Int. J. Dev. Biol. 47: 605-611 (2003)

Evolution of the *Hox/ParaHox* gene clusters

DAVID E.K. FERRIER*,[1] and CAROLINA MINGUILLÓN[2]

[1]*Department of Zoology, University of Oxford, U.K. and* [2]*Division of Developmental Biology, National Institute for Medical Research, London, U.K.*

ABSTRACT The *Hox* gene cluster is a guiding force within the field of Evolutionary Developmental Biology. In large part our understanding of this gene cluster comes from only a few model organisms in developmental biology. The situation is gradually changing. A comparative review of the organisation of the *Hox* and *ParaHox* gene clusters and, in particular, instances of cluster disintegration, leads us to the view that the phenomenon of Temporal Colinearity is the major constraining force in maintaining these gene clusters over such long evolutionary timespans.

KEY WORDS: *temporal colinearity, amphioxus, homeobox*

Introduction

The Hox gene cluster has been a key paradigm within Evolutionary Developmental Biology since the middle of the 1980's. Clustering of homeotic genes had been known in *Drosophila melanogaster* for many years prior to this (reviewed in Denell, 1994; McGinnis, 1994). With the discovery of the homeobox, a motif present in all of the clustered homeotic genes and coding for a sequence-specific DNA-binding domain, and the almost immediate discovery of homologous sequences in vertebrates, the door was opened to making detailed molecular comparisons across large phylogenetic distances (McGinnis *et al.*, 1984). A whole new swathe of data is being rapidly accumulated to address long-standing issues in Evo-Devo, that previously had been restricted to morphological comparisons alone.

The finding that galvanised the Evo-Devo community was that in the Hox genes we have homologous genes acting in an apparently homologous way across the animal kingdom. Furthermore these Hox genes were organised in a similar fashion. They are clustered in both flies and mice (McGinnis and Krumlauf, 1992). These clusters are not simply genes gathered together in the genome; they exhibit Colinearity. The genes at one end of the cluster are expressed, and pattern, the anterior end of the embryo, whilst the genes at the other end of the cluster pattern the posterior of the embryo. The genes in the central region of the cluster pattern the middle of the embryo. This is Spatial Colinearity. There is also a Temporal aspect to Colinearity. Genes at one end of the cluster are activated first and gene expression is initiated progressively through the cluster until the genes at the opposite end are turned on last of all (Duboule and Dollé, 1989; Graham *et al.*, 1989; Duboule, 1994). The organisation of the Hox gene cluster is intimately linked to the function of the genes in patterning the anterior-posterior axis of animals. More recently this whole phenomenon has found an evolutionary echo: the ParaHox gene cluster. The ParaHox cluster is the evolutionary sister, or paralogue, of the Hox cluster (Brooke *et al.*, 1998).

The above description of the Hox gene cluster is of course a generalization, and a closer look at the cluster from a comparative point of view is revealing with regards to the probable constraints on this mysterious organisation, and consequently the possible modes of regulation of these genes. As with any comparative approach a phylogeny is essential. The phylogeny of the metazoa has undergone a profound reorganisation in recent years, due to the impact of molecular data. The essential component of the 'new' metazoan phylogeny (Fig. 1) is the division of the protostomes into two major clades, the Ecdysozoa and the Lophotrochozoa (Aguinaldo *et al.*, 1997). The nematodes, which were previously held to be relatively basal within the Bilateria, are now considered to be quite closely allied to the arthropods along with a few other phyla that all moult, or undergo ecdysis. Hence their name: Ecdysozoa. The bulk of the remaining protostome phyla are now grouped within the Lophotrochozoan clade, so called because of the membership of the *Lopho*phorates (bryozoans, brachiopods and phoronids) and taxa with variations on the *trocho*phore larvae (e.g. annelids, sipunculans and molluscs).

From Fig. 1 it can be clearly seen that the characterisation of Hox clusters has been largely driven by conventional model systems in developmental biology: namely flies, nematodes, sea squirts and several vertebrates. Choice of taxa for study with a greater consideration of phylogenetic position reveals that many of these conventional model systems are rather derived in terms of the organisation and content of their Hox and ParaHox genes.

***Address correspondence to:** Dr. David E.K. Ferrier. Dept. of Zoology, University of Oxford, Tinbergen Building, South Parks Road, Oxford, OX1 3PS, U.K. Fax: +44-1865-271-184. e-mail: david.ferrier@zoo.ox.ac.uk

0214-6282/2003/$25.00

Printed in Spain
www.ijdb.ehu.es

The insects: cluster splitting and non-*Hox* genes

The Hox cluster of *D. melanogaster* is split into two; the ANT-C and BX-C, which are separated by approximately 8Mb on chromosome 3. The ANT-C consists of the Hox genes *labial* (*lab*), *proboscipedia* (*pb*), *Deformed* (*Dfd*), *Sex combs reduced* (*Scr*), and *Antennapedia* (*Antp*). The Hox genes of the BX-C are *Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*) and *Abdominal-B* (*Abd-B*). The split in the cluster lies in a different location in another species of *Drosophila*, *D. virilis*, where the separation is between *Ubx* and *abd-A* (Von Allmen *et al.*, 1996), and some *D. melanogaster* mutants have been generated that show that the BX-C can be split further and still produce viable flies (Struhl, 1984; Tiong *et al.*, 1987). The naturally occurring splits of the fly clusters are clearly a derived condition for insects, as more basal insects, and even more basal dipterans, have a single intact cluster (*Schistocerca* (Ferrier and Akam, 1996), *Tribolium* (Beeman, 1987) and *Anopheles* (Powers *et al.*, 2000; Devenport *et al.*, 2000)).

