Chapter 8

Are the Deuterostome Posterior Hox Genes a Fast-Evolving Class?

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Abstract

There has been a great deal of interest in analysing the molecular evolution of the Hox cluster using both bioinformatic and experimental approaches. The posterior Hox genes have been of particular interest to both groups of biologists for a number of reasons: they appear to be associated with the evolution of a number of morphological novelties; the protostomes appear to be have lost a highly-conserved and functionally important amino acid motif (the hexapeptide motif) from their posterior Hox genes; and deuterostome posterior Hox genes seem to be evolving more quickly than all other Hox genes. In this chapter I will discuss the last of these points.

The idea that Deuterostome posterior Hox genes were evolving more quickly than other Hox genes was first suggested by David Ferrier and colleagues.[1] In this chapter, I start by introducing the posterior Hox genes—their distribution among the animal phyla and the likely sequence of duplications that led to this distribution. I then introduce the idea of 'deuterostome posterior flexibility'[1] and examine this hypothesis in light of more recent phylogenetic and genomic work on the Hox cluster. Finally, I discuss some new approaches that could be used to test directly for differential rates of evolution among Hox genes and to assess what might underlie these differences.

The Distribution of the Posterior Hox Genes in the Metazoa

The posterior Hox genes exist in all the major bilaterian phyla examined so far, as well as in the Cnidaria (Fig. 1). To date no Hox genes of any kind have been found in any other phyla (either metazoan or otherwise), thus it seems reasonable to assume that the posterior Hox genes came into existence after the divergence of the poriferan lineage, but before the divergence of the Cnidaria and the other Metazoan phyla, roughly 650-850 million years ago.[2] Broadly speaking, the posterior Hox genes of the bilaterian phyla can be resolved into three major groupings, which are delineated along the same lines as the 'new' animal phylogeny[3,4] (Fig. 1): the Deuterostomia (chordates, echinoderms etc.) possess orthologs of *Hox9* to *Hox15* genes; and within the Protostomia the Lophotrochozoa (annelids, molluscs etc.) possess orthologs of the *Post-1* and *Post-2* genes; and the Ecdysozoa (insects, nematodes etc.) possess orthologs of the *Abd-B* gene. The posterior Hox genes of the acoel flatworms and the Cnidaria do not group robustly with any of the major groupings described above, although it is well established that they are indeed posterior Hox genes.[5-10] Despite occasional difficulties in assigning Hox genes to one of these three groupings, the major bilaterian groupings of posterior Hox genes have been repeatedly confirmed by different phylogenetic studies,[1,11-15] and are considered so robust that the possession of one type of posterior Hox gene or another is now considered good evidence on which to base the phylogenetic affinity of otherwise enigmatic taxa.[4,13] Unfortunately,

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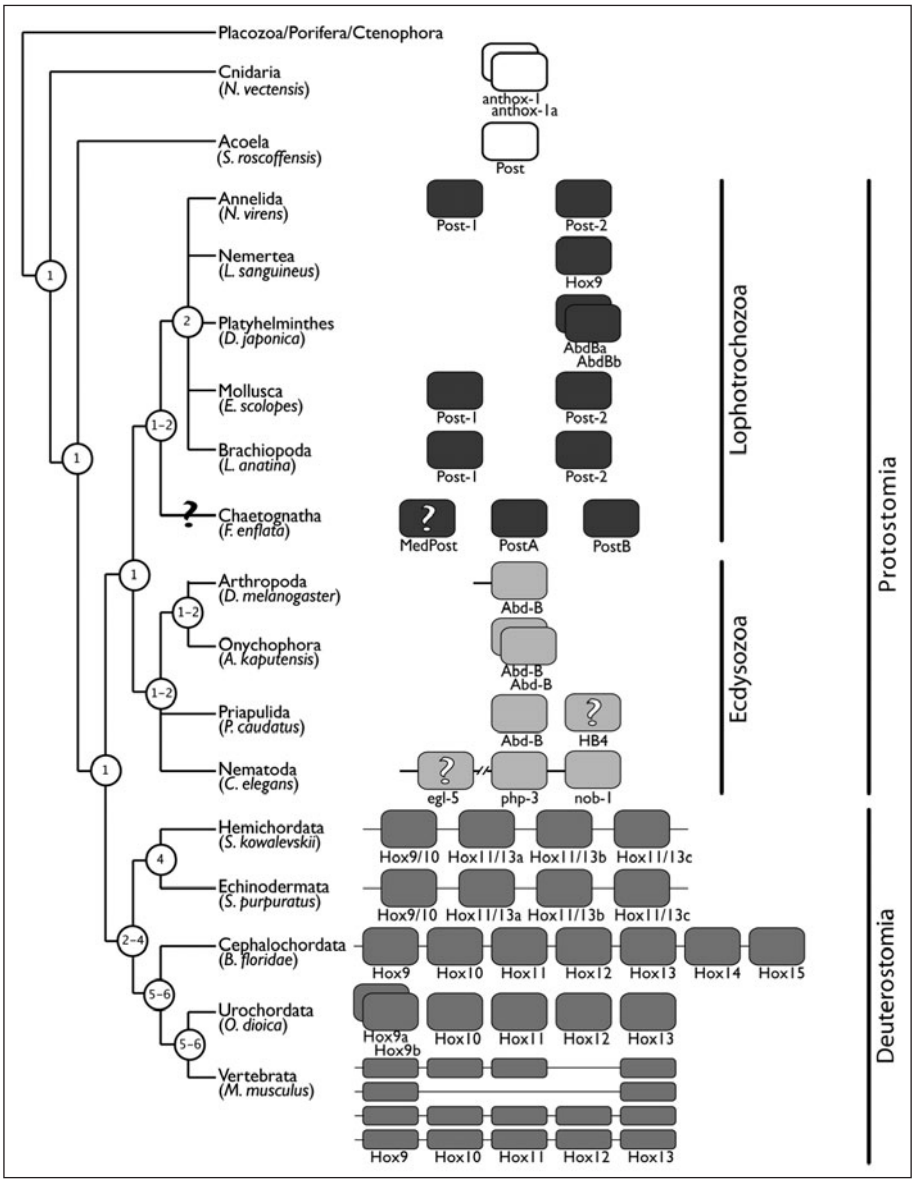

