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The anterobithorax and bithorax mutations of the bithorax complex

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The *antherobithorax (abx)* and *bithorax (bx)* genes together direct the development of the posterior second and anterior third thoracic segments of the fruit fly. We have characterized the phenotypes and DNA lesions of 19 *abx* and *bx* alleles. *abx* and *bx* mutations differ both in the nature and location of their DNA lesions, forming two clusters within a relatively small region of the *Ultrabithorax* transcription unit. Correlation between phenotype and DNA lesion suggests the presence of two or more genetic elements in this region distinct from the *Ultrabithorax* transcript. Mutant transformations do not strictly obey segmental or parasegmental boundaries. Most of the *bx* mutations result from insertions of the mobile element gypsy. The strength of these alleles varies in a regular way dependent on the position and orientation of the gypsy element. We propose models for gypsy element action and bithorax complex expression in the light of these results.

Key words: bithorax/homeotic mutations/gypsy element

Introduction

In 1915 Bridges isolated a mutation of *Drosophila melanogaster* which he called *bithorax (bx)*, because of its apparent partial duplication of the thorax. In the intervening years many other homeotic mutants have been found. All transform one body part towards another, and thus appear to affect genes which direct the course of development. Flies have a segmented body, with each segment following a unique developmental path. Bridges' *bx* mutant is now known to be part of a complex of genes which specify the identity of the second and third thoracic (T2 and T3) and the eight abdominal segments (A1-A8).

Many years of work, much of it by E.B.Lewis, have led to a genetic picture of the bithorax complex (BX-C; Lewis, 1978). The complex is an array of genes, each predominately responsible for specifying the identity of a given segment or part thereof. These genes are arranged on the chromosome in the same order as the body segments which they affect. Inactivating a given gene results in the affected segment following the developmental path of the adjacent more anterior segment. Thus, in the *bx* mutant mentioned above, the anterior part of T3 (T3a) is transformed towards the anterior part of T2 (T2a). This suggests that *bx* mutants affect sequences which code for or regulate the production of a product(s) necessary for the correct differentiation of anterior T3. Our current goal is to try to locate the genetic elements (regulatory or coding sequences) responsible for this effect.

The entire BX-C has now been cloned (Bender *et al.*, 1983a; Karch *et al.*, 1985). Mutant lesions spread over 300 kb of DNA. The BX-C can be split into the thoracic half, specifying posterior T2 (T2p), T3 and Ala, and the abdominal half, specifying A2 through A8. A map of the thoracic half is seen in Figure 1. Chromosomal rearrangement breaks within this half fall into two phenotypic classes, *Ultrabithorax (Ubx)* and *bithoraxoid (bxd)*. *Ubx* breaks fail to complement all of the recessive mutations of the thoracic half [*bx, anterobithorax (abx), postbithorax (pbx)*, and *bxd*]. *Ubx* homozygotes die as embryos or larvae, and show effects on all of the segments between T2 and A8. The *Ubx* breaks cover a region identical in extent to the '*Ubx*' transcription unit, as defined by several cDNAs (R.Saint and M.Goldschmidt-Clermont, personal communication; M.O'Connor, personal communication). Several transcripts share a 5' exon near -30 kb on the molecular map and a final exon near -105 kb, but they differ in their internal splicing pattern. One product is outlined in Figure 1. To the right of this transcripion unit lie the lesions responsible for the *bxd* and *pbx* mutations, which affect the differentiation of T3p, A1a, and (in the case of *bxd*), the larval abdominal segments. The *abx* and *bx* mutant lesions, which affect the development of T2p and T3a, lie within the transcription unit.

abx, bx, pbx and *bxd* mutations each result in a subset of the transformation seen in *Ubx* mutants. Each class of mutants must affect either regulatory or coding sequences active in all or part of a segment, while *Ubx* mutations must inactivate a product or products necessary in many segments. The identification of the genetic elements to the right of *Ubx* has begun. Different rearrangement breaks in the *bxd/pbx* region have graded effects on the fly's phenotype depending on their distance from the 5' end of *Ubx* (Bender *et al.*, 1985; Figure 1), suggesting the presence of multiple genetic elements in this region.

The differentiation of T2p and T3a is under the control of *abx* and *bx*. We have analyzed 19 different *bx* and *abx* mutations, comparing their phenotype and determining the nature of their DNA lesions. *bx* mutations are almost exclusively the result of mobile element insertions, mostly of the gypsy element. All three *abx* mutations result from deletions, which map to the left of most of the *bx* alleles. We will consider the mutations in classes sharing common DNA lesions, describing the phenotype and molecular lesion of each in detail.

Results

The thorax in wild type and Df(Ubx)^{109}/+

The thorax of the wild type fly, outlined in Figure 2, consists of three segments, the pro-, meso- and metathoraces (T1, T2 and T3). Ventrally all have a pair of legs, each pair unique in its bristle pattern and divided into anterior and posterior compartments (Steiner, 1976). Dorsal T1 includes a narrow collar in front of the notum, and perhaps the humeri (Figure 2). Dorsal T2 includes the notum, and the postnotum, a band of unbristled cuticle beneath the scutellum. The pre-sutural region, scutum, and scutellum, labeled in Figure 2, are subdivisions of the notum. T2 also carries the wings, which are divided into anterior and posterior compartments (Garcia-Bellido *et al.*, 1973), and the sternopleura, ventral cuticular plates with characteristic bristles. The notum and sternopleura are part of the anterior compart-

