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Hox Genes and the Evolution of Diverse Body Plans

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Hox genes and the evolution of diverse body plans

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SUMMARY

Homeobox genes encode transcription factors that carry out diverse roles during development. They are widely distributed among eukaryotes, but appear to have undergone an extensive radiation in the earliest metazoa, to generate a range of homeobox subclasses now shared between diverse metazoan phyla. The Hox genes comprise one of these subfamilies, defined as much by conserved chromosomal organization and expression as by sequence characteristics. These Hox genes act as markers of position along the antero–posterior axis of the body in nematodes, arthropods, chordates, and by implication, most other triploblastic phyla. In the arthropods this role is visualized most clearly in the control of segment identity. Exactly how Hox genes control the structure of segments is not yet understood, but their differential deployment between segments provides a model for the basis of segment diversity.

Within the arthropods, distantly related taxonomic groups with very different body plans (insects, crustaceans) may share the same set of Hox genes. The expression of these Hox genes provides a new character to define the homology of different body regions. Comparisons of Hox gene deployment between insects and a branchiopod crustacean suggest a novel model for the derivation of the insect body plan.

1. INTRODUCTION

The Hox gene clusters have become an icon of developmental genetics, symbolizing the conservation of genetic mechanisms across diverse taxa. These genes – first identified by homeotic mutations in flies – act as markers of position, defining different fates along the antero–posterior axis of animal embryos. They encode transcription factors that modify the differentiation of cells in many different tissues. Hox genes have now been described in many animal phyla, including cnidarians, platyhelminths, nematodes, hemichordates and vertebrates. This ubiquity has prompted the redefinition of animals as organisms with Hox genes (Slack *et al.* 1993), and has even lead to the suggestion that the origin of the Hox genes was a key event in triggering the Cambrian explosion.

I would not go that far, but I do believe that the Hox genes present some unique advantages for studying the evolution of development and the origins of diverse body plans. Here I illustrate this with reference to our studies of arthropods. Before turning to this topic, I consider the origin and position of the Hox genes within the larger family of homeobox genes.

2. HOMEOBOXES AND HOX GENES

The homeobox is a 180 b.p. DNA sequence motif that encodes a conserved DNA binding structure – the homeodomain – found within one class of transcription factors (Gehring *et al.* 1994). Genes with homeoboxes have been found in plants, fungi and many metazoa (Bürglin 1994). Many bacterial proteins have DNA binding domains with a similar overall structure to the homeodomain, but it is not clear that any of these should be regarded as the prokaryote homologues of the eukaryote homeodomain proteins (Gehring *et al.* 1994). Virtually nothing is known of the distribution of homeodomain proteins in unicellular eukaryotes other than fungi. As these encompass the greater part of the diversity of the eukaryotes, the origins of the homeobox gene superfamily remain obscure.

The homeobox genes of fungi are predominantly involved in the specification of mating type (e.g. Kues *et al.* 1994). In plants, it is too early to make any meaningful generalizations about the role played by homeobox genes (Langdale 1994). However, none of the fungal or plant homeobox genes fall within the subfamilies defined in animals (Bürglin 1994). Thus currently it seems that the diversity of homeobox genes in the metazoa derives from a radiation that occurred after the separation of this lineage from other Protistan eukaryotes, and is specific to the multicellular animals and their nearest relatives.

We can estimate the diversity of the homeobox gene family at different stages in the evolution of the metazoa by determining what specific subfamilies of homeobox gene are identifiable in lineages that diverged at different stages in the radiation of the metazoa. Good sampling is available for three groups: the chordates, the arthropods and the nematodes (Bürglin 1994). For our purposes, these groups can be taken to represent the diversity of triploblastic metazoans (Sidow & Thomas 1994). Their common ancestor clearly possessed a suite of homeobox genes that included many, perhaps most of the known subfamilies of animal homeoboxes. These include the *Evx*, *Pax*, *Cut*, *Msx*, *Dlx* and *Antp* classes (Kappen *et al.*

