Hox genes in the honey bee Apis mellifera

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ORIGINAL ARTICLE

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Hox genes in the honey bee *Apis mellifera*

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Abstract Hox genes are known to control the identity of serially repeated structures in arthropods and vertebrates. We analyzed the expression pattern of the Hox genes *Deformed* (*Dfd*), *Sex combs reduced* (*Scr*), *Antennapedia* (*Antp*), and *Ultrabithorax*/*abdominal-A* (*Ubx*/*abd-A*) from the honey bee *Apis mellifera*. We also cloned a cDNA with the complete coding region of the *Antennapedia* gene from *Apis*. Comparison with Antp proteins from other insect species revealed several regions of homology. The expression patterns of the isolated Hox genes from *Apis* showed that the original expression patterns of *Dfd*, *Scr*, and *Antp* appear between late blastoderm and early germ band stage in a temporal and spatial sequence. Each of them shows up as a belt, spanning approximately two segment anlagen, *Dfd* in the anterior gnathal region, *Scr* in the posterior gnathal and anterior thoracic region, and *Antp* in the thoracic region. Following expansion of the *Antp* domain in the abdomen as a gradient towards the posterior, *Ubx/abd-A* expression appears laterally in the abdomen. During gastrulation and in the germ band stage the domains of strong expression do not overlap any more, but touch each other. After gastrulation the borders of the expression domains partly correlate with parasegment and partly with segment boundaries. Laterally, gaps between the domain of each gene may show no expression of any of the genes examined.

Key words *Dfd · Scr · Antp · Ubx · abd-A*

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Introduction

In insects as well as in vertebrates, pattern formation processes are controlled by a class of regulatory genes, the Hox genes. They exert their function by activating different sets of downstream target genes responsible for distinct body structures (McGinnis and Krumlauf 1992; Carroll 1995). Hox gene functions have been studied most intensively in *Drosophila*, where these genes are located in two complexes, the *Antennapedia* complex (Kaufman et al. 1980) and the *Bithorax* complex (Lewis 1978).

In the last few years, orthologues of Hox genes from a variety of arthropod taxa have been identified and their expression patterns analyzed. Among these are other Diptera like *Musca* (Sommer and Tautz 1991), the beetle *Tribolium* (Stuart et al. 1993), the grasshopper *Schistocerca* (Tear et al. 1990; Kelsh et al. 1994; Hayward et al. 1995), the butterfly *Precis* (Warren et al. 1994), the moth *Bombyx mori* (Nagata et al. 1996), the firebrat *Thermobia* (Peterson et al. 1999), the chelicerates *Archegozetes* (Telford and Thomas 1998) and *Cupiennius* (Damen et al. 1998) as well as the centipede *Ethmostigmus* and the onychoporan *Acanthokara* (Grenier et al. 1997). A major goal of all these analyses was to understand the role of the Hox genes during evolution and the pathways of evolution, but also to compare the molecular mechanisms of pattern formation processes which created such a diversity of species.

Some time ago, we cloned the first Hox genes from a non-Drosophilid insect species, the honey bee *Apis mellifera* (Fleig et al. 1988; Walldorf et al. 1989). *A. mellifera* is a member of another large insect order, the hymenoptera, and diverged from *Drosophila* about 250 million years ago (Riek 1979). *Apis* is a long germ de-

veloper like *Drosophila*, but in contrast to the latter, the embryogenesis of *Apis* offers several advantages when studying expression patterns:

1. The blastoderm stage lasts about 24 h and the second half is without any mitoses (Fleig and Sander 1986). At this stage, one segment anlage is about 8 cells wide.
2. During all of gastrulation, segmental boundaries can be seen morphologically as grooves in the ectoderm, as well as in the mesoderm. Segmental and parasegmental boundaries can also be seen in this period using anti-En labelling (Fleig 1990).
3. The honey bee embryo does not perform germ band elongation and retraction.
4. *Apis* embryos do not show head involution like *Drosophila*. Therefore we expected to be able to follow the temporal and spatial pattern of homeotic genes in detail through all stages.

Comparison of Hox gene expression patterns from *Apis* and other insects may help to distinguish between special traits and general lines in insect development. A partial sequence and the expression pattern of the *Apis* orthologue from the *Drosophila Deformed* (*Dfd)* gene have been described previously (Fleig et al. 1988; Fleig et al. 1992). We extend our analysis now to the Hox genes *Sex combs reduced* (*Scr*), *Antennapedia* (*Antp*) and *Ultrabithorax/abdominal*-A (*Ubx/abd-A*) from the honey bee, and compare the sequence of the *Antennapedia* gene from *Apis* with orthologues from other species.

Materials and methods

Cloning of the *Apis Antennapedia* gene

An embryonic *Apis* cDNA library was constructed from 0–60 h old embryos (complete embryogenesis). mRNA was isolated using the Quick Prep mRNA Purification Kit (Pharmacia), and cDNAs were constructed using the ZAP cDNA synthesis Kit (Stratagene) and cloned into the Uni ZAP XR vector (Stratagene). A 0.7 kb genomic fragment from phage H90 (Walldorf et al. 1989) which includes *Antennapedia* sequences was used to screen 1.5×10^5 plaques of the embryonic cDNA library under high stringency conditions (Sambrook et al. 1989). Several positive phages were isolated and cloned by in vivo excision from the Uni ZAP XR vector.

DNA sequencing and sequence analysis

DNA was sequenced by the dideoxynucleotide procedure of Sanger et al. (1977). Sequencing was done on both strands of the DNA with the Sequenase Version 2.0 DNA sequencing kit (USB). Overlapping deletions were generated by using the exonuclease III-S1 method as described by the supplier (Pharmacia). In addition, gene-specific primers deduced from previously determined sequences were used. Sequences were analyzed by using the HUSAR/GCG sequence analysis software package from the University of Heidelberg.

Embryo staining

Bee keeping, egg collection, incubation and treatment of the embryos were as described earlier (Fleig and Sander 1986; Fleig 1990). Staining of mRNA was performed according to Tautz and Pfeifle (1989), and preparation of antibodies and antibody staining was performed according to Fleig et al. (1992). As probes for in situ hybridization experiments, a 0.7 kb *Bgl*II fragment from phage H90 (bee *Antp*) and a 1.5 kb *Xho*I-*Eco*RI fragment from phage H55 (bee *Scr*) (Walldorf et al. 1989) were used. For *Ubx/abd-A* stainings, we used the antibody FP6.87 kindly provided by M. Akam.

