

Genetic Analysis of the Homeotic Gene Complex (HOM-C) in the Beetle *Tribolium castaneum*

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Our laboratories have undertaken both genetic and molecular studies of the homeotic gene complex (HOM-C) of the beetle *Tribolium castaneum*, and this paper discusses results from our genetic analyses. We describe here the adult phenotypes and complementation behavior of over 50 new mutations. Many of these homeotic phenotypes resemble those of *Drosophila melanogaster*, but few precisely parallel the segmental transformations seen in this fly. Analysis of putative loss-of-function mutations affecting the head and thorax suggests that the *maxillopedia* and *Cephalothorax* genes most closely resemble *proboscipedia* and *Sex combs reduced* of *Drosophila*. In the abdomen, putative loss-of-function alleles of *Abdominal* affect a domain corresponding to those of the combined *abdominal-A* and *Abdominal-B* genes of *Drosophila*. In contrast to the situation in flies, *Abdominal* loss-of-function variants in *Tribolium* cause anteriorward transformations in A3-A5a, but posteriorward transformations in A5p-A7. The implications of the differences in developmental strategies evolved in *Tribolium* vs *Drosophila* are discussed. © 1989 Academic Press, Inc.

INTRODUCTION

Homeotic genes determine segment identity in *Drosophila*, and probably in all segmented animals. In *Drosophila* such "master switch" genes seem to regulate the transcription of subservient genes as a first course in the cascade of segment differentiation. Discoveries about the genetic organization, patterns of expression, and molecular structure of homeotic gene complexes are crystallizing into a fascinating story about how genes orchestrate development in *Drosophila* (e.g., Duncan, 1987; Kaufman and Abbott, 1984; Scott and Carroll, 1987).

How did such gene complexes evolve in the ancestors of *Drosophila*, and what became of the function of homologous genes in the higher animals? Lewis (1978) has suggested that homeotic gene clusters could have arisen during the evolution of segment specialization by tandem duplication of a progenitor gene, followed by divergence of function. Molecular biology has provided one line of insight into these questions, permitting genes with sequence similarity to *Drosophila* homeotic genes to be isolated from other species, even such distant relatives as mice and worms (McGinnis *et al.*, 1984).

Although library screens in any animal might reveal genes with sequence similarity to known *Drosophila* homeotic genes, it is generally not possible to know the biological function of such genes in the non-*Drosophilid* species, since genetic mutations are not available and

cannot easily be obtained. Homeotic genes from locusts, bees, moths, and other animals are eagerly being cloned in several laboratories in attempts to detect variation among species and to learn about the evolution of homeotic gene complexes. Comparisons are being pursued with respect to sequence similarity and patterns of expression of homeotic genes from these organisms.

We have overcome a major barrier to gaining an evolutionary perspective of homeotic gene complexes by developing a facile genetic approach to homeotic gene analysis in a distant cousin of the fruit fly. Our work on beetle genetics has turned up unexpected departures from the *Drosophila* theme.

In a previous report (Beeman, 1987) we showed that *Tribolium* contains a single homeotic gene complex (HOM-C) in linkage group 2 that incorporates the functions of two separate complexes, the antennapedia complex (ANT-C) and the bithorax complex (BX-C), in *Drosophila*. We also showed that the principle of colinearity holds in *Tribolium* as well as *Drosophila*. This principle, discovered by Lewis during his studies on the BX-C, states that the left-to-right sequence of homeotic genes on the chromosome corresponds to the anterior-to-posterior sequence of body segments controlled by those genes (Fig. 1). Finally, we showed that the types of homeotic transformations encountered in *Tribolium* could not have been predicted from previous results in *Drosophila*. This suggested that homologous genes in these two organisms may have been recruited for dif-

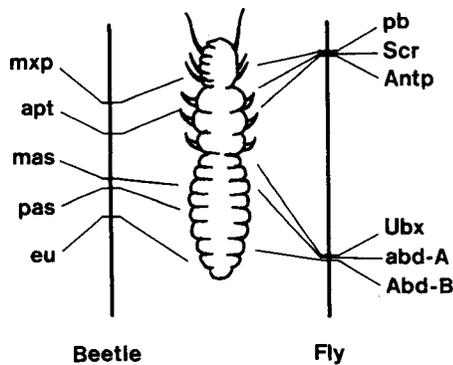


FIG. 1. Clustering of homeotic genes in beetle and fly. Recombination maps of linkage group 2 of *T. castaneum* (left) and chromosome 3R of *D. melanogaster* (right) are shown along with their associated segmental domains on a generalized insect. The beetle genes (abbreviation and domain of mutant expression in parentheses) are *maxillopedia* (*m xp*, labium and maxilla), *alate prothorax* (*apt*, prothorax), *missing abdominal sternite* (*mas*, segment A3), *pointed abdominal sternite* (*pas*, segment A4), and *extra urogomphi* (*eu*, segment A10). The fly genes are *proboscipedia* (*pb*, labium), *Sex combs reduced* (*Scr*, prothorax), *Antennapedia* (*Antp*, thorax), *Ultrabithorax* (*Ubx*, meta-thorax and segment A1), *abdominal-A* (*abd-A*, anterior abdomen) and *Abdominal-B* (*Abd-B*, posterior abdomen).

ferent functions or even that certain homeotic genes in one insect species may have no homolog in related species.

In this paper, we report the isolation of over 50 new mutations in the HOM-C, as well as their adult phenotypes, complementation relationships, and some recombinational mapping. These results confirm that the *Tribolium* homeotic genes differ in many ways from those of *Drosophila*. In addition, this work demonstrates that *Tribolium* offers the possibility of a level of genetic resolution unmatched by any insect except *Drosophila*, and points to the need for future molecular comparisons between homeotic genes of *Tribolium* and *Drosophila*.

MATERIALS AND METHODS

The approach used was to screen for mutations that would uncover one or more of the six known spontaneous recessive mutations in the complex. It was hoped that this approach would yield null alleles corresponding to these preexisting hypomorphic ones and would also yield deficiencies that would enable us to identify other genes in the complex. In this paper we describe new alleles for four of the six previously available recessive alleles, namely *maxillopedia* (*m xp*), *alate prothorax* (*apt*), *missing abdominal sternite* (*mas*), and *pointed abdominal sternite* (*pas*). Other HOM-C mutants described herein were found incidentally by virtue of their dominant phenotypes.

Beetles were reared in wheat flour containing 5% (w/w) brewer's yeast. The wild-type strain GA-1 (Beeman, 1983) was used for all mutageneses. Approximately 1-week-old adult males were starved overnight and then were mutagenized either by gamma irradiation (4 krad) from a Co^{60} source or by feeding overnight on a 0.01 M solution of ethylmethane sulfonate (EMS) in 1% sucrose. After mutagenesis, beetles were held for 1 day (EMS) or 2–4 days (gamma) at 30°C. They were then allowed to mate for 2 days with homozygous *m xp mas* or *m xp apt mas pas* females, after which time the males were discarded and the females were allowed to oviposit for 3–4 weeks at 34°C. The higher temperature was used to hasten development. (None of the variants found in this work are temperature-sensitive). F₁ adults were screened for visible phenotypes resulting from either new dominant mutations or recessives failing to complement *m xp apt mas pas* or *m xp mas* tester chromosomes. We switched to the latter after initial mutagenesis efforts produced dominant homeotic mutations with adult lethal effects in trans with *apt* or *pas*. New mutants were outcrossed for two consecutive generations to allow unlinked mutations to segregate away. The variants were then rendered homozygous or balanced lethal stocks were generated. All mutations described in this work were independently derived.

At present the balancers of choice are the homeotic variants *Stumpy* (*Stm*) and *Extra sclerite* (*Es*¹), described below. Both have good fertility, are homozygous lethal, are fully penetrant, and completely eliminate the recovery of progeny recombinants within the HOM-C.

RESULTS AND DISCUSSION

General Description of Wild-Type Morphology

For readers unfamiliar with beetles, we provide the following general description of some relevant features of *Tribolium* external morphology: The head of wild-type *Tribolium castaneum* (in contrast to that of *Drosophila*) is representative of the primitive insect condition. This includes five pairs of distinctly separated structures, namely eyes, antennae, and the three pairs of gnathal appendages (mandibles, maxillary palps, and labial palps). The gnathal regions derive from three separate body segments contiguous with the thoracic segments. In *Tribolium* the gnathal appendages retain their separate identities in the adult, whereas in adult *Drosophila* they become fused into a single proboscis.

The prothorax (T1) of *Tribolium* is modified into a large "hood" (pronotum) dorsally. In *Drosophila* the dorsal T1 is vestigial, being entirely overgrown by dorsal T2. Ventrally the T1 legs bear a pair of male-specific sex patches. As in *Drosophila*, these are absent from T2 and T3. The mesothorax and metathorax (T2 and T3) of

Tribolium bear a pair of protective wing covers (=elytra) and wings, respectively. Thus, the T2 nonflight wing covers of beetles are homologous to the wings of flies, whereas beetle T3 wings are homologous to the halteres of flies.

