

# SHARED DOMAINS OF INTEREST FOR DEVELOPMENTAL BIOLOGISTS AND PHYLOGENETICISTS

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**Abstract.** Larsen, E.; S. Monks; J. Stone; F. Marques; M. Godfrey; R. Pearson & D.R. Brooks. Shared Domains of Interest for Developmental Biologists and Phylogeneticists. *J. Comp. Biol.* 2(2):137-141. The presence of Hox genes in three model organisms, *Caenorhabditis elegans*, *Drosophila melanogaster*, and *Mus musculus*, has been used as an indicator of common ancestry for all Metazoa. Although this hypothesis has not been examined in a strict phylogenetic context, it has formed the basis of comparative developmental studies. Using data and phylogenies from the literature, we optimized Hox gene information onto different phylogenetic trees for the Metazoa. Character distribution was used to evaluate the presence of anterior, medial and posterior Hox genes and the presence of Hox gene clusters from an evolutionary perspective. We then compared the previously untested hypothesis of 'universality' of Hox genes with the phylogenetic interpretation of Hox gene evolution. We further discuss how interpretations may change as data from additional taxa become available. These results serve as a springboard to discuss the different perspectives of developmental biologists and phylogeneticists that must be acknowledged to facilitate two-way communication and collaboration between scholars of the two fields.

**Key words.** Phylogenetic methodology; Developmental Biology; Hox genes; Evolution.

## Introduction

Announcements of a renewed partnership between molecular/developmental biologists and phylogenetic systematists have been proclaimed at several meetings (Atkinson 1992; Purugganan 1996; Raff 1996; Wake et al. 1991). Likewise, Maynard Smith (1997; also Maynard Smith and Szathmary, 1995) has suggested that a new evolutionary synthesis was necessary, and perhaps imminent. The key to such a synthesis, according to Maynard Smith (1997), is the necessity for "geneticists" (microevolutionists) to provide a common ground for discussions of evolutionary diversification with "paleontologists" (macroevolutionists, which we will term "phylogeneticists" herein). Since it is generally perceived that developmental biologists approach questions about evolution from a different perspective than do phylogeneticists, we wondered if this union would be successful. In order to evaluate the benefits and problems associated with such interactions, a group of us working in each field met together to consider the implications of Hox gene attributes of metazoan phyla. Using Hox gene data and phylogenies from the literature, we evaluated the presence of anterior, medial and posterior Hox genes and the presence of Hox gene clusters from an evolutionary perspective.

## Antennapedia genes—a family united

The Antennapedia gene family, important for segment identity in *Drosophila melanogaster*, is present and plays a similar role in many taxa. This gene family has had a long and distinguished history in the annals of genetics and now is playing an important role in attempts to integrate patterns of development with patterns of phylogenetic change. The first Antennapedia family mutation found in *D. melanogaster* transforms the anterior part of the haltere to resemble the anterior part of the wing. Since the discovery of this allele, many other mutations have been found in which structures associated with one segment are transformed into structures resembling those found on another segment. Such mutations are called 'homeotic' mutations (Lewis 1994).

Two aspects of Antennapedia genes have attracted particular attention. First, both mutational analysis and the pattern of gene expression suggest that there is a corresponding linear relationship between the sequence of genes along a chromosome (a gene cluster) and the region of the body the genes affect. Second, the genes in a cluster each contain a nearly identical region of DNA sequence, dubbed a homeobox (Ruddle et al. 1994). The homeobox region has been found to code for a protein region which

has DNA-binding properties, indicating that it is involved in turning on or off other genes (McGinnis 1994).

Excitement continued to mount when genes with Antennapedia-like homeoboxes (Hox genes) were found in other genetic model organisms, such as the mouse (*Mus musculus*) and a nematode (*Caenorhabditis elegans*). These genes are clustered in much the same order, and are expressed along the body axis in a co-linear fashion, as are those in *D. melanogaster* (Ruddle et al. 1994)! Hox genes have been grouped into those which are expressed either anteriorly, medially or posteriorly in the embryo.

Slack et al. (1993) suggested that Hox gene expression provides relative information about position along the axes of all animals and furthermore, that it could be a shared homologous feature (synapomorphy) of the animal kingdom. Nevertheless, it should be noted that, although the three model organisms, *D. melanogaster*, *C. elegans*, and *M. musculus*, each have Hox genes expressed in all three body regions, posterior Hox genes have remained undetected in some other metazoan taxa (Ruddle et al. 1994). At present, clustering of Hox genes has been confirmed in relatively few taxa, although all of the taxa included in this study have been examined (Ruddle et al. 1994 and references therein).