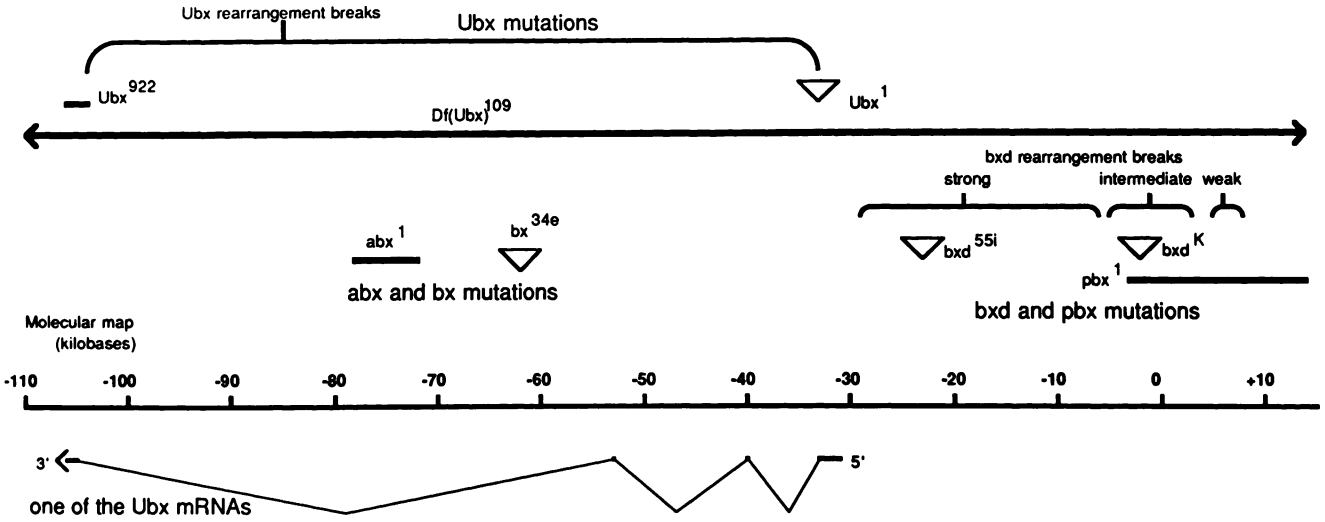

Fig. 1. Molecular map of the left half of the BX-C. The numbered line represents the DNA walk in kilobases from its starting point (Bender *et al.*, 1983a). Beneath this line is a diagram of one of the *Ubx* mRNAs, which is transcribed from right to left and has the four exons indicated (R.Saint and M.Goldschmidt-Clermont, personal communication; M.O'Connor, personal communication). Above are shown some representative mutations of the *Ubx*, *abx*, *bx*, *bxd* and *pbx* classes (Bender *et al.*, 1983a). Triangles indicate mobile element insertions; Ubx^1 has an insertion of the element Doc, while the others are gypsy elements. Thick black lines represent the extent of deletions. The regions interrupted by rearrangement breaks are indicated by brackets. Those in the *bxd* region are grouped by severity (Bender *et al.*, 1985).

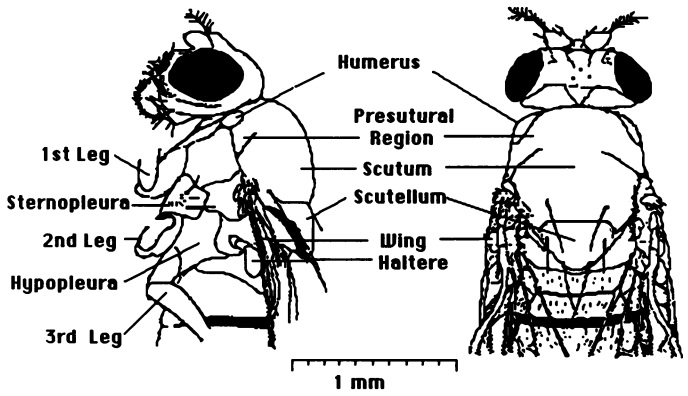

Fig. 2. Dorsal and lateral views of the thorax. On the left is a lateral view of the thorax of a wild-type fly, and a dorsal view is on the right. The legs are removed for clarity. Parts labeled are also described in the text. (Both diagrams are derived from a figure in Lindsley and Grell, 1968.)

ment of T2, while the postnotum is posterior (Lewis, 1951; Garcia-Bellido *et al.*, 1973; Steiner, 1976). T3 has little dorsal tissue. In place of the wing is the haltere, below which is the hypopleura which, unlike the sternopleura, has no bristles. The T2/T3 boundary runs through the hypopleura (Steiner, 1976).

bx and *abx* mutants transform T3a towards T2a to varying degrees. A complete transformation would change the anterior haltere to a copy of the anterior wing. The dorsal tissue of T3 would be transformed into a copy of the notum, referred to as the metanotum. Hypopleural plates would be replaced by sternopleural plates with their full complement of bristles. Finally, the anterior half of the third leg would have the bristle pattern characteristic of the second leg.

