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Exploring the myriapod body plan: expression patterns of the ten Hox genes in a centipede

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SUMMARY

The diversity of the arthropod body plan has long been a fascinating subject of study. A flurry of recent research has analyzed Hox gene expression in various arthropod groups, with hopes of gaining insight into the mechanisms that underlie their evolution. 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*

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 proteins, 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 are now known to be crucial players in the development of nearly all animals, both protostomes and deuterostomes (Manak and Scott, 1994). Furthermore, because the Hox genes coordinate a large suite of downstream targets that work together to create segmental identity, a shift in the expression pattern of a Hox gene can cause major morphological change without necessarily being disastrous to the animal. Thus, changes in Hox gene expression may provide a mechanism of relatively rapid macroevolutionary change.

Among the arthropods, the expression patterns of the Hox genes have been characterized in chelicerates, crustaceans and insects, with interesting implications for the evolution of the unique morphologies of those groups (see Fig. 10). Although

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; Boore et al., 1998; 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 Bortoletto, 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 pauropods, 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.

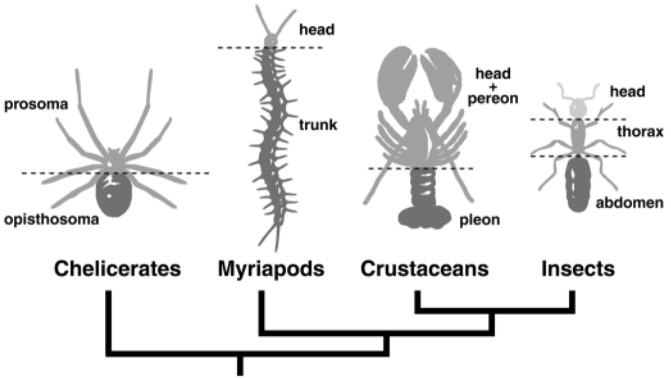

Fig. 1. Arthropod body plans and phylogeny. The four major groups of extant arthropods are illustrated here, with a tree based on several recent molecular phylogenies that group the insects with the crustacea (Giribet et al., 2001; Hwang et al,. 2001; Cook et al., 2001; Boore et al., 1998; Regier and 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.

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.

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*, AF434996; *Ultrabithorax*, AF435005; *abdominal-A*, AF434994; and *Abdominal-B*, AF434995.

Sequences of orthologs from other species used for alignments were retrieved from GenBank. The Accession Numbers are as follows: *Drosophila lab*, X13103; *Tribolium lab*, AF231104; *Porcellio lab*, AF148935; *Lithobius forficatus lab*, AF362084; *Cupiennius lab*, AJ007431; *Drosophila pb*, AAF54089; *Artemia pb*, AF363018; *Lithobius forficatus pb2*, AF362086, *pb1*, AF362085; *Archegozetes pb*, AAC35935; *Drosophila bcd*, P09081; *Drosophila zen*, P09089; *z2*, P09090; *Tribolium zen*, X97819; *zen2*, AF321227; *Schistocerca zen*, X92654; *Pachymerium Hox3*, CAB75744; *Cupiennius Hox3*, CAA06645; *Drosophila Dfd*, X05136; *Tribolium Dfd*, U81038; *Thermobia Dfd*, AF104005; *Artemia Dfd*, X70078; *Pachymerium Dfd*, AJ272191; *Lithobius forficatus Dfd*, AF362087;

Cupiennius Dfd, AJ007432; *Drosophila Scr*, X14475; *Tribolium Scr*, AF227628; *Artemia Scr*, X70080; *Ethmostigmus Scr*, AF010178; *Lithobius forficatus Scr1*, AF362088; *Scr2*, AF362089; *Archegozetes Scr*, AF071407; *Drosophila ftz*, X00854; *Tribolium ftz*, U14732; *Schistocerca ftz*, X73982; *Lithobius forficatus ftz*, AF362090; *Archegozetes ftz*, AF237818; *Drosophila Antp*, M20705; *Schistocerca Antp*, U32943; *Porcellio Antp*, AF241662; *Ethmostigmus Antp*, AF010175; *Lithobius forficatus Antp1*, AF362091; *Antp2*, AF362092; *Cupiennius Antp*, AJ007433; *Drosophila Ubx*, X76210; *Manduca Ubx*, U63300; *Artemia* Ubx, X70081; *Ethmostigmus Ubx*, AF010179; *Lithobius forficatus Ubx*, AF362093; *Cupiennius Ubx1*, AJ007434; *Ubx2*, AJ007435; *Junonia abd-A*, L41931; *Tribolium abd-A*, AF017415; *Artemia abd-A*, X70076; *Ethmostigmus abd-A*, AF010174; *Lithobius forficatus abd-A*, AF362094; *Cupiennius abd-A*, AJ007436; *Drosophila Abd-B*, A34220; *Tribolium Abd-B*, AF227923; *Schistocerca Abd-B*, S33375; *Lithobius forficatus Abd-B*, AF362095; *Cupiennius Abd-B*, AJ131397. Sequences were aligned using the Clustal function of MacVector software.

In situ hybridization

In situ probes were prepared using the Ambion MEGAscript or MAXIscript kits, with digoxigenin-UTP or biotin-UTP, and were mock-digested in carbonate buffer, then precipitated, resuspended and quantified. The optimal concentration of each probe was established empirically, by testing concentrations between about 0.01-1.0 μg/ml.

