

Structure and Function of the Homeotic Gene Complex (HOM-C) in the Beetle, *Tribolium castaneum*

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Summary

The powerful combination of genetic, developmental and molecular approaches possible with the fruit fly, *Drosophila melanogaster*, has led to a profound understanding of the genetic control of early developmental events. However, *Drosophila* is a highly specialized long germ insect, and the mechanisms controlling its early development may not be typical of insects or Arthropods in general. The beetle, *Tribolium castaneum*, offers a similar opportunity to integrate high resolution genetic analysis with the developmental/molecular approaches currently used in other organisms. Early results document significant differences between insect orders in the functions of genes responsible for establishing developmental commitments.

How Much Information from *Drosophila* Can Be Generalized?

Drosophila is uniquely accessible among higher eukaryotes for sophisticated genetic dissection of development. However, as our understanding of the genetic control of early embryonic events in *Drosophila* has deepened, so has the gulf between this knowledge and that available for other Arthropods. Given the remarkable diversity of insect forms, reproductive strategies, physiological systems and ecological niches, we cannot assume that any particular biological mechanism has been evolutionarily well conserved.

Segmental organization along the anterior/posterior axis is one of the most prominent features of Arthropod development⁽¹⁾. It is believed that the head of ancestral insects consisted of an asegmental acron and six segments. These included three pre-oral metameres: the labral, antennal and intercalary segments, and three gnathal metameres: the mandibular, maxillary and labial segments. The gnathal region often bore appendages specialized as mouthparts, and formed the posterior head. The remainder of the embryo consisted of a thorax with three segments, an abdomen with a maximum of eleven, and an asegmental caudal region termed the telson. Most insects resemble their presumed ancestors in establishing metamerism in the anterior-most portion of the embryo, and then adding more posterior segments successively. In contrast, the higher flies show 'long germ' development in which segmentation occurs essentially simultaneously along the entire anterior-posterior axis⁽²⁾.

Even in a taxon as morphologically diverse as the Insecta, the wormlike larva or maggot of the higher flies is considered the most derived⁽¹⁾. It lacks external thoracic and abdominal appendages and is essentially headless due to the involution of head primordia through the presumptive mouth. The adults of higher flies have also undergone major morphological modifications (fusion and/or loss of segments) in the head and caudal regions as well as the divergence and specialization of other body segments.

The technical advantages of *Drosophila* have yielded an unrivaled description of the genetic control of segmentation and early developmental commitments. A hierarchy of maternal and/or zygotically active genes establishes the segmental organization of the early embryo and the separation of each segment into anterior and posterior polyclonal lineages or compartments⁽³⁻⁵⁾. In the gnathal and trunk regions, the resulting metameric units are then committed to specific developmental fates by the functions of the homeotic selector genes⁽⁶⁾. These genes were originally identified by variants which cause the developmental replacement of one body region by another; such phenotypes can arise because loss-of-function mutations allow the region to follow a default pathway, or because gain-of-function mutations cause misregulation such that a gene is expressed outside its normal domain and results in an inappropriate developmental pathway superceding the normal one. The homeotic selector genes share a motif termed the homeobox, which encodes a 60-amino acid peptide (the homeodomain) required for sequence-specific DNA binding and thus for the functions of the homeoproteins as transcriptional regulatory factors. These genes are very ancient, and originated as a single cluster before the divergence of insects and vertebrates and probably before the first segmented animal arose. As is best described in *Drosophila* and the mouse, the genes are expressed in a linear order along the anterior-posterior body axis that parallels their order along the chromosome. Present evidence suggests that it is this feature, a cluster of genes encoding transcription factors which can be regulated along an axis, that has been so well-conserved, and that the regulation of the expression of the homeotic genes and the nature of the developmental pathways under their control has been highly modified during the evolution of animals with diverse body plans.