Figure 1. A sketch of the evolutionary history and current distribution of posterior Hox genes. Shading indicates approximate orthology relationships. Overlaid boxes (e.g., *Hox9a* and *Hox9b* in Urochordates) indicate recent duplication events. Question marks in boxes represent uncertain orthology relationships (see text) and question marks on the phylogenetic tree represent uncertain phylogenetic relationships. Where linkage relationships are known, they are indicated by connecting lines between boxes. Data references are as follows: Cnidaria,[6] Acoela,[5] Annelida,[12] Nemertea,[16] Platyhelminthes,[17] Mollusca,[18] Brachiopoda,[12] Chaetognatha,[13] Arthropoda,[19] Onychophora,[20] Priapulida,[12] Nematoda,[21] Hemichordata,[15] Echinodermata,[11] Cephalochordata,[22] Urochordata,[23] Vertebrata.[24]

the more or less robust grouping of many different types of posterior Hox genes is not reflected in their nomenclature and more often than not the existence of two posterior Hox genes with the same name is no indication of their relatedness (see e.g., Fig. 1 in which the major orthology groups are indicated by shading and the names are listed underneath).

Problematic Assignments of Hox Genes as 'Posterior'

Despite the relatively simple sketch of the distribution of posterior Hox genes given above, there are a number of instances in which the classification of a Hox gene as 'posterior' remains uncertain (indicated in Fig. 1 with a '?'). In some cases, orthology assignment is problematic because only very short fragments of the Homeobox have been sequenced and isolated.[25,26] In other cases however the situation can be somewhat more complex and the analysis of the whole homeodomain as well as its flanking sequences has proved insufficient to confidently ascertain whether some genes are posterior Hox genes at all, let alone to decide whether they fall into any of the three major groupings of posterior Hox genes described above. A case in point is the posterior Hox genes of the cnidarian *Nematostella vectensis*. Initial phylogenetic analyses of homeodomain sequences suggested that this species did not contain any true posterior Hox genes at all,[7] however a recent (and more thorough) re-analysis of precisely the same dataset, using the same phylogenetic procedures, suggests the opposite.[6] Despite disagreements about specific cases, however, it is well accepted that there exist a number of other posterior Hox genes in the Cnidaria.[10]

Another problematic case is the *MedPost* genes of chaetognaths. The homeodomains of these genes contain diagnostic residues of both the median (namely Q6, T7 and E59 and the LTR(R/K) RRI peptide at positions 26-32) and posterior (K3, A14, R18, Y20, Q36) Hox genes.[26] They were thus initially suggested to be mosaic genes that had arisen prior to the divergence of true posterior Hox genes from the other Hox genes. On the basis of this and the failure to find any unambiguous posterior Hox genes in chaetognaths, it was suggested that chaetognaths may have diverged from the bilaterians before the protostome/deuterostome split.[26] Recently however, a *Medpost* ortholog and two true Posterior genes (*PostA* and *PostB*) have been discovered in a different chaetognath species.[13] The discovery of true Posterior Hox genes (although they are difficult to resolve to one of the three major classes of posterior Hox genes mentioned above) suggests that the *MedPost* genes are likely to be a chaetognath-specific innovation, although their origin remains obscure. It is possible that they will end up being classified as true posterior Hox genes on the basis of data other than the sequence alone (e.g., data on their position in the cluster and on their developmental role).

Finally, the nematode Hox gene *egl-5* has also been the subject of some controversy. Although a number of studies have suggested that *egl-5* is a posterior Hox gene based on sequence analysis and its position in the remnants of the *C. elegans* Hox cluster,[21,27-29] others have suggested that *egl-5* cannot be classified as a posterior Hox gene with any certainty.[4,12,30]

Early Duplications of the Posterior Hox Genes

In order to examine whether the deuterostome posterior Hox genes are a fast-evolving class, it is helpful to first clarify the sequence of duplications that led to the current distribution of posterior Hox genes in the extant taxa. Figure 1 shows an attempt to do this, with predicted numbers of posterior Hox genes marked onto ancestral nodes of the tree. Three types of uncertainty limit the accuracy of this procedure: uncertainty in the phylogenetic placement of certain taxa (e.g., the chaetognaths), uncertainty as to the relationships between different posterior Hox genes (e.g., the *Hox9-15* genes of cephalochordates and the *Hox9-14* genes of most vertebrates) and uncertainty as to the classification of some genes as posterior Hox genes (see above).