The centipede in situ hybridization protocol was developed based on multiple protocols, especially that of O'Neill and Bier (O'Neill and Bier, 1994), with some critical added modifications. To make the fixed embryos permeable, it was necessary to start with a 50:50 heptane/ethanol soak for 20 minutes, followed by a 1 hour soak in RIPA detergent mix [150 mM NaCl, 1% NP-40, 0.5% Sodium Deoxycholate (DOC), 0.1% SDS, 1mM EDTA, 50mM Tris-HCl, pH 8.0]. These were followed by proteinase digestion of 7.5 minutes, a post-fixation for 20 minutes, and then hybridization for up to 48 hours at 56°C. After probe was removed, a long soak of 24-36 hours in hybridization buffer at 60°C helped to reduce background. Short washes in a lower-salt buffer [2×saline sodium citrate (SSC), 50% formamide, 0.1% Tween] also helped to reduce background. Anti-digoxigenen and anti-biotin antibodies conjugated to alkaline phosphatase were used (Roche), with overnight incubations at 4°C. The purplish-blue stain is the result of an NBT + BCIP color reaction. (Interested readers are encouraged to contact the authors for a full, detailed in situ protocol.)

Microscopy and images

Developmental stages of the centipede embryos were recorded using scanning electron microscopy (Jeol). Results of in situ hybridization were analyzed and photographed through a dissecting microscope (Nikon), using a blue filter (Tiffen 80A) to correct the color balance of the halogen illumination. DAPI-stained embryos and close-ups of in situ stained embryos were photographed on a transmission microscope (Zeiss). Images were prepared using Adobe Photoshop and Illustrator, with some minor image adjustments.

RESULT

Embryology

The extended-germband embryo of *Lithobius atkinsoni* 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 membrane expands to enclose the entire yolk mass. Following this ventral flexure, the appendages elongate and differentiate, and several weeks later the hatchling 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 Scolopendra, as even in early stages of embryogenesis a thin layer of cells connects the left and right halves of the germband.

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

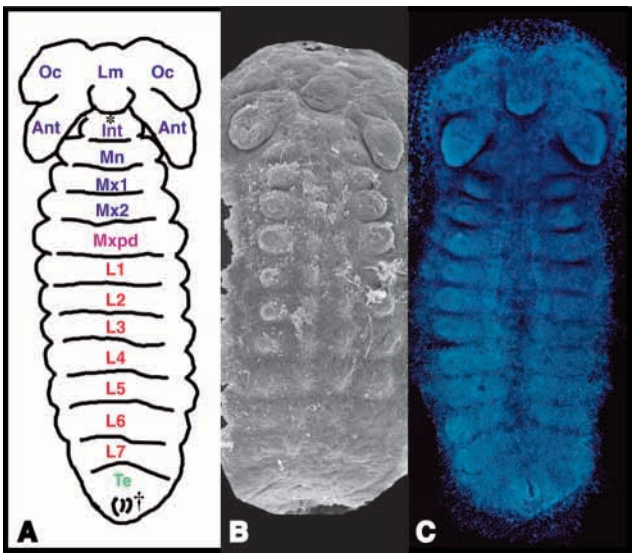

Fig. 2. The centipede extended-germband embryo is illustrated by a schematic diagram (A), a scanning electron micrograph (B) and a DAPI-stained embryo (C). Head segments are labeled in blue lettering: ocular, Oc; antennal, Ant; intercalary, Int; mandibular, Mn; maxillary I, Mx1; and maxillary II, Mx2. The labrum (Lm) probably represents the highly-modified, fused appendages of the intercalary segment (see Haas et al., 2001a; Haas et al., 2001b). The segment that will give rise to the poison fangs, or maxillipeds, is labeled in purple, as it is a trunk segment that has been co-opted into the head (Mxpd). The leg-bearing trunk segments are labeled in red (L1-L7). (The final L8 segment develops later in embryogenesis than is illustrated here.) The telson is labeled in green (Te). The stomadeum lies just behind the labrum (asterisk); the proctodeum lies to the posterior of the germband (dagger).

