The *Tribolium* homeotic gene *Abdominal* is homologous to *abdominal-A* of the *Drosophila* bithorax complex

Jeffrey J. Stuart¹*, Susan J. Brown², Richard W. Beeman¹ and Robin E. Denell²†

¹USDA, ARS, US Grain Marketing Research Laboratory, 1515 College Avenue, Manhattan, Kansas 66502, USA
²Division of Biology, Kansas State University, Manhattan, Kansas 66506, USA

*Present address: Department of Entomology, Purdue University, West Lafayette, Indiana 47907, USA
†Author for correspondence

**SUMMARY**

The *Abdominal* gene is a member of the single homeotic complex of the beetle, *Tribolium castaneum*. An integrated developmental genetic and molecular analysis shows that *Abdominal* is homologous to the *abdominal-A* gene of the bithorax complex of *Drosophila*. *Abdominal*-A mutant embryos display strong homeotic transformations of the anterior abdomen (parasegments 7-9) to PS6, whereas developmental commitments in the posterior abdomen depend primarily on *Abdominal-B*. In beetle embryos lacking *Abdominal* function, parasegments throughout the abdomen are transformed to PS6. This observation demonstrates the general functional significance of parasegmental expression among insects and shows that the control of determinative decisions in the posterior abdomen by homeotic selector genes has undergone considerable evolutionary modification.

Key words: *Tribolium castaneum*, *Drosophila melanogaster*, homeotic transformations, embryogenesis, evolution

**INTRODUCTION**

In *Drosophila melanogaster*, segmental identity is regulated by homeotic selector genes located in two clusters, the Antennapedia complex (ANT-C) (Kaufman et al., 1989) and the bithorax complex (BX-C) (Duncan, 1987). Genetic and molecular analyses of these genes have provided considerable insight into the regulatory mechanisms involved in the establishment of developmental fates in this organism. However, understanding the role that these homeotic genes played in animal evolution requires interspecific comparisons. Since *Drosophila* represents a highly derived and specialized species, comparisons within the Insecta should be informative. Thus, we have undertaken an investigation of homeotic genes of the red flour beetle, *Tribolium castaneum*, an insect that is in many ways more primitive than *Drosophila*. Beeman (1987) first showed that six spontaneous homeotic mutations in *Tribolium* map in a cluster (the Homeotic complex or HOM-C) on the second linkage group. In addition to their close linkage, these beetle genes resemble those of the ANT-C and BX-C with respect to their homeotic phenotypes and colinearity (that is, they map along the chromosome in the same order as their mutant effects lie along the anterior-posterior body axis (Lewis, 1978)). Beeman (1987) suggested that the HOM-C represents the homologs of the ANT-C and BX-C in juxtaposition, and that a single complex is the more ancient arrangement. The latter hypothesis is supported by the observation that mammals also have a single homeotic cluster (albeit in several divergent copies) which appears to have arisen before the divergence of protostomes and deuterostomes (Akam, 1989).

More recently, Beeman et al. (1989) isolated about fifty new mutations in the HOM-C, and characterized them with respect to complementation relationships and adult homeotic phenotypes. They suggested that a number of dominant mutations affecting the adult abdomen include both loss-of-function and gain-of-function alleles at a single locus called *Abdominal* (A). Moreover, they proposed that mutations associated with both a recessive, anteriorly directed transformation of the anterior abdomen and a dominant posteriorly directed transformation of the posterior abdomen represent null A alleles. This phenotype resembles that of the *Drosophila abdominal-A* (*abd-A*) gene in its more anterior aspects, but the posteriorly directed transformation is quite unexpected based on *Drosophila* studies (Duncan, 1987).

In this paper, we present results from reversion mutagenesis and dosage analysis, which establish allelism among these variants and clearly distinguish loss-of-function from gain-of-function *Abdominal* mutations. We also describe
Table 1. Dominant Abdominal mutant alleles and their revertants

<table>
<thead>
<tr>
<th>Class</th>
<th>No.</th>
<th>Abdominal recessive lethal*</th>
<th>Adult mutant phenotype</th>
<th>Allele Mutagenized</th>
<th>No. Revertants (frequency)</th>
<th>Revertant Abdominal lethal*</th>
<th>Revertant phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal</td>
<td>17</td>
<td>yes</td>
<td>A3→A2 A4→A3</td>
<td>none</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Extra sclerite</td>
<td>3</td>
<td>no</td>
<td>A4→A3</td>
<td>Es1</td>
<td>2 (0.0003)</td>
<td>yes</td>
<td>A3→A2 A6→A7 A4→A3</td>
</tr>
<tr>
<td>Socketless</td>
<td>2</td>
<td>no</td>
<td>A3→A4</td>
<td>Skl1</td>
<td>3 (0.0008)</td>
<td>yes</td>
<td>A3→A2 A4→A3 A6→A7</td>
</tr>
<tr>
<td>Mosaic sclerotization</td>
<td>3</td>
<td>no</td>
<td>A7→A6 A2,3→A4† A8→A7†</td>
<td>Mcs1</td>
<td>3 (0.0004)</td>
<td>yes</td>
<td>A3→A2 A4→A3</td>
</tr>
</tbody>
</table>

*Alleles were designated as having the Abdominal recessive lethality if they failed to complement A10 for viability to the adult stage. Mcs1+R4/A10 individuals infrequently survive to adulthood and resemble rare adult escapers heterozygous for A10 and the hypomorphic variant A5. Escapers homozygous for A5 also show recessive lethality at A57→A8.