There is another problem inherent in the estimation of ancestral gene content, which is distinct from those listed above—there is very likely to be an ascertainment bias in our knowledge of the distribution of posterior Hox genes among different taxonomic groups. The majority of Hox genes have been discovered by PCR surveys or the screening of genomic libraries, both

of which are limited techniques insofar as they are only able to recover sequences that are sufficiently similar to other known sequences. Because of this, it has often been the case that initial PCR surveys greatly underestimate the number of Hox genes in a given species. As such, we can only be sure of the Hox gene content of a given species once a fully assembled genome sequence is available and even when this is the case, current problems with whole-genome assembly methods mean that it is preferable to double-check the assembly using genomic walking. These methodological issues are neatly illustrated by the recent discovery of the amphioxus *Hox15* gene. This gene had gone undiscovered until the recent completion of the amphioxus genome, despite the fact that the amphioxus Hox cluster is among the most thoroughly studied of all Hox clusters[1,22,31] and that a previous study which had explicitly set out to look for a *Hox15* gene in amphioxus had concluded that it didn't exist.[32] This ascertainment bias in Hox gene identification will tend to favour the discovery of Hox genes in those clades for which we have more genome sequences—both due to the direct identification of Hox genes from the genome sequences themselves and by the indirect use of those genome sequences to fine-tune methods of 'fishing' for Hox genes in closely related species. Therefore, it is possible that a proportion of the excess of posterior Hox genes known in deuterostomes might be due to the effects of ascertainment bias in this clade.

Despite the difficulties inherent in such a procedure, it is still possible to estimate the posterior Hox gene complement of hypothetical ancestral species at important points in the history of the Metazoa. The number of posterior Hox genes at each ancestral node in Figure 1 was estimated by comparing phylogenetic trees of Hox genes from various sources and from the discussions of previous authors.[5,6,8,10-12,14,15,18,20,22,30,33-41] For instance the chordate ancestor (Fig. 1), likely posessed 5 or 6 genes (although other numbers are also conceivable): a minimum of 5 posterior Hox genes seems probable since all chordates have at least 5 posterior Hox genes which tend to group together (although with little resolution) on phylogenetic trees. However there is some evidence that the chordate ancestor may have possessed 6 posterior Hox genes as both cephalochordates and some vertebrates[42,43] possess a *Hox14* and it is quite possible (though difficult to show with any degree of certainty) that the vertebrate and cephalochordate *Hox14* genes are homologous and that the chordate ancestor therefore also possessed a *Hox14*.[37]

The 'Deuterostome Posterior Flexibility' Hypothesis

"Deuterostome posterior flexibility" refers to the hypothesis that the posterior Hox genes of Deuterostomes are evolving at a faster rate than other Hox genes.[1] This hypothesis was put forward to explain the fact that in phylogenetic analyses the posterior Hox genes of deuterostomes (*Hox9+*) tend to be poorly resolved, whereas the posterior Hox genes of protostomes tend to resolve with high support (into the *AbdB*-like genes for the Ecdysozoa and the *Post1*-like and *Post2*-like genes for the Lophotrochozoa). In this section, I introduce the original observations that the deuterostome posterior flexibility hypothesis was put forward to explain and discuss this hypothesis in light of recent phylogenetic and genomic studies.

Ferrier et al[1] undertook a genomic walk along the Amphioxus Hox cluster and discovered four new posterior Hox genes—*AmphiHox11*, *AmphiHox12*, *AmphiHox13* and *AmphiHox14*. Phylogenetic analysis of a large dataset of posterior Hox genes was carried out using maximum parsimony (MP) and neighbour-joining (NJ). These analyses showed that groupings of *AbdB*-like genes from the Ecdysozoa and *Post1*-like and *Post2*-like genes from the Lophotrochozoa were recovered with high bootstrap support. In contrast there was very low support for the grouping together of the deuterostome posterior Hox genes—individual orthology groups from within the vertebrates (e.g., vertebrate *Hox12*) were recovered with high support, but support for clustering of these groups with any other deuterostome posterior Hox genes was almost always less than 50%, well below the levels usually required for confident phylogenetic inference. The authors explored two possible evolutionary hypotheses for the origin of the amphioxus posterior Hox genes using a maximum likelihood (ML) based statistical significance test. The first hypothesis was that the amphioxus posterior Hox genes had arisen independently after the

split of the amphioxus and vertebrate lineages and the second was that each amphioxus gene was orthologous to a vertebrate gene (e.g., *AmphiHox10* is orthologous to vertebrate *Hox10*). Interestingly, the first hypothesis (independent duplication) was significantly rejected, whereas the second hypothesis (orthologous genes) was statistically indistinguishable from the ML tree.[1] Thus, the most parsimonious explanation by far (with respect to the number of gene duplication and loss events that have to be postulated to explain a given phylogenetic tree) is that the chordate ancestor possessed copies of *Hox9, Hox10, Hox11, Hox12* and *Hox13* and that amphioxus and the vertebrates each inherited copies of these genes. The puzzle therefore was why the protostome groupings of posterior Hox genes (e.g., the *AbdB*-like genes) could be recovered with high confidence, whereas the deuterostome posterior Hox genes could not. Ferrier and colleagues resolved this dilemma by suggesting that deuterostome posterior Hox genes were evolving at a faster rate than other Hox genes. A faster rate of evolution would in turn have led to a faster degradation of phylogenetic signal in these genes and could therefore explain their lack of resolution in phylogenetic trees.

Recent Analyses Broadly Support the Posterior Flexibility Hypothesis

The enormous interest in the evolution of the Hox cluster has meant that a number of recent studies have performed comparable phylogenetic analyses to those in the Ferrier et al[1] study in which the deuterostome posterior flexibility hypothesis was proposed. Five studies in particular have included a wide representation of metazoan posterior Hox genes and reported measures of clade support such as nonparametric bootstrap proportions (BP) or Bayesian posterior probabilities (BPP).[11,14,15,22,23] Three of these studies include a dataset sufficient to compare the phylogenetic resolution of the posterior Hox genes of protostomes (i.e., the *AbdB*-like, *Post1*-like and *Post2*-like genes) to the phylogenetic resolution of the posterior Hox genes of deuterostomes (i.e., the *Hox9+* genes). All of these three studies support the observation that the resolution of the protostome posterior Hox genes is far higher than that of the deuterostome posterior Hox genes.[11,14,15] Additionally, two more studies support the notion that there is low resolution among the deuterostome posterior Hox genes relative to the deuterostome anterior Hox genes, although neither of these include sufficient data to compare this to the resolution of the protostome Hox genes.[22,23] These five studies do not represent five completely independent tests of the deuterostome posterior flexibility hypothesis as all of the studies use somewhat similar datasets and methods. However, it has recently been shown that conclusions drawn from phylogenetic analyses of homeodomains can be extremely sensitive to small changes in dataset composition and phylogenetic methodology,[44] so the agreement of all comparable studies to date lends credence to some important aspects of the hypothesis.

