review article

A gene complex controlling segmentation in *Drosophila*

E. B. Lewis
Division of Biology, California Institute of Technology, Pasadena, California 91125

The bithorax gene complex in Drosophila *contains a minimum of eight genes that seem to code for substances controlling levels of thoracic and abdominal development. The state of repression of at least four of these genes is controlled by* cis*-regulatory elements and a separate locus (Polycomb) seems to code for a repressor of the complex. The wild-type and mutant segmentation patterns are consistent with an antero-posterior gradient in repressor concentration along the embryo and a proximo-distal gradient along the chromosome in the affinities for repressor of each gene's* cis*-regulatory element.*

THE segmentation pattern of the fly provides a model system for studying how genes control development. The phylogeny, although not the ontogeny, of that pattern is reasonably well understood. Flies almost certainly evolved from insects with four wings instead of two and insects are believed to have come from arthropod forms with many legs instead of six. During the evolution of the fly, two major groups of genes must have evolved: 'leg-suppressing' genes which removed legs from abdominal segments of millipede-like ancestors followed by 'haltere-promoting' genes which suppressed the second pair of wings of four-winged ancestors. If evolution indeed proceeded in this way, then mutations in the latter group of genes should produce four-winged flies and mutations in the former group, flies with extra legs. In *Drosophila*, not only have both types of mutation been observed, they have been shown to involve a single cluster of pseudoallelic genes known as the bithorax complex (BX-C)[1-7]. During evolution a tandem array of redundant genes presumably diversified by mutation to produce this complex[1,4]. During development the BX-C genes seem, on the basis of evidence to be summarised in this article, to control much of the diversification of the organism's thoracic and abdominal segments. It is as if during ontogeny the BX-C genes recapitulate their own phylogeny.

Each of the wild-type thoracic and abdominal segments has a unique pattern of differentiated structures (Fig. 1*a*) which constitutes a morphologically defined state or 'level of development' (L). Each BX-C mutant phenotype can be described in terms of the degree to which one or more segments, or portions thereof, are transformed from one such level to another. As shown in Fig. 1*b* a striking feature is that the metathoracic (MT) and first three abdominal segments (AB1, AB2 and AB3) can approach or achieve almost all segmental levels of development from a mesothoracic level (LMS), or 'primitive' level[1], to a third abdominal level (LAB3), depending upon the genotype. The wild-type allele of each BX-C gene (Fig. 2) will be assumed to code for a BX-C substance (S) which controls one or more components of an intersegmental transformation (Table 1). The various BX-C substances are presumed to act indirectly by repressing or activating other sets of genes which then directly determine the specific structures and functions that characterise a given segment. On such a model the level of development which a segment achieves depends upon the particular array of BX-C substances elaborated in that segment. When the wild-type and mutant phenotypes are interpreted in accordance with this model, it can be seen from Fig. 1*b* that the attainment of any level more

Table 1 Summary of the roles of BX-C substances (S) in controlling specific types of body segment transformations and specific structures in one or more segments of the larva

Gene	Substance	Segmental transformation	Larval structures affected	Genotypic comparisons (from Fig. 6)
bx$^+$	S_1*	LAMS→LAMT		
pbx$^+$	S_2	LPMS→LPMT		
Ubx$^+$	S_0*	LMS→LMT	DLT	*a* vs *b* *f* vs *h*
bxd$^+$	S_3	LMS→LAB1	DLT†, VP, KO, VSB	*g* vs *h*
iab-2$^+$	S_4	LMS→LAB2	DLT, KO, VSB	*b* vs *c* *c* vs *h*
iab-3$^+$	S_5	LAB2 or LMS→LAB3		*e* vs *f*
iab-5$^+$	S_7	LAB4 or LMS→LAB5		
iab-8$^+$	S_x	LAB7 or LMS→LAB8	DLT, PSP, CP, VP‡, KO‡	*a* vs *d*

* Substance, S_1, was originally postulated to be coded for by either *bx*$^+$ or *Ubx*$^+$ (ref. 4); in this article S_1 is assigned to *bx*$^+$ and S_0 to *Ubx*$^+$.
† Comparison of genotype *e* and *f* indicates that *bxd*$^+$ and/or *iab-2*$^+$ may be responsible for the continuity of dorsal tracheal trunk in AB1.
‡ Suppression of VP and KO may result from the presence of *iab-8*$^+$ and/or one or more *iab* genes located between *iab-3* and *iab-8*.

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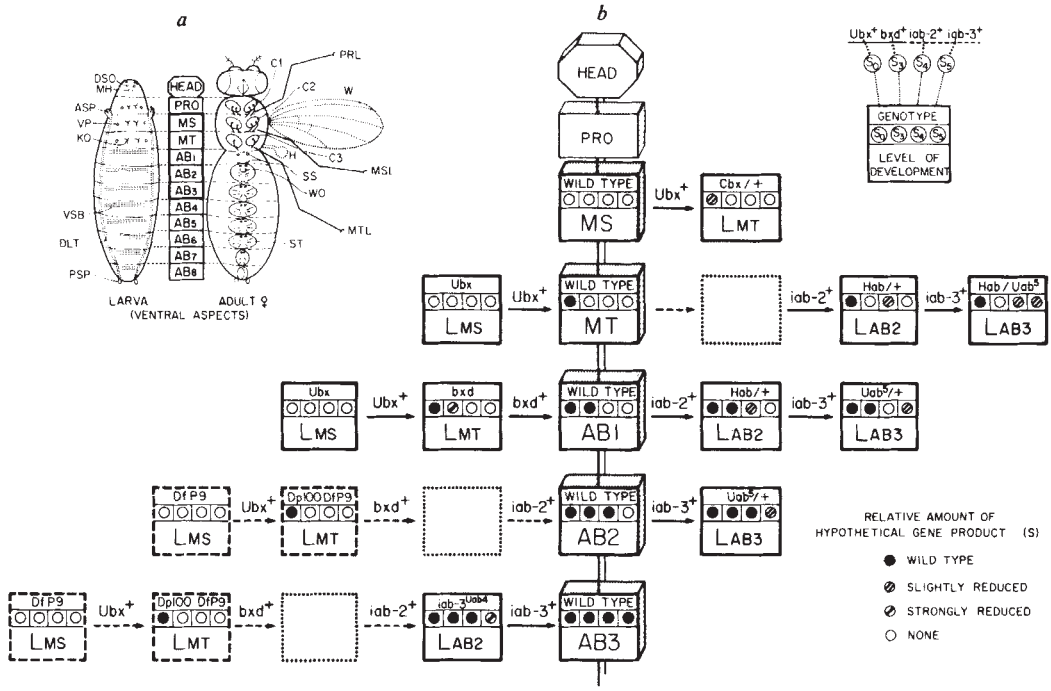