Embedded within the insect Hox clusters are some other homeobox genes that do not function as Hox genes. These genes in *Drosophila* are *zerknüllt* (*zen*) (of which there are two, *zen1* and *zen2*), *fushi-tarazu* (*ftz*) and *bicoid* (*bcd*). These genes are now known to have evolved within the cluster from Hox genes. Zen genes are involved in dorsoventral patterning in flies, and arose from a Hox3 gene (Falciani *et al.*, 1996). *Bcd* is the maternally-supplied morphogen for the anterior development of the fly embryo, and seems to have evolved from the zen/Hox3 genes (Stauber *et al.*, 1999). *Ftz* is a Pair-Rule segmentation gene in *Drosophila*, and probably evolved from one of the middle Hox genes (Telford, 2000).

In *D.melanogaster* there are also some non-homeobox genes within the cluster. Between *lab* and *pb* there is a cluster of cuticle genes, and between *bcd* and *Dfd* there is the *amalgam* gene (Seeger *et al.*, 1988; Powers *et al.*, 2000). There are no non-homeobox genes in the beetle Hox cluster in the region homologous to the fly ANT-C (Brown *et al.*, 2002), and the mosquito *amalgam* and cuticle gene homologues lie elsewhere in the genome, well away from the Hox cluster (*amalgam* is at 2.7Mb and cuticle protein-encoding genes are at 7.6Mb on chromosome arm 2R, whilst the Hox cluster begins at 61Mb on 2R, on the Ensembl release of 6th May 2003). Invasion of the fly Hox cluster by these non-homeobox genes may well be correlated with the splitting of the drosophilid Hox cluster, and be another indication of its recently derived state.

Spatial Colinearity is present in flies, and indeed *D. melanogaster* was the first animal in which the phenomenon was defined, but Temporal Colinearity is not apparent. The fly Hox genes are activated almost simultaneously during the cellular blastoderm stage. Temporal Colinearity was only really articulated once vertebrates were examined, with their more gradual elaboration of the Anterior-Posterior axis during embryogenesis (Duboule, 1994). The absence of Temporal Colinearity in flies is probably due, in large part, to the speed of their embryogenesis. In Short Germ insects, which form their segments gradually from a posterior growth zone, Temporal Colinearity is probably present (Kelsh *et al.*, 1993; Kelsh *et al.*, 1994)

Therefore *Drosophila* is derived, even within the insects, with regards to the Hox cluster. The ancestral insect condition was an intact cluster, with both Spatial and Temporal Colinearity. The presence of some homeobox genes with non-Hox functions does however seem to be a basal feature of insects, in stark contrast to the vertebrate condition (see below).

Vertebrates: cluster duplications, Quantitative Colinearity and 'clean' clusters

Mammals (e.g. human and mouse) have four Hox clusters, containing a total of 39 genes. These four clusters are paralogous, having arisen by duplications from a single ancestral cluster at the origin of the vertebrates (Garcia-Fernàndez and Holland, 1994). Each cluster contains a selection of thirteen paralogy groups, but no cluster contains all thirteen. There are gaps in the clusters due to gene loss.

In bony fish further duplications seem to have occurred, such that zebrafish has at least seven Hox clusters (Amores *et al.*, 1998) (see Fig. 1). Of all of the vertebrate Hox genes examined so far both Spatial and Temporal Colinearity are obeyed (apart from Hox 2 genes extending more anteriorly than Hox 1 genes in the hindbrain) (Dekker *et al.*, 1992; Duboule, 1994, and references therein; Prince *et al.*, 1998, and references therein). This Colinearity is even repeated in evolutionary novel structures such as the paired appendages, which arose during vertebrate evolution. Posterior Hox genes (Hox groups 9 and above) of the HoxA and HoxD clusters are expressed sequentially in nested domains in the limb buds (Duboule, 1992).

The nested expression in the digit domain of mice has recently been shown to be driven by a Global Control Region (GCR) located outside of the Hox cluster (Spitz *et al.*, 2003). The Colinearity of gene expression and chromosomal position occurs due to the enhancer activity being titrated out in digit development, by distance to the promoter and the number of intervening promoters. The genes further away from the GCR are activated at gradually lower levels. This is Quantitative Colinearity. A consequence of such Quantitative Colinearity is Spatial Colinearity of the Posterior Hox genes in digit development. It is now necessary to establish whether such a titration mechanism is also applicable to other instances of Colinearity outside of the limb, particularly the more ancient occurrence of nested expression in the trunk of the animal. It will also be interesting to see how widespread over the animal kingdom such Hox GCRs are.

By comparison to the insect Hox clusters those of vertebrates are 'cleaner'. In vertebrates there are no homeobox genes with non-Hox expression patterns, and there are no reports of invading non-homeobox genes, except for the *PRAC* gene which lies just upstream of *HoxB13* (Liu *et al.*, 2001). A further indication of cleanliness is that transposable elements seem to be excluded from the vertebrate clusters. The incidence of such elements is very low within the clusters but their density is clearly much higher immediately outside of the clusters (http://genome.ucsc.edu/). Although again *HoxB13* is an exception to this rule (Zeltser *et al.*, 1996). There may be a selective pressure to keep these repetitive elements out of the Hox clusters so that the chance of a genomic rearrangement is reduced (Ferrier and Holland, 2002).