**Putative dominant gain-of-function phenotypes.

the cloning and characterization of a portion of the Tribolium abd-A homolog and show that this genomic region originates from the genetically defined A locus. Finally, we show that, despite the marked morphological differences between beetles and flies in the thorax and anterior abdomen, the Abdominal lethal syndrome resembles that of abd-A in causing transformations of posterior parasegments to parasegment 6. This work presents direct functional and structural comparison between homologous homeotic selector genes in distantly related species, and demonstrates that Tribolium offers the possibility of integrated developmental, genetic and molecular analysis with a resolution presently unrivaled in any higher eukaryotic animal outside of the Drosophilids and Caenorhabditis.

MATERIALS AND METHODS

Beetle strains

For experimental crosses, beetles were reared at 32°C in wheat flour containing 5% (w/w) brewer’s yeast. The 25 Abdominal variants listed in Table 1 are described by Beeman et al. (1989) or (for five A and one Skl mutant alleles) isolated subsequently. Recessive lethal mutations were balanced with Ultrathorax (Utx1), maxillopedaStumpy (mpSm) or Eyeless (Ey), each associated with crossover suppression and recessive lethality. The HOM-C duplication Dp(2)Dachs was maintained in two stocks: Dp(2)Dachs/A10/Cx1 and Dp(2)Dachs/A5/Cx1.

Reversion mutagenesis

In order to assess whether A12, A10 and A5 are gain-of-function Abdominal alleles, males heterozygous for each were treated with 4-5 krad of gamma radiation from a 60Co source, held for 2-3 days at 30°C and then mated with tester females; males were discarded after two days and females were allowed to oviposit for 4-5 weeks at 32°C. A12/Utx1 and A10/mpSm irradiated males were crossed to mpSm A10 and sooty (s) females, respectively, whereas Amfs1/mpSm and Sk1/mpSm irradiated males were mated to s and blackborne (t) females, respectively. (The dark body mutations s and t are unlinked to the HOM-C and aid in separating sexes in order to discard irradiated males.) F1 adults were screened for a wild-type phenotype and, in one of the crosses, for new mutations that failed to complement Amax. The nature of new mutations was confirmed by showing that the putative ‘revertant’ chromosome fails to complement the recessive lethality associated with the original mutation and does complement the marker associated with the irradiated balancer chromosome.

Dosage analysis

The A12/A12 genotype was constructed using the mutation A12, the chromosome mpSm Cephalothorax2 (Cx2) and the HOM-C duplication Dp(2)Dachs. The latter contains wild-type copies of the A and Cx loci (Stuart et al., 1991). Therefore, in hyperploid beetles, it covers the haplo-insufficient dominant Cx2 phenotype, but not the dominant gain-of-function mpSm phenotype. A12/mpSm Cx2/Dp(2)Dachs beetles could thus be recognized among the progeny of A12/Ey males and A5/mpSm Cx2/Dp(2)Dachs females.

The A10/A10/A5+A+ genotype was observed while constructing the A10/A10/Dp(2)Dachs stock. A5mp2/A10/Dp(2)Dachs females, which express the dominant A5 phenotype but not the recessive A5mp2/A5 phenotype, were mated with A10/mpSm males. Among the progeny, the putative A10/A10/Dp(2)Dachs beetles expressed the A5 phenotype but neither the dominant mpSm nor recessive A5mp2/A10 phenotypes. The presence of Dp(2)Dachs in these aneuploid beetles was confirmed by further progeny testing.

The A5/Dp(2)Dachs/A5+A+ genotype was generated by mating A5mp2/A5/Dp(2)Dachs males with mpSm/A5+ females. The A5/mpSm/Dp(2)Dachs progeny showed both the dominant ‘short legs’ phenotype associated with mpSm and the dominant A2→A3 transformation associated with A5, A5/mpSm/A5/Dp(2)Dachs and A5mp2/A5/Dp(2)Dachs and A5mp2/A5/Dp(2)Dachs females were generated by mating A5mp2/A5/Dp(2)Dachs males with A5/mpSm and A5mp2/mpSm females, respectively. In each case, the presence of Dp(2)Dachs was confirmed by additional test crosses.