Beetles show a characteristic modification of abdominal segmentation. Ventrally, A1 and A2 are vestigial in the adult. The apparent first abdominal segment ventrally is actually A3. The anterior portion of ventral A3 projects inward and is modified to serve as a "socket" for the T3 leg attachments (=coxae). The coxae + socket function together as a hinge, allowing the coxae to rotate in a single plane. Some workers consider the socket to be A2, or A1 + A2, rather than anterior A3 (El-Kifi, 1953). A3 through A7 are prominent and heavily sclerotized ventrally. Segments posterior to A7 are membranous and are telescoped in.

The dorsal abdomen has not been well described in wild-type *Tribolium*. Detailed characterization is in progress in our laboratories. Briefly, the entire abdomen is membranous dorsally, with the exception of paired, dorsolateral sclerotized patches specific to A3. The entire dorsal abdomen is covered and protected by the elytra.

General Description of Mutations Affecting the Head and Anterior Thorax

Antennapalpus (Apl). The previously undescribed *Antennapalpus* mutation occurred as a single EMS-in-

duced variant, found among 10,200 F₁ screened for dominant traits affecting the antennae. *Apl* is dominant and homozygous viable. Homozygous or heterozygous *Apl* larvae are phenotypically normal, but adults have short antennae caused by fusion or deletion of antennal segments, making them resemble maxillary or labial palps (Fig. 2C and Fig. 3). The club (=distal antenna) is always affected; the middle of the antennae is usually affected; but the basal segments are never affected. Patches of cuticle near the tips of *Apl* clubs are often membranous, resembling the tips of wild-type maxillary and labial palps (Fig. 3). *Apl* thus seems to transform distal antenna toward distal palp. A similar mutation, *Spatulate (Spa)*, was previously described and mapped to linkage group 4 by Sokoloff and his students (Sokoloff, 1966; Sokoloff *et al.*, 1966) but was not recognized by them to be homeotic. We found that *Spa* also maps to the HOM-C in linkage group 2 (see below), so it apparently is associated with a T(2;4) translocation. *Spa* is lethal when homozygous and is indistinguishable from *Apl* in phenotype, but *Spa/Apl* beetles are viable and show no increased mutant effect. Since *Spa* is associated with a rearrangement and we do not know whether the homeotic and lethal effects are due to the same altered gene, it is currently unclear whether *Spa* and *Apl* are allelic. Analogous transformations are unknown in *Drosophila*.

Maxillopedia (mxp). This gene is defined by the previously described, spontaneous recessive allele of the

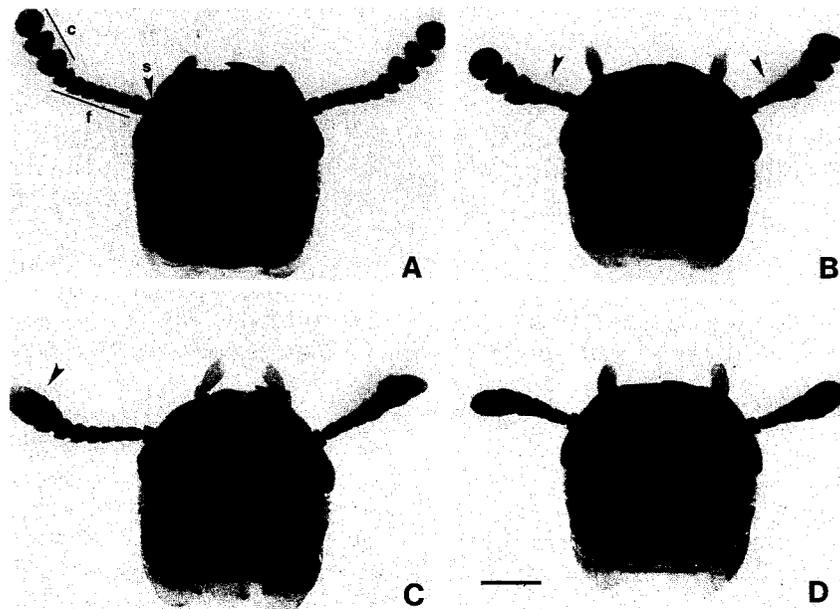


FIG. 2. Adult heads showing effects of "short-antenna" type HOM-C mutations. (A) Wild-type showing three-segmented club (c), six-segmented funicle (f), scape (s), and pedicel (p); (B) *mxp^{D8}/+* showing funicle-specific segment fusions and deletions (arrows); (C) *Apl/+* showing club-specific fusions (arrow); (D) *Stm/+* showing fusions and deletions in both club and funicle. Scale bar = 0.2 mm.

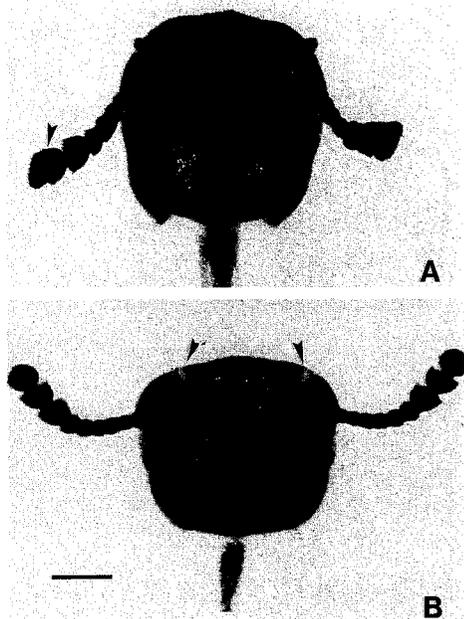


FIG. 3. Phenotype of *Antennapalpus* (*Apl*). (A) *Apl*/+ showing reiteration of palp-specific membrane on the transformed antennal tip (arrow); (B) wild-type showing membranous tips (appearing as white ovals) on maxillary palps (arrows) and labial palps. Scale bar = 0.2 mm.

same name (Hoy, 1966). *mzp* transforms the maxillary and labial palps of adults and larvae into legs, analogous to *proboscipedia* (*pb*) mutations in the ANT-C of *Drosophila*. In this work we found nine new independently derived alleles, among 114,000 F_1 screened for mutations that failed to complement *mzp*. All nine proved to be homozygous lethal and recessive with respect to the maxillopedia phenotype. One of these (*mzp^{lp}*) transforms only labial but not maxillary palps (Fig. 4D). Two of the nine (*mzp^{Ng-1}* and *mzp^{Ng-2}*) had a dominant effect on the anterior part of the head capsule, consisting of deep notches in the genae (Fig. 4C). Also, we discovered two new alleles (among 55,000 mutagenized chromosomes screened for dominant traits) representing a distinct class of *mzp* mutations that have a dominant "short antenna" phenotype in addition to the recessive *mzp* phenotype. These are the EMS-induced mutations *mzp^{Ds}* and *mzp^{Ds}* (Figs. 2B and 4B), the latter showing a specific transformation of maxillary but not labial palps. A third allele in this group is the previously described mutation *Dachs* (*Dch*) (Sokoloff, 1982), which is here renamed *mzp^{Dch}*. A fourth allele, *Stumpy* (*Stm*) (Fig. 2), was found in the same F_1 screen that yielded *Apl*. *Stm* is exceptional in that it largely fails to complement the recessive lethal effects of *mzp* mutations (except for occasional "escapers"), but does complement the recessive *mzp* effect. *Stm* homozygous

escapers are occasionally found but in most cases do not diminish the utility of *Stm* as a lethal balancer.

The short-antenna-type *mzp* alleles differ from *Apl* and *Spa* in the nature and specificity of the effect on the antennal club. None of the dominant *mzp* alleles produce the membranous (palp-like) antennal tips that are characteristic of *Apl* and *Spa*, although adult, homozygous *Stm* escapers do display this phenotype as a recessive effect. Also, the middle region of the antenna, rather than the distal club, is the major site of antennal segment fusion caused by *mzp* alleles. For example, antennal segment fusions and deletions associated with *mzp^{Ds}* always occur in the middle segments, are often absent in the club, and usually do not involve the terminal segment of the club (Fig. 2). In contrast, the terminal club segment is always involved in fusions in beetles carrying *Apl* or *Spa* (Fig. 2). Finally, short-antenna-type *mzp* mutations often cause fusion of not only the antennal but also the tarsal segments (producing short tarsi), whereas *Apl* and *Spa* affect only the antennae. We interpret all of these "short antennae" and "short tarsi" phenotypes as transformations of antennae and legs toward maxillary or labial palps. This inference is supported by the strong transformation of leg toward palp seen in a rare homozygous escaper of *mzp^{Dch-4}* (a *Dachs*-like allele recently found in our laboratory) (Fig. 5). Analogous transformations are unknown in *Drosophila*. *mzp* lethals complement the lethal effects of *Spa*, and effects on the antennae are generally additive. We suspect that *Apl*, *Spa*, *Stm*, *mzp^{Dch}*, and *mzp^D* variants all represent gain-of-function alleles of *mzp*.