Fig. 1 Genetic control of segmental levels of development in thoracic and abdominal segments. *a*, Anatomy of third instar larva. Head: dorsal sense organs (DSO) and protruding portion of mandibular hooks (MH). Thoracic segments: each bears a pair of Keilin's organs[13] (KO), three sensory hairs in a depression; ventral pits (VP) and ventral setal bands (VSB) with fine tooth-like setae. Abdominal segments: VSB with coarse tooth-like setae; KO and VP lacking. Each dorsal longitudinal trunk (DLT) terminates in an anterior (ASP) and posterior (PSP) spiracle. Anatomy of adult female. Thoracic segments: coxae (C1, C2 and C3) of prothoracic (PRL), mesothoracic (MSL) and metathoracic (MTL) legs; wing (W) and haltere (H), being dorsal, are shown in dotted outline. Abdominal segments: AB1 to AB7 bear sternital sensillae (SS); AB2 to AB7 bear bristled sternites (ST); AB2 has a raised lump, or Wheeler's organ[10] (WO). *b*. Levels of development (L), achieved in wild-type segments (vertical array of connected boxes) and approached in mutant segments, are depicted in panels containing genotype, phenotype and status with respect to relative amounts of hypothetical BX-C substances, S_0, S_3, S_4 and S_5, products of the wild-type alleles of Ultrabithorax (*Ubx*), bithoraxoid (*bxd*), infra-abdominal-2 (*iab-2*) and infra-abdominal-3 (*iab-3*). Panels to the left of wild type depict hemizygous genotypes for hypomorphic or amorphic mutants, the designated chromosome being opposite *Df-P9* (see Fig. 2) in all cases. For simplicity, a slight transformation of the posterior region of AB1 towards LMS in the *bxd* hemizygote is ignored. Panels to the right of wild type involve heterozygotes for hypermorphic (dominant-constitutive) mutants, Contrabithorax (*Cbx*), Hyper-abdominal (*Hab*) and Ultra-abdominal-5 (Uab^5). Panels in dotted outline represent theoretically possible LAB1 transformations for which definitive mutant genotypes are not available. Panels in dashed outline are genotypes dying in the first instar. All other genotypes shown survive to the adult stage except *Ubx* hemizygotes which die in either third instar or early pupal stages.