Results

The *Apis Antennapedia* gene

In our initial screen for homeobox-containing genes of the honey bee, we isolated genomic clones for seven genes, among them clones for *Apis* orthologues of *Deformed*, *Sex combs reduced*, *Antennapedia* and *abdominal-A* (Fleig et al. 1988; Walldorf et al. 1989). To characterize the *Antp* gene, we constructed an embryonic cDNA library and isolated several cDNA clones from the *Antp* gene of honey bees. The longest cDNA clone, of 2.1 kb, was further analyzed and sequenced.

An open reading frame, starting at position 118 and ending at position 1176, encodes a protein of 352 amino acids, including an *Antp* homeodomain. The protein has a predicted molecular weight of 39.8 kD and an isoelectric point of 9.85. A comparison of the homeobox sequence from the cDNA with the genomic sequence verified that the isolated clone corresponds to the *Antp* gene of honey bees. Further sequence conservations outside the homeodomain support this (Fig. 1).

A sequence comparison of the *Apis* Antp protein with proteins from other insect species is shown in Fig. 1. Here we used only species where the complete or almost complete open reading frame is known. In the homeodomain only one position is not conserved between the species analyzed, since in the honey bee a phenylalanine at position 23 is replaced by a tyrosine. Outside the homeodomain several conserved regions were found. They all agree with the regions 1–7 described by Hayward et al. (1995). The Antp protein of honey bees shows the closest relationship to the *Bombyx* Antp protein.

Expression of Hox genes from *Apis*

Deformed *(*Dfd*)*

The *Dfd* pattern has already been published (Fleig et al. 1992) except for the following details. However we de-

Fig. 1 Comparison of the Antennapedia protein of honey bees with other insect species. The deduced amino acid sequence of the Antennapedia protein of *Apis mellifera* (Genbank Accession No AJ276511) is compared to homologous sequences from *Schistocerca americana* (Genbank Accession No U32943), *Drosophila melanogaster* (Genbank Accession No M20704) and *Bombyx mori* (Genbank Accession No D16684). Amino acid sequences are shown in one letter code. Conserved regions (1–7) are indicated by *black bars* below the sequences. Amino acids which are conserved in at least one other species compared to *Apis* are shown in *bold* ▶

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          1                                                  50
AntpBee   MSSYFANSYI PDLRNGGVEH PHQHQQHYGA AVQVPQQTQS VQQQSQQAGD
AntpSch   MSSYFAVQQG PGG..... .......... .......... .......GAD
AntpDro   MTSYFTNSYM GAD..... ..MHHGHY.. .......... ..........
AntpBom   .......... .......... .......... .......... ..........
          ═1═

          51                                                 100
AntpBee   PCDPSLLRQG VPGHHYGAAG SQQ.DMPYPR FPPYNRMDMR NATYYQHQQD
AntpSch   PSSGAGAASW GGGGGGAGAQ QPPQGMPYPR FPPYDRMDIR SAAYYGAQQS
AntpDro   PGNGVTDLDA QQMHHYSQNA NHQGNMPYPR FPPYDRMPYY NGQGMDQQQQ
AntpBom   .MDGCDQQLR PAQHHYPAQP APG..MPYPR FPPYDRLGYY .......QQM
                                  ═══2═══

          101                                                150
AntpBee   HGSGMDGMGG YRSASPSP.. GMGHMGHTPT PNGHP..... ..........
AntpSch.  GGG......G GLDGSPGGGG GNGGGGGGGY RSGSP..... ..........
AntpDro   HQV......Y SRPDSPSS.. QVGGVMPQAQ TNGQLGVPQQ QQQQQQQPSQ
AntpBom   EQN......G YRPDSPS... QMGHMGPKTD GYGPN..... ..........

          151                                                200
AntpBee   .......... .......... ........S. ...TPIVYAS CKLQAAAVDH
AntpSch   .......... .......... .......MGG GGQTPVVYAS CKLQAAAAAA
AntpDro   NQQQQQAQQA PQQLQQQLPQ VTQQVTHPQQ QQQQPVVYAS CKLQAAVGGL
AntpBom   .......... .......... ........GH QPATPAVYTS CKLQAAAATG
                                                ═══3═══

          201                                                250
AntpBee   QGSVLD.... ..GPDSPPLV ESQMHHQMHT QHPHMQPQQG QHQSQAQQQH
AntpSch   AAAAAGNGGG AGGGGSPPLD VPPHQHQHHA HHAPPHLQQQ QQQQQQLYAD
AntpDro   GMVPE..... ...GGSPPLV DQMSGHHMNA QMTLPHHMGH PQAQLGYTDV
AntpBom   GAVP...... ....GSPPLE QAQQMPHHMH PQQHMVQHGV PPHQQH....
                      ═4═

          251                                                300
AntpBee   LQAHEQHMMY .....Q.... ...QQQQSQA ASQQSQPGMH PRQQQQAQQH
AntpSch   PAPQQQQQQQ PPPTQQPPPV PPPHQHQPPL GAGVPPPGHQ HQHQHPQQQQ
AntpDro   GVPDVTEVHQ .....NHHNM GMYQQQSGVP PVGAPPQGMM HQGQGPPQMH
AntpBom   .......... .......... .......... .LMYPVDDMQ HQTQMPPMHQ

          301                                                350
AntpBee   QGVVTSPLSQ QQQAAPQGAA SANLPSPLYP WMRSQF... .ERKRGRQTYT
AntpSch   Q......PPP QQ...HNGSP ANTLPSPLYP WMRSQF... .ERKRGRQTYT
AntpDro   QGHPGQHTPP SQ...NPNSQ SSGMPSPLYP WMRSQFGKC QERKRGRQTYT
AntpBom   QSMHAQQPPP QQ...PPPNT KPSLPSPLYP WMRSQF... .ERKRGRQTYT
                                  ═══5═══

          351                                                400
AntpBee   RYQTLELEKE FHYNRYLTRR RRIEIAHALC LTERQIKIWF QNRRMKWKKE
AntpSch   RYQTLELEKE FHFNRYLTRR RRIEIAHALC LTERQIKIWF QNRRMKWKKE
AntpDro   RYQTLELEKE FHFNRYLTRR RRIEIAHALC LTERQIKIWF QNRRMKWKKE
AntpBom   RYQTLELEKE FHFNRYLTRR RRIEIAHALC LTERQIKIWF QNRRMKWKKE
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          401                      428
AntpBee   NKSK...GTP GSGDGDTEIS PQTSPQG
AntpSch   NKSKPDAGQN GDGNAGSDIT PQTSPQ.
AntpDro   NKTK...GEP GSGGEGDEIT PPNSPQ.
AntpBom   NKTK...GEP GSGDEPDNMS PPTSPQ.
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scribe these data briefly for completeness. In 27-h-old embryos, which is an intermediate stage of the cellular blastoderm (Fig. 2A), a belt of nuclei can be seen labelled with anti-Dfd antibodies (Fleig et al. 1992). Its position is between 65–78% embryo length (posterior pole is 0%). The belt is about 16 cells wide dorsally, in tissue of the future serosa, but it is about 22 cells wide ventrally in embryonic parts of the blastoderm. This is ventrally equivalent to almost three segment anlagen. Labelling is strongest in the middle of the belt and be-

