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SHORT COMMUNICATION

Evolution of *Hox3* and *ftz* in arthropods: insights from the crustacean *Daphnia pulex*

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Abstract The *Drosophila melanogaster* genes *zerknüllt* (*zen*) and *fushi tarazu* (*ftz*) are members of the *Hox* gene family whose roles have changed significantly in the insect lineage and thus provide an opportunity to study the mechanisms underlying the functional evolution of Hox proteins. We have studied the expression of orthologs of *zen* (*DpuHox3*) and *ftz* (*Dpuftz*) in the crustacean *Daphnia pulex* (Branchiopoda), both of which show a dynamic expression pattern. *DpuHox3* is expressed in a complex pattern in early embryogenesis, with the most anterior boundary of expression lying at the anterior limit of the second antennal segment as well as a ring of expression around the embryo. In later embryos, *DpuHox3* expression is restricted to the mesoderm of mandibular limb buds. *Dpuftz* is first expressed in a ring around the embryo following the posterior limit of the mandibular segment. Later, *Dpuftz* is restricted to the posterior part of the mandibular segment. This is the first report of expression of a *Hox3* ortholog in a crustacean, and together with *Dpuftz* data, the results presented here show that *Hox3* and *ftz* have retained a Hox-like expression pattern in crustaceans. This is in accordance with the proposed model of *Hox3* and *ftz* evolution in arthropods and allows a more precise pinpointing of the loss of *ftz* "Hox-like behaviour": in the lineage between the Branchiopoda and the basal insect Thysanura.

Keywords *Hox3* · Zen · *ftz* · Arthropoda · *Daphnia pulex*

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Introduction

The Hox complex was originally characterised in *Drosophila melanogaster* (Lewis 1978; Kaufman et al. 1990). It contains eight genes with roles in anteroposterior (AP) patterning, which have all been shown to be related and to contain a homeobox motif. The molecular characterisation of the Hox complex revealed further genes containing the homeobox motif, which yet lack Hox-like expression or a homeotic function. These genes include *bicoid* (*bcd*), *zerknüllt* (*zen*; and the closely related *zen2* [*z2*]) and *fushi tarazu* (*ftz*). Comparisons of their amino acid sequences with those of Hox proteins from other animals have shown that *bcd*, *zen* and *z2* are closely related to each other (coming from duplications in the dipteran lineage) but are all orthologous to the *Hox3* genes of non-insect animals (Falciani et al. 1996) and that *ftz* is most likely an ortholog of the protostomian *Lox5* gene (Telford 2000). Importantly, both *Hox3* and *ftz*/*Lox5* genes from non-insects arthropods have the restricted AP expression pattern typical of *Hox* genes suggesting the homeotic function has been conserved (Abzhanov et al. 1999; Damen and Tautz 1998; Telford and Thomas 1998; Telford 2000; Hughes and Kaufman 2002a; Damen et al. 2005; Janssen and Damen 2006).

In insects, the *Hox3* ortholog shows a complex history of gene duplication as well as changes of function (see Panfilio et al. 2006), but the main step in its evolution of a novel function seems to involve a transition from a Hox-like role in AP patterning with discrete anterior and posterior boundaries to an involvement in patterning the extra-embryonic tissues (Hughes et al. 2004). The timing of this change of function has not been precisely determined as no *Hox3* expression data are yet available from a crustacean.

In *Drosophila melanogaster*, *ftz* has two functions: an early role in segmentation as a pair-rule gene (expressed in a series of seven stripes) and a later involvement in the specification of certain neurons (Wakimoto et al. 1984; Doe et al. 1998). The absence of homeotic function has been correlated with the loss of the hexapeptide motif (YPWM) upstream of the homeodomain (which is involved in the interaction with the cofactor Extradenticle, important for the homeotic function of *Hox* genes) and the acquisition of a LXXLL motif in the insect lineage, which is involved in the pair-rule function (Löhr and Pick 2005). It is unclear to what extent the pair-rule function and/or the role in segmentation are conserved within the insects, although the neurogenic expression is consistently observed (Stuart et al. 1991; Brown et al. 1994; Dawes et al. 1994; Hughes et al. 2004). In myriapods (millipedes and centipedes) and chelicerates (spider), *ftz* displays both a neurogenic and a Hox-like pattern of expression (respectively, Janssen and Damen 2006; Hughes and Kaufman 2002a; Damen et al. 2005). Although the Hox-like expression is observed, it is not known if *ftz* is expressed in the central nervous system (CNS) of the mite *Archegozetes* (Chelicerata, Telford 2000). Additionally, involvement in segmentation has been suggested in the case of *Lithobius* (Myriapoda, centipedes, Hughes and Kaufman 2002a). *ftz* has been studied so far in only one crustacean, the morphologically derived barnacle *Sacculina carcini* (cirripede), in which it is apparently restricted to the CNS (Mouchel-vielh et al. 2002). The current view, then, is that the homeotic function of *ftz* has been lost at an unknown stage in the lineage leading to the insects, probably associated with the gain and loss of some important cofactor interaction motifs (Alonso et al. 2001; Löhr et al. 2001; Hughes et al. 2004; Löhr and Pick 2005).

One important challenge for evolutionary biologists is to discover the genetic changes resulting in novel gene functions and to understand how a gene with an important role in development can lose that role without disastrously disrupting embryogenesis. *Hox3* and *ftz* are members of the *Hox* gene family, which have drastically changed their role in arthropods and thus provide an opportunity to study the mechanisms underlying the functional evolution of Hox proteins.