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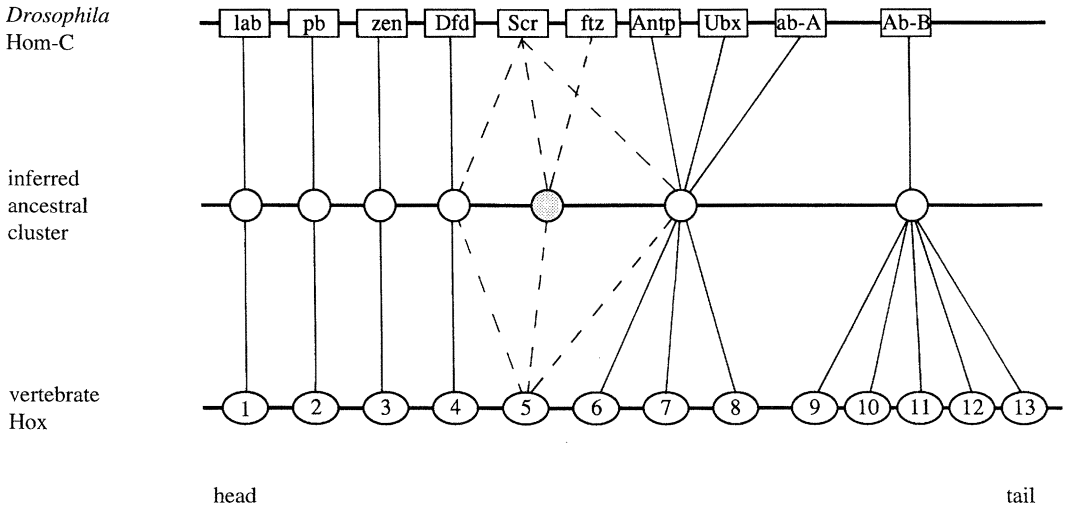

Figure 1. Minimal complexity of the Hox gene cluster in the ancestor of arthropods and vertebrates. The insect/crustacean Homeotic cluster (Hom-C), corresponding to the fusion of the Antennapedia and Bithorax complexes of *Drosophila*, is based on data from *Drosophila*, *Tribolium*, *Schistocerca* and *Artemia* (for reference see Akam *et al.* 1994). Abbreviations correspond to Drosophila gene names. For vertebrates, the diagram depicts the inferred structure of a cluster that gave rise to each of the four duplicated Hox clusters (McGinnis & Krumlauf 1992). Paralogy groups are numbered 1–13. Where vertebrate genes have well defined orthologues in insects, solid lines have been drawn to a gene in the inferred ancestral cluster. Less certain relationships are dotted.

1993; Bürglin 1994). A few of these genes are widely expressed, serving as ubiquitous transcription factors (e.g. *Oct1* of the POU class), but many more play some role in the specification of cell type, either characterizing particular organ primordia as they are first defined, or particular cell types during terminal differentiation (Duboule 1994; Manak & Scott 1994).

Sponges, cnidarians and platyhelminths clearly have diverse homeoboxes. Some of these can be related without difficulty to specific subclasses recognizable in the higher metazoa (Miles & Miller 1992; Schummer *et al.* 1992; Bürglin 1994; Seimya *et al.* 1994) but sampling of these basal metazoan groups is too limited to be representative. In no case is it yet clear what developmental role these homeobox genes are playing (c.f. Shenk *et al.* 1993). However, taken all together, these data suggest that the radiation of the homeobox genes within the metazoa occurred in concert with the evolution of complex multicellular bodies containing many differentiated cell types. It may well have played a significant role in this process.

(*a*) *Hox genes*

The Hom/Hox (hereafter Hox)† subfamily comprises only a small part of this larger family of homeobox genes (Akam 1989; De Robertis 1994). This subfamily is defined by a number of characteristics. The most striking is the remarkable property of colinearity. The genes are clustered on the chromosome in the same order as they are deployed along the axis of the body: genes at one end of the cluster are typically expressed just behind the head, genes at the opposite end are expressed near the posterior of the body. 'Anterior' and 'posterior' genes are recognizably homologous in vertebrates, arthropods and nematodes (see figure 1; for review see McGinnis & Krumlauf 1992).

Many Hox genes encode a homeodomain closely resembling the canonical sequence defined by the *Drosophila* homeotic gene, *Antennapedia* (*Antp*). They also share with *Antp* another sequence motif not found in other classes of homeobox gene. This 'Antennapedia' class of homeobox genes includes most of the central genes of the insect homeotic and vertebrate Hox gene clusters. However, the sequences of the most divergent members of the Hox clusters are barely more similar to *Antp* than they are to those of some other genes not in the Hox clusters (Bürglin 1994). It is the clustering and the conserved relation between chromosomal organization and expression that justifies the inclusion of labial and abdominal-B class homeobox genes among the set of Hox genes.

