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# Understanding the genetic basis of morphological evolution: the role of homeotic genes in the diversification of the arthropod bauplan

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**ABSTRACT Due to the segmental organization of their body plans, arthropods can be considered the paradigmatic modular organisms. In the past two decades, genetic studies of the homeotic *(Hox)* genes in *Drosophila melanogaster* have provided initial insight into the molecular mechanisms that govern the establishment of segmental identity. In this review, we will address the question of the possible role of four *Hox* genes: *labial (lab), proboscipedia (pb), Deformed (Dfd),* and *Sex combs reduced (Scr)* in the morphological evolution of arthropods, particularly with respect to the evolution of the head and head structures in insects. Overall, these preliminary studies illustrate the role that some of the *Hox* genes expressed in the insect head have played in the morphological evolution of hexapods and likely arthropods in general.**

KEY WORDS: *homeotic, evolution, arthropod, bauplan*

## Introduction

For millions of years, evolutionary forces and processes have been changing and adapting living organisms to their environments. But, of all of the evolutionary changes (behavioral, biochemical, physiological), perhaps none have been studied and discussed more than the changes in morphology and body organization. The natural histories of most animal groups provide us with numerous examples, sometimes truly wonderful and spectacular, of morphological evolution that has been occurring since ancient times (Gould, 1989). Because they were easy to observe and compare, the morphological differences among different taxa were the basis upon which zoology and animal taxonomy were built. And yet, despite the centuries of recording and classifying those differences, we are still lacking a basic understanding about their origins. How do new morphologies arise? What are their genetic bases? Only recently, with the maturation of the field of developmental genetics and evolution have we started to gain some insights into these fundamental biological questions. The reason for this long delay has been due to the fact that in order to study morphological evolution, we really need to combine information from the fields of both evolutionary and developmental biology. The need for bringing together these two fields becomes self-evident when one considers that for morphological evolution to take place, two separate events must occur. First, at the level of individual organisms, there must be some kind of change in a gene or genes that are responsible for the development of a particular morphological feature during embryogenesis. As a consequence, a modified structure ("morphological novelty") will develop. Second, at the population level, the morphological novelty should spread and eventually become established in natural populations. This second event clearly illustrates the fact that it is populations, not individuals, that have the ability to evolve (Futuyma, 1986).

Traditionally, these two events have been studied separately, the first being the domain of developmental biologists and embryologists, while the second was exclusively investigated by population geneticists (for more details on this topic, see Palopoli and Patel, 1996). The problem with this separate approach lies within the nature of the phenomenon itself: a detailed knowledge of the processes that take place during the first event is necessary for understanding the processes that occur during the second event. At present, evolutionary theory explains large phenotypic differences as a result of numerous gene substitutions, each with a relatively small effect (Futuyma, 1986). However, this explanation was not based on empirical evidence but on extrapolations from studies of "housekeeping" genes and the power of population genetics to predict the outcome of allelic frequency changes in natural populations. In contrast, laboratory studies conducted on genes that regulate developmental processes suggest that mutations in these genes can have a major and instantaneous effect on morphology. In order to bridge this gap between developmental and evolutionary processes, we should first learn the genetic basis of the morphological change before we can actually analyze how this change becomes established in nature (Palopoli and Patel,

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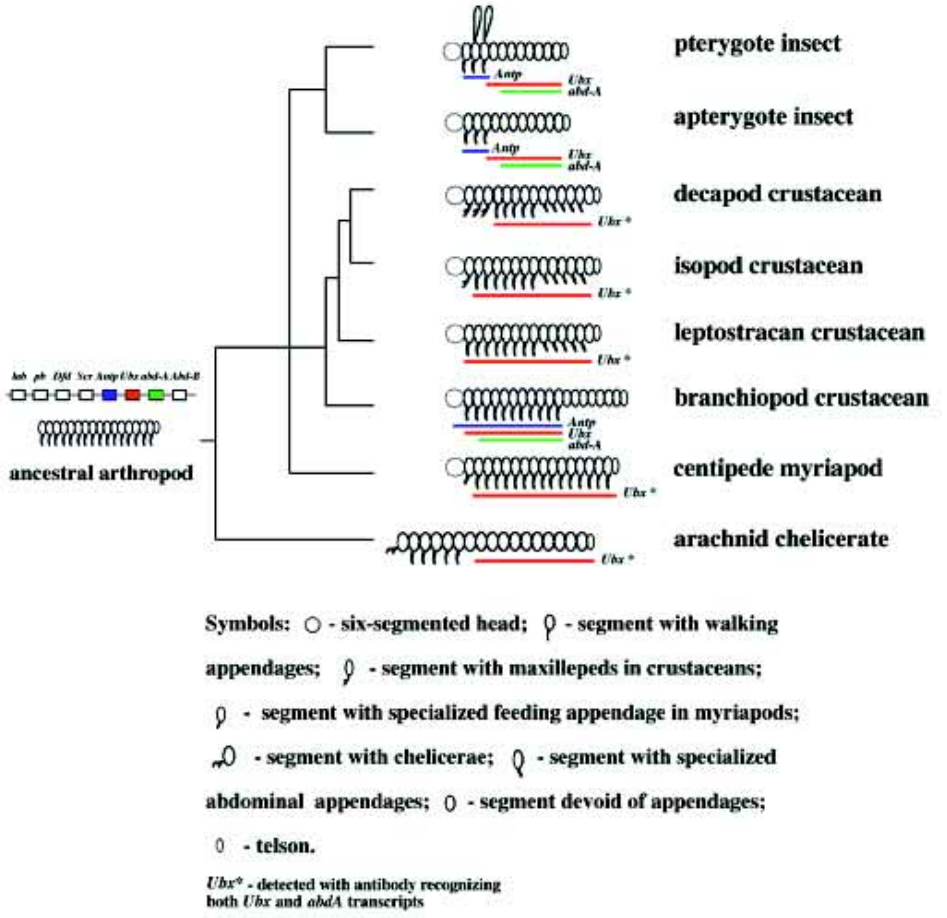


