Homeotic transformations of the abdominal segments of *Drosophila* caused by breaking or deleting a central portion of the bithorax complex

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**SUMMARY**

In *Drosophila*, genes in the centromere-proximal portion of the bithorax complex (*BX-C*) have been shown to control the development of the metathorax, and parts of the mesothorax and first abdominal segment. Here, we explore the roles of genes positioned more distally by examining the larval and adult phenotypes caused by a breakpoint and deletion in the middle of the complex. We find that both aberrations affect only abdominal segments, transforming the more anterior segments towards the first abdominal segment, and the remaining segments into a graded series of novel segment types which are partially transformed towards more anterior abdominal segments. Moreover, the adult transformations, which we have observed in somatic clones of mutant cells, are in close accord with the transformations observed in mutant first instar larvae, and appear to be expressed in a cell autonomous fashion. We discuss these results in the light of current views of the organization and function of the complex.

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**INTRODUCTION**

In *Drosophila melanogaster*, the morphological diversity of the thoracic and abdominal segments clearly depends on the normal activity of the bithorax complex (*BX-C*). In a series of genetic investigations spanning the past 30 years, Lewis (1951, 1954, 1955, 1963, 1964, 1967, 1978, 1981, 1982) has sought to define the individual genes within the complex and to determine their roles in specifying the unique developmental pathways followed by most of the thoracic and abdominal segments. These investigations have led to the isolation and characterization of (i) 'viable' mutations which cause homeotic transformations of the thoracic and abdominal segments of adult flies, and (ii) recessive lethal mutations and deletions which, when homozygous, result in homeotic transformations of

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the thoracic and abdominal segments of the larva. Each of these classes of mutations has provided valuable, though different, information about the wild-type functions of the complex. Thus, the many different recessive and dominant mutations affecting the adult have revealed the diversity of distinct functions encoded by the complex, while the deletions have revealed what happens when some or all of these functions are absent during embryogenesis. However, because the effects of viable and lethal mutations have generally been studied in different developmental stages, links between these two levels of analyses have depended on the few cases where genetic mosaics have been used to examine the phenotypes of lethal mutations in the adult cuticle (Lewis, 1963, 1964; Morata & Garcia-Bellido, 1976; Morata & Kerridge, 1981; Kerridge & Morata, 1982; Minana & Garcia-Bellido, 1982).

Putting together all the genetic and developmental information, Lewis (1978) proposed a general model for BX-C function which can be summarized as follows. The complete lack of BX-C function results in mesothoracic development, while full activity results in the pathway of development normally followed by the eighth abdominal segment. In between these extremes, the rest of the thoracic and abdominal segments are specified by particular combinations of active BX-C genes. This model predicts the existence of discrete genetic functions specifying the unique developmental paths followed by most of the thoracic and abdominal segments.

Until recently, most work on the bithorax complex has been focused on genes located in the centromere-proximal portion of the complex and known to control the development of the metathorax and parts of the mesothorax and first abdominal segment. Many dominant and recessive mutations, as well as rearrangement breakpoints and deletions have been obtained in these genes, and their phenotypic consequences in both the larva and adult have been described in detail. In contrast, our knowledge of genes in the more distal portions of the complex rests on the adult phenotypes caused by a small number of viable mutations, and the larval phenotypes of a few recessive lethal breakpoints and deletions (Lewis, 1978, 1981, 1982; Kuhn, Woods & Cook, 1981). Because the effects of the viable mutations on the functions of the altered genes are at present obscure (and likely to be minor) they are of limited value in assessing the normal roles of the wild-type genes. Consequently, it may be more informative to concentrate on phenotypic studies of breakpoints and deletions which are likely to greatly reduce or eliminate wild-type gene functions. Previous studies of such chromosomal aberrations have focused almost exclusively on the homeotic phenotypes observed in first instar larvae. An obvious extension of these studies would be to describe the adult transformations caused by these chromosomal aberrations and compare them with the larval transformations. However, this approach is confounded by the recessive lethality associated with virtually all breakpoints and deletions which disrupt the complex. One way around this problem is to study the phenotypes of somatic clones of cells that are mutant or
Homeotic transformations of Drosophila abdominal segments 321
deficient for the BX-C genes of interest and at the same time genetically marked. As Ripoll & Garcia-Bellido (1979) have shown, the majority of small chromosomal deletions are compatible with cell viability in somatic clones. Hence, by examining the phenotypes of mutant clones in all of the adult segments, it should be possible to obtain a piecemeal description of the effect of a particular BX-C deletion on the development of the adult fly. This method was first used by Lewis (1963, 1964) to study the effect of Ultrabithorax (Ubx) mutations in the adult cuticle and has been refined over the years (e.g., Morata & Garcia-Bellido, 1976). Because the generation of mutant clones rests on the elimination of the wild-type gene by X-ray-induced mitotic recombination, this method has the additional advantage that it permits the removal of the wild-type gene at virtually any time during development. It is therefore possible to detect gene functions which are only active during brief periods in early development (Morata & Kerridge, 1981).

Here, we describe the adult phenotypes associated with somatic clones that are mutant or deficient for genes within the central portion of the BX-C, and compare them with the phenotypes of larvae similarly mutant or deficient. Our results provide further evidence that the central portion of the BX-C contains a set of genetic functions which control the determined state of most of the abdominal segments. In addition, they show that these ‘abdominal’ functions are required in a cell autonomous fashion and that they play similar roles throughout development.

MATERIALS AND METHODS

Genotypes employed

BX-C aberrations

\( T(2;3)P10, Df(3R)Ubx^{109}, \) and \( Df(3R)P9 \) (called \( TP10, DfUbx^{109}, \) and \( DfP9 \) in the text) were isolated and described by E. B. Lewis (1978; 1980), and are illustrated in Fig. 1. Two other rearrangements \( (T(1;3)P115, \) and \( Tp(3;3)146) \) isolated and described by E. B. Lewis have been used. \( T(1;3)P115 \) is an insertion of a portion of the third chromosome (89B9-89E6,8) containing the entire BX-C into the base of the X chromosome. Both the \( Dp(3;3)P115 \) and \( Df(3R)P115 \) segregants are viable. \( Tp(3;3)146 \) (referred to as \( Tp(3;3)S462 \) in Lewis, 1980) is a transposition of a larger portion of the third chromosome (89D1,2-90D1) carrying the entire BX-C to the distal portion of the left arm of the third chromosome. Flies carrying a third chromosome bearing both \( Dp(3;3)P146 \) and \( Df(3R)P115 \) are viable and fertile.

Other mutations and chromosomal aberrations

The recessive mutations yellow (\( y \)), javelin (\( jv \)), and multiple wing hairs (\( mwh \)) affect respectively the colour and shape of bristles, and the number of hairs secreted by a single hair cell. \( Dp(1;3)sc^{14} \) is an insertion of a small portion of the
distal X chromosome containing the \( y^+ \) gene into the distal portion of the left arm of the third chromosome. \( M(3)r^{55} \) is a dominant Minute mutation which reduces bristle size and causes mutant cells to proliferate more slowly than wild-type cells (Morata & Ripoll, 1975). TM1 and TM3 are third chromosome balancers. See Lindsley & Grell (1968) for further descriptions.