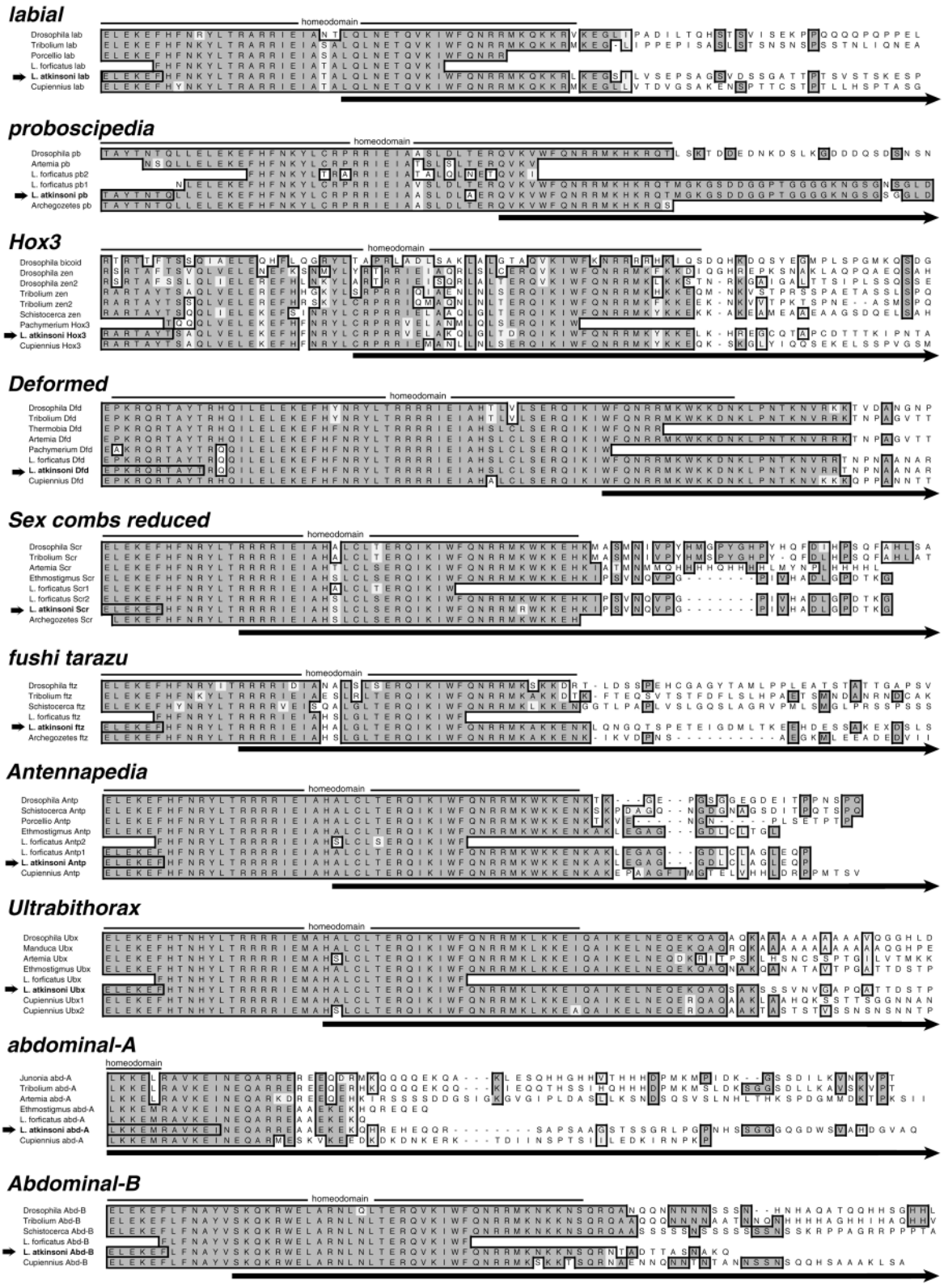

Fig. 3. *Lithobius* Hox gene sequences. The partial sequences of cloned portions of the *Lithobius* Hox genes are aligned with orthologs from a few other arthropod species. Small arrows highlight the centipede sequences (*Lithobius*). Regions of the homeobox within the clones are marked above the sequences. 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.

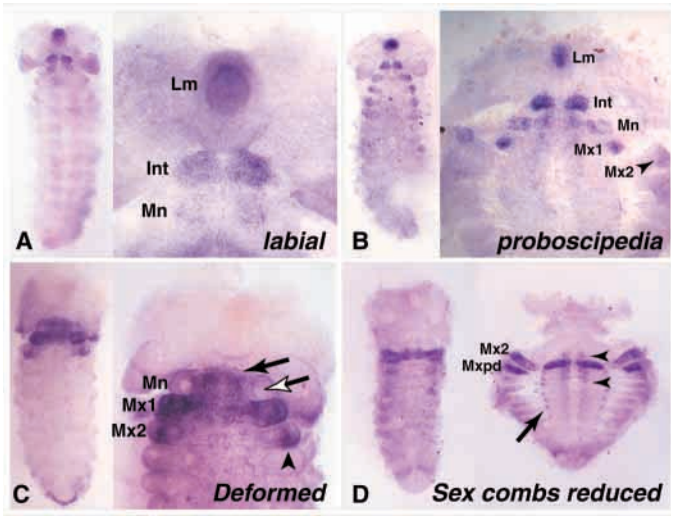

Fig. 4. The head Hox genes. (A) Two embryos stained for *labial* are shown, one full-length (left) and one magnified to show details of the expression pattern (right). Expression of *labial* is strong in the labrum (Lm) and the intercalary (Int), with weaker expression in the mandibular segment (Mn). (B) Expression of *proboscipedia* is shown in a younger (left) and older stage embryo (right). Staining of *pb* is strong in the labrum and intercalary segment, weaker in the mandibular segment and mandibular limb-buds, and strong in the maxillary I and II distal limb-buds (Mx1, Mx2). The maxillary II appendage is much longer than that of maxillary I. The arrowhead points out the expression of *pb* in distal maxillary II. (C) Expression of *Deformed* in two embryos shows expression to be across the mandibular segment, except for spots in the limb-buds (white arrow), in the segment and limb-buds of maxillary I and in a ring around the limb-bud of maxillary II (arrowhead). There is also some expression in the very posterior of the intercalary segment (black arrow). (D) Expression of *Sex combs reduced* is shown in a younger (left) and an older (right) embryo. In both stages, strongest expression is seen in the maxillary II segment and limb-buds, and the limb-buds only of the maxilliped segment (Mxpd). Expression near the ventral midline extends from the maxillary I to the first leg segment (arrowheads). Additional, presumptive neural expression is seen laterally in all the trunk segments (arrow).

