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The evolution of body plans: HOM/*Hox* cluster evolution, model systems, and the importance of phylogeny

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20.1 Introduction

Most evolutionary biologists wish to explain evolutionary patterns in the astonishing diversity among organisms. Developmental biologists ultimately hope to explain the developmental mechanisms that are at the basis for the wide array of Baupläne that characterize and differentiate phyla. In trying to understand biological diversification, albeit at different levels of inquiry, is where the interests of developmental biologists and evolutionary biologists meet. Moreover, developmental processes evolve just like other aspects of organisms. However, development is, in most evolutionary research, treated like a black box and most developmental biologists in turn typically do not recognize the potential contribution that evolutionary biology can make to the understanding of development. Historically, the connection between ontogeny and phylogeny was well appreciated and also reflected in the interchangeable use of the word 'evolution' (von Baer 1828, 1864; Haeckel 1866; Gould 1977). Research on the development–evolution connection lay dormant for over a century and was only recently re-established by evolutionary biologists considering 'developmental constraints' and the timing of developmental events as factors in shaping and constraining the evolution of adult phenotypes (for reviews see Baldwin 1902; Waddington 1957; Gould 1977; Alberch *et al.* 1979; Goodwin *et al.* 1983; Raff and Kaufman 1983; Arthur 1984; Northcutt 1990; Wray and Raff 1991, Hall 1992; Wray 1992, 1995; Wake 1995).

The recent establishment of powerful molecular genetics methods has allowed developmental biologists to identify developmental control genes and some of their interactions in early development (for example Nüsslein-Volhard and Wieschaus 1980; reviewed in Lawrence 1992). Because of the time-consuming and laborious need to establish baseline data on development, developmental investigations can typically focus on only a very small number of animal model systems. The major model organisms in developmental biology are mouse, frog, zebrafish, sea urchin, fly, and nematode (this is obviously an incomplete list and

other widely studied species include, for example, the salamander and the leech). These models are widely spread across the evolutionary tree of animals (Fig. 20.1) and their phylogenetic relationships are relatively undisputed. Developmental patterns and processes that are established from these model systems are assumed to be typical for a much larger number of species, at least those within the clade to which the model belongs. For example, lessons from 'the vertebrate models' are thought to apply to all vertebrates including man.

Currently, many developmental biologists place much importance in the development–evolution connection and attempt to explain the evolution of diverse body plans by changes in the HOM/*Hox* cluster architecture (e.g. Akam *et al.* 1988, 1994; Holland *et al.* 1994; Ruddle *et al.* 1994; Patel 1994 and references therein).

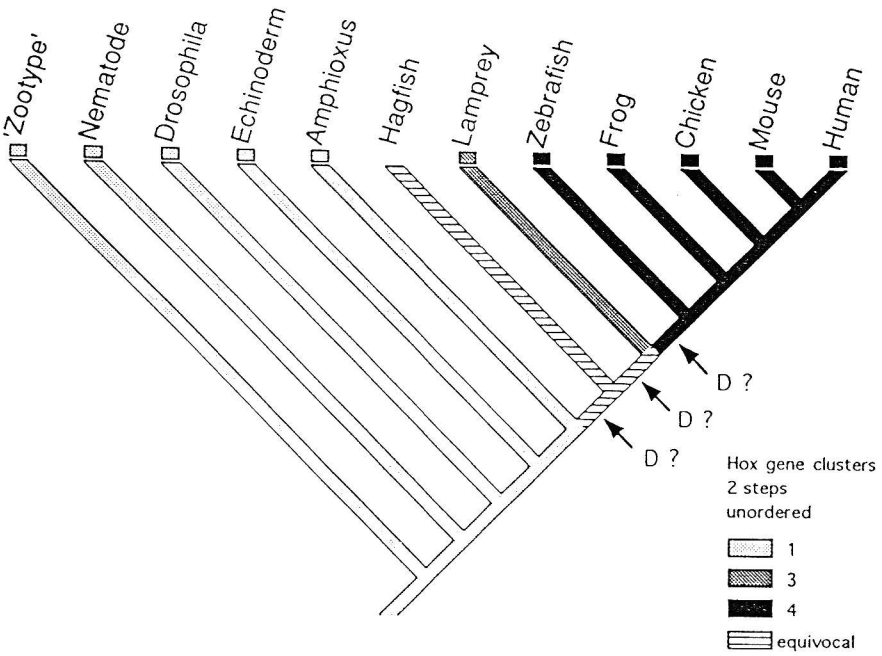

Fig. 20.1 Probable phylogenetic relationships among the major model systems used in developmental biology and some other crucial taxa for which the number of HOM/*Hox* clusters has been estimated or determined. At least two steps (evolutionary transitions) are required to go from the ancestral condition of a single HOM/*Hox* cluster to the presumed three HOM/*Hox* clusters in the lamprey and the presumed four-cluster condition in vertebrates. It is not known with certainty how many HOM/*Hox* clusters the hagfish and the lamprey (it is estimated to have three clusters) have, therefore the branch leading to the hagfish is drawn as 'equivocal'. This and several of the following figure are graphed and data analyzed with MacClade (Maddison and Maddison 1992). 'D' indicates where and when during metazoan HOM/*Hox* clusters might have been duplicated.

In evolutionary biology, the comparative method has long been the favoured approach for addressing many different kinds of questions (for example on adaptation) (Ghiselin 1984; see references in Harvey and Pagel 1991; Brooks and McLennan 1991). The basic idea is to study the evolution of phenotypic characteristics of taxa (species or phyla) based on knowledge of the phylogenetic relationships among them. Therefore, at the core of the comparative method is a phylogeny, ideally firmly established and based on characters that are not going to be studied with the comparative approach. From this phylogeny the tracing of character evolution, developmental or otherwise, can be attempted. The modern use of the comparative method is based on the development of rigorous statistical and cladistic approaches to both the reconstruction of phylogenetic relationships and the study of character evolution (for example Swofford 1991; Maddison and Maddison 1992). The arrival of the polymerase chain reaction (PCR) as a powerful molecular method which greatly facilitates the gathering of molecular data for phylogenetic work coincided with the development of statistical methods in the comparative approach (Felsenstein 1985*b*; reviewed in Harvey and Pagel 1991; Brooks and McLennan 1991). Surprisingly, so far the comparative method in evolutionary biology has not been used to predict the evolution of developmental processes from evolutionary patterns of phylogenetic relationships (reviewed in Brooks and McLennan 1991; Harvey and Pagel 1991). Both major reviews of the comparative consider the ontogeny–phylogeney connection only for the 'polarization' of characters, i.e. character states are treated as ancestral if they occur early, or derived if they occur later in development. The knowledge of the polarity of character state changes aids in the 'rooting' of phylogenetic trees.

