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Abd-B expression in *Porcellio scaber* Latreille, 1804 (Isopoda: Crustacea): conserved pattern versus novel roles in development and evolution

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SUMMARY The Hox genes are intimately involved in patterning the animal body during development and are considered to have had a pivotal role in the evolution of different body plans among the metazoans. From this perspective, crustaceans, a group that has evolved an extreme diversity of body structures, represent a choice group in which to study the evolution of these genes and their expression. The expression of one of these genes, *Abdominal-B* (*Abd-B*), has only been studied in two distantly related crustaceans, *Artemia* and *Sacculina*, where it shows dissimilar patterns, highly differentiated from the one described in other arthropods. Moreover, we have no information for the Malacostraca. Thus, we cloned the gene *Abd-B* and followed its expression through development by in situ hybridization in the isopod *Porcellio scaber*. We found a highly dynamic expression pattern of *PsAbd-B* during embryonic development. In early stages, it is expressed in the posterior-most part of the germ band, in a domain common to several arthropods studied to date, and later it is expressed in the developing limb buds of the pleon and still later in the endopodites of the third to fifth pleopodites. This raises the interesting possibility of the involvement of this gene in the later respiratory specialization of these appendages. In association with the above expression domain, *Abd-B* appears to be expressed in later stages also in the ventral ectoderm, raising the further suggestion of its possible involvement in patterning the developing nervous system. Moreover, we show that the first pleopod and the endopodite of the second pleopod, whereas present as limb buds in early embryonic stages, are later reduced and actually absent in the first postembryonic stage, although they reappear again in adults. These appendages thus represent an example of Lazarus appendages. Our data show strong plasticity in the use of a key developmental gene and point out the necessity of further research that may end with a revision of the current understanding of its role in animal evolution.

INTRODUCTION

Among the many genes involved in patterning the animal body during early development, the Hox genes are among the most extensively studied. This is, at least in part, because of their pivotal role in the specification of features along the anterior–posterior body axis. Following pioneering studies in *Drosophila*, a great deal of information about the embryonic expression patterns of the Hox genes has been obtained in recent years for several arthropods (for a review, see Hughes and Kaufman 2002a). This has allowed direct comparisons of the expression of at least the eight "canonical" Hox genes among a few representatives of each of the main arthropod groups: the insects, the crustaceans, the myriapods, and the chelicerates (Hughes and Kaufman 2002a). What is most striking is that not only are these genes highly conserved at the sequence level but also their expression patterns show clear homologies between different animals (not just arthropods) and often correlate with morphological boundaries between body regions.

It is important to note, however, that the expression patterns of the Hox genes are often dynamic over developmental time. Thus, following the changing expression pattern of a Hox gene over an extended developmental span, we can reduce the chance of erroneously concluding homologies, whereas at the same time exploring the possibility of novel roles for those genes being investigated. This approach should also allow the formulation of further hypotheses as to the nature and direction of change in the evolution of Hox function. It is with this aim in mind that we have studied the expression pattern of *Abdominal-B* (*Abd-B*) over an extensive period of embryogenesis in the isopod *Porcellio scaber* Latreille, 1804.

The Hox gene *Abd-B* is the 5′-most Hox gene of the Hom-C and, as a rule, is the most posteriorly expressed during embryonic development. Its expression has been studied in

two very distantly related crustacean species, the branchiopod *Artemia franciscana* and the rhizocephalan *Sacculina carcini* (Averof and Akam 1995; Blin et al. 2003; Copf et al. 2003). In *A. franciscana*, *Abd-B* expression is very different as compared with other arthropods in which expression is localized to a few of the most posterior body segments. Instead, in *Artemia*, *Abd-B* is expressed only in the genital segment, at the boundary between the thorax and abdomen (Averof and Akam 1995; Copf et al. 2003). In *S. carcini*, *Abd-B* is expressed in the vestigial abdomen and, in females, in an exceptionally long domain that covers the whole thorax (Blin et al. 2003). However, as *A. franciscana* and *S. carcini* represent just two among the many different arrangements and specializations of body regions found among the crustaceans, generalizations would seem to be ill advised at this point. Moreover, it is perhaps not surprising that the expression patterns of the Hox genes are significantly different in animals with such different regional organization (tagmosis).

Within the crustaceans, malacostracans present a more uniform body plan, with three distinct tagmata—a head of six segments, a pereion of eight segments (pereionites), and a pleon of six segments (pleonites), plus the terminal telson. A seventh pleonic segment is present only in the basal order Leptostraca (Schram 1986). Other than a comparative study with FP6.87, an antibody that recognizes both Ultrabithorax (Ubx) and abdominal-A (Abd-A) proteins in several species belonging to different crustacean lineages (Averof and Patel 1997), detailed studies on the developmental expression of the remainder of the Hox genes have been carried out on only two species representing two of the most successful malacostracan groups: the isopod *P. scaber* and the decapod *Procambarus clarkii* (Abzhanov and Kaufman 1999a,b, 2000a,b). It is reasonable to maintain that these two species are ecologically specialized because of their adaptation to terrestrial and freshwater environments, respectively. In addition, their development is direct, that is, without a planktonic larval stage. Although this character makes them experimentally amenable and allows for direct comparisons with other non-marine arthropod groups such as the insects, it also represents a further specialization that could be associated with alterations in Hox gene expression.