**Mosaic analysis of TP10 and DpP10; DfUbx\(^{109} \)** in the adult cuticle

**Production of DpP10; DfUbx\(^{109} \) clones**

\( y; Dp(3;2)P10/+; Dp(1;3)s^{14} Dp(3;3)146 M(3)r^{55} Df(3R)P115/TM3 \) males were crossed to \( y; mwh \ jv Df(3R)Ubx\(^{109} \)/TM1 females, and their progeny X-irradiated at various times during development with 500 or 1000 rad. One eighth of the progeny from this cross should be genotypically \( y; Dp(3;2)P10/+; mwh \ jv Df(3R)Ubx\(^{109} \)/\( Dp(1;3)s^{14} Dp(3;3)146 M(3)r^{55} Df(3R)P115 \). Flies of this genotype are phenotypically normal (with the exception of the Minute(3)\(^{55} \) phenotype) and hence distinct from the remaining progeny which are marked with the dominant mutations associated with the TM1 and TM3 balancer chromosomes, or with the dominant Ubx phenotype associated with haplo-insufficiency for this gene. Fig. 2 illustrates the chromosomal constitution of these flies and shows that a mitotic recombination event occurring on the left arm of the third chromosome proximal to the \( M(3)i \) locus can generate a \( y; Dp(3;2)P10/+; mwh \ jv Df(3R)Ubx\(^{109} \)/\( mwh \ jv Df(3R)P115 \) daughter cell. All of the descendents of this cell would constitute a clone of cells hemizygous for the \( DpP10; DfUbx\(^{109} \) deletion and marked by the \( y, mwh; \) and \( jv \) mutations. These cells also carry two copies of the \( M(3)i^{55} \) gene and therefore are fast-growing relative to the surrounding cells bearing the \( M(3)r^{55} \) mutation (Morata & Ripoll, 1975).

**Production of TP10 clones**

The procedure is essentially the same as that for \( DpP10; DfUbx\(^{109} \) clones except that \( DfP10 \) is used instead of \( DfUbx\(^{109} \).

**Production of control clones**

To control for the possibility that the homeotic phenotypes observed in mutant clones might have been caused by extraneous genetic factors unrelated to the loss of BX-C function, we produced TP10 clones exactly as described above except for the presence of an additional copy of the entire BX-C. To do so we irradiated larvae of the genotype \( y; Dp(3;1)P115/y; Dp(3;2)P10/+; mwh \ jv Df(3R)P10/\( Dp(1;3)s^{14} Dp(3;3)146 M(3)r^{55} Df(3R)P115 \). Marked clones arising in these flies are genotypically identical to those arising in the TP10 experiment except that they also carry a wild-type copy of the BX-C on the X chromosome thereby covering the loss of BX-C function resulting from the TP10 breakpoint.
Homeotic transformations of Drosophila abdominal segments

Analysis of larval cuticular phenotypes

Eggs from appropriate parents were collected on agar plates over 12 h periods, and allowed to mature for subsequent 24 h. They were then rinsed, dechorionated with dilute hypochlorite, fixed by incubation in glycerol–acetic acid (1: 4) for 30 min at 60 °C, and then mounted in Hoyer's mixture according to the procedure of Van der Meer (1977). After incubation of mounted preparations overnight at 60 °C, most of the internal tissues are dissolved allowing the cuticular features to be studied under bright-field, phase, Nomarski, or dark-field optics. When necessary, pharate first instar larvae were dissected out from the vitelline membrane prior to fixing in glycerol–acetic acid.

Each BX-C aberration was examined in the homozygous state. For example, DpPIO; DfUbx109 first instar larvae were obtained from a DpPIO/DpPIO; DfUbxm/TM3 stock. Homozygous mutant larvae were recognized by a characteristic mutant phenotype distinct from the wild-type phenotype and were found in approximately the correct frequency relative to phenotypically wild-type larvae (e.g., about one quarter of the progeny from the DpPIO; DfUbxm/TM3 stock had the characteristic mutant phenotype showed in Fig. 8B).

RESULTS

Rearrangement breakpoints and partial deletions of the BX-C

A diagram of the genetic map of the BX-C (modified from Lewis, 1978) is shown in Fig. 1. The leftmost (centromere-proximal) portion of the complex contains at least five genes, anterobithorax (abx), bithorax (bx), Ultrabithorax (Ubx), bithoraxoid (bxd), and postbithorax (pbx), which are required for the normal development of particular portions of the meso- and metathorax, and the first abdominal segment (Lewis, 1963, 1964, 1967, 1978, 1981, 1982). In general, abx, bx, bxd, and pbx mutations are viable when homozygous and transform particular anterior and posterior compartments of these segments towards the corresponding compartment in the adjacent anterior segment (Lewis, 1963, 1964; Morata & Garcia-Bellido, 1976; Lawrence, Struhl & Morata, 1979; Kerridge & Sang, 1981). Ubx mutations behave as if they lack the functions of the abx, bx, bxd, and pbx genes as well as an additional postprothorax (pxx) function (Lewis, 1963, 1964, 1978, Morata & Garcia-Bellido, 1976; Morata & Kerridge, 1981; Kerridge & Morata, 1982). For convenience, we will refer to the subset of BX-C genes which are inactivated by recessive lethal mutations of the Ubx gene as the 'thoracic' genes.

Most, if not all, of the BX-C genes which lie to the right of the thoracic genes appear necessary for the correct development of the abdominal segments and terminalia (Lewis, 1978, 1981, 1982; Duncan & Lewis, 1982). Here, we examine two rearrangements of the central portion of the BX-C which alter the function...
Fig. 1. Genetic map of the bithorax complex (after Lewis, 1978, 1981, 1982; Bender, et al. 1983). The complex can be subdivided into two functionally independent domains: the centromere-proximal domain contains the 'thoracic' genes (abx, bx, Ubx, bxd, pbx, and ppx (Morata & Kerridge, 1981; Kerridge & Morata, 1982) if it exists as a separable function); the centromere distal domain contains the abdominal genes (iab2–iab8). Separable dominant and recessive mutations of the iab2 and iab5 genes have been obtained; in addition the properties of several other mutations and chromosomal aberrations mapping to the abdominal gene domain suggest that other iab genes are present (see Lewis, 1978, 1980, 1981, 1982; Duncan & Lewis, 1982). Three chromosomal aberrations, T(2;3)P10, Df(3R)Ubx109, and Df(3R)P9 (referred to as TP10, DfUbx109, and DfP9; see Lewis, 1978, 1980, 1981, 1982; Duncan & Lewis, 1982) have been used in this study. TP10 is an insertional translocation which breaks within the BX-C and in a neighbouring centromere-proximal portion of the third chromosome, and translocates the material in between to the left arm of the second chromosome (the positions of these breakpoints are indicated by arrow heads above the representation of the wild-type BX-C region; the BX-C itself is shown as a broad bar, whereas neighbouring portions of the third chromosome are shown as a thin bar). The material translocated to the second chromosome (shown as a line) is referred to as DpP10 (=Dp(3;2)P10) and includes all of the thoracic genes; the reciprocal deletion in the third chromosome is referred to as DfP10 (=Df(3R)P10) and in addition to deleting all of the thoracic genes also appears to damage the iab2 gene. The precise position of the TP10 breakpoint relative to the iab2 gene is unknown. DfUbx109 deletes all of the BX-C genes deleted by DfP10 and extends further into the complex; the precise endpoint of this deletion within the complex is unknown, but appears to be near the iab5 gene. DfP9 deletes the entire BX-C.
Homeotic transformations of Drosophila abdominal segments