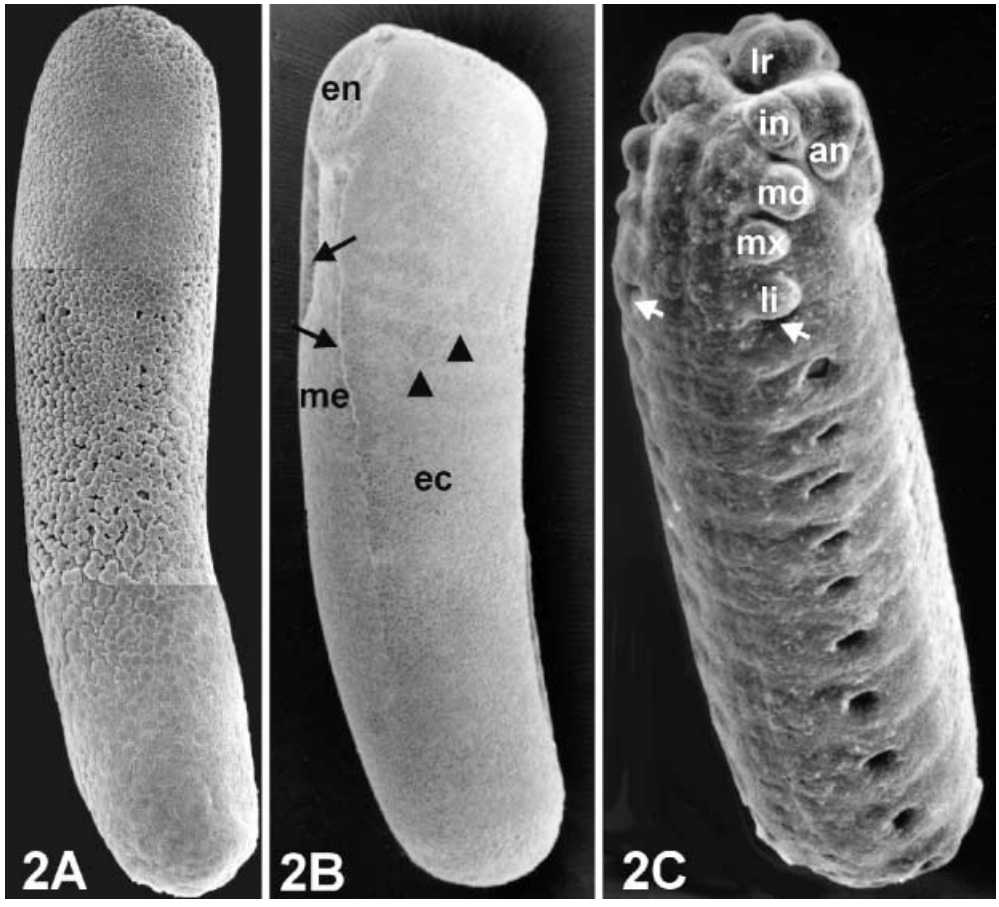
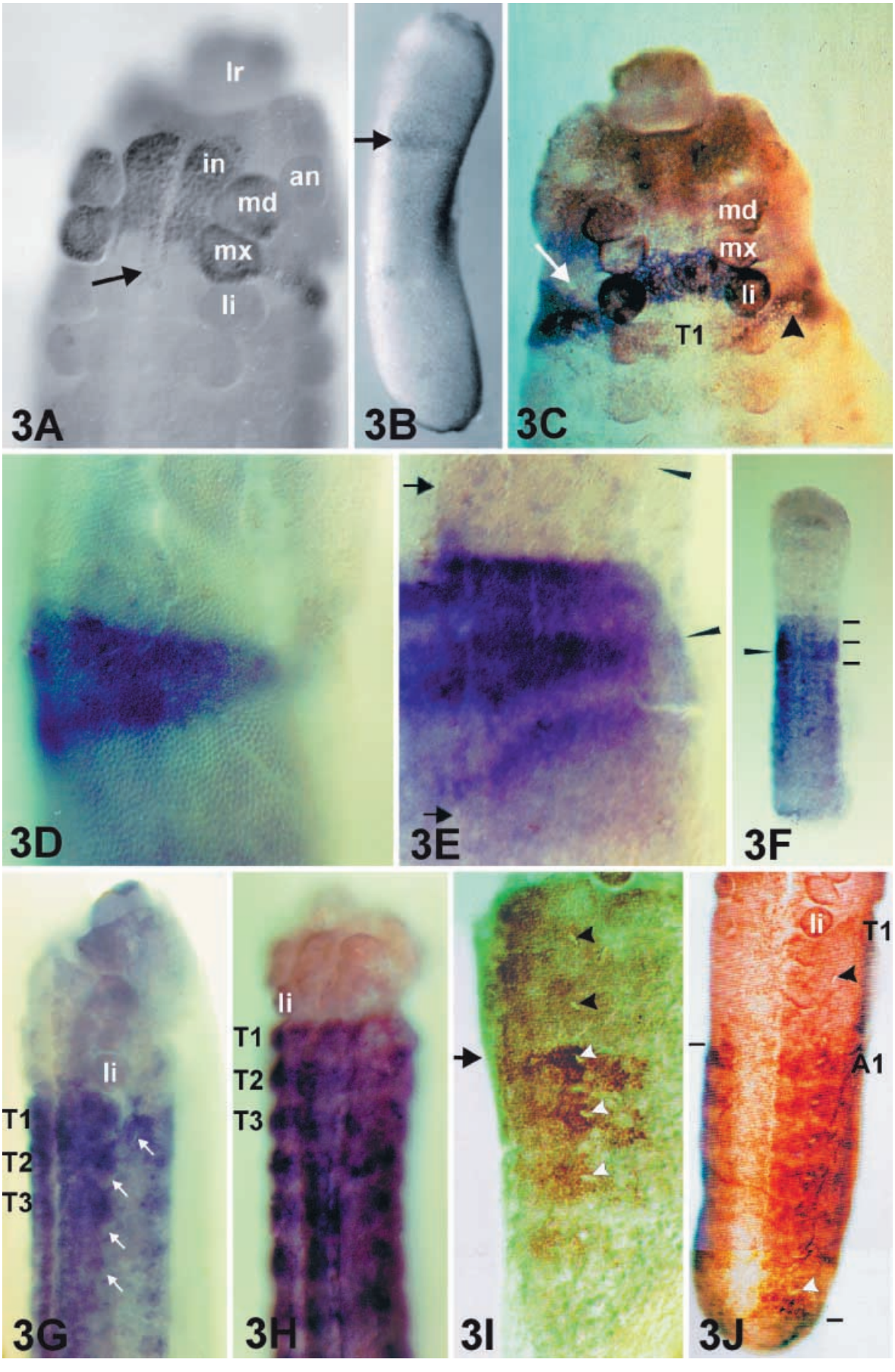


