EvoDevo and Its Significance for Animal Evolution and Phylogeny

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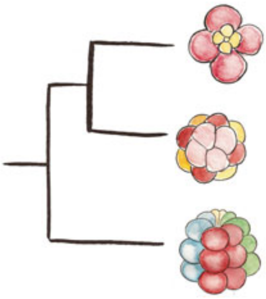

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ONTOGENY VS PHYLOGENY

Despite Steinböck's (1963, p. 49) dismissive statement that "ontogeny has only a very limited value for phylogenetic questions," successful attempts to infer phylogenetic relationships from comparative information about the developmental schedules of animal species are numerous, beginning with two well-known, eighteenth-century examples. One is Thompson's (1830) discovery of the crustacean nature of barnacles, based on his observation of nauplius larvae metamorphosing into sessile adults (see Vol. 4, Chapter 5) whose morphology deviates so strongly from the arthropod ground plan that Linné (1758) placed *Lepas* (inclusive of barnacles) in his Vermes Testacea (i.e., the shelled mollusks) rather than in his Insecta (a "class" broadly equivalent to present-day Arthropoda). The other example is Kowalewski's (1866) discovery of the affinities between vertebrates and ascidians, revealed by the presence of the notochord in the larva of the latter (Vol. 6, Chapter 4). This does not imply, however, that the relationships between ontogeny and phylogeny are always easy to discover or that these follow simple and perhaps universal principles such as Haeckel's (1866) "biogenetic law." Haeckel's recapitulationist views, indeed, have never been again much in favor since Garstang (1922) demonstrated that many larval adaptations are recent and independent; and a further strong blow to the theory was de Beer's (1930, 1940) demonstration of the pervasiveness of heterochrony. However, new opportunities to extract phylogenetic information from ontogenetic data have been emerging since the advent of evolutionary developmental biology (Telford and Budd 2003; Cracraft 2005; Minelli 2007, 2009; Minelli et al. 2007).

EVOLUTIONARY DEVELOPMENTAL BIOLOGY

Evolutionary developmental biology, or EvoDevo, is one of the most active frontiers of the life sciences, despite the fuzzy definition of its scope and its sometimes problematic boundaries in respect to the parent disciplines – evolutionary biology and developmental biology. Comprehensive overviews of origins, aims, and methods of evolutionary developmental biology can be found in Hall (1998) and Hall and Olson (2003); other useful book-size accounts, although more selective in their approach, are Wilkins (2001), Minelli (2003a), Carroll et al. (2005), and Minelli and Fusco (2008).

As one should expect for a newly established, or reestablished, field of study, EvoDevo is still struggling to define its own identity; short introductions to the internal debate have been provided by Arthur (2002) and Müller (2008).

Many researchers (e.g., Carroll et al. 2005) view EvoDevo essentially as comparative developmental genetics, that is, as the comparative study of the spatial and temporal expression patterns of genes controlling the establishment of body architecture: anterior-posterior and dorsoventral polarity; longitudinal patterning of the main body axis; segmentation, production, and patterning of appendages; and so on, down to details such as the differentiation of eyespots on butterfly wings or the rows of specialized bristles forming the sex combs on the forelegs of *Drosophila* males. This comparative approach to the study of gene expression pattern has produced the positive effect of rapidly increasing the number of organisms used in the lab as model species. In turn, the expanding taxonomic coverage of these studies has helped generating results of potentially high relevance for phylogenetic research. At the level of the genetic mechanisms controlling development, it has become meaningful, and operationally feasible, to address questions of homology between features of vastly divergent taxa.

Arguably, simply broadening the scope of comparison beyond the traditional bunch of model species, such as *Caenorhabditis elegans*, *Drosophila melanogaster*, and *Mus musculus*, would hardly justify the recognition of a new, distinct discipline. EvoDevo, however, is characterized by a problem agenda that could not be satisfactorily fulfilled within the premises of either evolutionary or developmental biology in isolation. This is true, for example, for the origin of evolutionary novelties (Müller 1990; Müller and Wagner 1991, 2003; Wagner 2000, 2011; Galis 2001; Müller and Newman 2003, 2005; Minelli and Fusco 2005;

Love 2008; Moczek 2008; Pigliucci 2008; Shubin et al. 2009; Brigandt and Love 2010, 2012; Hall and Kerney 2012; Peterson and Müller 2013). The most discipline-specific problem addressed by EvoDevo is the nature and the properties of *evolvability*, defined by Hendrikse et al. (2007) as "the capacity of a developmental system to evolve." This means that EvoDevo characteristically focuses on the *arrival* of the fittest rather than on the *survival* of the fittest. How far this shift of focus should be considered an extension, either marginal or substantial, of the evolutionary synthesis paradigm, or a radical alternative to the same, is still a matter of dispute (e.g., Laubichler 2010; Minelli 2010; Pigliucci and Müller 2010), but this is not relevant to our subject.

To introduce, instead, an overview of the possible significance of EvoDevo in the context of phylogenetic analysis, it is fair to repeat, at the outset, the comment made 10 years ago by Wiens et al. (2005) that up to now the overall contribution of EvoDevo to phylogenetics has been quite small. But this is arguably due to the limited awareness of EvoDevo by a large majority of phylogeneticists, and vice versa, rather than to the exiguity of the potential intersection between the two disciplines. Eventually discovering mutual foreignness between EvoDevo and phylogenetics would be ironic, indeed: let's recall that Gould's magisterial introduction to one of the roots of EvoDevo, namely, the study of heterochrony, was published in 1977 under the title *Ontogeny and Phylogeny*. There are instead several important areas to which EvoDevo can contribute to progress in phylogenetics. I will articulate these areas in the following sections, mainly taking examples from invertebrates.

RECAPITULATION VS CLADISTIC ASSESSMENTS OF CHARACTER POLARITY

In its earliest steps, long before getting its current name, EvoDevo contributed substantially to a critical revisitation of Haeckel's recapitulationism, the principle according to which ontogeny recapitulates phylogeny. As mentioned before, de Beer's books (1930, 1940) dissected the possible relationship between ontogeny and phylogeny in such a way that these eventually revealed the wealth of alternative patterns, recapitulation being only one among several possible scenarios and, arguably, not necessarily the most common among them. De Beer's analysis eventually resulted in the birth of the modern studies on heterochrony, especially after this area was popularized by Gould's (1977) book.

In the meantime, debates about the phylogenetic signal contained in ontogenetic sequences developed in cladistic circles. Some cladists, like Rieppel (1979), were critical of the independence of ontogenetic information from the morphological data used in outgroup comparisons. Others, however, thought otherwise.