Despite the broad support for the deuterostome posterior flexibility hypothesis in recent studies, one interesting caveat to the hypothesis has emerged—that the hypothesis might not apply to all posterior Hox genes in all deuterostome taxa.[14,15] Both echinoderms and hemichordates have at least four posterior Hox genes (*Hox9/10*, *Hox11/13a*, *Hox11/13b* and *Hox11/13c*; see Fig. 1) whose similar nomenclature in the two taxa represents the likelihood that they were all inherited from a common ancestor (although this is disputed[22]). Two of these genes (*Hox9/10* and *Hox11/13a*) seem to show phylogenetic resolution consistent with the posterior flexibility hypotheses—i.e., they are poorly resolved. The other two genes (*Hox11/13b* and *Hox11/13c*) however, have been shown to group together with strong support in recent analyses: Holland et al[22] report a BPP of 0.92, a ML BP of 95% and a NJ BP of 99% for the grouping of *Hox11/13b* and *Hox11/13c* sequences and Cameron et al[11] report a NJ BP of 88% for the same grouping. Interpretation of this situation is complicated by the lack of resolution within the *Hox11/13b* and *Hox11/13c* clade. It might be the case that an ancestral *Hox11/13b* gene duplicated independently and recently in the hemichordate and echinoderm lineages to form the *Hox11/13c* genes. This explanation is consistent with the deuterostome posterior flexibility hypothesis, in that the high support for the grouping can reconciled with fast rates of molecular evolution by the postulation of a recent duplication. Another explanation for

the same pattern, preferred by some authors,[14,15] is that both *Hox11/13b* and *Hox11/13c* were present in the hemichordate/echinoderm ancestor. This hypothesis is not consistent with deuterostome posterior flexibility, in that it requires these two genes to have been evolving much more slowly than other deuterostome posterior Hox genes (and thus retaining a greater proportion of their phylogenetic signal than other deuterostome posterior Hox genes, since both the hemichordate/echinoderm and the cephalochordate/vertebrate splits are predicted to have occurred at around the time of the Cambrian explosion[45,46]). Distinguishing among these possibilities will require detailed statistical tests of phylogenetic topologies, in order to compare trees consistent with each hypothesis.

In addition to phylogenetic studies, two recent genomic studies[47,48] have made observations that are consistent with the deuterostome posterior flexibility hypothesis. Both of these studies have shown that the intergenic regions between the posterior Hox genes tend to be less conserved than those between the anterior Hox genes. This is consistent with the notion that not only the coding sequences but also the regulatory regions of deuterostome posterior Hox genes are evolving at a faster rate than those of the deuterostome anterior and central Hox genes.

The Mechanistic Basis of Deuterostome Posterior Flexibility

In essence the deuterostome posterior flexibility hypothesis is based upon the observation that most deuterostome posterior Hox genes appear less well resolved than other Hox genes in molecular phylogenetic analyses. Explanations for this pattern of phylogenetic support can be split into two broad categories. The first category of explanations presupposes that most deuterostome posterior Hox genes are evolving faster than most other Hox genes and goes on to propose possible reasons why this might be the case. The second category of explanations proprose reasons why the observed patterns of phylogenetic support might have arisen in the absence of differential rates of evolution. Each of these categories is discussed in more detail below.

Faster Rates May Be Linked to Gene Duplications

There are a number of mechanisms that have been suggested to underlie a faster rate of molecular evolution in the deuterostome posterior Hox genes, of which perhaps the most convincing is that the increased rate is linked to gene duplication events.[1] Although exact numbers are hard to estimate (see Fig. 1), it is clear that there have been significantly more duplications of posterior Hox genes in the deuterostome lineage than in the protostome lineage. Following a gene duplication event, the most likely outcome is that one of the two 'daughter' genes quickly degenerates to become a pseudogene through the acquisition of deleterious mutations ('nonfunctionalisation'). It is also conceivable (though unlikely) that one of the two daughter genes acquires a beneficial mutation that confers a new function ('neo-functionalisation'). A third possibility is that the two daughter genes evolve in such a way that the functional repertoire of the original gene is divided between them ('sub-functionalisation', also known as the duplication-degeneration-complementation model).[49-52] Gene duplications can lead to an increase in the rate of molecular evolution in two ways. First, there may be a brief period of relaxed selective constraint immediately following a duplication event.[51,53] Second, both neo- and sub-functionalisation—which are likely to have occurred in the majority of the posterior Hox genes present in the extant taxa, by virtue of the fact that the genes are still operative—imply a period of positive selection as the genes evolve to operate with a new or subdivided functional repertoire.[54] Indeed, although it might well be impossible to demonstrate whether there had been relaxed or positive selection following ancient Hox gene duplications[55] (such as those duplications which created many of the posterior Hox genes), there is good evidence that positive selection has occured after more recent Hox-gene duplications.[49,56-58] Thus, given the excess of gene duplications in the posterior Hox genes of deuterostomes relative to other taxonomic groups, it is likely that there exists a link between these duplications and an increased rate of molecular evolution, thus potentially explaining the observation of low phylogenetic resolution among deuterostome posterior Hox gene sequences.