In *Drosophila*, the ancestral Homeotic complex has been spatially separated into two groups of genes: the Antennapedia complex (ANT-C) which is important in the more anterior portion of the embryo⁽⁷⁾, and the bithorax complex (BX-C) which controls more posterior decisions⁽⁸⁾. The genes comprising these two complexes are depicted in Fig. 1, and have been extensively characterized with respect to their structure, regulation and function. At least within the central portion of the embryo, the homeotic genes are regulated within domains which do not correspond to segments. Rather, their expression is limited by boundaries between anterior and posterior compartments, and the functional units of organization (termed parasegments) comprise the posterior compartment of one segment and the adjacent anterior compartment of the next. Thus, for example, the *Ultrabithorax* gene regulates normal development in parasegments 5

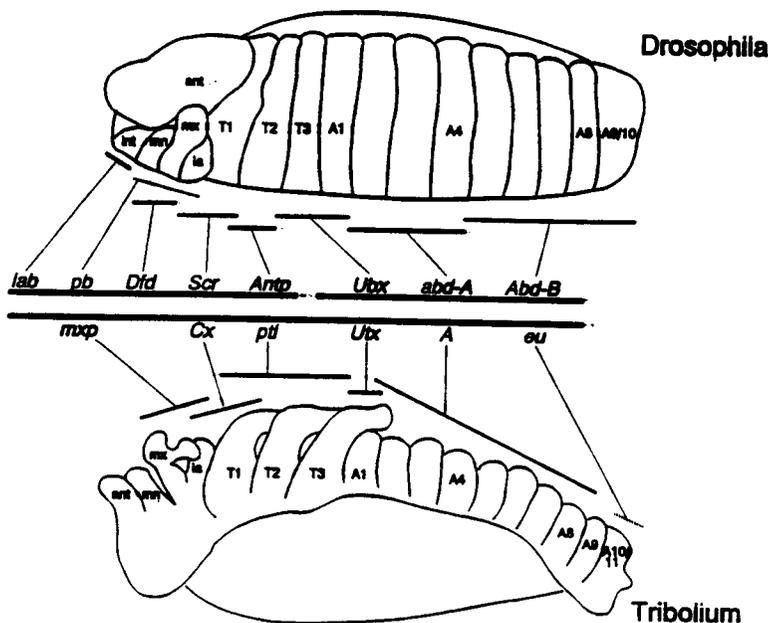


Fig. 1. Maps of homeotic selector genes currently recognized in *Drosophila* and *Tribolium*, and the mutant domains of each gene are indicated with respect to a schematic of an embryo of each insect. In each case, anterior is to the left and the ventral surface faces the maps. The mutant domains of *proboscipedia* (*pb*) and *extra urogomphi* (*eu*) are dotted to indicate that these variants do not affect embryos; *pb* causes adults to be homeotically transformed, whereas *eu* causes abnormal larvae and pupae. Segment abbreviations: ant, antennal; int, intercalary; mn, mandibular; mx, maxillary; la, labial; T, thoracic; A, abdominal.

and 6, comprising the region from the posterior compartment of the second thoracic segment (T2p) to the anterior compartment of the first abdominal segment (A1a). When *Ultrabithorax* function is missing, these parasegments resemble the more anterior PS4 (T1p/T2a)⁽⁹⁾. On the other hand, when *Ultrabithorax* functions are expressed ectopically in a more anterior domain, a posteriorly directed transformation results⁽¹⁰⁾.

The BX-C comprises the genes *Ultrabithorax* (*Ubx*), *abdominal-A* (*abd-A*) and *Abdominal-B* (*Abd-B*). Each is itself a very large transcription unit, but in addition each is associated with large and complex *cis*-regulatory regions. The ANT-C includes five homeotic selector genes: *labial* (*lab*), *proboscipedia* (*pb*), *Deformed* (*Dfd*), *Sex combs reduced* (*Scr*) and *Antennapedia* (*Antp*). It differs from the BX-C in including other genes (not shown in Fig. 1) that are not homeotic in nature⁽⁷⁾. Two of the best described are *bicoid*, which functions maternally to establish anterior positional information in the early embryo, and *fushi tarazu*, which is required for correct segmentation. Several features about the organization and function of the ANT-C suggest that important changes have occurred concomitant with the evolution of long germ development and advanced anterior morphology. Firstly, non-homeotic genes have not been reported in the complexes of any other taxon, and may have arisen during the evolution of the higher Diptera. It will be interesting to assess the phylogenetic distribution of such genes as *bicoid* and *fushi tarazu*. Secondly, despite the prevalent view that the ancestral function of homeotic selector genes among insects is in the establishment of embryonic developmental commitments, loss-of-function mutations of three of these genes (*lab*, *pb* and *Dfd*) do not result in recognizable larval homeotic phenotypes. Rather, such abnormal phenotypes are restricted to adults. Again, a reasonable working hypothesis is that their ancestral embryonic func-