Among the criteria to be used for polarizing characters, i.e., to distinguish the plesiomorphic from the apomorphic state of a character, Hennig (1966) had suggested ontogenetic character precedence. Somehow echoing Haeckel's biogenetic principle, this criterion postulated that the derived character states are to be found in late developmental stages, whereas similarities shared at earlier stages are generally symplesiomorphies (shared primitive character states) that cannot be used to infer phylogenetic relationships. The ontogenetic character precedence was regarded by some authors (e.g., Fink 1982) as reliable as the outgroup comparison, whereas others (e.g., Kluge 1985) pointed to its lack of general applicability and still others (e.g., Nelson 1978; de Queiroz 1985) suggested different reformulations of the principle, effectively taking distance from the original recapitulationist flavor of Hennig's principle. For example, Nelson (1978, p. 327) reformulated the "biogenetic law" in the following terms: "given an ontogenetic character transformation, from a character observed to be more general to a character observed to be less general, the more general character is primitive and the less general advanced." A different formulation was recently suggested by Martynov (2012, p. 833) as the main principle of his *ontogenetic systematics*, which should be based on "progressive (addition of stages and characters) or regressive (reduction of already existing stages and structures) modification of ancestral taxon, the diagnosis of which corresponds to the model of its ontogenetic cycle."

An unusual extension of the recapitulationist paradigm into the area of animal behavior has been recently proposed by Barrantes and Eberhard (2010) with a comparative study of the web-spinning behavior in spiders. These authors found that the design of the web spun by adults of three *Latrodectus* species is more divergent than the design of those spun by juveniles of the same species and more similar to those of young spiders of the genus *Steatoda* than to those of the adult of the latter genus.

But let's move to more explicit suggestions and recent examples of their application.

DEVELOPMENTAL GENES AND PHYLOGENETIC INFERENCE

Gene-Based Homology

The first step toward a phylogenetic analysis is getting informative data. How can EvoDevo contribute to filling a matrix?

The contribution of EvoDevo to the assessment of homology is controversial (see Chapter 2). On the one hand, it is right from early works in what was still to be named EvoDevo that biologists realized that traits firmly regarded as homologous by comparative morphologists can have quite a different developmental origin. On the other hand, one of the most visible successes of EvoDevo has been the discovery that homologous genes are often involved in building equivalent structures in the most disparate animals, although this equivalence has been generally regarded as nonhomologous by comparative morphologists. On homology, see Minelli and Fusco (2013), Wagner (2014), and Chapter 2 herein.

The Genotype→Phenotype Map

One of the most far-reaching results of EvoDevo studies is the growing awareness of the complexity (and, to a very large extent, unpredictability) of the genotype→phenotype map, that is, of the cascade of processes through which a given phenotypic trait is controlled by the expression of a given gene (e.g., Alberch 1991; Altenberg 1995; Mezey et al. 2000; Kell 2002; West-Eberhard 2003; Pigliucci 2010; Wagner and Zhang 2011). To put it in simple terms, this mapping is rarely, if ever, a one-to-one function (one gene→one phenotypic trait), but it is generally one-to-many (pleiotropy; e.g., Wagner and Zhang 2011, 2013; Paaby and Rockman 2013) or many-to-one (convergence and or redundancy) and eventually many-to-many.

In *Drosophila*, some 50 genes are the direct targets of transcription factors encoded by Hox genes (Pearson et al. 2005): some of these genes are involved in apoptosis and others in the control of cell cycle, cell motility, intercellular signaling, or cell adhesion (Davidson 2006), and there are hundreds of genes whose expression is downstream of the expression of one or more of the Hox genes (Mastick et al. 1995; Botas and Auwers 1996).

Convergence

A nice example of the intricacies of the genotype→phenotype map is the fact that the same genes can regulate the development of homologous structures through significantly different cellular processes. A recently studied example is offered by the sex combs of male *Drosophila* species. The key regulatory genes involved in the production of these rows of specialized bristles are the same in the different species that have been investigated, but the cellular mechanisms through which they operate are different, not only between members of different subgenera (*Sophophora* vs *Lordiphosa*) (Atallah et al. 2012) but also between quite closely related species that are classified in the same subgenus (*Sophophora*: species of the *obscura* and *melanogaster* species groups; Barmina and Kopp 2007; Tanaka et al. 2009, 2011).

Developmental genes, Hox genes included, are as prone to convergence as morphological characters are. An example is offered by the mechanisms controlling leg repression in one of the body regions (abdomen or opisthosoma) of some arthropod clades. In insects, the Hox genes *Ultrabithorax* (*Ubx*) and *abdominal-A* mediate leg repression, thus providing the most obvious difference between a leg-bearing thorax and a legless abdomen. Things are different in spiders, so far as the recent findings of Khadjeh et al. (2012) on *Achaearanea tepidariorum* will hold for the whole clade. Here, the gene *Antennapedia* (*Antp*) represses leg formation in the first segment of the opisthosoma, whereas both *Antp* and *Ubx* show their (redundant) effect in repressing leg formation in the following segment.

From Gene Phylogeny to a Comparison of Gene Expression Patterns

Most of the total output of EvoDevo research has been a growing knowledge of the identity, sequence, patterns of expression, and relative position in developmental control cascades of "developmental genes," i.e., of genes demonstrably involved in the control of specific ontogenetic events or in the deployment of specific traits of body architecture. Among these genes are those involved in body segmentation and those (the Hox genes) that specify positions along the anterior-posterior body axis. Indeed, the discovery of the high degree of conservation of these genes across the animal kingdom was one of the main successes that contributed to establishment of EvoDevo as a promising new biological discipline.

The potential phylogenetic signal contained in these genes can be studied at different levels, as shown here briefly on the example of the Hox genes.

A first level of analysis is the reconstruction of gene phylogeny, a necessary step, not only required to reveal duplications and thus to disentangle orthologous from paralogous sequences but also to establish relationships between gene families that may evolve either in concert or in divergent manner and eventually to polarize gene changes. The literature on the phylogenetic relationships of Hox genes is extensive (e.g., Finnerty and Martindale 1998; Kourakis and Martindale 2000; Ferrier and Holland 2001; Ferrier and Minguillon 2003; Garcia-Fernàndez 2005a, b; Duboule 2007; Ferrier 2007, 2010; Butts et al. 2008). A recent review by Holland (2012) highlights the phylogenetic relationships between the Hox family and other gene families also involved in development (*ParaHox*, *Evx*, *Dlx*, *En*, *NK4*, *NK3*, *Msx*, and *Nanog*), all together forming the ANTP class.

