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

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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 casta-neum*, an insect that is in many ways more primitive than *Drosophila*, and which has similar advantages for genetic and molecular studies (Beeman et al., 1989; Brown et al., 1990). The evolutionary distance between these two species is considerable; they represent insect orders that diverged over 300 million years ago.

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

Class	No.	*Abdominal* recessive lethal?*	Adult mutant phenotype		Allele Mutagenized	No. Revertants (frequency)	Revertant *Abdominal* lethal?*	Revertant phenotype	
			Recessive**	Dominant				Recessive**	Dominant
Abdominal	17	yes	A3→A2 A4→A3	A6→A7	none	-	-	-	-
Extra sclerite	3	no	A4→A3	A2→A3† A6→A7	Es^1	2 (0.0003)	yes	A3→A2	A6→A7 A4→A3
Socketless	2	no	none	A3→A4†	Skl^1	3 (0.0008)	yes	A3→A2 A4→A3	A6→A7
Miscaudal sclerotization	3	no	A7→A6	A2,3→A4† A8→A7†	Mcs^1	3 (0.0004)	yes	A3→A2 A4→A3	

*Alleles were designated as having the *Abdominal* recessive lethality if they failed to complement A^{10} for viability to the adult stage. Mcs^{1+R4}/A^{10} individuals infrequently survive to adulthood and resemble rare adult escapers heterozygous for A^{10} and the hypomorphic variant A^8. Escapers homozygous for A^8 also show recessive transformations of A6&7→A8.

**The recessive adult homeotic phenotypes were assessed by complementation with *mas* (A3→A2) and *pas* (A4→A3).

†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 *abdominal-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 $Ultrathorax^1$ (Utx^1), $maxillopedia^{Stumpy}$ (mxp^{Stm}) or *Eyeless* (*Ey*), each associated with crossover suppression and recessive lethality.The HOM-C duplication *Dp(2)Dachs* was maintained in two stocks: $Dp(2)Dachs/A^{10}/A^{Es1}$ and $Dp(2)Dachs/A^{pas2}/A^{Es1}$.

Reversion mutagenesis

In order to assess whether A^{Es1}, A^{Mcs1} and A^{Skl1} are gain-of-function *Abdominal* alleles, males heterozygous for each were treated with 4-5 krad of gamma radiation from a ^{60}Co 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. A^{Es1}/Utx^1 and A^{Es1}/mxp^{Stm} irradiated males were crossed to *mxp* A^{mas} and *sooty* (s) females, respectively, whereas A^{Mcs1}/mxp^{Stm} and A^{Skl1}/mxp^{Stm} irradiated males were mated to *s* and $black^{tawny}$ (b^t) females, respectively. (The dark body mutations *s* and b^t are unlinked to the HOM-C and aid in separating sexes in order to discard irradiated males.) F_1 adults were screened for a wild-type phenotype and, in one of the crosses, for new mutations that failed to complement A^{mas}. 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 $A^-/A^+/A^+$ genotype was constructed using the mutation A^{12}, the chromosome mxp^{Stm} $Cephalothorax^5$ (Cx^5) 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 Cx^5 phenotype, but not the dominant gain-of-function mxp^{Stm} phenotype. A^{12}/mxp^{Stm} $Cx^5/Dp(2)Dachs$ beetles could thus be recognized among the progeny of A^{12}/Ey males and A^{Es1}/mxp^{Stm} $Cx^5/Dp(2)Dachs$ females.

The $A^-/A^{Es1}/A^+$ genotype was observed while constructing the $A^{10}/A^{Es1}/Dp(2)Dachs$ stock. $A^{pas2}/A^{Es1}/Dp(2)Dachs$ females, which express the dominant A^{Es1} phenotype but not the recessive A^{pas2}/A^{Es1} phenotype, were mated with A^{10}/mxp^{Stm} males. Among the progeny, the putative $A^{10}/A^{Es1}/Dp(2)Dachs$ beetles expressed the A^{Es1} phenotype but neither the dominant mxp^{Stm} nor recessive A^{pas2}/A^{10} phenotypes. The presence of *Dp(2)Dachs* in these aneuploid beetles was confirmed by further progeny testing.

The $A^{Es1}/A^+/A^+$ genotype was generated by mating $A^{pas2}/A^{Es1}/Dp(2)Dachs$ males with mxp^{Dachs4}/A^{Es1} females. The $A^{Es1}/mxp^{Dachs4}/Dp(2)Dachs$ progeny showed both the dominant 'short legs' phenotype associated with mxp^{Dachs4} and the dominant A2→A3 transformation associated with A^{Es1}. $A^{Skl1}/A^{pas2}/Dp(2)Dachs$ and $A^{Mcs1}/A^{pas2}/Dp(2)Dachs$ beetles were generated by mating $A^{pas2}/A^{Es1}/Dp(2)Dachs$ males with A^{Skl1}/mxp^{Stm} and A^{Mcs1}/mxp^{Stm} females, respectively. In each case, the presence of *Dp(2)Dachs* was confirmed by additional test crosses.

