INTRODUCTION

Over 500 million years ago in the early Cambrian, a group of animals evolved a basic morphology that would allow them to take over the world, becoming one of the most populous and diverse phyla on the planet. This group, the Arthropods, includes over a million species of spiders, mites, ticks, centipedes, millipedes, crustaceans and insects. Their segmented body plan consists of a series of repeated morphological units, which are grouped into tagmata dedicated to specific functions. Each class of arthropods has a unique division of body tagmata. For example, while the insects have three tagmata, the head, thorax and abdomen, myriapods have just two, the head and trunk (see Fig. 1).

The process of tagmosis, as well as independent differentiation of individual segments, has allowed a great degree of specialization that can account for the great success of the arthropods. However, until recently, we have had little conception of the mechanism by which such body plan changes were accomplished. To understand the origin of the morphological diversity upon which natural selection acts, it is necessary to understand how the process of embryonic development evolves. We can infer the evolution of development by comparing the mechanisms of development in different species. The extensive work in Drosophila developmental genetics facilitates this, as it provides some basis for speculating about the developmental processes of other arthropods.

The body plan of Drosophila is encoded in part by the patterned expression of a set of transcription factors called the Hox genes, which divide the embryo into a series of unique domains from anterior to posterior, and thereby assign spatial identity to the segments. The Hox genes have been analyzed in insects, crustaceans and chelicerates. However, the expression patterns of the Hox genes have not yet been comprehensively analyzed in a myriapod. We present the expression patterns of the ten Hox genes in a centipede, Lithobius atkinsoni, and compare our results to those from studies in other arthropods. We have three major findings. First, we find that Hox gene expression is remarkably dynamic across the arthropods. The expression patterns of the Hox genes in the centipede are in many cases intermediate between those of the chelicerates and those of the insects and crustaceans, consistent with the proposed intermediate phylogenetic position of the Myriapoda. Second, we found two ‘extra’ Hox genes in the centipede compared with those in Drosophila. Based on its pattern of expression, Hox3 appears to have a typical Hox-like role in the centipede, suggesting that the novel functions of the Hox3 homologs zen and bicoid were adopted somewhere in the crustacean-insect clade. In the centipede, the expression of the gene fushi tarazu suggests that it has both a Hox-like role (as in the mite), as well as a role in segmentation (as in insects). This suggests that this dramatic change in function was achieved via a multifunctional intermediate, a condition maintained in the centipede. Last, we found that Hox expression correlates with tagmatic boundaries, consistent with the theory that changes in Hox genes had a major role in evolution of the arthropod body plan.

Key words: Body plan, Centipede, Chilopoda, Lithobius, Hox, labial, proboscipedia, Hox3, Deformed, Sex combs reduced, fushi tarazu, Antennapedia, Ultrabithorax, abdominal-A, Abdominal-B
fragments of the Hox genes have been cloned from the myriapods (centipedes and millipedes), the expression pattern of most of the Hox genes has not been determined (Cook et al., 2001; Grenier et al., 1997). As recent molecular phylogenies place the myriapods outside the insect-crustacean clade, the absence of Hox gene expression data for the group leaves a gap in the middle of the arthropod tree (Giribet et al., 2001; Hwang et al., 2001; Cook et al., 2001; Regier and Shultz, 1997; Friedrich and Tautz, 1995). Thus, it has been difficult to infer the full course of the evolution of these genes in the arthropods.

Besides the importance of the myriapods’ phylogenetic position, they also have an interesting body plan. As noted, the myriapod body is divided into two tagmata, the head and trunk. The long trunk is typically fairly homonomous. That is, there is little specialization among the many pairs of legs. Moreover, the trunk can vary greatly in length and number of segments, even within a species (Minelli and Bortolotto, 1988). This relatively unspecialized, homonomous trunk is probably similar to the body plan of the arthropod ancestor.

There are also interesting differences in body plan within the myriapods. The head may include two, three or four sets of mouthpart appendages (in millipedes and paupropods, symphylans, and centipedes, respectively). In centipedes, the last pair of ‘mouthparts’ – their notorious poison fangs – is actually a modified pair of legs co-opted from the trunk and are therefore referred to here as maxillipeds.

