In: Development Gene Expresión Regulation
Editor: Nathan C. Kurzfield
ISBN: 978-60692-794-6

Short Communication

Cloning and Expression Analysis of the Homeobox Gene *Abdominal-A* in the Isopod *Asellus Aquaticus*

Philipp Vick*[4]*, Axel Schweickert and Martin Blum
Institute of Zoology, University of Hohenheim,
Garbenstr. 30, 70593 Stuttgart, Germany

Abstract

Regulation of embryonic axis patterning by Hox genes has been shown to be widely conserved among metazoans. In *Drosophila melanogaster* the Hox gene *abdominal-A* (*abd*-A) is important for the development of the legless abdomen. In contrast to the clear tagmata-correlated activity in insects the analysis of Hox expression patterns during crustacean development has turned out to be more diverse. While in the branchiopod brine shrimp *Artemia franciscana* the posterior genes show a more ancestral overlapping arrangement, this is not the case for the malacostracan isopod *Porcellio scaber*. In this more modern species *abd-A* is mainly restricted to the developing pleon. Here we present the cloning and expression pattern of the *abd-A* gene from the freshwater crustacean *Asellus aquaticus*. In contrast to the related isopod *Porcellio scaber*, *Asellus aquaticus* differs in the regulation of posterior segment patterning. While *Porcellio scaber* displays distinct and separate segments in the pleon, posterior segments are partially fused in *Asellus aquaticus* to yield a pleotelson. The *abd-A* signal was significantly reduced or absent in the pleotelson of *Asellus aquaticus*, while *abd-A* was expressed in the free segments of *Porcellio scaber*. An additional correlation between *abd-A* gene expression and patterning in these two species was found in that the orientation of walking legs was towards the posterior pole in segments expressing *abd-A*. *Asellus aquaticus* thus may provide a highly interesting and novel arthropod model organism to study evolution of segment identity and patterning.

[4] email:pvick@uni-hohenheim.de

Introduction

Homeotic mutations, in which one body segment adopts the identity of another one, have fascinated naturalists for more than a century (Bateson 1894). For example, mutants in Drosophila are known in which antennae are changed into legs, or halteres into wings (Duncan and Montgomery 2002). Homeotic mutations have also been described in vertebrates, such as changes in identity of vertebrae (Jegalian and De Robertis 1992). The identification of homeotic genes as the targets of such mutations has opened the molecular analysis of body segment identity, and - as segment identity in many cases underlies changes of body plans during evolution - provided an entry point into the investigation of macroevolution at the molecular level. All homeotic genes encode transcription factors which are characterized by the presence of a conserved DNA binding motif, the homeobox (Gehring 1987).

Segment identity in Drosophila is the best studied paradigm to date. Clusters of homeotic genes in the Antennapedia and the Bithorax complex specify segment identity along the anterior-posterior body axis (Lewis 1978; Carroll 1995). The Antennapedia complex contains the five genes *labial*, *proboscipedia*, *Deformed*, *Sex combs reduced* (*Scr*) and *Antennapedia* (*Antp*), and the Bithorax complex comprises the three genes *Ultrabithorax (Ubx*), *abdominal-A* (*abd-A*) and *Abdominal-B* (*Abd-B*). In *Drosophila* five of these genes are involved in defining the trunk segments. *Scr*, *Antp* and *Ubx* are specifically expressed in the developing thoracic segments, whereas *abd-A* is important for conferring abdominal segments their identity in concert with *Abd-B* (Carroll 1995).

Differences in body plans between the different classes of the arthropods concern for example the subdivision into head, thorax and abdomen in insects as compared to prosoma and opisthosoma in spiders. Analysis of Hox expression revealed that genes active in the *Cupiennius salei* prosoma were exclusively found in the *Drosophila* head (Damen et al. 1998). In addition, mRNAs of these genes were also found in the head of crustaceans and myriapods (Abzhanov and Kaufman 1999; Abzhanov and Kaufman 1999; Hughes and Kaufman 2002). These findings have led to the conclusion that the chelicerate prosoma is homologous to the head tagma of other arthropods (Damen et al. 1998; Abzhanov and Kaufman 1999; Abzhanov et al. 1999; Hughes and Kaufman 2002; Carroll 2004). In general, these and other analyses have helped to resolve the evolution of arthropod lineages more clearly, at times confirming or rejecting morphology-based phylogeny (Akam 2000; Cook et al. 2001; Averof 2002; Schram and Koenemann 2004).