Embryonic analysis

Females were allow 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. For

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% paraformaldhyde 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_2O_2 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 Antennape - dia* homeobox sequence was purified from pA2015 (Scott et al., 1983) and random-primer labeled with [^{32}P]dCTP.The filters were hybridized at 56˚C in 6× SSC, 10× 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 2× 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 *Eco*RI-*Hin*dIII 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 1.4×10^6 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 5×10^5 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 *Bam*HI and *Xho*I 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 dTTP, 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 product was precipitated in ethanol, resuspended in 300 μl hybridization solution [50% formamide, 5× SSC, 50 μg/ml heparin, 100 μg/ml herring testes DNA and 0.1% Tween 20 (Tautz and Pfiefle, 1990)], and boiled for 1 hour. The fragmented probe was hybridized (Tautz and Pfiefle, 1990) in situ to RNA in embryos, laid over a 2-3 day period, which had been dechorionated, fixed and devitellinized as described above.

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 (A3→A2), and *pointed abdominal sternite* (*pas*) transforms A4→A3. 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 A6→A7, 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.

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^1, Skl^1 and Mcs^1 (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^1 and Skl^1 are indistinguishable from *A* variants in their adult phenotypes (Fig. 1) and fail to complement *mas*, *pas* and the recessive lethality of A^{10}. Although characterization of the revertants of Mcs^1 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 A6→A7 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*$^+$ doses. The hypothesis predicts that A^-/A^+ 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 A6→A7 transformation when heterozygous with a wild-type chromosome.

Moreover, if the A6→A7 transformation is indeed haploinsufficient in nature, it should be complemented by a duplication of *Abdominal*$^+$. We have recovered a duplication, *Dp(2)Dachs*, presumably as an aneuploid segregant from