Fig. 2A–C SEM view of honey bee embryos, ventral to the *left*. **A** Blastoderm formation; mitotic wave, compare the size of the blastoderm cells anterior (*top*) and posterior (*bottom*). **B** Early gastrulation: anterior endoderm (*en*), ectoderm (*ec*), mesoderm (*me*), gastrulation folds (*arrows*), intersegmental grooves (*arrowheads*). **C** Young germ band: labrum (*lr*), antenna (*an*), intercalary rudiment (*in*), mandible (*md*), maxilla (*mx*), labium (*li*), salivary gland opening (*arrows*), and ten tracheal openings. Total length of the embryos: 1.6 mm

Fig. 3. Expression of *Dfd* (**A**), *Scr* (**B–C**), *Antp* (**D–H**) and *Ubx/abd-A* (**I–J**) in honey bee embryos. **A** *Dfd* expression. Germ band stage, 45 h old, anterior half, ventral view. *Dfd* is ventrally in the posterior compartment of the intercalary segment rudiment, in the complete mandibular segment, laterally complete and ventrally only in the anterior compartment of the maxillary segment, and in the most ventral part of the labial segment only below the surface (*arrow*). **B** *Scr* expression. Gastrulation stage, 31 h old, lateral view. A faint belt of *Scr* transcripts appears in the posterior head anlage (*arrow*). **C** *Scr* expression. Germ band stage, 45 h old, anterior half, ventral view. Strong *Scr* is seen ventrally between the mouth part buds in the posterior compartment of the maxillary segment, and in the labial segment except for the area of the tentorium invagination (*arrow*), an unknown spot laterally in the middle of the labelled area (*arrowhead*), and the ventral part of the posterior compartment. Weak label is seen ventrally in the posterior compartment of the labial segment and in the first thoracic segment (*T1*) ventrally and ventrolaterally. **D** *Antp* expression. Blastoderm stage, 32 h old, anterior detail, lateral view. *Antp* transcripts can be detected in a belt between 55 and 68% embryo length. The belt is much wider ventrally than dorsally. **E** *Antp* expression. Gastrulation stage, 34 h old, anterior detail, lateral view. The *Antp* belt is now wider, especially on the lateral side, and it is subdivided into three stripes. *Arrowheads* indicate lateral margin of the germ band; *arrows* indicate gastrulation fold. **F** *Antp* expression. Young germ band stage, 40 h old, ventral view. *Antp* is found weakly in the ventral part of the posterior compartment of the labial segment and in the prothorax, very strongly in the mesothorax (*arrowhead*), and gradually more weakly in the metathorax and the abdominal segments. The *bars* indicate the position of the borders of pro- and mesothorax. **G** *Antp* expression. Intermediate germ band stage, 45 h old, anterior half, ventrolateral. *Antp* is weak ventrally in the posterior compartment of the labial segment, strong in the ventral and ventrolateral part of the prothorax, strong in the mesothorax, intermediate in the metathorax, and gradually weaker in the abdomen. In the abdomen it is confined to the ventral nerve cord and around the tracheal pits (*arrows*). **H** *Antp* expression. Old germ band stage, 55 h old, anterior half, ventral view. No *Antp* transcripts are in the head region, label is in the whole trunk, strongest in the ventral nerve cord. **I** *Ubx/abd-A* expression. Intermediate germ band stage, 42 h old, lateral view of thoracic and and first abdominal segments. Border between thorax and abdomen (*arrow*), thoracic tracheal pits (*black arrowheads*), abdominal tracheal pits (*white arrowheads*). Strong label is found in the first abdominal segment and gradually weaker towards the fourth abdominal segment. **J** *Ubx/abd-A* expression. Intermediate germ band stage, 45 h old, gnathal, thoracic and abdominal region, ventral view. Label is seen in the lateral parts of the abdominal segment A1 (*bar* on the *left*) to A7 (*bar* on the *right*), strongest around the tracheal pits, weak in the segmental grooves, and not present in the ventral parts of the segments. *Black arrowhead* denotes the tracheal pit of T1; *white arrowhead* denotes the tracheal pit of A7 ▶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comes gradually weaker towards both sides. After the beginning of gastrulation (Fig. 2B), 33 h after egg deposition, the weak label on the anterior and posterior side of the belt disappears, resulting in sharp borders between labelled and non-labelled cells (Fleig et al. 1992). The label also disappears in the mesoderm part of the belt ventrally between the two lateral parts of the ectoderm, and dorsally in the developing serosa. The remaining labelled area in the two halves of the ectoderm now covers the posterior compartment of the intercalary, the mandibular and the maxillary segment anlagen, as can be shown by double labelling with antibodies against Dfd and En (Fleig et al. 1992). During gastrulation, when both lateral halves of the ectoderm start fusing ventrally, the final pattern of *Dfd* is established when Dfd protein disappears in the ventral part of the posterior compartment of the maxillary segment. The resulting pattern of label in the posterior compartment, the ventral part of the intercalary rudiment, the mandibular segment, and the maxillary segment except the ventral part of the posterior compartment, remains stable in the epidermis and the central nervous system during the germ band stage (Fig. 2C) until the larval cuticle prevents labelling; it is only in the central nervous system of the germ band, where additional anti-Dfd labelling appears in the labial segment (Fig. 3A).