We present sequence and expression data for the Hox genes in the centipede Lithobius atkinsoni. Having established the Hox expression patterns in a myriapod, we now have data that represent all four extant classes of arthropods, and thereby are better able to infer the course of Hox evolution within this fascinating and diverse group.

**MATERIALS AND METHODS**

**Centipede husbandry**

Wild-caught centipedes from North Carolina were supplied through Carolina Biological Supply. They were identified as *Lithobius atkinsoni*, thanks to help from Gerald Summers. Adult animals were housed in plastic tubs with layers of pine bark wood chips over a poured plaster-of-Paris floor, with vented lids to maintain moderate humidity. Tubs were sprayed with water every few days, and crickets or mealworms were provided every few weeks. Intraspecific predation is minimal unless the animals are crowded or starved.

Eggs were collected periodically by rinsing out the wood chips and tubs with water and catching the eggs in a sieve (mesh number 60). Eggs are laid year-round, and are deposited individually in damp crevices. The mother often coats each egg in a sphere of detritus; however, this is easily recognized and removed without damaging the egg. The clear eggshells allow the embryos to be staged by simple observation under a dissecting microscope. Embryos were maintained until the desired stage in watchglasses with moistened, shredded coconut fiber, which is sold through pet shops as a substrate for reptiles (‘Bed-a-Beast’).

**Embryo preparation**

The extended-germband stage embryo can be seen through the eggshell at about a week after egg deposition, at room temperature. Embryos were fixed for 30-60 minutes in 4% paraformaldehyde. The fixative permeates the embryo through the eggshell. After fixation, embryos were dissected from the eggshell and stored in ethanol at –20°C.

![Arthropod body plans and phylogeny. The four major groups of extant arthropods are illustrated here, with a tree based on several recent molecular phylogenies that group the insects with the crustacea (Giribet et al., 2001; Hwang et al., 2001; Cook et al., 2001; Boore et al., 1998; Regier and Shultz, 1997; Friedrich and Tautz, 1995). In the tree shown, myriapods are retained within the Mandibulata with insects and crustaceans (Giribet et al., 2001). Tagmatic boundaries are indicated by broken lines; names for tagmata of different groups are also indicated. Note that some groups of arthropods, for example, the crustaceans, include species with a variety of tagmatic plans not illustrated here.](image-url)

**Cloning**

RNA was prepared from collections of mixed-stage embryos using Trizol reagent, following manufacturer’s instructions. Total RNA was poly-A selected with the Qiagen Oligotex kit. The Boehringer Mannheim 5′3′ RACE Kit and Ambion RLM RACE kits were used to produce cDNA, and PCR was performed using the Advantage2 PCR System (Clontech).

Sets of degenerate primers were used to amplify portions of the various Hox genes. The primers were designed based on the sequences of orthologs from other arthropod species; primer sequences are available upon request. From the clones of the homeobox regions, exact primers were designed for 3′ RACE, which produced longer clones suitable for making in situ probes. In the case of the *abdominal-A* gene, 3′ RACE primers were designed based on the *abd-A* sequence of a similar centipede (Genbank Accession Number, AF362094). A variety of annealing temperatures were tested to optimize PCR amplification. A short set of five initial ramp cycles (with a gradually increasing temperature between the annealing and extension steps), or alternatively, a set of five initial ‘touchdown’ cycles (with an extension temperature 5-10°C higher than the main cycles) were each found to improve amplification. The cloned *Lithobius* gene sequences are available through GenBank with the following Accession Numbers: *labial*, AF435002; *proboscipedia*, AF435003; *Hox3*, AF435001; *Deformed*, AF434997; *Sex combs reduced*, AF435004; *fushi tarazu*, AF435000; *Antennapedia*, AF343996; *Ultrabithorax*, AF343005; *abdominal-A*, AF343994; and *Abdominal-B*, AF343495.