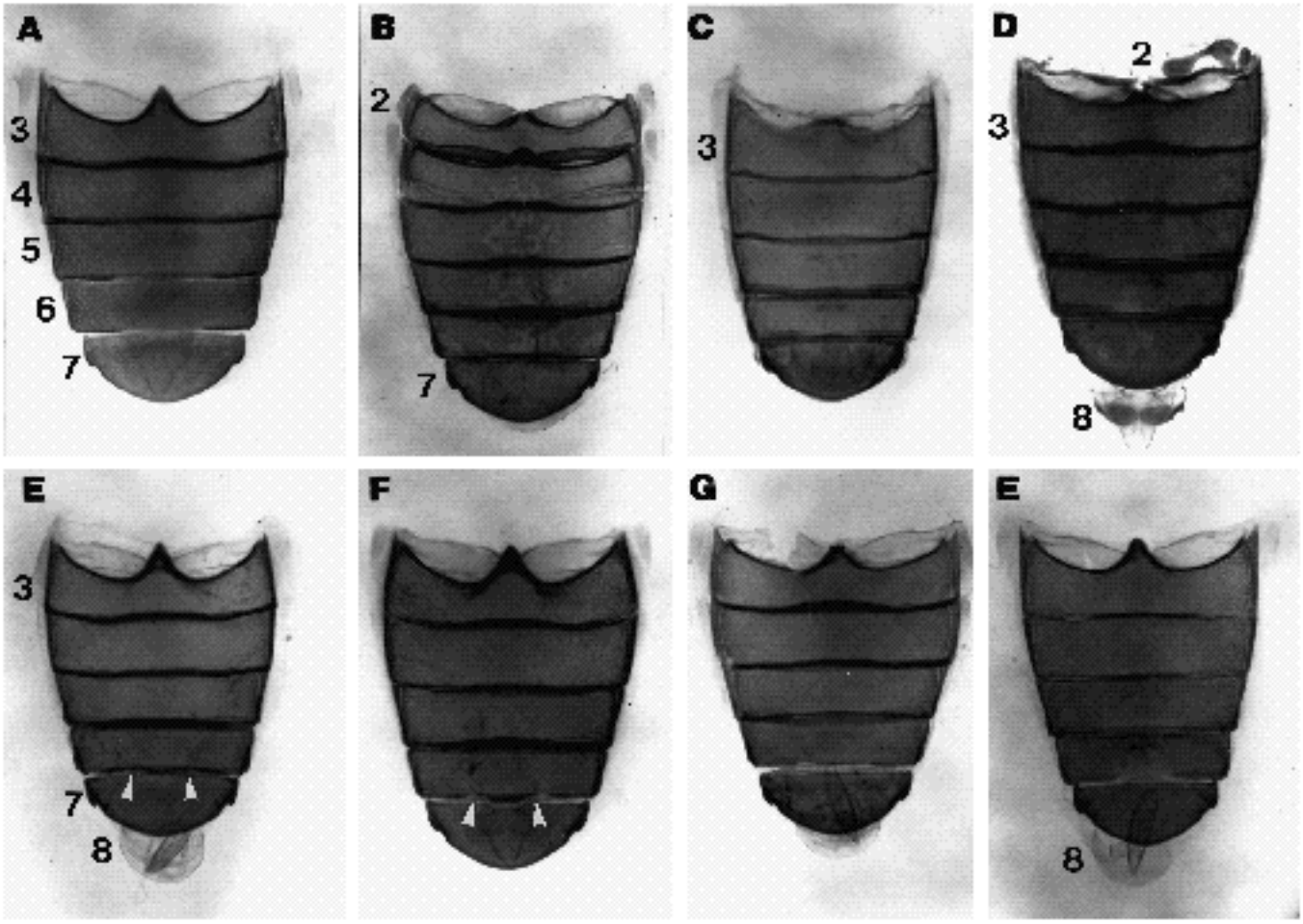

Fig. 1. Abdomens from wild-type and mutant adult beetles. (A) Wild type (*Georgia-1*). Sternites from A1, A2 and A8 are normally membranous; other sternites are numbered. (B) A^{Es1}/+, showing a sclerotized A2 sternite indicating an A2→A3 transformation. (C) A^{Skl1}/+. A transformation of A3→A4 is indicated by the loss of the mid-ventral point normally characteristic of A3. (D) A^{Mcs1}/+, showing partial posterior transformations of A2 and A3, as well as an A8→A7 transformation indicated by sclerotization of the normally membranous A8 sternite. (E) A^{10}/+, showing putative haplo-abnormal phenotypes. The 'notches' indicative of a dominant A6→A7 transformation are present at the positions indicated by arrowheads, this phenotype shows incomplete penetrance. (F, G, H) Revertants of A^{Es1}, A^{Skl1} and A^{Mcs1}, respectively. These specimens were chosen to demonstrate that, in each case, the putative gain-of-function dominant effect(s) is lost. Populations of revertants of A^{Es1} and A^{Skl1} show the incompletely penetrant notches phenotype, as indicated by arrows for the former. As described in the text, A^{Mcs1} revertants never show this effect and these mutations do not appear to be accompanied by a complete loss of *A* function.

the chromosomal rearrangement associated with the HOM-C mutation *maxillopedia*^{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 *Dp(2)Dachs*, beetles that are A^{-}/A^{+} or A^{-}/pas display no dominant A6→A7 effect or (in the latter case) recessive A4→A3 transformation. However, the duplication fails to complement the putative gain-of-function dominant effects of *Es*, *Skl* or *Mcs* beetles, showing that they do not arise because of diminished *Abdominal*$^{+}$ function (data not shown).

Given the strong evidence from these studies indicating that *mas*, *pas*, *Es*, *Skl* and *Mcs* variants are all *Abdominal* mutant alleles, we will hereafter assign them a genetic symbol indicative of that relationship. For example, *Extra sclerite*^{1} will now be referred to as *Abdominal-Extra sclerite*^{1} and symbolized A^{Es1}.

Recombination experiments show that the recessive, loss-of-function and gain-of-function *Abdominal* mutations are tightly linked (Table 2). Beeman (1987) determined that four HOM-C variants map in the order Cx^{apt} A^{mas} A^{pas} *eu*. A^{10} and A^{13} are putative null alleles which fail to complement A^{mas} and A^{pas}. A^{10} maps to the left of A^{mas} (toward *Cx*), whereas A^{13} lies to the right of A^{mas} (toward *eu*) and probably between the two recessive lesions. This interspersion of recessive hypomorphs and amorphs is similar to that reported for the *Drosophila* gene *Ultrabithorax* (Duncan, 1987). The gain-of-function mutations A^{Skl6} and A^{Mcs1} are tightly linked and to the right of A^{mas}, while A^{Skl1} is to the right of A^{pas}. These results are also consistent with the view that *Abdominal* is a single complex locus.

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

Cross	Informative recombinants	Total progeny
$\frac{+\ A^{10}\ +}{apt\ +\ pas}$ X $\frac{apt\ +\ pas}{apt\ +\ pas}$	1 $\frac{apt\ +\ +}{apt\ +\ pas}$ 1 $\frac{apt\ A^{10}\ +}{apt\ +\ pas}$	5,039
$\frac{+\ A^{10}\ +\ +}{apt\ +\ mas\ eu}$ X $\frac{apt\ +\ mas\ eu}{apt\ +\ mas\ eu}$	1 $\frac{apt\ +\ +\ +}{apt\ +\ mas\ eu}$	16,037
$\frac{+\ +\ A^{13}\ +}{apt\ mas\ +\ eu}$ X $\frac{apt\ mas\ +\ eu}{apt\ mas\ +\ eu}$	1 $\frac{+\ +\ +\ eu}{apt\ mas\ +\ eu}$	2,598
$\frac{+\ +\ Sk1^{6}\ +}{apt\ mas\ +\ eu}$ X $\frac{apt\ mas\ +\ eu}{apt\ mas\ +\ eu}$	4 $\frac{+\ +\ +\ eu}{apt\ mas\ +\ eu}$ 1 $\frac{+\ +\ Sk1^{6}\ eu}{apt\ mas\ +\ eu}$	6,214
$\frac{+\ +\ Sk1^{1}\ +}{apt\ mas\ +\ eu}$ X $\frac{apt\ mas\ +\ eu}{apt\ mas\ +\ eu}$	1 $\frac{+\ +\ +\ eu}{apt\ mas\ +\ eu}$	7,037
$\frac{+\ +\ Mcs^{1}\ +}{apt\ mas\ +\ eu}$ X $\frac{apt\ mas\ +\ eu}{apt\ mas\ +\ eu}$	2 $\frac{+\ +\ +\ eu}{apt\ mas\ +\ eu}$	8,377

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 Cx^{apt} but abbreviated *apt* here), and *extra urogomphi* (*eu*).