Although the studies on the above two species cover most of the Hox genes, we have no information about the spatial and temporal expression patterns of *Abd-B* in malacostracan crustaceans. This gene is of particular interest from the point of view of its relationship to the specification of the genitalia as *Abd-B* has been hypothesized to play a role whether this process in arthropods as well as in other animals (Kondo et al. 1997; Damen and Tautz 1999; Kagoshima et al. 1999). Therefore, it would be of some interest to determine whether this gene is expressed in the genital opening (at the level of the eighth pereionite) and/or the male gonopods (endopodites of the first and second pleopods) and thus perhaps involved in the specification of these reproductive structures in malacostracan crustaceans. More generally, it would be of interest to determine how a classical "posterior" Hox gene might be involved in the development of posterior appendages, that is, the pleopods, as at present little attention has been devoted to them. In general, the pleopods in malacostracans are very different from pereionic appendages. Specifically, in isopods, pleopods are often specialized for respiratory functions as they are in *Porcellio*.

Here, we extend the work started by Abzhanov and Kaufman (1999a,b, 2000a,c,d) on the Hox genes of *P. scaber*, the common sowbug/woodlouse. First, we describe aspects of the embryology and point out a possible Lazarus trait. Second, we have cloned the *Porcellio* homologue of *Abd-B* and by looking at its highly dynamic expression pattern during embryonic development, we report the possible involvement of this gene in patterning the pleon, in the specialization of the endopodites of the third to fifth pleopods (and possibly the first and second pleopods, which are also specialized, but in a completely different way) and, more tentatively, in the development of the nervous system.

MATERIALS AND METHODS

P. scaber husbandry

Colonies of the common woodlouse *P. scaber* (Isopoda, Oniscidea) were originally established from animals collected around Bloomington, IN, USA. Breeding colonies of several hundred animals were maintained in large plastic boxes at around 22°C with a constant light source in moistened soil with rotting leaves. Females of *P. scaber* brood some tens of embryos in a ventral brooding pouch formed by large plates (oostegites) corresponding to outgrowths of the pereiopods. It is easy to extract the eggs from the brood pouch by using dissecting forceps and tungsten needles. As the eggs are transparent, it is possible to stage them in situ. When further development is necessary, one can also culture them in crustacean saline according to Whitington et al. (1993) on 1% agar at room temperature.

Cloning and sequence analysis

RNA was prepared from collections of mixed stage embryos using Trizol reagent (GibcoBRL/Life Technologies, Gaithersburg, MD, USA), following the manufacturer's instructions. Total RNA was poly-A selected. cDNA was synthesized using the Clontech Smart RACE kit (BD Biosciences/Clontech, Palo Alto, CA, USA).

A fragment of *P. scaber Abd-B* was initially obtained by touchdown PCR from cDNA using degenerate primers directed against conserved positions within the homeodomain of *Abd-B*. The degenerate primers used were: EWTGQVTV (forward) and QVKIWFQN (reverse). All PCR were performed using the Advantage2 polymerase mix (BD Biosciences/Clontech) and cloning of candidate PCR products using the PCR-Script Amp Cloning kit (Stratagene, La Jolla, CA, USA). The sequence of the initial clone of the homeobox region allowed us to design exact primers for

3′ RACE. We were thus able to isolate a longer clone suitable for making in situ hybridization probes, a 441 base pair fragment (underlined in the following sequence), which begins at the homeobox (bold letters) and extends downstream to include some of the 3′ untranslated region from the middle of the homeobox:

CGAAAGAAACGGAAACCTTACTCCAAATTCCAGACGCT-GGAACTAGAAAAGGAATTTCTTTATAACGCGTACGTTTC-GAAACAAAAAAGATGGGAATTGGCGCGTAATTTAAATT-TAACGGAAAGGCAAGTGAAAATATGGTTTCAAAATAGA-AGGATGAAAAAAAAGAAAAATAGTCAAAGACAGGCCG-CTCAGGAAGGTCGGGGAGGCACCGGAGGGGGCACCCC-TTCGTCTGGTGGCGGAACCCCAGGTCACCAACCCACAA-CTCCACAGACTCCTAATCCAATTAAACCGTGACCGGAAT-CGGGGGCCGGGGGTGCCTCATGTAGTGGAAATAATAG-CAACATTGGGTGTCCTATGGTGCACGCCGAAGGAGGAA-TAGATCCTACTATGGAAACTCCTGAAGCTGCAATGATGG-GACCAGTTTACCAACAACCCTGGCTTTGTTGAGTGCATA-GTTTGTACCACAACCACGTTTCATTGACTCGAGTGAAGT-GTAGTGAATTACGCGCCAATTCCCATCTT.

The identity of the gene has been confirmed by BLAST and all nucleotide sequences were analyzed using Sequencer and MacVector software. Sequences have been deposited in GenBank with accession number AY779183.