We compared the *bx* and *abx* mutations as hemizygotes, in *trans* to $Df(Ubx)^{109}$. This deficiency removes the entire thoracic half and part of the abdominal half of the BX-C (Figure 1; Lewis, 1978; Karch *et al.*, 1985), deleting *Ubx*, *bxd* and *iab-2* through *iab-4*. Outcrossing to $Df(Ubx)^{109}$ intensifies the phenotype of the

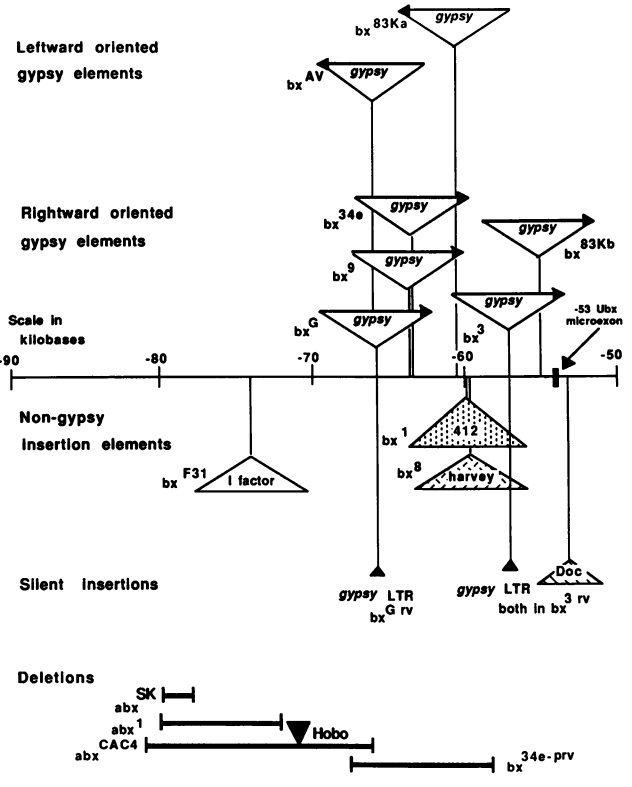

Fig. 3. Molecular map of the *abx* and *bx* lesions. At the center is an expanded part of the molecular map shown in Figure 1. The nature and position of mutant lesions are shown. Triangles indicate insertions of mobile elements, which are labeled according to the type of insertion. Heavy lines bounded by vertical bars represent deletions of DNA. Lesions are grouped according to their nature. Above the line are the mutations caused by gypsy element insertions. They are divided into two groups according to their direction of transcription, which is indicated by the arrows. Beneath the line are three other groups of mutations, those caused by the insertion of other mobile elements, the insertions without phenotypic effect, and the mutations which result from deletions. The deletion in abx^{CAC4} is accompanied by an insertion of the mobile element Hobo.

Table I. *abx* and *bx* mutations

Mutant	Discoverer	Origin	Location and nature of DNA lesion	Transformation of				No. showing *ppx* effect
				Haltere	Metanotum	Hypopleura	T3a leg	
$Df(Ubx)^{109}$	E.B.Lewis	X-rayed *gl e*	Deletion of thoracic half to + 90 Kb	3% wing size	None	None	Weak	1/53 2%
Gypsy insertions								
bx^{G}	M.Gans	Spontaneous	Gypsy at −66 kb oriented left to right	5−7.5% wing size	Patches or narrow interrupted band	3−5 bristles	Strong	1/47 2%
bx^{AV}	A.Rosenfeld	Probably spontaneous	Gypsy at −66 kb oriented right to left	Up to 15% wing size	Tripartite up to 2 × A1 tergite width	8−9 bristles	Strong	4/54 7%
bx^{9}	D.Kuhn and E.B.Lewis, 1981	Spontaneous	Gypsy at −64 kb oriented left to right	15% wing size	Small patches at most	5−6 bristles	Strong	8/55 15%
bx^{34e}	Schultz, 1934	Spontaneous	Gypsy at −63.5 kb oriented left to right	10−15% wing size	Band 1−1.5 × A1 tergite width	8−9 bristles	Strong	0/60 <2%
bx^{83Ka}	D.Kuhn, 1983	Spontaneous on *tuh-3*	Gypsy at −61.5 kb oriented right to left	15−20% wing size	Three-fourths have complete metanotum	8−10 bristles	Strong	2/53 4%
bx^{3}	C.Stern, 1925	Spontaneous on *al*	Gypsy at −57 kb oriented left to right + Doc element at −53 kb	20−25% wing size	Large tripartite or complete (<40%)	8−10 bristles	Strong	5/49 10%
bx^{83Kb}	D.Kuhn, 1983	Spontaneous on *tuh-3*	Gypsy at −55 kb oriented left to right	20−25% wing size	About half have complete metonotum	9−10 bristles	Strong	2/57 4%
Deletions								
abx^{1}	E.B.Lewis, 1959	X-rayed Canton S female	Deletion between −79 and −73 kb	Variable, often 15−20% wing size	Variable, often medium to large but tripartite	Usually 8−9 bristles	Strong	8/51 16%
abx^{SK}	S.Kerridge	X-ray mm[a]	Deletion between −79 and −77.5 kb	Quite variable, 3−20% wing size	Quite variable, none to large tripartite notum	Usually 4−8 bristles	Strong	19/42 45%
abx^{CAC4}	P.Adler	P−M dysgenesis	Deletion between −80 and −66 kb + Hobo insertion	Variable, usually 15−30% wing size	Variable, 1/3 have complete notum	Usually 8−10 bristles	Strong	8/57 14%
$bx^{34e\text{-}prv}$	E.B.Lewis	X-rayed bx^{34e}	Deletion between −66.5 and −57 kb	Variable 3−15% wing size	Variable, none to complete heminotum	0−2 bristles	Moderate	0/49 <2%
Other insertions								
bx^{1}	C.Bridges, 1915	Spontaneous	Insertion of 412 element at −60 kb	Variable 3−15% wing size	None to small	2−3 bristles	Weak	0/51 <2%
bx^{8}	E.B.Lewis, 1965	EMS treated Oregon R male	Insertion of Harvey element at −59.5 kb	20−30% wing size	Usually complete metanotum	9−10 bristles	Strong	6/55 11%
bx^{F31}	P.Adler, 1982	Spontaneous	Insertion of I factor at −74 kb	<10% wing size	Small patch	1−2 bristles	Weak	0/56 <2%
Complete revertants								
bx^{3rv}	E.B.Lewis	Spontaneous in sbd^{2} bx^{3}	Gypsy LTR at −57 kb + Doc element at −53 kb	Completely wild type				
bx^{Grv}	M.Peifer, 1984	Spontaneous in bx^{G}	Gypsy LTR at −66 kb	Completely wild type				
Other mutations								
bx^{4}	E.B.Lewis	Isoallele in RuPrica stock	No change detected	3−5% wing size	<5% have small patch	0−3 bristles	Weak	2/46 4%
bx^{1s}	G.Ising	Spontaneous	Identical to bx^{1}					
bx^{AF}, bx^{AF2} and bx^{X}	Caltech stocks	Spontaneous	All identical to bx^{34e}					