The emerging hypothesis from such studies has been that although Hox genes are highly conserved between different arthropod classes and species, changes in expression patterns were the driving force for alteration in body plan (Akam 2000; Cook et al. 2001; Averof 2002; Schram and Koenemann 2004). Crustaceans offer a particularly revealing case. Specifically, Hox genes have been used to visualize the differentiation of the major groups. Averof and Akam showed that in the ancestral branchiopod species *Artemia franciscana* the trunk Hox genes *Antp*, *Ubx* and *abd-A* were expressed in a broad overlapping fashion (Averof and Akam 1993; Averof and Akam 1995). Additionally Kaufman and colleagues showed in several studies differences in Hox gene expression patterns in two crustaceans, the isopod *Porcellio scaber* and the decapod *Procambarus clarkii* as well as in the myriapod *Lithobius*

atkinsoni (Abzhanov and Kaufman 2000; Abzhanov and Kaufman 2000; Hughes and Kaufman 2002). The crustacean studies revealed that in contrast to the obviously more primary Hox expression patterns of *Artemia*, those in *Porcellio* and *Procambarus* were more tagmata-confined. *Ubx* was mostly expressed in the pereon whereas *abd-A* was mostly restricted to the pleon. *Antp* showed a strong signal in the pereon, which was much weaker in the pleon. These studies demonstrated the differences of Hox expression between major crustacean groups and how these corresponded to the diverse adult morphologies.

Interestingly, Hox expression patterns could also be used to describe variations in external morphology within a class of arthropods. In crustaceans and especially the Malacostraca, anterior Hox gene comparisons were used to analyze the formation of maxillipeds. Maxillipeds originally represented anterior thoracic walking legs which have been converted into additional feeding appendages during evolution. In several crustacean groups these transformations occurred at different positions along the anterior-posterior body axis. In all cases analyzed the anterior expression domains of *Ubx* and *abd-A* correlated with the transition of maxillipeds to walking legs (Averof and Patel 1997; Abzhanov and Kaufman 1999).

The above mentioned examples demonstrated the power of Hox gene analysis for evolutionary studies at the level of phyla and classes. Examples of studies at lower taxonomic levels are scarce and restricted to dipterans (Yoder and Carroll 2006). We thus wondered whether such analyses could be extended to closely related species, which display significant differences in segment morphology. In the present study we chose two isopod species, namely the water louse *Asellus aquaticus* (LINNAEUS, 1758) and the wood louse *Porcellio scaber* (Abzhanov and Kaufman 2000).

The freshwater isopod *Asellus aquaticus* is widely distributed in standing and slow flowing freshwaters of the Palearctic region. Like in all higher crustaceans (Malacostraca) its body is divided into different functional regions, so-called tagmata. Isopods in general have a head fused to the first thoracic segment forming a cephalothorax, as well as seven free thoracic segments presenting the walking leg bearing pereon (Fig.1A+B). The posterior pleon consists of six segments bearing the taxon-specific limbs. The sixth pleonic segment is always fused with the telson and bears the uropods (Gruner 1965; Gruner 1993).

Differences in pleonic limb morphology and degree of fusion of pleonic segments reflect phylogenetic relationships inside the isopoda. Specifically, members of the Asellidae and the genus *Asellus* have two very small free pleonic segments, and the last four segments are fused to the telson forming a pleotelson (Fig.1A). The third pair of pleopods builds up an operculum covering the fourth and fifth pair which function as gills. In addition, although thoracomeres are quite similar in morphology, only the last three are orientated posteriorly. Consistent with this notion the posterior three pairs of thoracopods differ from the remaining one in that they are significantly longer and directed posteriorly while the anterior ones point to the front (Fig. 1A+B; von Haffner 1937; Gruner 1965; Gruner 1993).

In contrast, Porcellio scaber, a member of the Oniscidea differs significantly from the Asellota design. Porcellio also possesses seven free thoracomeres, but these and the thoracopods are nearly identical in morphology. The pleon shows no further fusion of segments, resulting in five free pleomeres of equal size and shape (Gruner 1965). Kaufmann and colleagues have described expression patterns for various Hox genes in developing

Porcellio embryos (Abzhanov and Kaufman 2000), and found a clear correlation of abd-A and pleonic segments. We therefore hypothesized that differences should exist in Hox gene expression between Porcellio and Asellus and started out to characterize Hox genes in the water louse.