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 *invected* 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

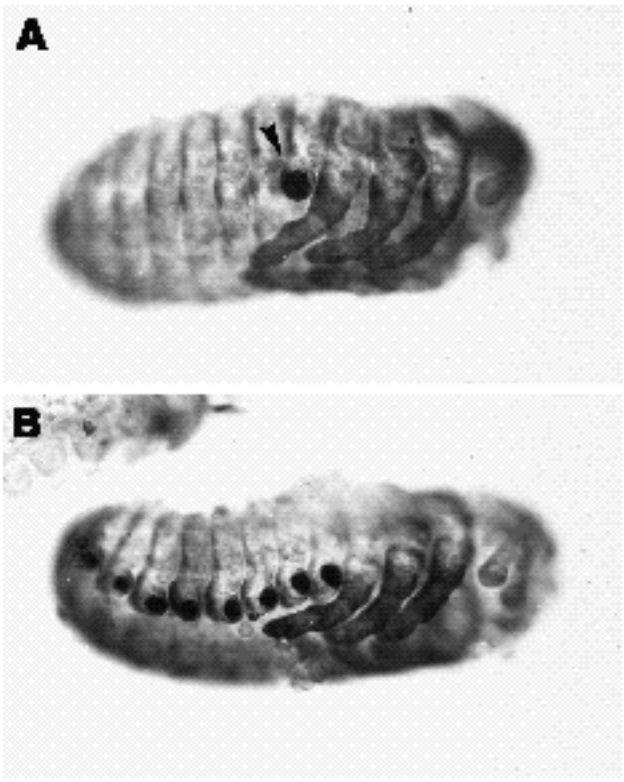

Fig. 2. The embryonic lethal syndrome of *Abdominal* homozygotes. (A, B) Wild-type and mutant embryos, respectively, after germ band retraction and immunostained with an antibody against the *Drosophila Antennapedia* protein. In this and following photomicrographs, embryos are shown with anterior to the right and dorsal up. Although this antibody recognizes a spatially restricted, nuclear antigen in young embryos, older embryos show apparently nonspecific, patchy immunostaining of the cuticle and intense staining of the pleuropodia (indicated by an arrowhead in A), which are unique to the A1 segment in wild-type individuals. Note that the homeotic pleuropodia that appear on A1-8 of mutant embryos are restricted to the anterior portion of each segment.

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* homeobox 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 mxp^{Stm} and the *GA-1*-derived mutation A^{83} for this experiment. Southern analysis of the parents and progeny of the mating between A^{83}/mxp^{Stm} males and *GA-1* females indicated that the *Lab-S*-derived restriction fragment always segregated with the mxp^{Stm} chromosome, whereas only the *GA-1* polymorphism was observed in A^{83} progeny (data not shown).

To correlate directly λJS16 and the *A* gene, we showed that the radiation-induced A^{12} 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 A^{12} (data not shown). When used to probe genomic blots, subclone pJS16-1 revealed RFLPs in A^{12} DNA digested with *Xho*I, *Hin*dIII or *Eco*RI (Fig. 6). Thus, A^{12} is associated with a DNA rearrangement breakpoint within the 419 bp *Xho*I/*Hin*dIII fragment, which contains 130 bp of putative *A* homeobox and an additional 204 bp of protein-coding sequence. These data strongly suggest that the A^{12} mutation was caused by a chromosomal rearrangement or transposon insertion.

The implication that the A^{12} 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 *Hin*dIII fragment in DNA from *GA-1* or *apt mas eu* beetles, and a 3.6 kb fragment in A^{12} DNA. This 3.6 kb fragment apparently represents homology to the left side of the A^{12} 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 $mxp^{Stm}/A^{12} \times Cx^{apt}\ A^{mas}\ eu/Cx^{apt}\ A^{mas}\ eu$ beetles, F_1 $A^{12}/Cx^{apt}\ A^{mas}\ eu$ males were backcrossed to $Cx^{apt}\ A^{mas}\ eu$ homozygous females. Progeny recombinant for visible markers were backcrossed once again to $Cx^{apt}\ A^{mas}\ eu$, and DNA was prepared from their offspring, restricted with *Hin*dIII and probed with the pJS16-3 insert. We observed 1.4% recombination between Cx^{apt} and A^{mas}, close to the 1.2% obtained by Beeman (1987) in an experiment in which no other *Abdominal* mutation was present. Thus, the A^{12} rearrangement does not markedly affect the rate of recombination in this interval. For the 12 beetles recombinant between Cx^{apt} and A^{mas} tested molecularly, there was complete coincidence between A^{12} and the 3.6 kb restriction fragment. These results indicate both lesions lie to the right of Cx^{apt}. No