Embryonic analysis

Females were allowed to oviposit in fine flour and embryos were isolated by sieving. Whole-mount preparations of cuticularized embryos were prepared by dissecting the specimens free of the egg membranes on double-sided cellophane tape, incubating them for approximately 24 hours at 40°C in a pool of 9:1 lactic acid:95% ethanol and then under coverslips for several additional days.
immunocytochemistry, embryos were treated for 2 minutes with commercial bleach to remove chorions and adhering flour particles. They were then fixed in a biphasic solution of 4% paraformaldehyde in PBS and n-heptane, and subjected to methanol shock at room temperature. This protocol (modified from Mitchison and Sedat, 1983) does not remove the vitelline membrane as it does in *Drosophila*, but does cause rents in the membrane, which aid in dissecting it free. Using standard protocols, endogenous peroxidase activity was destroyed by treating embryos with 1% *H*<sub>2</sub>O<sub>2</sub> in methanol for 15 minutes and, after washing, they were treated with 5% normal horse serum in PBS for 15 minutes. After overnight incubation at 4°C with primary antibody, the immunostaining pattern was revealed by horseradish peroxidase detection using the Vectastain mouse IgM ABC kit.

**Molecular analysis**

Construction and screening of a *Tribolium* genomic library in λGem-11 (Promega) was previously described (Brown et al., 1990). A 400 bp fragment containing the *Drosophila Antennapedia* - *dia* homeobox sequence was purified from pA2015 (Scott et al., 1983) and random-primer labeled with [³²P]dCTP. The filters were hybridized at 56°C in 6x SSC, 10x Denhardt’s, 0.1% SDS, 25 mM phosphate buffer, pH 7.0 and 0.1 mg/ml denatured sonicated herring sperm DNA. After 24 hours, the filters were washed twice for 15 minutes at room temperature in 2x SSC, 0.1% SDS.

Restriction fragments were subcloned into pGem 7F+ (Promega) by standard methods (Maniatis et al., 1982). Purified insert fragments of pJS16-1 and pJS16-3 (Fig. 6) were random-primer labeled and hybridized to genomic DNA from *Abdominal* mutants as previously described (Brown et al., 1990).

The 700 bp EcoRI-HindIII fragment containing the *Abdominal* homeobox was sequenced by the dideoxy method using double-stranded templates prepared from sets of overlapping nested deletions constructed in both orientations.

mRNA was isolated from eggs laid over a 2-3 day period and used to construct a cDNA library in Unizap (Stratagene). The primary library (with a base of 1.4×10⁶ plaques and 0.8 kb average insert size) was amplified and screened, and a single A clone with a 2.1 kb insert was identified among 5×10⁶ plaques. From this insert, single-stranded probes labeled with digoxigenin were prepared as suggested by N. Patel (personal communication). The cDNA plasmid was linearized at the 5’ end with BamHI and *XhoI* to make antisense and sense probes, respectively, and used as a template in a 50 µl PCR labeling reaction that contained 100 µM dATP, dCTP and dGTP, 65 µM dTPP, 35 µM digoxigenin-11-UTP, 1 µM T7 or SK (Stratagene) promoter primer, and 1.2 Units Taq polymerase in standard PCR buffer (Boeringher Mannheim). The 30 PCR cycles each consisted of 45 seconds at 95°C, 30 seconds at 55°C and 1 minute at 72°C. The resulting single-stranded insert fragments of pJS16-1 and pJS16-3 (Fig. 6) were random-primer labeled and hybridized to genomic DNA from *Abdominal* mutants as previously described (Brown et al., 1990).

A meaningful assessment of the functional significance of this locus requires that we clearly distinguish which effects are associated with null alleles. We have compiled several types of evidence confirming the interpretation of Beeman et al. (1989). The first is based on additional mutagenesis experiments. Many homeotic gain-of-function phenotypes are caused by the inappropriate expression of a gene in a region in which it is normally inactive (e.g., White and Akam, 1985; Frischer et al., 1986). Such a phenotypic effect can be ‘reverted’ by any mutational event that eliminates the inappropriate expression (e.g., Hazelrigg and Kaufman, 1983). Such events occur with frequencies typical of forward rather than reverse mutation and often result in total inactivation of the gene. We screened for mutations eliminating the dominant putative gain-of-function phenotype of the variants *Es*<sup>1</sup>, *Skl*<sup>1</sup> and *Mcs*<sup>1</sup> (Table 1). In all three cases, ‘revertants’ were isolated at rates typical of forward mutation to A variants under similar conditions. As predicted, revertants of *Es*<sup>1</sup> and *Skl*<sup>1</sup> are indistinguishable from A variants in their adult phenotypes (Fig. 1) and fail to complement *mas*, *pas* and the recessive lethality of A<sup>10</sup>. Although characterization of the revertants of *Mcs*<sup>1</sup> indicate that this lesion is also at the *Abdominal* locus, they differ in that they do not appear to represent complete A nulls: they share the A recessive lethality and fail to complement *mas* and *pas*, but do not display the dominant A<sub>6</sub>→A<sub>7</sub> phenotype expected (Fig. 1). Overall, these experiments provide strong evidence that all three classes represent gain-of-function mutations at the *Abdominal* locus.

The second type of evidence depends on manipulating the number of *Abdominal*<sup>+</sup> doses. The hypothesis predicts that A<sup>−</sup>/*A*<sup>+</sup> heterozygotes should be the equivalent of a deficiency heterozygote in phenotype. Stuart et al. (1991) described the generation of a deficiency of a large portion of the HOM-C, apparently by an exchange in the common region of two overlapping inversions. Such *Df(HOM-C)* chromosomes fail to complement *mas* and *pas*, and express a dominant A<sub>6</sub>→A<sub>7</sub> transformation when heterozygous with a wild-type chromosome.