We have studied the expression of *Hox3* and *ftz* in the crustacean *Daphnia pulex* (Branchiopoda). This model organism presents several advantages being easy to breed, producing a fairly large number of eggs and having an almost completely sequenced genome.

In the following discussion, we use our expression data to inform our model of *Hox3* and *ftz* evolution, but it is important to remember that the model would benefit from additional functional data.

Materials and methods

Husbandry

Daphnia pulex specimens were kindly provided by Dr John Colbourne of the Daphnia Genomics Consortium (http://daphnia.cgb.indiana.edu/). Animals are kept in a mixture of tap water and distilled water and fed with the algae *Scenedesmus acutus*, bought from the University of Toronto Transcript Center http://www.botany.utoronto.ca/utcc/.

Gene isolation and WMISH

The first draft of the *Daphnia pulex* genome (provided by DoE Joint Genome Institute & the Daphnia Genomics Consortium, http://daphnia.cgb.indiana.edu/) was used as a target for TBLASTX using arthropod Hox proteins as query sequences to retrieve orthologs of *Hox3* and *ftz*. Probes for Whole Mount In Situ Hybridisation (WMISH) were amplified using polymerase chain reaction (PCR) primers upstream of the homeobox to avoid non-specific signal because of homeobox sequence conservation. Primers used for *Hox3* were ACTGCTCGTCTGTTGATGTCGG (forward) and CGTTTGCTGGTTGTTCAGG (reverse) and for *ftz* GCTTCCAGCTGTACCTCACC (forward) and GTAGGCTTGCATCCAGCTTC (reverse). Amplified complementary DNAs were cloned into the pGEM-T easy vector (Promega). Accession numbers: EF363483 (*DpuHox3*), EF363484 (*Dpuftz*).

The protocol used for WMISH was as described by Shiga et al. (2002), with the following modifications: vitelline membranes were removed by peeling, no sonication step and antibody washes were overnight at 4°C. DIG-labelled probes were synthesised using the Roche DIG-labelling KIT. At the end of the WMISH, embryonic nuclei were fluorescently labelled using 4′-6-diamidino-2-phenylindole (DAPI).

Embryos were recorded using the ZEISS Imager M1 microscope equipped with a ZEISS AxioCam HRc and the program AxiVision 4.3. For some embryos (Fig. 2a–d) series of pictures at different focal planes were taken and merged using the stacks/Z-project option of ImageJ (Rasband, W.S., ImageJ, US National Institutes of Health, Bethesda, MD, http://rsb.info.nih.gov/ij/, 1997–2006) so that the entire expression pattern could be seen on a single picture.

Embryos were staged according to the study of late *Daphnia* embryogenesis by Kotov and Boikova (2001). Fifty hours after the beginning of development, the juvenile, which is essentially a miniature adult, is released from the mother's brood pouch.

Results and discussion

Expression of *DpuHox3*

We found only one copy of the Hox complex in *Daphnia*, and the *Hox3* sequence was PCR amplified and cloned as described. Comparison of the homeodomain sequence with those from other species demonstrates the high conservation typical of most Hox proteins (Fig. 1). The hexapeptide YPWM is present upstream of the homeodomain of *DpuHox3* (data not shown), whereas the various zen-specific motifs proposed in Falciani et al. (1996) and Stauber et al. (2002) could not be found.

WMISH with the *Daphnia pulex Hox3* gene (*DpuHox3*) revealed a dynamic expression profile (Fig. 2). In early embryos (12 h, Fig. 2a–c), only the second antennal grooves are visible. The first antennal grooves are very small, and the other appendages have not yet started to form, making it difficult to determine in which segments the gene is expressed. These embryos show a complex pattern of expression, with three symmetrical regions of expression, a dorsal band and an additional site of expression on the future location of the posterior furrow (Fig. 2a–c). At 14 h, when the mandibles enlarge, only the middle pair of cell groups still noticeably express *DpuHox3* (Fig. 2d). The other pairs of expression have nearly completely faded.