tions have been altered during the evolution of a largely headless maggot.

The Homeotic Complex of the Beetle, *Tribolium castaneum*

Our approach to the problem of homeotic gene evolution has been to develop a sophisticated genetic capability in an insect only distantly related to *Drosophila*, thus allowing high resolution genetic studies in concert with molecular and developmental approaches. This approach was made possible by the existence of several spontaneous homeotic mutations in *Tribolium*. Beetles and flies first appeared in the fossil record in the Permian and Triassic, respectively, suggesting that these orders separated over 250 million years ago⁽¹¹⁾. Initially, we performed linkage and recombinational analyses of the existing spontaneous homeotic mutations in the beetle, and showed that most of them are clustered on the second linkage group and show colinearity⁽¹²⁾. We proposed that this single complex thus defined (the Homeotic complex or HOM-C) represents the juxtaposed homologs of the ANT-C and BX-C of *Drosophila*, and that a single complex is ancestral. As noted above, more recent comparative molecular experiments have confirmed this idea, and the term HOM-C is now commonly used for complexes with such an ancestral organization.

Our first goal was to use the few existing spontaneous recessive homeotic mutations to isolate new ones which fail to complement them and/or have dominant phenotypes. This effort has led to the isolation of approximately eighty homeotic variants mapping to the HOM-C (reference 13 and unpublished results). At the same time, we developed good crossover-suppressing balancer chromosomes that enabled us to maintain recessive lethal HOM-C mutations as stocks and permitted unambiguous complementation analysis. Thus far, genetic evidence has revealed the presence of six

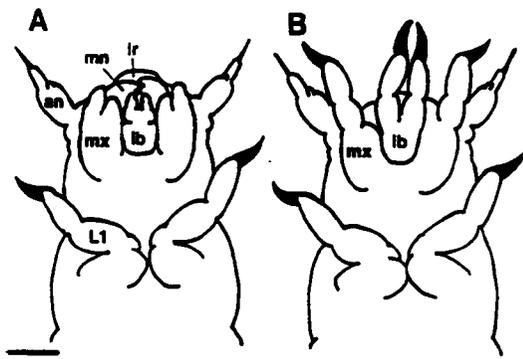


Fig. 2. Schematic representation of terminal phenotype of a lethal *maxillopedia* (*mxp*) loss-of-function mutation. (A) Ventral view of the head and T1 of a wild-type first instar larva. (B) Same of homozygous *mxp*^{Vs-1}. Note transformation of labial and maxillary palps into legs. an, antenna; mn, mandible; lr, labrum; mx, maxillary palp; lb, labial palp; L1, T1 leg. Tarsal claws are indicated with black shading. Bar in A, 0.05 mm.

homeotic genes in the HOM-C. These are *maxillopedia* (*mxp*), *Cephalothorax* (*Cx*), *prothoraxless* (*ptl*), *Ultrathorax* (*Utx*), *Abdominal* (*A*) and *extra urogomphi* (*eu*). They are depicted in Fig. 1 beneath their known or suspected *Drosophila* homologs, and their domains of mutant abnormalities (see below) are indicated as well. Probable nonfunctional (null) alleles of at least the first five are lethal. Probable null alleles of *eu* have not yet been isolated, although we have detected radiation-induced, dominant sterile *eu*-like variants. We have also molecularly cloned and at least partially characterized the *Tribolium* homologs of all of the *Drosophila* homeotic selector genes except *Scr*, although we will not emphasize molecular comparisons here. Rather, we will discuss the implications of their mutant phenotypes to our understanding of the evolution of the homeotic selector genes in the insects.