Cephalothorax (*Cx*). The previously described spontaneous recessive mutation, *alate prothorax* (*apt*) (Sokoloff, 1965), defines this gene. *apt* partially transforms T1 toward T2 both dorsally (pronotum toward mesonotum) and ventrally (reduced femoral sex patches) in adults, analogous to *Sex combs reduced* (*Scr*) mutations in the ANT-C of *Drosophila*. However, *Scr* mutations in *Drosophila* transform only ventral structures, whereas in *Tribolium* *apt* acts both ventrally and dorsally. In this work we recovered five new radiation-induced alleles among 38,000 F_1 screened for mutations that failed to complement *apt*. (The true *Cx* mutation frequency may have been considerably higher, since *Cx/apt* beetles often die during adult eclosion.) All have dominant effects on the head and pronotum, in addition to showing noncomplementation with *apt*. Dominant effects, typified by *Cx¹* and *Cx⁶*, consist of incomplete fusion of the labial sclerites at the gular sutures, growths of pronotum-like tissue on the occipital region of the head, and buds (everted elytryl discs?) on the posteriolateral margins of the pronota (Figs. 6 and 7). We regard these effects as posteriorward transformations of the labium

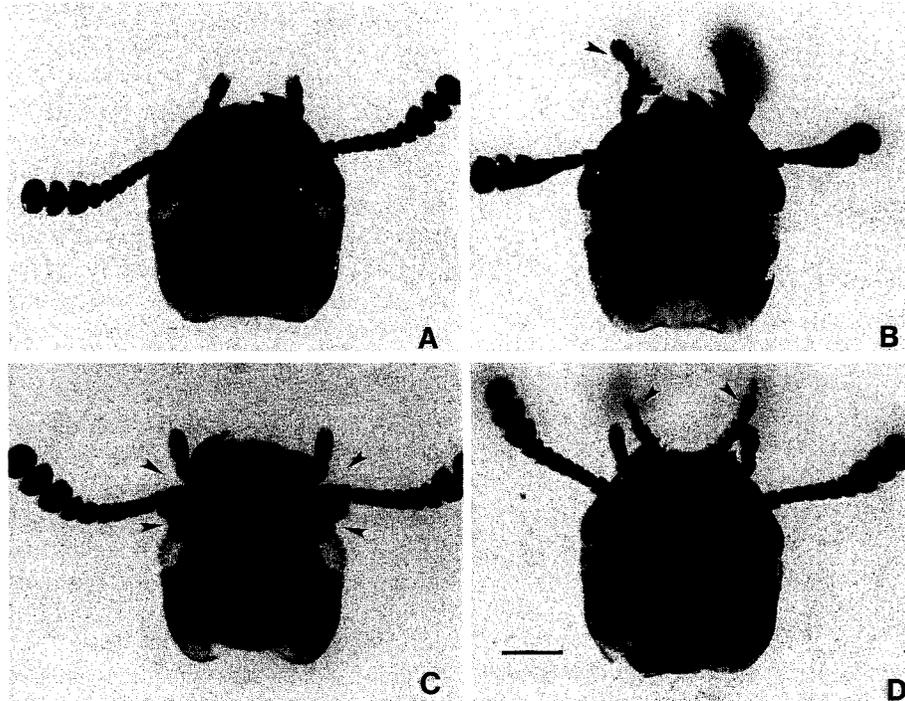


FIG. 4. Adult phenotypes of *maxillopedia* (*maxp*) alleles. (A) Wild-type; (B) *maxp^{Ds}/maxp* showing specific transformation of maxillary (but not labial) palps toward legs (arrow), and dominant "short-antennae"; (C) *maxp^{Ng-1}/+* showing notched genae (arrows); (D) *labiopedia* (*maxp^{lp}/maxp*) showing specific transformation of labial (but not maxillary) palps toward legs (arrows). Scale bar = 0.2 mm.

and prothorax. *Cx⁶/apt* beetles show a dramatic transformation of pronotum to mesonotum, including the presence of well-formed elytra extending from the transformed pronotum (Fig. 7, and Beeman, 1987). In contrast, *Cx¹/apt* beetles more closely resemble *apt* homozygotes, never developing supernumerary elytra. All

dominant *Cx* alleles are lethal when homozygous or in heteroallelic combination with *Cx⁶* (data not shown), but complement the lethal effects of other HOM-C mutations. It seems likely that *Cephalothorax* is a haploinsufficient locus, with *apt* representing a hypomorphic allele.

Prothoraxless (*ptl*). This gene is represented by the previously described, spontaneous, incompletely recessive allele *ptl* (Lasley and Sokoloff, 1960). In homozygous *ptl* mutants T1 development is suppressed both dorsally and ventrally in adults and larvae. In *Drosophila* a similar reduction in the normally vestigial dorsal T1 would be harder to detect, but ventrally acting, *ptl*-like transformations are unknown. *ptl* and *apt* are complementary, but tightly linked (see below). Heterozygotes bearing the EMS-induced dominant mutation *ptl^{D2}* (found incidentally in the same screen that yielded the *Apl* mutation) are indistinguishable from *ptl* homozygotes with respect to the prothoracic effect (Fig. 7). In addition there is an effect of *ptl^{D2}* on the labium not shared by *ptl*. This effect consists of a failure of the mentum to fuse along the ventral midline, causing the labium to be divided into paired structures, similar to the maxillae (Fig. 8). Although the prothoracic reduction caused by *ptl* is not overtly a homeotic transformation, we suggest that the *ptl* and *ptl^{D2}* phenotypes represent anteriorward transformations of prothorax to-

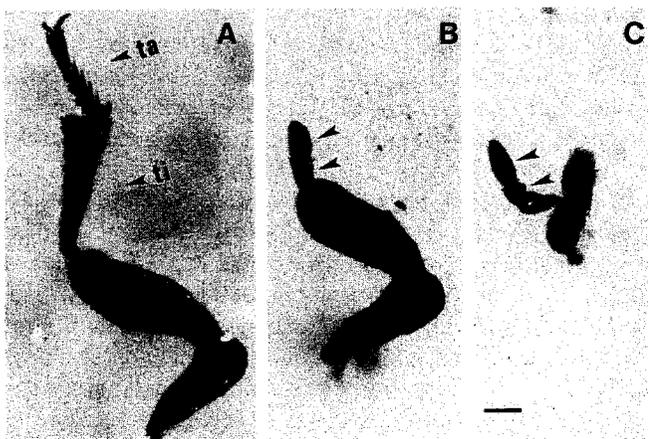


FIG. 5. Adult phenotype of *maxp^{Dch-4}* homozygous escaper. (A) Wild-type T1 leg with tibia (ti) and 5-segmented tarsus (ta) indicated; (B) T1 leg of *maxp^{Dch-4}* homozygote, with transformed tibia and tarsus indicated; (C) wild-type maxillary palp with terminal two segments (mimicked in *maxp^{Dch-4}*) indicated. Scale bar = 0.1 mm.

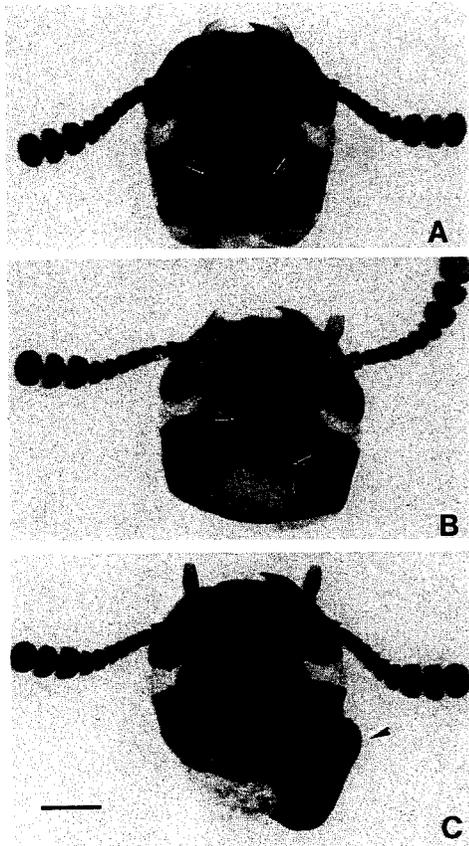


FIG. 6. Adult heads (ventral view) showing dominant phenotype of *Cephalothorax* (Cx^1). (A) Wild-type showing paired gular sutures (arrows); (B) $Cx^1/+$ showing malformation and incomplete fusion of ventral sclerites at the gular sutures (arrows); (C) $Cx^1/+$ showing pronotum-like growth on the occiput, or posterior head (arrow). Scale bar = 0.2 mm.

ward labium and labium toward maxilla. The ptl^{D2}/mcp^{D2} balanced stock breeds true except for occasional (ca. 2%) non- mcp^{D2} beetles. These invariably show extreme expression of ptl^{D2} , and are presumably homozygous ptl^{D2} "escapers". ptl^{D2}/ptl beetles are indistinguishable from ptl^{D2}/ptl^{D2} and have a stronger phenotype, on average, than $ptl^{D2}/+$. On this basis, ptl and ptl^{D2} are assumed to be allelic. On the basis of unpublished observations of recently found, strong ptl alleles, we believe that ptl is analogous to the *Antennapedia* (*Antp*) gene in the ANT-C of *Drosophila*.