Here we present the expression analysis of *abd-A* during development of *Asellus aquaticus*. We show by whole-mount *in situ* hybridization that beside very similar expression characteristics compared to *Porcellio* there are differences between these two isopod species which reflect differences in their family-specific posterior morphology. In *Asellus* the anterior and the posterior expression boundary each were shifted more anteriorly. Additionally we detected an expression domain in the epithelial cells of the developing early endodermal digestive gland tissue. This study therefore provides an example of how modest but highly significant differences in expression patterns of Hox genes reflect morphological differences between families of the same arthropod order.

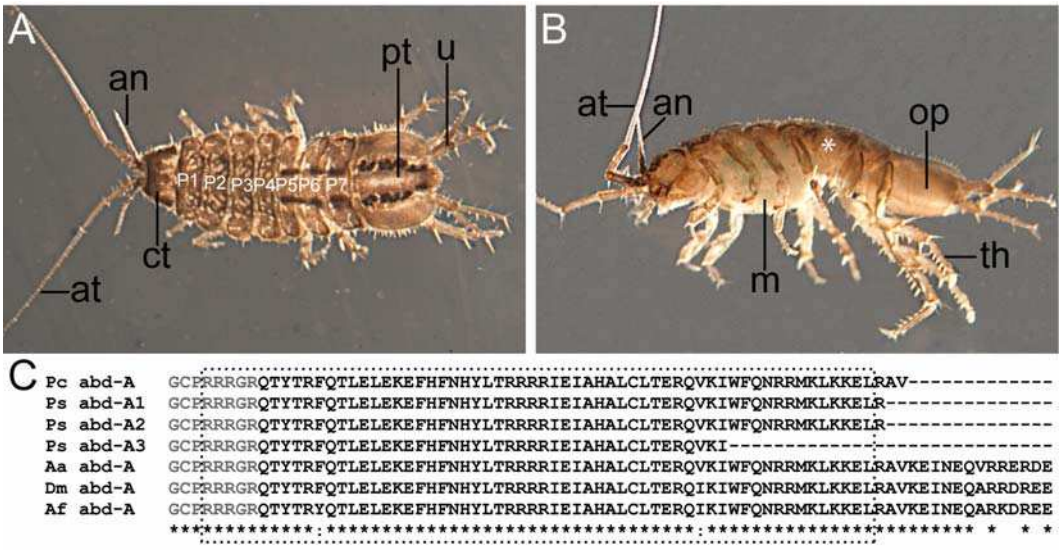

Figure 1. Morphology of the water louse *Asellus aquaticus* and amino acid sequence alignment of arthropod *abdominal-A* (*abd-A*) homeodomains.(**A**) Dorsal view of an adult female displaying a cephalothorax (ct) with a pair of antennules (an) and antennae (at), seven free pereonic segments (P1-P7), two free pleonic segments and the pleotelson (pt) bearing the uropods (u).(**B**) Lateral view of same specimen displaying the seven pairs of thoracopods (th), marsupium (m) and operculum (op). Please note the lost 5th pereopod marked by an asterisk. (**C**) Amino acid sequence alignment of the homeodomain and flanking sequences of the heomeobox gene *abd*-A from *Asellus aquaticus* (Aa), *Procambarus clarkii* (Pc), *Porcellio scaber* (Ps), *Drosophila melanogaster* (Dm) and *Artemia franciscana* (Af). Primer sequences are indicated in grey; the boundary of the homeobox is marked by the dotted box; asterisks mark identical amino acids, gaps indicate missing or un-matched residues, colons highlight conserved amino acid changes.

Results and Discussion

In order to correlate gene expression patterns with body segment differentiation, a number of Hox genes and other homeobox genes were selected for analysis. Following molecular cloning of cDNA fragments and preliminary expression analysis, *abd-A* was chosen for an in-depth study, as this gene proved to be the most promising candidate. Cloning and expression patterns of further genes will be described elsewhere (Vick and Blum, in preparation).