Sex combs reduced *(*Scr*)*

In 31-h-old blastoderm stages, shortly before the beginning of gastrulation, a belt of cells is found containing mRNA of *Scr* in the region of the labial segment anlage (Fig. 3B). Its position is between 62% and 75% embryo length. The labelled belt is approximately twice as wide on the ventral side as on the lateral. In the germ band stage the pattern of *Scr* is more complicated (Fig. 3C). Ventrally, between the mouth part buds, an area is strongly labelled. It corresponds to the posterior compartment of the maxillary segment and the anterior compartment of the labial segment. Only weak label can be found in the ventral parts of the posterior compartment of the labial segment and in the prothorax. Ventrolaterally strong label is found only in the labial mouth parts, i.e. in both compartments of the labial segment. Weak label is seen posterior to the labial mouth parts in the prothoracic segment. Laterally, between the mouth parts and the lateral border of the germ band, *Scr* expression can be seen in the posterior compartment of the labial segment and in the anterior compartment of the prothoracic segment. The cells around the invagination of the posterior part of the tentorium show no label. In the middle of the labelled prothorax area on both sides there is a peculiar spot of cells of unknown fate which are always devoid of *Scr* label. Weak label is also present in the stomodeum. *Scr* label is restricted to the ectoderm throughout. In the germ band stage, the strong *Scr* expression domain crosses two different segment borders, maxillary-labial (ventrally), and labial-prothoracic (laterally). The strong expression domain is only segmental in the mouth parts, whereas ventrally and laterally it is parasegmental.

Antennapedia (Antp)

In 32-h-old embryos, just before the beginning of gastrulation, an initial pattern of *Antp* mRNA can be detected in a belt between 55% and 68% embryo length (Fig. 3D). The labelled area is about three times wider ventrally than it is dorsolaterally, and no label can be found in the most dorsal extraembryonic serosa tissue. The anterior and the posterior borders of the labelled area are very sharp. The labelled area is ventrally approximately identical with the pro- and mesothorax anlage.

During gastrulation a second pattern develops (Fig. 3E). The original belt widens during gastrulation, when its posterior border shifts to 50% embryo length. It is now almost as wide dorsolaterally as ventrally and covers the complete thorax anlage. This wide belt is subdivided into three stripes which are strongly labelled and separated by two stripes showing only weak label. All parts of the embryo posterior to this belt at this stage show weak label. In contrast, in the head anlage we have not found any sign of *Antp* mRNA at this stage.

In the young germ band stage, 40 h after egg deposition, a third pattern shows segmental and parasegmental traits (Fig. 3F). Very strong label is seen in the complete second thoracic segment. From there to the posterior pole the label gradually weakens. In the first thoracic segment, the ventral part is labelled to an intermediate level, whereas the lateral parts as well as the ventral part of the posterior compartment of the labial segment exhibit low level of *Antp* mRNA. Anterior to that, no label is found in the head segments.

During the germ band stage a fourth pattern of *Antp* expression evolves (Fig. 3G). Weak label is still seen ventrally in the posterior compartment of the labial segment and strong label in the ventral and ventrolateral parts of the three thoracic segments. Gradually weaker label is seen in the ventral part of the abdominal segments, strong label in the lateral parts of the second thoracic segment, and intermediate label around the tracheal pits from T3 to A8. Transiently *Antp* completely disappears in the lateral parts of the first thoracic segment. It is only in the oldest stages during dorsal closure when *Antp* transcripts appear again in this area but they are not seen any more in the labial segment (Fig. 3H). In the second thoracic segment label remains strongest. In the third thoracic and abdominal segments it remains visible in an anterior-posterior gradient until dorsal closure about 55 h after egg deposition. In this late stage, a few hours before hatching, intermediate expression is found only in the ventral nerve cord and in domains close to the tracheal pits, whereas in the epidermis, only weak expression remains until staining becomes impossible when the larval cuticle seals off the surface of the embryo.

Ultrabithorax (Ubx) and abdominal-A (abd-A)

The antibody FP6.87 used here recognizes the products of the *Ubx* and *abd-A* genes via an epitope common to the carboxy-terminal region of the two proteins (Kelsh et al. 1994). Therefore we cannot distinguish between Ubx and Abd-A proteins. Whenever we use the term *Ubx/abd-A* expression, we mean the combined expression patterns of both genes. The antibody recognizes antigen in the first seven abdominal segments of the honey bee embryo. Staining is first detected in 45-h-old intermediate germ band stage embryos. In this stage label is found only in the first four abdominal segments, strongest in the first and weaker towards the posterior (Fig. 3I). In older germ band stages the expression domain was found to be expanded towards the posterior up to the seventh abdominal segment, inclusive (Fig. 3J). Expression intensity is now equal in the abdominal segments A1–A6 and weaker only in the abdominal segment A7. Label is only in the nuclei of the lateral epidermis. Nuclei of cells in the segmental grooves are only weakly labelled, whereas cells around the tracheal pits show the strongest labelling. The ventral parts of the abdominal epidermis, which cover the ventral nerve cord (where *Antp* is found), are never labelled by the antibody. Anterior to the first abdominal segment no label can be detected in any stage. This is also true for the abdominal segments A8–A10.

Discussion

Antennapedia sequence

We have cloned a cDNA from the *Antp* gene of the honey bee *A. mellifera*. The translation start site is in agreement with that from *Schistocerca* and not further upstream as predicted by Hooper et al. (1992) for *Drosophila*. Several conserved domains within the Antp proteins were already mentioned by Hayward et al. (1995) and verified by our analysis. For most of these domains no functions are known yet, except for the YPWM domain and of course the homeodomain. The YPWM motif is present in several homeotic proteins and mediates interactions with a cofactor encoded by the *extradenticle (exd)* gene (Rauskolb and Wieschaus 1994). Since residues flanking the YPWM motif of the vertebrate HoxD4 gene contribute to cooperative interactions with the vertebrate *exd* homologue PBX (Shanmugam et al. 1997), it is not surprising that residues flanking YPWM are conserved as well in different Antp proteins. The homeodomain represents the most highly conserved domain of the protein with only one alteration in the honey bee at position 23 (F to Y). The C-terminus shows conservations as well whereas the 3′ untranslated region is, like in *Schistocerca*, not conserved in honey bees compared to *Drosophila*.

Borders of expression domains, parasegments, and segments

Assuming that there is no spatial and only a short temporal difference between the patterns of mRNA and protein, the gene products of all four genes discussed here appear in an anteroposterior sequence in the last hours of the blastoderm stage. This is also the case for the formation of the blastoderm, the waves of blastoderm mitoses, the pattern of gastrulation (Nelson 1915; Schnetter 1935; Fleig and Sander 1986) and *Engrailed* expression (Fleig 1990). In contrast to *Dfd* and *Scr*, the patterns of *Antp* and later also *Ubx/abd-A* secondarily expand towards the posterior. Comparing the stable patterns of the two anterior genes with the expansion of *Antp* during gastrulation, and the expansion of *Ubx/abd-A* during early germ band stage, one might think of short germ developers like grasshoppers and crayfish, where at first only the anterior part of the body is established and the abdominal segments only appear later (Patel et al. 1989; Patel 1994).