General Description of Mutations Affecting the Posterior Thorax and Abdomen

Ultrathorax (*Utx*). This is a newly recognized gene represented by a single dominant, homozygous lethal allele found incidentally in the same screen that yielded the *Apl* mutation. Adult *Utx* heterozygotes have warped

or dented elytra, the deformations usually occurring along a longitudinal axis near the middle of the elytra (Fig. 7). Preliminary observations indicate that *Utx* homozygotes die as late embryos in which the first abdominal segment develops thoracic legs, reminiscent of *Ultrabithorax* (*Ubx*) mutations in *Drosophila*. Another new, dominant mutation, designated *Hairy wing* (*Hw*) (found incidentally in the same screen that yielded the *Cx* alleles), seems to represent a transformation of wing toward elytra (T3 toward T2), and thus may also be regarded as *Ubx*-like. The anterior margins of the wings of *Hw/+* adults, as well as the proximal part of the dorsal wing surfaces, are more densely setiferous than wild-type, causing them to resemble the anterior margins and ventral surfaces of wild-type elytra (Fig. 9). *Hw* shares with *Utx* a dominant effect on the elytra, consisting (in the case of *Hw*) of large blisters, usually near the tips of the elytra (Fig. 7). *Hw* is tightly linked to the HOM-C and is homozygous lethal (data not shown), but complements the lethal effects of *Utx*.

Abdominal (*A*). At the inception of this study three homeotic mutations affecting the abdomen were available: the spontaneous recessive, homozygous viable mutations, *missing abdominal sternite* (*mas*) (Hoy and Sokoloff, 1964), *pointed abdominal sternite* (*pas*, formerly *ppas*) (Sokoloff, 1963), and *extra urogomphi* (*eu*) (Wool and Medlinger, 1973) (see also Fig. 10, and Beeman, 1987). *mas*, *pas*, and *eu* transform A3 toward A2, A4 toward A3, and A10 toward A9, respectively. Ventrally, *mas* transforms the normally sclerotized A3 toward the normally membranous A2, while dorsolaterally the A3-specific tergites are reduced or eliminated. In beetles homozygous for *pas* the ventral A4 (and occasionally A5) sternite resembles that of A3 in having an anterior projection, while the dorsolateral A3 tergites are reiterated on A4 (Fig. 10). Further, *pas* homozygotes sometimes display a pair of membranous sacs or "hernias" protruding at the posterior margin of the A6 sternite. These correspond in position to a pair of "notches" and associated quinone glands normally characteristic of A7. We interpret these sacs as everted, supernumerary quinone glands. *mas* and *pas* show synergistic interactions: *mas pas* double homozygotes show an extension of the *mas* effect to A4 and of the pointed abdominal sternite effect to A5, as well as a more frequent expression of sacs on A6 (Fig. 10).

We have isolated 20 new dominant mutations which are associated with homeotic transformations of the abdomen and which map to the HOM-C. They fall into four groups, namely *Abdominal* (*A*), *Extra sclerite* (*Es*), *Socketless* (*Sk*), and *Miscaudal sclerotization* (*Mcs*). Their adult phenotypes are shown in Figs. 11-13. The *Abdominal* class is a frequently encountered type (13 independently derived alleles including two spontane-

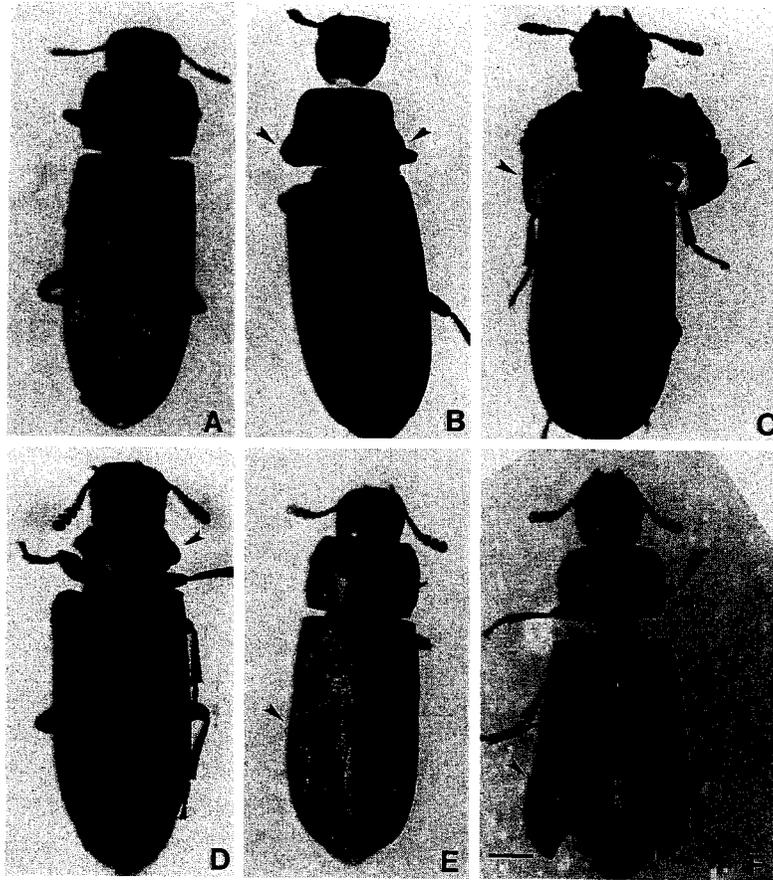


FIG. 7. Phenotypes of HOM-C mutations affecting the thorax. (A) Wild-type; (B) *Cx^l/+* showing bud-like outgrowths of pronotum (arrows); (C) *Cx^l/apt* showing reiterated elytra (arrows) projecting from transformed pronotum; (D) *ptl^{D2}/+* showing vestigial pronotum (arrow); (E) *Utx/+* showing warped elytra (arrow); (F) *Hw/+* showing blistered elytra (arrow). Scale bar = 0.4 mm.

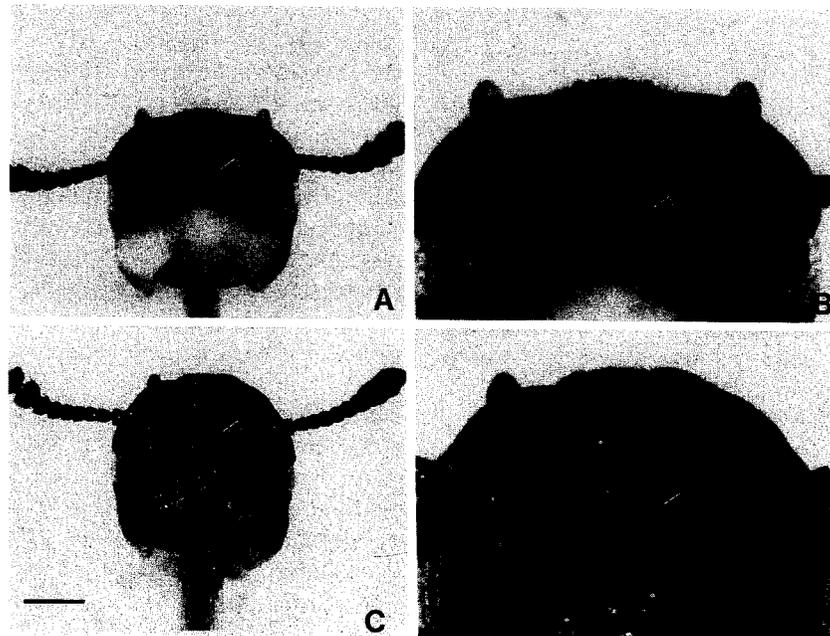


FIG. 8. Adult heads (ventral view) showing "split-mentum" phenotype of *prothoraxless* (*ptl^{D2}*). (A) Wild-type showing normal mentum (arrow), or fused basal attachment of labial palps; (B) enlargement of (A); (C) *ptl^{D2}/+* showing mentum unfused (arrow), creating bilaterally symmetric pair of sclerites; (D) enlargement of (C). Scale bar = 0.2 mm in (A and C), 0.1 mm in (B and D).