Sequences of orthologs from other species used for alignments were retrieved from GenBank. The Accession Numbers are as follows: *Drosophila lab*, X13103; *Trichobium lab*, AF231104; *Porcellio lab*, AF148935; *Lithobius forficatus lab*, AF362084; *Cupiennius lab*, AJ007431; *Drosophila ph*, AAF54089; *Artemia ph*, AF363018; *Lithobius forficatus phb*, AF362086, pb1, AF362085; *Archegozetes pb*, AAC59595; *Drosophila bcd*, P09081; *Drosophila zen*, p09089; z2, P09090; *Trichobium zen*, X97819; zen2, AF321227; *Schistocerca zen*, X92654; *Pachymerium Hox3*, CAB75744; *Cupiennius Hox3*, CA006645; *Drosophila Dfd*, X05136; *Trichobium Dfd*, U81038; *Thermobia Dfd*, AF104005; *Artemia Dfd*, X70078; *Pachymerium Dfd*, AJ272191; *Lithobius forficatus Dfd*, AF362087;
and folds in half ventrally, to form a ‘C’ shape, while the dorsal membrane expands to enclose the entire yolk mass. Following this ventral flexure, the appendages elongate and differentiate, and several weeks later the hatching emerges as a tiny centipede with eight pairs of legs. Additional leg-bearing segments are added at each molt during juvenile development, up to a final number of 15.

The observed development of this species of Lithobius is consistent with that previously described for a similar species (Hertzel, 1984). Lithobius embryogenesis in general is also similar to that of other centipede families. However, the embryo is not split along the ventral midline as in the Scolopendridae, as even in early stages of embryogenesis a thin layer of cells connects the left and right halves of the germ band.

**Hox gene sequences**

Degenerate PCR was used to acquire short clones of homeobox regions of the genes. Using these sequences to design exact primers, we then performed 3′ RACE to acquire longer clones suitable for making in situ hybridization probes. The sequences of these clones are shown in Fig. 3, aligned with homologous genes from other arthropod species. The sequences corresponding to each in situ probe are marked.

Gene homology was determined by alignment with other described arthropod Hox genes from GenBank. Sequences were retrieved that corresponded to the ten Hox genes: *labial*, *proboscipedia*, *Hox3/zen*, *Deformed*, *Sex combs reduced*, *fushi tarazu*, *Antennapedia*, *Ultrabithorax*, *abdominal-A* and *abdominal-B*.

**RESULT**

**Embryology**

The extended-germband embryo of *Lithobius forficatus* is illustrated in Fig. 2. The scanning electron micrograph shows the outer form of the embryo, while the DAPI staining reveals the nuclei. The identity of each segment is labeled in the diagram. The embryo at this stage lies along the surface of the yolk, just under the chorion, with the ventral side outwards in a crescent-shape. Soon after this stage, the embryo contracts and folds in half ventrally, to form a ‘C’ shape, while the dorsal
Fig. 3. Lithobius Hox gene sequences. The partial sequences of cloned portions of the Lithobius Hox genes are aligned with orthologs from a few other arthropod species. Small arrows highlight the centipede sequences (Lithobius). Regions of the homeobox within the clones are marked above the sequences. The primers used for Lithobius are marked with boxes, indicating that that region of the sequence is somewhat uncertain. The sequence corresponding to the 5' end of each in situ probe is marked by a bar. The arrow indicates that the probe sequence extends further to the 3' end of the transcript. All sequences except those of Lithobius atkinsoni were acquired from GenBank; for Accession Numbers, see Materials and Methods.
Abdominal-B. Note that although fushi tarazu and the Hox3 homologs zen, z2 and bicoid do not behave like typical Hox genes in Drosophila, they appear to have been more typical Hox genes ancestrally (see Discussion). No evidence for duplications of any of the genes was found in Lithobius atkinsoni; however, we cannot exclude the possibility of additional unrecovered Hox genes.

The head genes: lab, pb, Dfd and Scr

In other arthropods, the gene labial (lab) is the most anteriorly expressed of the Hox genes. Likewise, in the centipede, lab is expressed strongly in the labrum and intercalary segment, and weakly in the mandibular segment (Fig. 4A). The labrum is a thick structure that could potentially accumulate background staining as an artifact. However, staining in the labrum is seen consistently only with the lab and pb probes; therefore, we interpret this staining as a bona fide region of the expression domain for these genes. Interestingly, in both cases the labrum staining is seen in conjunction with staining in the intercalary segment. This result is consistent with a recent suggestion that the labrum represents the fused appendages of the intercalary segment (Haas et al., 2001a; Haas et al., 2001b). For the centipede embryos shown here, it should be noted that the occasional staining of the antennae is merely background accumulation. The antennae are cup-like, and in some embryos they accumulate chromagen with all probes tested, including negative control sense probes (not shown).

