Arthropod evolution: **Same *Hox* genes, different body plans**

Michalis Averof

Surveys of *Hox* genes in various arthropods and related phyla demonstrate that extensive duplication and diversification of *Hox* genes occurred long before the appearance of overt segmental diversity in arthropod body plans.

Address: EMBL, Meyerhofstrasse 1, 69117 Heidelberg, Germany.
E-mail averof@embl-heidelberg.de

Current Biology 1997, 7:R634–R636
http://biomednet.com/elecref/09609822007R0634

Morphological evolution is conventionally studied by direct comparison of morphological characteristics in different organisms. Recently it has also become possible to study the evolution of developmental processes and gene functions, and to speculate about links between the evolution of genes and the evolution of morphology. One of the cases that has attracted particular interest is the relationship between the evolution of *Hox* genes and the evolution of body plans in higher metazoans, particularly in arthropods.

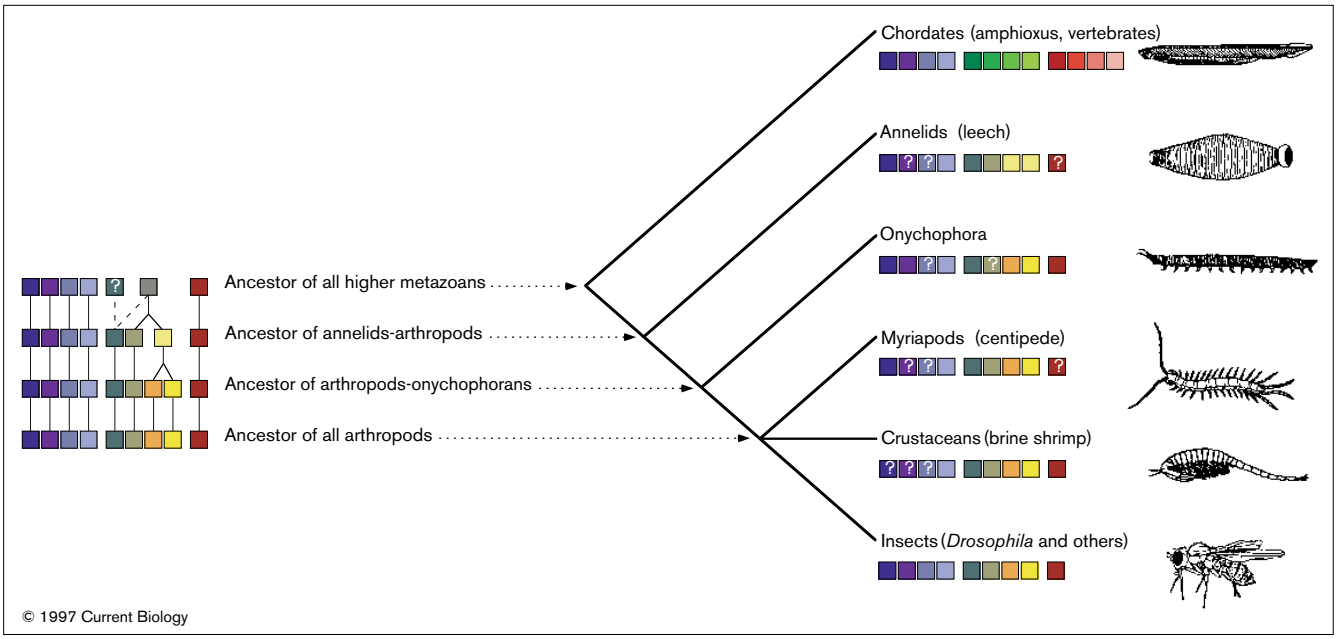
Hox genes are known to play a major role in specifying segmental differences within the body of arthropods [1–3]. These genes have been best characterized in *Drosophila*, where genetic studies have shown that *Hox* gene mutations can transform structures characteristic of one segment into the corresponding structures of another — for example, an antenna into a leg, or a haltere into a wing. Different *Hox* genes are expressed in different domains along the anterior–posterior axis of *Drosophila* embryos, where they are able to promote the development of segment-specific characteristics by regulating the activity of other, 'downstream', genes in a segment-specific manner.

Molecular studies have shown that all *Hox* genes are related to each other, and are likely to have arisen by a series of gene duplications from a common ancestral gene. Some of these duplications occurred in very distant ancestors and are shared by most higher animal groups, whereas others appear to have occurred later and gave rise to *Hox* genes that are restricted to particular animal groups [3,4]. A comparison of vertebrates and insects indicates that, although many of the *Hox* genes responsible for specifying head regions have distinctive and well-conserved homologues in these two groups, the *Hox* genes involved in specifying different regions of their trunk appear to have arisen and diversified independently [4]. It has therefore been tempting to hypothesize that duplication and diversification of those 'trunk' *Hox* genes may be directly linked to the evolution of different segmental patterns. If this were true, one might expect that the number and types of *Hox* genes present in different animals would somehow reflect the diversity of segment types observed in their bodies. A number of recent studies have set out to address this hypothesis by determining the types of *Hox* gene present in arthropods with very different patterns of segmental specialization.