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

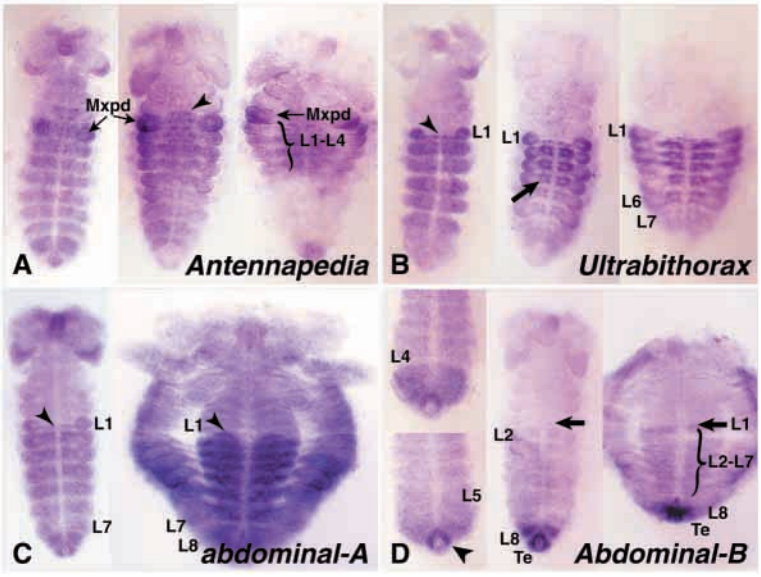

Fig. 5. The trunk Hox genes. (A) Three embryos illustrate expression of *Antennapedia*. Strongest expression in is the maxilliped limb-buds and segment (arrows). Weaker expression extends to the posterior in the youngest embryo (left), but extends only from L1 to L4 in the oldest embryo on the right (bracket). The anterior boundary of expression is in the posterior of the maxillary II segment (arrowhead). (B) Expression of *Ultrabithorax* is shown in three embryos. From the youngest stage shown here (left) to the oldest, expression begins in the limb-buds and the posterior region (arrowhead) of the first leg segment (L1), and extends through most of the trunk. In the later stage (right), expression is absent from the last few segments of the posterior. From late extended germband stage (middle) on, expression in the trunk segments takes the form of a rosette of patches of presumptive neural tissue (arrow). (C) An early- (left) and late-stage embryo (right) show expression of *abdominal-A*, which is similar to that of *Ubx*. Expression extends from the limb-buds of L1, with a ventral boundary in the posterior of the segment (arrowheads), and extends all the way along the trunk. Expression of *abd-A* does not fade from the posterior in older embryos. (D) Embryos of four stages show expression of *Abdominal-B*. In early embryos, expression comes on in the posterior, even in cells still in the growth zone (top left), with especially strong expression circumferential to the proctodeum (bottom left; arrowhead). In extended-germband embryos, expression is strongest in the last few segments (middle), fading from L8 in the oldest embryos and then limited to the telson (right; Te). Another, weaker domain of expression is seen in segments from extended-germband stage through older embryos (middle and right). This domain extends from the posterior of the L1 segment (arrow) on backwards through segments L2-L7 of the trunk (bracket).

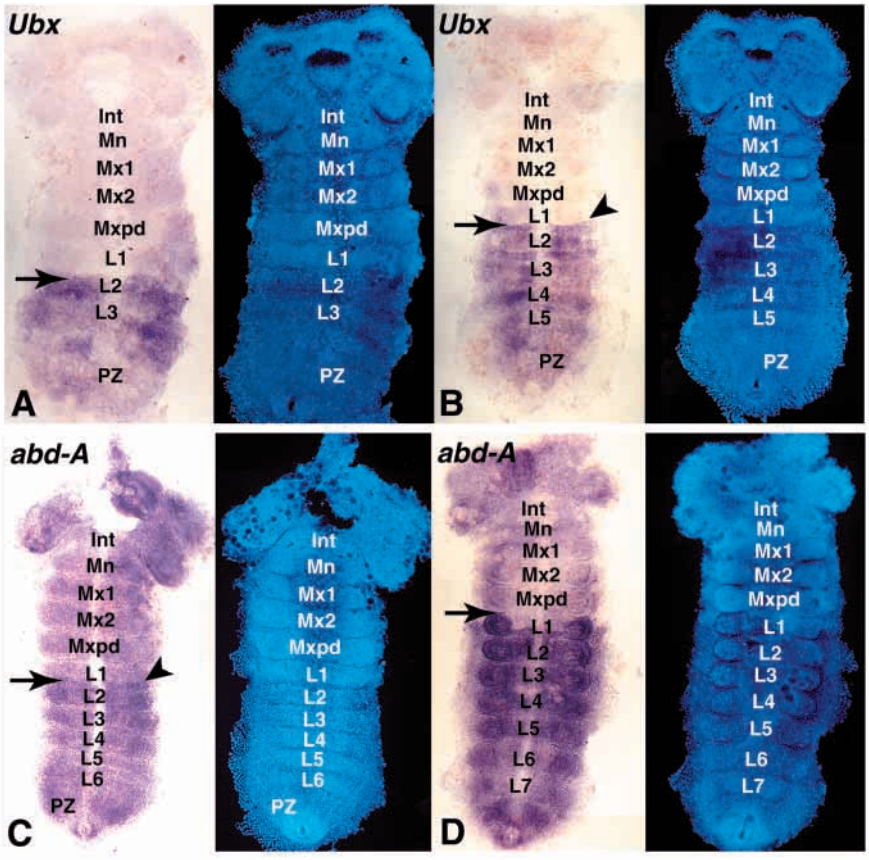

Fig. 6. *Ubx* and *abd-A* in early embryos. The same embryos are shown with *Ubx* or *abd-A* in situ hybridization staining (left) and with DAPI-staining (right) to facilitate identification of segments (labeled). (A) *Ubx* expression in a very young embryo, which has just formed the L3 segment. Expression is visible in the extreme posterior of the L1 segment (arrow), in the L2 and L3 segments, and further back in unsegmented tissue of the proliferation zone. Punctate expression is due to staining of nascent transcripts. (B) *Ubx* expression in an embryo that has formed five pairs of walking legs. The lateral expression is beginning to extend more anteriorly (arrowhead). (C) Expression of *abd-A* in an embryo that has just formed the L6 segment. The anterior boundary is at the posterior of L1 (arrow), even at the limb-bud (arrowhead). (D) Expression of *abd-A* in an extended-germband embryo. Now the expression domain extends into the L1 limb-buds (arrow). Abbreviations: Int, intercalary; Mn, mandibular; Mx1, maxillary I; Mx2, maxillary II; Mxpd, maxilliped; L1, first leg (etc.); PZ, proliferation zone.