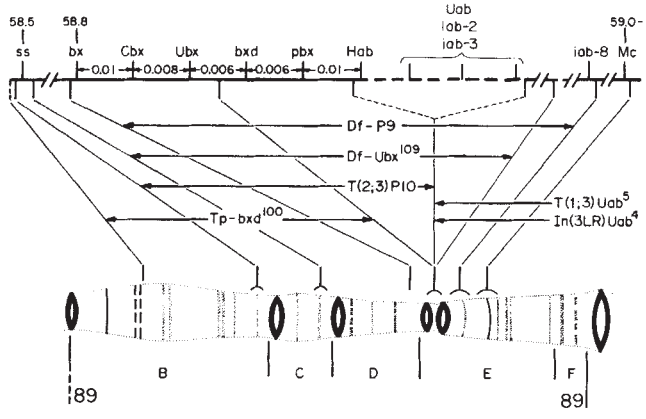

Fig. 2 Correlation of the linkage and salivary gland chromosome maps of BX-C and immediately surrounding regions in the right arm of the third chromosome. The solid line portion of the linkage map is based on half-tetrad[6], as well as on conventional, linkage analysis. The dashed line portion is based on unpublished cytogenetic studies of the designated rearrangements, the relative order of *Hab*, *Uab*, *iab-2* and *iab-3* with respect to each other being uncertain. The complex spans approximately 0.05 to 0.1 centimorgan and lies within the region of two heavy doublet structures and possibly an adjoining faint band of section 89E. Not shown is a spontaneous dominant mutant, Miscadestral pigmentation (*Mcp*), recently found by M. Crosby, which maps between *Hab* and Microcephalus (*Mc*)[15]. *DF-P9* in its interactions with BX-C mutants and rearrangements acts as if it is deleted for all of the BX-C genes. *Df-P9/+* has reduced male pigmentation in AB5 and AB6, is sterile, and has deformed genitalia in both sexes, which on the basis of other interactions and of larval studies (Fig. 6*d*) behaves as a deficiency extending from *bx* to *iab-3*, associated with a weakened dominance of genes to the right of *iab-3*. *Mc* is apparently a very small tandem duplication which partially restores fertility when *trans* to *Df-P9* but does not otherwise modify the *Df-P9/+* phenotype. *T(2; 3)P10* is an insertional translocation of the designated region of 89 to section 29C of chromosome 2; the breakage point in 89E is likely on genetic grounds to lie between *pbx* and *iab-2* and to be accompanied by polar position effects that weaken the dominance of $iab\text{-}2^+$ and $iab\text{-}3^+$. $Tp(3)bxd^{100}$ is a transposition of the designated 89 region to 66C of chromosome 3L; the breakage point lies between *Ubx* and *bxd* and is accompanied by a weakened dominance of bdx^+ and pbx^+ (ref. 1) but not, as far as can be detected, of genes to the right of *pbx*. $T(1; 3)Uab^5$ is a reciprocal translocation with a breakage point in section 1E of the X chromosome; a synthetic deletion analysis indicates that the Uab^5 phenotype is associated with the distal portion of the translocation; that is, the breakage point in 89E lies to the left of Uab^5 and falls either to the left or right of $iab\text{-}2^+$. $In(3LR)Uab^4$ is a complex inversion with additional breaks in 80C and in or near 85A or 3R. Analysis of X-ray induced revertants ot Uab^4 indicates that the breakage point in 89E probably lies just to the left or right of *iab-2* and is associated with a weakened dominance of $iab\text{-}3^+$ (designated in Fig. 1 $iab\text{-}3^{Uab4}$) and possibly of $iab\text{-}2^+$, as well. *Hab* was originally designated as the F locus of the complex[7]; and has also appeared in the literature as Contrabithoraxoid (*Cbxd*). The first two *Uab* mutants found, *Uab* and Uab^2, although cytologically apparently normal, are associated with a reduction in crossing over within the complex and are accompanied by position effects or point mutations at other loci within BX-C. Their dominant phenotypes resemble that of Uab^4 much more closely than that of Uab^5. Cellular determination of *Uab* has been extensively studied by Kiger and Davis[20,21].

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advanced than LMS is a stepwise process in which each step requires the presence of a specific BX-C substance. In keeping with the model, all of the genotypes involving recessive loss of function lie to the left or LMS side of the wild-type segments while those involving dominant gain of function lie to the right or LAB3 side of those segments.

Segment MT transforms towards LMS even in larval stages of homozygotes[1] and hemizygotes for Ultrabithorax (*Ubx*). The most prominent feature is the occurrence of an extra set of spiracles on MT (and also AB1) (Fig. 3*a*) compared to the usual single set on MS. Although such animals die in late larval or early pupal stages, adult *Ubx/Ubx* cuticular tissue in MT has been shown by somatic mosaic analysis to transform autonomously towards LMS[4,8]. A substance, S_0, effecting LMS→LMT, will be assumed to be the Ubx^+ product. The inability of MT to achieve LMT in *Ubx* hemizygotes or homozygotes is then consistent with the expected reduction in amount of S_0 in that segment. The adult MT cuticle, but not the larval tracheal system, strongly transforms toward LMS in a double mutant combination involving bithorax-3 (bx^3) and postbithorax (*pbx*), notably producing a four-winged fly[5,8]. Substances S_1 and S_2 are assumed to be the products of bx^+ and pbx^+, respectively (Table 1), and to be involved in effecting LMS→LMT; however, for the sake of simplicity, they are omitted from Fig. 1*b*. The wild-type phenotype, on these assumptions, corresponds to that expected if bx^+, Ubx^+ and pbx^+ are repressed in MS and derepressed in MT.

Segment MS takes on MT characteristics in flies heterozygous or homozygous for Contrabithorax (*Cbx*), the most conspicuous effect being a partial conversion of wings into halteres[5,9]. *Cbx* is known to regulate Ubx^+ in a *cis*-dominant fashion; that is, MS transforms towards LMT in *Cbx* + / + *Ubx* but is virtually wild type in *Cbx Ubx*/ + +[2,5]. *Cbx* presumably damages a regulatory element adjacent to Ubx^+ causing S_0 to be made constitutively in MS but in a relatively reduced amount compared with that made in MT when Ubx^+ is in the wild-type configuration.

Segment AB1 transforms primarily towards LMT in hemizygotes for bithoraxoid (*bxd*), producing flies with one or two MT-like legs on AB1, and on rare occasions a tiny haltere-wing as well[5]. This phenotype has been accounted for by postulating that bxd^+ codes for a substance, S_3, effecting LMS→LAB1[4]. Evidence to be represented later suggests that Ubx^+ is derepressed not only in MT but also in AB1 and segments beyond. Hence, in the *bxd* hemizogote, there is presumably enough S_0 in AB1 to transform that segment towards LMT. That AB1 remains at LMS in the *Ubx* hemizygote (Fig. 3*a*) is expected since *Ubx* is known to exert a strong polar effect on bxd^+ (ref. 1); that is, neither S_0 nor S_3 is expected to be made in sufficient amounts to transform AB1 appreciably towards LMT or LAB1. In wild type, S_0 as well as S_3 is presumably being produced in AB1; although S_3 function may override that of S_0, a more attractive possibility is that LAB1 actually corresponds to a mosaic of structures and functions controlled jointly by S_0 and S_3.

Segments MT and AB1 approach LAB2 in heterozygotes for a second type of dominant regulatory mutant, Hyperabdominal (*Hab*), producing flies with missing halteres and/or MT legs and with an AB2-type of sternite pattern[10,11] on MT and AB1 (Fig. 4*b*). These effects are variably expressed within a fly even though the penetrance of *Hab*/+ exceeds 99% in certain backgrounds.