Fixation of embryos and in situ hybridization

Embryos/eggs were fixed in 4% paraformaldehyde in PBT (PBS plus 0.1% Tween 20) for 60 min. During fixation, embryos were extracted from the eggshell with dissecting forceps. Embryos were then rinsed with PBT, dissected from the yolk, dehydrated, and stored in methanol at -20°C.

The in situ hybridization protocol was performed according to Liu and Kaufman (2004). To stain DNA, and allow a comparison between morphology and in situ staining, embryos were rinsed and soaked in the green nucleic acid stain SYTOX (Molecular Probes, Eugene, OR, USA) (0.5 μM in Tris 0.1 M), rocked for 1 h, and rinsed again.

Microscopy and images

Results of in situ hybridization and SYTOX staining on the same specimen were analyzed and photographed using a dissecting microscope (Nikon, Tokyo, Japan). Images were prepared using Corel Photo Paint, with some minor image adjustment.

RESULTS

Adult *P. scaber* are characterized by a more or less uniform pereion with seven pairs of walking pereiopods (the first pereiopod becomes a maxilliped during embryogenesis; see Abzhanov and Kaufman 1999b) and by six pairs of pleopods. The posterior-most pair of these latter appendages differentiates into uropods. All six pairs of pleonic appendages are biramous, characterized by an originally external (exopodite) and an internal branch (endopodite).

Embryology and embryonic expression of *PsAbd-B*

The staging of the embryos was based on Whitington et al. (1993) and Abzhanov and Kaufman (1999a,b, 2000a,c,d), although some of the stages described below were not examined in those studies.

P. scaber, as a typical member of the Isopoda, has yolky eggs. Early cleavage, as in most crustaceans, is superficial (McMurrich 1895). Segmentation is as in typical short germband arthropods, that is, segments are added in sequence from a posterior growth zone. At any given stage, an anterior–posterior gradient in the degree of specification and maturation of segments is observed.

At approximately 10% of embryonic development (Fig. 1, A and B, A′ and B′), the germ-band is well formed. However, although all the anterior (cephalic and pereionic) limb buds are already well established, the formation of the pleon is not yet completed. In situ hybridization with *PsAbd-B* shows that expression is strong in the subterminal growth zone, all along the six to eight posterior-most rows of orderly aligned cells (arrows in Fig. 1). In addition to this area, localized *PsAbd-B* staining is present in groups of few cells (four to eight) that will likely produce the limb buds of the first and second pair of pleopods (p1 and p2 in Fig. 1A′). The remaining pleopods are still undergoing formation (third) or are not yet formed (fourth–sixth).

At approximately the 30% stage (Fig. 2, A and B), all adult segments are present, although the limb buds of the last pleonic segment are still undeveloped. As a consequence, there is a clear gradient in the degree of development from the first to the sixth pleonic limb bud. The first one or two pleonic primordia already show the characteristic double bud of the typical crustacean biramous appendage (p1–p3, in Fig. 2A; p1-ur in Fig. 3). *PsAbd-B* staining is present in all the pleopod limb buds, both in those already formed and in the cells that are aggregating to give rise to those yet to be formed. At the same time *PsAbd-B* staining surrounds and marks the well-formed proctodeum (arrow in Fig. 2, A and B).

At approximately the 55% stage (Fig. 3, A and B), the pleonic biramous limb buds are all formed and are all stained by the *PsAbd-B* probes as is the region around the proctodeum (arrow in Fig. 3, A and B).

At the 75–80% stage (Fig. 4, A and B), all the cephalic and pereionic appendages as well as the hemitergites (the left and right parts of the tergites, which will fuse together at a later stage of development as the embryo closes dorsally over the remaining yolk) are well developed. At this stage, the first pereionic limbs, which until this point are similar to the other pairs, begin to diverge in their course of differentiation possibly because of alterations in the expression patterns of genes like *Scr* (Abzhanov and Kaufman 1999b) (mxp in Fig. 4B). At the same time, the last pleonic limbs assume an elongated shape, different from the more anterior appendages, and will