of a restricted subset of these 'abdominal' genes. The first rearrangement is the breakpoint associated with $T(2;3)P10$ (referred to subsequently as $TP10$) which is an insertional translocation that carries a centromere-proximal portion of the $BX-C$ as well as an adjacent portion of the third chromosome to the left arm of the second chromosome, leaving behind a complementary deletion (Lewis, 1978, 1980; see Fig. 1). The $TP10$ breakpoint within the complex behaves as a recessive lethal mutation which partially or completely inactivates at least one abdominal gene. The second rearrangement is a synthetic deletion, $Dp(3;2)P10; Df(3R)Ubx^{109}$ (referred to subsequently as $DpP10; DfUbx^{109}$), which begins at the $TP10$ breakpoint and extends rightwards until the distal breakpoint of $DfUbx^{109}$: this deletion eliminates one or more abdominal genes lying to the right of the $TP10$ breakpoint (Lewis, 1978, 1980, 1981, 1982; see Fig. 1).

Functional independence of the thoracic and abdominal genes

To clarify the functional relationships between the thoracic and abdominal genes we have constructed several genotypes involving mutations and breakpoints from both sets of genes. We find that recessive lethal $Ubx$ mutations that completely inactivate the thoracic genes are fully complemented by recessive lethal breakpoints and deletions affecting the abdominal genes. For example, flies carrying the $trans$ combination of $Ubx^{130}$ and $TP10$ are viable and phenotypically normal aside from the dominant Ultrabithorax phenotype resulting from haplo-insufficiency of the $Ubx$ gene. Similarly, flies carrying the $trans$ combination of $Ubx^{130}$ and $DpP10; DfUbx^{109}$ are viable and phenotypically normal (except for the dominant Ultrabithorax phenotype). Thus, the thoracic and abdominal genes appear to constitute separably mutable and functionally autonomous subgroups of the $BX-C$.

Adult phenotypes of $TP10$ and $DpP10; DfUbx^{109}$

When either homo- or hemizygous, both $TP10$ and $DpP10; DfUbx^{109}$ cause lethality just before or after hatching of the first instar larva. Hence, to study the adult phenotypes associated with each rearrangement, we have used X-ray-induced mitotic recombination to generate somatic clones of mutant cells (Fig. 2).

Clones of both genotypes were produced at different developmental stages ranging from blastoderm to pupariation, and found in all cuticular structures of the fly which can be marked by the $y$, $jv$, and $mwh$ mutations. Those found in the cephalic and thoracic segments as well as in the anterior compartment of the first abdominal segment and the terminalia (the genitalia and analia) differentiate normally. Moreover, they are similar both in size and frequency to clones generated in control flies. Thus, neither aberration appears to alter the development of imaginal cells giving rise to the adult head, thorax, terminalia, or first abdominal segment (anterior compartment). In contrast, clones in the remaining abdominal segments show homeotic transformations. Before
Fig. 2. Generation of marked clones deficient for abdominal gene function by X-ray-induced mitotic recombination. The genotype used for generating DpP10; DfUbx109 clones is shown; the genotype used for generating TP10 clones differs only in that DfUbx109 is replaced by DpP10. The left-hand side of the figure illustrates the genetic constitution of the animal prior to mitotic recombination. The right arm of the third chromosome lacks the BX-C genes deleted by DfUbx109. This deficiency is covered in part by DpP10 on the left arm of chromosome II, and in totality by DfPlO on the left arm of chromosome III. Thus, as shown in the lower part of the figure, this genotype carries a complete set of BX-C genes. An X-ray-induced mitotic recombination (MR) in the proximal portion of the left arm of the third chromosome will lead to the elimination of Dpsc34 (y+), Dpi46 and M(3)i65 from one of the daughter cells after one mitosis. This cell is now homozygous for the cuticle markers y, mwh, and jv, and because of the loss of the retarding M(3)i55 mutation, it proliferates more rapidly than surrounding cells. We represent in the lower part of the figure the different BX-C deficiencies and duplications present in each genotype to illustrate how the loss of Dpi46 results in a partial synthetic deletion extending from the TP10 breakpoint within the BX-C to the distal breakpoint of DfUbx109.

considering these phenotypes, it should be noted that even though each abdominal segment may be formed by an anterior and posterior compartment (Kornberg, 1981), the posterior compartments construct disproportionately small parts of each segment which generally cannot be marked by the y, jv, or mwh mutations. Our results therefore pertain only to the anterior compartments of the abdominal segments.

DpP10; DfUbx109 clones in the abdomen

As described above, clones found in the first abdominal segment appear normal. Virtually all of the clones in abdominal segments 2–5 are clearly transformed to
Homeotic transformations of Drosophila abdominal segments

segment 1 (Table 1; Figs 3, 4A, B); the few cases of untransformed clones are probably rare recombination events producing marked clones not deficient for the BX-C. The transformation appears to be complete in both the dorsal (tergites) and ventral (sternites) regions although some very rare cases (3) were found in which the transformation seems to be partial. Clones found in the tergites of segments 6 and 7 in females and of segment 6 in males (wild-type males do not have a seventh tergite) show a phenotype different from that found in more anterior segments. In most cases they produce only hairs but sometimes one or two bristles are also found. However, these hairs and bristles are different from those normally found in the first abdominal segment; they appear more like those of segments 2–5. In general the clones in tergites 6 and 7 are smaller than those produced in more anterior abdominal segments and are sometimes associated with abnormal cuticle differentiation. Since the number of clones found in these segments is low (Table 1), it is very likely that many do not develop or are eliminated. Clones in the sternites of segment 6 behave like sibling clones in the sternites of segments 2–5; namely they appear transformed into the first abdominal segment in that they do not produce bristles (Fig. 4C, D) and have a pattern of hairs like that of the first sternite. Frequently they extend from the sternite to the pleura (Lawrence, Green & Johnston, 1978). Note that clones in the sixth sternite behave differently from clones in the sixth tergite which differentiate structures appropriate to abdominal segments 2–5.