(*b*) *The Developmental Role of Hox genes*

In insects, the Hox genes of the Antennapedia and Bithorax complexes function as selector genes for segment identity, defining the appropriate fates of many different cell types within particular regions of the body (Lewis 1978; Kaufman *et al.* 1990; Lawrence & Morata 1994). The Hox genes in nematodes and vertebrates appear to play an analogous, and presumably homologous, role in development. Hox mutations in these organisms transform structures to those normally found at other positions along the body axis (Wang *et al.* 1993; Krumlauf 1994).

This model of Hox gene function is conceptually simple and perhaps deceptively simple. It is unusual for any transcription factor to serve only a single role during development. Most are used at several different times in development, to regulate processes that appear to have little or nothing in common. For example, in *Drosophila* the homeobox gene *even-skipped* is used

† The term Hom/Hox, never euphonic, is now redundant. The epithet Hox should be used only for the clustered genes homologous to the insect and vertebrate homeotic complexes, and not for other classes of homeobox genes (Scott 1992).

transiently during segmentation to define parasegment boundaries. It is used again during neurogenesis to define particular cell types, and again in certain muscles, where its role is not clear (Patel *et al*. 1992). It is only a convenient fiction to describe *even-skipped* as a segmentation gene. The same is true for most of the other genes involved in patterning the early *Drosophila* embryo, and for many transcription factors involved in later developmental processes. Pleiotropy, at this primary level of gene function, is the rule rather than the exception. Thus it would be particularly surprising to find, in the Hox gene products, a whole class of transcription factors whose function conformed solely to our external 'rational' definition of them as selector genes for the A–P axis. In fact, this is probably not the case.

Some *Drosophila* Hox genes play roles other than defining segment differences. *Labial* defines one cell type within the midgut (Hoppler & Bienz 1994). *Labial*, *proboscipedia* and *Antennapedia* are expressed in subsets of cells in many segments of the nervous system, not restricted to the domains where they define segment identity (Diederich *et al*. 1989; Mahaffey *et al*. 1989). Other homeobox genes of the Drosophila Antennapedia cluster are not (or are no longer) homeotic genes at all. At least some of these genes (*zen*, *fushi-tarazu*) probably derive from Hox genes, but they no longer show typical collinear expression, and no longer provide cells with 'addresses' defining segment differences (Akam *et al*. 1994).

In vertebrates, the Hox genes are used to pattern the limbs and other secondary axes. This role may well be analogous to that in the primary body axis, perhaps reflecting the recruitment of a whole patterning network to a new morphological structure (Graham 1994). It remains possible that they are also used for purposes unrelated to the specification of position along axes.

3. HOX GENES AND SEGMENT DIVERSITY IN ARTHROPODS

How do Hox genes make segments different? In what follows I make the simplifying assumption that all differences between one segment and another are mediated by the Hox genes. This is clearly not true for the most anterior and posterior segments of *Drosophila*. Here the differential expression of other genes (*caudal*, *spalt* etc.) is independent of Hox gene activity, and directly responsible for some aspects of segment differentiation (Jürgens & Hartenstein 1993). The same could be true for the central trunk segments. Segmentation genes of the gap and pair-rule classes are differentially expressed between different segments and so could directly influence segment differentiation, indepenent of the Hox genes (Akam 1987). However, the phenotype of mutants in the Hox genes suggests that such Hox-independent effects are minor, at least from the first thoracic (T1) to the seventh abdominal (A7) segment (Lewis 1978; Struhl 1983; Wakimoto *et al*. 1984). The animal does seem to channel most, if not all, specification of segment differences through the Hox gene cluster.

Segment diversification can be broken down into two quite distinct problems. The first is the problem of building the very different morphology of segments adapted for different purposes: biting mouthparts, walking legs, swimming paddles, breathing gills. This is essentially a problem that can be considered at the level of the single segment; how does gene regulation within any one segment elicit the development of a particular differentiated structure. This is not yet understood (despite rapid progress, see for example, Williams & Carroll 1993). We believe that the precise regulation of Hox gene expression within segments forms a part of this 'programme' (Castelli-Gair & Akam 1995), but in this respect the Hox genes are acting downstream of the genes that define position and cell type within segments, as intermediates in the hierarchy of control that leads to morphogenesis. Changes in this 'within-segment' regulation of Hox genes can play some role in the evolution of segment morphology (Kelsh *et al*. 1993; Warren *et al*. 1994), but I do not deal with that issue here.