Despite their recent interest in evolution, developmental biologists, typically have only made pairwise comparisons of developmental features; yet many of these comparisons have yielded highly interesting and often surprising results about evolutionary differences or similarities in development (for example *Drosophila* and *Tribolium* (Sommer and Tautz 1993) or nematodes (Sommer and Sternberg 1994); for similarity in early determination of polarity in *Drosophila* and *Caenorhabditis* see references in Kimble (1994)). However, pairwise comparisons have inherent limitations and do not provide nearly as much information as comparisons between more than two taxa, in an explicitly phylogenetic context (Garland and Adolph 1994). Figure 20.2 outlines one way in which a comparison involving more than two taxa in a phylogenetic context can be much more powerful than a pairwise comparison. A pairwise comparison between any two taxa (i.e. species or phyla for example) (from A to E) would not be able to establish whether two traits (for example the relative timing and domain of expression of two homeobox genes) evolved at the same time or in two consecutive steps, and, if the latter, in which order (Fig. 20.2a, b). However the comparison of the taxa under consideration (A–C) with more distantly related ones (D + E) might allow one to determine the sequence of evolutionary events. In this example (Fig. 20.2c) the comparisons of character state distributions between species of the two clades (A–C, D + E) might allow

one to do so. This example suggests that in a first event the 'black box' evolved (in the common ancestor of both clades) followed in a second step by the evolution of the 'white box' in the common ancestor of the clade A–C.

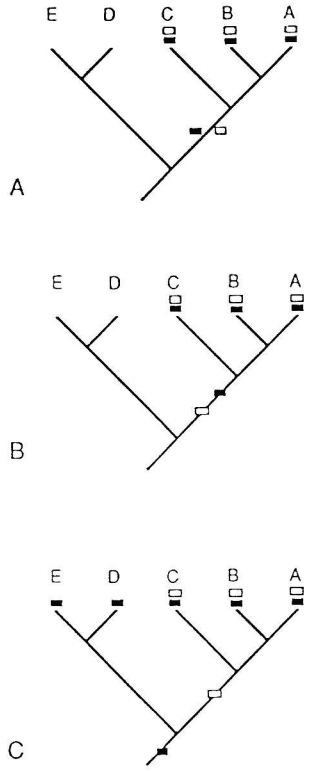

Fig. 20.2 Hypothetical phylogeny of five taxa (A–E). By comparing the character state distributions between more than two taxa the some hypotheses about the origin of two trait are less likely than others. The phylogeny and distribution of character states suggests that the 'black' trait evolved before the 'white' trait which evolved along the branch shared by A–C and their common ancestor. C: This information would rule out hypothesis A (the evolution of both traits at the same time in the common ancestor of A–C) and hypothesis B (the consecutive evolution of the 'black' and 'white' trait (in no particular order) along the branch leading to taxa A–C).

There are few studies that included phylogenetic and ontogenetic information for more than two species (DeSalle and Grimaldi 1993; Luk *et al.* 1994; Patel 1994; Wray and Bely 1994). A small number of excellent phylogeny-based developmental studies exist comparing developmental patterns and the evolution of body plans in arthropods (Patel 1994; Akam *et al.* 1994 and references therein) and echinoderms (Raff 1992; Wray and Bely 1994, and references therein); these studies tend to be concerned with distantly related species. However, it is known that developmental mechanisms can differ dramatically

between even closely related species, such as in direct and indirect developing sea urchins and amphibians (del Pino and Elinson 1983; Elinson 1990; Wray and Raff 1991; Raff 1992; Jeffrey and Swalla 1992; Wray 1992, 1995; Henry & Martindale 1994). There are known instances where fundamentally different embryological trajectories result in phenotypically similar adults (for example in congeneric sea urchins (Raff 1992; Wray and Raff 1991)) and conversely, similar developments can result in strikingly different adult phenotypes, as is illustrated by the many cases of large morphological differences among closely related species. Therefore, the importance of comparative developmental work among closely related species (such as different species of zebrafish) in an explicitly phylogenetic context was stressed recently (Meyer *et al.* 1995).

Developmental biologists have made much progress by incorporating evolutionary thinking into their research agenda. However, the acceptance of evolution's relevance for the understanding of development has been somewhat incomplete. There are several ways in which knowledge about the evolution of, and specifically the phylogenetic relationships among, model systems can aid in the understanding of developmental processes (Kellog and Shaffer 1993, Meyer *et al.* 1993, 1995). Here I wish to point out that the comparative method can predict the likely condition in common ancestors, might permit the reconstruction of intermediate stages, might be able to determine the historical sequence of evolutionary events, and has the capacity to falsify or support hypotheses about the evolution of developmental processes.

The combination of (1) the dramatic advances in developmental biology, (2) the elaboration of statistical tests in the comparative method, and (3) the power of molecular datasets for phylogeny reconstruction, might now significantly facilitate progress on the understanding of the development–evolution connection. Unfortunately, many phylogenetic hypotheses about the relationships of animal phyla are still hotly debated. Strongly supported phylogenetic estimates must underlie all further work on the ontogeny–phylogeny connection if we hope to establish a causal relationship between ontogenetic changes, i.e. in evolution of HOM/*Hox* clusters, and the evolution of body plans.

19.2 Bauplan evolution and phylogeny of chordates: is there a correlation with the evolution of the HOM/*Hox* cluster architecture?

Homeobox (HOM/*Hox*) genes are found in all metazoans, for example in platyhelminth (Kenyon and Wang 1991; Bartels *et al.* 1993) and annelid worms (Dick and Buss 1994), cnidarians (Schierwater *et al.* 1991), and even plants (for review see De Robertis 1994; Gehring 1994). They code for a class of transcription factors defined by a helix-turn-helix motif with a 183-nucleotide core sequence that are involved in the regulation of developmental genes in animals. There are (at least) 38 *Hox* genes in mouse and human which are organized into four clusters (termed A–D or 1–4) of up to 13 members per

cluster (reviewed in Scott 1990; Holland 1992; Garcia-Fernandez and Holland 1994; De Robertis 1994; Gehring 1994) (Fig. 20.3). Additionally, numerous individual homeobox genes that are not part of the HOM/*Hox* cluster are also present in most animals' genomes.

