespread among the Geophilomorpha. Only an unbelievably strong selection pressure against centipedes with an even number of leg pairs would explain the complete absence of specimens with 36 or 38 pairs of legs in a population where only specimens with 35, 37 and 39 pairs are recorded. But this is where the evolutionary explanation ends, and developmental biology may enter the scene: only an accurate knowledge of segmentation mechanisms may explain why centipedes with an even number of leg pairs are never produced[88]. The knowledge we have of segmentation in arthropods and in centipedes in particular is limited, but it is only from those studies that we eventually expect to understand why the 35 and the 39 pairs of legs conditions are, indeed, the closest possible to the 37 pairs of legs condition, in both developmental and evolutionary terms, whereas the apparently intermediate conditions, with either 36 or 38 pairs of legs, are 'infinitely' far away. With fairly high numbers of segments, the easiest jump may be even further away than just two segments up or down. For example, in the geophilomorph family Mecistocephalidae, where segment number is nearly always fixed within each species, most evolutionary changes have involved adding four segments

in one step[39], such as from 41 to 45 to 49. These evo-devo perspectives on the topology of the ontogenetically accessible morphospace are clearly useful in defining character states.

12.4.4 Developmental Processes As Characters for Phylogenetic Analysis

The fact that ascertaining homology among different developmental stages is not a simple thing supports the use of an evo-devo approach so as to allow proper discrimination between true homology and apparent homology. The latter may have a very pervasive effect in phylogenetic reconstruction, particularly when coupled with heterochrony. Heterochrony, the change in developmental timing[89,90], may affect, in different ways, the recognition of homology amongst developmental stages. The order of developmental events may be changed, and this aspect needs be properly addressed, but heterochrony may also stop the development of some taxa at a stage which is not comparable with that reached by other taxa. If this aspect is not taken into account and such characters are included in the phylogenetic reconstructions, the resulting trees are the product of analyses based on noncomparable developmental traits.

This point is well exemplified in phylogenetic reconstructions of salamanders determined on the basis of evidence which included developmental characters affected by paedomorphosis, that is, an arrest of some traits in a larval stage condition[91]. The inclusion of larval characters deeply affects inferences on the higher-level phylogeny of salamanders in three distinct ways. First, such traits, which are homoplastic, are shared by paedomorphic adults of different lineages that thus group together. Second, and a more difficult factor to be removed, the paedomorphic traits destroy the clade-specific synapomorphies that are present in the metamorphosed adults. This phenomenon causes the misplacement of the paedomorphic taxa in the phylogenetic reconstruction with respect to their nonpaedomorphic relatives. Finally, the aquatic life style of the larvae produces parallel adaptive changes that again cause an erroneous placement of paedomorphic taxa. Even worse, the strong bias introduced by heterochrony in the phylogenetic tree based on paedomorphic characters is corroborated by statistical support[91]. This clearly shows the strong value of an evo-devo approach in identifying the traits affected by heterochrony. In this way, we can include them in the data set after suitable corrections, accounting for the heterochronic effects, have been made.

During the last decade the burgeoning evo-devo approach to phylogenetics has moved even further, by trying to produce algorithms that allow the proper handling of the effect of heterochrony. The most widespread approach is based on developmental sequences. "A developmental sequence is a list of different events in the chronological order in which they happen in the ontogeny"[92]. A developmental sequence can be divided into a series of developmental events. "Developmental events may be regarded as series of morphological states which a given embryonic structure passes"[93]. The order of events along the sequence characterises the sequence itself. If we consider two events (A and B) in a sequence, they can only occur in one of the following temporal series: A occurs before B, or simultaneously with it, or after B.

Each of these timing relationships constitutes an event pair that can be given a numerical score[94,95]. For every species having a developmental sequence including N events, there are $^1/_2(N^2 - N)$ event pairs. Developmental sequences that have been scored according to the event pairs characterizing them may be assembled in a data matrix. The rows in the matrix are the developmental sequences, while every column of the matrix is occupied by the numerical scores of each event pair previously coded according to procedures such as developed by Smith[94] or Velhagen[95]. This matrix can be used under the criterion of maximum parsimony to reconstruct a phylogenetic tree[96]. Characters may be treated both as unordered or ordered. However, event pairs considered in the developmental sequence are not independent characters and thus violate one of the fundamental requirements of phylogenetic reconstruction, that is the independence of characters analysed.

There are two kinds of nonindependence in the event pairing approach: an ontogenetic dependence due to the fact that some events occurred according to a specific order during ontogeny and a coding

dependence due to the fact that scoring of event pairs is not independent. This double nonindependence may lead to highly inconsistent results, or even absurd results, from a logical perspective.

To circumvent this intrinsic flaw in the event pair approach, Schulmeister and Wheeler[92] developed a new method in which every developmental sequence is considered as a single multistate character. A search based optimisation is used to investigate changes within developmental sequences, and step matrices are used to account for changes within each sequence. The new method does not suffer from the nonindependence that characterises the event pair approach and thus appears to be a promising strategy for the use of developmental data in phylogenetic reconstruction when data are affected by heterochrony.

12.5 CONCLUSION

In the context of the current dialogue between evolutionary and developmental biology, the value of reliable phylogenetic reconstructions in comparative evaluation of developmental processes has been adequately demonstrated. In contrast, the relevance of evolutionary developmental biology in our effort to reconstruct the tree of life has been very poorly explored until recently. However, as shown in this article, the contribution of an evo-devo approach to the main steps of phylogenetic analysis, such as evaluation of homology, selection of characters and assessment of character polarity, can be critically important.

ACKNOWLEDGMENTS

We thank Ronald Jenner for insightful comments on a previous version of this work. The idiosyncratic views we present here are definitely ours. Alessandro Minelli was supported by a grant of the Italian Ministry of Education, University and Research.

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