The second problem is the allocation of different fates to segments at different positions along the body axis. For this, the deployment of Hox genes provides a working model. My favoured version of this model is as follows.

1. *Differences between segments depend on Hox gene expression.* Hox genes of insects (and vertebrates) typically show a sharp anterior boundary of expression at a particular point along the body axis. Behind this boundary, expression may be initially ubiquitous but later shows complex modulation, particularly in posterior regions of the body where several classes of Hox genes are coexpressed. The combination of Hox genes that are expressed in each segment has been defined as a Hox code (Struhl 1982). I would redefine the term Hox code to mean, not just the binary combination of genes that are on or off, but those particular spatial and temporal patterns of Hox gene expression that characterize segments. These patterns are important for normal development (Castelli-Gair *et al*. 1994; Castelli-Gair & Akam 1995).

Each different segment morphology implies a different Hox code. So far as we understand it, these codes are achieved by defining for each Hox gene (early in development) which subset of regulatory elements will be active in each particular segment, and which will be silent (Peifer *et al*. 1987; Akam *et al*. 1988; Müller & Bienz 1995). It is the state of Hox gene regulatory elements that defines and remembers segment identity. It may well be the ability of these regulatory elements to sense and remember this early information that makes the Hox clusters so special. In *Drosophila*, this information is provided by the distribution of the products of the gap and pair-rule segmentation genes (Akam 1987; Müller & Bienz 1992; Zhang & Bienz 1992).

2. *In any one species, groups of similar segments are likely to be specified by similar Hox codes.* In *Drosophila*, the regulation of the Hox genes is sufficiently complex to allow virtually every segment to develop a unique 'identity'. However, segments of the pregenital abdomen (A2–A7) are similar in many details. All

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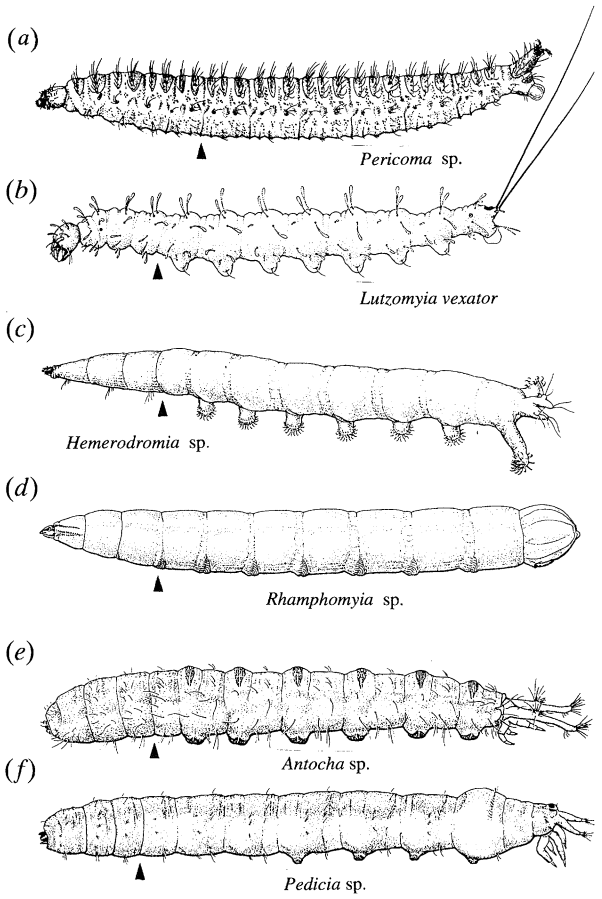

Figure 2. Patterns of segment diversification in dipteran larvae. Many dipteran larvae, in a wide range of families, have similar mid-trunk segments, though the terminal segments of the abdomen are distinct. (e.g. *a*). Others have diverse segments, particularly visible in the distribution of larval prolegs. Two common and widely distributed patterns are: (*b*) similar prolegs on A1–7; (*c*, *e*) similar prolegs in A2–A7, but A1 distinct. (*f*) A rare exception to the rule that segments A2–A7 look similar. (*a*, *b*: Larvae from two genera of the family Psychodidae; *c*, *d*: larvae from two genera of the family Empididae; *e*, *f*: larvae from two genera of the family Tipulidae. Illustrations from McAlpine *et al.* (1981). Arrowheads mark the anterior boundary of the second abdominal segment, A2).

express a similar Hox code, involving primarily the genes *Ultrabithorax* (*Ubx*) and *abdominal-A* (*abd-A*) (Akam *et al.* 1988).