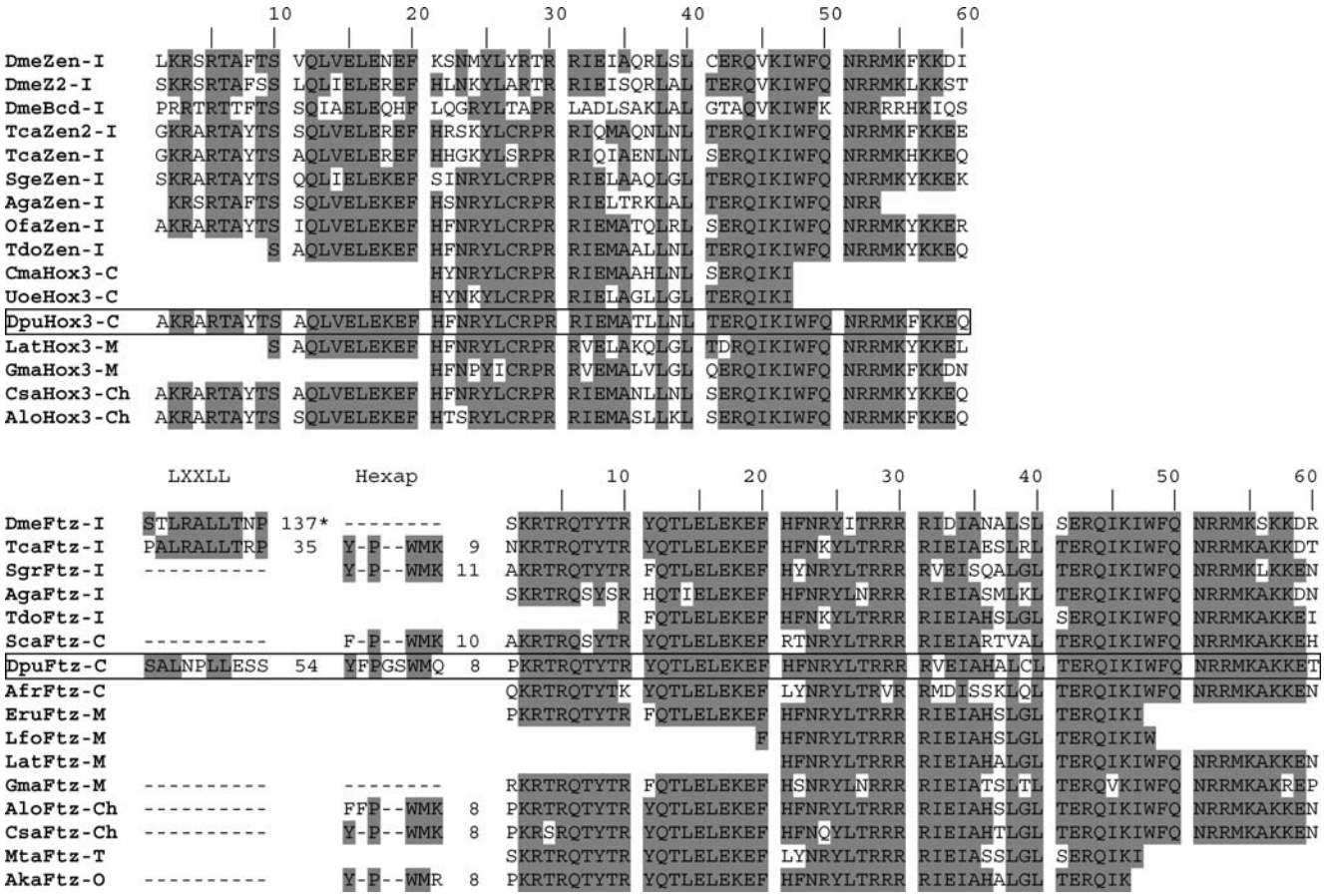


Fig. 1 *Daphnia pulex* Hox3 (*upper panel*) and Ftz (*lower panel*) homeodomain amino acid sequences compared with orthologs from other arthropods. Conserved residues are *shaded* and unknown residues are left *blank*. In the *lower panel*, the presence or absence (*dashes*) of the two cofactor-interactions motifs *LXXLL* and the hexapeptide (*Hexap*) are shown (see the text for discussion). *Numbers* indicate the length of the sequences separating the domains from each other and the hexapeptide from the homeodomain. The *Daphnia* homeodomain sequences were obtained from the genome data available on https://daphnia.cgb.indiana.edu. Abbreviations: *Hexap* Hexapeptide, *C* Crustacea, *Ch* Chelicerata, *I* Insecta, *M* Myriapoda, *O* Onychophora, *T* Tardigrada. Species used: *Aga Anopheles gambiae*, *Aka Acanthokara kaputensis*, *Alo Archegozetes longisetosus*, *Ate Achaearanea tepidorium*, *Cma Carcinus maenas*, *Csa Cupiennius salei*, *Dme Drosophila melanogaster*, *Dpu Daphnia pulex*, *Gma Glomeris marginata*, *Lat Lithobius atkinsoni*, *Mta Milnesium tardigradum*, *Ofa Oncopeltus fasciatus*, *Sge Schistocerca gregaria*, *Tca Tribolium castaneum*, *Tdo Thermobia domestica*, *Uoe Ulophysema oeresundense*. *Asterisk* in DmeFtz, the length corresponds to the number of positions between the LXXLL motif and the homeodomain as there is no hexapeptide

Around 15 h after the beginning of development (Fig. 2e), the pattern of *DpuHox3* expression includes a domain strictly restricted to the mouthparts, in the mandible buds, but not in the ventral ectoderm of the corresponding segment. This expression in the mandibles is conserved until at least 20 h of development (Fig. 2e–h).

As the peripheral layer of the mandible buds is free of transcripts (arrowheads in Fig. 2e′, f, f′, g and g′), it seems that *DpuHox3* is only expressed in the mesodermal layer of the limb. This is confirmed by series of pictures taken in parallel both with transmitted light and DAPI fluorescence; when the focal plane is on the external part of the embryo, the cells expressing *Hox3* are not in focus (Fig. 2h, h′), but when the focal plane is deeper within the embryo, external features such as appendage buds become blurry, while the cells expressing *DpuHox3* come into focus (Fig. 2i, i′, j and j′).

When compared to the *Hox3* expression described in other arthropods, the pattern in the present study raises interesting points.