[a]mm indicates the stock *mwh jv st red sbd* e^{11} *ro ca.*

mutations and neutralizes the effect of recessive modifiers. $Df(Ubx)^{109}$/+ flies have halteres which are 2−3 times their normal size (Figure 4; Table I), a result of *Ubx* haplo- insufficiency. Such flies never have hypopleural bristles or metanotal tissue, but many $Df(Ubx)^{109}$/+ flies show a weak transformation of anterior T3 leg to anterior T2 leg. We also crossed some *bx* and *abx* mutations to Ubx^{1} (Lindsley and Grell, 1968) or Ubx^{922} (Kerridge and Morata, 1982). These mutations result, respectively, from insertion of the mobile element Doc into the 5′ exon of the *Ubx* transcription unit, and from a deletion in the 3′ *Ubx* exon (Bender *et al.*, 1983a; Figure 1). They have slightly weaker effects than $Df(Ubx)^{109}$.

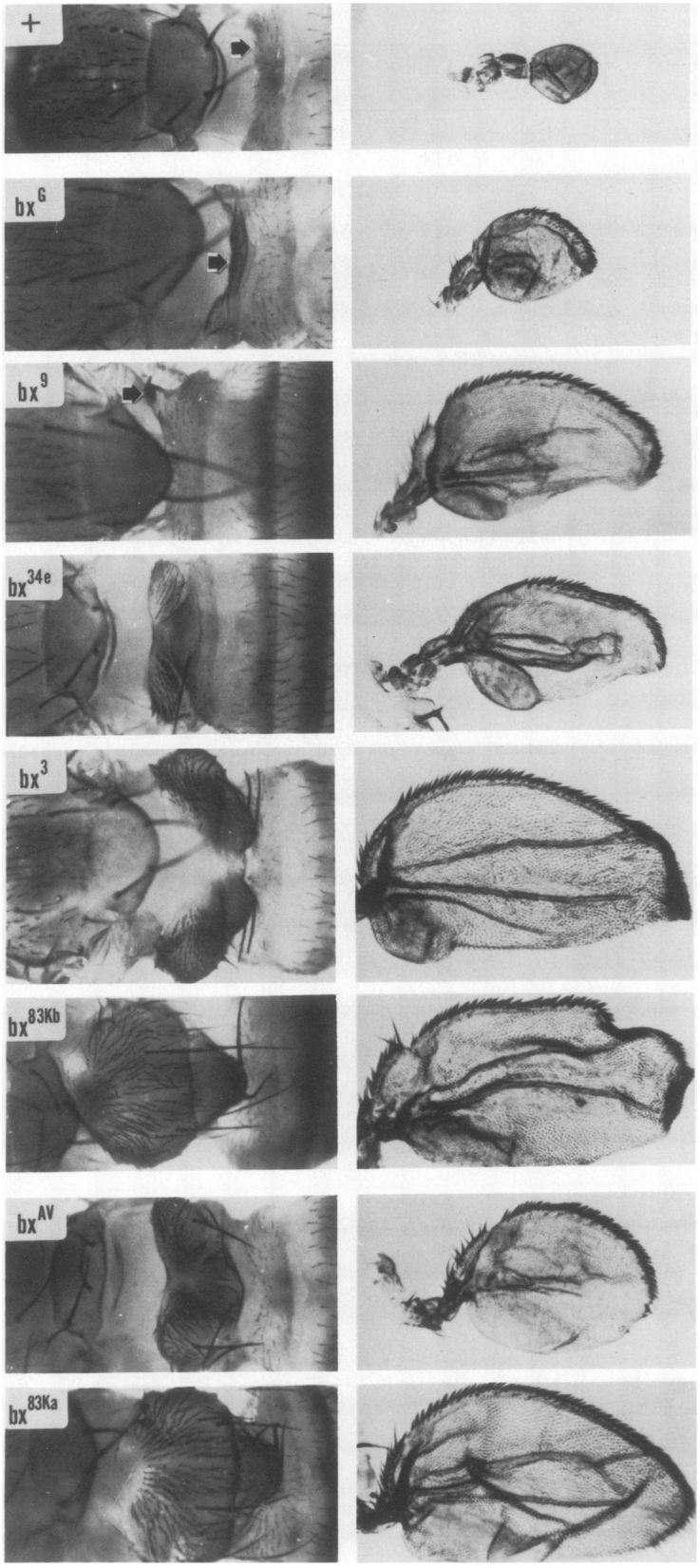

Fig. 4. Phenotypic comparison of the different gypsy-induced *bx* alleles. The left series of photographs compares the metanotal transformation in different mutants. The right series compares haltere transformation. +, the top pair of photographs are from $Df(Ubx)^{109}$/+ individuals. The arrow in the left photograph points to the junction between the postnotum of T2 and the A1 tergite, where metanotal tissue appears in the mutants. bx^x the gypsy-induced *bx* mutants are displayed as hemizygotes over $Df(Ubx)^{109}$. bx^G through bx^{83Kb} are mutants with gypsy elements in the left to right transcriptional orientation, while the bottom pair of photographs show bx^{AV} and bx^{83Ka}, mutants with gypsy elements in the opposite orientation (Figure 3). Arrows in the left photographs of bx^G and bx^9 point to the patches of transformed metanotal tissue seen in these individuals. Metanotal structures in the other mutants are more obvious.