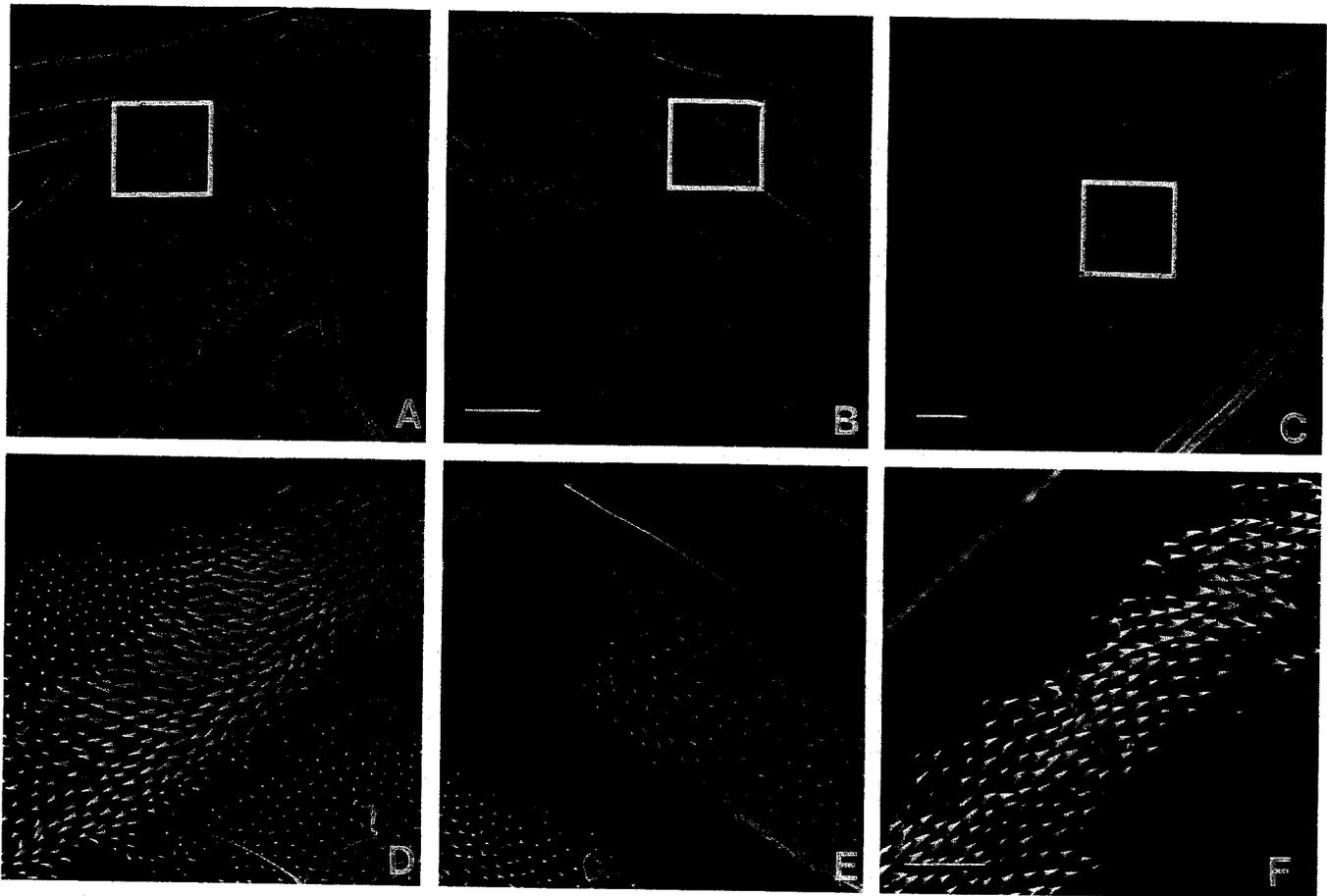


FIG. 9. Adult phenotype of *Hairy wing* (*Hw*). (A) Dorsal view of proximal portion of *Hw*/+ wing showing setiferous patch (boxed) and long hairs over general surface of wing; (B) same view of wild-type wing in mirror-image showing absence of setiferous patch (boxed) and absence of long hairs over general wing surface; (C) ventral view of wild-type elytron showing setiferous patch (boxed) mimicked in (A); (D) enlargement of boxed region of (A); (E) enlargement of boxed region of (B); (F) enlargement of region analogous to boxed region of (C), but in a different individual. Scale bar = 0.1 mm in (A and B), 0.1 mm in (C), and 0.02 mm in (D-F).

ous and 11 others found among 66,000 chromosomes screened for radiation-induced mutations that failed to complement *mas*). *A*/+ heterozygotes display a pair of notches in the posterior margin of the A6 sternite, resembling those normally found only on A7 (Fig. 11). The A6 notches are sometimes associated with hernias, similar to those seen in *pas* homozygotes. *A* mutations fail to complement *mas* and *pas* (i.e., *A*/*mas pas* adults display *mas* and *pas* phenotypes in the anterior abdomen and a "notch" phenotype in the posterior abdomen). Most *A* mutations are lethal when homozygous or in heteroallelic combination. The *A^s* allele is exceptional in that some homozygotes survive to the adult stage and display an extreme *mas* phenotype in which A2-A4 are nonsclerotized, and the anterior portions of A5 and A6 are poorly sclerotized. In addition, the "notch" and/or "sac" phenotypes appear on posterior A5 as well as A6, and A7 is strongly transformed toward A8. On the basis of the frequency of *Abdominal* mutations and the com-

plementation behavior just described, our working hypothesis is that there is a single gene for which mutants express a "notch" phenotype (transformation of A6 toward A7) as a haplo-insufficient effect; *mas*, *pas*, and *A^s* are then interpreted as partial loss-of-function variants. *Abd-B* in the BX-C of *Drosophila* has similarly been reported to be haplo-insufficient (Sanchez-Herrero *et al.*, 1985) but in *Drosophila* the haplo-insufficient phenotype is precisely opposite that of *Abdominal* in *Tribolium*, namely a transformation of A7 toward A6.

The remaining three categories of dominant, abdominal mutations each express some transformations opposite in direction from those of *mas*, *pas*, and *A* alleles, and we will argue that they represent gain-of-function mutations in the same gene. *Extra sclerite* (*Es*) (three alleles including one spontaneous, one radiation-induced found among 64,000 F₁ screened for dominant abdominal aberrations, and one radiation-induced found among 38,000 F₁ screened for mutations failing to

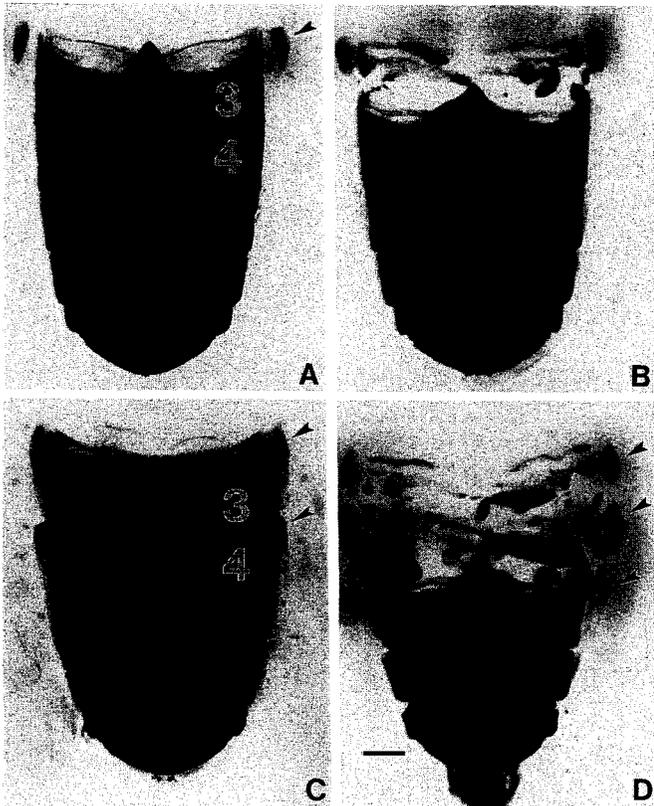


FIG. 10. Phenotypic interaction between *mas* and *pas*. (A) Ventral view of wild-type adult abdomen with segments 3 and 4 indicated. Also note the paired dorsolateral tergites (arrows), displayed by folding out the unsclerotized dorsal cuticle; (B) *mas/mas* showing ventral suppression of A3 sclerotization; (C) *pas²/pas²* showing anterior A4 to A3 transformation, including reiteration of A3 tergites on A4 (arrows); (D) *mas pas/mas pas* double homozygote showing synergistic interaction, including extension of the *pas* effect to A5. Scale bar = 0.2 mm.

complement *pas*) is a class of mutations in which the second abdominal segment is transformed toward the third (Fig. 11). *Es*/+ beetles also express the A6 "notch" phenotype. *Es* alleles complement *mas*, but fail to complement *pas* or the *pas* effect of *A* alleles. Although *Es*¹ homozygotes are embryonic lethal, *Es*¹/*A*¹⁰ beetles survive with reduced viability. Finally, *Es*¹ shows good viability in heterozygous combination with either of the other two categories of dominant mutations affecting the abdomen, *Mcs* or *SkI*. *Es*¹ suppresses crossing over in the vicinity of the HOM-C (data not shown), and we believe that an unrelated recessive lethal remains linked to it because of a rearrangement.