Faster Rates May Be Linked to Morphological Evolution

Another prominent feature in the recent literature is the hypothesised link between the evolution of Hox genes and the evolution of morphological novelties.[59-63] With respect to the deuterostome posterior flexibility hypothesis, there are tantalising correlations between the expansion of the chordate Hox cluster and the evolution of a chordate-specific features.[59] Among other novelties, the posterior Hox genes are involved in the patterning of the post-anal tail of all chordates,[64] the limbs and digits of vertebrates[65-69] and have been implicated in the evolution of the pelvis—a key adaptation for the tetrapod lineage.[64] Morphological novelties, and the genes that are involved in patterning them, might have fast rates of evolution for two reasons. First, both genes and morphology might be evolving under strong positive selection. Second the novel morphological features may not be as tightly constrained—either in a developmental or an evolutionary sense—as many other morphological features and thus many more mutations which affect the patterning genes are likely to be selectively neutral. In contrast to the posterior Hox genes, it has been argued that the structures that the anterior and central Hox genes are responsible for patterning tend to be highly constrained (e.g., the neural tube of cephalochordates and the rhombocephalon of vertebrates).[47,48]

It will be very difficult to test effectively whether there exists (or existed) a causal link between the rate of evolution of the posterior Hox genes and the development of new morphological features in certain taxa. However, there are two lines of evidence that are suggestive of such a link. First, the observation that the intergenic regions of deuterostome posterior Hox genes tend to be less conserved than the intergenic regions of other deuterostome Hox genes provides good evidence that the posterior Hox genes of chordates are less tightly constrained than either the anterior or central Hox genes. This observation seems to square well with the degree of evolutionary constraint of the structures which these genes pattern.[47,48] Second, the echinoderm/hemichordate clade is thought to have inherited a small post-anal extension from the deuterostome ancestor,[70] a feature that has been lost or obscured in echinoderms[71] and remains un-elaborated in hemichordates (despite the expression of all three hemichordate posterior Hox genes in this region[70]). It is interesting that the posterior Hox genes of these phyla are those that appear to buck the deuterostome posterior flexibility trend and have a slower rate of evolution than other deuterostome posterior Hox genes (see above). At present, this is just a coincidental observation, but genomic studies of the degree of conservation of intergenic regions in the hemichordate/echinoderm clade would be extremely informative with respect to a possible link between the rates of molecular and morphological evolution.

Processes Other Than Faster Rates Might Be Operating

It is possible that the observations that led to the proposal of the deuterostome posterior flexibility hypothesis could be explained without the need to posit differential rates of molecular evolution among Hox genes. No studies have explicitly compared the rates of evolution of different Hox genes. Indeed, in those cases where molecular branch lengths have been included in published analyses of Hox genes, there is no obvious trend for the deuterostome posterior Hox genes to have significantly longer branches than other Hox genes[22,23] as would be expected if they were evolving at a faster rate. It has been suggested that the observed patterns of phylogenetic support might be the result of nonphylogenetic signal in the data, rather than the result of differential rates of evolution among Hox genes.

Some models of the evolution of the Hox genes have been suggested in which there were long periods of stasis in the evolutionary history of certain genes.[26,30] Such periods of stasis contravene the assumption that phylogenetic distance will tend to increase with time. Although the implications of this for phylogenetic analyses have not been worked out in detail, it is conceivable (though perhaps unlikely) that such periods of stasis could contribute to the observed patterns of phylogenetic support among Hox genes.

A more plausible source of nonphylogenetic signal that could confound phylogenetic analyses involves the co-evolution of interacting proteins. It has been suggested that in those cases where

a group of genes interact with a given protein (for instance vertebrate posterior Hox genes all interact with Meis1 proteins[72]), changes in the given protein (Meis1 in this case) within a given lineage might lead to correlated changes in all of the interacting proteins (the posterior Hox genes in this case) in that lineage.[73] This is problematic for conventional phylogenetic analyses as a fundamental assumption of such approaches is that all genes are evolving independently in all lineages. Simulations suggest that in those cases where the gene duplications are ancient and the evolutionary rate of the given protein is slow relative to the interacting proteins (as might be the case for the posterior Hox genes and Meis1 respectively) a conventional phylogenetic analyses of the duplicated genes will tend to be poorly resolved.[73] Thus it is feasible, although it remains untested, that this kind of process might explain the observed pattern of phylogenetic resolution among the Hox genes.

Conclusion and Future Directions

In the eight years since it was proposed that the deuterostome posterior Hox genes might be a fast evolving class a great many new Hox gene sequences have been published and a number of genomic studies of Hox genes have been undertaken. Concomitantly, our understanding of phylogenetic methodology, genomics and molecular evolution has increased significantly. However, despite these advances it is still difficult to come up with a reliable answer to the question: "Are the deuterostome posterior Hox genes a fast evolving class?" In general the available evidence weighs in favour of the idea that the majority of deuterostome posterior Hox genes are fast-evolving and the most likely mechanistic explanation for this is (in my opinion) that it is largely a result of the effects of gene duplication. It is difficult to make more concrete conclusions than this as there are a number of key deficiencies in the available data which preclude taxonomically broad-scale comparisons of the rates of evolution of different Hox genes and thus also preclude meaningful comparisons of the mechanistic underpinnings of such rate variation. Below I indicate where the current deficiencies in our understanding lie and suggest some approaches that might be taken to remove these deficiencies.