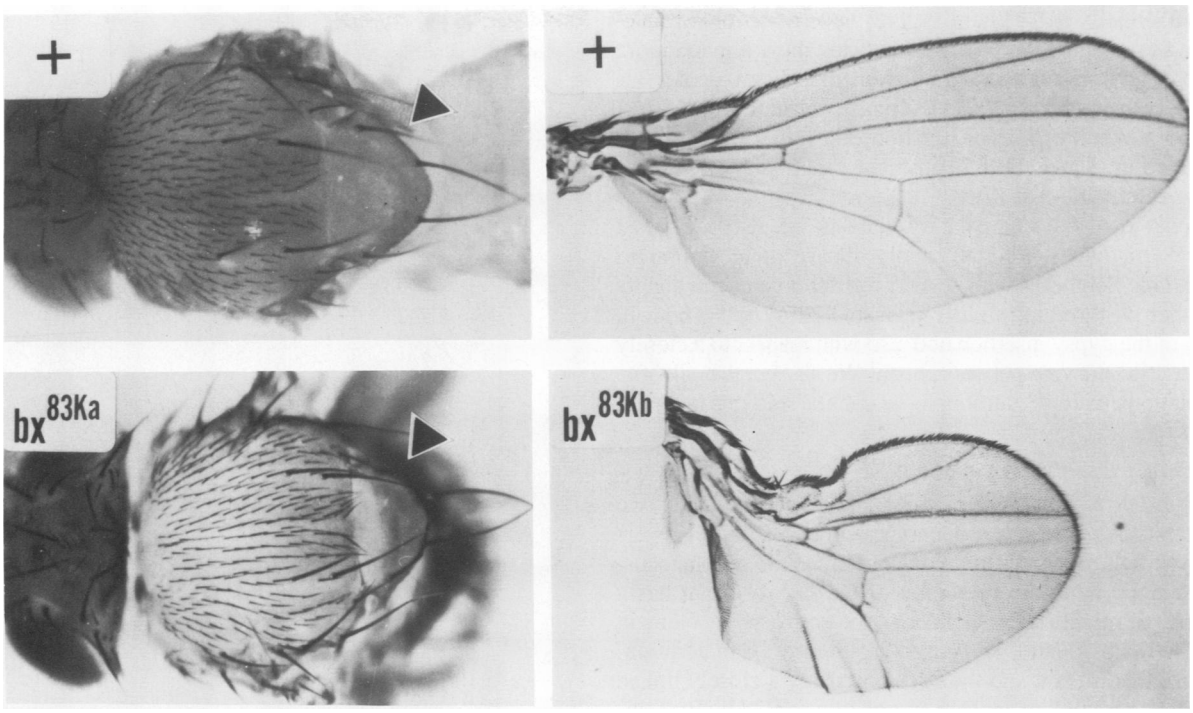

Fig. 5. Reduction of T2 by the gypsy-induced *bx* mutants. The left pair of photographs compares the mesonotum from a $Df(Ubx)^{109}$/+ adult (**top**) with a similar mesonotum from a $bx^{83Ka}/Df(Ubx)^{109}$ individual (**bottom**). Arrowheads highlight the scutellum of both flies, where the greatest difference is seen. The right pair of photographs compares a wing from a $Df(Ubx)^{109}$/+ adult (**top**) with a much reduced wing from a $bx^{83Kb}/Df(Ubx)^{109}$ individual (**bottom**). A minority of wings (10%) show such reduction, and the reduction in amount of wing tissue can occur in the anterior wing, posterior wing, or both.

Gypsy insertions

Many of the recessive mutations of the BX-C are suppressible by mutations of the *suppressor of Hairy wing* [su(Hw)] locus. Most mutants which *su(Hw)* suppresses are caused by the insertion of the mobile element gypsy (Modelell *et al.*, 1983; Bender *et al.*, 1983a). E.B.Lewis had previously examined many *bx* and *abx* mutations for their response to *su(Hw)*, checking abx^1, bx^G, bx^1, bx^3, bx^8, bx^9 and bx^{34e} (Lindsley and Grell, 1968, and personal communication). We tested the other *abx* and *bx* mutations, with the exception of abx^{SK}. Seven of the *bx* mutants, all the result of gypsy element insertions, are suppressed by *su(Hw)*. The other *abx* and *bx* mutations, which result from other mobile elements or deletions, are not suppressible.