Such blocks of similar segments are found in many arthropods. Frequently each block is adapted for some particular function (feeding, walking, swimming etc.) These blocks form well-defined body regions called tagmata (Brusca & Brusca 1990). We have previously suggested that tagmata identify groups of segments that express similar Hox codes (Akam *et al.* 1988). In insects the trunk is conventionally divided into three tagmata, mouthparts, thorax and abdomen. A more complex description may perhaps be justified. For example, the first abdominal segment is specified by a distinct Hox code (involving *Ubx* but not *abd-A*). In many insects, the appendages of this segment are uniquely specialized for a role during embryogenesis (Johannsen & Butt 1941).

Segments within a tagma tend to show coordinated evolution. By this I mean that, if the morphology of one segment changes, other segments within the tagma are likely to show parallel changes. In Dipteran larvae, for example, the detailed morphology of abdominal segments varies considerably from species to species, but with few exceptions, abdominal segments A2–A7 look very much alike (see figure 2) (McAlpine *et al.* 1981). A simple explanation would be that different closely related species interpret a conserved Hox code in different ways.

3. *Differences in tagmosis reflect differences in the allocation of Hox codes between segments*. Groups of segments that typically evolve as a unit are sufficiently noticeable, and sufficiently stable, to form the basis for much taxonomy within the arthropods. For example, the possession of prolegs on abdominal segments 3–6 is a distinctive character of larval Lepidoptera. This character depends on an alteration in the spatial pattern of Hox gene expression within these segments at a particular time in development (Warren *et al.* 1994).

At a higher taxonomic level, patterns of tagmosis characterize classes and subclasses within the arthropods. For example, the malacostracan crustaceans are characterized by the possession of a trunk divided into two major regions, a thorax of 8 segments and an abdomen of 6 or 7. Within the Malacostraca, further subdivisions within the thorax are used to define subordinate taxonomic groups. In the decapods, three of the thoracic segments are specialized to make feeding appendages (maxillipeds), leaving the five pairs of walking legs that give the group its name (Brusca & Brusca 1990). If these tagmata are based on shared Hox codes, then by implication the differences in tagmosis will reflect differences in the way that Hox codes are mapped onto the array of segments.

4. THE EVOLUTION OF SEGMENT DIVERSITY IN ARTHROPODS

It is generally agreed that the diversity of segment types seen in the lobster or the fruit fly is not primitive. The most primitive arthropods were probably homonomous, with a specialized front and back end, but with all the trunk segments similar to one another (Brusca & Brusca 1990). This is illustrated by the body plan of several Cambrian forms (e.g. Branchiocaris, Briggs & Fortey 1989), and among recent arthropods, by myriapods, and remipede crustaceans (though there are reasons for thinking that homonomy may sometimes be a derived character in recent arthropods, (Akam *et al.* 1994)).

If homonomy was a primitive character of the arthropods, segment diversity evolved within arthropod lineages. We expect its origin to involve the Hox genes. One hypothesis is that the increasing complexity of arthropod body plans depended on, and was perhaps driven by, Hox gene duplications (Lewis 1978).

To assess that hypothesis, we must know what the Hox cluster looked like in the last common ancestor of extant arthropods, existing before arthropod diversification (and possibly before arthropodization). Comparisons with vertebrate Hox clusters can provide a

