

An analysis of Abdominal-B expression in the locust *Schistocerca gregaria*

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SUMMARY

We have cloned a homologue of the homeotic gene *Abdominal-B* from the locust *Schistocerca gregaria* and raised antibodies against the homeodomain and C-terminal flanking region of the encoded protein. This antibody stains the posterior abdomen of the developing *Schistocerca* embryo from 26% of development onwards, a stage when only the anterior of the abdomen is visibly segmented. By the time segmentation has reached the posterior of the abdomen, ABD-B protein is detectable only in the terminal segment (A11). Expression extends anteriorly as development proceeds

to include the epidermis of all segments from A8p to A11, including the genital appendages of A9 and A10. The anal cerci, generally regarded as appendages of A11, remain unstained. This expression domain corresponds to the region within which the *Abd-B* r function of the *Drosophila Abd-B* gene is expressed. We detect no expression corresponding to the *Abd-B* m function in *Drosophila*.

Key words: Abdominal-B, homeotic gene, segment identity, short-germ insect, orthoptera

INTRODUCTION

The bodies of arthropods are composed of a reiterated unit, the segment. These segments, whilst sharing a common plan, may be modified to varying degrees to produce their unique specialisations. The class Insecta displays an enormous diversity of segmental specialisations. In the abdomen, with which we are chiefly concerned here, this diversity is principally in the apparent number and degree of reduction of the segments, and in the structure and origin of the external genitalia (Matsuda, 1976).

A large body of work, triggered by the findings of Lewis, showed that in *Drosophila* this process of segmental specification is under the control of a distinct class of genes, the homeotic genes (Lewis, 1963, 1978; reviewed in Akam, 1987). These genes reside in one of two clusters, the Antennapedia Complex (ANT-C) and the Bithorax Complex (BX-C) (Lewis, 1978; Sánchez-Herrero et al., 1985; Kaufman et al., 1990). The three genes of the BX-C control segment specification in the posterior thorax and throughout the abdomen, each functioning in distinct but partially overlapping domains (Lewis, 1978).

Homeotic genes have been defined in a number of other insects. In some species, they have been identified by homeotic mutations (e.g. *Bombyx* - Tazima, 1964; *Tribolium* - Beeman, 1987, Beeman et al., 1989; *Blattella* - Ross, 1964, 1966; and *Manduca* - Booker and Truman, 1989). In other species, presumed homeotic genes have

been identified by sequence homology (*Apis* - Fleig et al., 1988; Walldorf et al., 1989; *Manduca* - Nagy et al., 1991; *Bombyx* - Ueno et al., 1992). With the exception of *Blattella* (on which very little work has been done), all of the species examined in these studies are representatives of one relatively recent and derived lineage within the insects - the endopterygotes (Hennig, 1981).

We are studying homeotic gene function in the African Desert Locust, *Schistocerca gregaria* (Akam et al., 1988; Tear et al., 1990 and this work). *Schistocerca* is a member of an ancient and morphologically unspecialized insect order, the Orthoptera. In contrast to the highly specialised *Drosophila*, *Schistocerca* retains a number of apparently primitive insectan features. In the context of this paper three are of particular importance: (i) eleven abdominal segments are readily distinguishable in the embryo, and most of them are clearly defined even in the adult (only eight abdominal segments are distinct in *Drosophila*; the more posterior abdominal segments of *Drosophila* are fused and reduced); (ii) the segments form sequentially by growth from a posterior growth zone (i.e. *Schistocerca* is a short-germ insect), in contrast to *Drosophila*, where segments form by subdivision of a syncytium (the extreme long-germ mode of development; Anderson, 1972a,b) and (iii) the female external genitalia are derived from the appendages of segments A8 and A9 (whereas in *Drosophila* they are believed to form from a modified region of the trunk of A8).

Our interests focus on two broad questions. Firstly, to

what extent does the diversity of insectan morphology result from modifications of homeotic gene regulation? Thus, are specific modifications due to changed patterns of homeotic gene expression, or are they due to modification of the downstream response to the same homeotic gene prepattern? Secondly, how do the different developmental strategies of long- and short-germ development affect the establishment of segment identities, as reflected by homeotic gene expression? In the short-germ embryo, are regions of the body specified before segmentation; are individual segments specified as they are formed from the growth zone, or are they all formed as naive segments before being specified?

Here we address these questions through the study of a *Schistocerca* homologue of *Abdominal-B* (*Abd-B*). *Abd-B* is the most distal of the BX-C homeotic genes in *Drosophila* (Sánchez-Herrero et al., 1985 and Tiong et al., 1985). Acting in concert with *abd-A*, it controls segment specification in the posteriormost abdomen, from posterior A4 to anterior A10 (i.e. A4p-A10a; or parasegments 10-15; Martínez-Arias and Lawrence, 1985). It thus controls specification of the male (A9) and female (A8) genital segments (Karch et al., 1985; Sánchez-Herrero et al., 1985; Tiong et al., 1985; Casanova et al., 1986; Sato and Denell, 1986; Jürgens, 1988; Celniker et al., 1989). Molecular analysis of the genetically complex *Abd-B* region of the BX-C has revealed a single gene (with one homeobox) encoding two different proteins with distinct functions. These two distinct functional units are termed *Abd-B m* and *Abd-B r* (Fig. 1). The different ABD-B proteins are expressed in different spatial domains (A4p-A8a and A8p back, respectively) under the control of different promoters. However, their transcripts share the same 3 exons including the coding sequences for the homeodomain and carboxy terminal portion of the protein. The resultant ABD-B M and ABD-B R proteins differ only in the length of the amino-terminal region of the protein: the R protein is a truncated version of the M protein.

We describe the identification of a *Schistocerca Abd-B*

class homeobox and the isolation of antibodies directed against the product of the gene containing it. We use one of these antibodies to map the distribution of ABD-B protein during much of *Schistocerca* embryogenesis.

MATERIALS AND METHODS

Schistocerca gregaria

Embryos and DNA were obtained from a population of *S. gregaria* (Forsk.) maintained by the Department of Zoology at Cambridge for more than 20 years. Eggs were collected and maintained as described previously (Tear et al., 1990).

Library screening

400,000 phage from the original packaging of a *Schistocerca gregaria* genomic DNA library containing *Sau3A* partial-digest fragments in EMBL 3 (Tear et al., 1990) were screened under low-stringency conditions. Hybridisation was at 37°C in 5× SSPE, 0.5% SDS, 5× Denhardt's solution, 43% formamide and 100 µg/ml sheared single-stranded salmon sperm DNA, and filters were then washed to 65°C in 2× SSPE, 0.1% SDS (Maniatis et al., 1982). Clone 3A2 was one among four clones that hybridized to two or more homeobox probes, and proved subsequently to contain homeoboxes. None of the other characterized sequences were of the *Abd-B* class.

Sequencing

DNA fragments from 3A2 were subcloned into pBluescript KS (Stratagene, La Jolla, California) or M13mp18 and mp19 (Yanisch-Perron et al., 1985) and sequenced by dideoxy chain termination (Sanger et al., 1977). An Exonuclease III (Boehringer Mannheim) nested deletion series (Henikoff, 1984) was made from the 1.7 kb *KpnI/PstI* fragment of 3A2 and sequenced from double-stranded template. All sequencing was with Sequenase enzyme according to the manufacturer's instructions (USB Corporation, Ohio). The second strand of the presumptive homeobox exon of 3A2 was determined using custom primers. Sequence analysis was performed using the Staden package on a VAX computer (Staden, 1984).