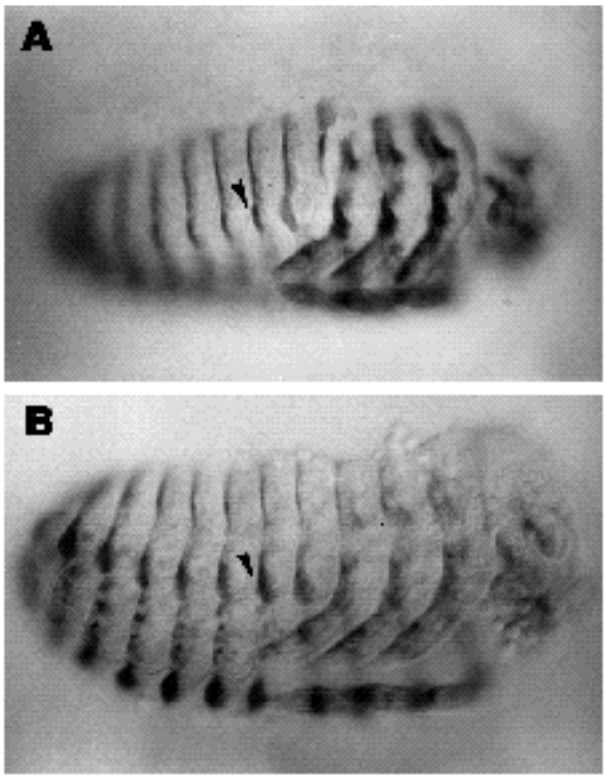

Fig. 3. Compartmental organization of wild-type and *Abdomina*l mutant embryos. (A) Wild-type embryo immunostained with an antibody against the *Drosophila* engrailed/invected proteins, and photographed with differential interference microscopy. (B) *Abdominal* homozygous embryo treated similarly to A and oriented to display more of the ventral surface. As indicated by arrowheads, this mutant specimen shows an enlargement of the posterior compartment in the region of A1 and ectopic pleuropodia compared to the wild-type embryo in A. This enlarged portion remains evaginated and develops cuticular structures not normally present on the abdomen but characteristic of the legs and other appendages.

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TGTCGAATGTTTTATTGCGCTTTTTCTCGACGTAAACAGGGCTTAGAGCC   50

GTAACGGTTATATTTTATAGCCGTCTCAGTGACAGTGATGCTGACTGTCT  100

CCCGAACCCTTTAGAAAGTCGCCCGAACGGTTTAAAGCATTTCTCCGGGT  150

                                          *  k  r  k
AGTTTTTTAGTTCTTTAATAAAGGGTTTACAGCGTCCGTAAAAGAGAAAG  200

s  p  f  i  d  f  r  r  f  v  a  G  P  N  G  C  P
TCTCCTTTTAttgacTTTCGGCGTTTTGTTgcaggTCCGAACGGGTGCC C 250

 R  R  R  G  R  Q  T  Y  T  R  F  Q  T  L  E  L  E
TCGACGACGCGGTCGGCAGACCTACACAAGGTTTCAAACTTTAGAACTCG  300

   K  E  F  H  F  N  H  Y  L  T  R  R  R  R  I  E
AGAAAGAGTTTCACTTTAATCACTATTTGACCCGGCGGAGACGCATTGAA  350

I  A  H  A  L  C  L  T  E  R  Q  I  K  I  W  F  Q
ATCGCACACGCCTTGTGTCTGACAGAGAGACAAATCAAAATCTGGTTTCA  400

 N  R  R  M  K  L  K  K  E  L  R  A  V  K  E  I  N
GAACAGGAGGATGAAGCTGAAGAAGGAGCTGCGGGCGGTGAAGGAGATCA  450

   E  Q  A  R  R  E  R  E  E  Q  E  R  H  K  Q  Q
ACGAGCAGGCCAGGCGGGAGCGGGAGGAGCAGGAGAGGCACAAGCAGCAG  500

Q  Q  E  K  Q  Q  K  I  E  Q  Q  T  H  S  S  I  H
CAGCAGGAGAAGCAGCAGAAGATCGAGCAGCAGACGCACTCCTCGATCCA  550

 Q  H  H  H  D  P  M  K  M  S  L  D  K  S  G  G  S
CCAGCACCACCACGACCCCATGAAGATGAGCTTGGACAAAAGTGGGGGCT  600

   D  L  L  K  A  V  S  K  V  P  T  *
CCGATCTACTCAAAGCCGTCTCGAAAGTACCTACATAATTGTGCAGTGAC  650

TCCGACAAGTGTCAGTGACTGGACTCTAGTGACGAGTGACTTGACCTTTT  700

GTTTTATACAAAGAAGCTT  719
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Fig. 4. DNA sequence and conceptual translation of pJS16-4. The DNA sequence of the putative non-coding strand of genomic subclone pJS16-4 is given in the 5′ to 3′ orientation, based on the conceptual translation of an open reading frame to give a homeobox region (underlined) and carboxyl terminus with strong sequence similarity to *abdominal-A* (see Fig. 5). The predicted amino acid residue is indicated in bold above the first base of each codon and the flanking stop codons are depicted as asterisks. Nucleotide bases in italics encode a potential branch point signal and splice acceptor site; the *Drosophila abd-A* gene has a similarly placed splice acceptor site. Since the portion of the open reading frame 5′ to this site encodes a peptide (depicted in lower case) with no similarity to that of the *Drosophila abd-A* cDNA (Karch et al., 1990), it is likely that this region is intronic and not translated. Double underlining indicates a group of amino acid residues possibly representing a degenerate M repeat (see text).

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^{apt} 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^{apt} 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.