Cloning and Sequence Analysis of the Homeobox Gene *Abd-A* from *Asellus Aquaticus*

Known arthropod sequences were retrieved from the databases and aligned to select degenerate primers for cloning of the *Asellus aquaticus* ortholog of *abdominal-A* by RT-PCR. First, a standard PCR was used to obtain a short stretch of *Asellus*-specific sequences. In a second step 3'-RACE PCR was performed with two *Asellus*-specific nested and one sequence-tagged unspecific primer targeted to the poly-A tail of messenger RNAs. Following PCR reactions, electrophoresis and sequencing we obtained a 238 base pair (bp) fragment of *abd-A* spanning the whole homeodomain and parts of the 3' region. Surprisingly, the 3' sequences recovered did not contain the poly-A tail or UTR sequences, indicating that the unspecific oligo dT anchor primer annealed to A-rich stretches within the coding region, which we found to be particularly AT-rich in coding regions of *Asellus* as compared to *Drosophila melanogaster*. In *Porcellio*, three different splice variants of *abd-A* were described (Abzhanov and Kaufman 2000). We only recovered one form, which does however not exclude that additional isoforms exist. We did not attempt to isolate additional variants, as the one we cloned should be able to detect all three potential isoforms in *in situ* hybridization experiments.

The alignment of the deduced amino acid sequence with those of *Drosophila melanogaster*, *Artemia franciscana*, *Procambarus clarkii* and *Porcellio scaber* revealed a very high degree of conservation inside the homeodomain as well as in the neighboring region (Fig. 1C). A single change was found to *Drosophila abd-A*, namely a conserved amino acid replacement from valine to isoleucine at position 45 of the homeodomain. Within the crustaceans, identity in all positions was detected except for *Artemia franciscana*, in which in addition to the change in position 45 a second conserved change from phenylalanine to tyrosine in position 11 of the homeodomain was found.

Embryonic Development and Gene Expression Pattern of Abd-A in *Asellus Aquaticus*

The development of isopods in general and of *Asellus aquaticus* in particular has been described in several studies in the 19th and 20th century (Rathke 1834; Dohrn 1867; McMurrich 1895; Dejdar 1930; Länge 1958; Weygoldt 1959). As with all Peracarida, *Asellus*

displays direct development. The female lays its eggs into the marsupium, a ventral brood pouch located between the sternites and the oostegites of the anterior thoracic segments, in which the whole development takes place (Fig. 1B; Gruner 1965). Following superficial cleavage and gastrulation, segments and limbs form from anterior to posterior, creating a gradient of mature body regions towards the posterior end (McMurrich 1895; Weygoldt 1959). These early stages of development were not analyzed for *abd-A* gene transcription, as embryos were fragile, and because development of the pleotelson had not yet been initiated at that stage.

With increasing length, head and telson meet inside the chorion and vitelline membrane. Because isopods have no ventral groove they form a dorsal curvature (Fig. 2A). When the embryo hatches from the chorion it has built up most segments as well as the cephalothoracic and pereonic limb buds. Shortly thereafter the pleonic limbs occur. At that stage *abd-A* was expressed in a defined domain at the posterior pole of the embryo (Fig. 2A). Discrete segment boundaries could not be distinguished at that stage in whole-mounts. A parasagittal section confirmed posterior-specific expression, and revealed signals to be present in pleonic segments 1-4 as well as pereonic segment P7 (Fig. 2A''). An additional expression domain was found in the paired primordia of the endodermal epithelia. Signals were faintly visible in ventral view of whole-mounts (Fig. 2A'), as the ectodermal epithelium was translucent at that stage. The histological analysis in Fig. 2A'' confirmed the specificity of this expression, which is further supported by the absence of signal in sense control hybridized specimens (Fig. 2B-B''). Probe trapping seems highly unlikely, as signals were clearly located inside the epithelial cells lining the endodermal cavity. This pattern came as a surprise, as no endodermal mRNA signals of *abd-A* have been reported in any model organism so far. However, *abd-A* was shown to be expressed in the midgut-surrounding visceral mesoderm and its loss of function influenced *labial* expression in the endodermal midgut of *Drosophila* (Manak et al. 1994; Staehling-Hampton and Hoffmann 1994).

As development progresses and the embryonic axis lengthens the inner membrane ruptures and sheds. This process is induced by the downward bending of the pleon (cf. Fig. 2A and C). At that stage (75% of development; Fig. 2C) signal intensity had markedly increased. Expression had begun to shift anteriorly, and was now predominantly found in pereonic segments P5-7, and – progressively fading – in the anterior-most pleonic segments (Fig. 2C). Additional signals were detected in transverse histological sections at the level of the fifth (Fig. 2C') and seventh (Fig. 2C'') pereonic segments. Staining was seen in the ventral nerve cord in a bilaterally symmetrical fashion (Fig. 2C', C''), likely representing differentiated neurons. This neural expression of *abd-A* correlates well with studies of *abd*-A expression in *Porcellio,* where neural signals were described to be localized specifically to pereonic segments P5-7 (Abzhanov and Kaufman 2000), in perfect agreement with our study. In addition to the segmental signals, transcription was also clearly present in pereonic limbs, with stronger staining in the proximal areas, fading towards the distal ends (Fig. 2C, C', C''). Strikingly, expression was restricted to pereonic limbs P5 and P6, i.e. limbs which orient themselves toward the posterior pole in the adult. In *Porcellio*, expression in P6 and P7 was also reported, however, much fainter signals compared to *Asellus*, which correlated with the only moderate posterior bending of pereopods in *Porcellio* (Abzhanov and Kaufman 2000).