First, it is noteworthy that the Hox-like *DpuHox3* expression is very restricted along the AP axis, ultimately being present only in the mandibles. As has happened with a number of *Hox* genes, particularly in the anterior region in insects, crustaceans and myriapods, the broad, overlapping pattern of expression seen in chelicerates is reduced and *Hox* expression is more restricted along the AP axis. This shrinking tendency may be linked to the diversification of feeding appendages in Mandibulata, notably in crustaceans and insects (Hughes and Kaufman 2002b).

Second, *Daphnia* is not the only arthropod in which a *Hox3* gene is expressed in a ring (the basal Thysanura insect *Thermobia* and the millipede myriapod *Glomeris*) and dorsally in *Glomeris* (Hughes et al. 2004; Janssen and Damen 2006).

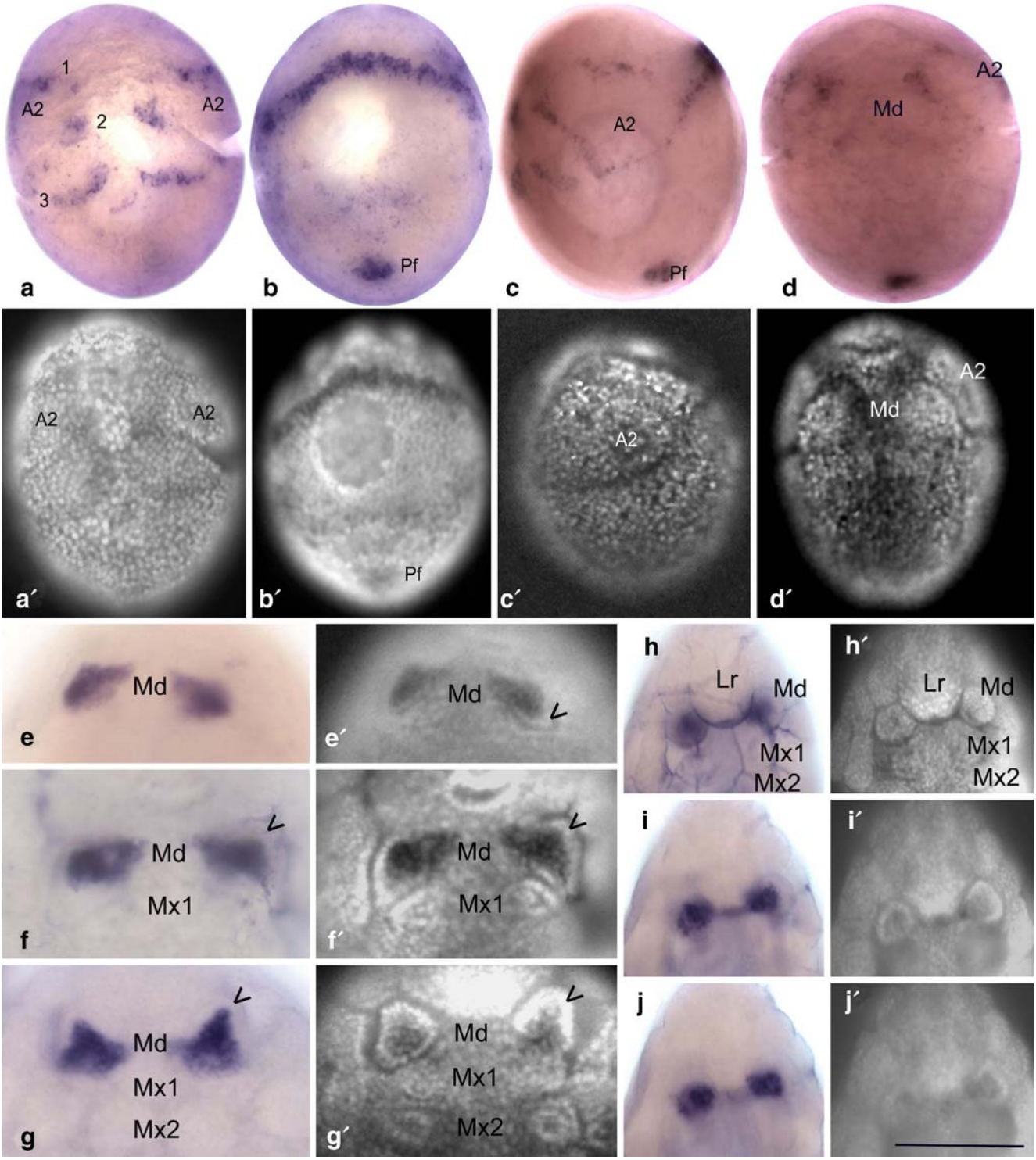
Although the posterior furrow of *Daphnia* and the posterior growth zone of other arthropods cannot be homologised, *Hox3* is similarly expressed in a posterior region in *Daphnia*, *Glomeris* and *Thermobia*. The *Hox3* expression in annelids also shares some of the above features such as a ring-shaped expression (*Chaetopterus*) or at least some dorsal domains (*Nereis* and *Platynereis*), as well as transcripts in the posterior prepygidial zone (*Chaetopterus* and *Nereis*; Irvine and Martindale 2000; Kulakova et al. 2007).