Cis-trans studies involving all possible double mutant combinations between *Hab* and bx^3, *Ubx*, *bxd* and *pbx* indicate that *Hab* does not derepress the wild-type alleles of any of these genes. *Hab* will be assumed to cause partial derepression of a wild-type infra-abdominal-2 ($iab\text{-}2^+$) gene, whose coding product, S_4, effects LMS→LAB2. On this assumption, the wild-type phenotype is that expected if $iab\text{-}2^+$ is repressed in MT and AB1 and derepressed in AB2. Similarly, the *Hab*/+ phenotype is that expected if $iab\text{-}2^+$ is partially derepressed in MT and AB1 and fully derepressed in AB2.

The failure of MS to be modified in *Hab*/+ requires explanation. The *Hab* mutation presumably damages a regulatory element adjacent to $iab\text{-}2^+$ in such a way as to reduce its affinity for a repressor. If there is an antero-posterior concentration gradient in that repressor, then conceivably the threshold concentration for keeping $iab\text{-}2^+$ repressed in the *Hab* chromosome is exceeded in MS but not in MT and segments beyond MT.

Segments AB1 and AB2 partially transform toward LAB3 in adults heterozygous for Ultra-abdominal-5, Uab^5, a third type of dominant constitutive mutant, inseparable from an X-3 translocation (Fig. 2). The transformation is most easily seen in the ventral regions of AB1 and AB2 which develop sternite patterns approaching those found in AB3 (Fig. 4*c*). The effect on AB2 can be accounted for if Uab^5 derepresses a gene, $iab\text{-}3^+$, which codes for a substance, S_5, effecting LAB2→LAB3. The wild-type pattern then corresponds to that expected if $iab\text{-}3^+$ is repressed in AB1 and AB2, but derepressed in AB3. The penetrance of Uab^5/+ is complete but the transformation of AB1 towards LAB3 is variable with that segment often progressing only towards LAB2. If, as certain larval findings discussed below suggest, $iab\text{-}2^+$ is normally weakly derepressed in AB1, then the presence of S_4 in AB1 even in subthreshold amounts may facilitate the transformation to LAB3 in Uab^5/+. Another possibility is that the Uab^5 rearrangement in some way partially derepresses $iab\text{-}2^+$ as well as $iab\text{-}3^+$; however, although MT transforms to LAB2 in *Hab*/+, MT is not modified in Uab^5/+.

Segment MT is able to approach LAB3, along with AB1 and AB2, in *Hab*/Uab^5 animals (Fig. 4*d*). Transformation to LAB3 in MT thus seems to require two sequential steps: LMS→LAB2→LAB3, the former controlled by S4 and the latter by S_5. The observed transformation of all three segments towards LAB3 is readily accounted for, since both S_4 and S_5 are expected to be produced in MT, AB1 and AB2 of *Hab*/Uab^5.

Additional evidence for an $iab\text{-}3^+$ gene comes from an analysis of another rearrangement-associated *Uab* mutant, Uab^4. When heterozygous, Uab^4 shows a dominant gain of function in which AB1 partially transforms towards LAB2. When hemizygous, or opposite *T(2; 3)P10* (Fig. 2), Uab^4 exhibits a new type of recessive loss of function; namely, the sternites of not only AB1 but AB3 to AB6, inclusive, transform towards LAB2 (Fig. 3*c*). These latter effects are attributed to a reduction in the amount of S_5 which would be expected if the Uab^4 and *T(2; 3)P10* rearrangements are accompanied by position effects on $iab\text{-}3^+$.

Gonads, normally located in AB5, are absent in Uab^4 hemizygotes, suggesting that BX-C genes control mesodermal as well as ectodermal tissues. Gonadal examination of Uab^4 genotypes was prompted by the report of gonadal defects in homozygotes for certain *Uab*-like mutants of the homologous 'E-locus' complex of Bombyx (see review by Tazima[12]).

Segment AB4 transforms strongly towards LAB5, both in pigmentation and bristle patterns in heterozygotes and homozygotes for Miscadestral pigmentation (*Mcp*), a dominant mutant recently discovered by M. Crosby (unpublished). Analysis of X-ray induced revertants indicates that *Mcp* probably derepresses a gene, $iab\text{-}5^+$, which codes for a substance, S_7, effecting either LAB4→LAB5 or LMS→LAB5.

A new dimension has been added to the analysis of BX-C with the discovery that a homozygous deficiency for the entire complex, *Df-P9* (Fig. 2) survives to the late embryonic or early first instar stage and exhibits a transformation of MT and all eight AB segments towards LMS. Pairs of Keilin's organs[13] (KO), ventral pits (VP) and thoracic-type ventral setal bands (VSB) appear on those segments (Fig. 5*a* and 5*b*) and in place of a continuous dorsal longitudinal tracheal trunk (DLT) each segment bears a section of DLT terminating presumably in an incipient anterior spiracle (ASP)[14] (Fig. 5*c*, vs. *d*); finally, the ventral nerve cord tends to retain its primitive embryonic pattern in that it extends to segment AB6 or AB7 instead of foreshortening to segment AB3 or AB4 as in wild type. Tiny