**Fig. 1. Phylogeny of major arthropod groups and the expression patterns of homeotic genes *Antp, Ubx* and *abdA* where known.** *The represented phylogeny illustrates the monophyly of arthropods and of mandibulates (based on Boore et al., 1995). The body plan of the ancestral arthropod is shown with all segments homonomous because no specific ancestral structures can be inferred from the morphology of the known arthropod groups. Results from PCR surveys and other studies suggest the presence of a single complete HOM-C drawn next to this hypothetical organism (Cartwright et al. 1993; Akam et al. 1995). The expression patterns of Antp, Ubx and abdA in pterygote insects is exemplified by Drosophila melanogaster (White and Wilcox, 1985; Kaufman, 1990) and for apterygote insects by the firebrat Thermobia domestica (Rogers and Kaufman 1997; M. Peterson unpublished). Insects have a rather conserved expression pattern of the homeotic genes in comparison with crustaceans. Expression patterns of Antp, Ubx and abdA have been shown to broadly overlap in the trunk of the branchiopod crustacean Artemia franciscana (Averof and Akam, 1995). Also several crustacean orders have been surveyed using an antibody recognizing both Ubx and Abd-A (Averof and Patel, 1997). The lack of accumulation of Ubx/Abd-A in the anterior thoracic segments has been found to correlate with transformation of these thoracic appendages to a gnathal identity (Averof and Patel, 1997). The Ubx/abdA expression pattern detected with the above mentioned antibody in the centipede Ethmostigmus rubripes is consistent with the hypothesis of ancestral role of these genes in identifying "trunk" segments in mandibulates (Grenier et al., 1997). The anterior border of Ubx/abdA expression in an arachnid chelicerate roughly corresponds to the boundary of opisthosoma and prosoma (Popadić, unpublished).*

1996). Thus, in order to study morphological evolution it is necessary to identify the potential candidate genes that control the development of a particular morphological structure in an organism. Then, by characterizing changes in these genes at the molecular level, we can gain insight into the origin of a particular morphological novelty.

Among the thousands of mutant phenotypes discovered in laboratory organisms that are being used in genetical research, none have a more dramatic morphological effect than the mutations in homeotic *(Hox)* genes of *Drosophila melanogaster*. Mutations in these genes can transform one part of the body of the fly into another (Lewis, 1978; Kaufman *et al.*, 1980). More than twenty years ago, Antonio García-Bellido proposed that the homeotic loci represent a group of "selector genes" that control a subset of subordinate target genes (or "realizator genes") that in turn encode cellular proteins that are directly required in differentiation processes (García-Bellido, 1975). If we think of the *Drosophila* embryo as being composed of a number of individual, but interacting modules (i.e., segments), then the "selector" genes could change the identity of a particular module without affecting neighboring modules. It is important to note that this kind of change would not necessarily perturb the synchronized cascade of developmental events that occur during embryogenesis. As a consequence, the change in the morphology of an organism theoretically could be accomplished without deleterious affects to its overall fitness. This concept would also suggest that at present, the whole general class of "selector" genes should be our first choice when searching for candidate genes that may be responsible for morphological evolution. We wish to make it clear that there are many genes that control the development of the distinct morphological features of any organism, the effect of which can range from minute to drastic. However, at present, only certain "selector" genes have been characterized in sufficient detail so as to allow us to start investigating their possible role in the morphological evolution of animals. As part of our tribute to Antonio's work, in this review we evaluate and discuss whether, and to what degree, *Hox* genes have played a part in the diversification of arthropod body plans. In particular, we focus on the development of the head and specific head morphology in the major arthropod taxa. We also provide a necessary framework that allows one to make broad comparisons and predictions with respect to the origins of particular head structures in arthropods.

## *Hox* genes and evolution of arthropod body plans

In terms of their structural and morphological diversity, as well as sheer numbers, arthropods represent the most successful animal phylum. Although the true phylogeny of arthropods is a

much debated topic, four major extant groups can be recognized: insects, myriapods (millipedes and centipedes), crustaceans (e.g., crabs, lobsters, shrimps), and chelicerates (e.g., spiders, ticks, mites, scorpions). With respect to their morphology, however, all arthropods share a common feature: a subdivision of their bodies into distinct segments (Brusca and Brusca, 1990). To a large degree, because of their complete body segmentation, arthropods can be considered to be the paradigmatic modular organisms (Raff, 1996). This modular organization has apparently facilitated and influenced the divergence of arthropod body plans in general as well as the divergence of individual segments in particular. In insects, it is the *Hox* genes that are involved in establishing segmental identity along the axis of the body (Lewis, 1978; Kaufman *et al*., 1990; Lawrence and Morata, 1994). On the basis of this connection between homeotic genes and segment identity it has been recently suggested that structural or regulatory changes in *Hox* genes may very well be responsible for the observed differences in morphology (Akam, 1995; Carroll, 1995).