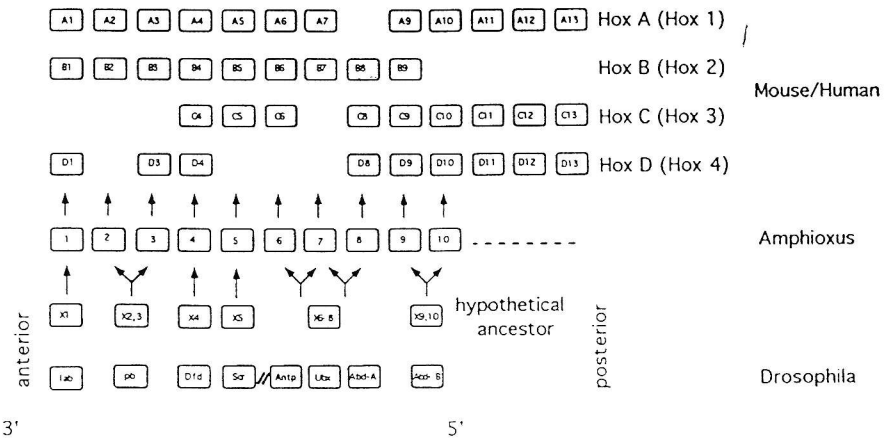

Fig. 20.3 Architecture of the HOM/*Hox* clusters of *Drosophila*, amphioxus, the mouse, and the presumed ancestral condition of (Redrawn after Garcia-Fernandez and Holland 1994.)

The major model systems in developmental biology differ in their number of HOM/Hox clusters (Figs. 20.1, 20.3), but how and when during evolution the number of clusters increased from the single ancestral cluster to the derived tetrapod condition of four is not known (see Fig. 20.1). The relative position of individual *Hox* genes in the HOM/*Hox* gene cluster defines, among other features, the sequence of transcription during development of the anterior–posterior axis, sensitivity to retinoic acid, and expression and relative positions of developing structures in the embryo (Scott and Carroll 1987; McGinnis and Krumlauf 1992; Slack *et al.* 1993; Marshall *et al.* 1994; Warren *et al.* 1994 and references therein). The increasing complexity in the homeobox cluster number and architecture has been hypothesized to be related to the increasing complexity in body plans among phyla of animals.

The assumed omnipresence of homeobox genes arranged into a HOM/*Hox* cluster of common architecture in animals recently led to the suggestion that a common, defining (in cladistic terminology, synapomorphic) character of all animals and their hypothetical ancestor (the 'zootype') is the presence of this *Hox* gene cluster (Slack *et al.* 1993) (Fig. 20.1). The 'zootype' concept, and that of the existence of phylotypic stages, are gaining in acceptance also outside the developmental biology community (for example Minelli and Schram 1994).

During metazoan evolution there were probably only three homeobox genes in the ancestral metazoan homeobox cluster (Akam *et al.* 1988; Kappen *et al.* 1989; Murtha *et al.* 1991; Holland 1992; Schubert *et al.* 1993). It is not known

exactly how many and which class of homeobox genes made up the single ancestral chordate HOM/*Hox* cluster (Garcia-Fernandez and Holland 1994). It probably included five (Schubert *et al.* 1993) or six homeobox genes (Garcia-Fernandez and Holland 1994) (Fig. 20.3). Based on sequence comparisons it has been established that during deuterostome evolution several tandem duplications of the most 5′ group of homeobox genes (from a single gene that is homologous to the *Drosophila Abd-B*) occurred. Echinoderms might have only one *Abdominal B* (*Abd-B*) related gene (cognate group 9) and amphioxus (a cephalochordate) has at least two (cognate groups 9 and 10) (Garcia-Fernandez and Holland 1994) in their single cluster. All vertebrates that have been investigated have up to five *Abd-B* related genes, increasing the total number of cognate groups to 13 and the cluster number to four (see for example Pavel and Stellwag 1994) (Fig. 20.3). Not all four clusters contain the same number (due to independent deletions and duplications of genes) or members of the same *Hox* 'cognate groups', and only group 4 and group 9 homeobox genes are found in all four homeobox gene clusters (Fig. 20.3). Therefore, the exact homologies between homeobox genes of the derived vertebrate condition and the ancestral chordate one, or even more distantly related insect and worm clusters, are not entirely clear (see for example Garcia-Fernandez and Holland (1994) and references therein).

The substantial similarity, in terms of relative timing and position of expression, of mouse, human, and *Drosophila* homeobox genes suggests that HOM/*Hox* cluster and function is strikingly conserved over huge evolutionary time spans (Graham *et al.* 1989). Evidence for the astonishingly conserved positional information during development of homeobox genes also comes from transgenic experiments. When mouse genes (*Hoxb-6*) were expressed in *Drosophila*, ectopic mutations (antennal legs) were induced (Malicki *et al.* 1991) showing that anterior–posterior axis determination in flies and vertebrates are similarly controlled and conserved. Elegant 'promoter swap' experiments in mice provide another similar approach (Lufkin *et al.* 1992). Here, the control of the expression of *Hoxd-4* gene was placed under the control of the promoter of the *Hoxa-1* gene in transgenic mice. The effect was that the expression of *Hox-4.2* (and probably also those of several downstream effectors), which typically occurs in the cervical vertebrae of mice, was moved anteriorly to a region including the occipital bones which were homeotically transformed into structures that resembled cervical vertebrae. Other characteristic homeotic transformations were discovered in earlier classic experiments on transgenic mice (involving *Hox-1.1*) and retinoic acid treated mice (Kessel *et al.* 1990 and references therein).

It seems paradoxical that, at the DNA level and at the level of gene order, the homeobox genes and HOM/*Hox* cluster architecture are evolutionarily so conserved, yet they are often said to be the cause of or at least correlated with the diversity of body plans that differentiate phyla of animals (Akam *et al.* 1988; Holland 1992; see references in Akam *et al.* 1994). The cause for Bauplan evolution therefore cannot simply be the gene order of homeobox genes within a

cluster, which, as far as is known, is conserved across species in different phyla with profoundly different body plans. It is more likely that the increase in HOM/*Hox* cluster number (and variation in the number of homeobox genes per cluster), changes in function of individual homeobox genes, co-option into new roles, changes in the regulation of expression of homeobox genes, and increased complexity in the nexus of communication between these individual transcription factors are all partly responsible for increased complexity of body plans during evolution (Akam *et al.* 1988; Holland 1992; references in Akam *et al.* 1994).

The increasing complexity at the genetic level both in terms of numbers of genes per cluster and in terms of the number of clusters could be responsible for the increasing complexity of development and adult morphology throughout evolution in several ways. Gene duplications, which free up the old or the new copy of a gene, or group of genes, to take on a new function are possibly one of the major forces of molecular evolution that can lead to the evolution of new function and novelty (Ohno 1970; Zuckerkandl 1994; Walsh 1995). Duplications of homeobox genes would free up these transcription factors to take on new functions. Alternatively, the regulatory control of the expression of genes is also likely to be involved, and may be an even more important force in evolution than gene duplications (see review in Wilson *et al.* 1977).