Three additional features of these clones are worthy of note. First, only cells marked with the y, jv, and mwh mutations, and hence unquestionably belonging to the mutant clone, show homeotic transformations. Taken together with the finding that virtually all the clones found in abdominal segments 2–7 are clearly transformed, this result indicates that the mutant phenotype is expressed in a cell autonomous fashion. Similar results have been obtained with mutations of

| Table 1. Number and phenotype of DpP10; DfUbx109 clones generated during the larval period and found in the different abdominal segments |
|---------------------------------|---|---|---|---|---|---|---|
| ABDOMINAL SEGMENT               | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| a) females                      |   |   |   |   |   |   |   |
| Dorsal clones                   | 179| 14| 20/22| 15/16| 21/21| 12/12| 8/8| 8/8|
| Ventral clones                  | 165| 4/4| 7/7| 3/3| 4/4| 6/6| - |
| b) males                        |   |   |   |   |   |   |   |
| Dorsal clones                   | 304| 23| 34/34| 20/31| 17/18| 12/12| 7/7 |
| Ventral clones                  | 261| 11/11| 10/10| 6/6| 6/6| 2/2 |

Clones in abdominal segment 1 are phenotypically wild type. Most of the remaining clones except for the dorsal clones in segments 6 and 7 are homeotically transformed to abdominal segment 1 (the number of transformed clones are shown together with the total number of clones found in each segment). Dorsal clones in segments 6 and 7 (bold figures) show partial transformations towards abdominal segments 2–5 (see text).
the thoracic genes of the \textit{BX-C} (Lewis, 1963, 1964; Morata & Garcia-Bellido, 1976) suggesting that \textit{BX-C} genes are generally required cell by cell in the segments in which they act. Second, clones in the tergites of segments 2–5 differentiate fine short bristles characteristic of the first abdominal tergite which are thinner and longer in the centre of the tergite than they are at the sides. This gradual change in bristle morphology according to mediolateral position is also found in the normal first abdominal segment. Similarly, another first tergite structure differentiated by the clones, the archlike putative apodeme, is also found laterally in segments 2–5, just as it is in segment 1 (e.g., Fig. 4B). These observations suggest a point-by-point spatial correspondence between the cuticular derivatives of the first abdominal segment and the derivatives of the next four abdominal segments. Similar correspondences have been observed between the derivatives of other adult segments (Postlethwait & Schneiderman, 1971; Morata & Garcia-Bellido, 1976; Morata & Lawrence, 1979; Lawrence et

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Fig. 3. Dorsal (left) and ventral (right) sides of the anterior adult abdomen. Black arrow heads mark the approximate anterior margins of the second, third, and fourth segments. The first abdominal segment is clearly distinct from all the others in several respects. The dorsal derivative (the first tergite) contains smaller and finer bristles, and the hairs are less pigmented and more densely packed, than in the more posterior tergites (note also that the bristles positioned in the centre of the first tergite are longer and thinner than those positioned laterally). In addition, an arch-like cuticular engrossment (probably an apodeme) is formed on the internal surface of the first abdominal tergite, but not on the tergites of any of the remaining abdominal segments. On the ventral side (the sternite and pleura), the differences are equally clear. Unlike the other segments, the first sternite does not contain bristles, the hairs are smaller, and there is no clear demarcation between the sternite and pleura. Thus, transformations between the first abdominal segment and the remaining abdominal segments can be easily detected and unambiguously classified. Magnifications: Dorsal = 100x; Ventral = 100x.

Fig. 4. Marked \textit{DpP10}; \textit{DfUbx109} clones in abdominal segments 2, 3, 4 and 6. (A) Clone in the fourth tergite which differentiates thin short bristles characteristic of the first tergite. Note that all of the hair-forming cells belonging to the clone (i.e., marked by the \textit{y} and \textit{mwh} mutations) form a coherent patch; however three of the five bristles belonging to the clone are separated from this patch indicating that they derive from single cells which migrated away from their hair-forming sibs after they became determined to form bristles. (B) Laterally positioned clone in the second tergite which differentiates the arch-like cuticular structure (arrow) as well as two short bristles normally found only in the first tergite (compare with the first tergite positioned immediately above). (C) and (D) Clones in sternites 3 and 6 which remove bristles indicating a transformation into sternite 1 which is normally bristleless. Magnifications: A = 400x; B = 200x; C = 400x; D = 200x.

Fig. 5. Marked \textit{TP10} clones showing partial segmental transformations. (A) Clone in the fourth tergite in which two of the three marked bristles are small and thin, showing a transformation towards tergite 1, whereas the remaining bristle (arrow) is large, and hence, appears untransformed. (B) Clone (arrow) in tergite 6 which differentiates hairs in a region normally devoid of hairs, and bristles which are larger than those normally formed in tergite 1. This clone therefore appears transformed towards a more anterior abdominal segment other than the first segment. Magnifications: A = 400x; B = 200x.
Fig. 6. Ventral cuticular patterns of wild type, DfP10, and DfP9 larvae. (A) wild type larva. The segmental pattern of the wild-type larva has been described previously in detail (Lohs-Schardin, Cremer & Nusslein-Volhard, 1979; Turner & Mahowald, 1979; Struhl, 1983b). In this study, we have concentrated principally on the cuticular structures of the ventral hypoderm, particularly the unique patterns of ventral hairs characteristic of each segment, and the presence or absence of specialized sensory organs and the posterior spiracles. Black arrow heads mark the approximate anterior margin of the thorax and the abdomen, as well as the eighth abdominal segment. The thoracic segments are clearly different from the abdominal segments in having much finer ventral hairs which are arranged in a few narrow rows that extend laterally to the dorsoventral midline; moreover, each of these segments bears bilaterally symmetric sense organs (ventral pits (v), dorsal pits, and Keilin's organs (k); see also Fig. 7) which are not normally present in any of the abdominal segments. The pattern of ventral hairs on the first abdominal segment is distinct from that of any other abdominal segment. For example, in abdominal segments 2–8, the anterior-most row of hairs forms a well-ordered line in which all the hairs are of similar size, and appear to point anteriorly. No such row of hairs is present in the first abdominal segment. It is difficult to distinguish between the patterns of any two neighbouring segments between the second and seventh abdominal segment. However, it is apparent that the pattern changes gradually as one proceeds posteriorly from segment to segment. The most notable difference is that the bands of ventral hairs are trapezoidal in the more anterior segments and become progressively more rectangular in the more posterior segments. The eighth abdominal segment is clearly different from the other abdominal segments in having a distinct rectangular pattern of ventral hairs and bearing the posterior spiracles (ps) as well as other unique cuticular structures on the dorsal surface. (B) DfP10 larva (see also Lewis, 1978; Struhl, 1981a). Several aspects of this larval phenotype should be noted. First, the metathorax and first abdominal segment appear transformed cleanly into mesothoracic segments (as they are in BX-C~ embryos), but abdominal segments 2–8 all appear to bear patterns of ventral hairs which are intermediate between the wild-type patterns and the mesothoracic pattern (that these segments are partly thoracic in character is shown by the presence of Keilin's organs and ventral pits in all but the eighth segment. Second, none of the segments with intermediate characteristics resemble a particular segment of the wild-type larva; all appear to be novel segment types. Third, as one proceeds posteriorly from segment 2 to segment 8, the bands of ventral hairs become trapezoidal and then progressively broader and more rectangular, just as they do in the wild-type larva. These aspects of the phenotype indicate that DfP10 eliminates all of the BX-C genes which normally function in the thorax and first abdominal segment, but that some genes which normally function in the second abdominal segment remain at least partly active in this segment. In addition, they show that the deletion partly or completely eliminates BX-C functions which are required for the normal development of all of the remaining abdominal segments. (C) DfP9 larva (see also Lewis, 1978; Struhl, 1981a, 1983b). The salient features are that the head and anterior thorax appear normal, but the metathorax and first seven abdominal segments appear transformed into mesothoracic segments. The eighth abdominal segment is also transformed into a thoracic segment, though it appears intermediate in character between the pro- and mesothorax and carries additional cuticular structures not normally found in either the thorax or abdomen (see Fig. 7A). Note also that the posterior spiracles are absent. DfP9 deletes the entire BX-C; hence this phenotype may be regarded as the ground state on which the BX-C genes normally act to specify the wild-type pattern. The particular nature of the segmental transformations observed when some of the BX-C genes are absent therefore provides a measure of the function of the remaining portions of the complex. Magnification = 150×.
al. 1979; Struhl, 1981b) and have led to the supposition that the unique patterns of each segment reflect different interpretations of a common field of positional information reiterated in each segment. Third, wild-type bristles not belonging to the clones are frequently present inside the clone surrounded by marked, mutant hairs (Fig. 4B). Conversely, mutant bristles belonging to the clone sometimes appear to have migrated outside of the clone territory (Fig. 4A). These results indicate that cells set aside to become bristles often move away from sibling, hair-forming cells during the process of bristle patterning. Similar observations have been made for the sex comb teeth (Tokunaga, 1962) and tarsal bristles (Lawrence et al. 1979) of the legs and for wild-type bristles found in the abdominal tergites (Garcia-Bellido & Merriam, 1971). Yet, here, the bristle-determined cells intermix with surrounding cells that have a different segmental specification. This result supports previous suggestions that affinities are similar between cells in neighbouring abdominal segments (Wright & Lawrence, 1981).