To date there have been no studies which have explicitly measured the rates of evolution of different Hox genes in different metazoan lineages. A comparative study of substitution rates in Hox genes is particularly important since it has been argued that not all deuterostome posterior Hox genes are fast evolving and that it is instead a phenomenon limited to the chordates.[14,15] The data and the methods to conduct a comparative study of rates of molecular evolution in the Hox genes are already available, although their application will be complicated by the very short alignable (60 amino acid) regions of different Hox genes.[74] Nevertheless, it might be possible to circumvent these difficulties by estimating the absolute rates of evolution of posterior Hox genes of closely related taxa using a dated molecular phylogeny and then comparing these absolute rates between different genes and taxa.

If a method can be found which allows the rates of evolution of different Hox genes in different lineages to be measured reliably, it may also be possible to compare the extent to which different putative explanatory variables (e.g., morphological evolution or gene duplication events) might be responsible for the observed variation in rates. For instance, methods which have been developed to test for links between rates of molecular evolution and speciation rates[75,76] could be adapted to test for a link between rates of molecular evolution and gene duplication events. Currently available methods to test for a link between morphological and molecular rates of evolution[77,78] would be much harder to apply to the Posterior Hox genes, however if such a study were carried out it would be the first study of its kind to systematically compare the rates of molecular evolution of developmental genes with the rates of evolution of the morphological features that those genes are responsible for patterning.

It is always difficult to rule out systematic bias in phylogenetic studies and it has been suggested that this might be a particular problem for studies of the Hox genes.[73] In particular, it has been suggested that co-evolutionary dynamics among Hox genes may confound conventional phylogenetic analyses, but thankfully there are existing methods that could be used to test for

the existence of such processes in Hox genes.[73,79] If robust conclusions are to be made about differential rates of evolution in Hox genes in the absence of comparative studies of substitution rates, it will be important to carry out such tests.

The results of genomic studies indicate that the intergenic regions of the chordate posterior Hox genes are evolving more quickly than those of the other Hox genes of chordates.[47,48,80,81] Although this observation is certainly consistent with the idea that the deuterostome posterior Hox genes are a fast evolving class, it is insufficient to assess whether the posterior Hox genes of all deuterostomes are evolving more quickly than all of the other deuterostome Hox genes and these data are also uninformative with respect to the relative rates of deuterostome Hox genes to Hox genes from other phyla. An extension of the genomic approach to the rest of the deuterostomes (i.e., the hemichordates and echinoderms) and to nondeuterostome taxa will be important in this respect. It would already be possible to carry out comparable studies on a number of publically available protostome genomes, although given the current limitations of the methodology[31] it might prove to be the case that some of the currently available genome sequences are too divergent, or the Hox clusters too large, for such methods to be applicable.

Finally, current analyses of the evolution of Hox clusters can be somewhat hampered by the difficulty of assigning Hox genes to particular orthology groups. This is a particular problem with the deuterostome posterior Hox genes and a key area of work in this respect is further sequencing of the Hox clusters of key deuterostome taxa—in particular the lamprey and hagfish and the *Xenoturbella*—which it might be hoped will further elucidate the evolutionary history of the Hox clusters of deuterostomes.

References

1. Ferrier DEK, Minguillon C, Holland PWH et al. The amphioxus hox cluster: deuterostome posterior flexibility and hox14. Evol Dev 2000; 2(5):284-293.
2. Peterson KJ, Butterfield NJ. Origin of the eumetazoa: testing ecological predictions of molecular clocks against the proterozoic fossil record. Proc Natl Acad Sci USA 2005; 102(27):9547-9552.
3. Aguinaldo AM, Turbeville JM, Linford LS et al. Evidence for a clade of nematodes, arthropods and other moulting animals. Nature 1997; 387(6632):489-493.
4. Balavoine G, de Rosa R, Adoutte A. Hox clusters and bilaterian phylogeny. Mol Phylogenet Evol 2002; 24(3):366-373.
5. Cook CE, Jimenez E, Akam M et al. The hox gene complement of acoel flatworms, a basal bilaterian clade. Evol Dev 2004; 6(3):154-163.
6. Ryan JF, Mazza ME, Pang K et al. Pre-bilaterian origins of the hox cluster and the hox code: evidence from the sea anemone, nematostella vectensis. PLoS One 2007; 2:e153.
7. Kamm K, Schierwater B, Jakob W et al. Axial patterning and diversification in the cnidaria predate the hox system. Curr Biol 2006; 16(9):920-926.
8. Finnerty JR, Martindale MQ. Ancient origins of axial patterning genes: Hox genes and ParaHox genes in the cnidaria. Evol Dev 1999; 1(1):16-23.
9. Finnerty JR, Martindale MQ. Homeoboxes in sea anemones (cnidaria; anthozoa): A PCR-based survey of nematostella vectensis and metridium senile. Biol Bull 1997; 193(1):62-76.
10. Ferrier DEK, Holland PWH. Ancient origin of the hox gene cluster. Nat Rev Gen 2001; 2(1):33-38.
11. Cameron RA, Rowen L, Nesbitt R et al. Unusual gene order and organization of the sea urchin hox cluster. J Exp Zoolog B Mol Dev Evol 2006; 306(1):45-58.
12. de Rosa R, Grenier JK, Andreeva T et al. Hox genes in brachiopods and priapulids and protostome evolution. Nature 1999; 399(6738):772-776.
13. Matus D, Halanych KM, Martindale MQ. The hox gene complement of a pelagic chaetognath, flaccisagitta enflata. Integ Comp Biol 2007; 47:854-864.
14. Peterson KJ. Isolation of hox and parahox genes in the hemichordate ptychodera flava and the evolution of deuterostome hox genes. Mol Phylogenet Evol 2004; 31(3):1208-1215.
15. Aronowicz J, Lowe CJ. Hox gene expression in the hemichordate saccoglossus kowalevskii and the evolution of deuterostome nervous systems. Integ Comp Biol 2006; 46:890-901.
16. Kmita-Cunisse M, Loosli F, Bierne J et al. Homeobox genes in the ribbonworm lineus sanguineus: Evolutionary implications. Proc Natl Acad Sci USA 1998; 95(6):3030-3035.
17. Nogi T, Watanabe K. Position-specific and noncolinear expression of the planarian posterior (abdominal-B-like) gene. Dev Growth Differ 2001; 43(2):177-184.