The second step is to use gene sequences to reconstruct the phylogeny of the organisms from which the sequences have been obtained. Will EvoDevo suggest to give preferences to selected gene families? In the past, several biologists looked at chromosome structure as at privileged morphological traits, insofar as chromosomes contain genes and genes are involved in determining the phenotype. In the same vein, some authors have looked at Hox genes – genes controlling aspects of the overall body architecture – as to privileged genes, possibly carrying important phylogenetic signal. Of course, the phylogenetic information potentially carried by the highly conserved homeobox sequence (the "morphological signature" of this gene class) will be very different from the phylogenetic information potentially carried by the remaining of the molecule, especially by regions distant from the homeobox. Eventually, Hox gene sequences have been used in reconstructing the mutual relationships of bilaterian phyla (de Rosa et al. 1999; Balavoine et al. 2002; Hueber et al. 2013) or to investigate phylogeny within large phyla such as Arthropoda (Cook et al. 2001). Other studies have contributed to fix affinities, e.g., of bryozoans (ectoprocts) as lophotrochozoans (Passamaneck and Halanych 2004). Apart from the Hox genes, specific signatures have been found in many other developmental genes. An example is provided by the *bone morphogenetic protein* genes, which are represented in all insects by *decapentaplegic* and *glass bottom boat* (*gbb*); a third gene, *screw* (*scw*), is found in *Drosophila melanogaster* and other flies, and recent comparative studies have placed the *gbb*/*scw* duplication in the interval between the origin of the Brachycera and the origin of the Cyclorrhapha, that is, between 200 and 150 Ma ago (Wotton et al. 2013).

The last step is to compare gene expression patterns in order to trace homologies, especially in cases where morphological evidence does not seem to allow a definitive assessment. This approach has been followed, for example, by Hughes and Kaufman (2002), Copf et al. (2003), and Angelini and Kaufman (2005) in comparing body regions of different arthropod groups and by Lichtneckert and Reichert (2005) in delineating homologies between vertebrate and arthropod brains. Jager et al. (2006) (see also Manuel et al. 2006) used the expression patterns of Hox genes to align the anterior appendages of sea spiders with those of other arthropods, thus yielding results that are in contrast with the morphological (neuroanatomical) evidence obtained by Maxmen et al. (2005). There are, however, examples of developmental genes whose expression patterns confirm the homologies suggested by morphology. One of these is *Brachyury* (*bra*): its expression in the notochord of chordates has been fittingly chosen by Ferrier (2011) as a good example of a homologous gene with a homologous function in a homologous morphological character, a far from marginal example, the presence of a notochord being an apomorphy of a phylum.

miRNA and Phylogeny

According to Wheeler et al. (2009), a substantial increase in morphological complexity along the evolutionary history of metazoans is linked to a corresponding increase in the number and specificity of action of miRNAs. The same authors stress the high phylogenetic value of these molecules, confirm the previously established (Hertel et al. 2006; Sempere et al. 2006; Prochnik et al. 2007) major expansion of the miRNA family at the base of the nephrozoan clade, and identify the presence of 34 miRNA families in the last common ancestor of protostomes and deuterostomes, to the exclusion of acoels. A few miRNAs have been discovered in sponges (Robinson et al. 2013) but none of these is shared with eumetazoans.

Conservation of Gene Function and Developmental System Drift

The value of detailed patterns of Hox gene expression as a base to establish homology of segments or positions along the main body axis has probably been overestimated (Abzhanov et al. 1999; Brenneis et al. 2008), because of the observable evolutionary shifts of the anterior boundary of expression of many Hox genes within arthropods, especially *Antennapedia*, *Ultrabithorax*, *abdominal-A*, and *abdominal-B* (Hughes and Kaufman 2002).

Shifts corresponding to a positional inversion along the main body axis are very unlikely. Morphologically, the *fore*wings of male strepsipterans (the females are wingless and mostly vermiform) are quite similar to the halteres of dipterans – their characteristically modified *hind*wings. However, whatever the mechanisms specifying the peculiar structure of the strepsipteran forewings, it is quite unlikely that these evolved from the dipteran condition, through a "macromutation" switching the haltere specification from the meta- to the mesothorax, in turn restoring the metathoracic wings to a more conventional morphology. This unconventional hypothesis was suggested by Whiting and Wheeler (1994) as a tentative EvoDevo counterpart of their phylogeny of holometabolous insects, in which the Strepsiptera turned out to be the sister group of the Diptera (for the putative Diptera+Strepsiptera monophylum, the name Halteria was also proposed) (Whiting et al. 1997). The need to demonstrate the actual occurrence of such a macromutation eventually vanished, as soon as subsequent phylogenetic analyses (e.g., Rokas et al. 1999; Wiegmann et al. 2009) refuted the monophyly of the Halteria, thus showing the independent evolution, in the two clades, of morphologically similar but positionally nonequivalent "halteres."

Evolving Gene Functions

Comparative developmental genetics has revealed many examples of evolutionary changes in gene function. For example, *fushi tarazu* and *oskar* may have initially functioned in the central nervous system but later became involved in the patterning of the early embryo, as seen today in *Drosophila* (Ewen-Campen et al. 2012; Heffer et al. 2013). The evolution of new functional roles has been documented to occur even if the phenotypic traits previously controlled by a gene are subjected to strong stabilizing selection; this is why the evolution of new functions in a lineage of orthologous genes (i.e., independent from gene duplications) has been called *developmental system drift* (DSD) (True and Haag 2001; Haag 2014). Even organs that are identical at the cellular level, because they are produced through an identical cell lineage, can experience rapid DSD. This has been shown by Verster et al. (2014) by comparing over 20 species of *Caenorhabditis* where functional divergence has been found in orthologous genes regulating sex determination, early embryonic patterning, vulva development, and excretory physiology.