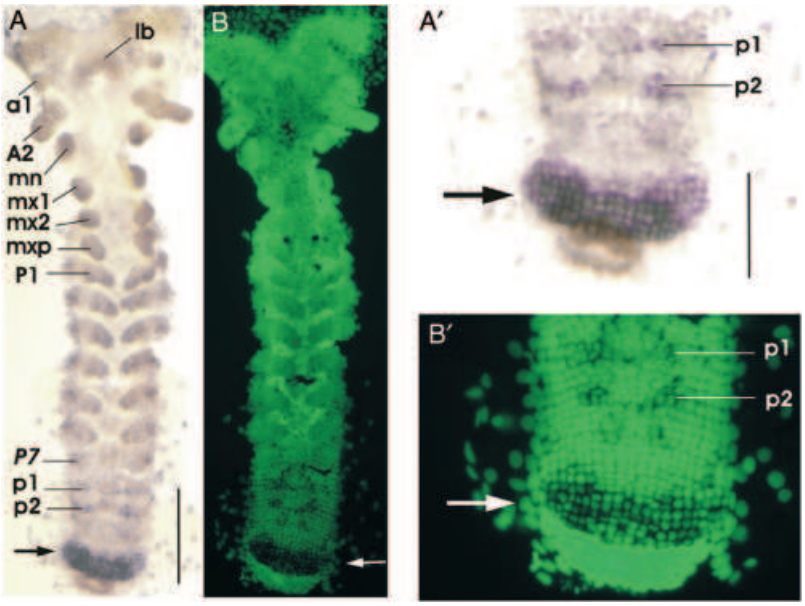

Fig. 1. Expression of *Abd-B* in *Porcellio scaber* at approximately 10% of embryonic development, as revealed with whole-mount in situ hybridization; ventral view with embryo oriented anterior to the top. (A, A′) Visible light illumination, showing staining for *PsAbd-B*; (B, B′) same specimen at the same scale under UV illumination showing SYTOX nuclear staining, used to represent general embryo morphology. (A′, B′) Detail of the same animal shown in A and B at higher magnification; *PsAbd-B* is expressed in the posterior-most area of the grid-shaped growth zone (arrow) and, separately, in the clusters of cells that likely will give rise to the first and second pleopods (indicated as p1, p2). Diffuse background staining, more evident in appendages, is present here and in Figs. 2 and 3 but is discernibly different from the signal. a1, first antenna; A2, second antenna; lb, labrum; mn, mandible; mx1, first maxilla; mx2, second maxilla; mxp, maxilliped/first trunk appendage; P1, first pereiopod; *P7*, seventh pereionite; p1 and p2, first and second pleopods; ur, uropod. Scale bar 200 μm in A and 100 μm in A′.

later become the uropods (ur in Fig. 4, A and B). As it is also clear from Fig. 4B (see also Fig. 2E in Abzhanov and Kaufman 1999b), the first pleopod buds (p1) have markedly diminished. The same behavior is exhibited by the endopodite of the second pleopod (p2 in Fig. 4B). All the pleonic limb buds that are still present show a clear *PsAbd-B* staining (p3 and p5 in Fig. 4A). There is also diffuse staining in the remaining portions of the pleon. In addition, we observe stronger staining along the midventral ectoderm of the whole embryo, involved in the development of the nervous system (Fig. 4A). This expression marks the limits of each segment (arrows in Fig. 4A). In the cephalic area, stain also accumulates

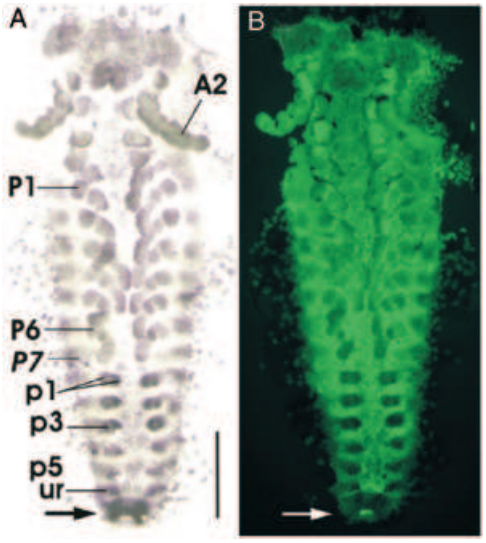

Fig. 2. Expression of *Abd-B* in *Porcellio scaber* at approximately 30% of embryonic development, as revealed by whole-mount in situ hybridization; ventral view with embryo oriented anterior to the top. (A) and (B) as in Fig. 1. *PsAbd-B* staining is present in the forming pleonic buds (p1 to ur) and in the proctodeum (arrow). A2, second antenna; P1, first pereiopod, at this early stage already clearly biramous; P6, sixth pereiopod; *P7*, seventh pereionite; p1, p3, and p5, first, third, and fifth pleopods; ur, uropod (sixth pleonic appendage). Scale bar: 200 μm.

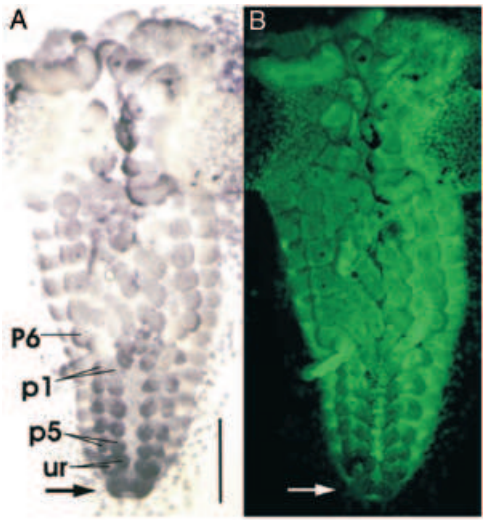

Fig. 3. Expression of *Abd-B* in *Porcellio scaber* at approximately 55% of embryonic development, as revealed by whole-mount in situ hybridization; ventral view with embryo oriented anterior to the top. (A) and (B) as in Fig. 1. *PsAbd-B* staining is still present in the pleonite buds (p1 to ur) and in the proctodeum (arrow); this staining is discernibly different from what seems to be background staining characterizing, e.g., the tip of the fifth and sixth pereiopods. P6, sixth pereiopod; p1 and p5, first and fifth biramous pleopods; ur, uropod. Scale bar: 200 μm.