Within the phylum Chordata, it was suggested that serial homology of fins in fishes and the origin of the tetrapod limb might be due to the ectopic expression and duplication of some *Abd-B* related genes (Ahlberg 1993; Tabin and Laufer 1993). Moreover, it was suggested that the evolutionary origin and transition from paired fins to the tetrapod pentadactyl limb is related to the above-mentioned cluster duplications and tandem duplications from a single to a final number of five *Abd-B* related genes per cluster (cognate groups 9–13) (Tabin 1992; but see Coates 1994; Favier *et al.* 1995). These hypotheses could be addressed in a phylogenetic framework since the duplications of these genes should not post-date the evolutionary origin of fins and pentadactyl limbs—if they are really causally related to the origin and increasing complexity of paired appendages. However, for this set of hypotheses to be tested much more information on the phylogeny of chordates, homeobox cluster architecture, and the mode and timing of cluster duplications during vertebrate evolution remains to be collected (e.g. Fig. 20.1). Since the homeobox cluster has been mapped in only a single cephalochordate it is unclear in which of several possible ways the postulated duplications of *Abd-B* related genes and the *Hox* gene clusters occurred.

During the evolution of chordates the ancestral chordate cluster was duplicated (in at least two duplication events) to the vertebrate condition of four clusters. The duplications from the one ancestral chordate cluster in amphioxus to the four clusters in all (?) vertebrates must have occurred after the evolution of cephalochordates (Garcia-Fernandez and Holland 1994). PCR-based approaches have been applied to lampreys, and these have suggested that it has at least three clusters (reviewed in Ruddle *et al.* 1994) (Fig. 20.1). It is not

clear which of the four vertebrate clusters (A–D) is the most ancient and most closely related to the ancestral chordate cluster (Garcia-Fernandez and Holland 1994) (Fig. 20.2). There are 15 possible relationships (bifurcating trees) among the four clusters that relate the four gene clusters to an ancestral one (Felsenstein 1978). Therefore, there would be 15 possible bifurcating relationships (discounting the possibility that more clusters first evolved and then were later deleted) if the four clusters arose by individual duplication events (similar to speciation or bifuraction events). However, only two evolutionary events would be required if whole-genome duplication events (increases in ploidy) caused the increase in HOM/*Hox* cluster number from the single-cluster ancestral condition to the presumed typical four-cluster vertebrate condition, with two clusters being intermediate. There are other duplicated genes up- and downstream of these clusters (for example keratin-coding genes) that are duplicated (R. Krumlauf, personal communication). This would seem to support the hypotheses that two whole-genome duplication events during the evolution of the chordates led to the presumed typical four-cluster condition in tetrapods. However, if HOM/*Hox* cluster evolution proceeded by whole-genome duplications then, if taxa with three HOM/*Hox* clusters were to be found it would seem to imply that the fourth cluster was subsequently lost. For example, if agnathan fish are monophyletic, and if the estimate of three clusters in lampreys is correct then probably one cluster was lost independently in lampreys. In this case, two duplication events must be postulated after the splitting off of amphioxus from the stem leading to higher chordates, and hagfish possibly already possessed four HOM/*Hox* clusters (Fig. 20.1). Alternative scenarios could be constructed based on the model of cluster evolution, and the phylogeny of chordates. However, since it is not firmly established (i) how many HOM/*Hox* clusters hagfish and lampreys have, (ii) how the cluster duplications occurred, and (iii) what the phylogeny of chordates is, these alternative scenarios remain just speculations.

A preliminary phylogenetic analysis using *Drosophila*, amphioxus, and the four mouse clusters based on cognate group 9 amino acid sequences of the homeobox domain and flanking regions from Garcia-Fernandez and Holland's study (1994) analyzed with PROTPARS in PAUP (Swofford 1991) found only weak support for one of the 15 possible relationship (Fig. 20.4). This very preliminary analysis suggests a gene tree relationship between the four clusters in which the B cluster is the most ancestral, the C+D clusters are the most derived and the A cluster is the next most closely related one to the C+D group (Fig. 20.4). The amino acids of group 4 *Hox* genes, which is the only other group of *Hox* genes that is present in all four vertebrate HOM/*Hox* clusters, did not allow any resolution since two equally parsimonious solutions were found, the consensus of which was a completely unresolved tree. Unfortunately, the *Hox* genes are highly conserved and do not contain much phylogenetic information at the DNA or amino acid level. Future phylogenetic work on this question will need to consider the inclusion of more extensive and more variable flanking regions.

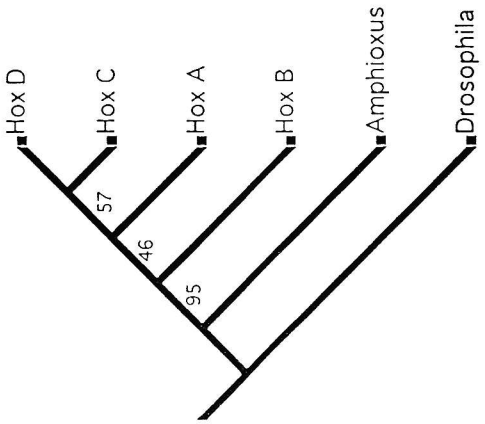

Fig. 20.4 The preliminary gene tree supported most strongly by a parsimony analysis. Numbers indicate bootstrap values for chordates (Felsenstein 1985) in 100 replications with PAUP (Swofford 1991).

In addition to the importance of phylogenetic knowledge (also see below) alternative models of character evolution need to be considered (an incomplete list is provided in Fig. 20.5) when one wants to attempts the reconstruction of historical events using the comparative method. Several alternative models of character evolution exist, the most unrestrictive is 'unordered' parsimony which permits the change from one character state to any other in a single evolutionary event (Fig. 20.5). Other more restrictive models of character change than 'unordered' parsimony, such as 'Dollo' parsimony, will always require more explanations, i.e. more steps in the reconstruction of evolutionary history (Figs. 20.5 and 20.6). For example, in 'ordered parsimony' the evolution from one character state to another can occur only in an ordered sequence in which evolution has to proceed through intermediate stages. In 'Dollo parsimony' it is assumed that a particular derived character state can only be gained once throughout the history of a group, but that it can be loss independently several times. As such 'Dollo parsimony' is less restrictive than 'irreversible parsimony' where no reversals at all are tolerated (Fig. 20.5). The 'Dollo' model might apply to the evolution of HOM/*Hox* clusters such that duplications of whole clusters or the whole genome are unlikely evolutionary events that are likely to have occurred only once during evolutionary history, but that the loss or inactivation of clusters or individual elements of the cluster are more likely to occur repeatedly. If such a model is invoked for the evolution of the HOM/*Hox* clusters then a different (more complex) explanation might be necessary than if the gain and loss of a particular character states were equally likely (see the simple example in Fig. 20.6).