A molecular characterization of the *Hox* gene complex (*HOM-C*) has been done in insects (Kaufman *et al*., 1990; Beeman *et al*., 1993), which possess a total of eight resident genes: four "head" genes that specify identity of head segments *[labial (lab), proboscipedia (pb), Deformed (Dfd)*, and *Sex combs reduced, (Scr)]*, followed by three "trunk" genes *[Antennapedia (Antp), Ultrabithorax (Ubx)* and *abdominal-A (abd-A)]*, followed by a posterior-acting "tail" gene *[Abdominal-B (Abd-B)]*. In light of the large divergence of body plans among major arthropod lineages, the finding of eight *Hox* genes in insects raises the question of the composition of the *HOM-C* complex in these other groups. Recently, several studies have tried to answer that question by using the polymerase chain-reaction to pull out short gene fragments that can be used to identify the presence of a particular *Hox* gene in the complex of a brachiopod crustacean, horse-shoe crab, centipede, onychophoran, spider, millipede and terrestrial isopod (Averof and Akam, 1993; Cartwright *et al*., 1993; Grenier *et al*., 1997; Popadić and Abzhanov, unpublished data). The results of all of these studies have shown that all major arthropod taxa have homologs of all eight insect *Hox* genes, with the single exception that in horse-shoe crabs multiple sets of these genes are found (Cartwright *et al*., 1993). Furthermore, the complement of all eight genes was also found in onychophorans. This finding of the identical complement of homeotic genes in arthropods and onychophorans has been deemed significant, because it implies that the existence of a single HOM-C comprised of eight genes predated not only the radiation of arthropods, but also the origin and radiation of the onychophoran/arthropod clade (Grenier *et al*., 1997). However, based on the fact that a full complement of *Hox* genes is also found in vertebrates, one might not necessarily find its presence in the progenitor of the arthropods too surprising as these genes apparently antedated the protostome -deuterostome divergence. Indeed, the apparent duplication of the *HOM-C* in a single chelicerate, the horse-shoe crab, similar to what has apparently occurred in the phylogeny of chordates would seem to be a more intriguing result.

What was the role of the *Hox* genes in the ensuing morphological evolution of arthropods? In contrast to the presumably undifferentiated, homonomous body of a proto-arthropod, insects have three well defined body regions: head, thorax, and abdomen (Fig. 1). While myriapods are characterized by a head and homonomous trunk, crustaceans show a great diversity in the morphology of their trunk (some of which are depicted in Fig. 1). In brachiopods, the trunk is divided into a homonomous "thorax", genital and postgenital region, whereas in malacostracans (which include isopods and decapods) the anterior thoracic segments have undergone a homeotic-like transformation and assumed gnathal (food gathering/handling) identities. Finally, chelicerates exhibit yet another type of body organization characterized by the lack of a defined head region. Instead, they have a prosoma (cephalothorax) with locomotory and feeding appendages and an opisthosoma (abdomen).

Theoretically, two kinds of changes in the *Hox* genes could occur: structural (involving changes at the protein level) and regulatory (involving changes in domains of expression as well as in time of expression). The former changes have been investigated in a series of experiments where the functionality of orthologous *Hox* genes (from a wide range of animals) was tested by transformation into *Drosophila* or mice (Bachiller *et al*., 1994). Typically, these experiments have shown that orthologs are capable of causing similar defects when ectopically expressed, and are capable of rescuing homeotic gene mutants (Lutz *et al*., 1996). For this reason, it is now generally believed that morphological evolution in arthropods was governed by regulatory, not structural, changes (Akam, 1995; Carroll, 1995). At present, however, the expression patterns of only a few *Hox* genes have been compared among arthropods. As shown in Figure 1, *Antp* is expressed exclusively in the thorax of *Drosophila*, whereas it is expressed throughout the thorax of the brine shrimp, *Artemia francisciana* (Carroll *et al*., 1986; Averof and Akam, 1995). Fortunately, for two of the trunk genes, *Ubx* and *abd-A*, there exists a cross-reacting antibody that can be used to study their pattern of expression across a broad range of taxa (Kelsh *et al*., 1994). In insects, these genes are expressed principally in the abdomen (White and Wilcox *et al*., 1985; Karch *et al*., 1990; Kelsh *et al*., 1994), although at later stages of development *Ubx* has been recruited to modify the third thoracic segment in flies (Castelli-Gair and Akam, 1995). In crustaceans, however, a recent study by Averof and Patel (1997) has shown that shifts in the anterior domains of expression of *Ubx/abd-A* correlates with a homeotic-like transformation of locomotive to mouthpart appendages in the anterior thorax. Similarly, in two myriapod taxa (Chilopoda, centipedes, and Diplopoda, millipedes), the *Ubx/abd-A* expression starts anteriorly in the second trunk segment and continues posteriorly throughout the trunk (Grenier *et al*., 1997; Popadić, unpublished data). This pattern correlates with the morphological differences between the first and second leg segments, the former having a distinct morphology and being involved in food gathering, and the latter being morphologically similar to the rest of the walking legs (Brusca and Brusca, 1990; Hopkin and Read, 1992). Overall, these studies show that changes in the expression pattern of *Ubx* and *abd-A* are correlated with the morphological changes in the arthropod body plans: in insects, these genes provide identity to a distinct body region - the abdomen (Vachon *et al*., 1992; Castelli-Gair and Akam, 1995); in crustaceans and myriapods, however, these genes seem to be involved in changing the morphology and function of anterior trunk segments from locomotory to feeding and food handling. On the basis of these results then, one might expect that the homeotic head genes may also have played a part in the morphological evolution of arthropods.