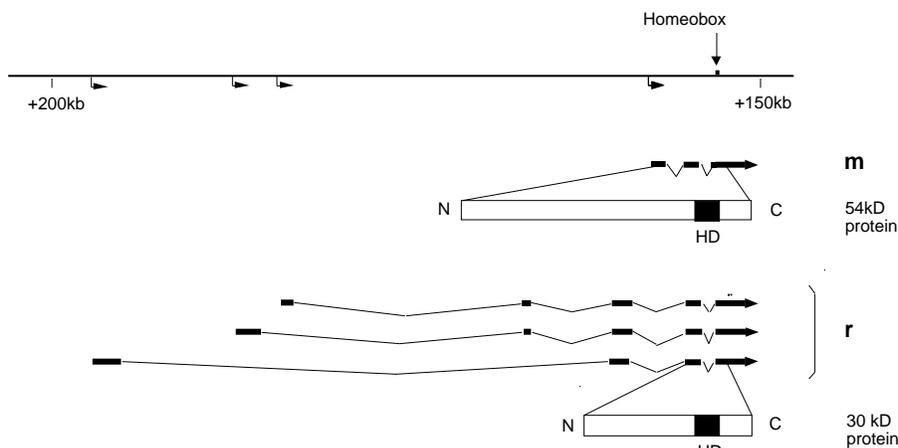


Fig. 1. Molecular organization of the *Abd-B* transcription units in *Drosophila*. Transcripts corresponding to the m and r functions are represented by arrowed lines. Their intron-exon structure is shown with respect to the genomic DNA. (Coordinates for the genomic DNA are taken from Karch et al., 1985). The corresponding M and R proteins that they encode are represented by open boxes; the position of the homeodomain (HD) near the C terminus is shown by a filled box. Note that all protein-encoding exons of the r transcript are shared by the m transcript; the R protein is thus an N-

terminally truncated version of the M protein. Based on data of Kuziora and McGinnis (1988), Sánchez-Herrero and Crosby (1988), Celniker et al. (1989), DeLorenzi et al. (1988) and Zavortink and Sakonju (1989).

Genomic Southern analysis

Locust genomic DNA Southern blots were prepared and probed by standard techniques as described in Sambrook et al. (1989). Locust DNA was digested with a variety of restriction enzymes, electrophoretically separated (40 µg/track) and blotted onto BA85 nitrocellulose membrane (Schleicher & Schuell). Probe fragments were radiolabelled by the random priming method (Feinberg and Vogelstein, 1983). Blots were hybridised under high-stringency conditions (50% formamide; 5× Denhardt's solution; 5× SSPE; 0.5% SDS; 100 µg/ml denatured salmon sperm DNA at 42°C) and washed to 68°C in 0.1× SSPE.

Production of antisera

A 1.0 kb *XhoI/SmaI* fragment of pB11.7KpPs (1.7 kb *KpnI/PstI* fragment of 3A2 subcloned into pBluescript KS) was partially end-filled and then subcloned into the partially end-filled *BamHI/SmaI* digested pGex-3X glutathione-S-transferase fusion vector (Amrad Corporation, Melbourne, Australia) to make pAbd-BFus. The frame of the construct was confirmed both by analysing the size of the resultant inducible fusion protein and by sequencing the subcloned 'joint'. This construction results in a 38×10³M_r IPTG-inducible fusion protein consisting of part of the glutathione-S-transferase of *Schistosoma japonicum* linked to 103 amino acids of the *Schistocerca Abd-B* homeodomain and downstream sequences. pAbd-BFus was transformed into *E. coli* strain TG1 and total bacterial protein extracts were made after 3-5 hour IPTG-inductions essentially as described by Smith and Johnson (1988).

Total bacterial protein samples from bulk cultures were prepared by SDS-boiling lysis (Harlow and Lane, 1988) and were run on 10% SDS-polyacrylamide preparative gels using a Protean II Slab Cell (Bio-Rad) according to the manufacturer's instructions. The fusion protein band was visualised, excised and electroeluted as described in Tear et al. (1990). The eluted protein was dialysed overnight against three changes of 8 M urea, and then against three changes of 1× PBS overnight.

One rabbit and three mice were immunised with these fusion protein preparations. The rabbit was immunised by Animal Biotechnology Cambridge Ltd; it was injected with 150 µg of fusion protein subcutaneously in complete Freund's adjuvant. Subsequently it was boosted subcutaneously with 150 µg of fusion protein in incomplete Freund's adjuvant at 2-3 week intervals; test bleeds were taken one week after each boost. A preimmune bleed was obtained before the initial immunisation. The mice were immunised by R. Smith in M. Wilcox's laboratory at the Laboratory of Molecular Biology, Cambridge; they were injected with 50 µg of fusion protein subcutaneously in complete Freund's adjuvant. Subsequently, they were boosted intraperitoneally with 50 µg of fusion protein in incomplete Freund's adjuvant at 2-3 week intervals; test bleeds were taken one week after each boost. All sera supplied were made up to 0.02% NaN₃ and stored at 4°C.

Sera were purified by affinity chromatography on a fusion protein column and then adsorbed against a bacterial extract (acetone powder) prepared from TG1 cells expressing glutathione-S-transferase from the parental pGex-3X vector as described in Tear et al. (1990).

The data presented here were all obtained using sera from mouse 1. Sera from mouse 2 gave an identical but weaker pattern of labelling on embryos. The rabbit sera also gave a good signal showing the same pattern, but superimposed upon this was a very strong pattern of staining not seen with the other animals. This labelled a complex pattern of scattered cells throughout the embryo, probably peripheral neurons. This unique pattern of staining could largely be eliminated by further absorption of the serum with bacterial protein. Presumably, it was generated against a bacterial epitope that fortuitously mimics a locust protein.

Western analysis

Locust protein samples were prepared by a modification of the protocol used for bacterial protein samples given above. *Schistocerca* embryos of approx. 30-45% of embryogenesis were dissected out of the egg, cleaned of yolk and then cut into anterior and posterior 'halves' by slicing with forceps at segment A6. These fragments were then transferred to Eppendorfs and the locust embryo saline replaced with protein loading buffer (10 µl per 'anterior', 2.5 µl per 'posterior' fragment; Harlow and Lane, 1988). After homogenisation with a disposable pestle (Kontes), samples were boiled for 5 minutes before spinning down cellular remnants with a microfuge. The supernatant was kept at 4°C; samples were boiled (5 minutes) before loading.

Equivalent protein samples (total bacterial protein extracts, total locust protein extracts and various semipurified fractions excised from preparative gels) were run on each of two 10% SDS-polyacrylamide gels on a Mini-Protean II Dual Slab Cell (Bio-Rad). Protein samples on one were stained with Coomassie blue and the gel then dried. The other gel was western blotted onto S&S BA85 nitrocellulose paper (Schleicher & Schuell) using a Bio-Rad Transblot apparatus (electrophoresis buffer: 190 mM glycine; 25 mM Tris base; 20% methanol) according to the manufacturer's instructions, before staining with Coomassie blue to check the transfer. The western blot was probed according to a published protocol (Wilcox, 1986), using 0.5 M Tris-HCl, pH 7.4; 300 mM NaCl; 0.05 M EDTA; 0.5% gelatin; 0.05% NaN₃ as a blocking buffer. Affinity-purified and acetone-powder-adsorbed mouse 1 serum (third test bleed) was used at 1:75 dilution as the primary antibody; a 1:1000 dilution of alkaline-phosphatase-conjugated rabbit anti-mouse (Dakopatts) was the secondary antibody.