The gene proboscipedia (pb) is expressed in very different
domains in crustaceans versus insects; thus, it was important to analyze the expression in a myriapod. The $pb$ probes reveals a pattern of expression that extends over four segments: intercalary/labrum, mandibular, maxillary I and maxillary II (Fig. 4B). The expression is strong in the intercalary segment and labrum. In the mandibular segment, staining extends across both the segment and the limb-buds, but is weak and spotty. Expression in maxillary I and II is limited to the distal appendages. Interestingly, this expression domain resembles a combination of the crustacean and insect expression patterns (see Discussion).

Expression of *Deformed* (*Dfd*) extends from the very posterior edge of the intercalary segment to the maxillary II limb-buds (Fig. 4C). *Dfd* is expressed across the mandibular segment and limb-buds, but is excluded from the central region of the limb-buds. In maxillary I, expression extends across the entire segment and limb-buds. In the maxillary II segment, expression is only seen in the middle region of the appendages. 

*Sex combs reduced* (*Scr*) is expressed primarily in maxillary II and maxillipeds (Fig. 4D). In the maxillary II segment, expression is strong in the segment and the limb-buds, but in the maxillipeds expression is limited to the limb-buds. Two additional domains of expression are seen: a medial domain just outside the ventral midline, which extends from the maxillary I segment to the L1 leg segment; and, more laterally, spots of presumptive neural expression in each of the trunk segments.

The trunk genes: *Antp, Ubx, abd-A and Abd-B*

The gene *Antennapedia* (*Antp*) is expressed most strongly in the maxilliped limb-buds and segment, but is also weakly expressed in the segments and limb-buds of more posterior legs (Fig. 5A). In early stages, the posterior expression fades gradually along the entire trunk, but in later embryos, the expression reaches only to L4. The Segmental expression has its anterior boundary in the extreme posterior of the maxillary II segment.

Expression of the gene *Ultrabithorax* (*Ubx*) is shown for extended-germband stages of embryogenesis in Fig. 5B (expression in earlier embryos for *Ubx* and *abd-A* is shown separately in Fig. 6). In extended-germband embryos, *Ubx* expression is strong in the limb-buds of the first leg segment (Fig. 5B; L1), with a distinct boundary along the posterior of the L1 segment. This expression pattern of *Ubx*, with an
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The anterior boundary in the first leg segment, is similar to that seen in a Scolopendran centipede (Grenier et al., 1997). In the early extended-germband stage, expression extends through all the segments and limb-buds of the trunk, but in later embryogenesis, expression fades from the extreme posterior. In addition, in later embryos, ventral trunk expression fades from regions of the segment, leaving rosette-like patches of expression that may be proneural.

The gene abdominal-A (abd-A) is expressed in a pattern very similar to that of Ubx (Fig. 5C). In both early and late extended germband embryos, expression starts in the limb-buds and segment of L1 (again with a boundary in the posterior of the segment), and extends along the trunk. Unlike Ubx, however, the expression of abd-A does not fade away from the posterior-most segments in older embryos.

Abdominal-B (Abd-B) comes on surprisingly early, in embryos still
forming segments (Fig. 5D), with expression in the growth zone and a bright ring of expression around the proctodeum. In later embryos, strongest expression is seen in the last few segments, eventually becoming restricted to the telson. There is another weak domain of expression of Abd-B along the segments of the trunk, with an anterior boundary in the posterior of the first leg segment.

**Ubx and abd-A in early embryos**

The anterior boundary of Ubx and abd-A expression is presumed to play an important role in determining tagmatic boundaries in crustaceans (Averof and Patel, 1997). In addition, there has been some indication of a dynamic shift in this boundary in a centipede (Akam, 2000). Therefore, we analyzed in more detail the anterior boundary of expression of these genes in early embryos still undergoing segment formation (Fig. 6).