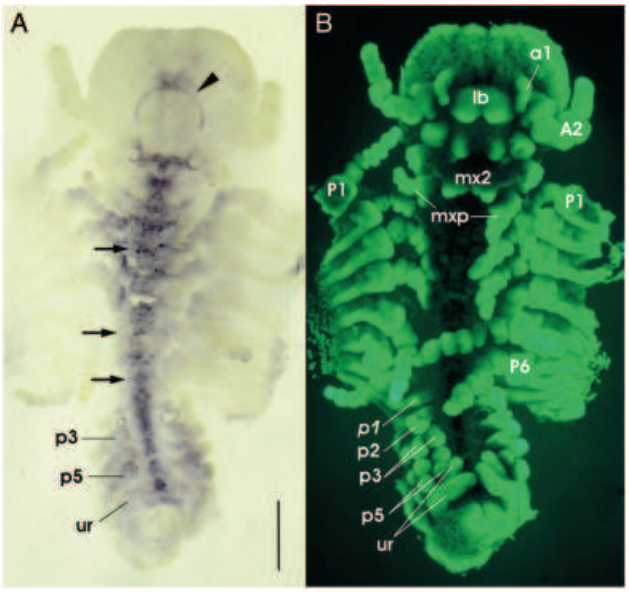

Fig. 4. Expression of *Abd-B* in *Porcellio scaber* at approximately 75–80% of embryonic development, as revealed by whole-mount in situ hybridization; ventral view with embryo oriented anterior to the top. (A) and (B) as in Fig. 1. First trunk and last pleonic appendages (maxillipeds, mxp; uropods, ur in B) are differentiating from the other trunk and pleonic appendages; first pleopod and the endopodite of the second pleopod are diminishing (*p*1 and p2 in B); *PsAbd-B* staining is present in the pleon, more clearly in the third to fifth pleonic limb buds (p3–p5 in A), and in the ventral ectoderm, along the body (arrows in A), forming, at its cephalic extremity, an open circle which borders the labrum bud (arrowhead in A; see comparison with B). a1, first antenna; A2, second antenna; lb, labrum; mx2, second maxilla; mxp, maxilliped/first trunk appendage; P1, first pereiopod; P6, six pereiopod; *p*1, p2, first and second pleopods; p3 and p5, third and fifth pleopods; ur, uropod (sixth pleonic appendage). Scale bar: 200 μm.

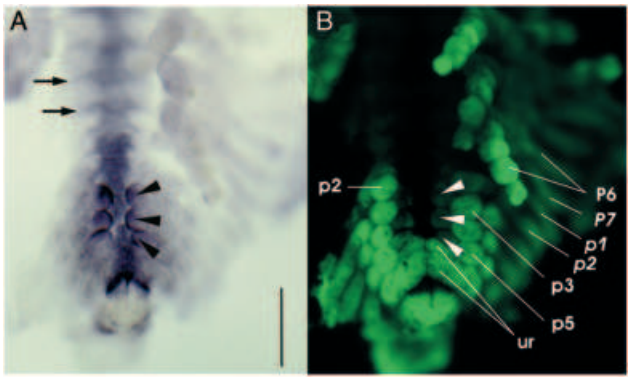

Fig. 5. Expression of *Abd-B* in *Porcellio scaber* at >80% of embryonic development but prior to deposition of cuticle, as revealed by whole-mount in situ hybridization; ventral view with embryo oriented anterior to the top. (A) and (B) as in Fig. 1. At this stage, both the seventh pereionite (*P7* in B) and the first pleonite (*p1* in B) lack limb buds, whereas the second pleonite (*p2* in B) has only the exopodite of the pleopod (p2); *PsAbd-B* staining pattern of the midventral ectoderm is the same as in the previous stage (Fig. 4), marking each segment (arrows in A); *PsAbd-B* staining is also present at the distal tip of the limb buds of the endopodites of the third to fifth pleopods (arrowheads in A; see B for a morphological comparison). P6, six pereiopod; *P7*, seventh pereionite; *p1*, *p*2, first and second pleonites; p2, exopodite of the second pleopod; p3 and p5, third and fifth pleopods; ur, uropod (sixth pleonic appendage). Scale bar: 200 μm.

in an open circle that borders, external to it, the anterior–lateral basal edge of the labrum bud (arrowhead in Fig. 4A). From our data, this staining seems to be different from the more diffuse background staining and does not seem to appear in control embryos.

After the 80% stage (Fig. 5, A and B), that is, slightly after the stage just described, the general morphology of the embryo does not change markedly, other than that the appendages are slightly more elongated. The *PsAbd-B* staining pattern of the ventral ectoderm/nervous system is the same as in the previous stage, but stronger in intensity (Fig. 5A). There are, however, novelties in the pleon: *PsAbd-B* staining has largely disappeared from this body region except for the ventral ectoderm (similar to the other body regions) and a strong staining at the distal tip of the limb buds of the endopodites of the third to fifth pleopods (arrowheads in Fig. 5).