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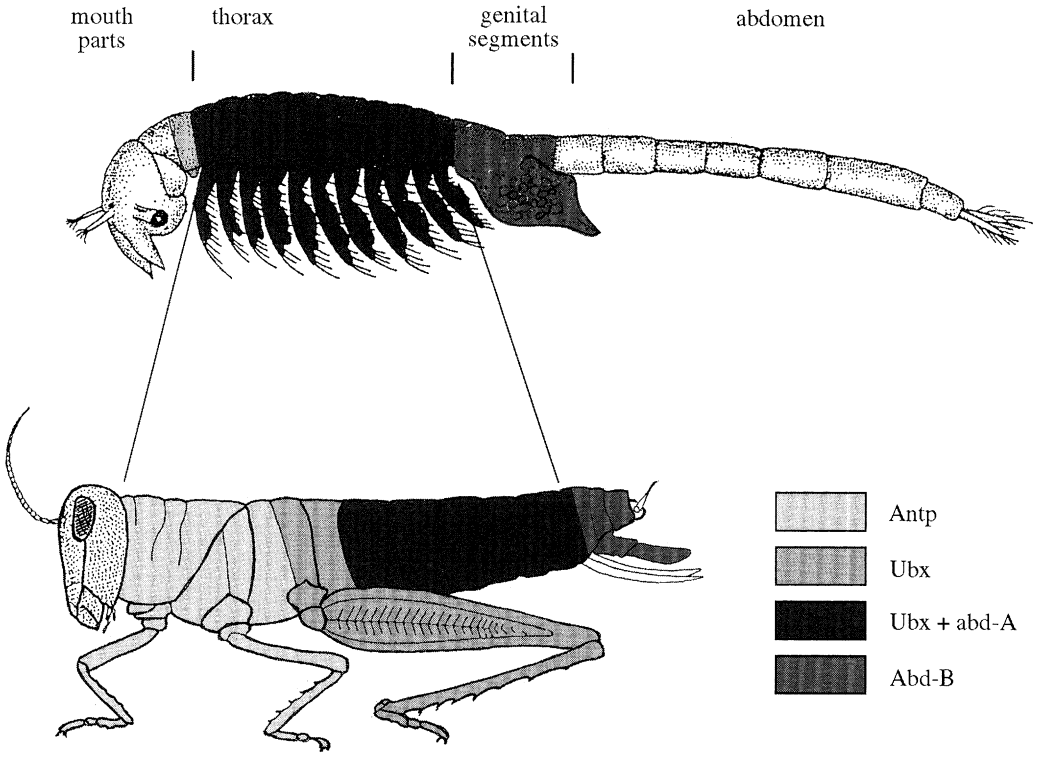

Figure 3. A comparison of the body plans of the branchiopod crustacean *Artemia* with that of insects. *Artemia* (top) has a thorax of 11 similar segments, followed by two genital segments and a post-genital abdomen of 7 segments. Comparing domains of Hox gene expression suggests that the Artemia thorax may be homologous to the whole pregenital trunk in insects (e.g. grasshopper, bottom), a region which includes the differentiated segments of the thorax and most of the abdomen (see text). Gene abbreviations: Antp, *Antenapedia*; Ubx, *Ultrabithorax*; abd-A, *abdominal-A*; Abd-B, *Abdominal B*.

minimal estimate of the complexity of this cluster, for we presume that vertebrates are not derived from within the arthropods. Such an ancestor clearly had multiple head genes because orthologues for each of the head gene classes are shared between vertebrates and insects (see figure 1). Clearly also it had distinct trunk (*Antp*-like) and tail (*Abd-B*-like) genes (Bürglin 1994). From insect-vertebrate comparisons alone, though, we cannot say whether multiple Antennapedia-like trunk genes were present. The minimal hypothesis is that there was a single trunk gene in the ancestral cluster. Annelid-arthropod comparisons suggest a minimum of two trunk genes in the ancestor shared between these two groups (Akam *et al.* 1994).

Comparisons within the arthropods suggest that most Hox gene duplications preceded the diversification of the major Arthropod groups. My colleague Michalis Averof has shown that the Branchiopod crustacean, *Artemia* shares with insects distinct representatives of all the trunk homeotic genes (Averof & Akam 1993). Therefore the Hox gene duplications and diversification that led to the modern insect cluster predate the insect-crustacean split. Short gene fragments isolated from the chelicerate *Limulus* suggest that this branch of the arthropods also contains many trunk genes (perhaps multiple clusters), although exactly which classes are represented is not yet clear (Cartwright *et al.* 1993).