Socketless (*SkI*) is represented by one radiation-induced allele, found in the same screen that yielded *Es*¹. *SkI*/+ adults have A3 sternites transformed to resemble A4; i.e., they lack the anterior medial projection and the lateral tergites normally characteristic of A3 (Fig. 11).

SkI is homozygous lethal, but complements the recessive lethality associated with all other dominant mutations affecting the abdomen.

The last three dominant mutations form a class termed *Miscaudal sclerotization* (*Mcs*) because of a dominant transformation of ventral A8 (not normally well-sclerotized) to resemble the heavily sclerotized A7 (Fig. 11). All three *Mcs* alleles also cause a dominant transformation of A2 and A3 toward A4, and a dominant fusion of antennal segments (gain of *A* function in the head?). Beetles homozygous or bearing heterozygous combinations of *Mcs* alleles show reduced viability, and *Mcs*¹ homozygous escapers surviving to the adult stage also show a recessive transformation of A7 toward A6. *Mcs* alleles are completely viable in heterozygous combination with *A* alleles, *Es*¹, or *SkI*. Only *Mcs*² shows a weak failure to complement *pas*, whereas all three alleles complement *mas*. *SkI* and two of the three *Mcs* alleles were found in the same screen that yielded *Es*¹. *Mcs*¹ was found incidentally among 38,000 F₁ screened for mutations that failed to complement *mas*.

Mapping and Complementation

The two new variants *Apl* and *Utx*, and the previously described variant *ptl* and its new allele *ptl*^{D2}, were partially mapped, as were the new *Abdominal* mutations *A*¹⁰ and *Mcs*¹ (Tables 1-3, Fig. 14, and data not shown). *Apl* is tightly linked to *m xp*, but recombines with *apt* and the abdominal variants. *ptl* and *ptl*^{D2} are tightly linked to *apt*, but recombine with *m xp* and with the abdominal variants. *Utx*, *A*¹⁰, and *Mcs*¹ are tightly linked to one another, but recombine with *apt* and *m xp*. A balanced *Hw/Es*¹ stock was established but died out after several generations. The viability of *Hw* is too low for facile recombinational mapping.

The *Tribolium* HOM-C is characterized by a large number of lethal complementation groups (data not shown). *Spa*, *m xp*, *Cx*, and *ptl* represent four distinct lethal groups. The two lethal *Ubx*-like variants *Utx* and *Hw* complement each other's lethality. Thus, head and thoracic HOM-C mutations define at least six lethal complementation units. However, many of the lethals used in this analysis may be gain-of-function or double mutants, consisting of a nonlethal homeotic mutation tightly linked to an unrelated lethal by chromosome rearrangement. Definitive conclusions regarding the number of vital genes in this portion of the HOM-C must await further genetic analysis.

Mutations within the *Abdominal* region show complex and overlapping complementation behavior. The viable mutations *mas*, *pas*, and *eu* are mutually complementary. The lethal mutants *Es*¹, *Mcs*, and *A* represent three separate lethal complementation groups, but

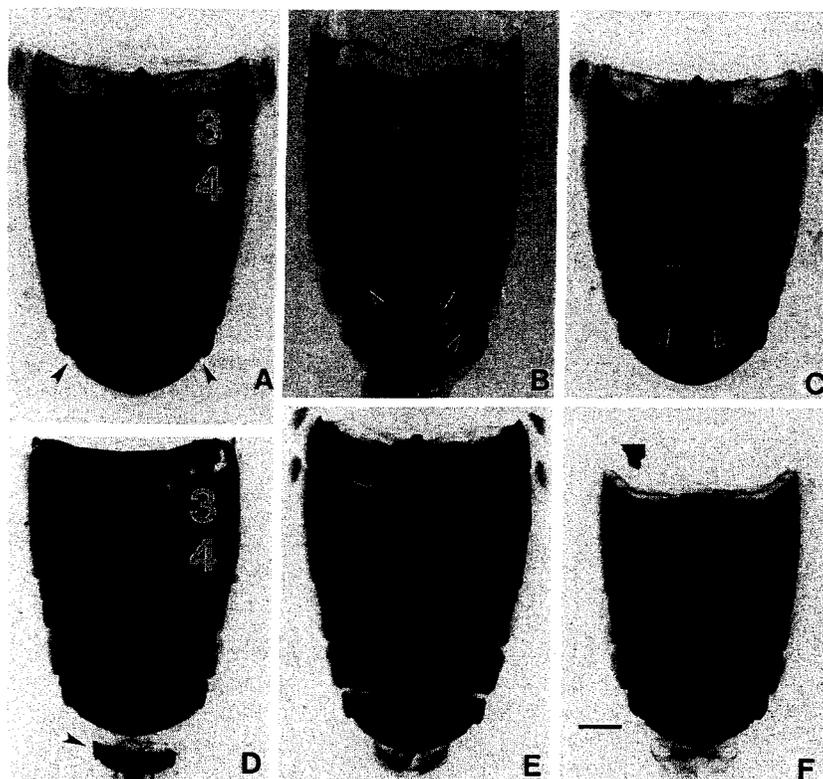


FIG. 11. Adult phenotypes of *Abdominal* mutations. (A) Wild-type abdomen (ventral view) showing "notches" in A7 (arrows); (B) *Es^s/pas* showing paired, everted sacs protruding from under the posterior margins of A5 and A6 (arrows), weak transformation of A6 toward A7 (rounded posterior margin of A6) and A7 toward A8 (reduced sclerotization on anterior A7). Note also the weak (dominant) A2 toward A3 transformation in addition to the strong (recessive) transformation of A4 toward A3; (C) *A¹/+* showing paired notches (arrows) on the posterior margin of A6; (D) *Mcs¹/+* showing transformation of A8 toward A7 (arrow), and A2 and A3 toward A4; (E) *Es²/+* showing strong A2 toward A3 transformation; (F) *Skl⁺* showing A3 toward A4 transformation. Scale bar = 0.2 mm.

variants in all three groups fail to complement *pas*; i.e., they have recessive *pas* effects in addition to their dominant effects on abdominal segments 2, 3, 6, and 8. *Es* alleles fail to complement alleles of *pas*, but do complement *mas* and *eu*. All three *Mcs* alleles complement both *mas* and *eu*, but one of the three shows weak failure of complementation with alleles of *pas*. *A* alleles fail to complement either *mas* or *pas*, but do complement *eu*.

Comparisons between the Mutant Phenotypes of the ANT-C of Drosophila and the Anterior Region of the HOM-C of Tribolium

In adult *Drosophila* the three gnathal segments are fused into a single, derived structure, the proboscis. The maxillary palps are still discernible as a pair of structures that branch off the proboscis. The larval head is only poorly differentiated (Jurgens *et al.*, 1986). In the primitive condition represented by *Tribolium*, the three gnathal segments are separate, paired structures (although the labial paired appendages are fused at their bases), and the anterior-to-posterior sequence (mandi-

ble-maxilla-labium) and separate segmental integrity are retained in both adults and larvae.

The ANT-C of *Drosophila* contains five homeotic complementation groups. In distal-to-proximal sequence, these are *labial (lab)*, *proboscipedia (pb)*, *Deformed (Dfd)*, *Sex combs reduced (Scr)*, and *Antennapedia (Antp)*. The four "anterior" lethal complementation units in the *Tribolium* HOM-C (*Spa*, *m_{xp}*, *Cx*, and *ptl*) seem to be the functional equivalent of the *Drosophila* ANT-C. We suspect that *m_{xp}*, *Cx*, and *ptl* are three distinct genes, and that *Spa* is a gain-of-function *m_{xp}* mutation, in which *m_{xp}⁺* is misexpressed in the head segment that bears the antennae. The lethality of the *Spa* translocation may stem from its LG4 breakpoint rather than that on LG2.

The *m_{xp}* gene in *Tribolium* may correspond to *proboscipedia (pb)* in *Drosophila*. The latter is normally expressed in the maxillary and labial segments, and its expression is required to prevent thoracic (leg) differentiation and promote proboscis differentiation in these segments.