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.

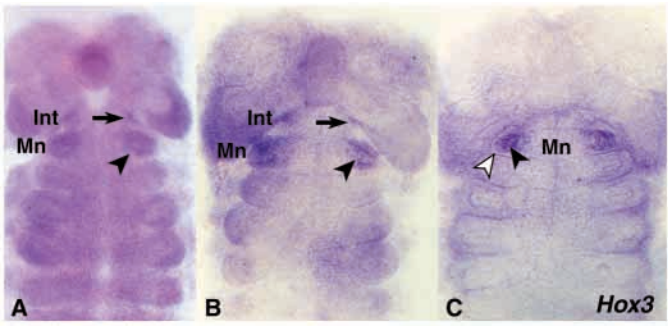

Fig. 7. Expression of *Hox3*. Three embryos illustrate sequential stages of Hox3 expression. In young embryos (A,B), expression is strong throughout the mandibular limb-buds (arrowheads), with small patches of expression in part of the intercalary segment (arrows). (Staining of the antennae in A is background accumulation.) In an older embryo (C), the intercalary expression is gone, and mandibular expression is seen only in the limb-bud mesoderm (black arrowhead), and is absent from the ectodermal layer (white arrowhead).

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

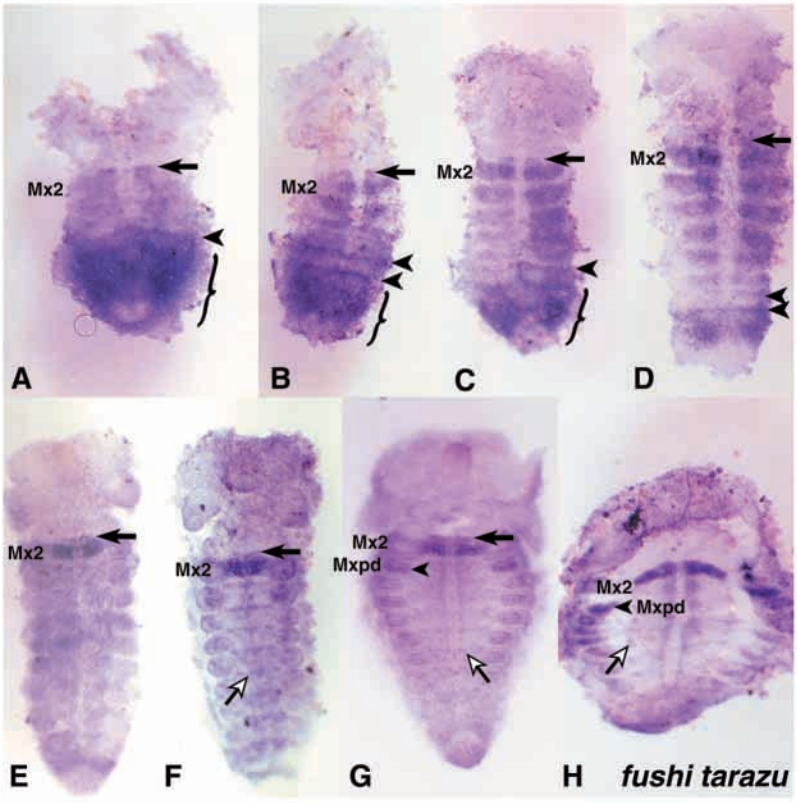

Fig. 8. Expression of *fushi tarazu*. Embryos of eight successive stages are shown to illustrate the dynamic changes in *fushi tarazu* expression during development. In the younger embryos (A-D), one can see strong expression in the proliferation zone (brackets), with stripes forming in the newest segments (arrowheads). The older segments have broad expression across them, from the posterior of the maxillary I segment (arrows), across maxillary II (Mx2), on back to the posterior. In older embryos (E-H), expression has faded from the proliferation zone and from across the trunk segments, leaving the strong expression in the maxillary I and II segments (arrows; Mx2), and a presumptive neural pattern in each trunk segment (white arrows). In the oldest embryos (G,H), expression has intensified in the limb-buds of the maxilliped (arrowhead), while strong expression is maintained in the maxillary II segment (Mx2).