maxillopedia

This gene appears to be homologous to the *proboscipedia* gene of *Drosophila*. In adult flies bearing null *pb* mutations, derivatives of the gnathal segments develop thoracic structures, including a transformation of labial palps to leg; hypomorphic mutations cause a palp-to-antenna transformation⁽⁷⁾. Surprisingly, normal *pb* function is completely dispensable with respect to larval development in flies. In beetles, the original, homozygous viable *mxp* mutation causes a variable transformation of the adult maxillary and labial appendages to legs. This allele appears hypomorphic, and homozygotes or transheterozygotes bearing any of a number of radiation-induced alleles die as first instar larvae displaying the same homeotic transformation (Fig. 2). Probable dominant gain-of-function *mxp* alleles transform antennae and/or legs to resemble maxillary or labial palps. The gain-of-function nature of one variant, *maxillopedia-Stumpy*, has been demonstrated by its failure to be complemented by a duplication as well as the observation that its dominant phenotype is eliminated by apparent knockout mutations. In contrast to relatively high frequency of gain-of-function *mxp* alleles encountered in beetles, no such *pb* alleles are known in *Drosophila*. Since motifs important to *pb* transcriptional control are included in the first intron⁽¹⁴⁾, it may be that chromosomal rearrangements which might bring such regions under altered regulatory control are invariably associated with loss-of-function, implying that this regulatory organization may not pertain in *Tribolium* (T. Kaufman, personal communication). The likelihood that *pb* and *mxp* are homologous is consistent with the idea that a function in larval development is primitive, and has been lost in the *Drosophila* lineage.

Cephalothorax

This gene appears functionally similar to the *Drosophila* gene *Sex combs reduced*, being required for normal development of the first thoracic segment (T1) and the posterior head. *Scr*

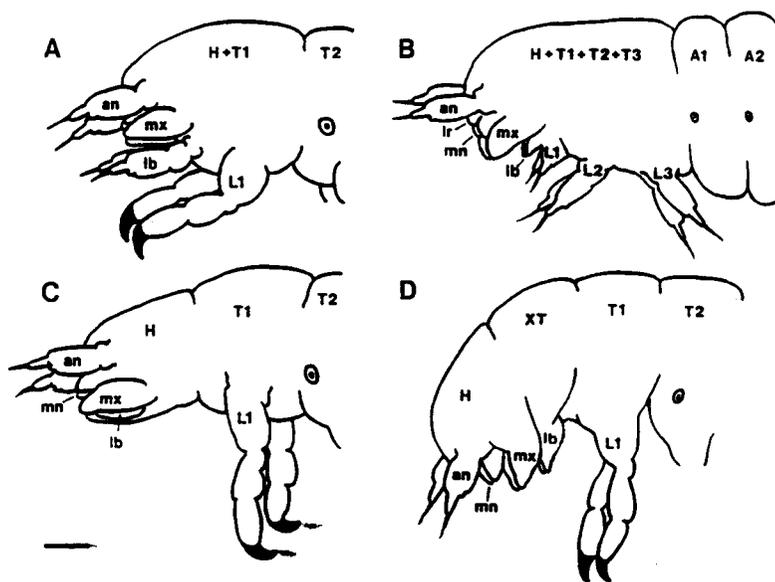


Fig. 3. Terminal phenotypes of lethal *Cephalothorax* (*Cx*) and *prothoraxless* (*ptl*) loss-of-function mutations. (A) Lateral view of head (H) and T1 and T2 of a first instar *Cx*^c homozygote. Note fusion of head and T1 into a single cephalothorax, and transformation of the labial palps (lb) into antennae (an). Other abbreviations same as Fig. 2. (B) Lateral view of head through A2 of a first instar *ptl*^{D60} homozygote. Note extensive fusion of head with entire thorax, absence of T2 spiracle, and transformation of all three pairs of thoracic legs into antennae. (C) Lateral view of head through T2 of wild-type first instar larva. (D) Same view of first instar *Cx*^l homozygote. Note the appearance of the dorsal portion of a super-numerary thoracic segment (XT) between the head and T1 at a position corresponding to the apparent dorsal labium. Note also the contrast between the phenotypes of *Cx*^l and its allele *Cx*^c (panel A). Bar in C, 0.05 mm.