This difference between the composition of the insect and vertebrate clusters perhaps reflects a difference in the mechanisms regulating the two types of cluster. Vertebrates use GCR's and shared enhancers to control their Hox genes, whilst flies tend to have gene-specific enhancers (Mann, 1997). The enhancer-sharing seen

in vertebrates would provide another selective pressure to prevent break-up of the Hox cluster, whilst splits of the *Drosophila* cluster may be viable as long as they occur between the genes and their associated neighbouring enhancers (Struhl, 1984; Tiong *et al.*, 1987).

Other deuterostomes: a single cluster, but how many genes?

The deuterostomes consist of the vertebrates, cephalochordates and urochordates (which together form the chordates), and the hemichordates and echinoderms. Hox clusters have been shown to exist in the cephalochordate amphioxus (*Branchiostoma floridae*) and the echinoderm *Stronglyocentrotus purpuratus* (Garcia-Fernàndez and Holland, 1994; Martinez *et al.*, 1999). The cluster of the sea squirt *Ciona intestinalis* seems to have disintegrated, and some Hox genes have been lost (Dehal *et al.*, 2002; Spagnuolo *et al.*, 2003).

Amphioxus is in the sister group to the vertebrates. It has a single contiguous Hox cluster, consistent with the hypothesis that the multiple Hox clusters of vertebrates arose during the genome-wide duplications that occurred at the origin of the vertebrates after the divergence of the amphioxus lineage. The amphioxus cluster has a pro-orthologue of each of the vertebrate paralogy groups (Sharman, 1999; Ferrier *et al.*, 2000), although the relationship of the Posterior Hox genes of amphioxus with the paralogy groups Hox9-13 of vertebrates is somewhat obscured. This lack of a clear relationship amongst the Posterior Hox genes of deuterostomes is probably due to higher rates of sequence evolution amongst these Posterior Hox genes than amongst other Hox genes, or the Posterior Hox genes of the protostomes; a phenomenon called Deuterostome Posterior Flexibility (Ferrier *et al.*, 2000). Amphioxus also has an 'extra' Hox gene, *AmphiHox14*, that has no counterpart in vertebrates as yet (Ferrier *et al.*, 2000). The first four Hox genes of amphioxus have had their expression analysed in detail. They obey Temporal Colinearity, but *AmphiHox2* breaks Spatial Colinearity (Wada *et al.*, 1999).

The single cluster of the sea urchin, *S. purpuratus*, is lacking one of the central Hox genes (probably Hox4 (Hano *et al.*, 2001)). This represents a gene loss in echinoderms, as homologues of Hox4 are present in the chordates and protostomes, such as the *Dfd* gene of flies. Also the precise number of Posterior Hox genes in echinoderms remains to be established. The genomic walk in *S. purpuratus* encompassed three Posterior Hox genes, but did not extend further. More than three Posterior Hox genes have been isolated in other echinoderms by PCR (Mito and Endo, 2000). The nature of their linkage and whether there are still further types of Posterior Hox to be found, that may be too divergent to have been detected by the degenerate PCR primers used so far, remains to be seen.

The deuterostome ancestor therefore had a single Hox cluster, which probably contained multiple Posterior Hox genes. The exact ancestral number will be very difficult to ascertain because of the levels of sequence divergence amongst the deuterostome Posterior Hox genes, and the consequent difficulty in assigning clear homologies.