A special experiment was performed to find out the temporal limit of the requirement for the deleted genes. The mutant clones were produced at different times after pupariation at intervals of 4 h. Even those generated as late as 16 h after pupariation show the characteristic segmental transformations observed in clones generated during larval development, indicating that the normal gene functions are required well into the pupal period.

TP10 clones in the abdomen

As in the previous experiment clones in abdominal segment 1 appear normal, in segments 2–5 they appear transformed towards segment 1, and in segments 6 and 7 they appear transformed towards abdominal segments 2–5. However, in contrast to the results with DpP10; DfUbx109 clones, the transformations caused by TpP10 clones appear incomplete. For example, many of the clones in both the tergites and sternites of segments 2–5 do not show a detectable transformation to segment 1, or differentiate both transformed and untransformed structures (Table 2; Fig. 5A). Note that the degree of incompleteness of the transformation depends on the particular segment in which the clone occurs: those in segment 2 are nearly always transformed, whereas those in segment 5 are almost never transformed (Table 2). In parallel with the DpP10; DfUbx109 experiment, TP10 clones in abdominal tergites 6 and 7 do not develop bristle or hair patterns characteristic of the first abdominal segment, but instead differentiate patterns resembling those of abdominal segments 2–5 (Fig. 5B). However, as in the more anterior abdominal segments, the transformations are not complete (Table 2).

Control clones in the abdomen

It is possible, in principle, that some of the homeotic transformations we observed in mutant clones could be due to extraneous genetic factors present on
Table 2. Number and phenotype of TP10 clones generated during the larval period and found in the different abdominal segments (only clones in the dorsal abdomen (the tergites) are considered)

<table>
<thead>
<tr>
<th>ABDOMINAL SEGMENT</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>females</td>
<td>No.</td>
<td>tl</td>
<td>p</td>
<td>nt</td>
<td>tl</td>
<td>p</td>
</tr>
<tr>
<td></td>
<td>132</td>
<td>9</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>males</td>
<td>89</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>total</td>
<td>221</td>
<td>15</td>
<td>1</td>
<td>0</td>
<td>11</td>
<td>7</td>
</tr>
</tbody>
</table>

tl = transformed towards abdominal segment 1; p = partially transformed towards abdominal segment 1; nt = no sign of transformation detected; t2 = transformed towards abdominal segments 2–5 (see text).

the chromosomes used for the mosaic analysis. To control for this possibility we generated clones in flies which were genotypically identical to flies in the TP10 experiment except that they carried an extra dose of the entire BX-C (see Materials and Methods). In this case, the y, jv, mwh, M(3)i+ clones are expected to be normal in all segments if all the effects observed in previous segments were due exclusively to the partial loss of BX-C function. In a sample of 67 flies irradiated as late second and early third instar larvae (72–96 h after egg laying) we found 20 clones in the second, third, fourth, and fifth tergites, 11 clones in the sixth and seventh tergites, and 17 clones in the sternites and pleura. None showed any sign of homeotic transformation.

**Larval phenotypes of TP10 and DpP10; DfUbx**

The TP10 and DpP10; DfUbx rearrangements consist of the centromere-proximal fragment DpP10 carrying the thoracic genes and the centromere-distal fragments resulting from the DfP10 or DfUbx deletions which remove all of the thoracic genes and partially or completely inactivate one or more of the abdominal genes. In order to assess the independent contributions of the BX-C material present in each of these fragments, we examined first the phenotypes caused by DfP10 and DfUbx alone, comparing homozygous larvae with wild-type and BX-C− larvae (Figs 6, 7). Then, we compared these phenotypes with the phenotypes of homozygous DfP10, DfUbx, and BX-C− larvae which also carried two copies of the DpP10 fragment (Figs 7, 8). Detailed descriptions of these phenotypes are provided in the legends to Figs 6–8. Here we summarize the principal results derived from this analysis.

**TP10 larval phenotype**

The head and thorax of TP10 larvae are indistinguishable from that of wild
type; however the first eight abdominal segments show segmental transformations (Figs 6A, 7A). These are (i) the anterior halves of the first and second abdominal segments appear similar to the anterior half of the normal first abdominal segment, but the posterior halves of these segments appear thoracic