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**Fig. 10.** Shifting Hox domains across the arthropods. The expression domains of Hox genes from studies of various arthropods are illustrated here in simplified fashion for ease of comparison. Solid bars indicate strong expression, while striped bars indicate weak or transient expression. As this diagram represents the temporal and spatial complexity of each gene as a single bar, in some cases using information from multiple species, it is necessarily highly simplified. Therefore, we have included the source references, listed on the right (1-43), in addition to special notes on the expression patterns (a-p). For this information see below. Different arthropod species often have differing numbers of segments; the segment-boxes illustrated here are based on the spiders *Cupiennius* and *Achaearanea* (Chelicerate); the centipede *Lithobius*, at hatching (Myriapod); the pillbug *Porcellio* (Crustacean); and the firebrat *Thermobia* (Insect). Question marks for Hox3 and ftz indicate that these genes have not yet been analyzed in a crustacean. In the insects, Hox3 homologs and ftz have highly diverged functions, so these are treated separately in Figs 11 and 12.
staining can be seen in each cell, indicative of a high level of transcription from the two chromosomal copies of the gene (not shown). At the anterior of the Ubx domain, there is a strict boundary between these Ubx-expressing cells and their neighbors that lack any detectable Ubx expression.

Expression of fushi tarazu

The expression pattern of the centipede fushi tarazu (ftz) gene is complex, and changes dramatically throughout development (Fig. 8). In the earliest embryos expression is very strong in the proliferation zone, with stripes apparently emanating off this area (Fig. 8A,B). There is also expression in the whole of each segment up to maxillary II, with a distinct set of bands just to the posterior of the maxillary I segment (Fig. 8C,D). At subsequent stages, the proliferation zone expression becomes weaker and limited to a chevron above the proctodeum. The segmental expression gradually fades as well, except for the maxillary I and II expression, which becomes more intense (Fig. 8E). As the broad expression across the trunk segments fades, it resolves into a presumably neural pattern of small dots in a line across the anterior of each segment (Fig. 8F). In the oldest embryos, there is strong expression maintained in the maxillary II segment and a bit of the posterior of maxillary I, accompanied by expression in the limb-buds of the maxillipeds. There is also possible weak expression in the limb-buds of more posterior trunk segments (Fig. 8G,H). Presumptive neural expression is still faintly visible in the segments of the oldest embryos examined (Fig. 8H). To summarize, ftz is expressed in the following domains: first, in the proliferation zone and the segments arising from it; then gradually stronger in the segments of maxillary I and II; later with expression in the limb-buds of the maxillipeds; and finally in the developing nervous system of the trunk.

DISCUSSION

The expression data for the centipede Hox genes is summarized in Fig. 9. The expression of each gene is shown

References


Notes

a) Ref. 3 also reports weak staining throughout the opisthosoma.
b) Ref. 2 also reports staining in the opisthosoma; Ref. 3 reports two paralogs of Dfd.
c) In early embryos, there is also some opisthosomal staining.
d) Ref. 1 reports two paralogs of Ubx, and Ubx-2 mRNA is expressed slightly more anteriorly than that of Ubx-I or protein.
e) Additional small spots of expression in the Op2 segment correspond to the future genital pores.
f) Only the ‘Hox’ domain of ftz is illustrated here.
g) In early embryos, expression of Antp extends along the entire trunk, but later fades from posterior segments.
h) Striped bars indicate that translation of Scr transcript in the Mx2 and T1(Mxpd) segments is delayed until late embryogenesis, where the appearance of Scr protein correlates with transformation of the maxillipeds (in Porcellio); expression is absent from Mx1 in Procambarus (Ref. 12).
i) Expression of Porcellio Antp is shown here; expression in Procambarus becomes restricted more to the anterior; expression in Artemia extends from posterior Mx1 to the end of the thorax (T11).
j) The anterior border of Ubx varies in correspondence with the number of maxillipeded segments (Ref. 15); in Artemia expression extends to the end of the thorax (T11) (Ref. 14).
k) The top bar indicates expression of abd-A in Porcellio and Procambarus (although Porcellio lack the extension of expression into T7 and T8); the bottom bar indicates expression of abd-A in Artemia.
l) Expression of Abd-B in Artemia is in genital segments I and II, which lie between the thorax and abdomen; the genital segments are followed by six abdominal segments that are not shown here.
m) The typical insect expression in the Mx and Lb segments is indicated here by a solid bar; the striped bar indicates that some insects have additional weak expression in the Mn and/or Int segments. Note that Oncopeltus lacks expression of pb in the Mx appendage, a change in expression that may be correlated to the unique sucking mouthparts of Hemipterans (Ref. 17).