Immunohistochemistry

Locust eggs laid in moist sand were cultured at 26°C. Embryos were dissected out and staged according to Bentley et al. (1979). Whole embryos were immunostained as follows. Those stained for *engrailed* expression were fixed and stained with mAb 4D9 exactly as described by Patel et al. (1989). Embryos stained for ABD-B expression were dissected out in locust embryo saline (150 mM NaCl; 3 mM KCl; 2 mM CaCl₂; 1 mM MgSO₄; 5 mM TES (N-tris[Hydroxymethyl]methyl-2-aminoethanesulfonic acid) (Sigma)) and cleared of adhering yolk. The enveloping amnion was ruptured and removed when possible before fixing the embryos for 45-60 minutes at 0°C in 1× PBS; 3.7% formaldehyde. After rinsing twice in PTX (1× PBS; 0.1-0.5% Triton X-100), then once in 1× PBTX (PTX with 0.1% BSA; 10 mM NaN₃), embryos were blocked in PBTX for 1.5 hours at 4°C. The PBTX was then replaced with PBTX containing 5% normal horse serum (Vecta Labs) for 2-4 hours. The affinity-purified ABD-B sera were added (at 1:50 for mouse sera; 1:50-1:500 for rabbit sera) and the embryos left overnight at 4°C. Next day, embryos were rinsed three times with PBTX, washed for 1.5 hours with PBTX and then for 1.5 hours in PBTX with 5% normal horse serum. Horse anti-mouse IgG (Vecta Labs) was added to 1:400 dilution and incubated at 20°C for 1.5 hours. After rinsing three times in PT (1× PBS; 0.1% Tween-20), embryos were washed for 30 minutes in PT and stained using the Vectastain Elite kit as per the manufacturer's instructions. Embryos were then dehydrated through an ethanol series (15 minutes each), cleared in xylene (15 minutes) and mounted in DPX mountant (BDH) ready for examination with a Zeiss Axioplan.

Material for sectioning was dissected out and staged as above. After fixation and washing as for whole mounts, the embryos were mounted in 1% LGT-agarose. They were then rolled overnight in 30% sucrose in 1× PBS. The blocks were then mounted onto a cryostat chuck using PolyFreeze mountant. 10-20 µm sections were cut on a cryostat. Sections were dried onto gelatine-subbed

slides overnight, and immunostained as follows. Sections were rehydrated in PBTX (with 0.01% Triton-X100) for 30 minutes. PBTX was then replaced with column-eluted, acetone-powder adsorbed serum at 1/50 dilution in PBTX (with 0.01% Triton-X100). After a two hour incubation with this primary antibody, the slides were rinsed briefly and washed for 10 minutes in PBTX (with 0.01% Triton-X100). Horse -mouse secondary antibody (Vector Labs, Peterborough) was used at 1/400 dilution for 2 hours. After a rinse and 10 minute wash with PBTX (with 0.01% Triton-X100), the sections were stained with the Vectastain Elite ABC Kit (Vector Labs) according to the manufacturer's instructions. The staining solution used was 0.12 mg/ml DAB in PT; 0.1% H₂O₂; usually with 0.3% w/v ammonium nickel sulphate. Sections were dehydrated through an ethanol series and mounted in DPX Mountant (BDH).

RESULTS

An Abdominal-B homologue from *Schistocerca gregaria*: isolation and characterization

A *Schistocerca gregaria* genomic library (Tear et al., 1990) was screened with homeobox probes derived from *Drosophila Abd-B*, *Schistocerca abd-A* (Tear et al., 1990) and two other *Schistocerca gregaria* type 1 homeoboxes (Dawson, 1989). One phage, 3A2, hybridised to all four probes and was further characterised. A restriction map of this phage is shown in Fig. 2. The 1.7 kb *KpnI/PstI* fragment with homology to the homeobox was subcloned in pBluescript KS+ and sequenced. The sequence revealed a complete homeobox showing 82% nucleotide identity to that of *Drosophila Abd-B* (Fig. 3). Regions of homology extend both 5 and 3 to the homeobox itself and include a downstream CAX repeat (M repeat; Wharton et al., 1985).

To confirm that phage 3A2 does represent a *Schistocerca* genomic fragment, a locust genomic Southern was probed with a 560 bp *BglII/PstI* fragment from the presumed 3 untranslated region downstream of the homeobox (see Fig. 2). Each of a series of different restriction digests gives a single hybridizing band under high stringency conditions (Fig. 4). Repeat probings of the same filter with fragments including parts of the homeobox and flanking sequences detected multiple bands, even after high-stringency washes. The intensity of some of these bands suggested that they

derive from repeated sequences, presumably detected by the very GC-rich regions of the homeobox.

Conceptual translation of the homeobox and flanking sequence in phage 3A2 predicts a polypeptide that shows extended homology to *Drosophila* ABD-B. The homeodomains are identical at 59/60 residues, the *Schistocerca* sequence matching the ABD-B class consensus at all positions (Scott et al., 1989; Fig. 3). Furthermore there is a 6/7 amino acid match upstream and a 4/4 amino acid match downstream from this homeodomain. These features clearly identify clone 3A2 as a *Schistocerca* homologue of *Abdominal B*.

In both species, an imperfect CAX repeat lies downstream of the homeobox. This translates into a region of partially repetitive amino acid sequence. Because of shifts in the reading frame, this region is dominated by serine in *Schistocerca*, asparagine in *Drosophila*. No other regions of the sequence are obviously similar between the two species.

Splicing of this *Schistocerca* gene appears to differ from that of *Abd-B* in *Drosophila*. In *Drosophila*, the *Abd-B* homeobox is interrupted by an intron. This is not present in the *Schistocerca* clone, which thus represents exons 7 and 8 of the *Drosophila* homologue (Celniker et al., 1989). Upstream of the homeobox, the limit of the exon is not clear. The proteins encoded by the *Drosophila* and *Schistocerca* sequences diverge only 6 amino acids upstream of the homeobox, at a possible splice acceptor in the *Schistocerca* sequence. However, conceptual translation of a genomic clone from a *Bombyx Abd-B* homologue (Ueno et al., 1992) matches the *Schistocerca* sequence for at least three further amino acids, suggesting that this region is protein coding.

Production of anti-*Schistocerca* Abdominal-B antibodies

To permit detailed analysis of the expression domain of ABD-B in *Schistocerca*, we generated antibodies against part of the protein. A fragment containing most of the homeobox and all the downstream coding region was cloned into the pGex-3X expression vector (see methods). This generated a predicted 38×10³M_r fusion protein containing 103 amino acids of *Schistocerca Abd-B*.