Moreover, if the A<sub>6</sub>→A<sub>7</sub> transformation is indeed haplo-insufficient in nature, it should be complemented by a duplication of *Abdominal*<sup>+</sup>. We have recovered a duplication, *Dp(2)Dachs*, presumably as an aneuploid segregant from

**RESULTS**

**The genetic nature of *Abdominal* variants**

Beeman (1987) described two recessive variants within the HOM-C of *Tribolium* associated with adult abdominal homeotic transformations: *missing abdominal sternite* (*mas*) causes a transformation of the third abdominal segment into the second (A<sub>3</sub>→A<sub>2</sub>), and *pointed abdominal sternite* (*pas*) transforms A<sub>4</sub>→A<sub>3</sub>. Subsequently, Beeman et al. (1989; R. W. B. and J. J. S., unpublished results) isolated several classes of dominant variants also linked to the HOM-C. Members of the most frequent class, *Abdominal* (A), fail to complement *mas* and *pas*, cause a dominant transformation of A<sub>6</sub>→A<sub>7</sub>, and share a common recessive lethality. Three other classes, *Extra sclerite* (*Es*), *Socketless* (*Skl*) and *Miscaudal sclerotization* (*Mcs*) complement the A recessive lethality and cause dominant transformations opposite in direction (along the anterior/posterior axis) to those of *Abdominal* mutants (Table 1 and Fig. 1). Beeman et al. (1989) suggested: (1) all of these dominant mutations are alleles of a common gene and (2) A variants are loss-of-function mutations of this haplo-insufficient gene, whereas the other dominants display gain-of-function effects.
the chromosomal rearrangement associated with the HOM-C mutation maxillopedia\textsuperscript{Dachs} (Beeman et al., 1989). Stuart et al. (1991) used restriction fragment length polymorphisms to demonstrate that a portion of the HOM-C is present in three copies in aneuploid beetles carrying this duplication. As expected, in the presence of \textit{Dp(2)Dachs}, beetles that are \textit{A}\textsuperscript{mas}/\textit{A}\textsuperscript{pas} or \textit{A}\textsuperscript{mas}/\textit{pas} display no dominant \textit{A}6→\textit{A}7 effect or (in the latter case) recessive \textit{A}4→\textit{A}3 transformation. However, the duplication fails to complement the putative gain-of-function dominant effects of \textit{Es}, \textit{Skl} or \textit{Mcs} beetles, showing that they do not arise because of diminished \textit{Abdominal}\textsuperscript{+} function (data not shown).

Given the strong evidence from these studies indicating that \textit{mas}, \textit{pas}, \textit{Es}, \textit{Skl} and \textit{Mcs} variants are all \textit{Abdominal} mutant alleles, we will hereafter assign them a genetic symbol indicative of that relationship. For example, \textit{Extra sclerite}\textsuperscript{1} will now be referred to as \textit{Abdominal-Extra sclerite}\textsuperscript{1} and symbolized \textit{A}\textit{Es}\textsuperscript{1}.

Recombination experiments show that the recessive, loss-of-function and gain-of-function \textit{Abdominal} mutations are tightly linked (Table 2). Beeman (1987) determined that four HOM-C variants map in the order \textit{C}e\textit{apt} \textit{A}\textit{mas} \textit{A}\textit{pas} \textit{eu}, \textit{A}\textsuperscript{10} and \textit{A}\textsuperscript{13} are putative null alleles which fail to complement \textit{A}\textit{mas} and \textit{A}\textit{pas}, \textit{A}\textsuperscript{10} maps to the left of \textit{A}\textit{mas} (toward \textit{Cx}), whereas \textit{A}\textsuperscript{13} lies to the right of \textit{A}\textit{mas} (toward \textit{eu}) and probably between the two recessive lesions. This inter-sppersion of recessive hypomorphs and amorphs is similar to that reported for the \textit{Drosophila} gene \textit{Ultrabithorax} (Duncan, 1987). The gain-of-function mutations \textit{A}\textit{Skl}\textsuperscript{6} and \textit{A}\textit{Mcs}\textsuperscript{1} are tightly linked and to the right of \textit{A}\textit{mas}, while \textit{A}\textit{Skl}\textsuperscript{1} is to the right of \textit{A}\textsuperscript{mas}. These results are also consistent with the view that \textit{Abdominal} is a single complex locus.
The **Abdominal** lethal syndrome resembles that of **abdominal-A**

The results presented thus far support the hypothesis that loss-of-function A alleles are associated with bidirectional adult abdominal transformations unexpected from *Drosophila* studies. However, examination of the A embryonic phenotype reveals that it is very similar to that of *Drosophila abd-A* mutations. Sokoloff (1972) has reviewed the events of embryogenesis in *T. confusum*, a sister species to *T. castaneum*, and we have followed embryonic development in the red flour beetle in whole-mount preparations. During germ band extension in normal embryos, appendage primordia appear on the antennal, gnathal, thoracic and A1 segments. Later in development, the A1 evaginations are retracted and develop the glandular pleuropodia. We have examined normal and mutant individuals immunostained with 4C3, a monoclonal antibody directed against a protein encoded by the *Drosophila Antennapedia* gene (Glicksman and Brower, 1988). In normal *Tribolium* embryos after germ band retraction, 4C3 immunostains the pleuropodia very intensely (Fig. 2A). (A complete account of the use of 4C3 to study beetle embryonic development will appear elsewhere.)