### The phylogenetic approach

One of the strengths of the phylogenetic approach is that it encourages the formulation of hypotheses which can be tested and subsequently refined (Brooks and McLennan 1991; Harvey and Pagel 1991). The methodology is basically a five-part process (Wiley 1981; Wiley et al. 1991). First, one begins by assuming that similarity of characters indicates homology. Second, one distinguishes ancient (plesiomorphic) from more recent (apomorphic) homologous characters through the use of outgroup comparisons. Third, one unites members of the ingroup based on their shared apomorphic traits (synapomorphies). Fourth, one adopts as the working hypothesis the one that requires the fewest number of gains and losses to explain the data, among other reasons, because this hypothesis is the one most easily falsified by additional data (see Brooks and McLennan (1991; 24-68) and references therein for a more comprehensive discussion). Finally,

one interprets as homoplasies, those traits that conflict with the most parsimonious solution. Thus, even though one begins with a presumption of homology, the overall weight of evidence can lead one to reinterpret some similarities as homoplasies.

Characters can be used in two ways in phylogenetic studies: (1) character information can be used to build cladograms; and, (2) the states of a given character can be plotted onto existing cladograms to analyze character evolution. One example of the former use of characters is the work by Holland and Garcia-Fernandez (1996), who examined chordate evolution, in an analysis based on Hox gene data. Our focus in the remainder of this communication is an example of the second use of characters. To demonstrate the power of integrating developmental genetics with phylogenetic systematics, we explored the evolutionary implications of homeobox data by plotting Hox gene information onto different phylogenetic trees for the metazoa. We will discuss: (1) the robust conclusions that can be made about Hox gene evolution in metazoans from the data currently available; (2) how interpretations may change as data from additional taxa become available; and (3) the different perspectives of developmental biologists and phylogeneticists that had to be acknowledged and discussed to allow our two-way communication.

### Our contribution

We focus on two problems: (1) at what point in metazoan evolution did anterior, medial, and posterior Hox genes originate?; and (2) when did Hox gene clusters originate (*i. e.* is Hox gene clustering an ancient or more recently derived feature)? Phylogenetic systematic methodology can help to answer these questions. The information we considered consisted of Hox gene expression patterns (anterior, medial and posterior) and the presence of clusters. This information was taken predominantly from Valentine et al. (1996) and Ruddle et al. (1994), who discussed the ubiquity of Hox genes and clusters, as well as the report by Finnerty and Martindale (1996) on posterior Hox genes in Cnidaria.

Currently, there are several different phylogenetic hypotheses for metazoan interrelationships, each based on a different data