These alternative scenarios of HOM/*Hox* cluster evolution can be tested by further sequencing of homeobox clusters in crucial taxa such as echinoderms, tunicates, hagfish, and lampreys. Once the phylogeny of these species is well established (see below) a likely model of evolutionary change in *Hox* clusters

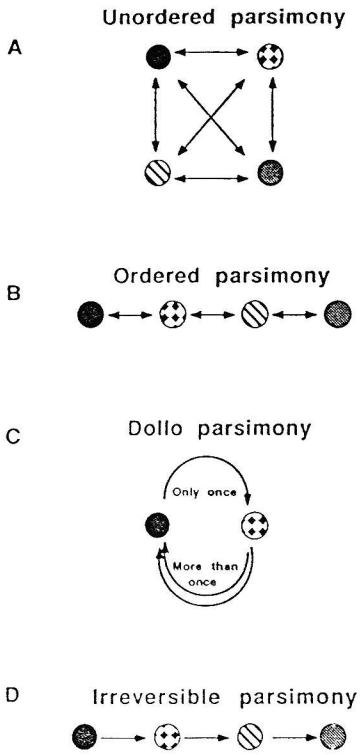

Fig. 20.5 Alternative models of evolutionary change. A: 'unordered parsimony' (also called Fitch parsimony) here changes between all observed character states can be made in a single step. B: 'ordered parsimony' (also called Wagner parsimony) requires that an order is maintained between transitions between different character states. Reversals are allowed. C: Dollo parsimony stipulates that derived character states only evolve once during the evolutionary history of a group, but that they can be lost more than once. D: irreversible parsimony (also called Camin–Sokal parsimony) requires that once a character state change occurred it cannot be reversed to a more ancestral state, but that it can change into a even more derived condition.

can be deduced. Depending on the outcome in these projects, other primitive fishes such as chondrostean and chondrichthyans may also need to be investigated for their HOM/*Hox* cluster architecture to further test the hypothesis of how HOM/*Hox* cluster evolution is linked to the increasing complexity of body plans in chordates. Current knowledge of homeobox cluster evolution, both in terms of within- and between-cluster evolution, is still too sketchy and too concentrated on a very small number of animal model systems to allow us to predict the mode and timing of deletion and duplication events during chordate evolution (Fig. 20.1). Without more comparative data on maps and sequences of complete homeobox clusters from a wider range of organisms from the 'tree of life', hypotheses into the unanswered questions surrounding the relationship of homeobox cluster evolution and body plan evolution in chordates will remain highly speculative.

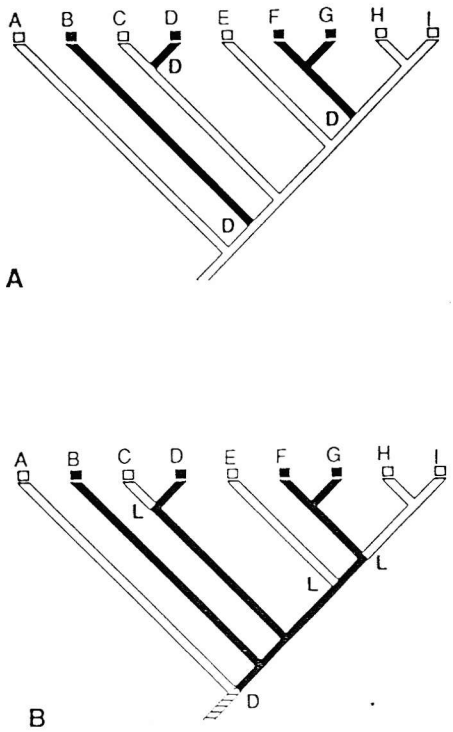

Fig. 20.6 A hypothetical phylogeny among taxa A–I. The reconstruction of the derived condition 'black' based on a model of A 'unordered parsimony' and B 'Dollo parsimony'. The A hypothesis requires only three steps, three independent duplications (D) of a trait. The B hypothesis assumes a more restrictive model of evolution 'Dollo parsimony' where a derived trait (the evolution from 'white' to 'black') can only occur once (D), but the loss of the trait (L) is allowed to occur more than once (here three times). A model of evolution that is more restrictive than the most simple one ('unordered parsimony') always requires more evolutionary explanations (steps).

19.3 The importance of phylogenetic knowledge and the evolution of HOM/Hox clusters

The phylogenetic relationships among most animal phyla are still largely unresolved, possibly due to their 'explosive' origin within a very short time period in the Cambrian (see Conway-Morris 1993, 1994*a*,*b*; Bergström 1994; Phillipe *et al.* 1994). Unfortunately, even the phylogeny of deuterostomes is still uncertain and new phylogenetic hypotheses are constantly being suggested. Most recently, the phylogenetic position of lophophorates (a presumed deuterostome phylum) was called into question (Halanych *et al.* 1995) and they were placed with some groups of protostomes, rendering the deuterostomes an unnatural (paraphyletic) group. In general, knowledge of phylogeny is crucial if we ever hope to understand the relationship between ontogeny and phylogeny.

As already mentioned, a firmly established phylogeny based on which the tracing 'up and down the tree' of character evolution, developmental or otherwise, can be conducted is necessary. A brief example might serve to outline how uncertainty in phylogenetic relationships can affect the power of hypothesis testing in the study of the development–evolution relationship. The evolutionary relationships of the two living groups of jawless (agnathan) fishes, lampreys and hagfish, to each other and to other chordates (Figs. 20.1 and 20.7) are still debated. It should be noted that there is support for both the paraphyly (Fig. 20.1) and the monophyly (Fig. 20.7) hypotheses of agnathan relationships (reviewed by Forey and Janvier 1993). Monophyly has been suggested by the recent phylogenetic analysis of 18S rRNA data (Stock and Whitt 1991), but traditionally paraphyly is rather strongly supported by several kinds of phenotypic datasets (reviewed in Forey and Janvier 1993). These comparisons underscore that we need to know the answer to this phylogenetic question in addition to knowing the actual homeobox cluster architecture of these two crucial species. Without this knowledge we could not decide if the lamprey condition is intermediate (in terms of homeobox cluster evolution as well as morphological evolution between the hagfish and the jawed-vertebrate condition (in the case of paraphyly) or possibly independently derived for some more advanced vertebrate features (in the case of agnathan monophyly). Depending on the (phylogenetic as well as *Hox* cluster architecture) results for these two primitive groups of vertebrates, groups of primitive cartilaginous and bony fish will possibly have to be investigated as well.