Strongest signals in *Porcellio* were restricted to more posterior segments, namely pleonic segments 1-5 (Abzhanov and Kaufman 2000).

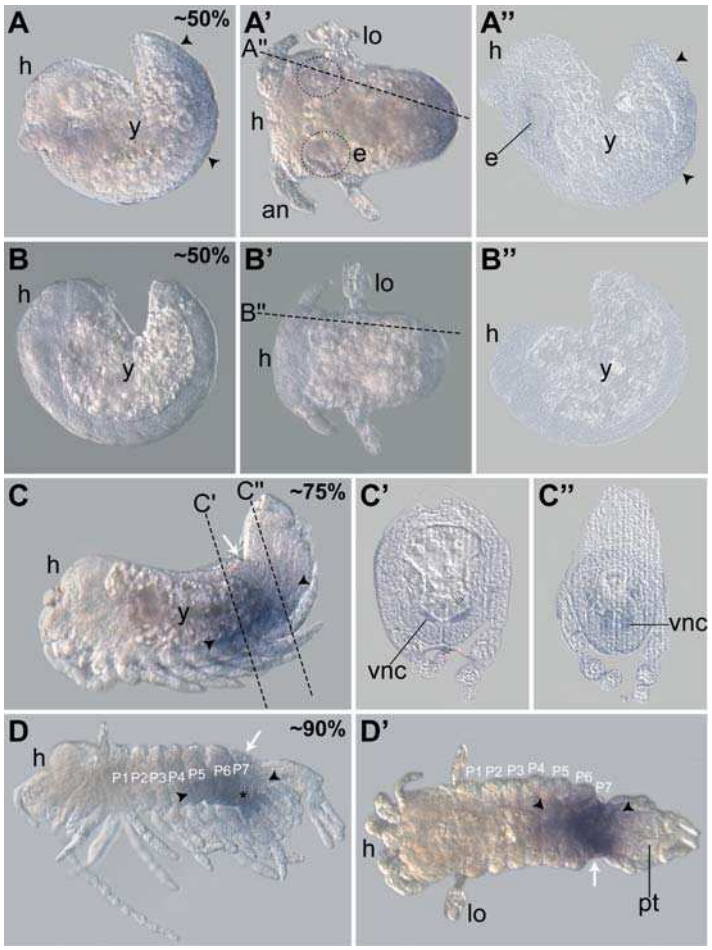

Figure 2. *Abdominal-A* mRNA expression during development of *Asellus aquaticus* embryos.Staging of embryos was according to Whitington et al. (1993). **(A-A'')** Embryo at ~50% of development showing *abd-A* mRNA expression mainly in the pleon and in the paired endodermal anlagen of the midgut (e); in (A) only the posterior expression domain can bee seen. (A') Ventral view showing lateral organs (lo), and endodermal primordia visible through the translucent ectoderm (dotted rings). A sagittal section in (A'') highlights expression domains.**(B-B'')** Control embryo at ~50% of development hybridized with a sense probe showing no expression in any tissue.**(C-C'')** *abd-A* mRNA expression in a ~75% embryo. Expression was located between the 5th pereonic segment and the anterior pleon with decreasing intensity towards the posterior pleon. Transversal sections (C'+C'') showed strong expression in the ventral nerve cord (vnc) and weaker signals in the proximal limbs and the remainder of the mesodermal and ectodermal trunk tissue, which gradually faded towards the dorsal side. No expression was detected in the dorsal most part and in the distal limbs.**(D+D')** Expression of *abd-A* in an embryo at ~90% of development. The signal was clearly restricted between P5 and pleonic segment 3 with strongest expression in P6+P7 (D'), the developing 7th pereopod (*) and the proximal limbs of the anterior pleonic segments.A, A'', B, B'', C, D lateral views, anterior to the left, dorsal to the top; A', B' ventral views, anterior to the left; D' dorsal view, anterior to the left; C', C'' transversal sections, dorsal to the top. White arrows mark tagmata boundary between pereon and pleon. Black arrowheads indicate approximate anterior and posterior expression boundaries. Asterisk marks 7th pereopod. Dashed lines indicate approximate planes of section. an, antennule; h, head; pt pleotelson; y, yolk.