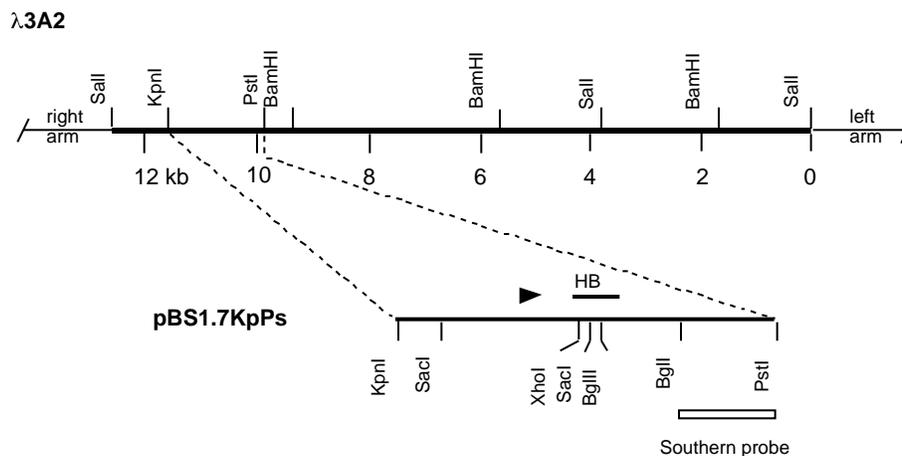


Fig. 2. Restriction map of the *Abdominal-B* genomic clone from *Schistocerca*, 3A2. The 1.7 kb *KpnI/PstI* fragment containing the homeobox homology (HB) is shown enlarged. The transcriptional orientation inferred from the homeobox sequence is left to right, indicated by the arrowhead. The 560 bp *BglII/PstI* fragment used for genomic Southern analysis is indicated. Scale is in kilobases.

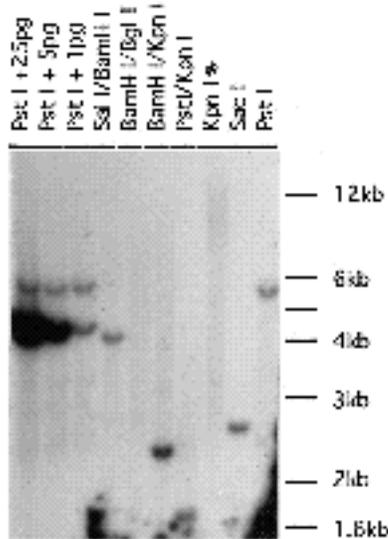


Fig. 4. Southern hybridization to *Schistocerca* genomic DNA, probed at high stringency with the 560 bp *BglII/PstI* fragment of 3A2 indicated in Fig. 2. Each lane carries 40 µg of genomic DNA, digested as indicated. The three left-hand lanes were spiked with the indicated amounts of linearized plasmid pBS 1.7KpPs (Fig. 2). Each lane shows a single hybridising genomic band. Note that the *PstI/KpnI* and *BamHI/KpnI* lanes are predicted from the restriction map of Fig. 2 to show single hybridising fragments of 1.7 kb and 2.3 kb respectively. For the other digests, only the minimum sizes of fragments can be predicted from the map in Fig. 2, but all observed fragments are consistent with this map. *, n.b. Genomic DNA in the *KpnI* digest lane was degraded.

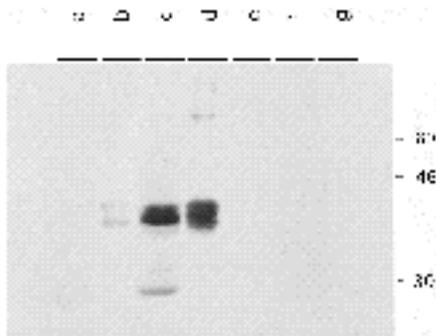


Fig. 5. Western blot analysis of anti-ABD-B serum from mouse 1 (third test bleed). Protein samples as follows. a: total bacterial protein from pGex-3X transformant, 1.5% of 1.5 ml culture. b: gel isolated bacterial protein bands of sizes flanking the fusion protein band, 0.6 µg. (contains only trace amounts of fusion protein). c: total bacterial protein from ABD-B fusion construct transformant, 1.5% of 1.5 ml culture. d: gel isolated ABD-B fusion protein band, 0.3 µg. e: BSA, 5 mg. f-g: total locust protein from anterior (f) or posterior (g) halves of locust embryos (8 embryo equivalents). Note that the serum detects the ABD-B fusion protein very strongly; it detects other bacterial proteins only weakly or not at all. Under these conditions Abd-B protein cannot be detected in *Schistocerca* embryos.

1937; Johannsen and Butt, 1941; Bentley et al., 1979). In the description below, embryonic stages are given as a percentage of the time from egg-laying to hatching, and are

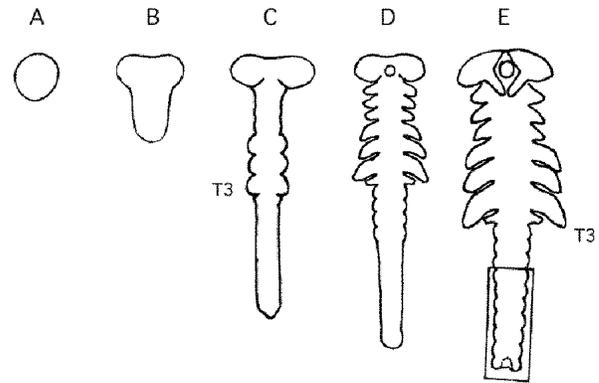


Fig. 6. Short-germ development: the formation of the abdomen in *Schistocerca*. (A) Approx. 10% - the embryonic primordium when first formed is just a disc of cells. (B) Approx. 15% - tail-like growth zone is formed posteriorly. (C, D) As growth proceeds segments become visible, first in the thorax (C: approx. 23%), then in the abdomen (D: approx. 28%). (E) Approx. 33% - abdominal segments form in anterior-posterior progression until all eleven segments are distinct. Box represents the region shown in Fig. 7B-F.

estimated from the descriptions and photographs of the development of *S. nitens* given by Bentley et al. (1979) and, for early stages, from the descriptions of Patel et al. (1989).

The embryonic primordium is a disc at 10% of embryogenesis, but by 15% the growth zone has formed a clear posterior 'tail' on the ventral side of the egg. The first morphologically visible segmentation appears at approx. 20% of embryogenesis and, by 26%, A1 is visibly segmented and *engrailed* stripes are visible in each of A1-A6 (Patel et al., 1989). There is no detectable staining with our anti-ABD-B antibody in 26% embryos, or in the few younger embryos (20%-25%) that we have examined (Fig. 7A).

Ectodermal expression of *Schistocerca* ABD-B begins in A11 and spreads anteriorly

From about 28% of development onwards, our antibodies detect ABD-B expression in the posteriormost abdomen. Expression continues until at least 55% of embryogenesis, when organogenesis is underway and cuticle deposition is beginning. Beyond this stage we have not looked carefully.

At approx. 28%, the embryo consists of a visibly segmented head, thorax and anterior abdomen with a thin unsegmented tail. A5 and A6 are forming and the rest of the posterior abdomen is unsegmented. *Engrailed* stripes representing each of A1-A8 are visible (Patel et al., 1989). At this stage, we see weak but reproducible ABD-B expression in the very posterior tip of the abdomen. Shortly afterwards, at approx. 30% (when A7 and A8 are forming morphologically and *engrailed* stripes are clear in each of A1-A9; Fig. 7C; Patel et al., 1989), there is clear nuclear staining in a posteriormost abdominal zone (Fig. 7B). In the absence of an A10 *engrailed* stripe, it is impossible to allocate this staining to a particular segment.