Extensive data now exist for at least three types of divergent arthropod (insects, branchiopod crustaceans and centipedes) and for members of a few related phyla (onychophorans and annelids). The most complete set of data is available for insects, where studies have been conducted for a number of years and in a few different species [5]. All insects apparently share the same set of eight *Hox* genes, each with well-conserved functions in establishing a set of segmental identities found in the bodies of all insects. Work on a brine shrimp, *Artemia*, has shown that the same set of *Hox* genes is carried by branchiopod crustaceans, although these animals have very different (and simpler) patterns of segmental specialization [6]. Similarly, there is preliminary evidence from a horseshoe crab that very similar types of *Hox* gene may exist in chelicerates [7]. The latest data bearing on this issue demonstrate that centipedes and onychophorans also share an identical set of eight *Hox* genes, with distinct homologues found for each of the *Drosophila Hox* genes [8].

The study on centipedes and onychophorans [8] is particularly interesting, for two reasons. First, because the trunk of centipedes and onychophorans consists of a long series of identical segments, with little or no specialization of individual segments. Yet this lack of segmental specialization is not at all reflected in the complexity of their *Hox* genes — all of the genes that specify the segmental diversity of the *Drosophila* trunk (thorax and abdomen) find clear homologues in these species (Figure 1). The second reason relates to their controversial phylogenetic positions. Centipedes and related myriapods have conventionally been thought of as being the closest living relatives of insects — although recent work has cast some doubts on this relationship — and are usually allied with both crustaceans and insects into a common clade called the mandibulates. Onychophorans, on the other hand, are thought to represent the closest living relatives of the arthropods as a whole. The study of *Hox* genes in these creatures is therefore particularly relevant if we are interested in finding out what types of *Hox* gene existed at the base of the mandibulate and arthropod trees, respectively.

Figure 1



Distribution of different types of *Hox* gene among arthropods, related protostomes, chordates, and their ancestors. *Hox* genes are represented by coloured boxes, with orthologous genes shown in the same colour. Question marks indicate genes that are likely to be present but have not yet been identified with certainty, or putative ancestral genes whose existence is uncertain. This is a 'minimal' representation of *Hox* genes found in different animal groups —- it is likely to be complete only for vertebrates and insects, which have been studied extensively. In other groups, additional gene duplications may have given rise to new, as yet unidentified, types of *Hox* gene. Homologies, ancestral genes, and the pattern of gene duplications are inferred by sequence comparisons, based on data in [4,6,8,12,21].

The overwhelming conclusion that one draws from these studies is that the number and types of *Hox* gene present in different arthropods do not parallel the diversity of segment types observed in their bodies — almost identical sets of *Hox* genes are found in arthropods with segmental patterns that vary greatly in complexity (Fig. 1). These results also imply that all of the gene duplications that gave rise to these basic types of *Hox* gene must have occurred some time before the divergence of the different arthropod subgroups — and so before the earliest known Cambrian radiation. Surveys for *Hox* genes in annelids [9–11] and leeches [12] suggest that some of these duplications occurred even before the divergence of the arthropod and annelid lineages, deep within the phylogeny of protostome animals.

Following these surprising findings, two interesting questions arise. What is the function of these multiple *Hox* genes in animals with limited segmental diversity — for example, in the trunk regions of centipedes, onychophorans and branchiopods? And, if segment diversification has not been driven by *Hox* gene duplication and diversification, which are the main genetic factors responsible? There are a number of possible answers to these questions.

One possibility is that *Hox* genes are not involved in the specification of segmental identities in most arthropods, and that this is a function they acquired specifically in insects. Although formally possible, this is unlikely, as an important role in regional specification has been assigned to *Hox* genes in more distantly related animals, such as vertebrates and nematodes [3]. Furthermore, the observed expression patterns of *Hox* genes, particularly in crustaceans but also in centipedes [8,13,14], do correlate with patterns of segment specialization, albeit in a different way to what has been observed in insects.

A second possibility is that a complex set of *Hox* genes reflects a complex pattern of segmental specialization in the ancestor of all arthropods and onychophorans. If this were true, one would have to assume that the homonomous body regions of onychophorans, centipedes and branchiopods have been secondarily simplified from more complex patterns. Although such simplification is conceivable in individual cases, the fossil record actually points to the opposite trend: the earliest arthropods have bodies with long series of indistinguishable segments; the great abundance of segmental diversity arises later (see [15]).

A third possibility is that the apparently uniform series of segments found in creatures like centipedes and onychophorans may still conceal a reasonable degree of segmental specialization. This specialization may not be

obvious in external morphology, but may exist, for example, in the fine specializations of the nervous system, or physiological attributes of different segments. In some animals, such 'cryptic' segmental differences may be the primary object of *Hox* gene regulation (see [16], for example).

Finally, a fourth possibility is that a number of *Hox* genes may initially — soon after the gene duplications that create them — have similar, or largely overlapping functions, and may acquire distinct roles in the specification of distinct segment types later in evolution and only in some animal lineages [13,17]. If this were true, one might expect that segment diversification would be associated, not with the gene duplications *per se*, but with the subsequent diversification of functions for each of the *Hox* genes produced — that is, with changes in gene regulation. This might involve changes in the expression patterns of *Hox* genes themselves and/or in their ability to regulate different downstream targets. Evidence in support of this last hypothesis has come from recent studies which document the overlapping function of different *Hox* genes [18,19], and comparative studies of expression patterns in a number of crustaceans and insects [13,14,20] which support the idea that changes in *Hox* gene regulation may be responsible for the diversification of body plans and the generation of new segment types.

More concrete answers to these questions will require not only the identification of types and numbers of *Hox* genes in diverse animals, but also a more detailed study of their expression patterns, regulation and functions.

Acknowledgements

I am grateful to Michael Akam, Graham Budd, and Maura Strigini for various discussions and comments.

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