The phylogenetically widespread involvement of *Pax6*/*ey* homologs in eye morphogenesis (e.g., Halder et al. 1995; Tomarev et al. 1997; Glardon et al. 1998; Kmita-Cunisse et al. 1998; Chow et al. 1999; Pineda et al. 2000) has led to the hypothesis of a monophyletic origin of bilaterian eyes (e.g., Gehring and Ikeo 1999; Gehring 2000), contrary to a well-entrenched opinion, based on gross morphological differences between ciliary- and rhabdomeric-type eyes, suggesting an at least diphyletic origin of eyes. More cautiously, Wagner (2001) suggested that ancestrally *Pax-6* homologs may have been involved in initiating the development of light-sensitive epithelia, eventually a key component of subsequently evolved eye types such as the compound eye of arthropods and the camera eye of squids, but the hypothesis of a monophyletic origin of the eye has been strongly rejected by others, among which Harris (1997) and Meyer-Rochow (2000). In addition to the arguments provided by comparative morphology, Harris (1997) remarked that the expression of *Pax-6* is not restricted to the eyes. For example, in vertebrates this gene is also expressed in the nasal placodes, the diencephalon, the latero-ventral hindbrain, and the spinal cord (Li et al. 1994; Amirthalingam et al. 1995). In *Drosophila*, its homolog *ey* is also expressed in the brain and the ventral nerve cord, and in the squid, *Pax6* expression extends to the brain and the arms (Tomarev et al. 1997). Even more intriguing is the fact that *Pax6* homologs are also present in eyeless animals. In the nematodes, for example, *vab-3* is involved in the differentiation of the cephalic body end and *mab-18* is expressed in the precursors of peripheral sense organs (Chisholm and Horvitz 1995; Harris 1997). In the sea urchins, a *Pax6* homolog is expressed in the tube feet (Czerny and Busslinger 1995). Summing up, *Pax6* is likely a patterning gene, expressed in the head, which has been repeatedly involved (or, better, co-opted; see below) in the regulation of eye development.

Two arthropod genes of the Hox family have undergone dramatic functional changes. In selected branches of the arthropod tree, both of them have lost their original function as specifiers of position along the main body axis. One of these genes is *fushi tarazu*, which is involved in segmentation and, in insects only, in neurogenesis. The other gene is *zerknuellt* (*zen*), which is involved in dorsoventral patterning. In the Diptera, a duplication of *zen* has given rise to *bicoid*, whose functional role has continued to evolve rapidly: in *Drosophila*, it is required for the normal development of the head and thorax, and in the phorid *Megaselia abdita*, it is additionally required for the development of four abdominal segments (Stauber et al. 2000).

Gene Regulatory Networks and Their Evolution

Eventually, following the rapidly increasing knowledge on gene control cascades, research focus has shifted from the evolution of individual genes, and of their expression, to the evolution of whole gene regulatory networks (Davidson 2006; see also Davidson et al. 2002, 2003; Davidson and Erwin 2006; see also Chapter 2). From the perspective of phylogenetic reconstruction, this means moving from the limited evidence of homology provided by single genes, whose involvement in a given developmental process is prone to convergent evolution (multiple independent co-option events), to the more robust evidence provided by whole sets of functionally integrated genes (Ferrier 2011).

Comparative developmental genetics is able to reveal the intricate nature of gene networks such as those underlying the architectural design of the nervous system of bilaterians (Denes et al. 2007), the segmented body of arthropods (Dray et al. 2010), and the notochord of chordates (Kugler et al. 2011), a kind of synapomorphy packages for the corresponding clades.

Davidson (2006) described the developmental regulatory genome as something like a computer, with four classes of subcircuits: (i) batteries of genes involved in cell differentiation, (ii) little invariant subcircuits repeatedly involved in less specific functions, (iii) switches, and (iv) "kernels," complex and highly conserved networks responsible for specifying morphogenetic fields from which particular body parts arise. One of those kernels, for example, would be responsible for the specification of the endoderm. Kernels would be most robust to change and are thus likely to be shared by distantly related clades. Davidson envisaged a phylogenetic hierarchy of regulatory networks, e.g., bilaterian kernels, protostome kernels, and ecdysozoan kernels.

However, selected parts of a gene regulatory network may show unequal rate of evolution. For example, within the gene regulatory network (GRN) controlling the specification of endomesoderm in nematodes, a preliminary analysis of genome sequences of *Haemonchus contortus* and *Brugia malayi* suggests that evolution is most rapid for some zygotic genes involved in the specification of blastomere identity (Maduro 2006).

If we accept that development is controlled by GRNs, it follows that the evolution of development and form is due to changes within GRNs (Carroll 2008), but this is arguably an excessive generalization.

An exceptional example of the evolvability of developmental gene networks has been revealed by Kugler et al. (2011) with a comparison of notochord development between the pelagic urochordate *Oikopleura* and the ascidian *Ciona intestinalis* (Vol. 6, Chapter 4). In the latter, some 50 genes are known to be activated downstream of *bra*, but 24 of them do not have a homolog in the small, very compact genome of *Oikopleura*. Some of the latter have undergone a lineage-specific duplication, but less than a half of them are apparently expressed in the context of notochord formation. For an extensive discussion on gene regulatory networks and their bearings on character identity and evolution, see Chapter 2.

Gene Loss and Character Loss

From the perspective of phylogenetic reconstruction, character loss is a frequent cause of problems.

In an important study of salamander phylogeny, Wiens et al. (2005) have shown the misleading effects of paedomorphosis on phylogenetic analysis, because of which a previous analysis by Gao and Shubin (2001), based on morphological data, had placed most paedomorphic families in a single clade. As demonstrated by the new analysis, problems are not solved by simply excluding from the data matrix the characters suspected to be paedomorphic and by taking into account the parallel evolution of adaptive changes associated with the aquatic habitat typical of salamander larvae generally and definitely retained in the paedomorphic lineages. A possibly more disturbing problem is the absence, in the paedomorphic lineages, of those synapomorphies that in non-paedomorphic taxa develop at metamorphosis.

In respect to regressive changes, EvoDevo has much to offer beyond a conceptual framework, especially in those cases in which a regressive change is apparently due to gene loss. This has been tentatively suggested (Aboobaker and Blaxter 2003a; Minelli 2009) as a possible explanation for the relatively simple organization of the nematodes, compared to most ecdysozoans, which possibly correlates with a reduction in the number of Hox genes (which is coupled, however, with a very high rate of evolution of the surviving members of this gene family; Aboobaker and Blaxter 2003b). The most intriguing example of a likely correlation between the loss of a gene and the loss of a body part is, however, the extreme reduction of the abdomen in the parasitic crustacean *Sacculina carcini*, matched by the loss of the Hox gene *abdominal-A* (Blin et al. 2003; cf. Vol. 4, Chapter 5).