Individuals homozygous for putative null *Abdominal* mutations die late in embryonic development without hatching from the egg. For five different *Abdominal* variants examined in homozygous and hemizygous condition, the first eight abdominal segments have appendage primordia normally restricted to segments anterior to A2 (data not shown). As development proceeds, a larger anterior portion of the evagination on each hemisegment undergoes retraction and forms a pleuropodium (Fig. 2B). Both the A1 and ectopically developing pleuropodia are smaller than normal because they are shortened posteriorly. The posterior-most portion of each hemisegment remains evaginated, and later becomes cuticularized and often decorated with campaniform sensillae and small sensory hairs. We suggest that this outgrowth represents a rudimentary posterior compartment of the T3 larval leg, which also bears such elements (albeit not uniquely). Our interpretation is that the beetle embryonic *Abdominal* transformation (similar to that of *abdominal-A*) is a reiteration of a unit corresponding to parasegment 6 (T3 posterior/A1 anterior) of *Drosophila*.

In other work, we have utilized monoclonal antibody 4D9 which recognizes an evolutionarily well-conserved epitope of the *Drosophila engrailed* and *inveorted* proteins (Patel et al., 1989) to delineate the putative anterior and posterior compartments in beetle embryos (R. E. D. and S. J. B., unpublished data). Most of each appendage (including the thoracic legs and the primordium giving rise to the A1 pleuropodium) of normal embryos is made up of cells from the anterior compartment and the posterior compartment is

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**Table 2. Recombinational mapping of dominant homeotic mutations affecting the abdomen**

<table>
<thead>
<tr>
<th>Cross</th>
<th><strong>Informative recombinants</strong></th>
<th>Total progeny</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ A10 ++ apt + pas</td>
<td>1 apt + + apt + pas</td>
<td>5,039</td>
</tr>
<tr>
<td>+ A10 ++ apt + pas</td>
<td>1 apt A10 ++ apt + pas</td>
<td></td>
</tr>
<tr>
<td>+ A13 ++ apt mas + eu</td>
<td>1 ++ ++ eu apt mas + eu</td>
<td>16,037</td>
</tr>
<tr>
<td>+ A13 ++ apt mas + eu</td>
<td>1 ++ ++ eu apt mas + eu</td>
<td></td>
</tr>
<tr>
<td>+ Sk16 ++ apt mas + eu</td>
<td>4 ++ ++ eu apt mas + eu</td>
<td>6,214</td>
</tr>
<tr>
<td>+ Sk16 ++ apt mas + eu</td>
<td>1 ++ ++ Sk16 eu apt mas + eu</td>
<td></td>
</tr>
<tr>
<td>+ Ms1 ++ apt mas + eu</td>
<td>1 ++ ++ eu apt mas + eu</td>
<td>7,037</td>
</tr>
<tr>
<td>+ Ms1 ++ apt mas + eu</td>
<td>2 ++ ++ eu apt mas + eu</td>
<td>8,377</td>
</tr>
</tbody>
</table>

The mutant alleles presented were ordered with respect to the *Abdominal* alleles mas or pas, and the flanking HOM-C markers Cephalothorax-alate prothorax (formally symbolized Ce but abbreviated apt here), and extra urogomphi (eu).
restricted to a narrow posterior stripe (Fig. 3A). Examination of A homozygotes immunostained with this antibody supports the interpretation that a T3 posterior/A1 anterior parasegment is reiterated. That is, the homeotic pleuropodia belong to the anterior compartment, and the posterior compartment is enlarged and includes each of the persistent outgrowths on A1-A8 (Fig. 3B).

The **Tribolium abdominal-A homolog is Abdominal**

We screened a Tribolium castaneum genomic library with a *Drosophila Antennapedia* homebox probe. Restriction maps of the resulting 16 recombinants revealed that they arise from six genomic locations. One group of four overlapping clones includes a region homologous to *abdominal-A*. Fig. 4 shows the DNA sequence of pJS16-4, a subclone of λJS16 (see Fig. 6). An open reading frame predicts an amino acid sequence identical to that of an *abd-A* cDNA (Karch et al., 1990) in the homeobox and flanking regions (Fig. 5). The carboxyl-most portions of each peptide are highly similar as well (Fig. 5). Within the homeobox, the *Tribolium* DNA sequence shows 80% identity to *abd-A*. Southern blots (see below) show this putative protein-coding region is present once per haploid genome. These observations indicate that we have cloned a portion of the *Tribolium* *abd-A* homolog.