n) The striped bar indicates that although in Drosophila expression of Scr is strong throughout the T1 segment, in other insects expression is limited to a few specific patches in the T1 segment (Ref. 27). Note that there is also expression of Scr in the mesoderm of the legs.

o) Expression is shown as for Thermobia; in later embryos of Drosophila expression of Antp becomes restricted to the thorax.
p) Expression shown is based on Thermobia and Schistocerca; in Drosophila, two Abd-B transcripts, m and r, have unique functions, and the m domain extends more anteriorly (Ref. 43).
in two diagrammatic forms. In Fig. 9A, the major expression domain of each gene is illustrated in cartoon form on an extended-germband embryo. In Fig. 9B, the extent of each gene expression domain is illustrated in bar form, below a diagram showing the segments and appendages of a larval centipede.

From the intercalary segment to the telson, all segments express at least one Hox gene (Fig. 9A,B). The expression domains of the Hox genes in the centipede follow their canonical order in the complex, as known from other species (Manak and Scott, 1994). Although the genes obey this 'rule of co-linearity', there is a certain amount of overlap between adjacent genes.

The expression of the Hox genes corresponds roughly with the tagmatic divisions in the centipede (Fig. 9B). The expression of the genes lab, ph, Hox3 and Dfd is confined to the head, while the trunk is apparently under the control of Antp, Ubx, abd-A and Abd-B. Interestingly, the maxilliped segment has expression of three genes that extend both into the head (Scr and ftz) and into the trunk (Antp). The maxilliped segment is thought to be homologous to the first trunk or thoracic segment of other mandibulate arthropods. The appendages of this segment in the centipede, however, have been highly modified. While their leg-like structure is still evident, they develop to become short and broad fangs, complete with a poison gland. Thus, the first legs of the...
centipede are modified to become more mouthpart-like, and are used for prey capture and manipulation. This mixed head/trunk identity of the segment seems to be reflected in the Hox code found there. While the segment itself has only a ‘trunk’ Hox gene (Antp), the appendages have expression of Antp as well as the ‘head’ genes Scr and ftz, which are also expressed in the maxillary II segment. It remains to be determined how these genes contribute to the development of the centipede fangs. It would also be interesting to know whether the evolution of this novel appendage is correlated with a shift in the expression of these genes. Further studies of Hox expression in other myriapods such as a millipede, or functional studies in the centipede, would be very interesting regarding these issues.

**Shrinking domains of head Hox genes**

Comparing the expression of the centipede Hox genes with those of other arthropods reveals significant variability in the observed patterns (Fig. 10). For example, in the chelicerate head the Hox expression domains broadly overlap. These same genes are expressed in much more restricted domains in the head segments of crustaceans and insects. Interestingly, the expression domains of these genes in the centipede are intermediate between these two extremes. For example, the gene lab is expressed over five segments in the spider, two segments in the centipede, and only a single segment in the crustaceans and insects (see Fig. 10). Likewise, the three-segment expression domain of centipede Dfd is intermediate between the four-segment domain in the spider and mite, and the two-segment domains of the crustaceans and insects. Most striking is the comparison between expression of pb among the four groups. In the spider, pb is expressed over five segments, from the pedipalps through the fourth walking leg. In the centipede, the expression domain covers four segments, from the intercalary to the maxillary II. In the crustaceans, the expression is restricted to the antennal II segment, which is homologous to the intercalary segment. In the insects, however, the expression of pb is more posterior, limited mainly to the appendages of the maxillary and labial segments (homologous to the maxillary I and II segments of the centipede). These expression patterns suggest that the centipede may retain some Hox expression domains in an intermediate state of their evolution, from the broad domains of the chelicerates to the more-restricted, less overlapping patterns of the crustaceans and insects. Moreover, the expression domain of pb apparently became differently subdivided in different lineages; towards the anterior in the crustaceans, and towards the posterior in the insects.