At later stages, the deposition of the cuticular matrix on the embryo's surface makes the penetration of the probes for in situ hybridization difficult. Morphologically, however, we found that the manca juveniles (the manca is the first post-embryonic stage, which has hatched but is still held by the mother within the brood pouch) were devoid of the last pereiopods (corresponding to the adult seventh pair) as well as the first pleopod and of the endopodite of the second (data not shown) and thus resembled the condition seen in these segments at the 80% stage.

DISCUSSION

The transient disappearance of the first pleopod and the endopodite of the second pleopod as "Lazarus developmental features"

It has been known for some time (e.g., Kaestner 1970; Schram 1986) that isopods hatch within the mother's brood pouch in the manca stage, with adult-like morphology, but lacking the eighth pair of thoracopods, that is, the seventh pereiopods. In *Porcellio*, the manca turns into the juvenile stage with a complete set of appendages, including the seventh periopods, after the third moult. However, to the best of our knowledge, nothing has yet been reported in the literature about similar behavior of the anterior pleopods of the Oniscidea (although the lack of—but not the embryonic development of—the first endopod has been reported for the manca larva and adult female of a marine species of Asellota; Elizalde and Sorbe

1992). Through the analysis of our specimens, both at the embryonic and manca stage, and of some published pictures (e.g., Abzhanov and Kaufman 1999b), it is clear that in contrast to the last pereionite (the seventh), which does not develop limb buds during any of the embryonic stages analyzed, the biramous limb buds of the first two pleonites are well developed in the early embryonic stages. Nevertheless, both branches of the first and the inner branch of the second pleopod are dramatically reduced in later embryonic stages (at about 75% development), but are found again, in well-developed form, in adults. At this later stage, they become either the typical pseudotracheae of the terrestrial Oniscidea, used for direct uptake of oxygen from the air (the large exopodites) and, in the male, the gonopods (the endopodites). These appendages thus represent an example of "Lazarus developmental features" (Minelli 2003), similar to the fourth pair of legs in mites: present in the embryo, lacking in the larval instars, and then reappearing in the nymphal and adult stages of most mites. Relevant to the present context is that several examples of Lazarus appendages have been recorded from decapod crustaceans. In the shrimp *Sergestes*, the appendages of the pereion (the maxillipedes as well as the locomotory legs), which are present in the previous mysis stage, lose their exopodites when reaching the mastigopus stage. Additionally, the last two pairs of locomotory legs are completely regressed. All of these structures, however, reappear during later stages. Similar phenomena have been observed for the maxillae and maxillipedes of a decapod larva of genus *Petalidium*. In scyllarid decapods, maxillae, first maxillipedes, and pereiopods IV–V, are formed in the embryo, partially reduced in late-embryonic and early postembryonic stages, but in later stages regain full growth again (Balss et al. 1940–1961; Schram 1986). Interestingly, the disappearance of the p1 and p2 appendages is correlated with a loss of *Abd-B* expression whereas accumulation is maintained in the p3–p5 appendages through the 80% stage. The cause–effect relationship is at present unclear and must await functional testing.

"Conserved" posterior expression of *PsAbd-B*

Abd-B is the most posteriorly expressed Hox gene in most arthropods. In *Drosophila*, the transcription of *Abd-B* gives rise to two different proteins, Abd-B m (m for "morphogenetic") and "Abd-B r (r for "regulatory") (Casanova and White 1987), differing by an additional sequence on the N terminus of the m protein. These two proteins act in two different and nonoverlapping domains: the r protein is expressed from the posterior of the eighth abdominal segment (pA8) to the anterior of the tenth abdominal segment (aA10), where it represses segmentation, whereas the m protein is expressed from pA4 to aA8 where it is involved in specifying and patterning the abdomen (e.g., Delorenzi and Bienz 1990).

The first appearance of *PsAbd-B* expression, within the growth zone, seems to clearly match the early-posterior pattern common to most of the arthropods studied to date (in addition to *Drosophila*, the desert locust *Schistocerca gregaria*; Kelsh et al. 1993, the firebrat *Thermobia domestica*; Peterson et al. 1999, the brown centipede *Lithobius atkinsoni*; Hughes and Kaufman 2002b, and the spider *Cupiennius salei*; Damen and Tautz 1999). This conserved posterior domain of expression is consistent with a conserved function possibly related or homologous to the Abd-B r protein's role in *Drosophila* (Casanova et al. 1986; Celniker et al. 1989; Kuhn et al. 1995). However, as our clone corresponds to a small region of the 3′ end of the *Abd-B* transcript, we are unable to identify possible 5′ splicing variants of the transcript—or variant gene products at all, if they exist in this animal—and the potential for an Abd-B m-type function.

Expression of *PsAbd-B* in the pleon

In two basal insects, *S. gregaria* and *T. domestica*, *Abd-B* is initially expressed in the last abdominal segments (A10 and A11). Subsequently, in later developmental stages, the expression pattern of *Abd-B* extends anteriorly to include the posterior half of the eighth abdominal segment (pA8) (Kelsh et al. 1993; Peterson et al. 1999). A similar dynamic extension of the expression domain is found in *Drosophila*. However, in this case, there is a difference in the extent of the abdominal spreading of the m protein from pA7 to pA4 (Delorenzi and Bienz 1990). In the spider *C. salei* (Damen and Tautz 1999), such an extension of expression is not clear, although, whereas segments are added at the rear of the animal, more and more opisthosomal segments are included in the *Abd-B* domain, that is, *Abd-B* expression is not confined to the growth zone but, once activated in the forming segments, it seems to remain in those segments in later developmental stages.