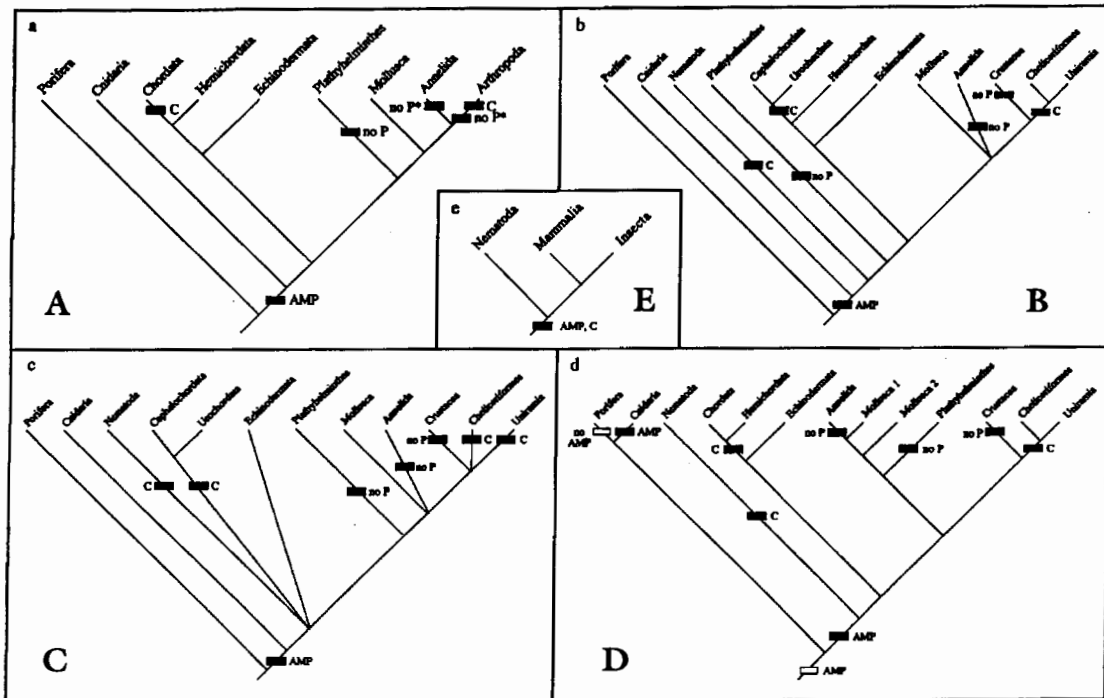


Fig. 1. Five phylogenetic hypotheses used for evaluating information about the evolution of Hox genes and gene clusters. Trees have been pruned to include only taxa for which information about Hox genes has been reported, but the relative relationships among taxa are retained from the original studies. Rectangles on trees indicate the most parsimonious estimates of evolutionary steps (gains or losses). A = anterior Hox genes; M = medial Hox genes; P = posterior Hox genes; no P = no posterior Hox genes; could be interpreted as either a loss or as never being present; C = Hox gene clusters present. (A) Phylogenetic tree modified from Brusca and Brusca (Brusca & Brusca, 1990). \* = Arthropoda considered as a single taxon, so absence of posterior genes in Crustacea and presence of posterior genes in Uniramia and Cheliceriformes was coded as a loss in the Arthropoda. The Nematoda were not included in the original analysis. (B) Phylogenetic tree modified from Meglitsch & Schram (Meglitsch & Schram, 1991). (C) Phylogenetic tree modified from Bäckeljau et al. (Bäckeljau et al., 1993). (D) Phylogenetic tree modified from Winnepenninckx et al. (1995). Open rectangles indicate a second equally parsimonious interpretation that anterior, medial and posterior Hox genes originated in the common ancestor of all Metazoans and were subsequently lost in Porifera, rather than having arisen twice independently, once in Cnidaria and once in the ancestor of all Metazoans except the Porifera + Cnidaria, as indicated by the solid rectangles. (E) Phylogenetic tree representing relative relationships of the model organisms used in study of Hox genes, *Caenorhabditis elegans* (Nematoda), *Drosophila melanogaster* (Arthropoda), and *Mus musculus* (Mammalia).

set resulting from different interpretations of similar information. As yet, none of them have received general acceptance. For this study, we arbitrarily chose four of the many competing hypotheses (Bäckeljau et al. 1993; Brusca and Brusca 1990; Meglitsch and Schram 1991; Winnepenninckx et al. 1995). Our selection of a particular tree was based only on recent citations of works in which they were presented and does not constitute support for any particular arrangement or groupings presented in the original works. We then modified these trees to include only taxa from the original studies for which Hox gene information is published. In other words, we arbitrarily pruned taxa from the various trees for which there is no Hox gene data available, while maintaining the original

relative placement of the other taxa (Fig. 1). All taxa represented on the trees have been examined for the presence of Hox genes and clustering as indicated in the literature examined for this study.

We then plotted Hox gene data onto the trees according to the simplest explanation of character evolution along the branches. We did not attempt to evaluate claims of homology of genes or clustering beyond what is presented here. In some groups, as discussed below, particular genes or evidence of clustering has not been found. This is interpreted by some as an artifact of the lack of specificity of current techniques and not as evidence of absence. We have chosen the more conservative interpretation and considered the negative results as evidence of absence of the particular feature. In some cases, there were

several equally parsimonious explanations, each requiring the same number of evolutionary steps. Each equally parsimonious optimization is presented (Fig. 1).

### Our interpretation

Differences of interpretation between developmental biologists and phylogeneticists began to emerge when the trees were examined. The tendency in published studies of developmental genetics has been to assume that a character present in several terminal branches has been inherited from a single common ancestor (Hanken 1993). This means a character is considered homologous, in a phylogenetic sense, and common to all members of a clade, even if it has only been studied in relatively few taxa. For instance, a three-taxon tree representing the interrelationship among nematodes, insects and mammals (Fig. 1E) commonly has been used to infer that the presence of Hox cluster type genes is an ancient (plesiomorphic) feature of all metazoans (Slack *et al.* 1993). However, as discussed below, this hypothesis was questioned by the phylogeneticists among us when the data were plotted onto trees which included the greater number of taxa for which Hox gene information has been published (Fig. 1A-D).