Gene and Gene Network Co-option vs Paramorphism

Since the last years of the past century, it has become fashionable to interpret major events in the evolution of the genetic control of development in terms of *co-option* of individual genes or even of whole gene regulatory networks. Gene co-option would be usually dependent on previous gene duplication. Following the latter event, neofunctionalization of a duplicate gene would add a new trait to the phenotypic features under its control. Co-option, for example, would explain the evolution of arthropod and vertebrate appendages (Tabin et al. 1999). According to Pires-daSilva and Sommer (2003), all developmental processes involved in the generation of new structures would necessarily depend on co-option.

However, we should probably advocate gene co-option only when an existing gene gets a new role in a developmental process in which it was not previously involved or in a body part where it was previously not expressed, only when the developmental process or the body part with which it now becomes involved was already in existence (Minelli 2009). This is the case of the wing eye spots of many butterflies, which are centered on a group of *Distal-less*-expressing cells (Carroll et al. 1994). *Distal-less* has a much older and phylogenetically much more general role in animal development, as an early marker of the sites where appendages will form, including insect legs, "polychaete" parapodia, vertebrate limbs, and sea urchin podia (Panganiban et al. 1997). In butterflies, *Distal-less* has been co-opted to mark the position of new "virtual axes," but the presence of wings does not depend on this novel expression of the gene.

The concept of co-option does not apply, however, when a novel pattern of expression of a gene, or of a whole gene regulative network, coincides with the origin of a new body part. It is possible, indeed, that the evolving phenotypic outcome of that gene's expression is a story of exaptation rather than one of co-option. This is arguably the case of *nanos*, originally a determinant of the posterior end of the trunk (cf. Rabinowitz et al. 2008), subsequently turned into a specifier of germ cell identity, and also of *Pax6*, perhaps exapted from pigment specifier to specifier of the eye (Kozmik 2005).

Genes involved in patterning the main body axis may have also a role in the proximo-distal patterning of appendages. This secondary expression is unlikely the result of co-option of these genes' function in patterning a new body feature (the appendage) that supposedly evolved prior to, and independent of, these genes' expression. If, on the contrary, this new gene expression evolved together with the origin of the appendage, this would be a case of paramorphism (Minelli 2000). With time, the patterning role of these genes in the appendage will likely diverge from the corresponding role in the trunk; nevertheless the appendage is likely to behave like a duplicate of the main body axis and thus to retain some characteristic traits of the latter. This may explain why the appendages of segmented animals are frequently segmented, while those of unsegmented animals never are. If we accept the hypothesis of axis paramorphism, we shall perhaps revise some popular interpretation of character polarity.

For example, is the arthropod (first) antenna a specialized leg, or vice versa? Dong et al. (2001) favored the antenna-first hypothesis, whereas Casares and Mann (1998) initially supported the "leg-first" hypothesis, but in a later paper (Casares and Mann 2001) they accepted that the appendages may have been already different (and segmented) since their very first expression. However, if the relationship between the (segmented) appendages and the (also segmented) main body axis of arthropods is one of paramorphism, the whole question of the primacy of the leg versus the antenna would become meaningless (Minelli 2003b; Minelli and Fusco 2005), and no scheme of character transition from one form to the other would be applicable (Minelli et al. 2007).

SEGMENTATION: GENES AND BILATERIAN PHYLOGENY

The first suggestion that arthropod and annelid segmentation may have evolved independently, thus shaking the solidity of one of the oldest "supraphyletic" assemblages – the one closely corresponding to Cuvier's (1812) old *embranchement* of the Articulata – was based on rudimentary EvoDevo arguments (Minelli and Bortoletto 1988). Shortly thereafter, the Articulata hypothesis was rejected by a phylogenetic analysis based on a for the time extensive matrix of morphological data (Eernisse et al. 1992). Eventually, a molecular analysis (Aguinaldo et al. 1997) confirmed the lack of close affinities between arthropods and annelids and revealed the existence of a clade of molting animals, segmented and unsegmented, which received the now popular name Ecdysozoa.

In the following years, the Articulata vs Ecdysozoa debate (e.g., Schmidt-Rhaesa et al. 1998; Wägele et al. 1999; Zrzavý 2001; Scholtz 2002, 2003; Giribet 2003; Nielsen 2003a, b; Schmidt-Rhaesa 2004, 2006; Pilato et al. 2005; Ivanova-Kazas 2013) was mostly centered on steadily revised interpretations of morphological evidence (including descriptive embryology), in the light of a growing set of phylogenetic analyses. The need of a contribution from EvoDevo, however, became increasingly important, insofar as a growing detail of segmentation processes was understood at the level of gene expression, in a few model organisms at least. It became thus critically important to determine what comparative developmental genetics could say about the single or multiple origin of segmentation. Eventually, the newly emerging phylogeny (e.g., Adoutte et al. 2000; Halanych 2004; Bourlat et al. 2008; Dunn et al. 2008; Telford and Littlewood 2009; Edgecombe et al. 2011; Mallatt et al. 2012), strongly based on molecular evidence, provided a background against which the problem of the evolution of segmentation could be framed in the following alternative terms: (i) segmentation evolved before the split between Ecdysozoa and Lophotrochozoa and perhaps even before the split between Protostomia and Deuterostomia, i.e., essentially, at the base of the Bilateria – if so, segmentation would have been secondarily lost several times – or (ii) segmentation evolved independently in the arthropod, annelid, and vertebrate lineages, from unsegmented ancestors, which were also the last common ancestor of all Bilateria and the last common ancestor of Ecdysozoa and Lophotrochozoa.

Discussions about the mono- vs polyphyletic origin of segmentation are far from settled. Comparative studies of the genetic control of segmentation have played an increasing role in the dispute. In the 1980s, the presence of regularly spaced stripes of *engrailed* (*en*) expression along the elongating main axis of the embryo emerged as a potentially reliable proof in favor of a segmentation mechanism shared by all segmented metazoans. In arthropods, indeed, *en* is expressed in transversal rows of cells immediately anterior to the future segmental margin. It is also expressed in a series of transversal stripes in the embryos of leeches, polyplacophoran mollusks, onychophorans, as well as in amphioxus and in the vertebrates (Jacobs et al. 2000). This does not mean, however, that in all these metazoans *en* is actually involved in segmentation. In *Drosophila*, *en* expression is limited to the ectoderm, where it marks compartment boundaries, besides being involved in the patterning of the nervous system. In the leech, its expression extends to the mesoderm but in the ectodermal derivatives it is not involved in patterning the nervous system into segmental units (Shankland 2003). Besides these spatial (germ layer or tissue level) differences, *en* expression is also diverse temporally. In vertebrates, *en* homologs are expressed in the segmental mesodermal units (somites), but only after these are formed (Holland and Holland 1998). Moreover, homologs of *en* are present and expressed during the embryonic development, also in non-segmented animals such as mollusks (*Patella*: Nederbragt et al. 2002; Vol. 2, Chapter 7). In a variety of segmented and unsegmented animals including arthropods, annelids, mollusks, and echinoderms, the ectodermal expression of *en* is associated with skeletal development (Jacobs et al. 2000). In polychaetes, *en* is regularly expressed in the chaetal sacs (Seaver et al. 2001). In mollusks, *en-expressing* cells surround the ectodermal cells producing shell material (Moshel et al. 1998; Wanninger and Haszprunar 2001). In ophiuroid echinoderms, *en*-expressing ectodermal cells delimit the areas where the ossicles are produced (Lowe and Wray 1997). It is thus quite possible that the association of segmentation with *en* expression is only an indirect one rather than evidence of a common origin of segmentation.