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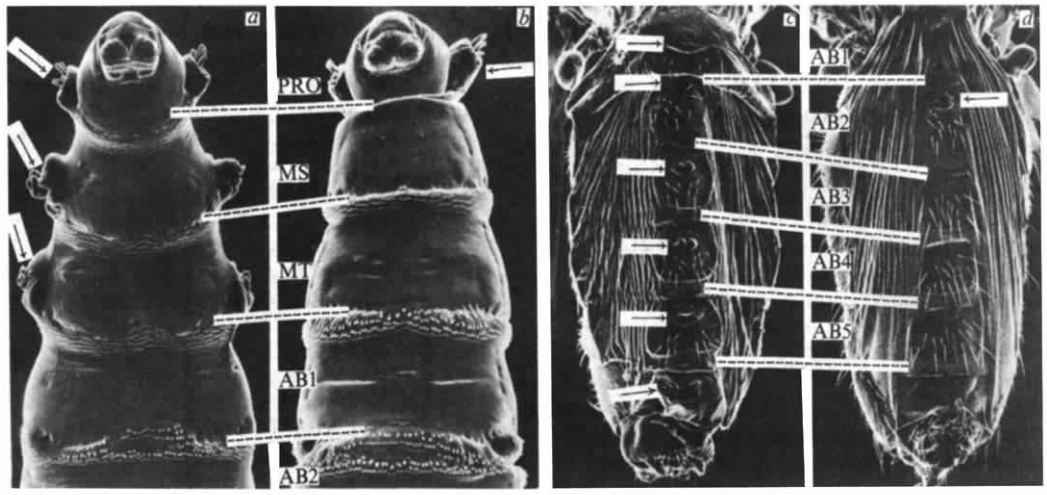

Fig. 3 Mutant phenotypes resulting from recessive loss of function compared with wild type. See Fig. 1(*a*) for legend. *a*, Late third instar larva hemizygous for *Ubx* (*Ubx/Df-P9*). Note extra pairs of anterior spiracles (ASP) (arrows) on MT and AB1, protruding into MS and MT, and thoracic type of ventral setal bands (VSB) on AB1 compared with abdominal type on AB2. *b*, Corresponding stage of homozygous wild-type (Canton-S) larva. Note single pair of ASP (arrow) which arises in MS but protrudes into the region of the prothorax (PRO). Note setae of VSB are coarse in AB1 and AB2, fine in MS, and intermediate in MT. (Specimens *a* and *b* were fixed in hot water and photographed with SEM, X40. *c*, Ventral aspect of male abdomen of genotype *In(3LR)Uab*^{4}*/T(2; 3)P10* (see Fig. 2). Note presence of WO (arrows) and sternital bristle pattern of AB2 type on segments AB1 through AB5. Gonads are rudimentary in specimens of this genotype or lacking entirely in *Uab*^{4}*/Df-P9*. *d*, Corresponding region of adult wild-type (Canton-S) male. Note that AB1 lacks a sternite and that only AB2 (arrow) has WO. (Specimens *c* and *d* were unfixed and photographed with SEM, ×50.)

chitinised plates (CP), resembling rudiments of mandibular hooks (MH), occur in AB8 and imply a head-like rather than LMS transformation of one or more of the embryonic segments that normally fuse to form AB8.

In order to delineate regional differences in morphological functions within BX-C, hemizygotes for deficiencies of portions of BX-C have been constructed. Figure 6 displays stylised phenotypes associated with genotypes hemizygous for five such deficiencies (*b–f*), for *Df-P9* (*a*), for *bxd* (*g*) and for wild type (*h*).

A direct demonstration that *Ubx*$^+$ effects LMS → LMT can be seen by comparing genotypes *a* and *b*, which lack all BX-C genes except that *b* retains one dose of *Ubx*$^+$ (and *bx*$^+$). All nine segments from MT to AB8 remain at LMS in *a* and attain LMT in *b*; that is, in *b* they acquire a pattern of attributes unique to the larval MT (namely, a continuous DLT, VP, KO and thoracic-type VSB). Furthermore *Ubx*$^+$ behaves as if derepressed not only in MT and AB1, but in the remaining abdominal segments as well. That many other BX-C genes share with *Ubx*$^+$ the ability to establish continuity of DLT is evidenced by the presence over several segments of continuous DLT in larval genotypes, *d*, *e* and *f*, which lack *Ubx*$^+$ but have one or more other BX-C genes still present.

All abdominal segments approach, but do not quite achieve, LAB1 in animals of genotype *c*, which is a hemizygote for *bxd*$^+$ (and *pbx*$^+$) as well as *Ubx*$^+$. Such animals are seen to have most of the characteristics of wild-type AB1 (absence of ASP and VP and presence of DLT and VSB, but incomplete suppression of KO). Failure of *bxd*$^+$ to effect a perfect transformation to LAB1 is not understood. One possibility is that *iab-2*$^+$ and *bxd*$^+$ are