By approx. 31%, the abdomen is fully segmented. It is now clear that only A11 is staining (Fig. 7D). In this and in all subsequent stages, the posterolateral regions of A11

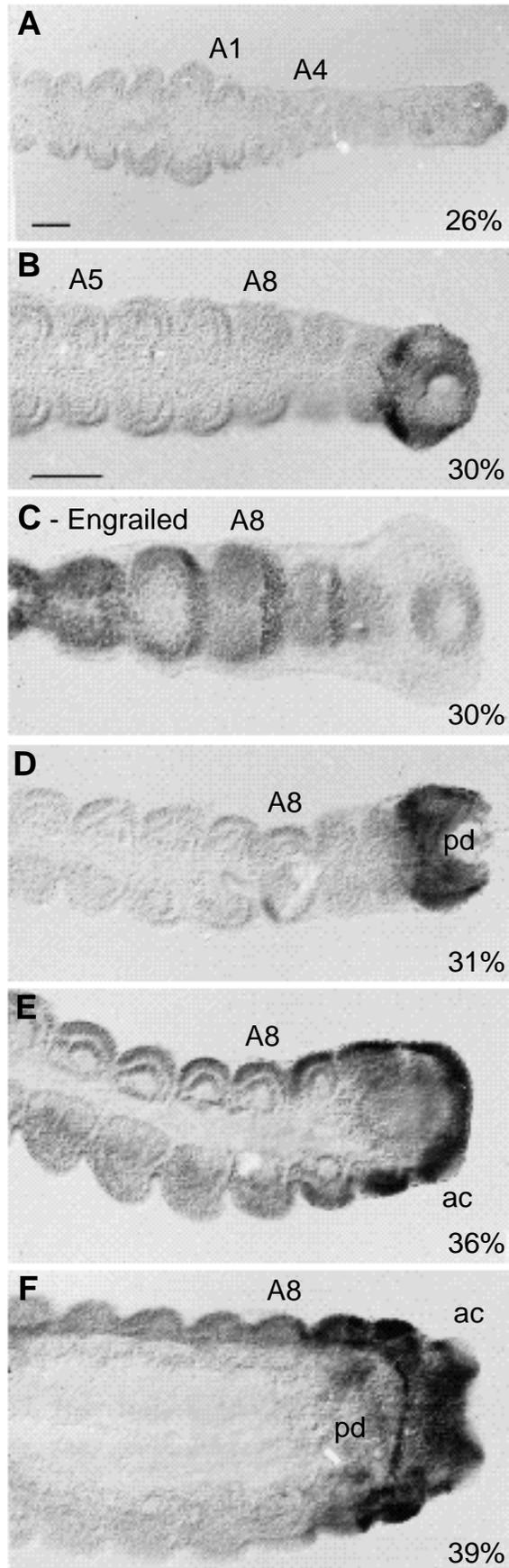


Fig. 7. Epidermal expression of ABD-B in *Schistocerca* embryos stained with ABD-B (A-B, D-F) or EN (C) specific antibodies. All embryos except (A) are at the same magnification, anterior is to the left, posterior to the right. (A) Approx. 26%, (B, C) approx. 30%, (D) approx. 31%, (E) approx. 36% and (F) approx. 39% of development. Abdominal segments A1 and A4, or A8 are indicated. No specific staining is visible up to 26% development (A). By approx. 30% of development specific nuclear staining is clearly visible in the posteriormost abdomen (B), although as yet the A10 and A11 segments are not expressing EN (EN stripes in A6-A9 are seen in C) and so the segmental localisation of this early expression cannot be ascertained. By approx. 31% of development A11 is morphologically distinct; ABD-B staining is restricted to A11 (D). ABD-B expression then spreads anteriorly, by approx. 36% strongly to posterior A9, and more weakly in much of anterior A9. By approx. 39% staining is very intense in posterior A9 to A11, and ABD-B is expressed at lower levels in the rest of A9 and also in posterior A8 (F). This expression domain is maintained from now until the latest stage examined (approx. 55%). From the moment they are distinguishable the cercal primordia (A11 appendages; ac) are unstained; this is very striking by approx. 39% of development (F). Scale bar: 100 μ m.

remain unstained. These are the cercal rudiments. At no stage do they express ABD-B.

From this point onwards, the ectodermal expression of ABD-B extends anteriorly (Fig 7). Thus by approx. 32% A11 and posterior A10 stain; by approx. 37.5% the ectoderm of posterior A9 is also staining. From approx. 40% the strongest expression is in posterior A9 and A10, but staining extends at low levels throughout anterior A9 and posterior A8; from this time onwards A11 stains relatively weakly. Nevertheless, from 40% to at least 55% development, the ectodermal expression domain remains the same. There is no sign of the high levels of ABD-B in A7 and A8 that are so prominent a feature in *Drosophila* (Celniker et al., 1989; DeLorenzi and Bienz, 1990; Boulet et al., 1991).

In each of A9 and A10 expression is first visible in the ventrolateral appendage primordia (data not shown). This is typical of two other homeotic genes that we have studied in *Schistocerca* (Tear et al., 1990; Kelsh et al., unpublished data).

The posteriormost ganglion in the CNS expresses Abdominal-B

In *Drosophila*, ABD-B expression is a prominent feature of the CNS, throughout the neuromeres of A5 to A8* (We use the term A8* to denote the fused terminal segments of *Drosophila*). In *Schistocerca* too ABD-B is expressed strongly in the posterior CNS. The posteriormost ganglion (ganglion VIII) has many nuclei expressing very high levels of ABD-B (Fig. 8A). This ganglion is a fusion of the neuromeres innervating each of the segments A8 to A11 (Snodgrass, 1935).

Expression in the mesoderm

The mesoderm at approx. 33% is distinct but does not express ABD-B. ABD-B expression in the mesoderm is first visible at approx. 35%; moderate levels of staining are seen in much but not all of the somatic mesoderm of A10 (Fig. 8B). Though little mesoderm is present in A11, there are a few ABD-B-positive nuclei which may be from the meso-