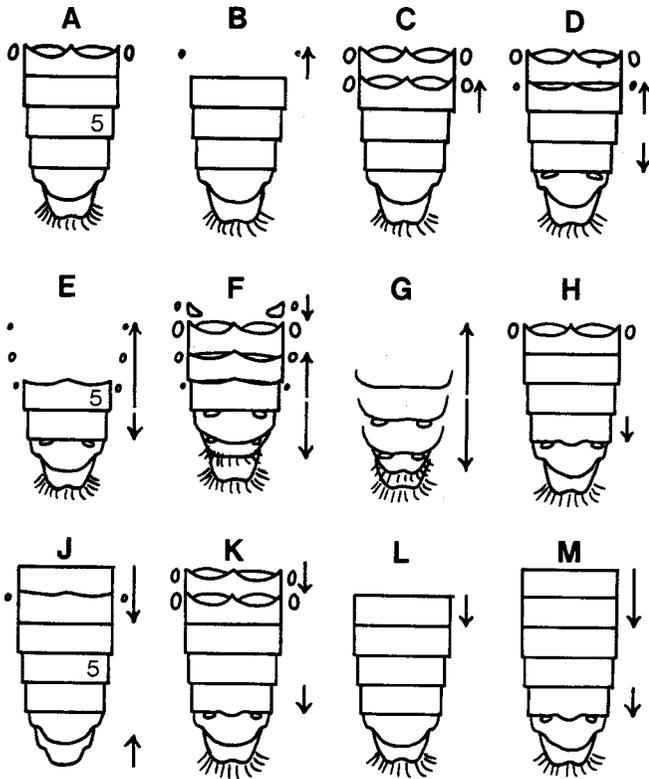


FIG. 12. Summary of all existing types of Abdominal homeotic transformations in *T. castaneum*. Arrows indicate direction and domain of transformation. (A) Wild-type showing coxal "sockets" just anterior to A3, paired A3 tergites, A8 telescoped out to show general shape and posterior fringe; (B) *mas/mas*; (C) *pas²/pas²*; (D) *pas/pas* showing pair of everted sacs protruding from the A6-A7 intersegmental membrane at positions comparable to the notches in A7; (E) *mas pas/mas pas*; (F) *Es²/Es²*; (G) *A⁸/A⁸* escaper showing strong posteriorward transformation of A5 toward A7, *mas pas* phenotype in A3-A4, and absence of sclerotization in the anterior half of all segments; (H) *A¹/+* showing notches and associated sacs; (J) *Mcs¹/+*; (K) *Es¹/+* showing the sometimes-expressed "notch" phenotype in A6; (L) *Skl/+*; (M) *Skl/Es* showing additive interaction.

TABLE 1
LINKAGE ANALYSIS OF *ApI*^a

Progeny phenotype	Number of progeny
+ <i>mxp apt mas pas</i>	687
<i>Apl + + + +</i>	923
+ <i>mxp + + +</i>	6
<i>Apl + apt mas pas</i>	10
+ <i>mxp apt + +</i>	16
<i>Apl + + mas pas</i>	17
+ <i>mxp apt mas +</i>	2
<i>Apl + + + pas</i>	0
+ + <i>apt mas pas</i>	44 ^b
All others	0
Total	1705

^a The testcross (en masse) was *Apl + + + + / + mxp apt mas pas* ♂♂ X + *mxp apt mas pas / + mxp apt mas pas* ♀♀.

^b All variants except *mxp* are fully penetrant. The 44 + + *apt mas pas* apparent recombinants were probably all nonpenetrant + *mxp apt mas pas* nonrecombinants.

A similar relationship between *mxp*⁺ expression and labial + maxillary palp development seems to exist in *Tribolium*. However, no dominant gain-of-function *pb* mutations are known in *Drosophila*, whereas putative gain-of-function *mxp* alleles (viz. *Apl*, *Spa*, *mxp^{Dch}*, *Stm*, *mxp^{Dz}*) are frequently encountered in *Tribolium*. *Cx* in *Tribolium* may correspond to *Scr* in *Drosophila*, since wild-type function in both is required to prevent T2 development in T1. However, at least two independent *Cx* alleles transform larval labial palps toward antennae (unpublished observations), reminiscent of *pb* mutations in *Drosophila*. As previously stated, we believe *ptl* to be analogous to *Antp* in *Drosophila*. A recessive mutation denoted *antennapedia* has been described in *Tribolium*, but it is not linked to the HOM-C, and may be homologous to the *aristapedia* gene of *Drosophila*.

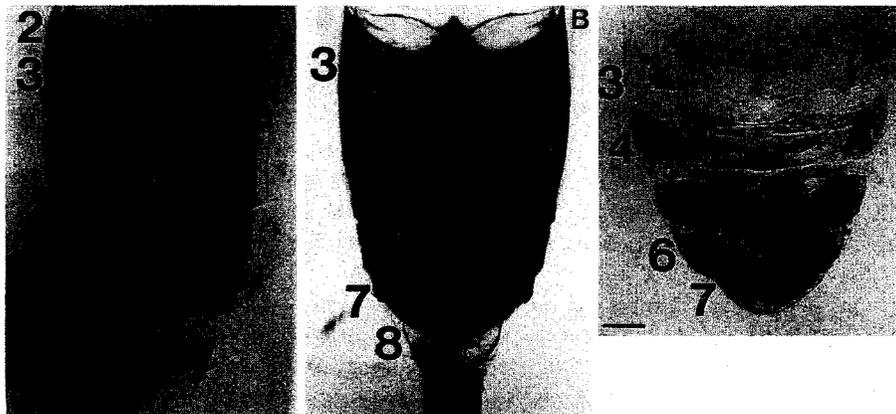


FIG. 13. Adult phenotypes of strong gain-of-function (A) and strong loss-of-function (C) Abdominal mutations. (A) *Mcs¹* homozygous escaper showing A2 → A4, A3 → A4, A8 → A7, and A7 → A6 transformations; (B) wild-type showing A8 telescoped out; (C) *A⁸* homozygous escaper showing A7 → A8, A6 → A8, A5 → A7, A4 → A2, and A3 → A2 transformations. Scale bar = 0.2 mm.

TABLE 2
LINKAGE ANALYSIS OF *Spa* AND *Utx*

Testcross ^a	Progeny phenotype	Number of progeny
A	<i>apt</i> + <i>mas pas</i>	680
	+ <i>Utx</i> ++	723
	<i>apt Utx</i> ++	9
	++ <i>mas pas</i>	11
	++++	36 ^b
	All others	0
	Total	1459
B	<i>Spa</i> +	102
	+ <i>pas</i> ²	100
	All others	0
	Total	202

^a Testcrosses (en masse) were (A) + *Utx* ++/*apt* + *mas pas* ♂♂ X *apt* + *mas pas*/*apt* + *mas pas* ♀♀; and (B) *Spa* +/+ *pas*² ♂♂ X + *pas*²/*pas*² ♀♀.

^b The 36 +++++ apparent recombinants were probably all nonpenetrant + *Utx* ++ nonrecombinants. *Utx* is ca. 90-95% penetrant. All others are 100% penetrant.

Interpretation of HOM-C mutant phenotypes may be facilitated if we can correctly sort them into gain-of-function vs loss-of-function categories. This may now be feasible because of the recent discovery of a HOM-C duplication in our laboratories (unpublished observations) and because of our current efforts to revert dominant HOM-C mutations. All currently available types of HOM-C variants are listed in Table 4.

Comparisons between Mutant Phenotypes of the BX-C of *Drosophila* and the Abdominal Region of the HOM-C of *Tribolium*

Number of subunits. The BX-C of *Drosophila* is believed to consist of three major subunits, namely *Ubx*, *abd-A*, and *Abd-B*. A corresponding division into three subunits is not apparent for the abdominal portion of the HOM-C in *Tribolium*, despite some similarities be-

TABLE 3
LINKAGE ANALYSIS OF *ptl*^a

Crossover chromosomes recovered in progeny	Number of progeny
<i>mxp</i> + <i>ptl</i> ++	3
+ <i>apt</i> + <i>mas pas</i>	1
<i>mxp apt</i> +++	5
++ <i>ptl mas pas</i>	3
+ + <i>ptl</i> + <i>pas</i>	1
all others	0

^a The testcross (en masse) was *mxp apt* + *mas pas*/++ *ptl* ++ ♂♂ X *mxp apt* + *mas pas*/*mxp apt* + *mas pas* ♀♀. Several hundred progeny were screened, and genotypes of all recombinants were determined by testcrosses to *ptl*/*ptl*.

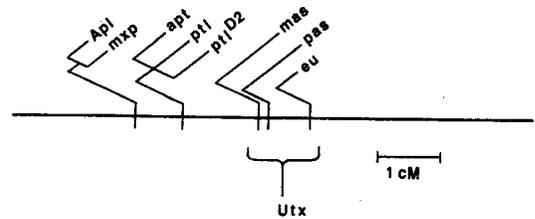


FIG. 14. Linkage map of the HOM-C. Brackets indicate region in which no recombination involving the indicated mutation (*Utx*) has yet been observed. Bracket at the right indicates 1 cM (=1% recombination).

tween the two complexes. Since *Utx* and *Hw* transform A1 and T3, they may correspond to *Ubx* in *Drosophila*. The Abdominal (A) mutations in *Tribolium* seem to represent the partial or complete loss-of-function of a complex gene that controls the differentiation of abdominal segments 2-7. *mas* and *pas* produce loss of different subfunctions within this complex locus. However, unlike *abd-A* mutations in *Drosophila*, which strongly transform only abdominal segments 2-4, the *Tribolium* A mutations strongly transform segments 2-7, and therefore seem to combine the mutant domains of *abd-A* and *Abd-B*.