Moreover, the most anterior limit of *DpuHox3* seems to be the anterior boundary of the second antenna, which corresponds to the most anterior limit of *Hox3* in chelicerates (pedipalps) and myriapods (premandibular or intercalary segment) (Abzhanov et al. 1999; Damen and Tautz 1998; Telford and Thomas 1998; Hughes and Kaufman 2002a; Janssen and Damen 2006). This strongly suggests that this anterior boundary is ancestral. The symmetrical patch of expression at the anterior limit of the second antenna in *Daphnia* is also very similar to the early expression in the premandibular segment of *Lithobius* (Hughes and Kaufman 2002a).

Fig. 2 *Hox3* expression in *Daphnia pulex*. *A–C*, 12-h embryo. *A*, ventral view. The first antennal grooves are very small, and the other appendages have not yet started to form, making it difficult to determine in which segments the gene is expressed. Three symmetrical pairs of region expression are observed: the most anterior pair is probably on the anterior part of the second antennae (*1*), the middle pair is more central and probably in the posterio-proximal part of the second antenna at the intersection with the antero-proximal region of the mandibles (*2*), the most posterior region of expression may be at the posterior limit of the future mandibles (*3*). *B*, dorsal view of the same embryo as *A*. There is a stripe of expression at the level of the second antennae and an additional region of expression corresponding to the future posterior furrow (*Pf*). *C*, left-side view of a different 12-h embryo. Ventral is to the left, dorsal to the right. When the dorsal band reaches the antero-distal part of the second antenna, it appears to divide. One band follows the anterior limit of the second antenna (*1* in *A*), while the other one goes around the distal part of the same appendage. This latter band divides again into two, one following the posterior limit of the second antenna (eventually becoming stronger at the end, which corresponds to the group of cells *2* in *A*), and the other one corresponds to pair of stripes *3* in *A*. *D*, ventral view of a 14-h embryo. The mandibles are forming, and only the middle groups of cells are still noticeably expressing *DpuHox3*. The other paired regions of expression observed earlier have nearly completely faded while the dorsal domains of expression have not changed (data not shown). *E–F*, expression can only be detected in the limb buds of the mandibular segment in 15-h (*E*), 16-h (*F*), 17.5-h (*G*) and 20-h (*H*) embryos. Absence of mRNAs in the outer layer of the mandible buds (*arrowhead* in *E′*, *F*, *F′*, *G* and *G′*) suggests that *DpuHox3* is only expressed in the mandibular mesoderm. *H–J*, series of focal planes from a 20-h embryo. Comparisons to the same series with DAPI counterstaining (*H′–J′*) show that the *DpuHox3* mRNAs are not localised in the external layer of the embryo, supporting an expression in the mesoderm of the mandible buds. *A′–G′* DAPI counterstaining of the embryos shown in *A–G*. Abbreviations; *A2* second antenna, *Lr* labrum, *Md* mandibles, *Mx1* first maxilla, *Mx2* second maxilla, *Mxz* maxillary zone, *Pf* posterior furrow, *TS* trunk segments. In all embryos, anterior is up. *Scale* represents 100 μm in *A–D* and *H–J*, 50 μm in *E–G*

The mandibular expression of *Hox3* in *Lithobius*, *Thermobia* and *Daphnia* shows an additional level of similarity, in that transcripts are expressed only in the presumptive mesoderm of the limb bud and not ectodermally as with most *Hox* genes (Hughes and Kaufman 2002a; Hughes et al. 2004). This could represent a shared and derived pattern of expression associated with the origin of the mandibles—the defining character of the mandibulate clade.

Finally, in terms of the evolution of *Hox3* in arthropods, the results presented here are in accordance with the model of evolution proposed by Hughes et al. (2004), with the conservation of the Hox-like expression in most arthropods, and its loss, together with the appearance of the extra-embryonic expression, in the lineage leading to Neoptera after the divergence of Thysanura. The Hox-like expression can be correlated with the presence of the hexapeptide



motif upstream the homeodomain in *Daphnia* and the spider *Cupiennius* (as well as in *Thermobia* [Panfilio and Akam 2007]; it is not known if the hexapeptide is also present in the mite and the myriapods *Glomeris* and *Lithobius*, as the sequences available lack this portion of the protein) and other features that differentiate Hox-like *Hox3* genes from zen-like ones (in higher insects) such as a more C-terminal position of the homeodomain in the protein and a longer protein length (Panfilio and Akam 2007).

Expression of *Dpuftz*

The homeodomain of *Dpuftz* is very similar to those of other arthropods sequences, however, the full length protein

presents two interesting features. First, the hexapeptide is very derived (Fig. 1), even compared to the other available crustacean sequence (*Sacculina*). It is noteworthy that no hexapeptide could be found in *Glomeris* Ftz. In addition, the putative cofactor-interaction motif LXXLL (LNPLL in *Daphnia*) is present upstream of the homeobox at roughly the same position as in the *Drosophila* and *Tribolium* proteins. This is surprising as this motif is involved in the interaction of Ftz with its cofactor Ftz-F1 (which has been shown to be important for the pair-rule function of Ftz in insects) and is not present in *Schistocerca gregaria* (Alonso et al. 2001). Whether this motif has been lost in *Schistocerca* or there have been independent acquisitions in higher insects and Branchiopoda is uncertain. Although the motif lies at the expected position in the protein, and it cannot be found anywhere else in any other arthropod Ftz sequence, it should be stressed that the consensus sequence of this motif (LXXLL) is not very complex, and random substitution could explain its presence in *Daphnia* Ftz protein.