To determine if the λJS16 insert corresponds to a portion of the genetically defined A locus, we first examined whether the *Tribolium* sequence from which the clone was derived is linked to the HOM-C. Linkage was assessed using a restriction fragment length polymorphism (RFLP) identified between two wild-type *Tribolium* stocks, Lab-S and Georgia-1 (GA-1), using the insert from subclone pJS16-3 (see Fig. 6) as a probe. We used the Lab-S-derived balancer chromosome mex83 and the GA-1-derived mutation A83 for this experiment. Southern analysis of the parents and progeny of the mating between A83/mex83 males and GA-1 females indicated that the Lab-S-derived restriction fragment always segregated with the mex83 chromosome, whereas only the GA-1 polymorphism was observed in A83 progeny (data not shown).

To correlate directly λJS16 and the A gene, we showed that the radiation-induced A12 mutation is associated with a DNA rearrangement breakpoint within λJS16 and within the HOM-C. Southern analysis of DNAs isolated from 16 A mutant stocks, using the λJS16 insert as a probe, revealed a unique restriction fragment associated with the radiation-induced mutation A12 (data not shown). When used to probe genomic blots, subclone pJS16-1 revealed RFLPs in A12 DNA digested with XhoI, HindIII or EcoRI (Fig. 6). Thus, A12 is associated with a DNA rearrangement breakpoint within the 419 bp XhoI/HindIII fragment, which contains 130 bp of putative A homeobox and an additional 204 bp of protein-coding sequence. These data strongly suggest that the A12 mutation was caused by a chromosomal rearrangement or transposon insertion.

The implication that the A12 mutation was caused by this rearrangement breakpoint was further supported by demonstration of tight linkage between the genetic lesion and RFLP. On genomic Southern blots, inserts from pJS16-1 and -3 each identify a 2.8 kb HindIII fragment in DNA from GA-1 or apt mas eu beetles, and a 3.6 kb fragment in A12 DNA. This 3.6 kb fragment apparently represents homology to the left side of the A12 rearrangement breakpoint, whereas a 1.0 kb fragment (identified only by pJS16-1) represents homology to the right side of the same breakpoint. From a mating of mex83/A12 × Cxopt Amax eu/Cxopt Amax eu beetles, F1 A12/Cxopt Amax eu males were backcrossed to Cxopt Amax eu homozygous females. Progeny recombinant for visible markers were backcrossed once again to Cxopt Amax eu, and DNA was prepared from their offspring, restricted with HindIII and probed with the pJS16-3 insert. We observed 1.4% recombination between Cxopt and Amax, close to the 1.2% obtained by Beeman (1987) in an experiment in which no other *Abdominal* mutation was present. Thus, the A12 rearrangement does not markedly affect the rate of recombination in this interval. For the 12 beetles recombinant between Cxopt and Amax tested molecularly, there was complete coincidence between A12 and the 3.6 kb restriction fragment. These results indicate both lesions lie to the right of Cxopt. No
recombination between $A^{12}$ and $A^{mas}$ was observed. Only one of the two classes arising from recombination in the interval between $A^{mas}$-$A^{12}$ and $eu$ was recognized, and neither of these $Cx^{opt} A^{mas}$ individuals received the 3.6 kb fragment. These data, as well as molecular cytogenetic results described by Stuart et al. (1991), indicate that the RFLP lies between $Cx^{opt}$ and $eu$, consistent with the idea that it arose as a consequence of the mutational event which generated the $A^{12}$ variant.

Expression pattern of the Abdominal gene

We have begun to assess the pattern of expression of the Abdominal gene by performing in situ hybridization to the transcripts in embryo whole mounts (Tautz and Pfeifle, 1989), using digoxigenin-substituted single-stranded DNA antisense and sense probes synthesized using an Abdominal cDNA template. Thus far, we have focused on germ band-extended embryos; a complete description of the temporal and tissue specificity is presently underway. At the fully extended germ band stage of Tribolium, it is possible to identify 11 abdominal segments (Stanley and Grundmann, 1970); A11 and to some extent A10 are reduced in size in comparison to more anterior abdominal segments. Consistent with the mutant phenotype, the antisense probe generates no signal in the head or thorax, and Abdominal expression in A1 is restricted to the posterior compartment (Fig. 7). However, in contrast to the lack of a mutant phenotype posterior to A8, the transcript is expressed in segments A2 through A11. Controls with the sense probe yield no consistent signal.