Direction of transformations. Individual BX-C mutations in *Drosophila* produce unidirectional transformations, either anteriorward or posteriorward. In contrast, individual Abdominal mutations in *Tribolium* often reverse their direction of transformation once or even twice along the longitudinal axis of the abdomen. Doubly homozygous *mas pas* adults (Fig. 10) and adult homozygous *A*⁸ escapers (Figs. 12 and 13) lack sclerites or show only partial sclerotization on abdominal segments 2-5; i.e., these segments resemble the normally unsclerotized first or second abdominal segment. Similarly, transformation of abdominal segments 2-6 toward 1 is characteristic of complete loss of *abd-A* function in *Drosophila* (Sanchez-Herrero *et al.*, 1985). The major difference between A mutations in *Tribolium* and complete loss-of-function *abd-A* or *Abd-B* mutations (such as *abd-A*^{M1} or *Abd-B*^{M1}, see Sanchez-Herrero *et al.*, 1985) in *Drosophila* is that the *Tribolium* A mutations reverse polarity in abdominal segment 5. That is, the *Tribolium* loss-of-function mutants produce anteriorward transformations in segments 2-5a, but posteriorward transformations in segments 5p-7. The same reversal of polarity at segment 5 is seen in the recessive phenotypes of *pas*, *Es*², and *Es*³. It is intriguing to note that the discontinuous-domain, dominant (gain-of-function?) mutations *Mcs*¹, *Mcs*², and *Mcs*³ produce the inverse syndrome to that of *A*⁸, namely posteriorward transformation in segments 2-3 and anteriorward transformation in segments 7-8 (Figs. 11-13 and 15).

Position of ground states. In the BX-C of *Drosophila*, loss-of-function mutations cause anteriorward trans-

TABLE 4
CATALOG OF EXISTING HOM-C MUTATIONS IN *T. castaneum*^a

Mutation	No. of alleles	Recessive phenotype	Origin	Reference
<i>Apl</i>	1	l	EMS, GA-1	This work
<i>Spa</i> [T(2;4)]	1	l	s	Sokoloff <i>et al.</i> (1966)
<i>mzxp</i> ^{Dch} [In(2)?]	1	l	X-ray	Sokoloff (1982)
<i>mzxp</i>	1	v, f	s	Hoy (1966)
<i>mzxp</i> ^{Nu}	2	l	EMS or γ , GA-1	This work
<i>mzxp</i> ^r	10	l	EMS or γ , GA-1	This work
<i>mzxp</i> ^{pr}	1	l	γ , GA-1	This work
<i>mzxp</i> ^D	2	l	EMS or γ , GA-1	This work
<i>Stm</i>	1	l	EMS, GA-1	This work
<i>Cx</i>	5	l	γ , GA-1	This work
<i>apt</i>	1	v, f	s	Sokoloff (1965)
<i>ptl</i>	1	v, f	s	Lasley and Sokoloff (1960)
<i>ptl</i> ^D	1	l	EMS, GA-1	This work
<i>Utx</i>	1	l	EMS, GA-1	This work
<i>Hw</i>	1	l	γ , GA-1	This work
<i>mas</i>	1	v, f	s	Hoy and Sokoloff (1964)
<i>pas</i>	2	v, f	s or γ , GA-1	Sokoloff (1963) and this work
<i>Es</i>	3	l or sl	γ , GA-1 or s	This work
<i>Skl</i>	1	l	γ , GA-1	This work
<i>Mcs</i>	3	l	γ , GA-1	This work
<i>A</i>	13	l	s or γ , GA-1	This work
<i>eu</i>	1	v, f	s	Wool and Medlinger (1973)

^a Abbreviations: v, viable; f, fertile; l, lethal; sl, semilethal; s, spontaneous; EMS, ethylmethane sulfonate; γ , gamma (from Co⁶⁰ source). *Es*² and *A*⁴ alleles occurred as spontaneous revertants of *mas*.

formations, whereas gain-of-function mutations cause posteriorward transformations in abdominal segments 2-8. The ground state for *abd-A* (controlling segments 1-4) is segment 1, or more precisely, A1a + T3p. The ground state for *Abd-B* (controlling segments 5-8) is segment 4. In *Tribolium* the ground state for genetic

control of segments 2-5 is segment 1 or 2, but the ground state for genetic control of segments 5-8 seems to be segment 8 (Fig. 15). Segment 5 is a "watershed," since a reversal of directionality occurs there, both for gain-of-function and for loss-of-function mutations. This is clearly seen in adult *A*⁸ homozygous escapers, which in our judgment manifest the loss-of-function (or diminution of function) of *Abdominal* in segments 3-7. It is also apparent in *Mcs*¹, *Mcs*², and *Mcs*³, which seem to be gain-of-function mutations, and in *Es*² and *Es*³, which seem to be gain-of-function in segment 2 (a completely dominant effect) and loss-of-function in segments 4-7 (a largely recessive effect).

In addition to the process of cell specification directed by homeotic selector genes, there exists during animal development a system of positional information which divides cell layers into "fields" or units of differentiation (Wolpert, 1969). In insects, head and thoracic appendages (including antennae, maxillary and labial palps, and legs) are serially homologous (Rempel, 1975). Evidence from studies of *Drosophila melanogaster* and other insects (French *et al.*, 1976; Bryant, 1978) suggests that the underlying positional information is fundamentally similar in each appendage, which show segment-specific differences in cytodifferentiation in response to common positional values. In support of this view, Postlethwait and Schneiderman (1971) showed that in the homeotic transformations associated with

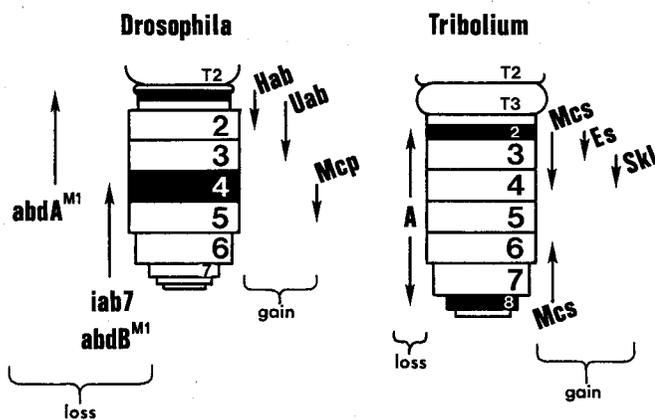


FIG. 15. Comparison of the *Abdominal* region of the HOM-C of *Tribolium* with the BX-C of *Drosophila*. Direction and domain of transformation are indicated for loss and gain-of-function mutations in *Drosophila*, and for the putative loss and gain-of-function analogs in *Tribolium*. Segmental ground states for *abdA* and *AbdB* in *Drosophila* (parasegment T3p + A1a, and segment A4) are indicated by dark shading, as are the proposed ground states (segments 2 and 8) in *Tribolium*.

the *Antennapedia* mutation, any given antennal region always gives rise to a specific part of the leg pattern. The phenotypes of a number of *Tribolium* mutants are also consistent with the existence of segmentally reiterated systems of positional information. In addition to the global-effect *mxp* alleles described in this work, other single mutations with pleiotropic effects on legs and antennae have been described. These include *Fta*, *Sa*, *Troll*, *pegleg*, *sta*, and *ab* (Sokoloff, 1977; Vasquez and del Castillo, 1985). There is also genetic evidence for homology between specific segments or regions (proximal, middle, and distal) of the legs and corresponding segments or regions of the antennae and palps. For example, *mxp^{Dch}* affects only the middle and distal regions of antennae, palps and legs. The recently discovered mutation, *antena bifurcada* (*ab*), affects only the proximal segments of legs and antennae (Vasquez and del Castillo, 1985). Finally, we have recently discovered a homeotic mutation that transforms the basal segment (scape) of the antenna toward the basal region (including the galea) of the maxillary palp (unpublished).

It has become apparent in recent years that the homeotic gene complexes of *Drosophila* comprise a vital network of interdependent "master switches" regulating key developmental decisions in the differentiating embryo. Similar developmental switch genes with more specialized or limited functions are being recognized in a variety of other biological systems. Molecular sequences similar to *Drosophila* homeotic gene probes are being isolated from a variety of animal species in several laboratories. However, insights into the normal functions of such complementary sequences, and thus evolutionary insights into the spectrum of adaptations among related species, may be difficult to gain unless molecular sequences can be correlated with biological functions with the aid of genetic mutations. These limitations have now been overcome in *Tribolium* with regard to a major category of developmental switches. Our experience to date has convinced us that saturation mutagenesis of the homeotic gene complex of *Tribolium* can be achieved in a relatively short time. Molecular sequence comparison between *Tribolium* and *Drosophila*, when combined with comparison of mutant phenotypes, promises to yield unprecedented evolutionary perspective into an important area of invertebrate development.

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