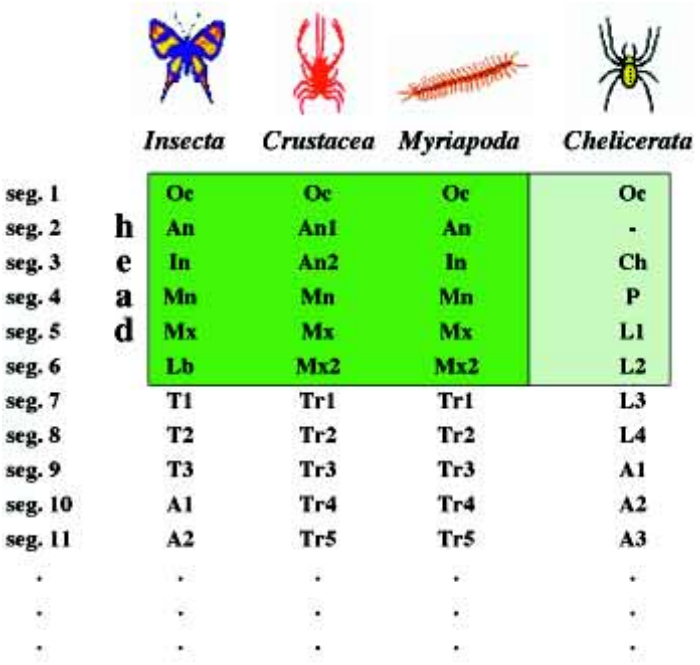


**Fig. 2. Alignment of the anterior segments across the arthropod lineages.** *Those arthropods that make up the subphylum Mandibulata possess a clearly defined head that consists of the first six segments (dark green shading). Chelicerates have no structure which corresponds to the mandibulate head and have lost the segment which is homologous to the antennal segment in insects (light green shading) (Beklemishev, 1994). Oc, Ocular; An, Antennal; In, Intercalary; Mn, Mandibular; Mx, Maxillary; Lb, Labial; T, Thoracic; A, Abdominal; Tr, Trunk; Ch, Cheliceral; P, Pedipalpal; L, Leg.*

## Homology and alignment of head segments in arthropods

Knowledge of the basic organization and homologies of the structures in the head are prerequisites for any study about evolutionary changes in this region. This is particularly true in the case of the *Hox* genes, which have distinct domains of expression. In order to interpret and understand the changes in patterns of expression between different arthropods, we need some sort of "common map" of the head region. But to do that, we first have to know the number and identity of head segments in the major arthropod taxa. A second and equally important question is: Are these segments at all homologous? These two questions have been vigorously debated for more than a century and are literally "the stuff that books are made of". In this review we present just a brief summary of our views and do not attempt to provide a detailed treatment of the subject.

With respect to the number of segments in insect head, the status of the three gnathal segments (mandibular, maxillary and labial) was never seriously questioned (Rempel, 1975). Instead, the debate and controversy concerned the status of the more anterior pre-gnathal segments, such as the intercalary, antennal, ocular and pre-antennal (labral) segments. In total, more than a dozen hypotheses have been proposed (Rempel, 1975), but because all of these studies used the same methodological approach (comparative embryology and neural innervation) it has been difficult to justify why any particular hypothesis would be superior to all others. Recently, however, an additional and more objective insight into origins of these structures was provided by the expression patterns of the segment polarity genes *wingless (wg)* and *engrailed (en),* which are expressed in the anterior and posterior compartments of each segment respectively and thus can be used to define segments on a molecular level (Baker, 1988; Martinez Arias *et al.*, 1988). The analysis of *en* expression in *Drosophila* and other insects has provided unambiguous evidence for the existence of six head segments: ocular, antennal, intercalary, mandibular, maxillary and labial (Patel *et al.*, 1989a,b; Rogers and Kaufman, 1996). Additionally, the study of *en* expression in four species of crustaceans also detected the same six segments (Scholtz, 1995). In contrast, these studies could not confirm the segmental status for the labrum, with the possible exception of flies (Schmidt-Ott and Technau, 1992; Schmidt-Ott *et al.*, 1994a,b). We should note here that the existence of the labrum as a morphological structure is not an issue - all arthropods have a labrum. The question revolves around its status as a *bona fide* segment, and in our view, this remains to be demonstrated (Rogers and Kaufman, 1996; Popadić *et al.*, 1998). At present, there are no data on *en* expression in myriapods, but previous embryological work agrees with the existence of the same six segments identified in insects and crustaceans (Anderson, 1973; Dohle, 1980; Kraus and Kraus, 1994). Overall, we conclude that both the molecular and the embryological data provide strong evidence in support of the well defined, six segmented head region in these arthropods. This leaves us with the issue of the segmental identities in the prosomal region of chelicerates. As is evident in Figure 1, chelicerates are the only major arthropod taxa without a distinct head region. Instead, their body is divided into an anterior (prosomal) and posterior component (opisthosomal). The question is, which of the prosomal segments correspond to the head segments of other arthropods? Traditionally, by following the neural innervation of each segment as well as other more esoteric morphological criteria, it has been suggested that the middle region of the arthropod brain (deuterocerebrum) was lost in chelicerates (Beklemishev, 1964; Brusca and Brusca, 1990). This would imply that the segment that is normally associated with the deuterocerebrum (the antennal in insects or antenna 1 in crustaceans) is missing in chelicerates. As a consequence, the next most posterior prosomal segment, the cheliceral, would correspond to the intercalary segment of insects and antennal 2 of crustaceans. Following this argument, and on the basis of the previously described molecular and embryological evidence, it is possible to establish the alignment and correspondence of head segments in arthropods that is depicted in Figure 2.