DISCUSSION

We have molecularly cloned a portion of the Tribolium abdominal-A homolog, and demonstrated that the mutant allele $A^{12}$ is associated with a rearrangement breakpoint in or just 3' to the homeobox. RFLP mapping confirms that this cloned region originates at or near the Abdominal locus.
Given this evidence that Abdominal and abdominal-A are homologs, it is important to compare their patterns of expression and functional roles, as assessed by mutant phenotypes. The loss-of-function lethal phenotypes associated with mutations of these two genes show strikingly similar homeotic transformations which are, however, different in their domains. In Drosophila, the abdominal-A gene plays an important role in the anterior abdomen, and abd-A− embryos display transformations of PS7-9 to PS6, as well as more subtle effects posteriorly through PS13 (Sánchez-Herrero et al., 1985; Tiong et al., 1985; Karch et al., 1985; Busturia et al., 1989). abd-A transcripts are abundant in PS7-12 and are detectable in PS13 and 14 (Harding et al., 1985; Regulski et al., 1985; Rowe and Akam, 1988); the protein accumulates through PS14 (see below). Tribolium embryos homozygous for A resemble abd-A mutants in displaying abdominal transformations to PS6 (Fig. 8), which clearly have a parasegmental limit in the anterior abdomen. That is, A1 develops a pleuropodium in its anterior compartment, but its posterior compartment is enlarged and elaborates what we interpret as the posterior portion of the T3 larval leg. In situ hybridization confirms that the anterior limit of A expression is the A1 anterior/posterior border, as it is for the expression of homologous genes in the grasshopper Schistocerca gregaria (Tear et al., 1990) and the moth Manduca sexta (Nagy et al., 1991). The strong transformation to PS6 is reiterated through PS7-13 (compared to PS7-9 in Drosophila), and the posterior compartment of A8 is also homeotically transformed. This mutant phenotype provides a direct demonstration that parasegmental expression is of true developmental relevance among non-Drosophilid insects. In addition, the strong differences in PS6 larval morphology between beetles and flies shows that homologous homeotic selector genes can act to regulate remarkably diverse downstream developmental events.

The posterior limit of abdominal-A expression and functional significance varies among the insects examined thus far. As just noted, Abdominal mutants show strong transformations through the A8/A9 segmental border, compared to the weak effects through PS13 (A8a) shown by abdominal-A mutants. In Drosophila, protein accumulates strongly through the A7/A8 segmental boundary (Karch et al., 1990), although some positive nuclei have been reported in A8a as well (Macias et al., 1990). In contrast, transcript and protein accumulation in Manduca and Schistocerca, respectively, occur through A10 and respect a segmental boundary. We have observed Abdominal transcripts in Tribolium through the end of the segmented germ band (A11). It may be that the phylogenetic variability of the posterior limit of expression of abdominal-A homologs will be elucidated by a better understanding of Abdominal-B function in these insects (see below).

Akam et al. (1988) speculated that early in the insect lineage an abdominal-A homolog was expressed in the abdomen (A2-A7 or PS7-PS13), whereas an Abdominal-B homolog was restricted to a more posterior tail region. Moreover, they suggested that the evolution of new regulatory mechanisms subsequently led to the role of Abd-B in PS10-12 of Drosophila. Our observation of a strong
Abdominal homeotic phenotype through A8 (PS13) is consistent with their view of the primordial abdominal-A function in the insect lineage. How might Abdominal be regulated in the posterior beetle abdomen, a domain in which its homolog has little significance in Drosophila? In the fruit fly, the effects of Abd-B in PS10-12 are regulated by iab-5, iab-6 and iab-7, regions that include enhancer-like elements and are envisaged to vary in their chromatin organization (and availability for interaction with trans-acting factors) in different abdominal metameres (Peifer et al., 1987). One possibility is that the primitive roles of iab-5 to iab-7 were to regulate the ancestral abdominal-A rather than Abdominal-B gene. This hypothesis is consistent with the subtle effects of abdominal-A mutations on the posterior abdomen mentioned previously and with several aspects of Abdominal-B structure and expression in Drosophila: (1) iab-5 to iab-7 are located 3′ to the Abd-B transcription unit (and 5′ to abd-A); (2) relative to other BX-C functions, Abd-B expression in PS10-12 is unusual in its relatively late appearance and increasing abundance in progressively more posterior parasegments and (3) iab-5 and possibly iab-6 have some influence on abd-A as well as Abd-B function (Celniker et al., 1990; Sánchez-Herrero, 1991). The putative beetle homolog of Abd-B is extra urogomphi, which is presently represented by a single, incompletely penetrant recessive mutant allele associated with transformations of A11 to A10 (Beeman et al., 1989 and unpublished results). We have molecularly cloned the Tribolium Abd-B homolog, and future genetic and molecular studies should elucidate the function of this gene in beetles.