More recently, the idea of a single origin of segmentation in bilaterians has been floated anew, based on the common involvement, shared between arthropods and vertebrates, of a periodic, oscillatory behavior in the expression of genes involved in the Notch/Delta signaling pathway (Stollewerk et al. 2003). To be more precise, this oscillating behavior is now firmly established as central to the segmentation process in vertebrates (e.g., Jiang et al. 2000; Holley et al. 2002; Mara et al. 2007; Özbudak and Lewis 2008; Lewis et al. 2009; Oates et al. 2012). In annelids, there is some positive evidence for the involvement of Notch signaling in segmentation in the leech *Helobdella robusta* (Rivera and Weisblat 2009), but not in the polychaete *Capitella* sp. 1 (Thamm and Seaver 2008). In arthropods, where it has been detected in several lineages (e.g., in the spider *Cupiennius salei*: Stollewerk et al. 2003; the cockroach *Periplaneta americana*: Pueyo et al. 2008; the flour beetle *Tribolium*: Sarrazin et al. 2012), this mechanism does not seem to be universally present or, at least, universally required for segmentation (Kainz et al. 2011: *Gryllus*), but this condition might well be secondary. However, the recent discovery of oscillatory transcription in *Arabidopsis*, with patterning effect on the positioning of the lateral root primordia (Moreno-Risueno et al. 2010), suggests that a “segmentation clock” is a general principle governing patterning in growing tissues, but this also suggests its multiple evolution in multicellulars (Richmond and Oates 2012); even among the metazoans, it has possibly evolved multiple times through the parallel co-option of ancestral gene regulatory networks (Chipman 2010).

RETHINKING EMBRYOLOGICAL EVIDENCE OF PHYLOGENETIC RELATIONSHIPS

The Phylogenetic Signal of Cleavage Patterns

Acoels (Chapter 9) are characterized by duet spiral cleavage; hydrozoans and other cnidarians (Chapter 6) have variable (Beklemishev 1963), unstable cleavage patterns, but this character is not easily coded in a matrix.

Synapomorphies of annelids, mollusks, entoprocts, nemerteans, and rhabditophorans are quite likely their quartet spiral cleavage, with the typical orientation of the mitotic spindles during the earliest mitoses and their characteristic cell lineage (reviewed in Nielsen 2008; cf. Vol. 2, Chapters 3, 6, 7, 8, and 9). The phylogenetic value of sharing spiral cleavage is likely strengthened by the low probability of multiple independent transitions to such an idiosyncratic cleavage pattern. The opposite transition (spiral to radial cleavage) is possibly quite easier, as shown by the coexistence of both patterns in a member of an otherwise typical spiralian group, the Rhabditophora. At the eight-cell stage, some embryos of the lecithoepitheliate *Prorhynchus stagnalis* have eight blastomeres of equal size, but others have four macromeres and four micromeres, as in radial and spiral cleavage, respectively (Steinböck and Ausserhofer 1950).

Germ Layer Homology

Rather than on objective morphological or molecular evidence, germ layers have being often identified in terms of their prospective fate. This theory-laden approach (Hall 1998) has invited comparisons even between embryos with clearly distinguishable germ layers as individualized cell sheets and embryos where germ layers are not distinguishable as morphological units. As a consequence, what had been called germ layers became the initial pools of cells eventually fated to produce specific tissues or organ systems rather than objectively recognizable morphological units in the embryo before organogenesis. Eventually, however, comparative developmental genetics has led to the identification of genes selectively expressed in one or the other of the germ layers, thus suggesting a more objective criterion upon which to compare features of embryos with morphologically identifiable germ layers with those without. For example, in their effort to homologize endomesoderm across eumetazoans – diplo- as well as triploblastic ones – Technau and Scholz (2003) have focused on *GATA 4-6*, *twist*, *snail*, and *brachyury*.

Interestingly, endoderm-specific genes have been found in *Caenorhabditis elegans*, where the distinct germ "layers" are not discernible, due to the very small total number of cells in the embryo (Maduro and Rothman 2002). Other genes, such as *snail* and *twist*, are characteristically expressed in the mesoderm. Eventually, a *snail* homolog has been found in the coral *Acropora millepora* (Hayward et al. 2004) and in the sea anemone *Nematostella vectensis* (Martindale et al. 2004), where it arguably contributes to the specification of the endoderm in respect to the ectoderm (Ball et al. 2004; Martindale et al. 2004; Chapter 6). A *twist* homolog has been found in the hydrozoan *Podocoryne carnea* (Spring et al. 2000). This is potentially of interest in respect to the repeatedly floated question of the possible presence of mesoderm in the Cnidaria, which are traditionally described as diploblastic (but see Boero et al. 1998; Seipel and Schmid 2005, 2006; Burton 2008; Chapter 6).

Persisting difficulties in finding reliable homologies between cnidarian germ layers and those of bilaterians are deepened by the diverse behavior of hydrozoans, whose germ cells generally differentiate from ectodermal interstitial cells, but in *Protohydra* and *Boreohydra*, germ cells originate instead from the endoderm (Van de Vyver 1993). Moreover, nervous cells originate from the endoderm in the hydrozoan *Phialidium gregarium* (Thomas et al. 1987) but from the ectoderm in scyphozoans (Nielsen 2001). Problems, however, are not restricted to Cnidaria. Malpighian tubules are ectodermal in insects but endodermal in chelicerates, and in tardigrades the midgut is of mesodermal origin (Kristensen 2003) rather than endodermal, as it would be expected to be.