Like *DpuHox3*, *Dpuftz* has a dynamic expression (Fig. 3). Early on (13-h embryos, Fig. 3a–d), the transcripts form a ring around the antero-dorsal part of the embryo, at a similar position than *DpuHox3*. This ring then follows, on the ventral side, the posterior boundary of the mandibular segment (anterior to the future maxillary zone). At 14 h of development (Fig. 3e), *Dpuftz* transcripts accumulate in a restricted part of the earlier pattern, on the ventral midline, between the mandibles and the first maxillae, while the circum-embryonic part of the expression starts to fade, particularly on the ventrolateral parts of the embryo. Comparison with the expression of the segmental marker gene *engrailed* (Fig. 3h) reveals that in later stages (15.5-, 16- and 17-h embryos, Fig. 3f–g, i and j, respectively), *Dpuftz* is restricted to the posterior part of the mandibular segment in the ventral ectoderm. It is not known whether this position corresponds to the first ventral ganglion as the formation of the CNS in *Daphnia pulex* is poorly known, hence, there is no direct evidence of a neurogenic expression for *Dpuftz*.

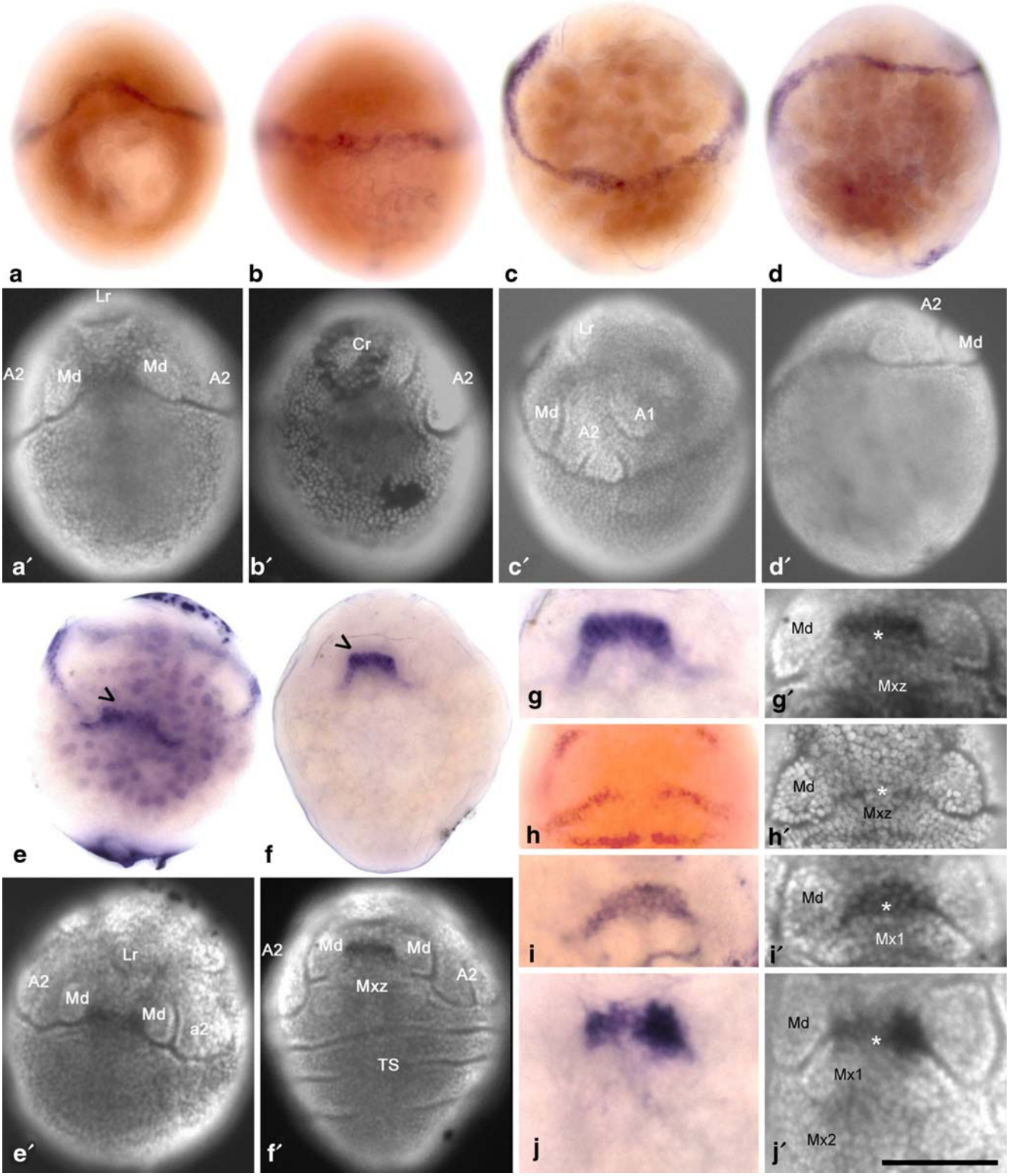
In *Daphnia*, as mentioned above about *DpuHox3*, we found a much more restricted domain of *ftz* expression than is seen in chelicerates (Fig. 3; Telford 2000; Damen et al. 2005). It is noteworthy that the expression of *ftz* in *Daphnia* is completely different to that seen in the crustacean *Sacculina*, where there is no Hox-like pattern, only expression in reiterated symmetrical pairs of spots located in the head and trunk ectoderm, in what are thought to be neural cells (Mouchel-vielh et al. 2002).