Fig. 7. Some aspects of the DfUbx$^{109}$, DpP10; DfP9, and DpP10; DfUbx$^{109}$ larval phenotypes. (A, B, C) Comparisons of the posterior abdominal segments of DfP9 and DfUbx$^{109}$ larvae (see also Lewis, 1978). The segmental phenotypes of DfP9 and DfUbx$^{109}$ larvae appear indistinguishable in all segments anterior to the fourth of fifth segment; however they differ in the remaining posterior segments. A shows the ventral aspect of a DfP9 larva, and B and C show ventral and lateral aspects of DfUbx$^{109}$ larvae (black arrowheads mark the approximate anterior margins of abdominal segments 5, 6, 7 and 8). The ventral hair patterns in segments 5, 6 and 7 of the DfP9 larva appear consistently like that of the mesothorax; in contrast fewer rows of hairs are present in each of these segments in the DfUbx$^{109}$ larva, and in addition, the hairs appear thicker. Similarly, the dorsal hair patterns of segments 5, 6 and 7 are also different. In DfP9 larvae, the anterior portions of these segments bear an even pattern of dorsal hairs while the posterior portion is bald. As shown in C, a few hairs appear in the bald region of segment 5 and then progressively more such hairs appear in the bald regions of segments 6 and 7. The greatest difference between DfP9 and DfUbx$^{109}$ larvae is apparent in the eighth segment. In DfP9 larvae, this segment differentiates a ventral pattern intermediate between that of the meso- and prothorax (including Keilin's organs and dorsal and ventral pits), lacks spiracles, and bears plates of sclerotized cuticle (s.c.). In contrast, in DfUbx$^{109}$ larvae it carries one or two rows of hairs which do not extend to the dorsal side (as they do in DfP9 larvae), lacks Keilins organs, and dorsal and ventral pits, and carries rudimentary posterior spiracles (ps). Thus, it appears intermediate between the normal eighth abdominal segment and the eighth abdominal segment of DfP9 larvae. On the basis of this comparison one may conclude that DfUbx$^{109}$ deletes all BX-C genes normally active in segments anterior to the fourth or fifth abdominal segment, and either deletes, or reduces, the function of some BX-C genes normally required in the more posterior segments. (D, E, F, G) The DpP10; DfP9 larval phenotype. As shown in Fig. 8C the head, thorax and first abdominal segment of DpP10; DfP9 larvae appear to be normal, whereas segments 2–8 appear to be transformed into first abdominal segments. However, on closer inspection, it appears that only the anterior portions of all of these abdominal segments develop as in the wild-type first abdominal segment while the posterior portion appears to develop like a thoracic segment (probably the metathorax). D and E show the dorsal hair patterns of a wild type and DpP10; DfP9 larva respectively. Black arrow heads mark the approximate anterior margins of the first and second abdominal segments. In wild type larvae, a small cluster of thicker dorsal hairs is usually found in the posterior region of the segment whereas in the thoracic segments the corresponding region is bald. Progressively more such hairs are found in the more posterior segments. In contrast, these hairs are absent in abdominal segments 1–8 of DpP10; DfP9 larvae. F and G show that the ventral pattern of the reiterated first abdominal segment is also partially thoracic. F shows the normal Keilin's organ (which consists of three sensory hairs) and ventral pit of the mesothorax; G shows a 'monohair' formed in the seventh segment of a DpP10; DfUbx$^{109}$ larva (similar monohairs are also found in the abdominal segments of DpP10; DfP9 larvae (Lewis, 1978) and appear to be rudimentary Keilin's organs). Thus, in DpP10; DfP9 larvae, abdominal segments 1–8 appear like mosaic segments in which the anterior portions develop as in the first abdominal segment and the posterior portions develop as in a thoracic segment. Magnifications: A, B, C = 180×; D, E = 200×; F, G = 450×.
Homeotic transformations of Drosophila abdominal segments

in character, and (ii) beginning in segment 3 and proceeding until segment 8, the segments display a graded sequence of novel patterns which appear intermediate between the first abdominal segment and the normal segment (compare Figs 6A, 8A). Because the characteristic patterns of the wild-type abdominal segments differ from each other principally in the arrangement of otherwise similar hairs, we cannot say whether the intermediate patterns are mosaics of cells of different segment types, or alternatively, whether they comprise cells which themselves have intermediate segmental characteristics. To a first approximation, the phenotype of TP10 larvae appears to reflect the additive

Fig. 8. Ventral cuticular patterns of TP10, DpP10; DfUbx$^{109}$, and DpP10; DfP9 larvae. (A) TP10 larva. The head, thorax, and first abdominal segment appear normal, abdominal segment 2 appears like segment 1, and abdominal segments 3–8 appear to form a series of graded intermediate segment types in which the pattern of each segment appears intermediate between that of the first abdominal segment and that of the wild-type segment (compare with Fig. 6A). TP10 consists of two BX-C fragments: DpP10 and the centromere distal fragment complementary to DfP10. Comparing the phenotypes of TP10, DpP10; DfP9 (C) and DfP10 (Fig. 6B), the TP10 phenotype appears to reflect the additive properties of the DpP10; DfP9 and DfP10 phenotypes. Note however that the cuticular patterns are not strictly additive; that is, superimposing the ventral hair patterns of, for example, the eighth abdominal segments of DpP10; DfP9 and DfP10 larvae would give a somewhat different hair pattern than is observed in the eighth abdominal segment of the TP10 larva. Occasionally, monohairs (see Fig. 7G) are found in abdominal segments 1–7. On the dorsal side, the hair pattern of the first abdominal segment appears like that of DpP10; DfP9 larvae (see Fig. 7A, B); however, in more posterior segments, first a few, and then progressively more, thick dorsal hairs are found in the posterior portion of this segment. (B) DpP10; DfUbx$^{109}$ larva. As in A, the head, thorax and first abdominal segment appear normal. In addition, abdominal segments 2–7 appear like abdominal segment 1, and the eighth abdominal segment looks like an intermediate between the normal first and eighth segment (note in particular that the ventral hairs of the eighth segment do not extend to the dorsal side as they do in the first abdominal segment, and that the posterior spiracles are present). On closer inspection, the first seven abdominal segments are often found to bear monohairs, as observed in DpP10; DfP9 larvae (see Fig. 7G). In addition, the pattern of dorsal hairs resembles that of DpP10; DfP9 in lacking thick dorsal hairs in the posterior portion of the segment (see Fig. 7E); however, in segments (5), 6 and 7, additional thin dorsal hairs are found in this portion of the segment as in DfUbx$^{109}$ larvae (see Fig. 7C). Thus, the phenotype of DpP10; DfUbx$^{109}$ larvae appears to reflect the additive phenotypes of DpP10; DfP9 (C) and DfUbx$^{109}$ (Fig. 7B, C) larvae. Note however, that as in the case of TP10 larvae, the phenotypes are not strictly additive (compare the eighth segments of all three genotypes). (C) DpP10; DfP9 larva (see also Lewis, 1978). The head and thorax appear indistinguishable from wild type; however all eight abdominal segments appear to develop like the first abdominal segment. As described in Fig. 7, closer inspection suggests that each abdominal segment is a mosaic in which the anterior portion of the segment appears like the corresponding portion of the first abdominal segment, and the posterior portion of the segment appears like the corresponding portion of a thoracic segment. Note that the eighth abdominal segment lacks posterior spiracles and bears plates of sclerotized cuticle as does the eighth abdominal segment of DfP9 larvae (Figs 6C, 7A). Magnification = 150×.
effects of the independent contributions of the \textit{DpP10} fragment and the centromere–distal fragment resulting from the \textit{DfP10} deletion. Thus, in the absence of all the \textit{BX-C} genes, most of the abdominal segments develop like mesothoracic segments (Fig. 6C). The \textit{DpP10} fragment appears to raise most of the abdominal segments from the mesothoracic state towards the first abdominal state (Fig. 8C), whereas the distal fragment complementary to the \textit{DfP10} deletion plays no apparent role in the development of the first abdominal segment, but appears to raise abdominal segments 2–8 from the mesothoracic state to a state intermediate between that of the mesothorax and that of the normal segment (Fig. 6B). Together, the fragments appear to raise all the abdominal segments to intermediate states between that of the first abdominal segment and that of the normal segment (Fig. 8A).