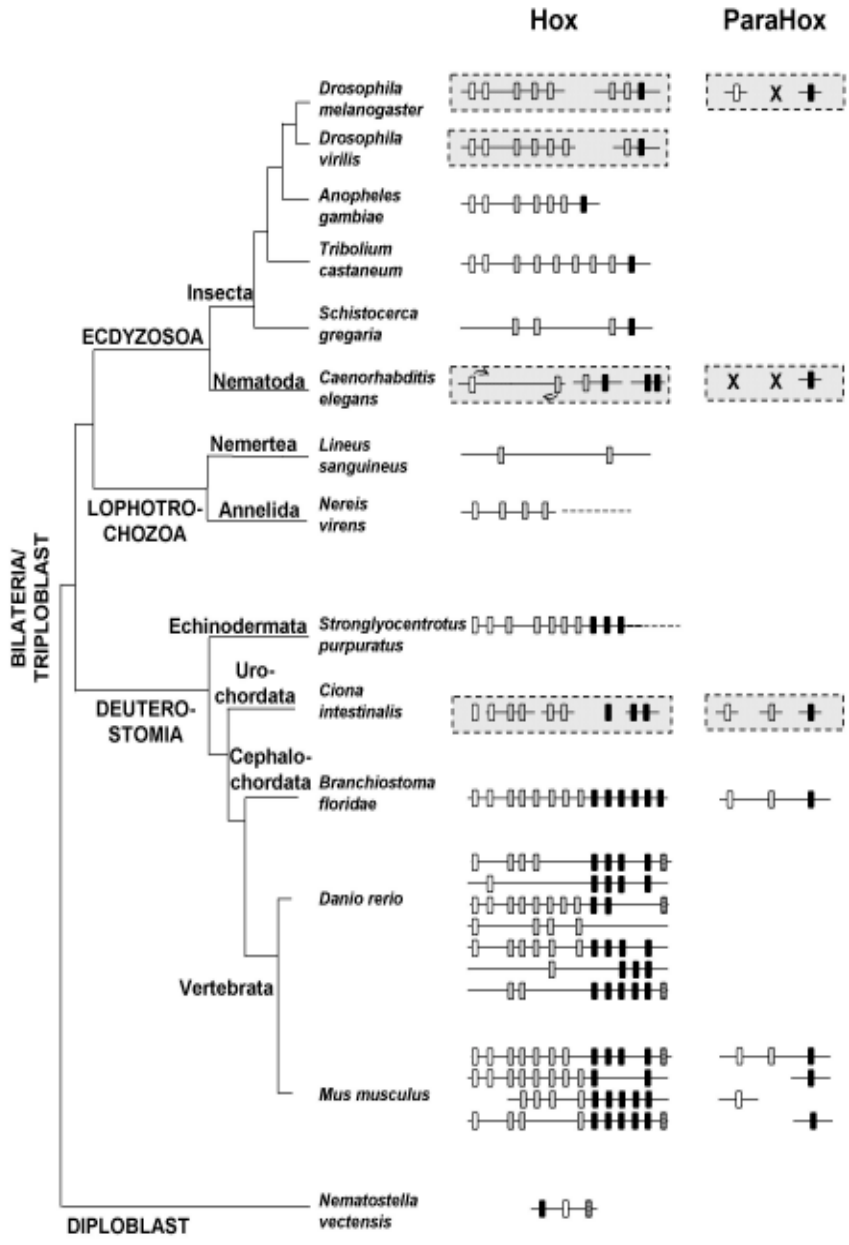


Fig. 1. Metazoan phylogeny with schematics of the genomic organisation of *Hox* and *ParaHox* genes. *The* Hox *and* ParaHox *clusters that have broken up are highlighted in grey boxes. The gene shading shows* Anterior Hox *and* Gsx *in white; group 3 central* Hox *and* Xlox *in grey; and Posterior* Hox *and* Cdx *in black. Striped boxes are* Evx *genes linked to the* Hox *clusters of vertebrates and cnidarians. X represents loss of a* ParaHox *gene. The gaps in the* Hox *clusters of* S. gregaria *and* L. sanguineus *represent lack of information on the intervening genes and not gene absence.*

Other bilaterian taxa: a gap waiting to be filled

In addition to the Hox clusters that have been characterised in deuterostomes and insects we have clustering information from only a few other bilaterian taxa: nemertines, annelids and nematodes. The data for nemertines and annelids so far consists of different Hox gene fragments hybridising to common Southern bands representing large fragments of DNA (several hundred kilobases) separated by Pulsed Field Gel Electrophoresis (PFGE). The nemertine *LsHox3* and *LsHox7* genes seem to be within 200-300kb of each other (Kmita-Cunisse *et al.*, 1998). In the polychaete annelid *Nereis virens* the Hox genes *Nvi-lab*, *Nvi-Hox3, Nvi-Dfd* and *Nvi-Scr* seem to be on the same760-780kb fragment (Andreeva *et al.*, 2001).

The data in nematodes largely comes from *C. elegans*. Six Hox genes have been found in this nematode. This represents a reduced set of Hox genes, resulting from several gene loss events within the nematode lineage (Aboobaker and Blaxter, 2003). The six *C. elegans* genes do not exist as a contiguous cluster. There are three pairs of Hox genes distributed over 6.5 Mb, with numerous non-homeobox genes in between (Van Auken *et al.*, 2000). Furthermore the anterior two genes (*ceh-13* and *lin-39*) are inverted with respect to the other Hox genes. *Ceh-13* is the orthologue of *lab, lin-39* of *Scr, mab-5* of an Antp-like gene and the remaining three *C. elegans* Hox genes are probably all Posterior Hox genes. *Egl-5, php-3* and *nob-1* all show an affinity with *AbdB*, but *php-3* and *nob-1* are thought to be a tandem duplication specific to the nematode lineage (Van Auken *et al.*, 2000).

The Hox genes of nematodes have relatively divergent sequences compared to those of other animals. The extensive gene loss combined with rearrangements in this taxon, mean that much important information with regards to the ancestral nematode or even ecdysozoan condition has been lost. Other less derived taxa will be required to fill this hole. The nemertine and annelid PFGE results show that the Hox clusters are probably intact in these animals. But these data are preliminary, and more detailed work will be needed to see whether all of the Hox genes are within these clusters, and whether the gene order, orientation and expression meets with expectations. If the gene order within the polychaete annelid Hox cluster is as expected from comparisons to other phyla, then Temporal Colinearity is apparent in this taxon as well (Irvine and Martindale, 2000).

Diploblasts: Evx, and the ProtoHox has duplicated

The animals discussed so far are all bilaterally symmetrical and triploblasts, with three germ layers (echinoderm adults are pentamerally symmetrical, but develop from a bilateral larva). The taxa with only two germ layers, the diploblasts, lie more basally within the animal kingdom (see Fig. 1). Of these diploblast taxa two have yielded information on Hox gene clustering: the coral *Acropora formosa* and the sea anemone *Nematostella vectensis*. In both of these animals Evx is tightly linked to Hox genes (Miller and Miles, 1993; Finnerty, 2001). Taken together with the close location of Evx to the Hox clusters of vertebrates, this is evidence that Evx was a member of the ancestral Hox cluster very early in animal evolution.