The early expression domains of the homeotic genes *Dfd*, *Scr*, and *Antp* show a simple pattern of belts covering about two segment anlagen each; the domains overlap, especially on the ventral side of the embryo where they are broader than dorsally (Fig. 4A). The original domain borders may partly be consistent with parasegmental or segmental boundaries, but at least those of *Antp* are oblique to these boundaries, which become visible at this stage (Fleig 1990).

In the blastoderm stage the expression domains of all four genes are similar in fruit fly and honey bee embryos. Certainly these genes play very similar roles in establishing the general character of that part of the insect body where they have their main expression domain. Even in Chelicerata the orthologues show comparable patterns (Damen et al. 1998; Telford and Thomas 1998). However, some differences should be mentioned. In the fly, the borders of the *Dfd* domain are segmental (Chadwick and McGinnis 1987; Regulski et al. 1987). In the bee, the anterior expression border is initially parasegmental in the rudiment of the intercalary segment, and changes from segmental to parasegmental during gastrulation in the ventral part of the maxillary segment (Fleig et al. 1992). The steady *Engrailed* pattern shows that this change is not a result of cell movement (Fleig et al. 1992). The same development of that pattern is reported for *Tribolium* (Brown et al. 1999). This is most clearly seen ventrally in the parasegmental *Scr* pattern in the germ band of the bee, corresponding to segmental in that area in the fly (Mahaffey and Kaufman 1987; LeMotte et al. 1989; Mahaffey et al. 1989; Rogers et al. 1997). While some differences in the *Dfd* pattern are consistently observed between the bee and the fly, the complicated posterior borders of the *Scr* and the *Antp* patterns of young and old embryos of flies (Levine et al. 1983; Hafen et al. 1984; Carroll et al. 1986; Martinez-Arias 1986; Wirz et al. 1986; Riley et al. 1987; LeMotte et al. 1989; Bermingham et al. 1990; Hayward et al. 1995; Rogers et al. 1997) and bees are identical. The

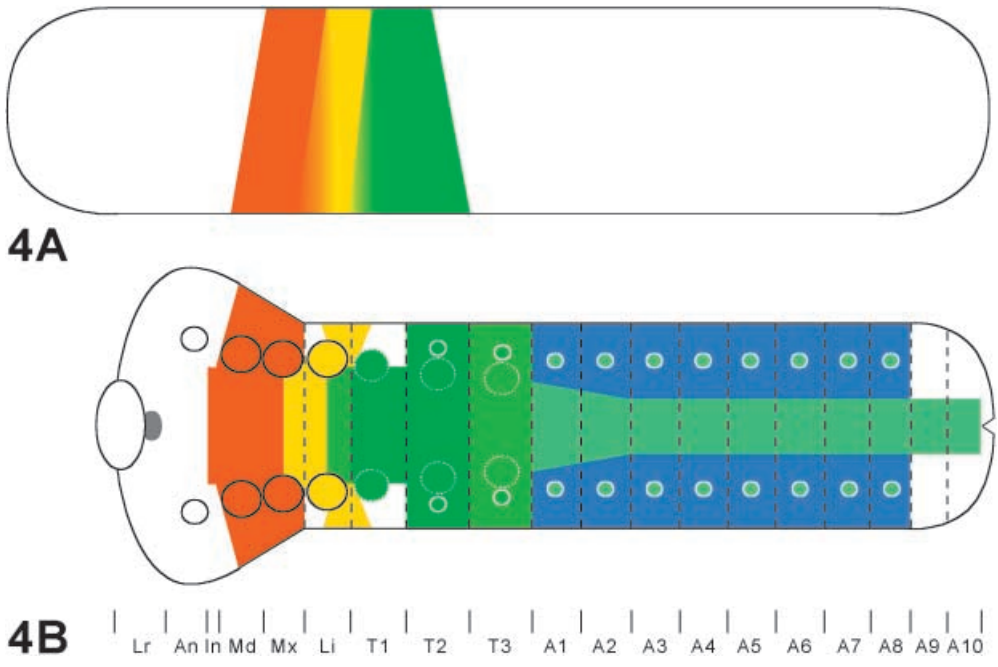

Fig. 4A, B Schematic drawings of expression domains of *Dfd* (*orange*), *Scr* (*yellow*), *Antp* (*green*) and *Ubx/abd-A* (*blue*). **A** Blastoderm stage, 32 h old, lateral view; intermediate colours show overlapping zones. **B** Germ band stage, 45 h old, ventral view. The anterior part of the blastoderm moves during gastrulation around the pole towards the dorsal side and is therefore not seen in the ventral view of the germ band (see Fig. 2)

same *Antp* pattern is also found in *Thermobia domestica* (Peterson et al. 1999). However, in *Manduca sexta* the anterior border of *Antp* is the compartment border of T1 (Zheng et al. 1999). It may be that some of these differences are only the result of different sensitivity levels of the stainings. Probably the ventral switch from segmental to parasegmental is more general in insects but is not seen very clearly in some cases, such as in the anterior part of the fruit fly head, due to the subsequent head involution. The resulting ventrolateral corners of the expression borders of *Dfd*, *Scr*, and *Antp* correlate fairly well with the position of the appendage anlagen and may help to pin down their position.

Expression patterns in the germ band

During germ band development the expression patterns of the four genes examined here become more complicated and presage specific body structures (Fig. 4B). No overlapping of strong expression domains can be found any more, except in the areas around the abdominal tracheal pits, where both *Antp* and *Ubx*/*abd-A* can be detected. The borders of each domain touch each other almost everywhere. A gap remains only laterally between *Dfd* and *Scr* in the anterior compartment of the labial segment. Most of this non-labelling tissue invaginates to form the posterior part of the tentorium. The anterior part of that head skeleton is from the anterior part of the intercalary segment anterior of the *Dfd* domain (Nelson 1915; Fleig et al. 1988; Fleig et al. 1992). A transient expression gap is also seen laterally during early germ band stage between the *Scr* and the *Antp* domains, where no tracheal invaginations form in the prothorax. Here *Antp* disappears during the period when the tracheal system invaginates in the other two thoracic segments and in the abdomen. In all other instances the strongly labelled domains fit perfectly together. This is seen most convincingly ventrally and in the areas of the mouth parts, where the borders of the strong expression domains jump from segmental to parasegmental boundaries.