The lamprey (*Petromyzon marinus*) appears to already have three to four HOM/*Hox* clusters, based on a homeobox PCR-based survey (Pendleton *et al.* 1993) (Figs. 20.1 and 20.7) whereas the number of HOM/*Hox* clusters in the hagfish is unknown. PCR-based approaches that use degenerate PCR primers recognizing homeobox motifs are a powerful labour-saving shortcut; however, it appears that the estimates of the number of homeobox clusters and homology assignments to cognate groups of PCR clones within homeobox clusters may not always be accurate with this technique. For example, the cluster number in amphioxus had been estimated to be two based on this technique (Pendelton *et al.* 1993) whereas it was later shown to be a single one (Garcia-Fernandez and Holland 1994). Ideally, the lamprey estimate should be confirmed by genomic DNA mapping of the kind that was conducted to get the information for amphioxus (Garcia-Fernandez and Holland 1994).

19.4 The future of the investigation of the development–evolution relationship

Wolpert (1994) argues that in the next 20 years we will come to understand how development constrains and directs the form of organisms and that some of this understanding will come from the study of homology. By that he means similarities and generalities about development will be gathered by compar-

isons from a wide variety of organisms. This seems an optimistic but attainable goal, judging by the surprising similarities that emerge in developmental processes and control systems in astonishingly different model organisms. The sensational discovery of *eyeless* (Halder *et al.* 1995) which was interpreted to be a master control gene for the development of eyes in flies seemed to suggest that this gene is also instrumental in the development of eyes of all other animals from which a homologue of *eyeless* has been discovered. This discovery might herald the advent of even more amazing discoveries of genes high up in the cascade or nexus of control of developmental control genes.

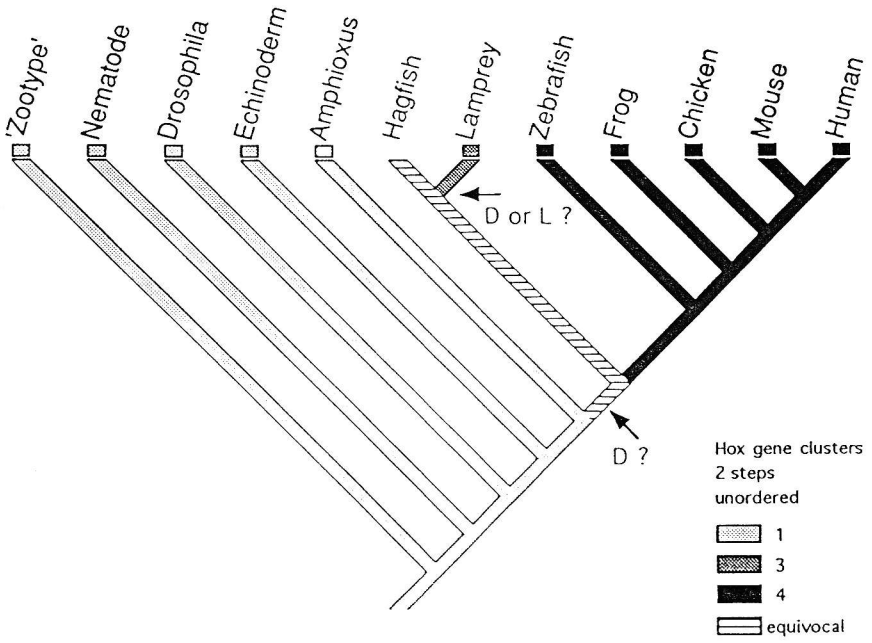

Fig. 20.7 Monophyly relationship (supported by the phylogenetic analysis of 18S rRNA (Stock and Whitt 1992)) of agnathan fish and their phylogenetic relationships to the most commonly used model systems in developmental biology. Other taxa for which HOM/*Hox* cluster numbers have been estimated or established are also included in this figure just as in Fig. 20.1. 'D' represents potential duplication events of HOM/*Hox* clusters and 'L' the potential loss of one HOM/*Hox* cluster in lampreys if they really had three clusters and the hagfish had four. In the latter case the 'L' is likely if the hagfish had two HOM/*Hox* clusters the 'D' might be likely if this phylogenetic hypothesis were correct.

In these studies, care has to be taken here, however, that homology and similarity of structure are not equated. Homology, is still an unsolved problem at the phenotypic as well as the genetic level (Patterson 1988). The issue of homology had been debated among evolutionary biologists for a long time and it proved notoriously intractable—no common definition has be agreed upon so far (Wake 1994; reviewed in Hall 1994). Homologous structures can be

phenotypically very similar or quite different (for example hands in humans and wings in bats are homologous, but wings in birds are not considered to be homologous to wings in bats). Similarity in morphology may be due to common descent or due to convergence, the independent evolution of similar morphological structures. Convergence cannot be predicted and can only be determined if phylogenetic relationships are known. For most evolutionary biologists, similarity of developmental processes is not part of the definition of whether or not structures are homologous (Hall 1992, 1994). This is, for example, because the ontogenetic mechanisms of the formation and induction of the eye in salamanders and frogs are different yet these are indisputably homologous structures. However, developmental biologists seem to turn homology on its head by arguing that because, for example, *eyeless* may be pivotal in the formation of morphological structures that are distinctly different in shape and make-up, but that serve the same function, such as compound eyes in flies and camera eyes in vertebrates, that these structures are therefore homologous. To an evolutionary biologist a fly eye is not homologous to a vertebrate eye even if their ontogenies are controlled by an astonishingly ancient set of homologous genes.

Without a doubt, the homology debate will, through the exciting discovery of master control genes such as *eyeless*, receive new impetus from developmental biology. The combination of phylogenetics and developmental genetics will allow the determination of whether traits that are considered to be homologous arose by the same or a different developmental mechanism. Developmental and evolutionary biologists seem to ask different kinds of questions; however, their approaches can be reciprocally elucidating and it is to be regarded as a positive development that the evolutionary and developmental biological communities have initiated this important dialogue.

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References

Ahlberg, P. E. (1993). Coelacanth fins and evolution. *Nature*, **358**, 459.

Akam, M., Dawson, I., and Tear, G. (1988). Homeotic genes and the control of segment diversity. *Dev. Suppl.*, **104**, 123–33.

Akam, M., Averof, M., Castelli-Gair, J., Dawes, R., Falciani, F., and Ferrier, D. (1994). The evolving role of Hox genes in arthropods. *Dev. Suppl.*, 209–15.

Alberch, P., Gould, S. J., Oster, G. F., and Wake, D. B. (1979). Size and shape in ontogeny and phylogeny. *Paleobiology*, **5**, 296–317.

Arthur, W. (1984). *Mechanisms of morphological evolution.* Wiley, Chichester.

Baer, K. E. von (1828). *Entwicklungsgeschichte der Thiere: Beobachtungen und Reflexion.* Bornträger, Königsberg.

Baer, K. E. von (1864). *Reden gehalten in wissenschaftlichen Versammlungen.* Karl Röttger St Petersburg.

Baldwin, J. M. (1902). *Development and evolution.* Macmillan, New York.