**The centipede trunk**

Expression of genes along the centipede trunk is, like the morphology of the trunk, fairly homonomous. Antennapedia extends along the whole trunk in early stages, and later retracts to cover legs one through four (Fig. 5A). It is not clear whether this later, more restricted domain imparts any developmental difference to these segments, as none is evident morphologically. It is intriguing to note that this restriction to the anteriormost segments of the trunk is reminiscent of a similar restriction of Antp expression in the pleon of malacostracan crustaceans and the thorax of insects (see Fig. 10). Perhaps the domain of Antp expression was restricted to the anterior portion of the trunk in the myriapod-like mandibulate ancestor, but was only exploited fully in the specialized differentiation of the crustaceans and insects. In the centipede, Ubx and abd-A expression patterns are similarly expressed along the trunk, although Ubx expression fades from the extreme posterior segments. Expression of Abd-B is strongest in the telson, but faint expression extends over the mid-region of leg segments two to seven. As the genes Ubx, abd-A and Abd-B are likely to have similar roles in patterning the trunks of all mandibulates, we suggest that the myriapods have developed their unique body plan largely by expanding the number of segments under the control of the ‘trunk’ genes. This is a similar scenario to that provided by recent findings that snakes seem to have created an elongated body by increasing the numbers of somites under the control of thoracic Hox genes (Cohn and Tickle, 1999).

**Genes with changing roles**

Those familiar with the developmental genetics of Drosophila may find it odd to refer to zen and fushi tarazu as ‘Hox genes’. In fact, only recently have these been recognized as such. Yet recent studies indicate that these genes were probably typical Hox genes in the arthropod ancestor, but have undergone remarkable functional transitions in some arthropod lineages.

The expression of the insect orthologs of Hox3 – bicoid, zen, and zen2 – reflects a remarkably versatile repertoire of functions (see Fig. 11). The gene bicoid encodes an anterior-specifying morphogen deposited maternally into the Drosophila egg (Frohnhofer and Nüsslein-Volhard, 1986). The gene zenknullt (zen) plays a role in the specification of Drosophila extra-embryonic tissues, whereas zen2, the adjacent duplication of zen, has a similar expression pattern but no discernable function (Pultz et al., 1988; Rushlow and Levine, 1990). When homologs were cloned from other insects, it was realized that these genes had sequence similarity with both the Hox3 genes of vertebrates as well as the zen gene of Drosophila; thus, zen is actually a highly derived homolog of Hox3 with a novel function (Falciani et al., 1996). More interesting still, when bicoid and zen homologs were cloned from another fly, it was discovered that these genes have sequence similarity as well (Stauber et al., 1999). Therefore it is likely that, despite its all-important role in early Drosophila development, bicoid may actually be a fairly recent duplication of the zen gene that has diverged greatly in function. Thus, the Hox3 gene has apparently been ‘caught in the act’ of changing function drastically in evolution – twice! To those interested in understanding the mechanisms of gene evolution, such a gene is worthy of much study. Researchers are currently working to clarify the timing of the zen to zen + bicoid duplication and divergence in the higher insects (Stauber et al., 1999; Stauber et al., 2000).

The results we present are relevant to the earlier functional change, from Hox3 (with a Hox-like role) to zen (with a role in extra-embryonic tissues). In spiders and a mite, the Hox3 gene has a typical Hox-like expression pattern, with a broad domain whose anterior boundary is approximately co-linear with the other Hox genes (Telford and Thomas, 1998b; Damen and Tautz, 1998; Abzhanov et al., 1999). The homologous genes of the grasshopper Schistocerca and the beetle Tribolium apparently have zen-like roles, with expression in the extra-embryonic serosa (Falciani et al., 1996). The centipede Hox3
gene presented here has a Hox-like expression pattern in the segments of the embryonic germ band, with no hint of an extra-embryonic domain. Thus, we have narrowed the window of the change in developmental function from Hox3 to zen to somewhere in the insect–crustacean clade. Further studies on crustaceans and lower insects may be able to pinpoint more precisely the phylogenetic timing of the change, and perhaps shed light on the context and the process by which this rogue Hox gene escaped from its role in determining segment identity.