It is important to note, however, that the reliability and robustness of the proposed alignment in Figure 2 is dependent upon our ability to determine if arthropods are a monophyletic or polyphyletic group. If they are indeed monophyletic, then the head segments of the major arthropod taxa can be considered homologous and the proposed correspondence of segments between different taxa is justified. Although studies based on comparative morphology and embryology have provided general support for monophyly (Snodgrass, 1938; Boudreaux, 1979; Weygoldt, 1979), they were also used to further the opposing view (Manton, 1977). For this reason, we tend to view the phylogenetic analyses that are based on molecular data as having a decisive influence in deciding which hypotheses is correct. Some of the earlier molecular studies turned

out to be somewhat controversial (for a review see Fryer, 1996), but the most recent investigations provide strong evidence in support of monophyly (Boore *et al.*, 1995; Friedrich and Tautz, 1995). What is convincing about this evidence is the fact that it includes several different molecules as well as an analysis of gene rearrangements in mitochondrial genomes. The latter data is particularly informative because these rearrangements are extremely rare and immune to selective pressures, which makes them ideal characters for phylogenetic analysis. In addition to supporting monophyly, the molecular phylogenies also separate mandibulates (insects, crustaceans and myriapods) from chelicerates, in agreement with previous morphological and embryological evidence (Snodgrass, 1938; Weygoldt, 1979). This grouping of mandibulates argues against one of the major premises of the polyphyletic view, which considers mandibles of insects and myriapods as being fundamentally different from the crustacean mandibles (Manton, 1977). According to Manton's view, the former taxa have mandibles of a whole-limb type (which bite with the tip), whereas crustacean jaws have been regarded as being formed from a limb base only (gnathobasic type). Recently, however, we have provided independent molecular evidence that shows that all three of these taxa actually have the same mandibular structure (Popadić *et al.*, 1998). We view all of these results as providing impressive support for the monophyly of arthropods in general and for the common origin of the head region in mandibulates in particular.

In our view, the alignment of segments in Figure 2 provides us with a kind of a "common map" of a the head region that is crucial for our ability to interpret the results of future evolutionary developmental studies. Especially within the mandibulates, the homology of head segments can be used as an independent reference for comparing and understanding the patterns of expression of *Hox* and other regulatory genes.

## Expression patterns of head *Hox* genes in insects

The molecular characterization of the Hox genes was first done in *Drosophila melanogaster*, the genetic utility of which offers unsurpassed features for detailed studies of interactions among genes that govern developmental processes. However, with respect to its morphology, *Drosophila* is a representative of a highly derived insect order (Diptera, flies), which makes it a poor representative of the ancestral morphological characters that characterize insects. Thus, in order to elucidate the role of homeotic genes in the morphological evolution of insects, it has been necessary to include representatives of additional insect orders into our survey. We also wish to emphasize the importance of the phylogenetic context for evolutionary developmental studies, just like any other kind of evolutionary research (Futuyma 1986; Raff 1996). For example, only by mapping the expression patterns of a particular *Hox* gene onto an established phylogeny, can we infer the directionality of changes in these patterns. Thus, for this reason, in addition to *Drosophila*, two other species were included in a survey: the firebrat, a primitively wingless insect (*Thermobia domestica*, Thysanura), and the milkweed bug (*Oncopeltus fasciatus*, Hemiptera). The firebrat belongs to a basal insect lineage that is a sister group to the Pterygota (winged insects). The milkweed bug, on the other hand is a representative of hemipterans, which occupy an intermediate position in the phylogeny of insects (Fig. 3). In this review, we will focus on only the four most anteriorly expressed *Hox*