The appearance of *PsAbd-B* in the cells that will give rise to the pleonic limb buds in *P. scaber* is reminiscent of the expression pattern in the spider *C. salei*, where *Abd-B* is found in the second opisthosomal (op2) limb buds, surrounded by unstained cells (Damen and Tautz 1999). Thus, the expression of *Abd-B* in the opisthosomal and pleonic limb buds is consistent with the conclusion that it may function to specify the formation of at least some of these appendages. Moreover, in later stages, *PsAbd-B* clearly extends to the whole pleon, suggesting a possible role in the specification of the appendages first, and of the whole tagma later. This biphasic expression suggests that both m and r functions might be present in *P. scaber*, similar to the situation in the basal insect *T. domestica* (Peterson et al. 1999). In the isopod, however, the expression domain of *Abd-B* is broader and overlaps with the expression of another Hox gene *abd-A* (Abzhanov and Kaufman 2000a), a condition that is common in more basal arthropods. In contrast, the expression patterns of *Drosophila abd-A* and

Abd-B do not extensively overlap (see Hughes and Kaufman 2002a). The cypris larva of *S. carcini* provides another example of overlapping expression in crustaceans, where *Abd-B* extends not only through the whole vestigial abdomen but in females seems also to extend to the whole thorax (Blin et al. 2003; Deutsch and Mouchel-Vielh 2003). Yet again among the crustaceans, an exceptional situation is found in the anostracan branchiopod *A. franciscana*. In this species, both the *Abd-B* RNA transcript and the Abd-B protein are expressed solely in the two genital segments (Averof and Akam 1995; Copf et al. 2003), which are located between the thorax and the abdomen. This circumstance has led Copf et al. (2003) to hypothesize that the "postgenital segments," which are not only posterior to *Abd-B*, but do not show any Hox gene expression, represent a unique body region without a homologue in the other crustaceans discussed here.

"Neural-like" expression

The *P. scaber* embryonic ventral nerve cord consists of a pair of longitudinal connectives, with a single anterior and posterior commissure and paired segmental and intersegmental nerves, a condition similar to the insect ventral nerve cord (Whitington et al. 1993). Whitington et al. (1993) described three subesophageal, seven "thoracic," and seven "abdominal" ganglia in *Porcellio*. As Whitington et al.'s (1993) study was focused principally on embryonic axonogenesis, we lack a description of the general anatomy of the nervous system in the *P. scaber* embryo. It is, thus, difficult for us to make a direct comparison between the expression of *PsAbd-B* in the ventral ectoderm and the corresponding stages of nervous system development. Although *Abd-B* accumulates in a neural-like pattern, we lack direct evidence that this expression is actually neuronal. Moreover, we cannot rule out the possibility that this is an artifact corresponding to the rims of sclerites, although our control embryos do not seem to show this pattern of expression. However, in addition to specifying segmental identity, the Hox genes are also required for patterning the nervous system (Doe and Scott 1988; Prokop et al. 1998; Heuer and Kaufman 1992). In fact, the regulation by the Hox genes of such a basic component of metazoan anatomy is so evolutionarily widespread that patterning the nervous system may have been the original function of these genes, before being co-opted for patterning the anterior–posterior body axis (the "neuronal zootype hypothesis" of Deutsch and Le Guyader 1998). Limited attention has been paid to the involvement of *Abd-B* in the organization of the nervous system, as *Abd-B* is expressed, in most cases, in a more limited domain as compared with other Hox genes such as *Ubx*. Nevertheless, its function in specifying the posterior peripheral nervous system of *Drosophila* has been reported, for example, by Heuer and Kaufman (1992). In later larval stages of *A. franciscana*, outside the genital domain, *Abd-B* is expressed in some cells of the most posterior thoracic/trunk segments (Copf et al. 2003), which could represent forming ganglia. In *S. gregaria*, the posterior-most ganglion has many nuclei that express high levels of *Abd-B* (Kelsh et al. 1993). No comparable data have been reported for *S. carcini*, *T. domestica*, or *C. salei*, but, for these latter species, this may result from a lack of analysis of more advanced embryonic stages (*T. domestica*) or from the fact that no ganglia are present in the opisthosoma (*C. salei*).

In any case, if the observed *Abd-B* expression is indeed real and, specifically, neural, then the potential involvement of *PsAbd-B* in the whole developing nervous system represents a novel feature and raises the possibility that this Hox gene has extended its expression domain from a typical positional one—involved in patterning the animal body plan—to a domain circumscribed to a tissue type—in this case, the nervous system.