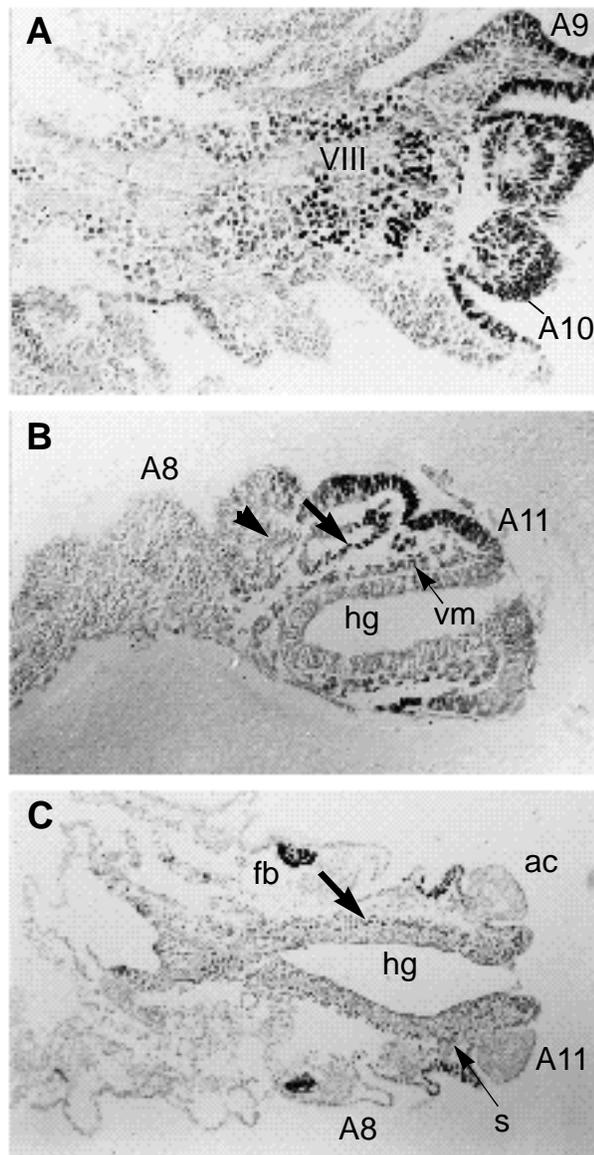


Fig. 8. ABD-B expression in other *Schistocerca* tissues as revealed in cryostat sections of staged embryos stained with ABD-B antibody. All sections are orientated such that anterior is to the left. In B and C segments A8 and A11 are labelled. (A) Expression in the posteriormost CNS. Ganglion VIII of approx. 55% embryo shows many intensely stained nuclei in the most posterior regions, and a gradient of decreasing levels more anteriorly. (B) Expression in the somatic mesoderm of approx. 37.5% embryo. The somatic mesoderm of A10 (arrow) shows prominent staining in many nuclei, but that of A9 is unstained (arrowhead). Some stained nuclei posterior to the A10 mesoderm may represent staining in the A11 mesoderm. The developing hindgut (hg) shows no ABD-B, although its associated visceral mesoderm is weakly labelled (vm). (C) Expression in the hindgut visceral mesoderm of approx. 55% embryo. Darkly stained visceral mesoderm nuclei are indicated with arrows. Some somatic mesodermal cell nuclei are labelled in A10 (s). Ectodermal expression is visible in A9 to A11, although the cerci (ac) are completely unstained. Note that the lower levels of staining in A8 and anterior A9 seen in whole mounts are not detectable in cryostat sections (B, C). The prominent cytoplasmic staining in the fat body (fb) of A8 is an artefact observed also with control sera.

derm of A11. Expression in the somatic mesoderm persists until at least 55% of embryogenesis (Fig. 8C). In *Drosophila*, the hindgut visceral mesoderm is one of the most prominent sites of ABD-B expression. In *Schistocerca*, the nuclei of the hindgut visceral mesoderm are weakly labelled (Fig. 8C). These nuclei are not labelled by an antibody detecting *Schistocerca* UBX and ABD-A (Kelsh et al., unpublished data).

Expression of *Abd-B* in the genital primordia

Drosophila mutants defective in *Abd-B* function show pronounced defects in either or both the male and female genitalia. Furthermore, *Abd-B* transcripts have been observed in the genital discs of *Drosophila* (Celniker et al., 1987). In the hemimetabolous *Schistocerca*, the genital primordia are more readily observed since their development begins during embryogenesis. The external genitalia are derived from the appendages of A8 and A9 in females and of A9 and A10 in males (Snodgrass, 1935; Richards and Davies, 1979; Matsuda, 1976). We examined these appendages for *Abdominal-B* expression. Embryos can be sexed from about 50% of embryogenesis onwards (Karandikar, 1942; Bentley et al., 1979). As with the rest of the epidermis, ABD-B is expressed in the genital appendages of A9 and A10. Hence in females only the posteriormost genital lobes (A9) are labelled, while in males both pairs (A9 and A10) are clearly stained (Fig. 9).

DISCUSSION

We have isolated the homeobox exon of an *Abdominal-B* gene from *Schistocerca*. The amino acid sequence encoded within and immediately flanking the homeobox of this clone is virtually identical to that of its *Drosophila* counterpart. This degree of conservation is similar to that observed for other homeotic genes within the class Insecta (Tear et al., 1990; Fleig et al., 1988; Walldorf et al., 1989; Ueno et al., 1992). It allows clones to be identified unambiguously as homologues for a specific *Drosophila* gene - a situation that is not true for all comparisons between such genes in insects and vertebrates (McGinnis and Krumlauf, 1992).

The intron-exon structure implied by the sequence of this clone differs from that of the *Drosophila Abd-B* gene. We think it unlikely that our sequence derives from a processed pseudogene or other rearranged fragment. One other *Abd-B* homologue has been described from an insect species - a genomic fragment from the *Bombyx* E complex (Ueno et al., 1992). The *Bombyx* and *Schistocerca* sequences resemble one another in that neither contains an intron in the homeobox, and they encode proteins that are somewhat more similar to one another than either is to *Drosophila* (note that their homeodomains are identical, Fig. 3B). These similarities in two distantly related species suggest that it is the structure of the *Drosophila* gene that has diverged from an ancestral pattern.

Abdominal-B expression domains in *Drosophila* and *Schistocerca*

A striking result of this study is the apparent discrepancy between the epidermal domains of ABD-B expression in

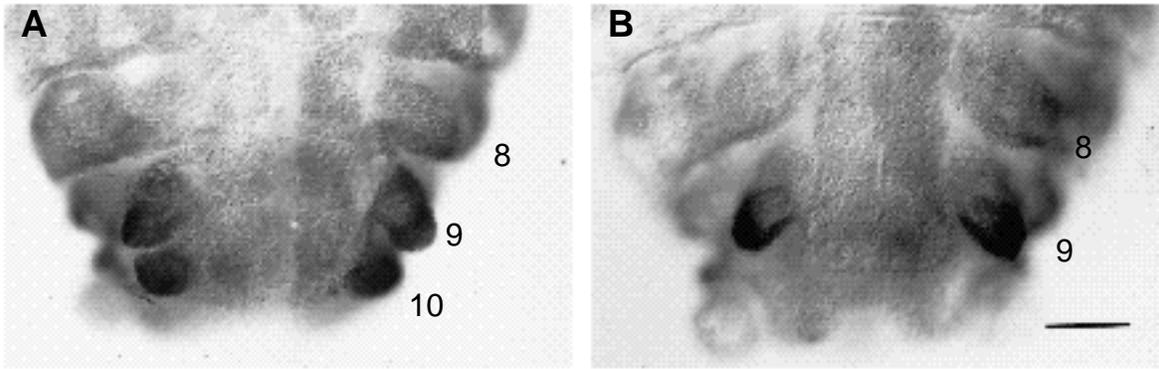


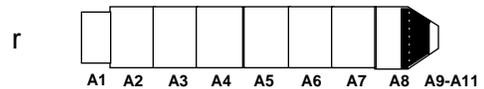
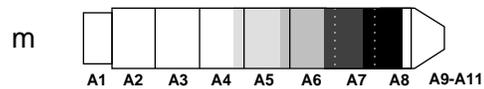
Fig. 9. ABD-B staining in the developing genital appendages of older (approx. 50%) embryos. In both panels posterior is down, and the genital appendages are labelled according to their segment of origin. (A) Male, (B) female. The appendages of A9 and A10 form the external genitalia of males: both label strongly with ABD-B antibodies (A). At this stage the A8 appendage in males is still present (it is resorbed by approx. 55% of development). In females the A10 appendages have been resorbed, but those of A8 and A9 are retained and form the external genitalia (ovipositer). A9 is strongly labelled. Staining is difficult at this stage due to the formation of cuticle (compare lack of staining in A11 with staining of sectioned embryo of similar stage in Fig. 8C); thus it is not clear whether A8 shows any staining, but there may well be low levels at this stage in both sexes. Scale: 100 μ m.