Genomic Southerns of mutant DNA were done for each *abx* and *bx* mutant between −80 kb and −50 kb on the molecular map. We constructed genomic libraries from nine mutants. Each mutant had only one lesion. Five others, bx^1, bx^3, bx^{34e}, abx^1 and abx^{SK}, were described by Bender *et al.* (1983a). Libraries were screened for phage with inserts from the appropriate region, and these phage were characterized by restriction mapping and heteroduplex analysis.

Figure 3 shows the composite map of all of the identified *abx* and *bx* lesions. All seven suppressible mutants have a gypsy element inserted between −66 and −55 kb. Five of the gypsy elements are inserted such that they are transcribed left to right on the map; two others are inserted in the opposite orientation. All seven gypsy elements are identical in restriction map, with one exception. The element bx^{34e} has a deletion of about 110 bp near the 5′ end of the internal region, relative to the other elements, a difference also found in other copies of gypsy in the genome. Comparison of two such gypsy variants inserted at the same site in the *bxd* region suggests that the longer variant is stronger in its effect on *bxd* (unpublished data). bx^{83Ka} and bx^{83Kb} arose simultaneously in the *tuh-3* stock of D.Kuhn. Six of the 14 gypsy-induced mutations of the BX-C arose in this stock; five of these occurred in two clusters (bx^9, bxd^9 and $iab\text{-}2^K$ arose together; Kuhn *et al.*, 1981; and E.B.Lewis, personal communication). The *tuh-3* stock has a highly elevated copy number of gypsy elements (unpublished data).

The gypsy-induced mutations form a monotonic phenotypic series that varies with the orientation and position of the gypsy insertion. A detailed description of the mutant phenotype of each allele is given in Table I, and transformed halteres and metanota are shown in Figure 4. The weakest mutation is that with the leftmost insertion, bx^G, while the rightmost gypsy, bx^{83Kb}, has the strongest phenotype. The two mutations with insertions in the right to left orientation are stronger than expected from their positions, but between them the rightmost is most extreme. The one major deviation from this rule involves bx^9 and bx^{34e}. bx^9 hemizygotes have a more extreme haltere transformation, while bx^{34e} hemizygotes have a more extreme metanotal effect. When examined over Ubx^1 or Ubx^{992}, however, bx^{34e} is clearly more severe than bx^9 for both transformations (data not shown). This deviation may be due at least in part to the difference in the structure of the gypsy elements in these two mutants mentioned above.

A number of the most severe gypsy-induced *bx* alleles also show effects on T2 (Figure 5). Lewis (1981a) noted that bx^3 has a dominant effect on the mesonotum, resulting in a slight reduction of the pre-sutural region. We observe a subtle reduction of the entire mesonotum, most noticeable as a reduction in the length of the scutellum, in bx^3, bx^{83Ka} and bx^{83Kb} hemizygotes. A small number (10%) of bx^{83Ka} and bx^{83Kb} hemizygotes have wings which are reduced in size. This reduction in the amount of wing tissue is clearly distinguishable from failure of wing expansion following eclosion, and is not seen in abx^{CAC4}/Ubx^{109} flies, which have comparably severe transformations of haltere and

metanotum. Finally bx^{83Ka} hemizygotes have occasional reduction of the sternopleura. bx^{83Kb} homozygotes show a much more severe notal and sternopleural reduction than hemizygotes, as Lewis observed with bx^3 (1981a). This suggests that the effect on T2 is not a result of loss of function, but rather due to some gain of function. Lewis (1985) postulates that such effects are due to hyperactivation of *abx*.

In the course of our survey of *bx* alleles we also examined bx^{AF}, bx^{AF2} and bx^{X}. Phenotypically all are quite similar to bx^{34e}. Genomic Southerns with several restriction enzyme digests revealed that all three are indistingushable from bx^{34e}, both in the region of the gypsy insertion and also with respect to a closely linked restriction enzyme polymorphism. We suspect that all three are re-isolations of bx^{34e}, although we cannot rule out independent events.

Revertants of gypsy-induced mutants

We compared three revertants of gypsy-induced *bx* mutants, two of which restore a wild type phenotype and one a partial revertant. The two full revertants are similar. Both arose spontaneously. DNA cloned from the *bx* region of the bx^3 revertant has a single gypsy terminal repeat in place of the entire gypsy element in bx^3 (at −57 kb; Bender *et al.*, 1983a; Figure 3). In addition, both the original mutant and the revertant carry a closely linked copy of the mobile element Doc at position −53 kb. Both inserts remaining in the revertant are without noticeable phenotypic effect. Analysis of the bx^{G} revertant by genomic Southerns suggests that it also retains a single gypsy terminal repeat at position −66 (Figure 3; data not shown). Similar revertants have occurred for the gypsy in *bxd* (Bender *et al.*, 1983a and unpublished data) and in *scute* (Campuzano *et al.*, 1985), as well as for the copia element in w^a (Carbonare and Gehring, 1985).