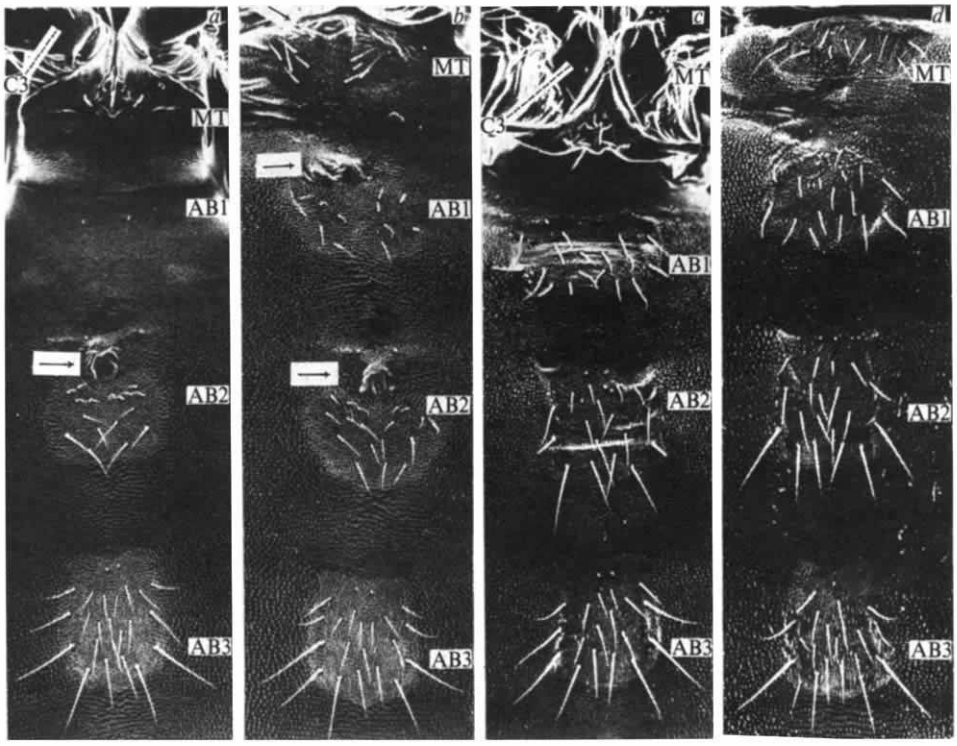

Fig. 4 Mutant phenotypes resulting from dominant gain of function compared with wild type. Mid-ventral cuticular regions of adults female with MT, AB1, AB2 and AB3 approximately parallel to one another. (All specimens unfixed and photographed with SEM.) *a*, Wild type. A portion of coxa-3 (C3) is visible on MT; AB1 lacks a sternite; AB2 has WO (arrow) and a reduced (compared to AB3) sternital bristle pattern, ×80. *b*, *Hab/+*. The specimen had four legs and no halteres. WO (arrows) and bristle pattern on MT and AB1 approach those of Ab2, ×80. *c*, *Uab*^{5}*/+*. C3 resembles that of wild type. AB1 and AB2 sternites lack WO and approach in bristle pattern that of AB3, ×90. *d*, *Hab/Uab*^{5}. The specimen had four legs and no halteres. Sternites on MT, AB1 and AB2 have little or no trace of WO and approach in their bristle patterns that of AB3, ×80.

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both involved in the suppression of KO and that *iab-2*$^+$ is weakly derepressed in AB1 (depicted in Fig. 6*h* by indicating a strongly reduced amount of S_4 in that segment of wild type). Complete suppression of KO can occur in genotypes which lack full *bxd*$^+$ function; namely, *f* and *g*. In such cases, it seems likely that *iab-2*$^+$ and possibly *iab-3*$^+$ are able to bring about suppression of KO when fully derepressed.

Suppression of VP seems to be exclusively controlled by *bxd*$^+$, these organs being absent from abdominal segments only when *bxd*$^+$ is present, as in genotypes *c* and *h*. It is therefore directly evident from *h* that *bxd*$^+$ is normally derepressed not only in AB1, but in all segments posterior to AB1.

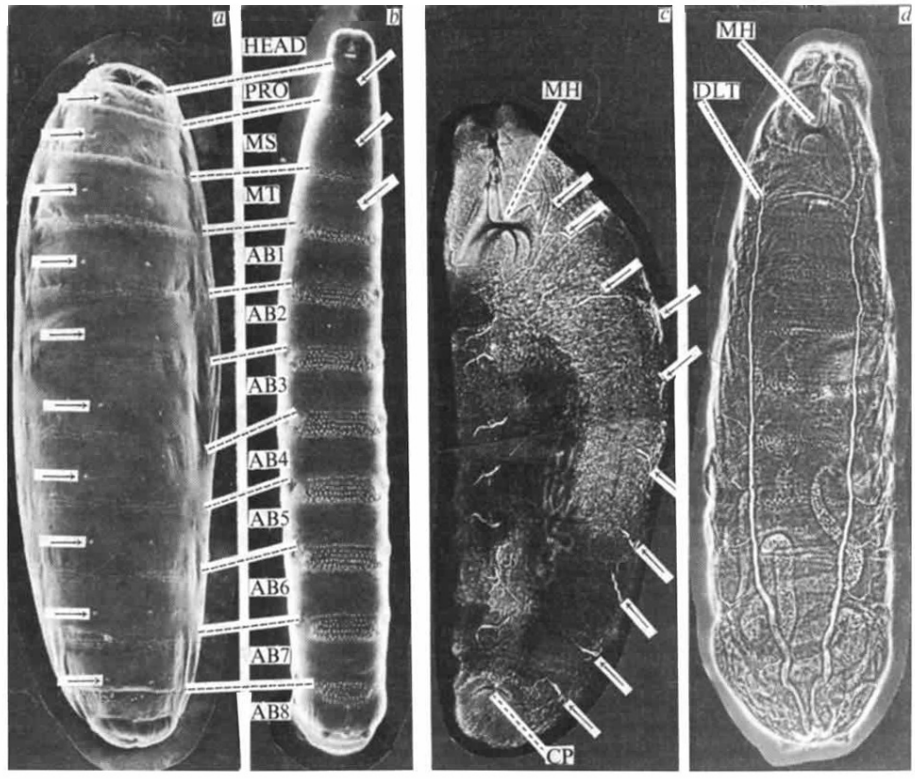

Fig. 5 Comparison of cuticular and tracheal patterns in homozygotes for a deletion of BX-C (*a* and *c*) compared with those of wild type (*b* and *d*). See Fig. 1 for legend. *a*, *Df-P9* homozygote. Animals die within the egg or after partly emerging from egg membranes. Note thoracic-like VSB, KO (arrows) on segments AB1 through AB7, as well as on thoracic segments; AB8 has a reduced VSB. KO not visible in AB8 of specimen shown but are visible in whole mounts examined under phase contrast, ×160. *b*, Wild-type first instar larva. Note more prominent VSB on abdominal vs. thoracic segments, with VSB on MT somewhat intermediate between those on MS and AB1. KO (arrows) are restricted to thoracic segments as are also VPs, which are not resolved at the magnification shown, ×60. (Specimens *a* and *b* were fixed in hot water and photographed with SEM.) *c*, Whole mount of mature embryo of *Df-P9* homozygote. Note separate tracheal sections (arrows) of DLT occur in each segment from MS through AB8, and tiny chitinized plates (CP) in AB8. *d*, Whole mount of wild-type first instar larva. Note continuous DLT. (Specimens *c* and *d* mounted in Zeiss W15 medium and photographed under phase contrast, ×120.)