The Hox cluster of *N. vectensis* has been shown to contain at least two Hox genes so far, in addition to Evx (Finnerty, 2001). Other Hox-like genes are present in this animal's genome. Whether these other genes are linked to the *Anthox6/anth-eve/anthox1* cluster, or have been dispersed around the genome awaits further work.

The cnidarian lineage diverged from that leading to the triploblasts over 600 MYA, at a conservative estimate. Such a deep time has contributed to the difficulty in assigning cnidarian Hox-like genes to specific bilaterian homologues. However, it is clear from the cnidarian sequences that the Gsx ParaHox gene is present, and possibly a Cdx gene (reviewed in Ferrier and Holland, 2001; Finnerty, 2001). Consequently the duplication that gave rise to the Hox cluster and its evolutionary sister, the ParaHox cluster, occurred very early in animal evolution, before the origin of the cnidarians.

ParaHox clusters

Information on the ParaHox cluster is so far restricted to the chordates. This gene cluster, consisting of Gsx, Xlox and Cdx, was discovered in amphioxus, and was revealed by mapping and more recently by genome projects in mammals (Brooke *et al.*, 1998). The ParaHox cluster seems to have disintegrated in the urochordate *C. intestinalis*, a situation reflected in this animal's Hox cluster (Dehal *et al.*, 2002; Ferrier and Holland, 2002; Spagnuolo *et al.*, 2003). Conventional model systems (*D. melanogaster* and *C. elegans*) are not helpful in understanding ancestral features of the ParaHox cluster because they have lost the cluster and deleted some of the genes (Ruvkun and Hobert, 1998). Again this cluster disintegration parallels the Hox situation in these two animals.

The amphioxus ParaHox cluster exhibits both Spatial and Temporal Colinearity. However, the Temporal Colinearity is inverted with respect to the pattern in the Hox cluster. In the ParaHox cluster the posterior gene Cdx is activated first, followed by Xlox and then finally the anterior gene Gsx is expressed last of all. This contrasts with Temporal Colinearity in the Hox cluster where the anterior genes are activated first and the posterior genes are the last to be turned on. This is important because with the Hox genes it could be argued that Temporal Colinearity is merely a by-product of a mechanism that is concerned with producing Spatial Colinearity along a gradually developing anterior-posterior axis (and indeed the two are probably intimately entwined) (Duboule, 1994). With the 'inversion' of Temporal Colinearity in the ParaHox cluster however, this perhaps represents a divorce of Temporal and Spatial Colinearity, and may allow us to tease apart the mechanisms underlying the two processes.

We need more ParaHox clusters to show us how accurate the amphioxus situation is as a proxy for the ancestral condition.

Temporal Colinearity as the constraining force on clustering?

From Fig. 1 it can be seen that several Hox clusters have been broken (*Drosophila*, *C. elegans, C. intestinalis*). Ferrier and Holland (2002) have pointed out how these taxa are probably also

derived with respect to their ParaHox genes; flies and nematodes having lost genes, and *C. intestinalis* probably having a broken-up ParaHox cluster. Such cluster break-up is correlated with a very rapid mode of embryogenesis in these three animals, this rapid embryogenesis being a derived condition in each lineage. Such rapid development may no longer allow the time or opportunity for a temporal progression in the activation of Hox and ParaHox genes. Temporal Colinearity does not have a sufficient time period in which to occur in these animals, whilst Spatial Colinearity still exists (although talking of Colinearity in a cluster that has disintegrated is somewhat of an anachronism!). Consequently any regulatory mechanisms that were ancestrally required to produce Temporal Colinearity became dispensable in the fly, nematode and sea squirt lineages. If such regulatory mechanisms required the genes to be tightly linked, then loss of the mechanisms due to lack of a constraining force on their maintenance, could permit the gene clusters to be broken during the course of evolution with no selectively deleterious effects.