PRIMARY VS SECONDARY LARVAE

Among the synapomorphies of clades such as the Holometabola among the Insecta and the Epimorpha among the Chilopoda are characters of their postembryonic development, holometaboly ("complete metamorphosis"), and epimorphosis (postembryonic development without addition of segments or appendages). Other "higher" taxa have been tentatively characterized by the presence of specific larval types, e.g., the trochophore or the tornaria. Larval morphology is however liable to profound and even rapid change, up to complete disappearance. EvoDevo can thus offer a valuable contribution to phylogenetics, insofar as it can provide reliable scenarios of the evolvability of larvae and determine the degree to which larval and adult traits can actually evolve independently – a property likely to be different in different major clades of metazoans.

Quite long ago, Steinböck (1963) argued that the phylogenetic significance of the larvae has been considerably overestimated. Today, in the context of cladistic methods and language, we can say that even coding larval characters in matrices intended for the reconstruction of "higher" group relationships is fraught with problems. First, we have not even a satisfactory definition of larva (for a discussion, see Minelli 2009). Second, across the metazoans, larvae certainly evolved several times. Third, the widely accepted distinction between primary and secondary larvae is far from obvious and perhaps unwarranted. This is briefly discussed here.

When proposing a distinction between primary and secondary larvae, it is necessary to specify the node(s) of the phylogenetic tree corresponding to ground plans we credit with possessing either larval type. In the literature it seems often to be implicitly accepted that the last common ancestor of all recent metazoans, the Urbilateria, was an indirect developer. This does not rule out, however, the possibility that some clades re-evolved a secondary larva after having lost the primary one.

According to the phylogenetic scenario proposed by (Davidson 1991; see also Peterson et al.

1997; Cameron et al. 1998; Peterson and Davidson 2000), ancestral bilaterians would have lacked the later evolved genetic circuitry responsible for the complex body structure of their modern descendants. Their simpler genetic networks were only capable to produce little animals with a bodily organization directly comparable to that of the larva of many living invertebrates. In this scenario, the modern bilaterian adult is interpreted as an evolutionary novelty, a terminal addition grafted onto the original body plan, which is eventually conserved in the larva. As a consequence, larvae such as the trochophore and the tornaria would be primary because they would be older and recapitulative in respect to the corresponding adults. An often implied corollary is their supposed monophyletic origin.

However, there are problems with phylogeny (Valentine et al. 1999; Jenner 2000; Sly et al. 2003). Mollusk veligers are probably homoplastic (Ponder and Lindberg 1997; Waller 1998; Lindberg et al. 2004). Transitions from one larval type to another are frequent and often reversible. Planktotrophic larvae corresponding to the "primary" larva of Davidson and others are often lost and acquired again (Haszprunar et al. 1995; McEdward and Janies 1997; McHugh and Rouse 1998). Independent transition from planktonic to non-planktonic larvae occurred many times even within one genus, as in the case of *Conus* (Duda and Palumbi 1999).

The opposite idea that all larvae are secondary has been championed by many authors (e.g., Garstang 1922; de Beer 1954; Hadži 1955; Steinböck 1963; Conway Morris 1998; Valentine and Collins 2000; Collins and Valentine 2001; Hadfield et al. 2001), although often without a precise reference to a specific node in the metazoan tree.

The most serious difficulty with Davidson's scenario is the implied polyphyletic origin of the "zootype," that is, of the anterior-posterior patterning of the main axis of the bilaterians controlled by the Hox genes (Slack et al. 1993). Nothing like a zootype organization is found in any of the putative "primary" larvae. For example, in the pluteus of the sea urchin *Strongylocentrotus purpuratus*, Hox gene expression is limited to the adult rudiment (Arenas-Mena et al. 2000). Similarly, in the trochophore of the polychaete *Chaetopterus*, Hox gene expression is limited to future adult tissues, while it does not show up in any of the larval structures that are fated to disappear at metamorphosis (Peterson et al. 2000). Things are broadly similar in other polychaetes, although in the late trochophore of *Platynereis dumerilii*, *Hox1* is expressed in the apical tuft cells (Kulakova et al. 2007).

Nielsen (2003a, b) regarded the lack of Hox gene expression in these larvae as an argument in favor of their primary nature. However, it is also possible (Minelli 2009) that the anterior-posterior patterning of the main body axis is a very old feature. If so, the lack of Hox gene expression in the larval tissues may indicate that the larva has been secondarily intercalated in the developmental schedule, in correspondence to an early developmental phase where Hox genes were still silent. We should not rule out, however, that other larvae may correspond to a later, Hox-expressing developmental phase. Let's remark in this context that trochophore-like larvae may have evolved repeatedly (Haszprunar et al. 1995).

TEMPO AND MODE IN EVOLUTION

Heterochrony in Phylogenetics: Noise or Data?

From the perspective of Haeckelian recapitulation, heterochrony is exception to the rule; in inferring phylogeny from ontogeny, it turns straight into noise. Indeed, it was right by showing the pervasiveness of heterochrony throughout the animal kingdom that de Beer, as mentioned before, was able to refute the "biogenetic law." However, de Beer was also able to provide a first classification of the possible kinds of change in ontogenetic sequences, thus remotely introducing two ideas that could be subsequently exploited in phylogenetics.

On the one side, de Beer's analysis suggested at least some degree of modularity of ontogenetic sequences. Anticipation, postponement, and changes in relative speed can only be predicated of "units," be these individual developmental processes or individual developmental stages.

This could suggest that homologs of which we can trace the evolution are not necessarily the organs – more generally, the structural features – of adult animals but perhaps also those of earlier stages or, better, (i) ontogenetic stages as such (e.g., the gastrula or the germband stage in arthropod embryonic development) and (ii) developmental processes as such (e.g., gastrulation, or a particular sequence of cell lineage).

On the other hand, the very possibility to classify heterochronies could invite a search for the phylogenetic signal possibly present in heterochronies as such. Patterns of heterochrony may contain useful phylogenetic signal, as demonstrated by, e.g., Guralnick and Lindberg (2001), who produced a phylogenetic tree of several lophotrochozoan taxa based on the timing of cell lineage events and found that the phylogenetic hypothesis thus obtained replicated patterns found in more traditional analyses. In another study, patterns of heterochrony in the developmental sequences of Branchiopoda were used to identify the origin of Cladocera (Fritsch et al. 2013).

Growth Heterochrony vs Sequence Heterochrony

At the beginning of this century, a decisive enhancement of the use of heterochrony as a source of data for phylogenetic reconstruction was obtained following a shift of focus from growth heterochrony to sequence heterochrony, to use a terminology introduced by Smith (2001). Virtually all of the traditional literature on heterochrony (e.g., Gould 1977; Alberch et al. 1979; McNamara 1986, 1995; McKinney 1988; McKinney and McNamara 1991) refers to *growth heterochrony*, i.e., to developmental changes in size and shape relationships.