Fig. 5. Comparison of the predicted amino acid sequence encoded by pJS16-4 to that of a *Drosophila abdominal-A* cDNA (Karch et al., 1990) and genomic sequences from homologs of *Schistocerca* (Tear et al., 1990), *Manduca* (Nagy et al., 1991), *Bombyx* (Ueno et al., 1992) and *Apis* (Walldorf et al., 1989). Identities to the *Drosophila* sequence are depicted by asterisks, and the homeodomains and the *Drosophila* M repeat are indicated. All sequencies begin in the vicinity of an intron/exon junction known or suspected to exist just 5′ to the homeobox of each. Sequences presented for *Manduca*, *Bombyx* and *Apis* are all that are currently available. For the other three insects, there is strong conservation in the extended homeodomain region and at the C terminus, but the intervening regions have diverged in both sequence and length. For the top group, they have been aligned through the end of M repeats for *Drosophila* and *Schistocerca*, and a putative degenerate M repeat for *Tribolium* (see Fig. 4). For the bottom group, they have been aligned from the C terminus of each predicted protein; one amino acid gaps have been introduced into the *Drosophila* and *Tribolium* sequences to optimize this alignment.

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

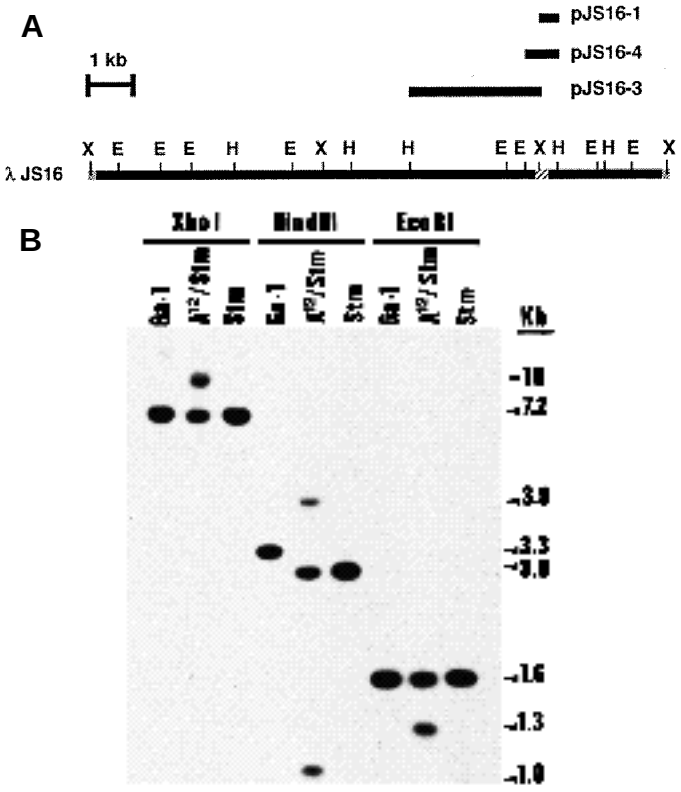

Fig. 6. Restriction map of cloned fragments and demonstration of restriction fragment length polymorphisms. (A) A restriction map of the λJS16 genomic insert is shown, as well as the extents of several subclones utilized as probes. The homeobox region is crosshatched, and flanking vector sequences (including the indicated *Xho*I sites) are stippled. (B) A Southern hybridization experiment in which genomic DNAs from the indicated stocks were restricted as shown and probed with the insert of pJS16-1. These results demonstrate that the A^{12} mutation is associated with a DNA rearrangement with respect to its parental chromosome (*Georgia-1*) and the balancer mxp^{Stm}. Sizes of the hybridizing fragments are indicated. E, *Eco*RI; H, *Hin*dIII; X, *Xho*I.

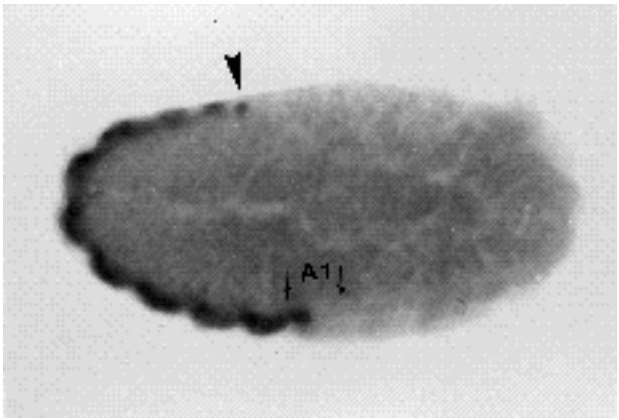

Fig. 7. The distribution of *Abdominal* transcripts in wild-type embryos. An extended germ band embryo utilized for in situ hybridization to *Abdominal* transcripts was photographed with bright-field optics. The extent of the A1 epidermis as assessed by differential contrast microscopy is indicated. The signal extends from the posterior compartment of A1 through the posterior end of the germ band; A11 is indicated by an arrowhead.