Drosophila and *Schistocerca*. In *Drosophila*, the *Abd-B* gene is expressed from the posterior of the fourth abdominal segment (A4p) back to the fused terminalia (A8*), with high levels of protein prominent in parasegments 13 and 14 (pA7-A8*) throughout development (Celniker et al., 1989; DeLorenzi and Bienz, 1990; Boulet et al., 1991). The phenotype of mutants shows that *Abd-B* function is necessary throughout this region for normal development. In contrast, our antibody detects ABD-B protein in *Schistocerca* only in segments A8p to A11; we cannot rule out the presence of low levels of protein more anteriorly, but prominent staining in the equivalent of *Drosophila* parasegment 13 (pA7-aA8) is clearly absent.

It is relevant to consider this difference in relation to the domains of the *Drosophila Abd-B m* and *r* functions (Fig. 10; Casanova et al., 1986; Celniker et al., 1989). The *m* and *r* functions of *Abd-B* are transcribed from different promoters, under the control of quite distinct *cis*-regulatory regions and in response to different *trans* regulators (see below). This results in essentially non-overlapping domains of expression and function: *Abd-B m* functions in segments A4p-A8a (parasegments 10-13); *Abd-B r* from A8p back (parasegments 14 back). These two functional units encode proteins with different morphogenetic specificities. The M protein acts with UBX, ABD-A and trunk-specific genes (e.g. *teashirt*; Fasano et al., 1991) to specify a range of mid to posterior abdomen segments in the trunk. The R protein is the only BX-C gene to function in the reduced 'tail' region of the abdomen, where it acts with a different region-specific regulator *spalt* (Lewis, 1978; Jurgens, 1988).

In *Schistocerca*, the region in which we detect epidermal expression of ABD-B corresponds precisely with the *Abd-B r* domain in *Drosophila*. The region in which expression appears to be absent is the *Abd-B m* domain. One conservative interpretation of this difference is that the function of *Abd-B* is essentially similar in the two insects, but that our antibodies fail to detect the M protein. We did not expect this, for the sera were raised against the region of the protein that is common to M and R forms in *Drosophila*.

Drosophila ABD-B



Schistocerca ABD-B

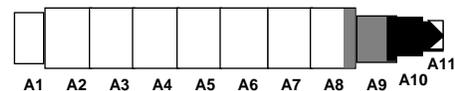


Fig. 10. The ectodermal expression domains of ABD-B in *Schistocerca* and *Drosophila* are shown schematically. The distinct abdominal structures of the two insects are cartooned to indicate their differences. Thus in *Drosophila* the posteriormost abdominal segments (A8 to A11) are all reduced to various degrees and are fused to form a single unit. In *Schistocerca*, all of the abdominal segments are morphologically distinct (those of A10 and A11 are partially fused in adults, but are distinct throughout the embryonic stages considered in this paper); the posteriormost segments (A9 to A11) show increasing degrees of reduction in an anterior-posterior progression. The expression domains of the *Drosophila* ABD-B M and R functions are shown separately to emphasise the correspondence of the *Schistocerca* ABD-B domain to that of the R function in *Drosophila*. The relative levels of expression have been indicated very coarsely. Note that there is no sign in *Schistocerca* of the high levels of ABD-B M so prominent in A8 and posterior A7. *Drosophila* data after Celniker et al. (1989), DeLorenzi and Bienz (1990) and Boulet et al. (1991).

Sera from at least three animals of two species gave essentially the same pattern in *Schistocerca*, suggesting that the pattern of reactivity described here is consistently observed for sera raised against the product of this *Schistocerca* sequence. Failure to detect the equivalent of an *m* function could result from the M proteins being expressed at much lower levels than R proteins, or being modified in such a way as to make them less likely to react with sera raised against bacterially synthesized protein. A further possibility, consistent with a conserved developmental role, is that the ABD-B M and R proteins may be encoded by different genes in *Schistocerca*. This would result if the *Abd-B* 3 exons that are common in *Drosophila* are duplicated in *Schistocerca*, allowing the distinct regulatory elements of *m* and *r* functions to act on independent transcription units. We know that our serum reacts with regions of the ABD-B protein that are not highly conserved, for it does not react with *Drosophila* ABD-B. Thus it may well fail to react with a second, independently diverging variant of the ABD-B protein in *Schistocerca*.

An alternative, and more radical, possibility is that *Drosophila* and *Schistocerca* use their homeotic proteins in different ways to specify segment development and that *Schistocerca* indeed lacks an *Abd-B m* function in the epidermis of more anterior abdominal segments. We have previously proposed that such changes in homeotic gene regulation might correlate with the increasing specialization of segments during the diversification of the insects (Akam et al., 1988). Further experiments are required to resolve this issue, but one observation specifically supports this latter interpretation. In *Drosophila*, the levels of *UBX* and *ABD-A* proteins are reduced where *ABD-B* protein is present at high levels (Struhl and White, 1985; Macias et al., 1990). Thus *UBX* is expressed at very low levels in parasegment 13, but this repression is released in an *Abd-B* mutant. In *Schistocerca*, the levels of *UBX* and *ABD-A* protein in A7p/A8a (parasegment 13) are similar to those in the more anterior abdominal segments (Tear et al., 1990; Kelsh et al., in preparation). The repression of *abd-A* that is evident in A9 and A10 (see below) does not occur in A8.

The process of segmentation and segment specification in *Schistocerca*

In *Drosophila* the body segments are formed in the syncytial blastoderm, almost simultaneously: it is thus a typical long-germ insect. In contrast, *Schistocerca* is an extreme short-germ insect: most segments are formed sequentially, after gastrulation (Anderson, 1972a,b; Sander, 1976). In the abdomen, this process follows an anterior-to-posterior sequence. This can be seen directly by the appearance of visible segmentation, and has been further confirmed at the molecular level by the use of a probe for Engrailed protein (Patel et al., 1989). As in *Drosophila*, the *engrailed* gene is expressed in the posteriormost region of each segment. Engrailed protein can first be detected shortly before each segment becomes morphologically distinguishable. By the time the germ band is completed, the patterns of Engrailed expression in *Drosophila* and *Schistocerca* are remarkably similar.

Homeotic genes are also expressed in broadly similar regions of the mature germ bands of *Drosophila* and *Schis-*

tocerca. The differences in ABD-B expression discussed above are striking, but are the exception rather than the rule. Overall similarity is best documented in the case of the *abd-A* gene. In the fully segmented embryo (33%), the anterior boundary of ABD-A expression is the same in *Schistocerca* and *Drosophila*, precisely in register with the anterior limit of *engrailed* expression in the first abdominal segment (Tear et al., 1990).

While the mature patterns are rather similar, the generation of these patterns differs significantly between the two species. In *Drosophila*, the pattern is generated before cell membranes have formed; patterning depends on the intracellular diffusion of gap and pair-rule gene products. In *Schistocerca*, segment patterning appears after cellularization; at least some of the genes that act early to segment the *Drosophila* embryo (e.g. *eve*) appear to play no corresponding role in *Schistocerca*; (Patel et al., 1992; Akam and Dawes, 1992).