18. Callaerts P, Lee PN, Hartmann B et al. HOX genes in the sepiolid squid euprymna scolopes: implications for the evolution of complex body plans. Proc Natl Acad Sci USA 2002; 99(4):2088-2093.
19. Akam M. Hox and HOM: homologous gene clusters in insects and vertebrates. Cell 1989; 57(3):347-349.
20. Grenier JK, Garber TL, Warren R et al. Evolution of the entire arthropod hox gene set predated the origin and radiation of the onychophoran/arthropod clade. Curr Biol 1997; 7(8):547-553.
21. Van Auken K, Weaver DC, Edgar LG et al. Caenorhabditis elegans embryonic axial patterning requires two recently discovered posterior-group hox genes. Proc Natl Acad Sci USA 2000; 97(9):4499-4503.
22. Holland LZ, Albalat R, Azumi K et al. The amphioxus genome illuminates vertebrate origins and cephalochordate biology. Genome Res 2008; 18(7):1100-1111.
23. Seo HC, Edvardsen RB, Maeland AD et al. Hox cluster disintegration with persistent anteroposterior order of expression in oikopleura dioica. Nature 2004; 431(7004):67-71.
24. Duboule D, Boncinelli E, DeRobertis E et al. An update of mouse and human HOX gene nomenclature. Genomics 1990; 7(3):458-459.
25. Fritzsch G, Bohme MU, Thorndyke M et al. PCR survey of xenoturbella bocki hox genes. J Exp Zoolog B Mol Dev Evol 2008; 310(3):278-284.
26. Papillon D, Perez Y, Fasano L et al. Hox gene survey in the chaetognath spadella cephaloptera: evolutionary implications. Dev Genes Evol 2003; 213(3):142-148.
27. Aboobaker A. Hox gene evolution in nematodes: novelty conserved. Curr Opin Genet Dev 2003; 13(6):593-598.
28. Aboobaker AA, Blaxter ML. Hox gene loss during dynamic evolution of the nematode cluster. Curr Biol 2003; 13(1):37-40.
29. Wang BB, Muller-Immergluck MM, Austin J et al. A homeotic gene cluster patterns the anteroposterior body axis of C. elegans. Cell 1993; 74(1):29-42.
30. Ferrier DEK. Evolution of hox gene clusters. In: Papageorgiou S, ed. Hox Gene Expression. New York: Springer, 2007:53-67.
31. Amemiya CT, Prohaska SJ, Hill-Force A et al. The amphioxus hox cluster: characterization, comparative genomics and evolution. J Exp Zoolog B Mol Dev Evol 2008; 310(5):465-477.
32. Minguillon C, Gardenyes J, Serra E et al. No more than 14: the end of the amphioxus hox cluster. Int J Biol Sci 2005; 1(1):19-23.
33. Swalla BJ, Smith AB. Deciphering deuterostome phylogeny: molecular, morphological and palaeontological perspectives. Philos Trans R Soc Lond B Biol Sci 2008; 363(1496):1557-1568.
34. Lemons D, McGinnis W. Genomic evolution of hox gene clusters. Science 2006; 313(5795):1918-1922.
35. Amores A, Force A, Yan YL et al. Zebrafish hox clusters and vertebrate genome evolution. Science 1998; 282(5394):1711-1714.
36. Chourrout D, Delsuc F, Chourrout P et al. Minimal ProtoHox cluster inferred from bilaterian and cnidarian hox complements. Nature 2006; 442(7103):684-687.
37. Ferrier DE. Hox genes: Did the vertebrate ancestor have a Hox14? Curr Biol 2004; 14(5):R210-211.
38. Finnerty JR, Martindale MQ. The evolution of the hox cluster: insights from outgroups. Curr Opin Genet Dev 1998; 8(6):681-687.
39. Garcia-Fernandez J. Hox, ParaHox, ProtoHox: facts and guesses. Heredity 2005; 94(2):145-152.
40. Kourakis MJ, Martindale MQ. Combined-method phylogenetic analysis of hox and paraHox genes of the metazoa. J Exp Zool 2000; 288(2):175-191.
41. Ogishima S, Tanaka H. Missing link in the evolution of hox clusters. Gene 2007; 387(1-2):21-30.
42. Powers TP, Amemiya CT. Evidence for a hox14 paralog group in vertebrates. Curr Biol 2004; 14(5):R183-184.
43. Kuraku S, Takio Y, Tamura K et al. Noncanonical role of hox14 revealed by its expression patterns in lamprey and shark. Proc Natl Acad Sci USA 2008; 105(18):6679-6683.
44. Lanfear R, Bromham L. Statistical tests between competing hypotheses of hox cluster evolution. Syst Biol 2008; 57(5):1-11.
45. Schubert M, Escriva H, Xavier-Neto J et al. Amphioxus and tunicates as evolutionary model systems. Trends Ecol Evol 2006; 21(5):269-277.
46. Peterson KJ, McPeek MA, Evans DAD. Tempo and mode of early animal evolution: inferences from rocks, hox and molecular clocks. Paleobiology 2005; 31(2):36-55.
47. Pascual-Anaya J, D'Aniello S, Garcia-Fernandez J. Unexpectedly large number of conserved noncoding regions within the ancestral chordate hox cluster. Dev Genes Evol 2008; 218(11-12):591-7. Epub 2008 Sep 13.
48. Santini S, Boore JL, Meyer A. Evolutionary conservation of regulatory elements in vertebrate hox gene clusters. Genome Res 2003; 13(6A):1111-1122.