Between 75% and 90% of development, the embryo hatches again and thereby finalizes limb development (Dohrn 1867). At ~90% the embryo shows already movements, and the remaining yolk has been fully absorbed by the paired midgut glands. *abd-A* gene expression now was most clearly restricted to P5-7 and to pleonic segments 1-3, with no remaining signals in the more posterior region, where the pleotelson had begun to form (Fig. 2D'). Expression in the neural tissue persisted until this stage (not shown), while signals in pereonic limbs, which by now had completed their posterior orientation, were hardly detectable. The expression in pleonic segment 3 was restricted to the ventral aspect of the segment (not shown). Strikingly, this expression again correlated with a morphogenetic event, namely the fusion of segments at the dorsal aspect in pleonic segments 3-5, resulting in the formation of the pleotelson. In contrast, expression was clearly present in anterior pleopods (Fig. 2D), and in the newly developing pereopod 7 (asterisk in Fig. 2D).

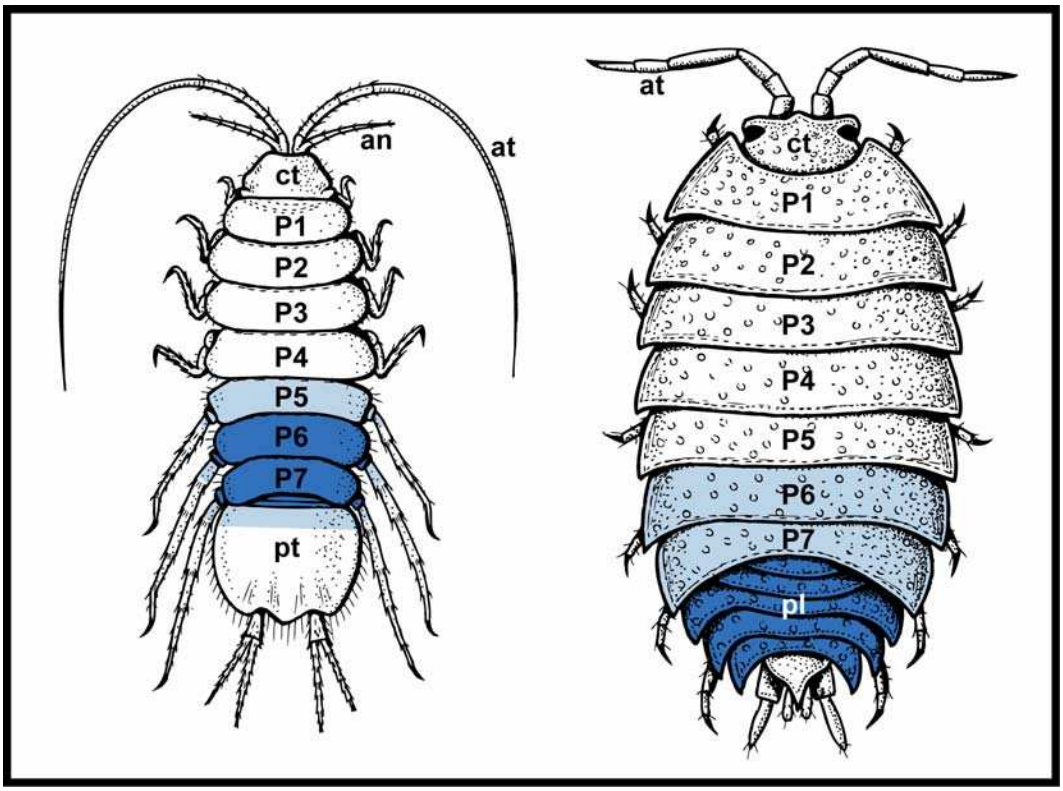

Figure 3. Schematic comparison of embryonic *abdominal-A* mRNA expression domains in *Asellus aquaticus* (left) and *Porcellio scaber* (right) in relation to adult morphology. In both cases, dark blue shading marks strong expression and light blue coloring indicates weak expression. *Asellus* showed strong expression of *abd-A* in P6, P7 and the free pleonic segments 1 and 2, and weaker expression in P5 and pleonic segment 3. In contrast, strong expression in *Porcellio* was restricted to the pleonic segments 1-5. In P6 and P7 a weak expression was reported (Abzhanov and Kaufman 2000). at, antennae; an, antennule; ct, cephalothorax; pl, pleon; pt, pleotelson.