With regard to *fushi tarazu*, we think we may have discovered just such a process of Hox gene change. Although *ftz* has a role in segmentation in *Drosophila*, ancestrally in the arthropods it seems to have been a more typical Hox gene. Our results here suggest that the transition between a Hox-like role and a role in segmentation may have occurred via an intermediate state in which the gene played multiple roles in development, and that this transition state was maintained in the centipede lineage.

Among the chelicerates, the sequence and expression of *ftz* was analyzed in the mite *Arachnogetes*. The sequence of mite *ftz* revealed its homology to the Lox5 gene of annelids, and the expression pattern is that of a typical Hox gene (see Fig. 12) (Telford, 2000). Yet in *Drosophila*, *ftz* is a pair-rule gene, with a striking pattern of seven stripes in alternating segments of the embryo (Carroll and Scott, 1985). In *Tribolium*, *ftz* has a modified pair-rule pattern, with stripes that appear out of the growth zone in alternate segments (Brown et al., 1994). In *Schistocerca*, the gene is expressed strongly in the posterior of the embryo, with additional expression in the nervous system, and some weak expression in the thorax (Dawes et al., 1994). It is unclear whether or not the expression in the region of the posterior growth zone of the grasshopper is related to a role in segment formation.

Two recent studies have explored the biochemical functions of *Schistocerca*, *Tribolium* and *Drosophila* Ftz proteins by misexpressing them in *Drosophila* (Lörh et al., 2001; Alonso et al., 2001). Lörh et al. found that the *Schistocerca* and *Tribolium* Ftz proteins retain some ability to function as a Hox protein when misexpressed, whereas the *Drosophila* protein does not. Expression data suggest that neither *Schistocerca* nor *Tribolium* *ftz* play a Hox-like role in their native context; yet apparently the YPWM-motif and homeodomain present in each gene can confer homeotic phenotypes and affect Hox target genes when misexpressed in the *Drosophila* environment.

These studies have also explored the ability of misexpressed *Schistocerca* and *Tribolium* Ftz proteins to mimic the disrupted-segmentation phenotype of misexpressed *Drosophila* Ftz. *Tribolium* Ftz could partially mimic this effect, while *Schistocerca* Ftz could not. Probably owing to the absence of the LXXLL motif, the *Schistocerca* Ftz protein has only weak interaction with *Drosophila* Ftz-F1, which is a necessary co-factor for the segmentation phenotype in the *Drosophila* environment. Thus, the acquisition of the LXXLL-motif in the insects may have led to an integral role for the Ftz-F1 interaction for the *Drosophila* segmentation process. However, these results do not rule out a role in segmentation for the *ftz* genes of other arthropods by an LXXLL-motif independent mechanism.

In fact, our results suggest that a role for *ftz* in the process of segmentation may have an ancient origin, and may be conserved across the mandibulate arthropods (myriapods, crustaceans and insects). In early centipede embryos, the pattern of expression in the posterior growth zone plus stripes in new segments (not unlike that of *even-skipped*; C. L. H. and T. C. K., unpublished) suggests a role in segment formation. But in later embryos, a clear Hox-like domain in the maxillary II and maxilliped segments emerges. Thus, we suggest that *fushi tarazu* made its evolutionary transition from a Hox-like role to a role in segmentation via an intermediate stage that is retained in the centipede. Based on its combined domains of expression, it would appear that *ftz* may be able to play multiple roles in the same embryo, one of which was lost in the insects (perhaps owing to redundancy with *Scr*) (Telford, 2000). Further studies of *ftz* homologs in the crustaceans and insects should clarify where in arthropod evolution the Hox role was lost.

The results we report suggest that the complex, dynamic expression domains in the centipede reflect multiple roles for the centipede *ftz* gene. The observed expression domains of this gene in the centipede suggest that major transitions in the function of a developmentally important gene may happen gradually via a multifunctional intermediate, and not necessarily only by duplication and divergence of two copies of a gene.

**Further explorations**

Our results, compared with others, suggest a dynamic role for the Hox genes in arthropod evolution. However, many more studies are needed to test the hypotheses presented here. Comparison of the expression patterns of other species, such as a millipede, for example, would be informative. Ultimately, we would like to bring functional techniques to bear on these questions. Currently, comparisons of development between different arthropods relies heavily on correlating expression pattern with inferred and presumed function, but expansion of knockout and misexpression techniques to more species will allow us to test our models of evolution directly.

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