As with *DpuHox3*, some possible conserved features have been observed between *Dpuftz* and its ortholog in annelids (*Lox5*) such as a dorsal and ring-shaped expression pattern (Kulakova et al. 2007).

Fig. 3 *ftz* expression in *Daphnia pulex*. *A–D*, 13-h embryos. *A* and *B*, ventral and dorsal sides of the same embryo. *C* and *D*, left and right sides of the same embryo. On the ventral side, the ring-shaped expression follows the posterior boundary of the mandibular segment (anterior to the future maxillary zone) and extends dorsally at the level of the distal limit of the second antenna. On the dorsal side, the band is similar to that observed with *DpuHox3*. *E*, 14-h embryo, ventral side. *Dpuftz* transcripts accumulate in the middle region of the ventral expression (*arrowhead*). The ring around the embryo starts to fade, particularly ventrolaterally. *F*, 15.5-h embryo. Strong expression can be seen only in a central region (*arrowhead*) in the posterior part of the mandibular segment, probably in the ventral ectoderm. *G*, *I*, *J*, close up on the region of *Dpuftz* expression in 15.5-, 16- and 17-h embryos. *H*, expression of *Daphnia pulex engrailed* mRNAs in the same region, showing that *Dpuftz* is expressed in the posterior part of the mandibular segment (*asterisks*). *A′–J′*, DAPI counterstaining of the embryos shown in *A–J*. Abbreviations: *Cr* Carapace. All other abbreviations are the same as in Fig. 2. In all embryos, anterior is up. *Scale* represents 100 μm in *A–F*, 50 μm in *G–J*

The pattern of *Dpuftz* described here has several implications regarding *ftz* evolution in arthropods. First, it seems that the Hox-like expression (and, by extrapolation, the homeotic function) has been retained in *Daphnia*. It is now accepted that the insects derive from a paraphyletic assemblage of crustaceans, and the possible positioning of Branchiopoda as a close sister group to the Hexapoda (insects and relatives; Mallatt and Giribet 2006; Glenner et al. 2006) allows an even more precise pinpointing of this loss of "Hox-like behaviour": probably in the lineage between the Branchiopoda and the basal Thysanura (Hughes et al. 2004). Given the present data, it can be proposed that the situation in *Daphnia* represents an intermediate evolutionary stage of *ftz* loosing its typical Hox function. Although this should be further investigated with functional studies, this conclusion is supported by the derived sequence of the hexapeptide in Dpuftz. The unexpected presence of the LXXLL motif upstream of the homeodomain (Fig. 1), which is thought to be important for the segmentation function, is quite puzzling, as there is no evidence of a role in segmentation for *ftz* in *Daphnia*.

Second, if the expression pattern of *ftz* in the myriapod *Lithobius* is indeed linked to a segmentation function, as claimed by the authors (Hughes and Kaufman 2002a), there are two evolutionary scenarios. If this role is homologous to the putative segmentation function in basal insects (a function different to the pair-rule function, which is specific to higher insects, and probably to Drosophilids), then one would be forced to conclude that this segmentation function has been lost in crustaceans and in millipedes (this study; Mouchel-vielh et al. 2002; Janssen and Damen 2006). Alternatively, the segmentation-like pattern and the proposed segmentation function in myriapods could be convergent on that in insects. We favour this last hypothesis. At this point, it is also interesting to mention first, that it is postulated that the expression pattern observed in *Schistocerca* is not related to a segmentation function (Dawes et al. 1994) and second,



deletion or RNA interference knock down of *Tribolium ftz* fail to cause a segmentation defect (Stuart et al. 1991; Choe et al. 2006); these are the only functional assays of *ftz* outside *Drosophila*.

Our examination of *ftz* and *Hox3* in *Daphnia* reveals characteristics of expression that show differing degrees of phylogenetic distribution and hence conservation. First, an aspect of expression likely peculiar to the water fleas is the continuous stripe of both *DpuHox3* and *Dpuftz* expression around the circumference of early embryos; this is not a general feature of arthropods or even crustaceans and presumably depends on the morphogenetic movements

involved in the formation of the embryonic blastoderm. Second, we find that expression of *Hox3* in the mandibles of all three groups of mandibulate arthropods (insects, crustaceans and myriapods) is ultimately restricted to the mesoderm; a detail that differs from the chelicerates and might give some support to the homology of mandibles across these taxa. Finally, the anteriormost boundary of *DpuHox3* expression corresponds with that seen in myriapods and chelicerates—the second antennal/premandibular/pedipalpal segment. Although our results suggest that these two *Daphnia Hox* genes have retained their ancestral function in AP patterning when compared to the novel roles seen in insects, it is nevertheless clear from these observations that their use as *Hox* genes has been subject to numerous more or less subtle alterations.