\textbf{DpP10; \textit{DfUbx}109 larval phenotype}

The phenotype of \textit{DpP10; DfUbx}109 larvae differs from that of \textit{TP10} larvae chiefly in the following respects: (i) abdominal segments 3–7 appear similar to segments 1 and 2 (i.e., the anterior halves develop as in the first abdominal segment, while the posterior halves develop as in the meso- or metathorax), and (ii) the pattern of ventral hairs in segment 8 appears further transformed towards the normal first abdominal segment (compare Figs. 8A, B). As in the case of \textit{TP10} larvae, the phenotype of \textit{DpP10: DfUbx}109 larvae appears to a first approximation to reflect the additive contributions of the two \textit{BX-C} fragments involved (see Figs. 7B, C and 8B, C).

\textit{Correlation between the adult and larval phenotypes of TP10 and DpP10; \textit{DfUbx}109}

Bearing in mind that we probably examined only the anterior compartments of the adult abdominal segments, we find that the larval and adult transformations are generally in very good agreement. Neither rearrangement affects the development of the head, thorax, anterior first abdominal segment, or terminalia. In both \textit{TP10} larvae and adults, abdominal segment 2 developed like segment 1, segments 3–5 developed as a graded series of novel segment types intermediate between segment 1 and segments 2–5, and segments 6 and 7 appeared as intermediates between segments 2–5 and segments 6 and 7. Similarly, in both \textit{DpP10; DfUbx}109 larvae and adults, the anterior portions of segments 1–5 developed as in segment 1, as did the ventral anterior portion of segment 6. In addition the dorsal portions of segments six and seven appear to have characteristics of abdominal segments posterior to the first abdominal segment.

\textbf{DISCUSSION}

We have described the larval and adult phenotypes resulting from a breakpoint and an internal deletion in the central portion of the \textit{BX-C}. Here, we
Homeotic transformations of Drosophila abdominal segments

discuss our results in the light of current views of the organization and function of the different genes of the complex.

**Thoracic and abdominal genes**

As described in Fig. 1 and Results, the bithorax complex can be subdivided into centromere-proximal and centromere-distal portions which appear to carry functionally distinct sets of genes. The set of genes located in the proximal portion appears to be involved principally in the development of the meso- and metathorax, and the first abdominal segment. This set of genes includes the \( abx, bx, Ubx, bxd, \) and \( pbx \) genes, and possibly the \( ppx \) gene (if it exists as a separate gene). Because mutations in all of these genes have the general property that they transform portions of the meso- and metathorax, and the first abdominal segment into corresponding portions of an anteriorly situated thoracic segment, we refer to them as the 'thoracic' genes. Mutations of the \( Ubx \) gene behave as if they partially or completely inactivate all of the thoracic genes and hence allow us to define their realm of action. In adults, all of the \( Ubx \) mutations which have been studied to date (Lewis, 1963, 1964; Morata & Garcia-Bellido, 1976; Morata & Kerridge, 1981; Kerridge & Morata, 1982; Minana & Garcia-Bellido, 1982; Sanchez-Herrero & Morata, unpublished) affect at most the posterior compartment of the mesothorax, the entire metathorax, and the anterior compartment of the first abdominal segment; no phenotype is observed in any other segment. It is not yet clear why the limits of \( Ubx \) gene function in the adult coincide with anteroposterior compartment boundaries within segments rather than with segmental boundaries. However, mutations in most of the subsidiary thoracic genes (e.g., \( bx, bxd, pbx, \) and \( ppx \) ) seem to affect only anterior or posterior compartments within a segment, suggesting that BX-C genes may be deployed compartment by compartment, rather than segment by segment. To a first approximation, the phenotype of \( Ubx \) larvae reflects the adult phenotype; however, there are also slight, but significant, segmental transformations in abdominal segments 2–7 (Lewis, 1978; Struhl, submitted). Thus, at least some thoracic genes appear to act in cells giving rise to most of the abdominal segments of the larva.

The 'abdominal' genes present in the complementary distal portion of the complex are considerably less well characterized than the thoracic genes. The majority of mutations affecting these genes are dominant or weak recessive mutations which appear to partially deregulate, or partially inactivate, one or more genes (Lewis, 1978, 1981, 1982; Kuhn et al., 1981). Both rearrangements we have studied retain all of the thoracic genes but break in or delete some of the abdominal genes. Though the breakpoint itself may not completely inactivate any of the abdominal genes (Lewis, 1978, 1981, 1982; here, Fig. 6A, B), it is virtually certain that the deletion does. Here we show that in both the larva and adult, neither aberration affects the development of the head, thorax, or anterior compartment of the first abdominal segment. However, both rearrangements cause anteriorly directed segmental transformations in all the remaining abdominal segments.
(excluding the terminalia). These results establish that abdominal genes of the \textit{BX-C} are not required for the normal development of the head and thorax.

Thus, in the adult, the thoracic and abdominal genes appear to be required in mutually exclusively domains of the thorax and abdomen. Moreover, these domains appear to meet in the middle of the first abdominal segment, possibly at the anteroposterior compartment boundary. Though the distinction is not quite so clear in the larva, the anteroposterior compartment boundary in the first abdominal segment again seems to represent a boundary of some kind between thoracic and abdominal gene function (Struhl, submitted).

One perplexing result we obtained is that in a few cases the segmental transformations caused by either the breakpoint or the deletion appeared to be out of register in the dorsal and ventral derivatives of a given segment (e.g., clones of the deletion caused the sixth tergite to be transformed towards the tergites 2–5, whereas they caused the sixth sternite to be transformed towards sternite 1). We do not understand why these differences occur. They may reflect that the entire segment is of intermediate segment type which has properties of different segments in different regions. Alternatively, it is possible that the dorsal and ventral realms of action of some of the abdominal genes may be out of register by a segment.

\textit{Temporal and cellular requirements for the abdominal genes}

The segments of the adult abdomen develop in a markedly different fashion from the segments of the adult head and thorax. Whereas the latter derive from imaginal disc cells which proliferate during most of the larval period, the abdominal derivatives are formed by the descendants of small nests of histoblasts which form part of the larval epidermis and do not divide until the pupal period. Clones of cells hemizygous for the deletion consistently showed segmental transformations even when induced 16 h after the onset of the pupal period at which time the histoblasts would already have undergone several rounds of proliferative divisions (Madhavan & Madhavan, 1980). Moreover, most if not all of the cells belonging to the clones were transformed whereas surrounding \textit{BX-C} cells were invariably normal. These results establish that the deleted \textit{BX-C} genes are normally required in proliferating histoblasts during the pupal period, moreover, they indicate that these genes are required autonomously in all of the cells in the affected segments which could be marked by the clones. Finally, the larval and adult phenotypes of the breakpoint and the deletion were in close accord, suggesting that the broken or deleted genes in each case played similar roles during embryogenesis and metamorphosis. Thus, in terms of their cellular and temporal requirements, the abdominal genes appear to be analogous to the thoracic genes which are required continuously and autonomously in all cells of the appropriate compartments and segments.