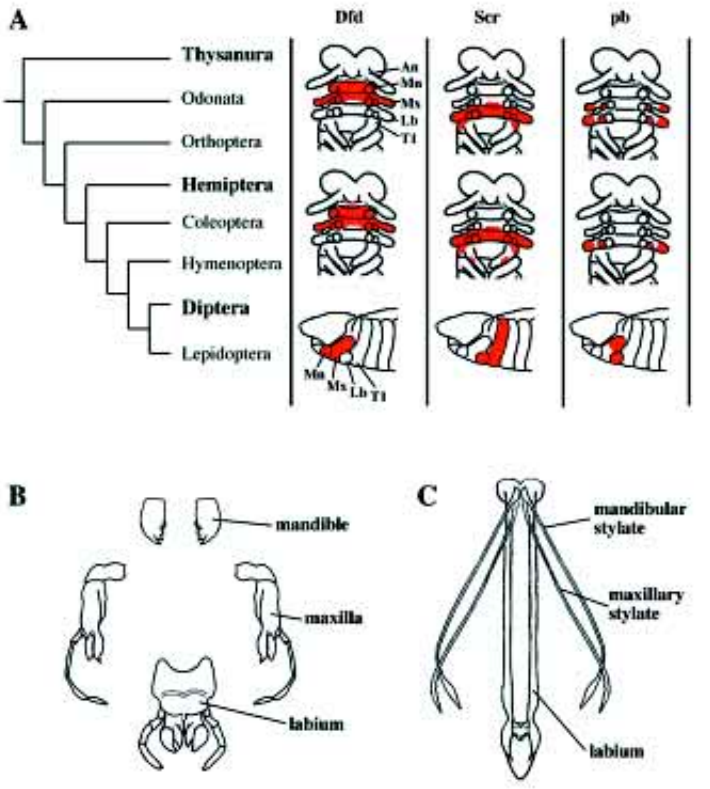


**Fig. 3. (A) Diagram of expression patterns of homeotic genes *Dfd, Scr* and *pb* in three different insect orders.** *The phylogenetic relationship of a few major orders of insects is diagrammed on the left to demonstrate the position of each depicted group (Kristensen, 1991). The anterior halves of embryos of three insects are cartooned: primitive apterygote Thermobia domestica, order Thysanura (top); milkweed bug Oncopeltus fasciatus, order Hemiptera (middle); fruit fly Drosophila melanogaster, order Diptera (bottom). Names of the illustrated orders are shown in bold on the phylogenetic tree. The expression patterns for genes Dfd, Scr and pb are shown in red. An, antenna; Mn, mandible; Mx, maxilla; Lb, labium; T1, first thoracic segment.* **(B) Schematic drawing of mandibulate mouthpart structures**. *Mandibles of this type of insect mouthparts are simple jaws structurally different from both maxillae and labium. Maxillae and labium are structurally similar but labium appendages fuse to form a lower lip.* **(C) Schematic drawing of stylate-haustellate mouthparts of hemipterans.** *Mandible and maxilla of this type develop into nearly identical styla as opposed to a trough-like labium. Mandibles and maxillae form a sharp-edged sucking tube placed inside the labium. Both drawings are after Brusca and Brusca, 1990.*

genes (*lab*, *pb*, *Dfd*, and *Scr*). The primary question we are interested in is the role of these genes in the development and evolution of the insect head, as inferred from the analysis of insect orders which represent three reference points in the insect phylogeny (basal, intermediate and highly derived).

## Conservation of expression patterns: *lab*, *Dfd*

Studies of HOM-C gene expression in insects suggest that at least three different stages in ectodermal gene expression patterns can be distinguished: an "initiation" stage, a "segment-specific"

stage, and a "modulated expression stage" (Rogers and Kaufman, 1997). In this review, we will primarily discuss the ectodermal expression patterns at the "segment-specific" stage. The domains of *Hox* expression at this point are thought to be established by the early embryonic segmentation gene functions and define the spatial context within which the developmental programs that delineate segment specific structures are initiated. In *Drosophila*, *lab* is expressed primarily in the intercalary segment (Diederich *et al.*, 1989). This same expression domain is found in both milkweed bug (Rogers and Kaufman, 1997) and firebrat embryos (Peterson, unpublished data). Thus, *lab* is an example of a homeotic gene whose domain of expression has apparently remained unchanged during insect phylogeny. Similarly, at the segment-specific stage *Dfd* in the firebrat, milkweed bug and *Drosophila* shows a very conserved pattern of expression: in all three species, this gene is expressed in the mandibular and maxillary segments (Fig. 3A). These highly conserved patterns of expression that characterize the *lab* and *Dfd* genes suggest that the function of these genes may be under strong selection. Interestingly, in *Drosophila* the mutant phenotypes of *lab* and *Dfd*, while showing striking defects, do not reveal obvious homeotic transformations (Merrill *et al.*, 1987,1989). Thus, it will be important to recover and analyze mutations in the homologous genes in *Tribolium* which has recently been developed as a genetic system (Beeman *et al.*, 1993) and possesses a less derived and more typically organized insect head.

## Modification of expression pattern, followed by the possible acquisition of a novel function: *Scr*

In contrast to *lab* and *Dfd*, the expression pattern of *Scr* has been modified during the course of insect evolution. In the firebrat, *Scr* is expressed throughout the labial segment and in a dorsal patch of epidermis in the prothorax (T1) (Fig. 3A). This labial expression of *Scr* is also found in the milkweed bug and *Drosophila*, in agreement with the proposal that one of the original functions of this gene in insects was to provide the specific identity to the labial segment by controlling the fusion of labial lobes (Rogers *et al.*, 1997). This feature, fused labial lobes, distinguishes the labium from all other gnathal segments. In the milkweed bug, in addition to the dorsal patch of expression in T1, *Scr* is also expressed in a patch on the tibia of the T1 leg. In *Drosophila*, on the other hand, *Scr* is expressed throughout the T1 segment (Pattatucci *et al.*, 1991; Gorman and Kaufman, 1995; also Fig. 3A).

While all modern insects lack wings on the prothorax, the fossil record shows that Paleozoic insects had wing-like appendages on all thoracic and abdominal segments (Kükalova-Peck 1987). Therefore, one of the important events in the evolution of modern winged insects was the repression of wing development on the prothorax. On the basis of the molecular and genetic evidence that *Scr* suppresses T1 wing development in *Drosophila* and *Tribolium* and considering the location of the dorsal patch of expression in the T1 segment, Rogers *et al.* (1997) suggested that this dorsal expression in T1 could explain the loss of T1 wings in winged insects. However, because *Scr* is expressed in the identical domain in a primitively wingless insect (the firebrat), the original role for this expression does not appear to have been in the repression of wing development. This novel function of *Scr* had to be acquired later, during the evolution of the winged insect lineages.