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

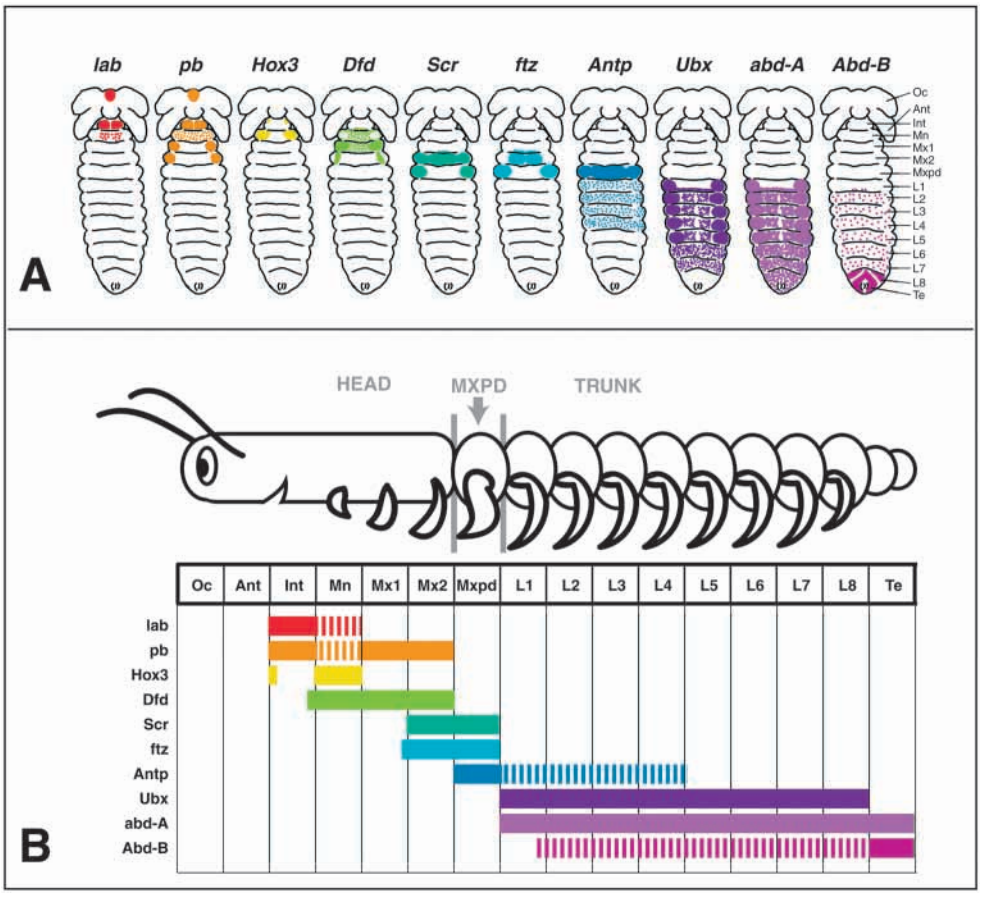

Fig. 9. Summary of centipede Hox expression. (A) The expression patterns of the ten centipede Hox genes are illustrated in cartoon form for an extended-germband embryo. Note that only the expression domains presumably corresponding to a segment identity function are illustrated here (e.g. for *ftz*). (B) The same expression data is shown diagramatically, for comparison of domain boundaries with each other and with tagmata and appendages of the centipede (shown for a newly-hatched larva, with seven full-size legs and an eighth not yet full length). Striped patterns indicate weaker expression.

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).

We found that for both *Ubx* and *abd-A*, expression in early embryos is restricted slightly more towards the posterior than in older embryos. For both genes, the initial expression domain has its anterior boundary in the second leg segment (L2 in Fig. 6A-C). As the embryos age, expression becomes apparent in the posterior of the first leg segment, and eventually expression is seen in the limb-buds of the first leg segment (Fig. 6D). At none of the stages examined, from embryos with newly formed L3 segments to embryos past germband flexure, did we see expression in the maxilliped segment or limb-buds [contrary to a previous report in a similar centipede (Akam, 2000)]. Interestingly, in newly formed segments, accumulation of *Ubx* transcripts is low in the cytoplasm, but two distinct spots of