The third revertant examined was a partial revertant of bx^{34e} ($bx^{34e\text{-}prv}$), which arose after treatment with X-rays (E.B.Lewis, personal communication). It is quite variable in phenotype, but is on average less severe than the original mutant (Table I). The variability in phenotype is reflected in a number of ways. The degree of transformation differs dramatically between flies of this genotype, between the two sides of a fly, and also between neighboring groups of cells within a given structure (Figure 6). Genomic Southern analysis, and the examination of a clone from a genomic library of this mutant revealed that reversion results from the deletion of the gypsy element found in the original mutant, along with 9.5 kb of wild-type DNA between positions −66.5 and −57 kb (Figure 3).

Non-gypsy insertions

Three of the *bx* alleles have insertions of DNA other than the gypsy element. One of them, bx^1, has a weak and variable phenotype, detailed in Table I. bx^1 has a copy of the transposable element 412 (Finnegan *et al.*, 1978) at position −60 kb (Bender *et al.*, 1983a; Figure 3). We also examined another allele, bx^{Is}, which resembles bx^1 in phenotype. Genomic Southerns and recloning reveal a lesion indistinguishable from that in bx^1; the two mutants also share two closely linked restriction site polymorphisms. bx^{Is} is probably a re-isolation of bx^1.

A second allele, bx^8, arose after ethyl methanesulphonate (EMS)-treatment of Oregon-R (Lewis, 1980). It is much stronger and less variable than bx^1, resembling the strongest of the gypsy-induced *bx* alleles (Table I). Like these alleles, it shows wing reduction, and also has occasional fusion of the metanotal tissue of T3 with the mesonotum of T2, resulting in a hybrid notum. Clones derived from a library of this mutant have 7.2 kb of DNA inserted at position −59.5 kb relative to Oregon R (Figure 3).

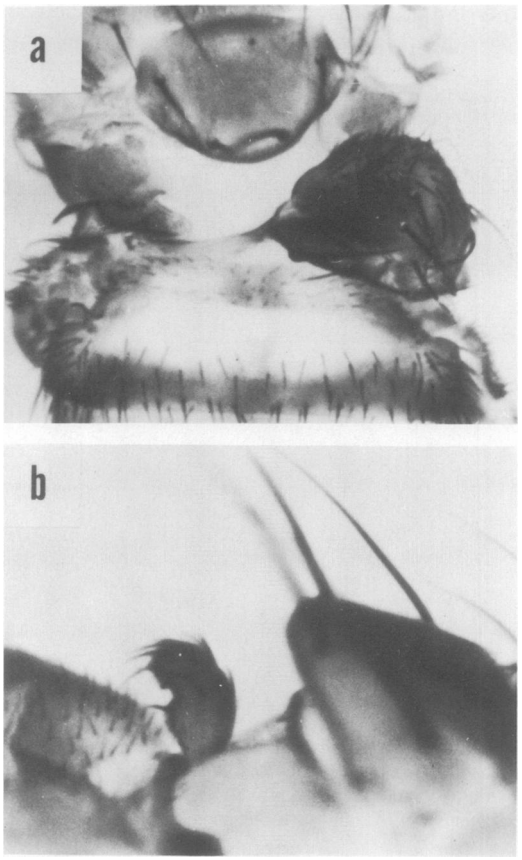

Fig. 6. Phenotypic variability, between and within flies, is associated wtih deletions (**a**) $abx^{SK}/Df(Ubx)^{109}$ adult. This individual shows extreme metanotal transformation on the right side and little transformation on the left. (**b**) $bx^{34e\text{-}prv}/Df(Ubx)^{109}$ adult. A small group of dorsal T3 cells are transformed toward mesonotum, while their neighbors are untransformed.

The insert is repetitive and its internal map is conserved (data not shown), and thus it is probably a mobile element. As it differs in restriciton map from known mobile elements, we named it Harvey. bx^8 has no other change in the *bx* region not explicable as a restriction enzyme polymorphism, implicating Harvey as the cause of the mutation.

The third is a spontaneous mutant, bx^{F31}, isolated by P.Adler. It is quite weak but consistent in phenotype (Table I). Recombinant phage from a library of mutant DNA have an insertion of the I factor (Bucheton *et al.*, 1984) at position −74 kb (Figure 3). Because this lesion is to the left of all of the other *bx* mutations, we examined recombinant phage covering the rest of the *bx* region. These show no change not explicable as a polymorphism, supporting the conclusion that the I factor is the cause of the *bx* phenotype.

abx deletions

E.B.Lewis renamed bx^7 as abx^1, because it can be distinguished from the *bx* alleles by several criteria (Lewis, 1980, 1981a,b). Its effect on the most proximal tissue of the haltere and the metanotum is stronger than that of the *bx* alleles. In particular, abx^1 produces a well formed costal region and a humeral crossvein on the transformed haltere, and a well formed pre-sutural region of the metanotum. When combined with *Rg-bx*, an enhancer of recessive BX-C transformations, hemizygotes have an extra humerus. Hemizygotes hold their wings straight up, suggesting an effect on the flight muscles. All but the last of these

transformations are extremely variable, between and within flies. Finally, *bx* and *abx* alleles partially complement; in other words, *trans*-heterozygotes are quite a bit less severe than either respective homozygote.

Two other mutants share these characteristics; abx^{SK}, isolated by S.Kerridge (called abx^{2} by Bender *et al.*, 1983a) and abx^{CAC4}, isolated by P.Adler. We compared the three mutants as heterozygotes with abx^{CAC4}, Ubx^{1}, or Ubx^{922}, and as hemizygotes. While their variability as hemizygotes obscures this comparison, heterozygotes with Ubx^{1} or Ubx^{922} allow easy differentiation among the three mutants, since such flies are much less variable than *abx* hemizygotes. Both notal and haltere transformation are clearly most severe for abx^{CAC4}, and least severe for abx^{SK}. This order of severity is supported by the other mutant combinations (Table I).