Bartels, J. L., Murtha, M. T., and Ruddle, F. H. (1993). Multiple *Hox*/HOM homeoboyes in platyhelminthes. *Mol. Phyl. Evol.*, **2**, 143–51.

Bergström, J. (1994). Ideas on early animal evolution. In *Early life on earth. Nobel Symposium no. 84*, pp. 460–6. Columbia University Press, New York.

Brooks, D. R. and McLennan, D. A. (1991). *Phylogeny, ecology, and behavior: a research program in comparative biology.* Chicago University Press.

Coates, M. I. (1994). The origin of vertebrate limbs. *Dev. Suppl.* 169–80.

Conway-Morris, S. (1993). The fossil record and the early evolution of the Metazoa. *Nature*, **361**, 219–25.

Conway-Morris, S. (1994*a*). Early metazona evolution: first steps to an integration of molecular and morphological data. In *Early life on earth. Nobel Symposium no. 84*, pp. 450–9. Columbia University Press, New York.

Conway-Morris, S. (1994*b*). Why molecular biology needs palaeontology. *Devel. Suppl.* 1–13.

De Robertis, E. M. (1994*b*). The homeobox in cell differention and evolution. In *Guidebook to the homeobox genes*, (ed. D. Duboule), pp. 13–23. Oxford University Press.

DeSalle, R. and Grimaldi, D. (1993). Phylogenetic pattern and developmental process in *Drosophila*. *Syst. Biol.*, **42**, 458–75.

Dick, M. H. and Buss, L. W. (1994). A PCR-based survey of homeobox genes in *Ctenodrilus serratus* (Annelida: Polychaeta). *Mol. Phyl. Evol.*, **3**, 146–58.

Elinson, R. P. (1990). Direct development in frogs: wipping the recapitulationist slate clean. *Sem. Dev. Biol.*, **1**, 263–70.

Favier, B., Le Meur, M. Chambon, P., and Dollé, P. (1995). Axial skeleton homeosis and forelimb malfunctions in HOxd-11 mutant mice. *Proc. Natl. Acad. Sci. USA*, **92**, 310–14.

Felsenstein, J. (1978). The number of evolutionary trees. *Syst. Zool.*, **27**, 27–33.

Felsenstein, J. (1985*a*). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, **39**, 783–91.

Felsenstein, J. (1985*b*). Phylogenies and the comparative method. *Am. Nat.*, **125**, 1–15.

Forey, P. and Janvier, P. (1993). Aganthans and the origin of jawed vertebrates. *Nature*, **361**, 129–34.

Garcia-Fernandez, J. and Holland, P. W. H. (1994). Archetypal organization of the amphioxus *Hox* gene cluster. *Nature*, **370**, 563–6.

Garland, T. jun and Adolph, S. C. (1994). Why not to do two-species comparative studies: limitations on inferring adaptation. *Physiol. Zool.*, **76**, 797–828.

Gehring, W. J. (1994). A history of the homeobox. In *Guidebook to the homeobox genes*, (ed. D. Duboule), pp. 3–10. Oxford University Press.

Ghiselin, M. T. (1984). *The triumph of the Darwinian method.* University of Chicago Press.

Goodwin, B. C., Holder, N., and Wylie, C. C. (ed.) (1983). *Development and evolution.* Cambridge University Press.

Gould, S. J. (1977). *Ontogeny and phylogeny*. Harvard University Press, Cambridge, MA.
Graham, A., Papalopulu, N., and Krumlauf, R. (1989). The murine and Drosophila homeobox gene complexes have common features of organization and expression. *Cell*, **57**, 367–78.
Haeckel, E. (1866). *Generelle Morphologie der Organismen: Allgemeine Grundzüge der organischen Formen-Wissenschaft, mechanisch begründet durch die von Charles Darwin reformierte Descendenz-Theorie*. George Riemer, Berlin.
Halanych, K. M., Bacheller, J. D., Aguinaldo, A. M. A., Liva, S. M., Hillis, D. and Lake, J. A. (1995). Evidence from 18S ribosomal DNA that the lophophorates are protostome animals. *Science*, **267**, 1641–3.
Halder, G., Callaerts, P., and Gehring, W. J. (1995). Induction of ectopic eyes by targeted expression f the *eyeless* gene in *Drosophila*. *Science*, **267**, 1788–92.
Hall, B. K. (1992). *Evolutionary developmental biology*. Chapman and Hall, London.
Hall, B. K. (1994). *Homology: the hierarchical basis of comparative biology*. Academic, San Diego.
Harvey, P. H. and Pagel, M. D. (1991). *The comparative method in evolutionary biology*. Oxford University Press.
Henry, J. Q. and Martindale, M. Q. (1994). Establishment of the dorsoventral axis in nemetean embryos: evolutionary considerations of spiralian development. *Dev. Genet.*, **15**, 64–78.
Holland, P. W. H. (1992). Homeobox genes in vertebrate evolution. *BioEssays*, **14**, 267–73.
Holland, P. W. H., Garcia-Fernández, J., Williams, N. A., and Sidow, A. (1994). Gene duplications and the origins of vertebrate development. *Dev. Suppl.* **125–33.**
Jeffrey, W. R. and Swalla, B. J. (1992). Evolution of alternate modes of development in ascidians. *BioEssays*, **14**, 219–26.
Kappen, C., Schughart, K., and Ruddle, F. H. (1989). Two steps in the evolution of Antennapedia-class vertebrate homeobox genes. *Proc. Natl. Acad. Sci. U.S.A.*, **86**, 5459–63.
Kellog, E. A. and Shaffer, H. B. (1993). Model organisms in evolutionary studies. *Syst. Biol.*, **42**, 409–14.
Kenyon, C. and Wang, B. (1991). A cluster of *Antennapedia*-class homeobox genes in a nonsegmented animal. *Science*, **253**, 516–17.
Kessel, M., Balling, R., and Gruss, P. (1990). Variations of cervical vertebrae after expression of a *Hox 1.1* transgene in mice. *Cell*, **61**, 301–8.
Kimble, J. (1994). An ancient molecular mechanism of establishing embryonic polarity? *Science*, **266**, 577–8.
Lawrence, P. A. (1992). *The making of a fly*. Blackwell, London.
Luk, S. K.-S., Kilpatrick, M., Kerr, K., and McDonald, P. M. (1994). Components acting in localization of ibicoid mRNA are conserved among *Drosphila* species. *Genetics*, **137**, 521–30.
Lufkin, T., Dierich, A., LeMeur, M., Mark, M., and Chambon, P. (1991). Disruption of the Hox-1.6 homeobox gene results in defects in a region corresponding to its rostral domain of expression. *Cell*, **66**, 1105–19.
Maddison, W. P. and Maddison, D. R. (1992). *MacClade: analysis of phylogeny and character evolution. Version 3.01*. Sinauer, Sunderland, MA.
Malicki, J., Schugart, K., and McGinnis, W. (1990). Mouse *Hox-2.2* specifies thoracic segmental identity in *Drosophila* embryos and larvae. *Cell*, **63**, 961–7.
Marshall, H., Studer, M., Pöpperl, H., Aparicio, S., Kuroiwa, A., Brenner, S., and Krumlauf, R. (1994). A conserved retinoic acid response element required for early expression of the homeobox gene *Hoxb-1*. *Nature*, **370**, 567–71.