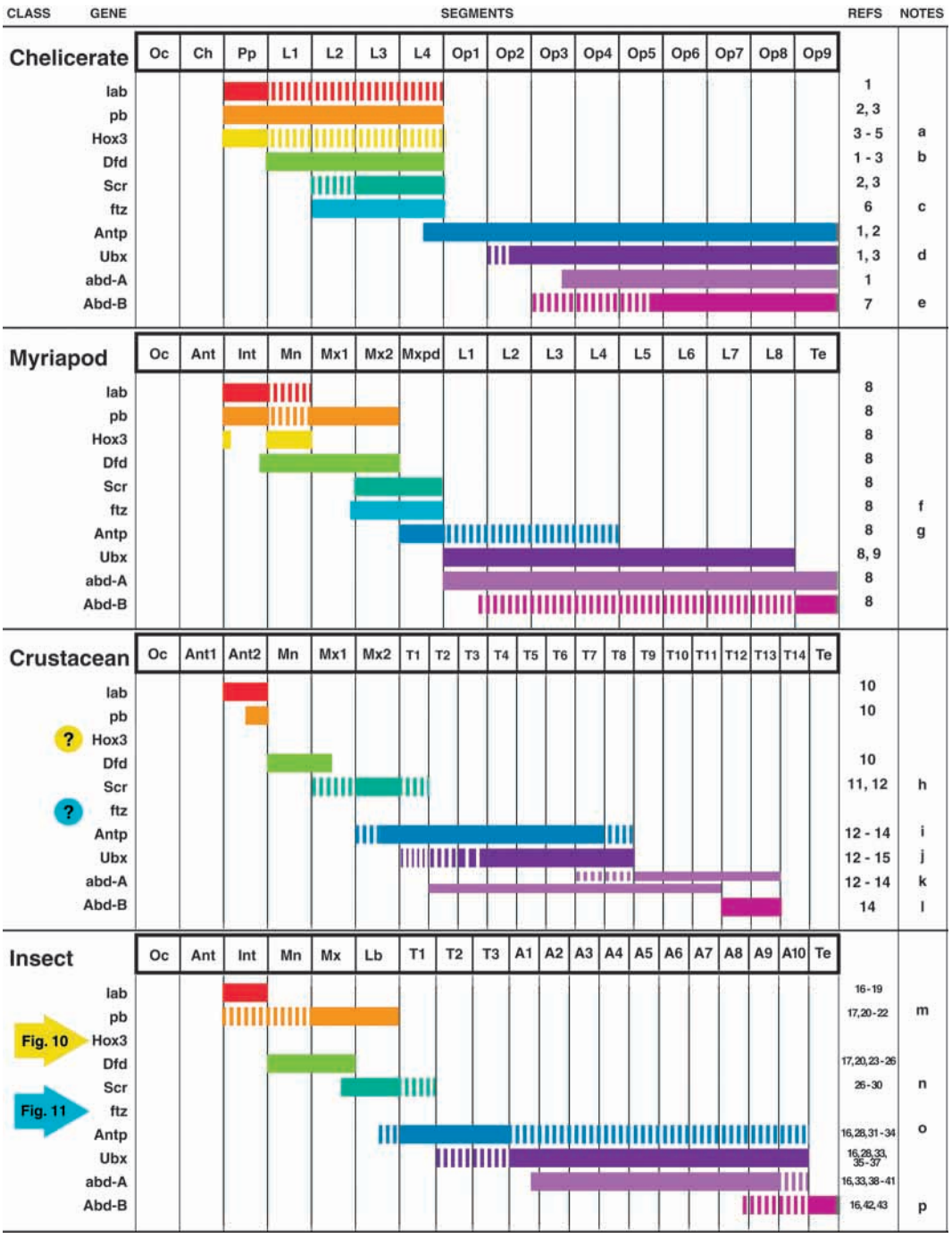

Fig. 10. Shifting Hox domains across the arthropods. The expression domains of Hox genes from studies of various arthropods are illustrated here in simplified fashion for ease of comparison. Solid bars indicate strong expression, while striped bars indicate weak or transient expression. As this diagram 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 *Hox3*

The *Hox3* gene is expressed in the centipede in a pattern limited to the intercalary and mandibular segments (Fig. 7). Throughout embryogenesis there is strong expression in the mandibular limb-buds, with no expression within the segment. In early embryos, this domain fills the developing limb-buds (Fig. 7A), but in older embryos this domain is restricted to the interior mesodermal layer of the mandibles, with no expression in the overlying ectoderm (Fig. 7C). In addition, young embryos show expression in two small lateral patches in the anterior of the intercalary segment, just under the antennae (Fig. 7A,B). This expression is absent from older embryos (Fig. 7C).

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 maxilliped segment. 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

(1) Damen et al., 1998; (2) Telford and Thomas, 1998a; (3) Abzhanov et al., 1999; (4) Telford and Thomas, 1998b; (5) Damen and Tautz, 1998; (6) Telford, 2000; (7) Damen and Tautz, 1999; (8) this work; (9) Grenier et al., 1997; (10) Abzhanov and Kaufman, 1999a; (11) Abzhanov and Kaufman, 1999b; (12) Abzhanov and Kaufman, 2000b; (13) Abzhanov and Kaufman, 2000a; (14) Averof and Akam, 1995; (15) Averof and Patel, 1997; (16) Peterson et al., 1999; (17) Rogers and Kaufman, 1997; (18) Nie et al., 2001; (19) Diederich et al., 1989; (20) Rogers et al., 2002; (21) Shippy et al., 2000; (22) Pultz et al., 1988; (23) Fleig et al., 1992; (24) Brown et al., 1999; (25) Chadwick and McGinnis, 1987; (26) Kokubo et al., 1997; (27) Rogers et al., 1997; (28) Walldorf et al., 2000; (29) Curtis et al., 2001; (30) Martinez-Arias et al., 1987; (31) Wirz et al., 1986; (32) Hayward et al., 1995; (33) Zheng, 1999; (34) Nagata, 1996; (35) Kelsh et al., 1994; (36) Bennett et al., 1999; (37) White and Wilcox, 1985; (38) Tear et al., 1990; (39) Shippy et al., 1998; (40) Nagy et al., 1991; (41) Macias et al., 1990; (42) Kelsh et al., 1993; (43) Delorenzi and Bienz, 1990.

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-1* 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 maxilliped segments (Ref. 15); in *Artemia* expression extends to the end of the thorax (T11) (Ref. 14).

[k]The top bar indicates expression of *abdA* in *Porcellio* and *Procambarus* (although *Porcellio* lack the extension of expression into T7 and T8); the bottom bar indicates the 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).