The lateral expression of *Scr* in the prothorax of the bee may cause wing suppression as in fly, cricket and bug (Rogers et al. 1997). In the prothoracic segment the relationship between *Scr* and *Antp* seems to be essential for the differentiation of middle (*Antp*) or anterior (*Scr*) thorax characters. The overlapping presence of different levels of mRNA of both genes in the germ band of the honey bee may show that the decision is made late and can easily switch from one to the other, as has been shown for crustaceans (Abzhanov and Kaufman 1999).

The differences in the details of the expression patterns of the four genes examined here may be the result of secondary roles of the genes. They may be needed again for the development of special segmental parts of the embryos such as mouth parts, silk/salivary gland, legs, wings, and the tracheal system. Functions of homeotic genes different from those in *Drosophila* are reported for the beetle *Tribolium castaneum* (Beeman et al. 1989). Using such fine-tuning mechanisms, the numerous insect species do not need many genes to generate the enormous variety of morphological differences of homologous segments. The significance of spatial and temporal regulation has been shown in *Drosophila* (Castelli-Gair and Akam 1995).

The coexpression of *Antp* and *Ubx/abd-A* in the cells around the tracheal pits may demonstrate a role of *Antp* in the development of the tracheal system in the thoracic segments T2, T3 and the abdominal segments A1–A8. Consistent with that assumption is the disappearance of

Antp in the lateral area of the first thoracic segment during the stage when the tracheal pits develop; no insect has tracheal pits there. In *Manduca sexta*, *Antp* is also seen around the tracheal pits, and it is missing in that area in the third thoracic segment, where no tracheal openings develop (Zheng et al. 1999). This is different in *Drosophila*, where the expression of *Antp* in the lateral parts of the abdomen is missing and the development of spiracles is suppressed (Martinez-Arias 1986; Castelli-Gair et al. 1994). The expression patterns of *Ubx/abd-A* in the *Apis* abdomen are essentially the same as in *Drosophila* (Karch et al. 1990; Macias et al. 1990; Kelsh et al. 1994), *Manduca sexta* (Nagy et al. 1991; Zheng et al. 1999), *Tribolium* (Stuart et al. 1993), *Schistocerca* (Tear et al. 1990), *Precis coenia* (Warren et al. 1994), and *Thermobia domestica* (Peterson et al. 1999). Therefore a similar role in abdomen specification is very likely, as has been worked out originally for *Drosophila* (Lewis 1978; Tiong et al. 1985; Duncan 1987; Mathog 1991; Vachon et al. 1992; Warren et al. 1994; Carroll 1995; Castelli-Gair and Akam 1995). In the honey bee embryos we found *Ubx/abd-A* in the whole first abdominal segment, but not in the thorax. This does not coincide completely with other insects. In *Schistocerca* and *Drosophila Ubx* is also found in meso- and metathorax (Tear et al. 1990; Kelsh et al. 1994) whereas in *Thermobia abd-A* is found only from the posterior compartment of the first abdominal segment on towards the posterior (Peterson et al. 1999). We have no convincing explanation for these differences; with the antibody we used, we could not distinguish between *Ubx* and *abd-A*. Neurulation and tracheal system invagination show no differences in thorax and abdomen in the honey bee embryo except the time gradient (Fig. 2).

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References

Abzhanov A, Kaufman TC (1999) Novel regulation of the homeotic gene *Scr* associated with a crustacean leg-to-maxilliped appendage transformation. Development 126:1121–1128

Beeman RW, Stuart JJ, Haas MS, Denell RE (1989) Genetic analysis of the homeotic gene complex (HOM-C) in the beetle *Tribolium castaneum*. Dev Biol 133:196–209

Bermingham JR, Martinez-Arias A, Petitt MG, Scott MP (1990) Different patterns of transcription from the two *Antennapedia* promoters during *Drosophila* embryogenesis. Development 109:553–566

Brown S, Holtman S, Kaufman TC Denell R (1999) Characterization of the *Tribolium Deformed* ortholog and its ability to directly regulate *Deformed* target genes in the rescue of a *Drosophila* null mutant. Dev Genes Evol 209:389–398

Carroll SB (1995) Homeotic genes and the evolution of arthropods and chordates. Nature 376:479–485

Carroll SB, Laymon RA, McCutcheon MA, Riley PD, Scott MP (1986) The localization and regulation of *Antennapedia* protein expression in *Drosophila* embryos. Cell 47:113–122

Castelli-Gair JE, Akam M (1995) How the Hox gene *Ultrabithorax* specifies two different segments. The significance of spatial and temporal regulation within metameres. Development 121:2973–2982

Castelli-Gair JE, Greig S, Micklem G, Akam ME (1994) Dissecting the temporal requirements for homeotic gene function. Development 120:1983–1995

Chadwick R, McGinnis W (1987) Temporal and spatial distribution of transcripts from the *Deformed* gene of *Drosophila*. EMBO J 6:779–789

Damen WGM, Hausdorf M, Seyfarth EA, 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 USA 95:10665–10670

Duncan I (1987) The Bithorax complex. Annu Rev Genet 21:285–319

Fleig R (1990) *Engrailed* expression and body segmentation in the honey bee *Apis mellifera*. Rouxs Arch Dev Biol 198:467–473

Fleig R, Sander K (1986) Embryogenesis of the honey bee *Apis mellifera* L. (Hymenoptera: Apidae): a SEM study. Int J Insect Morphol Embryol 15:449–462

Fleig R, Walldorf U, Gehring WJ, Sander K (1988) In situ localization of the transcripts of a homeobox gene in the honey bee *Apis mellifera* L. (Hymenoptera). Rouxs Arch Dev Biol 197:269–274

Fleig R, Walldorf U, Gehring WJ, Sander K (1992) Development of the *Deformed* protein pattern in the embryo of the honey bee *Apis mellifera* L. (Hymenoptera). Rouxs Arch Dev Biol 201:235–242

Grenier JK, Garber TL, Warren R, Whitington PM, Carroll S (1997) Evolution of the entire arthropod Hox gene set predated the origin and radiation of the onychophoran/arthropod clade. Curr Biol 7:547–553

Hafen E, Levine M, Gehring WJ (1984) Regulation of *Antennapedia* transcript distribution by the Bithorax complex in *Drosophila*. Nature 307:287–289

Hayward DC, Patel NH, Rehm EJ, Goodman CS, Ball EE (1995) Sequence and expression of grasshopper *Antennapedia*: comparison to *Drosophila*. Dev Biol 172:452–465