The insects and crustaceans are variously seen as near neighbours or distant relatives within the arthropods (Averof & Akam 1995*a*). However, all conventional schemes agree that insects derive from some myriapod-like ancestor, and higher crustaceans from a homonomous, perhaps remipede-like crustacean. Therefore whatever phylogeny is preferred, their last common ancestor would parsimoniously be pictured as a homonomous arthropod. Finding that the Hox gene duplications and diversification that led to the modern insect cluster predate the insect–crustacean split raises two questions: (i) What were diverse trunk Hox genes doing in the last common ancestor; and (ii) What did Hox genes have to do with the increasing complexity of the body plan?

One approach to address these questions is to use the Hox genes as markers to relate the body plans of insects and crustaceans. Within insects, this approach works well. With minor exceptions, the boundaries of Hox gene expression are conserved in a range of different insects, even when the morphology is quite different. For example, the anterior boundary of *abd-A* expression maps within the first abdominal segment in all insects tested – flies, moths, beetles and grasshoppers (for references see Akam *et al.* 1994). Within insects, however, the homologies between trunk segments are not controversial, whereas between insects and crustaceans they are.

Artemia is not a homonomous crustacean, but it has a relatively simple body plan (see figure 3) that has

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been considered primitive within the Crustacea (Fryer 1992). How should we relate this body plan to that of insects? Do the words thorax and abdomen refer to homologous regions of the trunk?

Hox genes help, or at least they enable us to compare the two body plans in a new light. We have recently documented the expression of four Hox genes in *Artemia* – *Antp*, *Ubx*, *abd-A*, and *Abd-B*. (Averof & Akam 1995*b*). *Antp*, *Ubx* and *abd-A* are expressed throughout the 'thorax' of Artemia, whereas in insects *Ubx* and more particularly *abd-A* specify the pregenital abdomen. The genital segments of *Artemia* express these trunk genes only transiently. They parallel the insect genitalia in expressing *Abd-B*.

These results suggest that the thorax of *Artemia* might be homologous with the whole insect thorax and pregenital abdomen, and that the genital segments are homologous regions of the body in these two groups. If so, the last common ancestor of insects and crustaceans did not have a homonomous trunk. However, thorax/abdomen tagmosis in insects would be derived from a homonomous region of the common ancestor, represented by the thorax in *Artemia*.

According to this model, the role of the Hox genes in trunk diversification involved, not gene duplications, but changes in gene regulation. We envisage that the ancestor already had differential antero–posterior regulation of the Hox cluster as a whole, but that the middle trunk genes were initially expressed in overlapping domains. This is certainly not unreasonable if the three genes *Antp*, *Ubx* and *abd-A* arose by gene duplication. Subsequently, in the insect lineage, each trunk gene acquired a distinct A–P domain of expression, concomitant with the acquisition of differential downstream targets, thus allowing segments within the trunk to develop distinct morphologies.

This model makes many assumptions, which remain to be tested. For example, that other more anterior Hox genes will 'fit' in their predicted places (*Dfd* in the mandibular segment, *Scr* in the maxillary segments), and that the posterior region of *Artemia* is homologous to the post-genital abdomen of insects, a hypothesis that might be tested by analysing expression of *caudal* homologues in *Artemia*. It also assumes that the differentiated trunk of higher crustaceans like the lobster represents an independent origin of spatial diversity in the pattern of Hox gene expression.

The model also has its problems. For example, it is not obvious that the genital segments should be regarded as homologous between widely disparate groups of arthropods. Within the myriapods alone, the genitalia can be located either at the anterior (in the diplopods, symphylans and pauropods) or the posterior of the trunk (in the chilopods). Other crustaceans have no specialized genital segments at the posterior of the trunk. Only more data, from phylogenetically diverse organisms, will tell us whether our model is useful.

In effect, this model uses domains of Hox gene expression to define homologous regions of the insect and crustacean trunk. We think this reasonable because the Hox genes are not just markers for homology, they are part of the mechanism that defines it. Even so, we would be foolish to think that the expression of one gene, or even one family of genes, will provide a certain guide to homology. Homology is a slippery concept. The closer we come to studying its molecular basis, the more difficult it may be to decide just what we are trying to describe with it. No one part of a developmental mechanism is immutable, so no single gene can define homology.