The sea urchin paradox

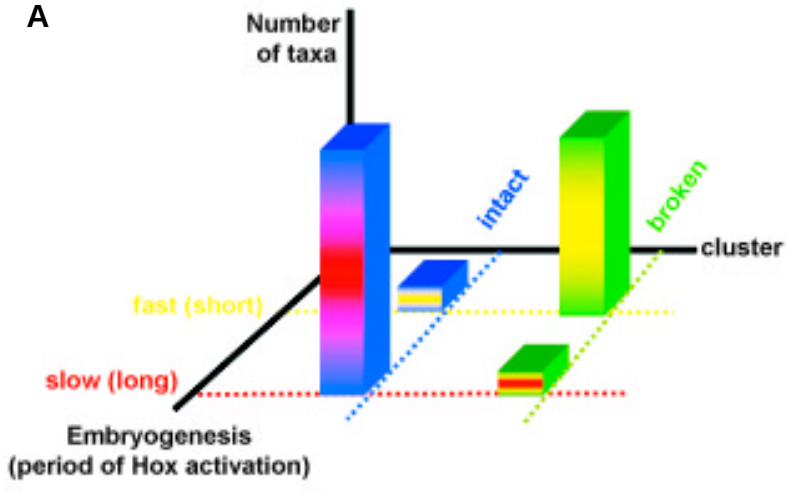
There is a fly (or rather a sea urchin) in the ointment however. Sea urchins have an intact Hox cluster, and a form of Spatial Colinearity has been observed (Arenas-Mena *et al.*, 2000), whilst Temporal Colinearity seems to be broken by *SpHox7* and *SpHox11/13b*, which are the only Hox genes found to be significantly expressed during embryogenesis so far (Arenas-Mena *et al.*, 1998). As the Hox and ParaHox clusters of other taxa are examined with regards to their organisation and expression, then a more rigorous assessment of the correlation between speed of embryogenesis (or more precisely the opportunity for Temporal Colinearity) and cluster integrity can be made. Is the sea urchin an isolated case, with an intact Hox cluster and violations of Hox gene Temporal Colinearity? Or the extreme form of indirect development present in echinoderms leading us astray, and the early embryonic expression of *SpHox7* and *SpHox11/13b* is not comparable to Hox expression in other animals, whilst the expression only in the adult rudiment is of concern from a comparative context? Perhaps the expression of *SpHox7* and *SpHox11/13b* is analogous to the non-Hox expression of zen and ftz homologues in insects, which seem to be insulated from, or immune to any mechanism of Temporal Colinearity governing the surrounding Hox genes. If it is indeed only the echinoderm adult rudiment expression that is relevant in this context, then the issue as to whether Temporal Colinearity can be observed during the metamorphosis of the adult rudiment needs to be addressed.

Molecular mechanisms

Models of the progressive gene activation implicit in Temporal Colinearity commonly involve protein complexes containing the Polycomb protein, or its homologues. Polycomb proteins act in large complexes and can alter chromatin conformation (Orlando, 2003). They are known to regulate the Hox genes of flies, nematodes and mice (Ross and Zarkower, 2003), and so probably of all animals. Of these model systems in which regulation of Hox genes by Polycomb has been established, only mice have Temporal Colinearity. If Polycomb complexes are involved with the mechanism of Temporal Colinearity, then the regulation of Hox genes by these proteins in flies and nematodes may largely be an evolutionary remnant from their ancestors which had Temporal Colinearity.

Conclusions

As more taxa are studied with regards to their Hox and ParaHox clusters it will be interesting to see how the diagrams in Fig. 2 develop. From the description above we would predict that animals with a rapid embryogenesis, and only a short period over which Hox and ParaHox gene expression is initiated, will be the taxa in which broken clusters are found (this being the rule that seems to be developing from the data so far, see Fig. 2A). Conversely, animals with a slower embryogenesis and a longer drawn out period in which the Hox and ParaHox genes are initiated should maintain their clusters intact. An alternative way of framing future studies of Hox and ParaHox organisation is



B

		Hox gene 1	2	3	4	5	… cluster
order of activation	(a)	1st	2nd	3rd	4th	5th	———
	(b)	1st	4th	2nd	5th	3rd	– – – ·
	(c)	1st	2nd	3rd	4th	5th	– – – ·
	(d)	1st	5th	3rd	2nd	4th	———

Fig. 2. Predictions for patterns of *Hox/ParaHox* gene organisation in relation to period and order of gene activation if Temporal Colinearity provides a major constraining force on *Hox/ParaHox* clustering. (A) *3D representation of the expected number of taxa exhibiting an intact (blue) or a broken (green) cluster in relation with the short (yellow) or long (red) period over which their* Hox *gene expression is initiated.* **(B)** *Relationship between the order of activation of* Hox/ParaHox *genes and their genomic organisation. The prediction is that lines (a) and (b) will be the predominant forms of* Hox/ParaHox *organisation in relation to their temporal sequence of activation. The alternatives in (c) and (d) should be rare (see text).*

outlined in Fig. 2B. If the mechanisms that produce Temporal Colinearity are the major constraining forces on cluster maintenance, then the situations in Fig. 2B (a) and (b) should prevail; taxa with Temporal Colinearity (Fig. 2B(a)) should have intact clusters, whilst taxa without Temporal Colinearity (Fig. 2B(b)) can have broken clusters. If examples begin to accumulate that take the form of Fig. 2B(c) and (d), with Temporal Colinearity and broken clusters or no Temporal Colinearity and intact clusters, then the link between cluster maintenance and Temporal Colinearity that we have postulated here will need to be modified.

Acknowledgments.

The authors would like to thank Jordi Garcia-Fernàndez and Peter Holland in whose labs we have been working and forming these ideas, and Peter Holland for comments on the manuscript. The authors research is funded by the BBSRC (D.E.K.F.) and MRC (C.M.)

References

ABOOBAKER, A.A. and BLAXTER, M.L. (2003). Hox gene loss during dynamic evolution of the nematode cluster. *Curr. Biol.* 13: 37-40.

AGUINALDO, A.A., TURBEVILLE, J.M., LINFORD, L.S., RIVERA, M.C., GAREY, J.R., RAFF, R.A. and LAKE, J.A. (1997). Evidence for a clade of nematodes, arthropods and other moulting animals. *Nature* 387: 489-493.

AMORES, A., *et al*., (1998). Zebrafish hox clusters and vertebrate genome evolution. *Science* 282: 1711-1714.

ANDREEVA, T.F., KUK, CH., KORCHAGINA, N.M, C'IKC'M, M. and DONDYA, A.K. (2001). Cloning and analysis of structural organization of Hox genes in the polychaete *Nereis virens. Ontogenez* 32:225-233.