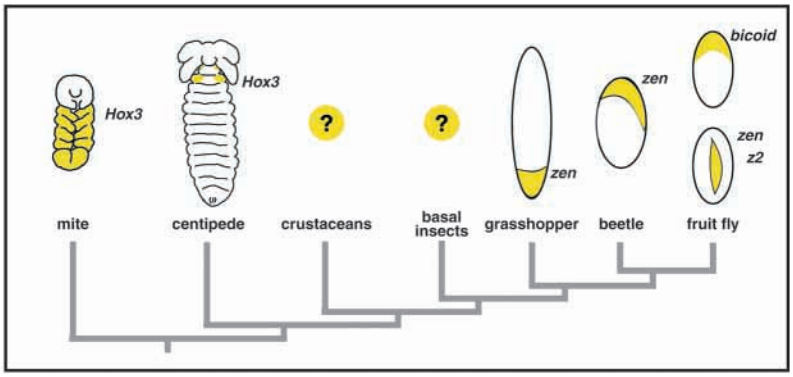

Fig. 11. Evolution of *Hox3* expression and function. The expression domains of *Hox3* homologs from work in other species are illustrated here in cartoon-form for comparison to that of *Lithobius*. In the mite, expression of *Hox3* is in a typical Hox-like segmental domain, extending from the pedipalps into the opisthosoma (Telford and Thomas, 1998b). A similar Hox-like pattern is seen in spiders as well (Damen and Tautz, 1998; Abzhanov et al., 1999). *Lithobius* expression is also Hox-like, but is limited to the mandibular segment, plus a small anterolateral region of the intercalary segment. As indicated by the question marks, the expression of *Hox3* has not yet been analyzed in a crustacean or a basal insect. Within the insects, the *Hox3* ortholog *zen* is expressed in the extra-embryonic tissues of the grasshopper *Schistocerca*, the beetle *Tribolium* and the fruit fly *Drosophila* (Falciani et al., 1996; Rushlow and Levine, 1990). The extra-embryonic tissue is located primarily at the posterior pole of the *Schistocerca* egg, at the anterior and dorsal edge of the *Tribolium* egg, and along the dorsal surface of the *Drosophila* egg. There is also a duplicate copy of the *zen* gene in *Drosophila* called *z2*, which has a very similar expression pattern (Rushlow and Levine, 1990). In *Drosophila*, the *Hox3* ortholog *bicoid* is maternally loaded into the anterior of the egg (Frohnhöfer and Nüsslein-Volhard, 1986). Thus, three separate functions are illustrated for homologs of Hox3 in the arthropods: a Hox-like segmental identity function (*Hox3* in the mite and centipede), a function in extra-embryonic tissues (*zen* in the insects) and a function in early anteroposterior polarity (*bicoid* in *Drosophila*).

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*, *pb*, *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

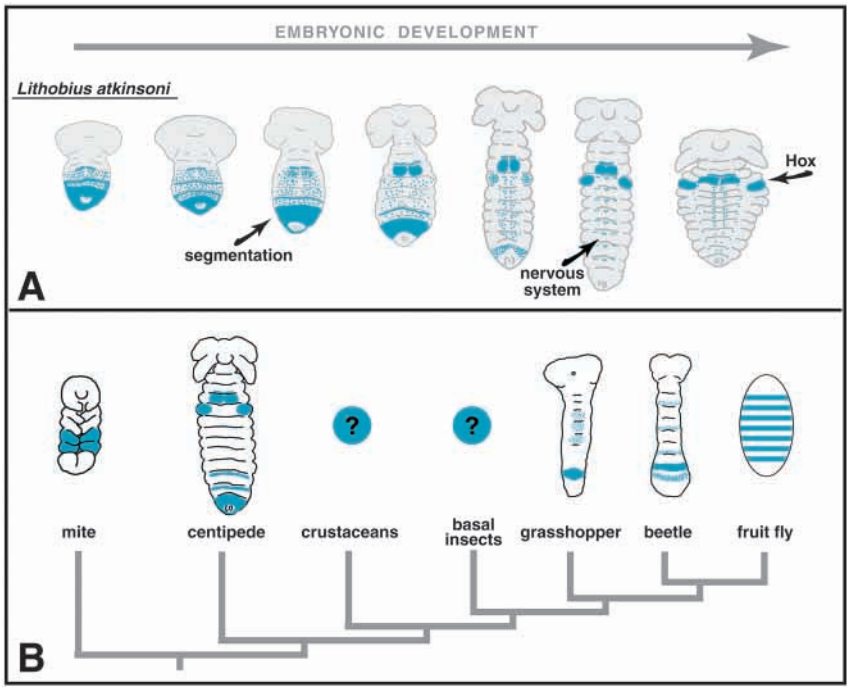

Fig. 12. Temporal dynamics of *ftz* in the centipede, and evolution of *ftz* expression. (A) The changing expression patterns of *ftz* during *Lithobius* embryonic development are shown in cartoon form. Three major expression domains are seen: expression in the posterior, probably related to segmentation; expression in the developing nervous system; and a Hox-like expression domain in the maxillary II segment and maxillipeds. (B) The expression of *ftz* homologs from work in arthropod species are illustrated for comparison to *Lithobius*. In the mite, expression of *ftz* extends from the second to the fourth leg, in a typical Hox-like segmental domain (expression in the fourth limb-bud, and earlier expression in the opisthosoma are not illustrated here) (Telford, 2000). In *Lithobius*, both a Hox-like and a segmentation-related expression domain are seen (representations of the two expression patterns are combined onto an extended germband embryo for simplicity). In the grasshopper *Schistocerca*, expression is mostly in the posterior region of the growth zone, but without stripes. There are also patches of expression in the three thoracic segments, in addition to strong nervous system expression that is not illustrated here (Dawes et al., 1994). In the beetle *Tribolium*, expression of *ftz* has a pair-rule pattern, with stripes appearing out of the growth zone in alternate segments, and fading out in the anterior (Brown et al., 1994). In the fruit fly *Drosophila*, seven pair-rule stripes of *ftz* expression appear in alternating segments synchronously along the germband (Carroll and Scott, 1985).

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 *z2* – reflects a remarkably versatile repertoire of functions (see Fig. 11). The gene *bicoid* encodes an anterior-specifying morphogen deposited maternally into the *Drosophila* egg (Frohnhöfer and Nüsslein-Volhard, 1986). The gene *zerknüllt* (*zen*) plays a role in the specification of *Drosophila* extra-embryonic tissues, whereas *z2*, 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 germband, 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 *Archegozetes*. 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* (Löhr et al., 2001; Alonso et al., 2001). Löhr 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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