Similar to the dorsal T1 pattern the expression of *Scr* in the tibia of the milkweed bug T1 leg may also represent an example of an expansion of an original domain of expression, followed by an acquisition of a new function (Rogers *et al.*, 1997). In contrast to the firebrat, both in the milkweed bug (Fig. 3A) and the cricket (Rogers *et al.,* 1997) *Scr* is expressed in the tibial region of the T1 leg. Furthermore, the position of the *Scr* tibial patch in the milkweed bug correlates with the position where a leg comb is formed. Since crickets (Orthoptera) do not have a leg comb, Rogers *et al.* (1997) suggested that this novel function was acquired only in the more recent insect orders, starting with the hemipterans (see phylogeny in Fig. 3A). The possible role of *Scr* in controlling the development of the T1 combs in these lineages relies on the fact that in *Drosophila,* proper development of the sex combs on the T1 leg requires *Scr* function. A way to further test this hypothesis would be to study the pattern of expression of *Scr* in additional hemipteran species, several of which have multiple combs on the T1 leg (as suggested by Rogers *et al.*, 1997).

## Direct correlation between change in the expression pattern and change in morphology of the mouthparts: *pb*

There are no better examples of the tremendous diversity in the organization of the insect head than the variation in the structure and morphology of mouthparts. Furthermore, the evolutionary success of insects has depended in good part on their ability to utilize different food sources, which in turn were facilitated by changes in the morphology of their mouthparts. Understanding the morphological evolution of insect mouthparts will also help us get a better understanding of the adaptive radiation of this group of arthropods.

In insects, the mouthparts are composed of the appendages of the three most posterior head segments: mandibular, maxillary and labial (Brusca and Brusca, 1990). In general, there are two basic types: mandibulate, specialized for chewing and biting; and haustellate, specialized for piercing and sucking (Matsuda, 1965). The mandibular type represents the ancestral form, and is characteristic of most of the basal hexapod lineages (*Collembola, Thysanura, Odonata, Orthoptera*). The main feature of this type is that while mandibular appendages have a separate function and distinct morphology in the "jaw", the maxillary and labial appendages are structurally very similar (Fig. 3B). In contrast, in the haustellate mouth type, it is the mandibular and maxillary appendages that are structurally similar, whereas the labial appendages have a distinct morphology (Fig. 3C).

In *Drosophila* and *Tribolium*, pb mutants display a homeotic transformation of their mouthparts towards walking limbs, suggesting that this gene plays an important role in specifying palps in anterior appendages (Kaufman, 1978; Randazzo *et al.*, 1991; Beeman *et al.*, 1993). For this reason, *pb* is an excellent candidate for a homeotic gene that may have played a critical role in the evolution of the insect mouthparts. In the firebrat, which has a mandibulate mouth type, *pb* is expressed principally in the maxillary and labial appendages (Fig. 3A, Peterson, unpublished data). This pattern is consistent with the fact that these two segments are structurally very similar (Fig. 3B), and different from the mandibular segment (where pb is not expressed). However, in a milkweed bug which is characterized with a stylate-haustellate mouth parts, *pb* is expressed only in the labial appendages (Fig. 3C, Rogers and Kaufman, 1997). This reduction in the domain of expression correlates well with a change in morphology of the maxillary and

labial segments that is characteristic for this mouth type (Fig. 3C) i.e., maxillary segment development and morphology parallels the mandibular, not the labial segment. These findings, then, provide us with a first example of a correlation between a change in an expression pattern of a homeotic gene and the corresponding change in the morphology of insect mouth parts.

## From insects to other arthropods

Classes Crustacea, Insecta and Myriapoda are believed to belong to a monophyletic subphylum Mandibulata (see Fig. 1). Based on an alignment of segments (as in Fig. 2) in these classes it is possible to conclude that their head structures are indeed homologous. Assuming that the *HOM-C* genes are playing similar roles in other mandibulates as they do in insects, it is possible to make some simple predictions regarding functions and expression patterns of these genes in crustaceans and myriapods. We can, for example, predict that *pb* should be expressed in the limbs of both maxillary segments of chilopod and symphylan myriapods as well as crustaceans, since these arthropods also have two pairs of maxillary appendages. As we already mentioned, there is a striking homeotic-like transformation of anterior thoracic appendages to maxillipeds (specialized feeding appendages) in many crustacean lineages (Averof and Patel, 1997). The *pb* gene, which has been shown to specify mouthparts in insects (see above), may be involved in this transformation. If this is correct one might expect to see a novel expression pattern of *pb* in the anterior trunk = >maxillepedal segments correlated with the observed absence (retraction?) of *Ubx/abdA* expression.

Some further interesting predictions can also be made about the *HOM-C* gene *Scr* since it is known that this gene is needed for the ventro-medial fusion of the appendages derived from the labial segment of insects. Curiously, a similar fusion of the maxillary appendages is known to occur during head development of some myriapods (Brusca and Brusca, 1990). For example, in symphylan myriapods the 2nd maxillary appendages are fused into a labium (as in insects), whereas in diplopod and pauropod myriapods the 1st maxillary appendages are fused instead to form a flap-like structure called gnathochilarium. The later case may imply an expansion of expression of *Scr* into the 1st maxillary segment since *Scr* is expressed in only a few cells of the maxillary segment in insects (Rogers *et al.*, 1997). In chilopods, neither pair of maxillary appendages are fused or missing. It is unknown if this condition represents an ancestral situation or a derived one through a loss of this particular aspect of *Scr* function.