ARENAS-MENA, C., MARTINEZ, P., CAMERON, R.A. and DAVIDSON, E.H. (1998). Expression of the Hox gene complex in the indirect development of a sea urchin. *Proc. Natl. Acad. Sci. USA* 95: 13062-13067.

ARENAS-MENA, C., CAMERON, A.R. and DAVIDSON, E.H. (2000). Spatial expression of Hox cluster genes in the ontogeny of a sea urchin. *Development* 127: 4631-4643.

BEEMAN, R. (1987). A homeotic gene cluster in the red flour beetle. *Nature* 327: 247-249.

BROOKE, N.M., GARCIA-FERNÀNDEZ, J. and HOLLAND, P.W.H. (1998). The ParaHox gene cluster is an evolutionary sister of the Hox gene cluster. *Nature* 392: 920-922.

BROWN, S.J., FELLERS, J.P., SHIPPY, T.D., RICHARDSON, E.A., MAXWELL, M., STUART, J.J. and DENELL, R.E. (2002). Sequence of the *Tribolium castaneum* homeotic complex: the region corresponding to the *Drosophila melanogaster* Antennapedia complex. *Genetics* 160: 1067-1074.

DEHAL, P., *et al*. (2002). The draft genome of *Ciona intestinalis*: insights into chordate and vertebrate origins. *Science* 298: 2157-2167.

DEKKER, E-J., PANNESE, M., HOUTZAGER, E., BONCINELLI, E. and DURSTON, A. (1992). Colinearity in the *Xenopus laevis* Hox-2 complex. *Mech. Dev.* 40: 3-12.

DENELL, R. (1994). Discovery and genetic definition of the *Drosophila* Antennapedia complex. *Genetics* 138: 549-552.

DEVENPORT, M.P., BLASS, C. and EGGLESTON, P. (2000). Characterization of the Hox gene cluster in the malaria vector mosquito, *Anopheles gambiae. Evol. Dev.* 2: 326-339.

DUBOULE, D. (1992). The vertebrate limb: a model system to study the Hox/HOM gene network during development and evolution. *BioEssays* 14: 375-384.

DUBOULE, D. (1994). Temporal colinearity and the phylotypic progression: a basis for the stability of a vertebrate Bauplan and the evolution of morphologies through heterochrony. *Development* (Supplement): 135-142.

DUBOULE, D. and DOLLÉ, P. (1989). The structural and functional organization of the murine HOX gene family resembles that of *Drosophila* homeotic genes. *EMBO J* 8:1497-1505.

FALCIANI, F., HAUSDORF, B., SCHRÖDER, R., AKAM, M., TAUTZ, D., DENELL, R. and BROWN, S. (1996). Class 3 Hox genes in insects and the origin of *zen*. *Proc. Natl. Acad. Sci. USA* 93: 8479-8484.

FERRIER, D.E.K. and AKAM, M.E. (1996). Organization of the Hox gene cluster in the grasshopper, *Schistocerca gregaria*. *Proc. Natl. Acad. Sci. USA* 93: 13024-13029.

FERRIER, D.E.K., MINGUILLÓN, C., HOLLAND, P.W.H. and GARCIA-FERNÀNDEZ, J. (2000). The amphioxus Hox cluster: deuterostome posterior flexibility and *Hox14*. *Evol. Dev.* 2: 284-293.

FERRIER, D.E.K. and HOLLAND, P.W.H. (2001). Ancient origin of the Hox gene cluster. *Nat. Rev. Genet.* 2: 33-38.

FERRIER, D.E.K. and HOLLAND, P.W.H. (2002). *Ciona intestinalis* ParaHox genes: evolution of Hox/ParaHox cluster integrity, developmental mode, and temporal colinearity. *Mol. Phyl. Evol.* 24: 412-417.

FINNERTY, J.R. (2001). Cnidarians reveal intermediate stages in the evolution of Hox clusters and axial complexity. *Amer. Zool.* 41: 608-620.

GARCIA-FERNÀNDEZ, J. and HOLLAND, P.W.H. (1994). Archetypal organisation of the amphioxus Hox gene cluster. *Nature* 370: 563-566.

GRAHAM, A., PAPALOPULU, N. and KRUMLAUF, R. (1989). The murine and *Drosophila* homeobox gene complexes have common features of organisation and expression. *Cell* 57: 367-378.

HANO, Y., HAYASHI, A., YAMAGUCHI, S. and YAMAGUCHI, M. (2001). Hox genes of the direct-type developing sea urchin *Peronella japonica*. *Zool. Sci.* 18: 353-359.

IRVINE, S.Q. and MARTINDALE, M.Q. (2000). Expression patterns of anterior Hox genes in the polychaete *Chaetopterus*: correlation with morphological boundaries. *Dev. Biol.* 217: 333-351.

KELSH, R., DAWSON, I. and AKAM, M. (1993). An analysis of Abdominal-B expression in the locust *Schistocerca gregaria*. *Development* 117: 293-305.

KELSH, R., WEINZERL, R.O.J., WHITE, R.A.H. and AKAM, M. (1994). Homeotic gene expression in the locust *Schistocerca*: an antibody that detects conserved epitopes in Ultrabithorax and Abdominal-A proteins. *Devel. Genet.* 15: 19-31.