\textit{Organization and function of the abdominal genes}

Fig. 9 shows a simplified view of the organization of the \textit{BX-C} genes along the
Homeotic transformations of Drosophila abdominal segments 337

chromosome and their corresponding realms of action along the body axis, and is intended to illustrate the main features of Lewis's model for the complex (Lewis, 1978, 1981, 1982). These are: (i) that the BX-C consists of a series of discrete genes which are active only in particular segments, (ii) that the order of these genes on the chromosome directly corresponds with the anteroposterior order of the segments in which they are active, (iii) that a given gene (say gene 4) is active in all segments posterior to the most anterior segment in which it is active (i.e., abdominal segments 4–8), and (iv) that the development of each segment is dictated either by the particular combination of active BX-C genes, or by the highest numbered gene which is active (e.g., gene 4 in abdominal segment 4). For the sake of the argument, let us assume that the TP10 breakpoint inactivates gene 2 and the DpP10; DfUbx109 deletion inactivates genes 2–5, but that all other BX-C genes function normally. Fig. 10 shows the combinatorial code words of active and inactive BX-C genes which would be present in each segment. In the case of the breakpoint, all segments anterior to the second abdominal segment would have the normal code word, segment 2 itself would have the normal code word for segment 1, and the remaining posterior segments

<table>
<thead>
<tr>
<th>Segments</th>
<th>BX-C genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 1 2 3 4 5 6 7 8</td>
<td></td>
</tr>
<tr>
<td>Head</td>
<td>0000000000</td>
</tr>
<tr>
<td>Thorax</td>
<td>0000000000</td>
</tr>
<tr>
<td>Abdomen</td>
<td>0000000000</td>
</tr>
</tbody>
</table>

Fig. 9. Simplified model of the BX-C intended to illustrate the general features of Lewis's model (Lewis, 1978; see text). The complex has been arbitrarily subdivided into nine genetic units and their realms of activity displayed as a matrix in which each gene is a column and each segment is a row. As described in the text, the BX-C genes may function combinatorially, such that the particular combination of active genes in each segment would specify the developmental pathway followed. Alternatively, they may function in a hierarchical fashion such that the highest numbered gene functioning in a segment dictates the development of that segment irrespective of whether or not the lower numbered genes are active. ● = active; ○ = inactive.
would have novel code words not normally found in any segment. If it is the combination of active genes rather than the highest numbered gene which is active that determines segmental stage, one might predict that all segments anterior to abdominal segment 2 would be normal, segment 2 would develop like segment 1, and the remaining posteriorly situated segments would be transformed into novel segment types intermediate in character between what they would normally be and the first abdominal segment. The predictions for the deletion would be similar except that abdominal segments 2–5 would all develop like the first abdominal segment. In general these predictions are borne out by our results in both the larva and the adult thereby providing further support for the general features of Lewis’s model. However, when examined in detail, they raise several questions about more specific features of the model some of which we consider below.

First, a major unresolved question is whether the \textit{BX-C} genes work in a combinatorial fashion as described above, or alternatively, in a hierarchical fashion such that the highest numbered gene active in a segment dictates the development of that segment irrespective of the activity of the lower numbered genes. Several aspects of our results can be interpreted as support for a combinatorial mechanism. One of the most striking results we observe is that all of the rearrangements we describe cause abdominal segments which one would predict are expressing a novel combination of \textit{BX-C} genes to develop into intermediate segment types. Moreover, we show that the particular intermediate pattern formed in a given segment depends on which, and how many, of the

\begin{verbatim}
H H H H H
T1 T1 T1 T1 T1
T2 T2 T2 T2 T2
T3 T3 T3 T3 T3
A1 A1 A1 A1 A1
(A3) (A3) (A3) (A3) (A3)
(A4) (A4) (A4) (A4) (A4)
(A5) (A5) (A5) (A5) (A5)
(A6) (A6) (A6) (A6) (A6)
(A7) (A7) (A7) (A7) (A7)
(A8) (A8) (A8) (A8) (A8)
\end{verbatim}

\begin{verbatim}
H H H H H
T1 T1 T1 T1 T1
T2 T2 T2 T2 T2
T3 T3 T3 T3 T3
A1 A1 A1 A1 A1
(A3) (A3) (A3) (A3) (A3)
(A4) (A4) (A4) (A4) (A4)
(A5) (A5) (A5) (A5) (A5)
(A6) (A6) (A6) (A6) (A6)
(A7) (A7) (A7) (A7) (A7)
(A8) (A8) (A8) (A8) (A8)
\end{verbatim}

Fig. 10. Predicted phenotypes of \textit{TP10} and \textit{DpP10; DfUbx\textsuperscript{109}} according to Lewis’s model. As described in the text, \textit{TP10} and \textit{DpP10; DfUbx\textsuperscript{109}} are assumed for the sake of simplicity to inactivate genes 2 and genes 2–5 respectively. The activity of each of the \textit{BX-C} genes is diagrammed as in Fig. 9 assuming either that the aberrations affect only genes 2 or 2–5 (left-hand matrix for each genotype) or that they have polar effects on more distal genes such that their activities are reduced in proportion to the proximity to the chromosomal breakpoint (right-hand matrices). If the \textit{BX-C} genes work combinatorially, one would predict the segmental transformations shown to the left of each pair of matrices. However, if they work in a hierarchical fashion, then one would predict these transformations only if both aberrations have polar effects as diagrammed.
Homeotic transformations of Drosophila abdominal segments

BX-C genes are present. These additive phenotypes suggest the possibility that each BX-C gene normally active in a segment influences the development of that segment in a qualitative fashion, and that the combination of these discrete influences normally dictates the particular pattern differentiated by the segment. Finally, we find that in both the larva and the adult, the intermediate segments form a graded series in which each mutant segment appears more like the normal abdominal segment than the preceding anterior segment. These phenotypic gradations may reflect the fact that each segment would be expected to approach the normal combination of active genes more closely than the preceding anterior segment.

If one could make the simplifying assumption that breakpoints and deletions of the BX-C affect the expression of only the genes actually broken or deleted, it is clear that these results would rule out a hierarchical model for BX-C gene function. However, this assumption is not valid because several examples of position effects between mutations in separable BX-C genes have been described (Lewis, 1954, 1955, 1963, 1964, 1978, 1981, 1982). Thus an alternative possibility is that the BX-C genes function in a hierarchical fashion, but that breakpoints or deletions lower the activity of distally situated genes in a polar fashion which decreases with distance. Accordingly, intermediate phenotypes would result from partial activity of the highest numbered gene which is active in a given segment. Fig. 10 illustrates how all of the particular phenotypes which can be accounted for by a combinatorial model can be equally well explained by a hierarchical model in which chromosomal rearrangements cause position effects on distal genes.

Recently Lewis (1981) has argued that the BX-C genes may function combinatorially by each specifying a particular subset of pattern elements; accordingly, the unique pattern of each segment would reflect a literal reading out of the cuticular elements specified by the active BX-C genes. If this view is correct, one would predict that the segmental patterns specified by two non-overlapping fragments of the complex would be strictly additive. However, we find that many of the intermediate segmental patterns resulting from TP10 and DpP10; DfUbx$^{109}$ are not strictly additive element by element (e.g., compare the eighth abdominal segments of DfP10, DpP10; DfP9, and TP10 larvae: Figs 6B, 8C and 8A). We therefore argue that the BX-C genes do not act by specifying subsets of discrete pattern elements, but that they discretely influence the developmental pathways of each segment as a whole. Hence the final patterns may be viewed as Gestalten with field properties rather than as assemblages of particular combinations of discrete elements.

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REFERENCES


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