However, many interesting evolutionary changes in developmental schedules are not changes in either size or shape. This is why Smith (1996, 2001, 2002, 2003) and Velhagen (1997) have suggested a different approach, termed *sequence heterochrony*, in which heterochrony is identified in the changes in the position of a developmental event relative to other events in the same ontogenetic sequence. Several techniques have been proposed to analyze sequence heterochronies. Any two events A and B in a developmental sequence occur in one of the following orders: (i) A occurs before B, (ii) A and B are simultaneous, or (iii) A occurs after B. These timing relationships, or event pairs, are given a numerical score. Data are thus assembled in a matrix that can be analyzed under maximum parsimony. In these efforts, the major problem to be addressed is how to dissect ontogeny into reasonably independent units, as required by a cladistic analysis. This difficulty was acknowledged since the earliest studies in this area (e.g., Velhagen 1997; Bininda-Emonds et al. 2002). Schulmeister and Wheeler (2004) remarked that the optimization of developmental event sequences on a given cladogram based on event pairing may lead to unacceptable results because event pairing treats interdependent features as if they were independent. To overcome this problem, they suggested a method of character optimization treating the entire developmental sequence as a single character and aiming to determine the transformation cost between pairs of character states. Parsimov, another method for examining heterochronies in a phylogenetic framework, was introduced by Jeffery et al. (2005). In this parsimony-based method, the least number of event displacements (heterochronies) that explains all the observed event-pair changes is identified for each branch of the tree, thus eventually obtaining all alternative, equally parsimonious explanations, out of which a consensus is derived that contains the developmental changes that form part of every equally most parsimonious explanation.

Hot Points of Change Along the Developmental Schedule

One of the reasons to abandon von Baer's (1828) scenario of morphological divergence regularly increasing with the embryos progressing along their developmental trajectory and Haeckel's recapitulationist view according to which the evolutionary novelties are essentially terminal additions to the largely invariant earlier developmental stages is the fact that some developmental stages are more conservative (or more variable) than others, although not in a monotonic relationship with developmental age.

It is now fashionable to describe embryonic development in terms of the so-called hourglass model, to signify that the earliest stages (especially, but not exclusively, those under exclusive or prevailing control of maternal genes) are more extensively and easily divergent than later embryonic stages (Duboule 1994; Raff 1996; Hall 1997; Galis and Metz 2001). From initially different starting points (first discussed for insects by Sander 1976), developmental trajectories converge toward a much more conserved stage, often recognizable as characteristic for an individual phylum, which is called the phylotypic stage (Sander 1983) or at least a largely conserved segment of the developmental trajectory that has been termed the phylotypic period (Richardson et al. 1997). As expected, gene expression is maximally conserved around the phylotypic period (*Drosophila*: Kalinka et al. 2010).

Early-stage divergence, especially between closely related species, is often a direct consequence of the different amount of yolk stored in the female gamete during oogenesis; for example, thus is the case of two sea urchin species, the lecithotrophic *Heliocidaris erythrogramma* and the planktotrophic *H. tuberculata* (e.g., Parks et al. 1988; Wray and Raff 1991; Henry et al. 1992). More interesting, however, are other examples of early-stage divergence that cannot be explained in such a simple "mechanistic" way. The most dramatic case is the nematodes, among which the pattern of cleavage, the spatial arrangement, and the differentiation of cells have diverged dramatically during the history of the phylum, without producing corresponding changes in the adult phenotype (Schierenberg and Schulze 2008; Schulze and Schierenberg 2011).

Early divergence is sometimes noticeable even at intraspecific level, as shown by Tills et al. (2011) for the pond snail *Radix balthica*.

Heterochrony is not limited to the embryonic segment of the developmental schedule, but its occurrence along the postembryonic development is not frequently studied and is still less used to infer phylogenetic relationship. A promising example is the crustacean genus *Niphargus*: a preliminary study by Fišer et al. (2008) has revealed extensive sequence heterochrony along the postembryonic development, independence between events being more pronounced in mid-aged instars.

Saltational Evolution and Discontinuous Variation

Continuous variation is notoriously difficult to handle when we are confronted with the problem of partitioning it into bins to be differently coded in a data matrix used in a phylogenetic analysis. However, from the perspective of evolutionary change, continuous variation fits well within a gradualistic neo-Darwinian paradigm. The opposite is true when the observed character states are widely separated. In this case, there is no problem in partitioning our set into unambiguously distinct classes (unless the differences are so big that we may have problems recognizing two states as homologous). However, from an evolutionary point of view, we would not expect closely related taxa to be separated by an apparently unbridgeable gap. In other terms, we do not expect evolution to be saltational. However, this expectation is due for revision, in the light of facts that are possibly intractable in a traditional evolutionary scenario, but may become reasonable in the light of EvoDevo.

Major phenotypic differences may not necessarily depend on major changes or even rearrangements, at the genetic or genomic level. As mentioned above, the genotype→phenotype map is not necessarily simple or obvious, and a single instance of saltational evolution may require a reassessment of the phylogenetic signal carried by a given character. For example, the presence of 21 or 23 pairs of legs in the adult was long regarded as a reliable synapomorphy of the Scolopendromorpha, the other "higher" clades among the Chilopoda having instead either 15 (Scutigeromorpha, Craterostigmomorpha, Lithobiomorpha) or at least 27 (Geophilomorpha) pairs of legs. Recently, a scolopendromorph species with either 39 or 43 pairs of legs has been described (Chagas et al. 2008). What most matters (Minelli et al. 2009) besides the obvious need to reformulate the diagnosis of the clade Scolopendromorpha is that the newly discovered species (*Scolopendropsis duplicata*) is not the sister group to all remaining scolopendromorphs, or at least to a substantial subclade within them, but a very close relative of a "normal" species (*Scolopendropsis bahiensis*), to the same genus of which it has been thus assigned. The nature of the change in developmental mechanisms that in this case has broken a long entrenched phenotypic stability (the Carboniferous *Mazoscolopendra* had 21 pairs of legs; Mundel 1979) is not known, but it is not difficult to hypothesize a point mutation potentially responsible for this one-shot duplication of segment number.

Patterns of "saltational" variation are perhaps less rare than our gradualistic tradition has thus far invited to expect. EvoDevo is the obvious tool for accommodating them within our growing hypotheses of phylogenetic relationships.

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