null individuals die as embryos and show a bidirectional transformation of labium-to-maxilla (anteriorward) and T1-to-T2 (posteriorward)⁽⁷⁾. In beetles, *Cx* larvae often hatch from the egg but soon die, apparently without feeding. There are two classes of such lethal alleles (reference 13 and unpublished observations). The first transforms labial palps into antennae and simultaneously causes cephalization of T1, so that the apparent head is actually a cephalothorax consisting of the head and T1, with the T1 legs assuming an orientation characteristic of mouthparts (Fig. 3). When this type of *Cx* allele is heteroallelic with the recessive hypomorphic allele *Cx^{apt}*, the resulting adult beetles show a dramatic transformation of T1 towards T2, including well-developed supernumerary elytra (wing covers). The second type of *Cx* mutation causes a labium-to-maxilla transformation, but T1 is not cephalized. Instead, the dorsal portion of a supernumerary T1 segment develops between the head and normal T1 (Fig. 3). This type of allele does not produce a dramatic T1-to-T2 transformation when heteroallelic with *apt*, although the pronotum is reduced. Mixed or intermediate *Cx* mutant alleles also exist. The first class of lethal allele described displays the same phenotype in the homozygous and hemi-zygous condition, and may represent nulls.

prothoraxless

This gene is the apparent homolog of the *Drosophila* gene *Antennapedia*⁽¹⁵⁾. *Antp* mutations cause different homeotic transformations in larvae and adults⁽⁷⁾. Lethal embryos bearing loss-of-function *Antp* mutations express partial transformations of parasegments 4 and 5 to resemble PS3 (LABIUMp/T1a), although clones of *Antp*⁻ cells in the adult T2 leg develop as antennae. In *Tribolium*, larvae homozygous or hemizygous for putative loss-of-function *ptl* alleles display transformations of all three pairs of thoracic legs to antennae, associated with a dramatic reduction in the size of the dorsal thorax as well (Fig. 3). The *ptl* gene appears haplo-insufficient, and in adults heterozygous for a probable null allele there is a variable reduction of T1. Further, adults heterozygous for a hypomorphic and a putative null allele display fusion of a rudimentary ventral T1 with the labium. We interpret this adult phenotype as a partial transformation of T1 to posterior labium, which is normally rather vestigial in *Tribolium* adults. We speculate that the thorax-to-antennal transformation seen in beetle larvae and adult fly clones represents the primitive function of the insect *Antennapedia* gene, whereas the transformations to more proximate structures seen in beetle adults and fly larvae are adaptations which arose during the evolution of each insect order. While *Cx* and *ptl* represent two distinct lethal complementation groups, their mutations show complex interactions, suggesting a closer functional or regulative interdependence than is known for their putative *Drosophila* homologs *Scr* and *Antp*.

Ultrathorax

This gene is the likely homolog of the *Drosophila* *Ultrathorax* gene, since both are required for the normal development of T3 and A1^(8,13). Embryos homozygous for *Utx* variants die before hatching, and the pharate larvae show a transformation of A1 to resemble T3 (Fig. 4). It is presently