In *Schistocerca*, the appearance of ABD-A protein follows the appearance of segments, starting in A2 and extending both slightly forwards and progressively backwards to A10, in each segment slightly delayed with respect to Engrailed (Tear et al. 1990). The first appearance of ABD-B protein follows a quite different pattern: It appears first at the extreme back end of the growing abdomen, before the onset of Engrailed expression in A10 and A11. The subsequent changes in the expression domain consist of an anteriorwards expansion, into segments that were formed rather earlier. Thus the first appearance of ABD-B does not appear to depend on the sequential formation of the posterior segments.

We think that, in *Schistocerca*, the *Abd-B* gene is responding to a signal that derives from the posterior end of the embryo and is independent of segmentation. Two observations support the existence of such a signal. First, at least one specific posterior structure, the hindgut primordium, is clearly distinguishable before segmentation is complete (Bentley et al., 1976). The second observation derives from studies of the effects of high doses of X-irradiation on embryos of another Orthopteran, the cricket *Gryllus*. Irradiation at 70-80 hours of embryogenesis (prior to elongation or segmentation of the embryonic primordium) often results in complete or virtually complete repression of the abdomen (Heinig, 1967, reviewed by Sander, 1976). However, if at least two segments of the abdomen do form then structures characteristic of the first and last segments (A1 and A11) are present, even if all the rest are absent.

In *Drosophila*, *Abd-B r* expression is regulated primarily by the terminal patterning system, mediated by the gap gene *tailless* acting in the absence of *huckebein* (Casanova, 1990). This patterning system, which is independent of the localised maternal RNAs contained in the pole plasm, also specifies the posterior mid-gut and hind-gut primordia; it seems a likely candidate for an early acting patterning mechanism that may be conserved in *Schistocerca*. In contrast, *Abd-B m* expression depends also on the sequence of gap gene expression that is initiated by the *hunchback* morphogenetic gradient, and culminates in the expression of the zygotic genes *giant* and *knirps* adjacent to the *tailless* domain (Harding and Levine, 1988; Reinitz and Levine,

1990). In *Drosophila*, the unfolding of this pattern is dependent on localised RNA within the pole plasm (the *nanos* gene product). It is not yet clear if a similar gap gene patterning system exists in *Schistocerca* and, if it does, whether it is involved in segment generation or only in regional specification. If similar diffusion mechanisms generate a gap pattern in *Schistocerca*, then they must operate prior to segmentation.

Conservation of tissue specificity of *Abd-B*

From this study, it is clear that all the principal tissues expressing *Abdominal-B* in *Drosophila* also do so in *Schistocerca*. The posterior abdominal epidermis, the CNS, the somatic mesoderm and the hindgut visceral mesoderm all express *Abd-B* at some point in embryogenesis. Study of later embryonic stages will be required to ascertain whether *Abd-B* is expressed in the mesoderm of the developing posterior mid-gut and gonads.

Abdominal-B and segment specification in the post-abdomen

The reduction and fusion of post-abdominal segments in *Drosophila* frustrates any attempt to define the role of homeotic genes in segment specification posterior to A8. In some respects, the situation is much clearer in *Schistocerca*. ABD-B is expressed throughout the post-abdomen from A8p to A11, but it is apparently not expressed more than fleetingly in the appendages of the A11 segment - the anal cerci. From their first appearance, the primordia of the anal cerci remain stubbornly free of staining with the anti ABD-B antibody. This is in contrast with the appendage buds of other segments, which are generally the most prominent sites of expression of homeotic genes during early embryogenesis. (Tear et al., 1990; this work and R. Kelsh, unpublished data).

Drosophila have no anal cerci. In the adult, the anal plates are derived from the most posterior parts of the genital disc, and may be the homologous structures in flies (McAlpine et al., 1981). Their formation requires expression of *caudal*, a homeobox gene that is not located within the ANT-C or BX-C. In the embryo, *caudal* is expressed and required for the development of the anal pads and tuft - extreme posterior structures whose segmental origin is unclear (Macdonald and Struhl, 1986; Jürgens, 1987). Initially *caudal* expression overlaps with that of the *Abd-B r* function but, in later development, mutually exclusive sets of cells express these two genes (Sanchez-Herrero and Crosby, 1988). We guess that *caudal* may specify the anal cerci of *Schistocerca*.

The most controversial aspect of the insect abdomen, and developmentally one of the most interesting, is the origin of the genitalia (Snodgrass, 1935; Matsuda, 1976). Primitively, both male and female genitalia are believed to have been derived from modified segmental appendages, as is the case in modern *Schistocerca*. Different segments are involved in the two sexes - A8 and A9 in females, A9 and perhaps A10 in males. In many more recently evolved groups, the whole posterior abdomen has been modified as a mating and egg-laying apparatus - the appendicular ovipositor of the female frequently being replaced by a tubular post-abdomen. This is the case in *Drosophila*, where

much of A8 and possibly A9 are modified to form the ovipositor. Thus the extent of homology between the genitalia of *Schistocerca* and *Drosophila* is hard to determine.

In *Drosophila*, *Abd-B m* and *r* functions are both essential for the development of the female genitalia, but only *Abd-B r* function is necessary for the development of the male genitalia (Casanova et al., 1986). This, and a comparison with other Diptera in which the genital primordia form separately and do not fuse, suggests that the male genitalia derive exclusively from A9, and indeed constitute the only derivatives of this segment in the adult male (Epper and Nothiger, 1982; French, 1983).

In *Schistocerca*, the appendage buds of the ninth and tenth segments express ABD-B prominently. The A9 buds develop into the male genitalia or, in the female, into part of the ovipositor. The A10 buds are resorbed in the female, but in the male they develop into parts of the external genitalia. In the appendage buds of A8, we can detect only very low levels of *Abd-B*, principally in A8p. These buds regress in the male, but in females they form the major part of the ovipositor - a developmental pathway very distinct from that of the transient appendage buds of the more anterior abdominal segments. Either low levels of ABD-B protein specify this difference, or it is controlled by some product other than that identified by our sera.

One other striking difference between the abdomens of *Drosophila* and *Schistocerca* is the retention of typical articulated abdominal sclerites in the post-abdomen of *Schistocerca*, but their reduction and loss in *Drosophila*. As a result, *Schistocerca* has a recognizably segmented post-abdomen, whilst in *Drosophila* only minute observation reveals the remaining hints of segmentation. This difference correlates with a difference in the expression of homeotic genes. In *Drosophila*, *abd-A* is never normally expressed in parasegment 14* (A9 back), though it becomes expressed here in *Abd-B* mutants, when an additional distinct A9 segment develops. In *Schistocerca*, *abd-A* is transiently expressed in A9 and A10 (Tear et al., 1990), but is repressed at about the time that *Abd-B* expression extends anteriorly. By analogy with known regulatory circuits in *Drosophila*, we suggest that this is a direct interaction of ABD-B protein with the *abd-A* promoter. We suggest that the window of expression of *abd-A* during the early development of A9 and A10 in *Schistocerca* may be sufficient to ensure that these segments develop the basic structure typical of a pre-abdominal segment, whereas in *Drosophila*, the genes that specify this developmental pathway (*Ubx* and/or *abd-A*) are never expressed.

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