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References

Abzhanov A, Popadic A, Kaufman TC (1999) Chelicerate Hox genes and the homology of arthropod segments. Evolut Develop 1:77–89

Alonso CR, Maxton-Kuechenmeister J, Akam M (2001) Evolution of Ftz protein function in insects. Curr Biol 11:1473–1478

Brown SJ, Hilgenfeld RB, Denell RE (1994) The beetle Tribolium castaneum has a fushi tarazu homolog expressed in stripes during segmentation. Proc Natl Acad Sci USA 91:12922–12926

Choe CP, Miller SC, Brown SJ (2006) A pair-rule gene circuit defines segments sequentially in the short-germ insect Tribolium castaneum. Proc Natl Acad Sci USA 103:6560–6564

Damen WG, Tautz D (1998) A Hox class 3 orthologue from the spider Cupiennius salei is expressed in a Hox-gene-like fashion. Dev Genes Evol 208:586–590

Damen WG, Janssen R, Prpic NM (2005) Pair rule gene orthologs in spider segmentation. Evolut Develop 7:618–628

Dawes R, Dawson I, Falciani F, Tear G, Akam M (1994) Dax, a locust Hox gene related to fushi-tarazu but showing no pair-rule expression. Development 120:1561–1572

Doe CQ, Hiromi Y, Gehring WJ, Goodman CS (1998) Expression and function of the segmentation gene fushi tarazu during Drosophila neurogenesis. Science 239:170–175

Falciani F, Hausdorf B, Schroder R, Akam M, Tautz D, Denell R, Brown S (1996) Class 3 Hox genes in insects and the origin of zen. Proc Natl Acad Sci USA 93:8479–8484

Glenner H, Thomsen PF, Hebsgaard MB, Sorensen MV, Willerslev E (2006) Evolution. The origin of insects. Science 314:1883–1884

Hughes CL, Kaufman TC (2002a) Exploring the myriapod body plan: expression patterns of the ten Hox genes in a centipede. Development 129:1225–1238

Hughes CL, Kaufman TC (2002b) Hox genes and the evolution of the arthropod body plan. Evolut Develop 4:459–499

Hughes CL, Liu PZ, Kaufman TC (2004) Expression patterns of the rogue Hox genes Hox3/zen and fushi tarazu in the apterygote insect Thermobia domestica. Evolut Develop 6:393–401

Irvine SQ, Martindale MQ (2000) Expression patterns of anterior Hox genes in the polychaete Chaetopterus: correlation with morphological bounderies. Dev Biol 217:333–351

Janssen R, Damen WG (2006) The ten Hox genes of the millipede Glomeris marginata. Dev Genes Evol 216:451–465

Kaufman TC, Seeger MA, Olsen G (1990) Molecular and genetic organization of the antennapedia gene complex of Drosophila melanogaster. Adv Genet 27:309–336

Kotov AA, Boikova OS (2001) Study of the late embryogenesis of Daphnia (Anomopoda, 'Cladocera', Branchiopoda) and a comparison of development in Anomopoda and Ctenopoda. Hydrobiologia 442:127–143

Kulakova M, Bakalenko N, Novikova E, Cook CE, Eliseeva E, Steinmets PR, Kostyuchenko RP, Dondua A, Arendt D, Akam M, Andreeva T (2007) Hox gene expresion in larval development of the polychaetes Nereis virens and Platynereis dumerilii (Annelida, Lophotrochozoa). Dev Genes Evol 217:39–54

Lewis EB (1978) A gene complex controlling segmentation in Drosophila. Nature 276:565–570

Löhr U, Pick L (2005) Cofactor-interaction motifs and the cooption of a homeotic Hox protein into the segmentation pathway of Drosophila melanogaster. Curr Biol 15:643–649

Löhr U, Yussa M, Pick L (2001) Drosophila fushi tarazu:a gene on the border of homeotic function. Curr Biol 11:1403–1412

Mallatt J, Giribet G (2006) Further use of nearly complete 28S and 18S rRNA genes to classify Ecdysozoa:37 more arthropods and a kinorhynch. Mol Phylogenet Evol 40:772–799

Mouchel-vielh E, Blin M, Rigolot C, Deutsch JS (2002) Expression of a homologue of the fushi tarazu (ftz) gene in a cirripede crustacean. Evolut Develop 4:76–85

Panfilio KA, Akam M (2007) A comparison of Hox3 and Zen protein coding sequences in taxa that span the Hox3/zen divergence. Dev Genes Evol 217 (in press) DOI 10.1007/s00427-007-0133-8

Panfilio KA, Liu PZ, Akam M, Kaufman TC (2006) Oncopeltus fasciatus zen is essential for serosal tissue function in katatrepsis. Dev Biol 292:226–243

Shiga Y, Yasumoto R, Yamagata H, Hayashi S (2002) Evolving role of Antennapedia protein in arthropod limb patterning. Development 129:3555–3561

Stauber M, Prell A, Schmidt-Ott U (2002) A single Hox3 gene with composite bicoid and zerknullt expression characteristics in non-Cyclorrhaphan flies. Proc Natl Acad Sci USA. 99:274–279

Stuart JJ, Brown SJ, Beeman RW, Denell RE (1991) A deficiency of the homeotic complex of the beetle Tribolium. Nature 350:72–74

Telford MJ (2000) Evidence for the derivation of the Drosophila fushi tarazu gene from a Hox gene orthologous to lophotrochozoan. Lox5 Curr Biol 10:349–352

Telford MJ, Thomas RH (1998) Of mites and zen:expression studies in a chelicerate arthropod confirm zen is a divergent Hox gene. Dev Genes Evol 208:591–594

Wakimoto BT, Turner FR, Kaufman TC (1984) Defects in embryogenesis in mutants associated with the antennapedia gene complex of Drosophila melanogaster. Dev Biol 102:147–172