Novel role: expression in the endopodites of third to fifth pleopods

In the extreme diversity of body architectures found in the Crustacea, many different kinds of appendages are involved in respiration and their co-option for such a function is so diversified that homology among them is often easily ruled out. In the Malacostraca, gills, in general, derive from different proximal lateral elements of the pereiopods (the epipodite, the coxopodites, or even the pleura). However, in stomatopods and isopods, pleopods form the gills (Brusca and Brusca 2003). A striking adaptation of the terrestrial isopods, the Oniscidea, is the evolution of the pseudotracheae, developed from the first and second pleopod exopodites. These structures allow absorption of oxygen directly from the air. Additionally, the endopodites of the third to fifth pleopods, characterized by soft exoskeleton and an increased surface area because of folding, retain their respiratory function as gills, connected in some species, like *Porcellio*, to a cuticular water transport system that collects water from the environment (Kaestner 1970).

Based on the similar expression of the two developmental genes *pdm/nubbin* (*pdm/nub*) and *apterous* (*apt*), as well as fossil evidence, a common evolutionary origin for wings, breathing organs, and spinnerets has been suggested (Averof and Cohen 1997; Damen et al. 2002). This hypothesis implies that the common ancestor of insects, crustaceans, and spiders had a uniform trunk presumably characterized by similar biramous appendages in the different tagmata.

Decapod gills are derived from epipodites or from pleural protrusions, whereas the gills of isopods are derived from the endopodites of the pleopods and have a very different origin, and are thus not homologous. Nevertheless, some parallels in gene expression can be found. Although the expression pattern of *apt* in *Porcellio* has not been reported, *Ps-nub* is clearly

expressed in the pleopods, with a stronger signal in the third to fifth endopodite (Abzhanov and Kaufman 2000d). Stronger and more clearly confined to those endopodites is the expression of the *ventral veins lacking* (*vvl*) gene (Abzhanov and Kaufman 2000d), which in *Drosophila* is also involved in the formation of tracheal placodes and in tracheal elongation (Anderson et al. 1995; de Celis et al. 1995). Neither *nub* nor *vvl* are expressed as strongly or as exclusively as *PsAbd-B* in the third to fifth endopodites in later embryonic stages (these results). In addition to the above genes, *Drosophila Abd-B* seems also to be involved in the development of respiratory structures as it directs the formation of the posterior spiracles (Castelli-Gair 1998). Thus, it seems that several genes that are involved in the development of respiratory organs in arthropods are exclusively, or preferentially, expressed in the limb buds of what are destined to be the gills of isopods. However, as we lack direct functional evidence, we cannot establish a direct causal relationship between the transcription of these genes and the final morphological outcome. Moreover, the presence of those transcripts does not imply homology of these structures, but likely represents an independent co-option of these genes in broadly equivalent processes. With respect to a possible role of *Abd-B* in the respiratory organs, it may be that, in the salient endopodites, it simply represses the development of a more conventional appendage, as it does in the genital disc of *Drosophila* (Estrada and Sánchez-Herrero 2001) rather than having a constructive responsibility.

Abd-B is expressed and apparently involved in the development of genitalia, and in early embryos, *PsAbd-B* is expressed in those limb buds that will eventually give rise to the gonopods albeit not exclusively so. Although we cannot rule out additional exclusive postembryonic expression of *Abd-B* in the same appendages, this gene would not appear to be solely involved in this kind of "posterior" reproductive appendage. This observation raises doubts as to the possibility of a strict correlation of *Abd-B* expression and function to the genitalia.

CONCLUSIONS

The evidence presented in this article shows the developmentally dynamic expression pattern of the Hox gene *Abd-B* in *P. scaber*. The gene's expression begins in a localized pattern in the posterior growth zone, which expands to include domains in the pleopods, and then subsequently in the whole pleon. This expansion is followed by a restriction to detectable appendage expression in only the third through fifth endopodites of the pleopods. Additionally in the later stages, the gene is also expressed in what appears to be the developing nervous system. These dynamic changes in expression emphasize the utility of examining the entirety of embryonic development. This also cautions against an overreliance on convenient but overly simplistic summary diagrams of expression patterns when comparing different species. An injudicious choice of a single stage would hide the diversity of expression and a possible variety of functions and lead to erroneous comparisons. Additionally, one should also keep in mind that asserting cavalier homologies between different parts and different appendages should be avoided because, as discussed by Schram and Koenemann (2001) and by Boxshall (2004), complexity and diversification can render evolutionary interpretations difficult and often subjective.

Despite the fact that *Abd-B* does appear generally to be expressed in a specific posterior position according to well-entrenched developmental pathways, based on the evidence presented here, it may also be co-opted, within that major portion of the body and utilized to specify more specialized traits, such as particular appendages. In the case of the arthropods, these may be genital, respiratory, or other specialized types of abdominal/pleonic appendages. This occurs in a strictly context-dependent way, following a trend from more general body patterning to more derived and specialized structures as can be seen for other Hox genes (see, e.g., Stern 1998).

We would add a final short note to stress the value—from an evolutionary point of view—of the pleonic appendages. Unfortunately, these limbs have been less well studied than is their due. They clearly represent an important aspect of the diversification seen in crustacean appendages and whereas perhaps less obtrusive than their larger more anterior pereionic cousins they have played just as important a role in the evolution and diversification of this group of organisms.

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