49. Prince VE, Pickett FB. Splitting pairs: the diverging fates of duplicated genes. Nat Rev Genet 2002; 3(11):827-837.
50. Mazet F, Shimeld SM. Gene duplication and divergence in the early evolution of vertebrates. Curr Opin Genet Dev 2002; 12(4):393-396.
51. Lynch M, Conery JS. The evolutionary fate and consequences of duplicate genes. Science 2000; 290(5494):1151-1155.
52. Force A, Lynch M, Pickett FB et al. Preservation of duplicate genes by complementary, degenerative mutations. Genetics 1999; 151(4):1531-1545.
53. Kondrashov FA, Rogozin IB, Wolf YI et al. Selection in the evolution of gene duplications. Genome Biol 2002; 3(2):RESEARCH0008.
54. Kondrashov FA, Kondrashov AS. Role of selection in fixation of gene duplications. J Theor Biol 2006; 239(2):141-151.
55. Van de Peer Y, Taylor JS, Braasch I et al. The ghost of selection past: rates of evolution and functional divergence of anciently duplicated genes. J Mol Evol 2001; 53(4-5):436-446.
56. Taylor JS, Raes J. Duplication and divergence: the evolution of new genes and old ideas. Annu Rev Genet 2004; 38:615-643.
57. Lynch VJ, Roth JJ, Wagner GP. Adaptive evolution of hox-gene homeodomains after cluster duplications. BMC Evol Biol 2006; 6:86.
58. McClintock JM, Carlson R, Mann DM et al. Consequences of hox gene duplication in the vertebrates: an investigation of the zebrafish hox paralogue group 1 genes. Development 2001; 128(13):2471-2484.
59. Holland PWH, GarciaFernandez J. Hox genes and chordate evolution. Dev Biol 1996; 173(2):382-395.
60. Wagner GP, Amemiya C, Ruddle F. Hox cluster duplications and the opportunity for evolutionary novelties. Proc Natl Acad Sci USA 2003; 100(25):14603-14606.
61. Budd GE. Does evolution in body patterning genes drive morphological change—or vice versa? Bioessays 1999; 21(4):326-332.
62. Hughes CL, Kaufman TC. Hox genes and the evolution of the arthropod body plan. Evol Dev 2002; 4(6):459-499.
63. Gellon G, McGinnis W. Shaping animal body plans in development and evolution by modulation of hox expression patterns. Bioessays 1998; 20(2):116-125.
64. Burke AC, Nelson CE, Morgan BA et al. Hox genes and the evolution of vertebrate axial morphology. Development 1995; 121(2):333-346.
65. Zakany J, Duboule D. The role of hox genes during vertebrate limb development. Curr Opin Genet Dev 2007; 17(4):359-366.
66. Zakany J, Kmita M, Duboule D. A dual role for hox genes in limb anterior-posterior asymmetry. Science 2004; 304(5677):1669-1672.
67. Davis AP, Witte DP, Hsieh-Li HM et al. Absence of radius and ulna in mice lacking hoxa-11 and hoxd-11. Nature 1995; 375(6534):791-795.
68. Deschamps J. Developmental biology. Hox genes in the limb: a play in two acts. Science 2004; 304(5677):1610-1611.
69. Galis F, Kundrat M, Metz JA. Hox genes, digit identities and the theropod/bird transition. J Exp Zoolog B Mol Dev Evol 2005; 304(3):198-205.
70. Gerhart J, Lowe C, Kirschner M. Hemichordates and the origin of chordates. Curr Opin Genet Dev 2005; 15(4):461-467.
71. Smith AB. Deuterostomes in a twist: the origins of a radical new body plan. Evol Dev 2008; 10(4):493-503.
72. Shen WF, Montgomery JC, Rozenfeld S et al. AbdB-like hox proteins stabilize DNA binding by the Meis1 homeodomain proteins. Mol Cell Biol 1997; 17(11):6448-6458.
73. Campos PR, de Oliveira VM, Wagner GP et al. Gene phylogenies and protein-protein interactions: possible artifacts resulting from shared protein interaction partners. J Theor Biol 2004; 231(2):197-202.
74. Welch JJ, Waxman D. Calculating independent contrasts for the comparative study of substitution rates. J Theor Biol 2008; 251(4):667-678.
75. Pagel M, Venditti C, Meade A. Large punctuational contribution of speciation to evolutionary divergence at the molecular level. Science 2006; 314(5796):119-121.
76. Xiang QY, Zhang WH, Ricklefs RE et al. Regional differences in rates of plant speciation and molecular evolution: a comparison between Eastern Asia and Eastern North America. Evolution 2004; 58(10):2175-2184.
77. Bromham L, Woolfit M, Lee MS et al. Testing the relationship between morphological and molecular rates of change along phylogenies. Evolution Int J Org Evolution 2002; 56(10):1921-1930.

78. Davies TJ, Savolainen V. Neutral theory, phylogenies and the relationship between phenotypic change and evolutionary rates. Evolution Int J Org Evolution 2006; 60(3):476-483.
79. Goh CS, Bogan AA, Joachimiak M et al. Co-evolution of proteins with their interaction partners. J Mol Biol 2000; 299(2):283-293.
80. Chiu CH, Amemiya C, Dewar K et al. Molecular evolution of the HoxA cluster in the three major gnathostome lineages. Proc Natl Acad Sci USA 2002; 99(8):5492-5497.
81. Chiu CH, Dewar K, Wagner GP et al. Bichir HoxA cluster sequence reveals surprising trends in ray-finned fish genomic evolution. Genome Res 2004; 14(1):11-17.