Conclusions

In summary, our *abd-A* expression analysis in *Asellus aquaticus* demonstrated a clear correlation of expression domains with distinct morphogenetic units in two cases (Fig. 3): pleotelson fusion (absence of *abd-A* transcription) and pereonic limb orientation (strong *abd-A* expression during the process of posterior bending). Compared to *abd-A* expression in *Porcellio*, the adult morphology divergence is mimicked by a corresponding change in gene expression, i.e. *abd-A* activity throughout the pleonic segments (which do not fuse), and lack of activity in posterior pereopods which do not orient posteriorly (von Haffner 1937; Gruner 1965). Thus, even between highly related crustaceans, shifts of Hox gene expression boundaries correlate with adult morphology variations (Fig. 3). It could, therefore, be quite beneficial to analyze the promoter regions of *abd-A* in these two species, as these changes should be represented in a comparably small number of nucleotide changes. Further, it might be worthwhile to extend this analysis to the most basal isopods, i.e. the suborder Phreatoicidea, which show an even more pronounced dimorphism of leg orientation (von Haffner 1937). Finally, the hypothesis put forward here, namely that temporal-spatial changes in *abd-A* gene expression are responsible for morphogenetic variations in adult morphologies of *Asellus aquaticus* and *Porcellio scaber* might be testable in the future, provided techniques for genetic manipulation of embryos become available.

Material and Methods

Animals and Embryos

Embryos bearing adult females were collected from a local pond during the month of May to August. Typically, 15-40 embryos were recovered from females by dissecting the marsupium. Embryos were staged in analogy to the crustacean staging system described by Whitington et al. (1993), fixed in 4 % paraformaldehyde and stored in methanol at –20°C until further analysis.

Whole-Mount *in Situ* Hybridization and Histological Analysis

In situ hybridization followed standard procedures. For histological analysis embryos were embedded in a gelatine-albumin mix and sectioned at 30 µm using a vibratome. Sections were embedded and photographed using Nomarski optics.

Accession Number

The *abdominal-A* sequence was deposited at gene bank, accession number EU882729.

References

Abzhanov, A. and Kaufman, T. C. (1999). "Homeotic genes and the arthropod head: expression patterns of the labial, proboscipedia, and Deformed genes in crustaceans and insects." *Proc Natl Acad Sci U S A* **96**(18): 10224-9.

Abzhanov, A. and Kaufman, T. C. (1999). "Novel regulation of the homeotic gene Scr associated with a crustacean leg-to-maxilliped appendage transformation." *Development* **126**(6): 1121-8.

Abzhanov, A. and Kaufman, T. C. (2000). "Crustacean (malacostracan) Hox genes and the evolution of the arthropod trunk." *Development* **127**(11): 2239-49.

Abzhanov, A. and Kaufman, T. C. (2000). "Embryonic expression patterns of the Hox genes of the crayfish Procambarus clarkii (Crustacea, Decapoda)." *Evol Dev* **2**(5): 271-83.

Abzhanov, A., Popadic, A. and Kaufman, T. C. (1999). "Chelicerate Hox genes and the homology of arthropod segments." *Evol Dev* **1**(2): 77-89.

Akam, M. (2000). "Arthropods: developmental diversity within a (super) phylum." *Proc Natl Acad Sci U S A* **97**(9): 4438-41.

Averof, M. (2002). "Arthropod Hox genes: insights on the evolutionary forces that shape gene functions." *Curr Opin Genet Dev* **12**(4): 386-92.

Averof, M. and Akam, M. (1993). "HOM/Hox genes of Artemia: implications for the origin of insect and crustacean body plans." *Curr Biol* **3**(2): 73-8.

Averof, M. and Akam, M. (1995). "Hox genes and the diversification of insect and crustacean body plans." *Nature* **376**(6539): 420-3.

Averof, M. and Patel, N. H. (1997). "Crustacean appendage evolution associated with changes in Hox gene expression." *Nature* **388**(6643): 682-6.

Bateson, W. (1894). *Materials for the Study of Variation Treated with Especial Regard to Discontinuity in the Origin of Species*. London, Macmillan and co.