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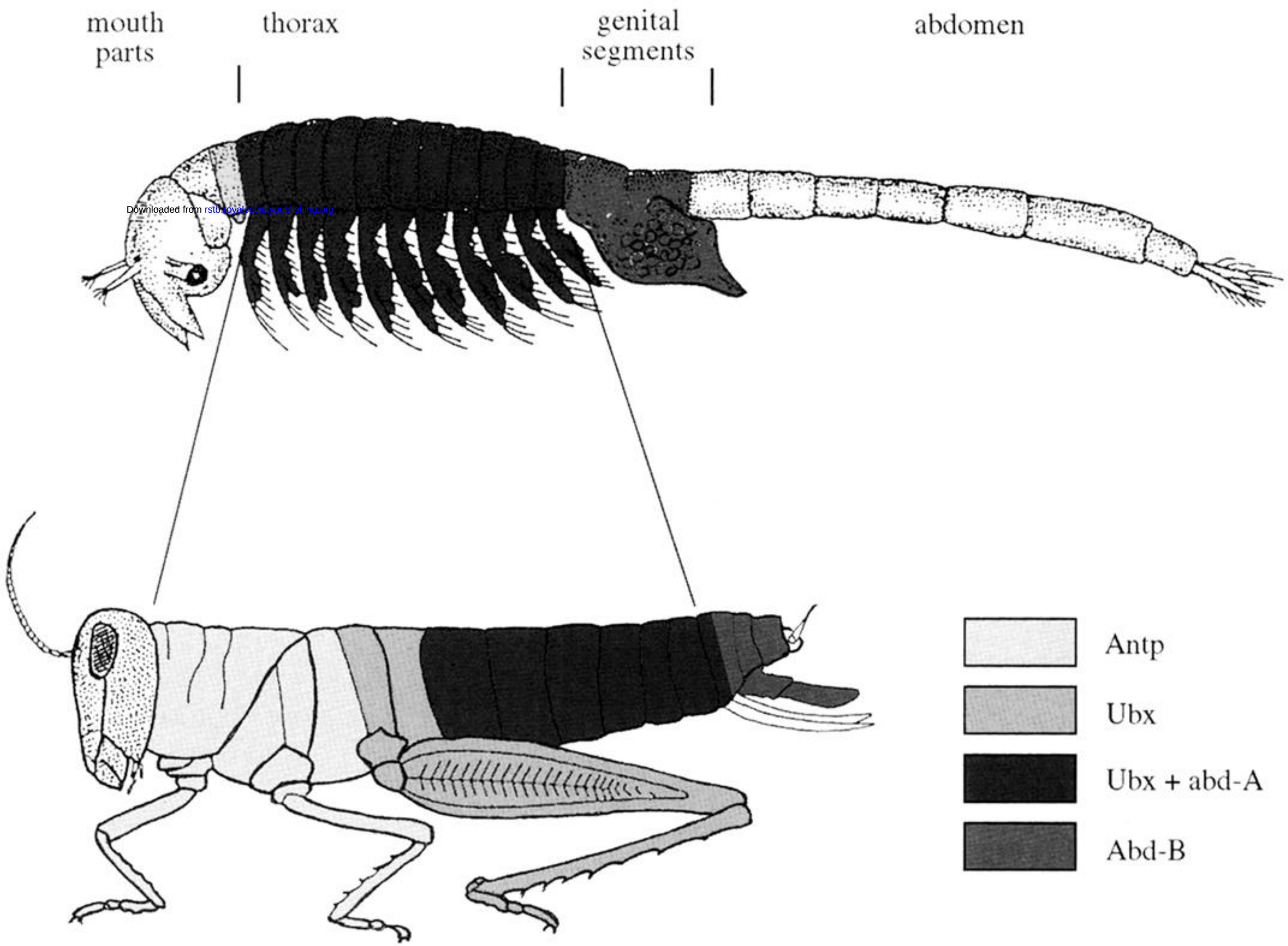
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



gure 3. A comparison of the body plans of the branchiopod crustacean *Artemia* with that of insects. *Artemia* (top) s a thorax of 11 similar segments, followed by two genital segments and a post-genital abdomen of 7 segments. mparing domains of Hox gene expression suggests that the Artemia thorax may be homologous to the whole egenital trunk in insects (e.g. grasshopper, bottom), a region which includes the differentiated segments of the orax and most of the abdomen (see text). Gene abbreviations: Antp, *Antenapedia*; Ubx, *Ultrabithorax*; abd-A, *dominal-A*; Abd-B, *Abdominal B*.