Hooper JE, Perez-Alonso M, Bermingham JR, Prout M, Rocklein BA, Wagenbach M, Edstrom JE, de Frutos R, Scott MP (1992) Comparative studies of *Drosophila Antennapedia* genes. Genetics 132:453–469

Karch F, Bender W, Weiffenbach B (1990) *abd-A* expression in *Drosophila* embryos. Genes Dev 4:1573–1587

Kaufman TC, Lewis R, Wakimoto BT (1980) Cytogenetic analysis of chromosome 3 in *Drosophila melanogaster*: the homoeotic gene complex in polytene chromosome interval 84A-B. Genetics 94:115–133

Kelsh R, Weinzierl ROJ, White RAH, Akam M (1994) Homeotic gene expression in the locust *Schistocerca*: an antibody that detects conserved epitopes in *Ultrabithorax* and *abdominal-A* proteins. Dev Genet 15:19–31

LeMotte PK, Kuroiva A, Fessler LI, Gehring WJ (1989) The homeotic gene *Sex combs reduced* of *Drosophila*: gene structure and embryonic expression. EMBO J 8:219–227

Levine M, Hafen E, Garber RL, Gehring WJ (1983) Spatial distribution of *Antennapedia* transcripts during *Drosophila* development. EMBO J 2:2037–2046

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

Macias A, Casanova J, Morata G (1990) Expression and regulation of the *abd-A* gene of *Drosophila*. Development 110:1197–1207

Mahaffey JW, Kaufman TC (1987) Distribution of the *Sex combs reduced* gene products in *Drosophila melanogaster*. Genetics 117:51–60

Mahaffey JW, Diederich RJ, Kaufman TC (1989) Novel patterns of homeotic protein accumulation in the head of the *Drosophila* embryo. Development 105:167–174

Martinez-Arias A (1986) The Antennapedia gene is required and expressed in parasegments 4 and 5 of the *Drosophila* embryo. EMBO J 5:135–142

Mathog D (1991) Suppression of abdominal legs in *Drosophila melanogaster*. Rouxs Arch Dev Biol 199:449–457
McGinnis W, Krumlauf R (1992) Homeobox genes and axial patterning. Cell 68:283–302
Nagata T, Suzuki Y, Ueno K, Kokubo H, Xu X, Hui C, Hara W, Fukuta M (1996) Developmental expression of the *Bombyx Antennapedia* homologue and homeotic changes in the *Nc* mutant. Genes Cells 1:555–568
Nagy L, Brooker R, Riddiford LM (1991) Isolation and embryonic expression of an *abdominal-A*-like gene from the lepidopteran, *Manduca sexta*. Development 112:119–131
Nelson JA (1915) The embryology of the honey bee. Princeton University Press, Princeton
Patel NH (1994) The evolution of arthropod segmentation: insights from comparisons of gene expression patterns. Dev 1994 [Suppl]:201–207
Patel NH, Kornberg TB, Goodman CS (1989) Expression of *engrailed* during segmentation in grasshopper and crayfish. Development 107:201–212
Peterson MD, Rogers BT, Popadic A, Kaufman TC (1999) The embryonic expression pattern of *labial*, posterior homeotic complex genes and the *teashirt* homologue in an apterygote insect. Dev Genes Evol 209:77–90
Rauskolb C, Wieschaus E (1994) Coordinate regulation of downstream genes by extradenticle and the homeotic selector proteins. EMBO J 13:3561–3569
Regulski M, McGinnis N, Chadwick R, McGinnis W (1987) Developmental and molecular analysis of Deformed; a homeotic gene controlling *Drosophila* head development. EMBO J 6:767–777
Riek EF (1979) In: The Insects of Australia, vol 1. Melbourne University Press, Carlton, Victoria, pp168–186
Riley PD, Carroll SB, Scott MP (1987) The expression and regulation of *Sex combs reduced* protein in *Drosophila* embryos. Genes Dev 1:716–730
Rogers BT, Peterson MD, Kaufman TC (1997) Evolution of the insect body plan as revealed by the *Sex combs reduced* expression pattern. Development 124:149–157
Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Plainview, NY
Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467
Schnetter M (1935) Morphologische Untersuchungen über das Differenzierungszentrum in der Embryonalentwicklung der Honigbiene. Z Morphol Ökol Tiere 29:114–195
Shanmugam K, Featherstone MS, Saragovi HU (1997) Residues flanking the Hox YPWM motif contribute to cooperative interactions with PBX. J Biol Chem 272:19081–19087
Sommer R, Tautz D (1991) Segmentation gene expression in the housefly *Musca domestica*. Development 113:419–430
Stuart JJ, Brown SJ, Beeman RW, Denell RE (1993) The *Tribolium* homeotic gene *Abdominal* is homologous to *abdominal-A* of the *Drosophila* bithorax complex. Development 117:233–243
Tautz D, Pfeifle C (1989) A non-radioactive in situ hybridisation method for the localization of specific RNAs in *Drosophila* embryos reveals translational control of the segmenation gene *hunchback*. Chromosoma 98:81–85
Tear G, Akam M, Martinez-Arias A (1990) Isolation of an *abdominal-A* gene from the locust *Schistocerca gregaria* and its expression during early embryogenesis. Development 110:915–925
Telford MJ, Thomas RH (1998) Expression of homeobox genes shows chelicerate arthropods retain their deutocerebral segment. Proc Natl Acad Sci USA 95:10671–10675
Tiong SYK, Bone LM, Whittle JRS (1985) Recessive lethal mutations within the Bithorax-complex in *Drosophila melanogaster*. Mol Gen Genet 200:335–342
Vachon G, Cohen B, Pfeifle C, McGuffin ME, Botas J, Cohen SM (1992) Homeotic genes of the Bithorax complex repress limb development in the abdomen of the *Drosophila* embryo through the target gene *Distalless*. Cell 71:437–450
Walldorf U, Fleig R, Gehring WJ (1989) Comparison of homeobox-containing genes of the honeybee and *Drosophila*. Proc Natl Acad Sci USA 86:9971–9975
Warren RW, Nagy L, Seleque J, Gates J, Carroll SB (1994) Evolution of homeotic gene regulation and function in flies and butterflies. Nature 372:458–461
Wirz J, Fessler LI, Gehring WJ (1986) Localization of the *Antennapedia* protein in *Drosophila* embryos and imaginal discs. EMBO J 5:3327–3334
Zheng Z, Khoo A, Fambrough D, Garza L, Booker R (1999) Homeotic gene expression in the wild-type and a homeotic mutant of the moth *Manduca sexta*. Dev Genes Evol 209:460–462