The larval effects of *iab-2*$^+$ can be indirectly inferred by comparing tracheal and cuticular patterns in AB2 of genotypes *d*, *e* and *f* (Fig. 6), which involve loss, weakened dominance and dominance, respectively, of *iab-2*$^+$. Continuity of DLT is seen in both *e* and *f*, suppression of KO occurs only in *f*, and VSB become progressively more abdomen-like in proceeding from *d* to *e* to *f*. These effects are not necessarily solely due to the *iab-2*$^+$ gene since it is possible that *iab-3*$^+$ is weakly derepressed in AB2 of the larva. Unfortunately, definitive genotypes are not available that would resolve *iab-2*$^+$ and *iab-3*$^+$ larval functions.

A comparison of larvae of genotypes *a* and *d* (Fig. 6) shows that the latter retain the wild-type cuticular pattern in AB8 (except for thoracic-type VSB) and have DLT continuity between AB7 (or AB6) and AB8. These effects are most simply accounted for by assuming that in contrast to *Df-P9*, *Df-Ubx*^{109} still retains an *iab-8*$^+$ gene, whose product, S_x, effects LAB7→LAB8 or LMS→LAB8. The ability of genotype *d* to restore a tracheal trunk in the region from AB6 to AB8 suggests that *Ubx*^{109} retains additional *iab* loci to the right of *iab-3*, besides *iab-8*.

The above findings point to an antero-posterior gradient within the organism in BX-C gene action. A regulatory gene(s) might therefore be expected to exist which, when activated, would derepress or activate BX-C genes in all segments of the body. The properties of Polycomb (*Pc*), a mutant found by P. H. Lewis[15], and of a more extreme allele, *Pc*^{3}, suggest that *Pc*$^+$ (locus 3-47.1)[16] is such a gene. Thus, hemizygotes and homozygotes for *Pc* or *Pc*^{3} have recently been found to have their thoracic and first seven abdominal segments partially transformed towards LAB8. Such animals, which die as late embryos, also have reduced head structures, including poorly developed and incompletely sclerotised MH. As the doses of BX-C are increased from two to three to four, the transformation towards LAB8 becomes increasingly more extreme with many of the abdominal segments containing rudimentary posterior-type spiracles. The LAB8 transformation is not observed unless at least one dose of BX-C is present; that is, *Pc*^{3} *Df-P9* homozygotes closely resemble *Df-P9* homozygotes (Fig. 5) except that the former have reduced head structures and incompletely sclerotised CP in AB8 (suggesting that the latter structures are indeed rudiments of MH). A dependence of BX-C phenotypic effects on BX-C dosage is seen also in *Pc*^{3} heterozygotes: for example, *Cbx*-like effects occur in *Pc*^{3}/+ animals (with two doses of BX-C) and become increasingly more extreme with three and four doses of BX-C. Gene dosage studies of R. Denell (personal communication) indicate that *Pc* represents the inactivated state of the gene. Therefore, *Pc*$^+$ in all likelihood is coding for a repressor of BX-C.

Regulatory rules

The BX-C genes are assumed to control the organism's thoracic and abdominal segmentation pattern by producing substances which in turn regulate other genes that actually determine segmental structure and function. Regulation of the BX-C genes, themselves, seems to be governed by the following rules: (1) state of repression or derepression of a given gene is controlled (in at least four instances) by a *cis*-regulatory element; (2) the genes tend to be individually, rather than coor-

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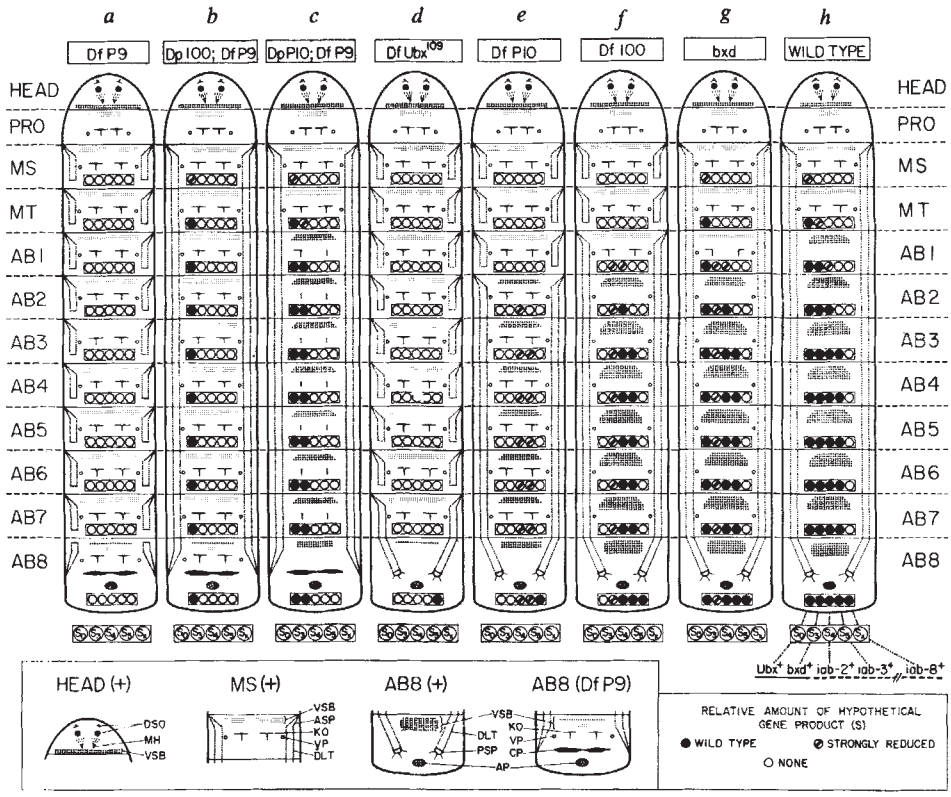