McGinnis, W. and Krumlauf, R. (1992). Homeobox genes and axial patterning. *Cell*, **68**, 283–302.

Meyer, A., Biermann, C. H., and Orti, G. (1993). The phylogenetic position of the zebrafish (*Danio rerio*), a model system in developmental biology: an invitation to the comparative method. *Proc. R. Soc.*, **B252**, 231–36.

Meyer, A., Ritchie, P., and Witte, K-E. (1995). Predicting developmental processes from phylogenetic patterns: a molecular phylogeny of the zebrafish (*Danio rerio*) and its relatives. *Phil. Trans. R. Soc.* (In press.)

Minelli, A. and Schram, F. R. (1994). Owen revisited: a reappraisal of morphology in evolutionary biology. *Bidragen tot de Dierkunde*, **64**, 65–74.

Murtha, M. T., Leckman, J. F., and Ruddle, F. H. (1991). Detection of homeobox genes in development and evolution. *Proc. Natl. Acad. Sci. USA*, **88**, 1071–15.

Northcutt, R. G. (1990). Ontogeny and phylogeny: a re-evaluation of conceptual relationships and some applications. *Brain Behav. Evol.*, **36**, 116–40.

Nüsslein-Volhard, C., and Wieschaus, E. (1980). Mutations affecting segment number and polarity in *Drosophila*. *Nature*, **287**, 795–803.

Ohno, S. (1970). *Evolution by gene duplication*. Springer, Berlin.

Patel, N. H. (1994). Developmental evolution: insights from studies of insect segmentation. *Science*, **266**, 581–9.

Patterson, C. (1988). Homology in classical and molecular biology. *Mol. Biol. Evol.*, **5**, 603–25.

Pavell, A. M. and Stellwag, E. J. (1994). Survey of Hox-like genes in the teleost *Morone saxatilis*: implications for evolution of the *Hox* gene family. *Mol. Mar. Biol. Biotech.*, **3**, 149–57.

Pendelton, J. W., Nagai, B. K., Murtha, M. T., and Ruddle, F. H. (1993). Expansion of the *Hox* gene family and the evolution of chordates. *Proc. Natl. Acad. Sci. USA*, **90**, 6300–4.

Phillipe, H., Chenuil, A., and Adoutte, A. (1994). Can the Cambrian explosion be inferred through molecular phylogeny? *D. Suppl.*, 15–25.

Raff, R. A. (1992). Direct-developing sea urchins and the evolutionary reorganization of early development. **BioEssays**, **14**, 211–18.

Raff, R. A. and Kaufman, T. C. (1983). *Embryos, genes, and evolution. The developmental–genetic basis of evolutionary change*. Macmillan, New York.

Ruddle, F. H., Bentley, J. L., Murtha, M. T., and Risch, N. (1994). Gene loss and gain in the evolution of vertebrates. *Dev. Suppl.* 155–61.

Schierwater, B., Murtha, M., Dick, M., Ruddle, F. H., and Buss, L. W. (1991). Homeoboxes in cniderians. *J. Exp. Zool.*, **260**, 413–16.

Schubert, F. R., Nieselt-Struwe, K., and Gruss, P. (1993). The antennapedia-type homeobox genes have evolved from three precursors separated early in metazoan evolution. *Proc. Natl. Acad. Sci. USA*, **4**, 143–7.

Scott, M. P. (1992). Vertebrate homeobox gene nomenclature. *Cell*, **171**, 551–3.

Scott, M. P. and Carroll, S. B. (1987). The segmentation and homeotic gene network in early *Drosophila* development. *Cell*, **51**, 689–98.

Slack, J. M. W., Holland, P. W. H., and Graham, C. F. (1993). The zootype and the phylotypic stage. *Nature*, **361**, 490–2.

Sommer, R. J. and Sternberg, P. W. (1994). Changes of induction and competence during the evolution of vulva development in nematodes. *Science*, **265**, 115–18.

Sommer, R. J. and Tautz, D. (1993). Involvement of an orthologue of the Drosophila pair-rule gene *hairy* in segment formation of the short germ-band embryo of *Tribolium* (Coleoptera). *Nature*, **361**, 448–50.

Swofford, D. L. (1991). *Phylogenetic analysis using parsimony (PAUP Version 3.0s)*. Illinois Natural History Survey, Champaign.

Tabin, C. J. (1992). Why we have (only) five fingers per hand: Hox genes and the evolution of paired limbs. *Development*, **116**, 289–96.
Tabin, C. J. and Laufer, E. (1993). Hox genes and serial homology. *Nature*, **361**, 692–3.
Waddington, C. H. (1957) *The strategy of genes*. Allen and Unwin, London.
Wake, D. B. (1994) Comparative terminology. *Science*, **265**, 268–9.
Wake, D. B. (1995). Evolutionary developmental biology—Prospects for an evolutionary synthesis at the developmental level. *Proc. Calif. Acad. Sci.* (In press.)
Walker, C. and Streisinger, G. (1983). Induction of mutations by γ-rays in pregonial germ cell of zebrafish embryos. *Genetics*, **103**, 125–36.
Walsh, J. B. (1995). How often do duplicated genes evolve a new function?
Warren, R. W., Nagy, L., Selegue, J., Gates, J. and Carroll, S. (1994). Evolution of homeotic gene regulation and function in flies and butterflies. *Nature*, **372**, 458–61.
Wilson, A. C., Carlson, S., and White, T. J. (1977). Biochemical evolution. *Ann. Rev. Biochem*, **46**, 573–639.
Wolpert, L. (1994). Do we understand development? *Science*, **266**, 571–2.
Wray, G. A. (1992). Rates of evolution in developmental processes. *Am. Zool.*, **32**, 123–34.
Wray, G. A. (1995). Puctuated evolution of embryos. *Science*, **267**, 1115–16.
Wray, G. A. and Bely, A. E. (1994). The evolution of echinoderm development is driven by several distinct factors. *De. Supp.* 97–106.
Wray, G. A. and Raff, R. A. (1991). The evolution of developmental strategy in marine invertebrates. *Trends Ecol. Evol.*, **6**, 45–50.
Zuckerkandl, E. (1994). Molecular pathways to parallel evolution: I. gene nexuses and their morphological of correlates. *J. Mol. Evol.*, **39**, 66–78.