As previously mentioned, these predictions require the *HOM-C* genes to have acquired most of their morphological functions prior to divergence of the mandibulates. Because of this condition, it is nearly impossible to predict the expression patterns or functions of *HOM-C* genes in non-mandibulate chelicerates. Based on morphology, no gnathocephalic limbs of chelicerates are clearly homologous with any head appendages of mandibulates, although we believe that this does not preclude the homology of the segments. However, some appendages of the chelicerate prosoma such as chelicerae and pedipalps are specialized for feeding. Therefore, it is conceivable that identity of segments in the prosoma can be specified by the *HOM-C* genes. Nevertheless, as discussed later in this review, homology should not be judged solely or even primarily on the bases of the expression patterns of the *HOM-C* genes.

## Homeotic genes and homology of arthropod structures

Homeotic genes have been shown to be important in the development of insects and other arthropods and are now beginning to be used as tools to answer evolutionary questions. Since homeotic genes are involved in establishing the identity of large body regions and/or single segments with specialized morphology and function, they are thought to be significant in morphological evolution (Akam *et al.*, 1994; Valentine *et al.*, 1996). As already discussed, the *HOM-C* genes are organized into a cluster that has persisted in its basic composition and structure for hundreds of millions of years both in vertebrate and invertebrate lineages. Therefore, it is easy to perceive these genes as something fixed in an otherwise remarkably plastic and diverse group. *HOM-C* genes have attracted a lot of attention from evolutionary and developmental biologists in the last few years. As the field develops, however, it is becoming increasingly clear that drawing broad conclusions from the example of only a small number of genes, even developmentally important ones, should be done with great caution. This is especially true when one considers the long and complex evolution of the arthropods.

To illustrate this point we will present one of several possible difficulties with using homeotic genes for evolutionary studies. Many (if not all) researchers in this field are sorely tempted to use information gleaned from the expression patterns of homeotic genes to determine evolutionary relationships, such as homologies. Homology, or inheritance of specific structures from a common ancestor is often difficult to determine in arthropods many of which are highly derived from their ancestors. It is easy to imagine that truly homologous structures, for example mandibles, would share the expression of several genes and even whole developmental programs. Thus, the structural differences observed in the mandibles among insect species might be caused by variations in genes at the end of the developmental hierarchy while the more fundamental differences between the mandibles of insects and myriapods could be associated with alterations in genes earlier in the ontogenic cascade although leaving the “basic mandible” vs “maxillary” or “thoracic” program intact. Homeotic genes would seem to be good candidates for such “basic” genes and one might conclude their expression patterns to be inviolate. Thus they could be used as markers defining homologous body regions and/or segments. However, it must be remembered that homeotic genes themselves are subject to evolutionary forces and their functions and expression patterns surely evolve and should not be used blindly as landmarks in determining homologies of specific structures in all arthropods. For example, it is becoming clear that co-option has played a critical role in evolution and the homeotic genes are not exempt in this regard. To demonstrate this point we can point to the expression pattern of the homeotic gene *Ubx* in various arthropod groups and the most basic morphology of the segments within its expression domains. The abdomen of insects, the thorax of crustaceans, the trunk of myriapods (Fig. 1) and the ophistosoma of chelicerates can hardly be described as identical structures. Each has its own set of basic features such as shape, cuticle, presence or absence of appendages etc. Therefore homeotic genes are not necessarily associated with specific structures and their control over other genes could be gained, modified or lost as could the regulation of the *HOM-C* genes themselves. It is obvious that *Ubx* was co-opted multiple times to control the identity of novel morphological structures evolving within its expression domain in each

arthropod lineage. Its original function in the arthropod ancestor was likely a basic one, for example specification of CNS in the posterior part of the body. Accordingly, homeotic genes should be seen as *evolving* landmarks that were used to distinguish segments in an otherwise genetically homonomous body and changes in their expression patterns could provide new opportunities for further specialization of arthropod body plans and generation of their enormous diversity. It is very important to understand the functions of *HOM-C* genes in extant arthropods and how and when these functions have been acquired during evolution. Only with this knowledge can we understand fully their role in morphological evolution. There are surely rules governing the evolution of morphologies and the *HOM-C.* However, we will need many more data points before we can begin formulating those rules. The wonderful thing is that due to the efforts of those who have gone before we now have the tools to find the answers.

## Conclusion

The presently available data suggest that at least some of the homeotic genes were involved in the morphological evolution of arthropods. To be sure not all of the morphological diversity found in this group involves *Hox* gene variation. But, as illustrated by the correlation between the change of *pb* expression and the change in the morphology of mandibulate and haustelatte mouthparts, at least some of the major morphological differences are associated with alterations in the pattern of expression of these genes. Thus, at present, the study of *Hox* genes offers an excellent starting point for obtaining insights into the genetic basis of phylogenetic change in the morphology of the arthropods. What we must learn next is the nature of the genetic changes that govern the observed changes in expression of genes like *pb*. This will require a detailed characterization of the regulatory elements of each gene in question. Only with these kind of data will we be able to gain a true understanding of the mechanisms of morphological evolution and bridge the gap between evolutionary processes and developmental patterns.

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