Fig. 6 Stylised phenotypes detectable in first instar larvae or mature embryos. See Fig. 1(*a*) for legend, Fig. 2 for description of rearrangements and Table 1 for roles of postulated BX-C substances (S). All drawings based on whole mounts of animals examined with phase contrast microscopy. Mounting fluid: 9 parts lactic acid to 1 part 95% ethanol for revealing cuticular structures; and Zeiss W15 for tracheal structures. *a*, *Df-P9* homozygote (see also Fig. 5*a* and *c*). *b*, *Df-P9* homozygote containing one dose of *Dp-100*, abbreviated symbol for the duplication derived from *Tp(3)bxd*^{100} (Fig. 2). Note identity to *a* except for presence of DLT. *c*, *Df-P9* homozygote containing one dose of *Dp-P10*, abbreviated symbol for the duplication derived from *T(2; 3)P10* (Fig. 2). Note suppression of VP and partial suppression of KO in segments AB1 to AB7. Although VP and KO appear to be absent in AB8, they may be obscured by CP and other structures in that segment. *d*, *Df-Ubx*^{109}*/Df-P9*. Note DLT between segments AB7 and AB8, and presence of PSP in AB8; and absence of CP in AB8; occasionally DLT also forms between AB6 and AB7. *e*, *Df-P10/Df-P9*. *Df-P10* is an abbreviated symbol for the deficiency derived from *T(2; 3)P10* (Fig. 2). DLT restored in AB2 through AB8. The pair of separate sections of that trunk in AB1 frequently lie in AB2, parallel to DLT in that segment. Note partial restoration of abdominal-type VSB, weakly developed in AB2 and moderately developed in AB3 through AB8. *f*, *Df-100/Df-P9*. *Df-100* is an abbreviated symbol for the deficiency derived from *Tp(3)bxd*^{100} and has moderately weakened dominance of *bxd*$^{+}$ as a position effect of the 89E breakage point. Note retention of DLT and failure of suppression of VP in AB1 through AB7 (with VP status in AB8 uncertain) and full restoration of abdominal type of VSB in all segments from AB2 through AB8. *g*, *bxd/Df-P9*. Although not deleted for any known genes of BX-C this genotype is depicted since it has effects on several larval (as well as adult) cuticular structures. Note failure of suppression of VP on AB1 through AB7; thoracic-type VSB on AB1; and only partial suppression of KO on AB1. *h*, Wild-type homozygote or hemizygote (the latter having possibly somewhat narrower VSB on AB segments than the former) (see Fig. 5*b* and *d*). All individuals of genotype *a* through *f* die in the late embryonic or early first instar stage. ASP are lacking in first instar larvae according to Bodenstein[14]. Separate sections of DLT send out a branch to the cuticle but no anterior spiracles as such have been resolved. A number of larval phenotypic expressions are not readily explained unless it is assumed that there is weak derepression of *Ubx*$^{+}$ (to establish continuity of DLT between MS and MT), *bxd*$^{+}$ (to make very slightly larger VSB teeth in MT than MS) and *iab-2*$^{+}$ (to suppress KO in AB1) in the segment anterior to that in which each is fully derepressed. Whether *iab-3*$^{+}$ or *iab-8*$^{+}$ are also similarly weakly derepressed is uncertain.

dinately, derepressed; (3) BX-C is under negative control maintained by a major regulatory gene, *Pc*$^{+}$ (along with perhaps other regulatory genes[7,17]); (4) a gene derepressed in one segment is derepressed in all segments posterior thereto; (5) the more posterior the segment (starting with MT) the greater the number of BX-C genes that are in the derepressed state; and (6) the more proximal the locus of a gene in the complex the more likely it is that that gene is in a derepressed state. Rules (5) and (6) suggest that two types of gradient are involved in BX-C regulation: an antero-posterior gradient within the organism in repressor concentration and a proximo-distal gradient along the chromosome in relative affinity for repressor of *cis*-regulatory elements. Presumably in MT repressor concentration is so high that the only genes derepressed are those whose *cis*-regulatory elements have relatively low repressor affinities, while in AB8 repressor concentration is so low that all of the genes of the complex escape repression.

During ontogeny the above rules presumably result in each segment having a specific array of BX-C substances, at the right time, at the right place. In AB8, for example, the rules predict a minimum of eight such substances, the number of BX-C genes being very likely to exceed the eight identified thus far. Whether such a multiplicity of substances act in a hierarchical or a compartmentalised manner[18,19] to establish the final pattern or level of development of a segment may well be accessible to analysis at the cellular level.

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