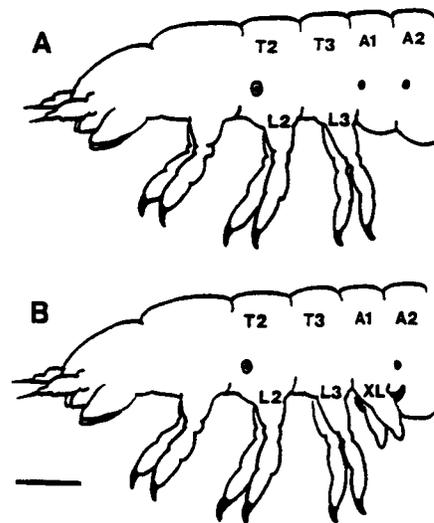


Fig. 4. Terminal phenotype of a lethal *Ultrathorax* (*Utx*) loss-of-function mutation. (A) Lateral view of head through A2 of wild-type first instar larva, showing abdominal spiracles. (B) Same view of lethal (unhatched) *Utx*⁻¹ homozygote, showing extra pair of leg-like appendages (XL) on A1. Note the well-developed tarsal claws and their non-terminal location on these appendages, as well as the absence of the A1 spiracle. Bar in B, 0.1 mm.

unclear whether this homeotic transformation is parasegmental.

Abdominal

Molecular evidence shows that this gene is the homolog of *abdominal-A* of the BX-C⁽¹⁶⁾. *Drosophila* embryos homozygous for *abd-A* mutations show transformations of parasegments in the anterior abdomen to resemble PS6 (T3p/A1a), whereas parasegmental identities in the posterior abdomen depend primarily on the *Abdominal-B* gene⁽⁸⁾. Although many mutant alleles of the *Tribolium* *Abdominal* (*A*) gene have been isolated, we have been able to distinguish between gain-of-function and loss-of-function variants by dosage analysis using an available duplication and deficiency and by so-called 'reversion' analysis, in which gain-of-function dominant phenotypes are reverted by mutational events causing loss-of-function of the gene. Several loss-of-function alleles also have molecular rearrangements which should render their products nonfunctional. Comparisons of the loss-of-function phenotypes of *Tribolium* *A* and *Drosophila* *abd-A* variants reveal clear similarities but also some remarkable differences. *Tribolium* embryos homozygous for *A* null alleles show transformations of parasegments throughout the abdomen to resemble PS6 (Fig. 5). Although observations on morphology and domains of gene expression have argued for the general significance of parasegmental organization among insects or even arthropods⁽¹⁷⁻¹⁹⁾, this represents the first direct evidence for the functional importance of such expression patterns. It should be emphasized that beetle and fly larvae differ dramatically with respect to the morphology of PS6, so the developmental pathways under control must differ considerably despite the conservation of regulatory

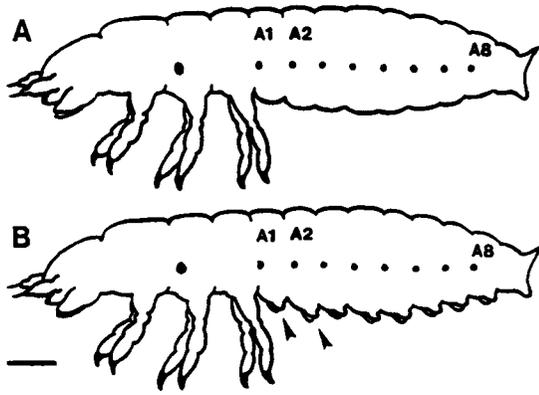


Fig. 5. Terminal phenotype of a lethal *Abdominal (A)* loss-of-function mutation. (A) Lateral view of wild-type first instar larva. (B) Same view of lethal (unhatched) *A1* homozygote, which shows a reiteration of the normal parasegment 6 comprising T3p/A1a. The posterior protuberances are interpreted as the posterior compartment of the T3 leg. Not shown is a reiteration of the anterior portion of the pleuropodium, a glandular organ normally unique to A1. Bar in B, 0.1 mm.