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^{mas} and A^{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-

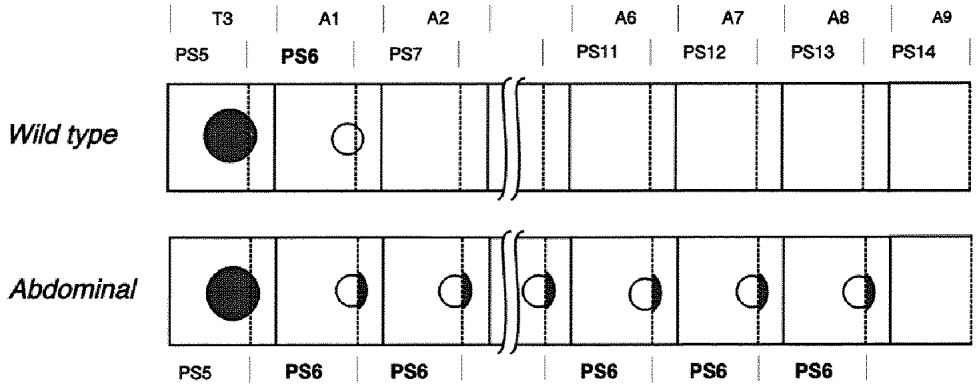

Fig. 8. A schematic comparison of the organization of the abdomens of wild-type and homozygous *Abdominal* embryos. The normal T3 leg is indicated by a large, shaded circle, whereas the normal A1 pleuropodium is depicted by a smaller open circle. Segmental boundaries are shown with solid lines and parasegmental 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.

ically different pattern of adult regulation can be superimposed upon the embryonic mechanisms discussed above.

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 $abd\text{-}A^{C26}$, 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

Akam, M. (1989). *Hox* and HOM: Homologous gene clusters in insects and vertebrates. *Cell* **57**, 347-349.

Akam, M., Dawson, I. and Tear, G. (1988). Homeotic genes and the control of segment diversity. *Development* **104 Supplement**, 123-133.

Beeman, R. W. (1987). A homoeotic gene cluster in the red flour beetle. *Nature* **327**, 247-249.

Beeman, R. W., Stuart, J. J., Haas, M. S. and Denell, R. E. (1989). Genetic analysis of the homeotic gene complex (HOM-C) in the beetle *Tribolium castaneum. Dev. Biol.* **133**, 196-209.

Beverley, S. M. and Wilson, A. C. (1984). Molecular evolution in *Drosophila* and the higher Diptera II. A time scale for fly evolution. *J. Mol. Evol.* **21**, 1-13.

Brown, S. J., Henry, J. K., Black, W. C. IV and Denell, R. E. (1990). Molecular genetic manipulation of the red flour beetle: Genome organization and cloning of a ribosomal protein gene. *Insect Biochem.* **20**, 185-193.

Busturia, A., Casanova, J., Sánchez-Herrero, E., González, R. and Morata, G. (1989). Genetic structure of the *abd-A* gene of *Drosophila. Development* **107**, 575-583.

Celniker, S. E., Sharma, S., Keelan, D. J. and Lewis, E. B. (1990). The molecular genetics of the bithorax complex of *Drosophila*: *cis*-regulation in the *Abdominal-B* domain. *EMBO J.* **9**, 4277-4286.

Duncan, I. (1987). The Bithorax complex. *A. Rev. Genet.* **21**, 285-319.

Frischer, L. E., Hagen, F. S. and Garber, R. L. (1986). An inversion that disrupts the *Antennapedia* gene causes abnormal structure and localization of RNAs. *Cell* **47**, 1017-1023.

Gibson, G., Schier, A., LeMotte, P. and Gehring, W. J. (1990). The specificities of sex combs reduced and Antennapedia are defined by a distinct portion of each protein that includes the homeodomain. *Cell* **62**, 1087-1103.

Glicksman, M. A. and Brower, D. L. (1988). Misregulation of homeotic gene expression in *Drosophila* larvae resulting from mutations at the *extrasexcombs* locus. *Dev. Biol.* **126**, 219-227.

Hama, C., Zehra, A. and Kornberg, T. B. (1990). Region-specific recombination and expression are directed by portions of the *Drosophila engrailed* promoter. *Genes Dev.* **4**, 1079-1093.

Harding, K., Wedeen, C., McGinnis, W. and Levine, M. (1985). Spatially regulated expression of homeotic genes in *Drosophila. Science* **229**, 1236-1242.

Hazelrigg, T. and Kaufman, T. C. (1983). Revertants of dominant mutations associated with the Antennapedia gene complex of *Drosophila melanogaster*: Cytology and genetics. *Genetics* **105**, 581-600.

Karch, F., Weiffenbach, B., Peifer, M., Bender, W., Duncan, I., Celniker, S., Crosby, M. and Lewis, E. B. (1985). The abdominal region of the bithorax complex. *Cell* **43**, 81-96.

Karch, F., Bender, W. and Weiffenbach, B. (1990). *abdA* expression in *Drosophila* embryos. *Genes Dev.* **4**, 1573-1587.

Kassis, J. A., Poole, S. J., Wright, D. K. and O'Farrell, P. H. (1986). Sequence conservation in the protein coding and intron regions of the *engrailed* transcription unit. *EMBO J.* **5**, 3583-3589.

Kaufman, T. C., Seeger, M. A. and Olson, G. (1989). Molecular and genetic organization of the Antennapedia gene complex of *Drosophila melanogaster*. *Adv. Genetics* **27**, 309-362.