Beeman et al. (1989) observed bidirectional abdominal transformations in adult beetles bearing putative loss-of-function mutant alleles: recessive anterior transformations of A3 and 4, and a dominant posterior transformation of A6. We have shown here that these effects are indeed associated with mutations that impair or eliminate Abdominal function. Given the parasegmental nature of embryonic homeotic changes, it is interesting that the A\textsuperscript{max} and A\textsuperscript{pas} transformations appear to affect the entire A3 and A4 sternites, respectively. It may be that the cuticular elements being scored derive largely or completely from the anterior compartment of each segment, as is true for the tergites of Drosophila abdominal segments (Hama et al., 1990). The caudally directed dominant posterior transformation associated with Tribolium A mutants is quite unexpected. That is, the observations that the Drosophila abdominal-A gene is haplo-sufficient, and associated with loss-of-function mutant effects that are only cephalically directed and largely affecting the anterior abdomen, make the bidirectional Abdominal adult phenotype a dramatic divergence from the Drosophila paradigm. The similarities in the embryonic functions of Abdominal and abdominal-A suggest that the divergent adult transformations represent an adaptation in the beetle lineage. In general, it is thought that the adult cuticle of beetle abdominal segments is secreted by the same cells (or their mitotic descendants) that secrete the cuticle at earlier life stages (Wigglesworth, 1972), and it will be fascinating to study how this dramat-
Abdominal-A cDNA (Karch et al., 1990) and homologous functional significance (Beverley and Wilson, 1984). Tribolium has an appropriately placed match (see Fig. 4), (CTRAY) for a branch point signal (Senapathy et al., 1990). Studies showing the importance of such regions to protein lines and parasegmental boundaries by dashed lines. As described in the text, in Abdominal homozygotes parasegments 7-13 are transformed to resemble PS6, and the A8 posterior compartment is also transformed to resemble that of T3.

**The Abdominal protein**

The nucleotide base sequence of the genomic Abdominal region examined thus far is given in Fig. 4, and Fig. 5 compares the predicted amino acid sequence to those of an abdominal-A cDNA (Karch et al., 1990) and homologous genes from Schistocerca (Tear et al., 1990), Manduca (Nagy et al., 1991), Bombyx (Ueno et al., 1992) and Apis (Walldorf et al. 1989). The long evolutionary time since these insects diverged implies that conserved features in these genes and encoded proteins correspond to domains of functional significance (Beverley and Wilson, 1984).

Among these six insects there is very strong conservation in an 'extended homeodomain' region extending from at least four residues on the N-terminal side through about 20 residues on the C-terminal side. The impressive phylogenetic conservation of such extended homeodomains has been observed for a number of genes and is consistent with studies showing the importance of such regions to protein function (Kuziora and McGinnis, 1989; Mann and Hogness, 1990; Gibson et al., 1990).

The abdominal-A gene has an intron-exon junction five codons 5' of the homeobox. At this position, the amino acid sequences predicted by the abdominal-A cDNA and Abdominal genomic DNA diverge completely. In addition (see Fig. 4), Tribolium has an appropriately placed match to a consensus splice acceptor site (CAGR preceded by a pyrimidine-rich region) and, beginning 20 bp upstream of the putative splice site, a good match to the consensus (CTRAY) for a branch point signal (Senapathy et al., 1990). This organization suggests that beetles resemble Drosophila with respect to the position of an intron 5' to the homeobox, and it is likely that this feature is well conserved among insects. Downstream from the homeobox abdominal-A has a 70 bp intron which is lacking in other insects for which data are available. Thus, this intron is likely to have originated relatively recently in the lineage leading to Drosophila.

Drosophila, Tribolium and Schistocerca share similar amino acid sequences near the C terminus as well (corresponding results from the other insects listed in Fig. 5 are not yet available). However, the proteins encoded by these three insect genes differ considerably in the intervals between the extended homeobox and the C terminus. We have isolated a 2.1 kb A cDNA from a Tribolium embryonic library; sequence data indicates that the 5' end of this partial cDNA lies within the homeobox and shows that the putative protein coding sequence presented in Fig. 5 represents a single exon. This peptide is 65 residues shorter than the corresponding abd-A sequence and 23 residues shorter than the grasshopper homolog. Both Tribolium and Schistocerca lack a domain rich in glycine and other nonpolar aliphatic amino acids present near the C terminus of the Drosophila protein. Moreover, Tribolium lacks an extensive polyglutamine tract (M or opa repeat) present in Drosophila and Schistocerca. Tribolium does have a peptide of 23 amino acids (double-underlined in Fig. 4) which includes nine glutamines (each encoded by CAG) and 12 additional residues with codons which can be generated from CAG by a single base pair substitution. Manduca, Bombyx and Schistocerca have similar domains, whereas the corresponding portion of the abdominal-A gene shows no enrichment of codons related to CAG. These observations, as well as the presence of additional uninterrupted polyglutamine tracts in holometabolous (Drosophila) and hemimetabolous (Schistocerca) insects, suggest that an M repeat existed in a common ancestor, which has been modified to various extents. The functional significance of such polyamino tracts is presently uncertain. They are common to many transcription factors and have been suggested to mediate protein interactions important to transcriptional activation (Pirrotta et al., 1987). Other examples of the phylogenetic conservation of these tracts (albeit of different length) have been reported (Kassis et al., 1986; Treier et al., 1989; Seeger and Kaufman, 1990), implying that they are maintained by selection pressure. On the other hand, in at least some developmental contexts they appear relatively dispensable (Mann and Hogness, 1989; Gibson et al., 1990). Particularly relevant are the observations that in Drosophila the mutant allele abd-A<sup>C26</sup>, a small deficiency that removes the polyglutamine repeat, encodes a protein that is expressed (at reduced intensity) in a normal spatial pattern (Karch et al., 1990) and is partially functional (Busturia et al., 1989). Future experiments to assess the functional implications of the differences in these insect proteins are an exciting prospect.
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REFERENCES


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