KMITA-CUNISSE, M., LOOSLI, F., BIERNE, J. and GEHRING, W.J. (1998). Homeobox genes in the ribbonworm *Lineus sanguineus*: evolutionary implications. *Proc. Natl. Acad. Sci. USA* 95: 3030-3035.

LIU, X.F., OLSSON, P., WOLFGANG, C.D., BERA, T.K., DURAY, P., LEE, B. and PASTAN, I. (2001). PRAC: a novel small nuclear protein that is specifically expressed in human prostate and colon. *Prostate* 47: 125-131.

MANN, R.S. (1997). Why are Hox genes clustered? *BioEssays* 19: 661-664.

MARTINEZ, P., RAST, J.P., ARENAS-MENA, C. and DAVIDSON, E.H. (1999). Organization of an echinoderm Hox gene cluster. *Proc. Natl. Acad. Sci. USA* 96: 1469-1474.

MCGINNIS, W., GARBER, R.L., WIRZ, J., KUROIWA, A. and GEHRING, W.J. (1984). A homolgous protein-coding sequence in *Drosophila* homeotic genes and its conservation in other metazoans. *Cell* 37: 403-408.

MCGINNIS, W. and KRUMLAUF, R. (1992). Homeobox genes and axial patterning. *Cell* 68: 283-302.

MCGINNIS, W. (1994). A century of homeosis, a decade of homeoboxes. *Genetics* 137: 607-611.

MILLER, D.J. and MILES, A. (1993). Homeobox genes and the Zootype. *Nature* 365: 216-216.

MITO, T. and ENDO, K. (2000). PCR survey of Hox genes in the crinoid and ophiuroid: evidence for anterior conservation and posterior expansion in the echinoderm Hox gene cluster. *Mol. Phyl. Evol.* 14: 375-388.

ORLANDO, V. (2003). Polycomb, epigenomes, and control of cell identity. *Cell* 112: 599-606.

POWERS, T.P., HOGAN, J., ZHAOXI, K., DYMBROWSKI, K., WANG, X., COLLINS, F. and KAUFMAN, T.C. (2000). Characterization of the Hox cluster from the mosquito *Anopheles gambiae* (Diptera: culicidae). *Evol. Dev.* 2:311-325.

PRINCE, V.E., MOENS, C.B., KIMMEL, C.B. and HO, R.K. (1998). Zebrafish Hox genes: expression in the hindbrain region of wild-type and mutants of the segmentation gene, *valentino*. *Development* 125: 393-406.

ROSS, J.M. and ZARKOWER, D. (2003). Polycomb group regulation of Hox gene expression in *C. elegans*. *Dev. Cell* 4: 891-901.

RUVKUN, G. and HOBERT, O. (1998). The taxonomy of developmental control in *Caenorhabditis elegans*. *Science* 282:2033-2041.

SEEGER, M.A., HAFFLEY, L. and KAUFMAN, T.C. (1988). Characterization of *amalgam*: a member of the immunoglobulin superfamily from *Drosophila*. *Cell* 55: 589-600.

SHARMAN, A.C. (1999). Some new terms for duplicated genes. *Semin. Cell Dev. Biol.* 10:561-563.

SPAGNUOLO, A., RISTORATORE, F., DI GREGORIO, A., ANIELLO, F., BRANNO, M. and DI LAURO, R. (2003). Unusual number and genomic organization of Hox genes in the tunicate *Ciona intestinalis*. *Gene* 309: 71-79.

SPITZ, F., GONZALEZ, F. and DUBOULE, D. (2003). A Global Control Region defines a chromosomal regulatory landscape containing the *HoxD* cluster. *Cell* 113: 405-417.

STAUBER, M., JÄCKLE, H. and SCHMIDT-OTT, U. (1999). The anterior determinant *bicoid* of *Drosophila* is a derived Hox class 3 gene. *Proc. Natl. Acad. Sci. USA* 96: 3786-3789.

STRUHL, G. (1984). Splitting the bithorax complex of *Drosophila*. *Nature* 308: 454-457.

TELFORD, M.J. (2000). Evidence for the derivation of the *Drosophila fushi-tarazu* gene from a Hox gene orthologous to lophotrochozoan Lox5. *Curr. Biol.* 10: 349-352.

TIONG, S.Y.K., WHITTLE, J.R.S. and GRIBBIN, M.C. (1987). Chromosomal continuity in the abdominal region of the bithorax complex of *Drosophila* is not essential for its contribution to metameric identity. *Development* 101: 135-142.

VAN AUKEN, K., WEAVER, D.C., EDGAR, L.G. and WOOD,W.B. (2000). *Caenorhabditis elegans* embryonic axial patterning requires two recently discovered posterior-group Hox genes. *Proc. Natl. Acad. Sci. USA* 97: 4499-4503.

VON ALLMEN, G., HOGGA, I., SPIERE, A., KARCH, F., BENDER, W., GYURKOVICS, H. and LEWIS, E. (1996). Splits in fruitfly Hox gene complexes. *Nature* 380:116.

WADA, H., GARCIA-FERNÀNDEZ, J. and HOLLAND, P.W.H. (1999). Colinear and segmental expression of amphioxus Hox genes. *Dev. Biol.* 213: 131-141.

ZELTSER, L., DESPLAN, C. and HEINTZ, N. (1996). *Hoxb-13*: a new Hox gene in a distant region of the HOXB cluster maintains colinearity. *Development* 122: 2475-2484.