Carroll, S. (2004). *From DNA to Diversity*, Blackwell Science Inc.

Carroll, S. B. (1995). "Homeotic genes and the evolution of arthropods and chordates." *Nature* **376**(6540): 479-85.

Cook, C. E., Smith, M. L., Telford, M. J., Bastianello, A. and Akam, M. (2001). "Hox genes and the phylogeny of the arthropods." *Curr Biol* **11**(10): 759-63.

Damen, W. G., Hausdorf, M., Seyfarth, E. A. and Tautz, D. (1998). "A conserved mode of head segmentation in arthropods revealed by the expression pattern of Hox genes in a spider." *Proc Natl Acad Sci U S A* **95**(18): 10665-70.

Dejdar, E. (1930). "Die Funktion der "blattförmigen Anhänge" der Embryonen von *Asellus aquaticus* (L.)." *Zeitschrift für Morphologie und Ökologie der Tiere* **19**.

Dohrn, A. (1867). "Die embryonale Entwicklung von *Asellus aquaticus*." *Zeitschrift für Morphologie und Ökologie der Tiere* **17**.

Duncan, I. and Montgomery, G. (2002). "E. B. Lewis and the bithorax complex: part I." *Genetics* **160**(4): 1265-72.

Gehring, W. J. (1987). "Homeo boxes in the study of development." *Science* **236**(4806): 1245-52.

Gruner, H.-E. (1965). *Die Tierwelt Deutschlands und der angrenzenden Meeresteile - 51. Crustacea, V. Isopoda*, Gustav Fischer Verlag Jena.

Gruner, H.-E. (1993). *Lehrbuch der speziellen Zoologie - Band 1, Teil 4: Arthropoda*, Gustav Fischer Verlag.

Hughes, C. L. and Kaufman, T. C. (2002). "Exploring the myriapod body plan: expression patterns of the ten Hox genes in a centipede." *Development* **129**(5): 1225-38.

Jegalian, B. G. and De Robertis, E. M. (1992). "Homeotic transformations in the mouse induced by overexpression of a human Hox3.3 transgene." *Cell* **71**(6): 901-10.

Länge, H. (1958). "Bau und Entwicklung der blutbildenden Organe von *Asellus aquaticus* (L.)." *Zeitschrift für wissenschaftliche Zoologie* **161**: 144-208.

Lewis, E. B. (1978). "A gene complex controlling segmentation in Drosophila." *Nature* **276**(5688): 565-70.

Manak, J. R., Mathies, L. D. and Scott, M. P. (1994). "Regulation of a decapentaplegic midgut enhancer by homeotic proteins." *Development* **120**(12): 3605-19.

McMurrich, J. P. (1895). "Embryology of Isopod Crustacea." *Journal of Morphology* **11**.

Rathke, H. (1834). "Recherches sur la formation et le développement de l'Aselle d'eau dpuce." *Annal. des Sci. Nat., 2^{me}sér.* **ii**.

Schram, F. R. and Koenemann, S. (2004). Developmental genetics and arthropod evolution: On body regions of crustacea. *Evolutionary Developmental Biology of Crustacea*. G. Scholtz, Aa Balkema.

Staehling-Hampton, K. and Hoffmann, F. M. (1994). "Ectopic decapentaplegic in the Drosophila midgut alters the expression of five homeotic genes, dpp, and wingless, causing specific morphological defects." *Dev Biol* **164**(2): 502-12.

von Haffner, K. (1937). "Untersuchungen über die ursprüngliche und abgeleitete Stellung der Beine bei den Isopoden." *Zeitschrift für wissenschaftliche Zoologie* **149**: 513-536.

Weygoldt, P. (1959). "Beitrag zur Kenntnis der Malakostrakenentwicklung. Die Keimblätterbildung bei *Asellus aquaticus* (L.)." *Zeitschrift für wissenschaftliche Zoologie* **163**: 342-354.

Whitington, P. M., Leach, D. and Sandeman, R. (1993). "Evolutionary change in neural development within the arthropods: axonogenesis in the embryos of two crustaceans." *Development* **118**(2): 449-61.

Yoder, J. H. and Carroll, S. B. (2006). "The evolution of abdominal reduction and the recent origin of distinct Abdominal-B transcript classes in Diptera." *Evol Dev* **8**(3): 241-51.

Reviewed by Dr. Matthias Gerberding, Max-Planck-Institute of Developmental Biology, Tübingen, Germany.