domains in these two insects. Moreover, the region of the abdomen in which this gene is important extends much further toward the posterior in beetles than in flies. In fact, Akam et al.⁽²⁰⁾ have proposed that among ancestral insects, an *abdominal-A* homolog specified determinative decisions throughout the entire abdomen and an *Abdominal-B* homolog was important for a post-abdomen or tail region, and further that the *Abd-B* homolog has a co-opted role in the posterior abdomen in the lineage leading to *Drosophila*. Our observations on the role of the *abd-A* homolog in beetles are largely consistent with this scenario, and raise interesting questions about the evolution of regulatory mechanisms in this region. One possibility, which should be directly testable, is that *cis*-regulatory elements controlling parasegment-specific homeotic gene expression are conserved in these two insects, but that elements which regulate the *Abdominal-B* homolog in the posterior abdomen of less advanced insects evolved in the *Drosophila* lineage to control *abdominal-A* in the same domains⁽¹⁶⁾.

extra urogomphi

This gene is presently identified only by a recessive, incompletely penetrant variant associated with an A10-to-A9 transformation in pupae⁽¹³⁾. The hypothesis that *eu* is the *Abd-B* homolog, based on its phenotype and position within the HOM-C, makes the recovery of null mutations and assessment of its domain of functional significance an interesting prospect.

Df(HOM-C)

We have isolated a deficiency of a considerable portion of the Homeotic complex which arises spontaneously at a relatively high frequency by an apparent exchange between two mutations associated with chromosomal rearrangements⁽²¹⁾. *Df(HOM-C)* deletes at least the interval including *Cx* and *A*,

but does not extend as far as *mxp*. Homozygous lethal embryos display a spectacular mutant phenotype in which all trunk and gnathal segments are homeotically transformed to develop antennae. This phenotype can reasonably be explained by the mutant phenotypes described above and extrapolation from *Drosophila*. That is, deletion of *Utx* and *A* would be expected to transform abdominal segments to resemble thorax, whereas deletion of *Cx* and the *unformed* homolog (known by molecular evidence to be *unformed*) might reasonably cause the gnathal segments to resemble thorax as well. Since normal *ptl* function is necessary for thoracic development, with an antennal identity occurring in its absence, the additional deletion of this gene could lead to antennal development throughout the gnathal and trunk region. We have further speculated that the ancestral function of the *Antennapedia (prothoraxless)* gene was to specify trunk, with head as a default state⁽²¹⁾, and that the Homeotic complex then arose by duplication and divergence of function as has been suggested in many contexts. The specific reiteration of the antennal segment presumably reflects the effects of HOM-C genes not deleted and probably others outside of the complex⁽⁶⁾. One additional interesting point is that if *Tribolium* were to have a *fushi tarazu* homolog in an equivalent position in the complex, it would be deleted in *Df(HOM-C)* embryos. Nevertheless, there is no indication of segmentation defects in such zygotes. This observation suggests that no *fushi tarazu* homolog exists in the HOM-C, or that it is present in the complex but does not play a role in segmentation. In *Drosophila*, *fushi tarazu* also has a role in neural development. Our result suggests that this function could be ancestral, and that an additional function in segmentation may have been acquired during the evolution of long germ behavior⁽²²⁾.

Conclusions

We feel that evidence is rapidly accumulating that the functions of the *Drosophila* homeotic selector genes (and segmentation genes as well^(16,23)) in many ways reflect advanced aspects of early fly development. Thus, comparative studies utilizing other insects offer an opportunity to trace the evolution of regulatory mechanisms important to establishing the embryonic body plan. Although a phylogenetically broad perspective will be crucial to this objective, the study of an insect offering the possibility of genetic as well as developmental and molecular approaches is particularly powerful. We have tried to emphasize here the utility of *Tribolium* in this context. In the example of the beetle *abdominal-A* homolog, the availability of mutations has allowed a demonstration of the functional significance of parasegmental expression in a non-*Drosophilid* insect, and provided evidence for dramatic differences between insect orders in abdominal homeotic selector gene function and regulation. Although other *Tribolium* homeotic selector genes have not been genetically characterized as thoroughly, we have provided many other examples of likely fundamental differences from *Drosophila*. The evidence suggesting that the primitive function of *Antennapedia* was in establishing a trunk rather than head identity may prove particularly

important. Further developmental genetic and molecular studies of beetle homeotic and segmentation genes should provide exciting insights into the evolution of mechanisms which establish embryonic organization in insects.

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