Recognizing that the following hypotheses are tentative because of the paucity of Hox gene data and the absence of a generally accepted metazoan phylogeny, we found that the distribution of information on all trees used in our analysis is consistent with the following phylogenetic hypotheses: (1) anterior, medial and posterior Hox gene expression patterns arose together early in the history of the metazoa; (2) gene clusters have arisen independently multiple times (in Nematoda, Chordata, Cheliceriformes, and Uniramia); and (3) posterior Hox genes have been lost independently multiple times within the metazoa (in Platyhelminthes, Annelida, and Crustacea). We have not evaluated these conclusions in relation to the many other phylogenetic hypotheses available for the Metazoa, by we suspect that they would be equally true for any of the present arrangements.

### Discussion

The first hypothesis was acceptable to both developmental biologists and phylogeneticists, because we all interpreted the presence of these

characters as homologies. Where we disagreed was on how to interpret the absence of these characters in some taxa. For example, to the developmental biologists, it seemed vanishingly improbable that the same linked sequence of genes found in Hox gene clusters of nematodes, arthropods and vertebrates could have arisen independently and, because of the probable requirement of Hox gene expression for normal development (Lewis 1994), that they could be lost from any taxa. This view of clusters as a plesiomorphic metazoan character is implicit in the izootypeî of Slack *et al.* (1993). Similarly, the presence of posterior Hox genes in these widely divergent taxa suggested to the developmental biologists that these genes are ubiquitous and their putative absence in some taxa was due only to insufficient research. To the phylogeneticists, two possibilities had to be considered in interpreting the absence of these genes. One possibility was that the genes occurred in all taxa but remained undetected in some, and the another possibility is that posterior Hox genes are truly absent in some taxa. For the neophytes in phylogenetic thinking among us, it was surprising that the extent of perceived homology depended upon the scope of the analysis and that inferences were inconclusive when data were unavailable for a taxon.

One of the satisfying results of our analysis was the delineation of which taxa should receive attention in order to resolve questions of the evolutionary origin and universality of both posterior Hox genes and gene clustering. To our knowledge, neither posterior Hox genes nor clustering has been found in the data that has been published for Annelida, Crustacea or Platyhelminthes, hence a more firm data base for these taxa would help to resolve issues of character gains and losses. Of course, it would also be desirable to examine those taxa not included in this study for which no data concerning Hox genes is known, as well as the taxa mentioned above in which researchers failed to find evidence of Hox genes. Furthermore, our collaborative analysis of Hox gene data suggests that, to expedite the family reunion, phylogeneticists should work toward providing a uniformly accepted hypothesis for the phylogenetic interrelationships among and within metazoan phyla, because any optimization of developmental characters can be only as robust as the phylogeny on which it is based. Finally, developmental biologists must be made aware

that many taxa must be studied in the testing of evolutionary hypotheses. Because of the effort required to obtain developmental data, developmental biologists have concentrated much of their efforts on a few model organisms, but, because the model organisms are distantly related, any finding general to the model organisms has been assumed to have been conserved in evolution. To substantiate such an assumption, more taxa must be investigated; yet only in rare instances may the resources be available to do so. For Hox genes, data on fewer than half of all invertebrate phyla are found in print; thus, any conclusions drawn from the data currently available must be made with caution and viewed as tentative, pending further support or refutation by future studies. To maximize the evolutionary information produced from the research effort expended, studied taxa must be selected carefully, but this task is made difficult by the lack of well-supported metazoan phylogenies.

Will a reunion between molecular/developmental biologists and phylogenetic systematists be successful? Based on our experience, the likelihood will be increased if we learn each others' languages and understand each others' modes of thinking. As it stands today, the two groups of researchers, each with different methodological constraints and interpretations of the existing data, have largely ignored the other's research program. By mutual understanding of both the strengths and limitations of each approach, the necessary balance between the practicalities of data acquisition and the rigor of phylogenetic systematics may be found to achieve the common goal: a fuller understanding of the nature and origin of animal diversity.

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