Kuziora, M. A. and McGinnis, W. (1989). A homeodomain substitution changes the regulatory specificity of the *Deformed* protein in Drosophila embryos. *Cell* **59**, 563-571.

Lewis, E. (1978). A gene complex controlling segmentation in Drosophila. *Nature* **276**, 141-152.

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

Maniatis, T., Fritsch, E. F. and Sambrook, J. (1982). *Molecular Cloning: A Laboratory Manual*. New York: Cold Spring Harbor Press: Cold Spring Harbor.

Mann, R. S. and Hogness, D. S. (1990). Functional dissection of Ultrabithorax proteins in D. melanogaster. *Cell* **60**, 597-610.

Mitchison, T. J. and Sedat, J. (1983). Localization of antigenic determinants in whole Drosophila embryos. *Dev. Biol.* **99**, 261-264.

Nagy, L. M., Booker, R. and Riddiford, L. M. (1991). Isolation and embryonic expression of an *abdominal-A-like* gene from the lepidopteran, *Manduca sexta*. *Development* **112**, 119-129.

Patel, N. H., Kornberg, T. B. and Goodman, C. S. (1989). Expression of *engrailed* during segmentation in grasshopper and crayfish. *Development* **107**, 201-212.

Peifer, M., Karch, F. and Bender, W. (1987). The bithorax complex: Control of segment identity. *Genes Dev.* **1**, 891-898.

Pirrotta, V., Manet, E., Hardon, E., Bickel, S. E. and Benson, M. (1987). Structure and function of the *Drosophila zeste* gene. *EMBO J.* **6**, 791-799.

Regulski, M., Harding, K., Kostriken, R., Karch, G., Levine, M. and McGinnis, W. (1985). Homeo box genes of the Antennapedia and bithorax complexes of Drosophila. *Cell* **43**, 71-80.

Rowe, A. and Akam, M. (1988). The structure and expression of a hybrid homeotic gene. *EMBO J.* **7**, 1107-1114.

Sánchez-Herrero, E. (1991). Control of the expression of the bithorax complex genes abdominal-A and Abdominal-B by *cis*-regulatory regions in *Drosophila* embryos. *Development* **111**, 437-449.

Sánchez-Herrero, E., Vernos, I., Marco, R. and Morata, G. (1985). Genetic organization of the *Drosophila* bithorax complex. *Nature* **313**, 108-113.

Scott, M. P., Weiner, A. J., Hazelrigg, T. I., Polisky, B. A., Pirrotta, V., Scalenghe, F. and Kaufman, T. C. (1983). The molecular organization of the *Antennapedia* locus of Drosophila. *Cell* **35**, 763-776.

Seeger, M. A. and Kaufman, T. C. (1990). Molecular analysis of the *bicoid* gene from *Drosophila pseudoobscura*: identification of conserved domains within coding and noncoding regions of the *bicoid* mRNA. *EMBO J.* **9**, 2977-2987.

Senapathy, P., Shapiro, M. B. and Harris, N. L. (1990). Splice junctions, branch point sites, and exons: sequence statistics, identification, and applications to the genome project. *Meth. Enzymol.* **183**, 252-278.

Sokoloff, A. (1972). *The Biology of* Tribolium, Vol. 1. Oxford: Clarendon Press.

Stanley, M. S. M. and Grundmann, A. W. (1970). The embryonic development of *Tribolium castaneum*. *Annals Entomol. Soc. Amer.* **63**, 1248-1256.

Stuart, J. J., Brown, S. J., Beeman, R. W. and Denell, R. E. (1991). A deficiency of the Homeotic complex of Tribolium. *Nature* **350**, 72-74.

Tautz, D. and Pfeifle, C. (1989). A non-radioactive in situ hybridization method for the localization of specific RNAs in *Drosophila* reveals translational control of the segmentation gene *hunchback. Chromosoma (Berl)* **98**, 81-85.

Tear, G., Akam, M. and Martinez-Arias, A. (1990). Isolation of an *abdominal-A* gene from the locust *Schistocerca gregaria* and its expression during early embryogenesis. *Development* **110,** 915-925.

Tiong, S., Bone, L. M. and Whittle, J. R. S. (1985). Recessive lethal mutations within the *bithorax* complex in *Drosophila melanogaster. MGG* **200**, 335-342.

Treier, M., Pfeifle, C. and Tautz, D. (1989). Comparison of the gap segmentation gene hunchback between Drosophila melanogaster and Drosophila virilis reveals novel modes of evolutionary change. *EMBO J.* **8**, 1517-1525.

Ueno, K., Hui, C.-C., Fukuta, M. and Suzuki, Y. (1992). Molecular analysis of the deletion mutants in the E homeotic complex of the silkworm *Bombyx mori. Development* **114**, 555-563.

Walldorf, U., Fleig, R. and Gehring, W. J. (1989). Comparison of homeobox-containing genes of the honeybee and *Drosophila*. *Proc. Natl. Acad. Sci. USA* **86**, 9971-9975.

White, R. A. H. and Akam, M. E. (1985). *Contrabithorax* mutations cause inappropriate expression of *Ultrabithorax* products in *Drosophila. Nature* **318**, 567-569.

Wigglesworth, V. B. (1972). *The Principles of Insect Physiology*, 7th Ed. London: Chapman & Hall.

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