All three *abx* mutations have the same sort of DNA lesion. Both abx^{1} and abx^{SK} result from deletions of DNA; abx^{SK} deletes 1.5 kb between −79 and −77.5 kb and abx^{1} deletes 6 kb between −79 and −73 kb (Figure 3; reported by Bender *et al.*, 1983a). We recloned DNA from the *bx* region of abx^{CAC4}. It deletes 16 kb between −82 and −66 kb, but also has 1.5 kb of DNA inserted between the deletion endpoints (Figure 3). This insertion cross hybridizes with a cloned copy of the Hobo element (McGinnis *et al.*, 1983). As abx^{CAC4} arose during a P−M dysgenic cross, this provides further evidence that Hobo as well as P is mobilized during dysgenesis.

A possible point mutant

The weakest *bx* allele among those we examined is bx^{4} (Lewis, 1980). It maps near or in the BX-C by recombination. It is nearly wild type as a homozygote, but hemizygotes have a weak *bx* phenotype (Table I). We have examined the *bx* region of bx^{4} flies by genomic Southerns and have found no noticeable change from the restriction map of the wild type Canton S, other than loss of the *Bam*HI site at position −58 kb and the gain of a *Sal*I site at position −77 kb. Both are polymorphisms observed in other stocks. It is thus possible that bx^{4} is a true point mutation, although our analysis could have missed small (≤200 bp) deletions, insertions or other rearrangements. It also remains possible that bx^{4} is a mutation in some other part of the BX-C, such as *Ubx*, or even outside but closely linked to th BX-C. We consider these possibilities less likely, because of the congruence in phenotype between bx^{4} and other *bx* alleles.

The ppx transformation and larval effects

Homozygous clones of *Ubx* tissue in the posterior compartment of the T2 and T3 legs are transformed toward the posterior compartment of the T1 leg (Morata and Kerridge, 1981). This *postprothorax (ppx)* effect also occurs to a variable degree in *abx* and *bx* alleles (Casanova *et al.*, 1985). We examined each mutant for its effect on the posterior compartments of the T2 and T3 legs, to extend this examination. Legs were scored for *ppx* transformation by looking for characteristic T1 bristles on the T2 femur; if two or more such bristles were seen the leg was considered transformed. As in the alleles examined by Casanova *et al.*, no effect was seen on the posterior third leg, but as hemizygotes many of the mutants show a variable transformation of the posterior second leg, as summarized in Table I. In contrast to previous results, however, there seemed to be only a slight correlation between the class of mutant and the extent of the *ppx* transformation of hemizygotes. abx^{SK} had a much more penetrant leg transformation than any of the other mutants, but there was little difference between the other two *abx* mutants and several of the gypsy-induced *bx* mutants. There seemed to be no correlation between the severity of a gypsy-induced *bx* as measured by other transformations, and the penetrance of its *ppx* transformation, but the weaker non-gypsy *bx* mutants showed little if any *ppx* effect. The difference between our results and those of Casanova *et al.* may reflect differences in genetic background of the mutant combinations tested.

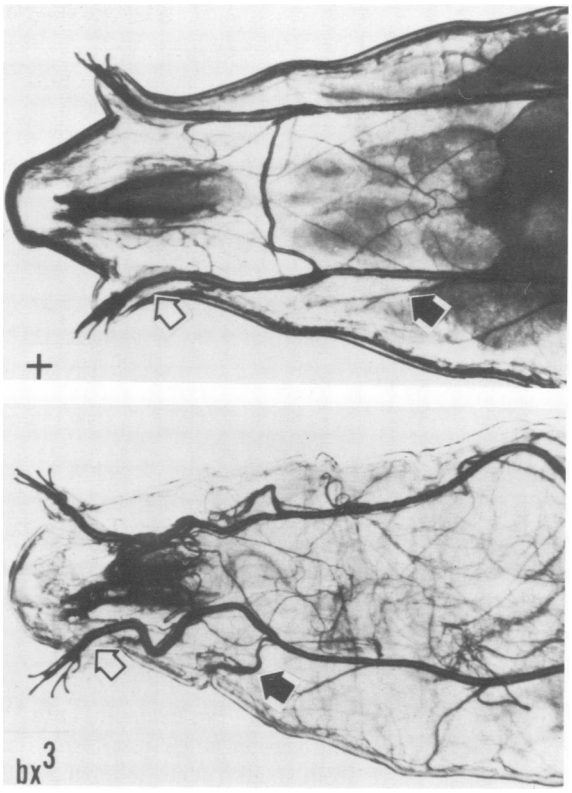

Fig. 7. Larval transformation. +, $Df(Ubx)^{109}$/+ third instar larva. A white arrow points to the tracheal trunk leading to the anterior spiracle at the anterior edge of T2, while a solid arrow indicates the analogous tracheal branch in T3. bx^{3}, $bx^{3}/Df(Ubx)^{109}$ third instar larva. A white arrow again indicates the tracheal trunk leading to the anterior spiracle, while a solid arrow indicates the partially transformed tracheal trunk found in T3.,

We also scored two of the mutants for larval effects. Homozygous *Ubx* larvae show a reiteration of the anterior spiracles, usually found at the anterior edge of T2, in T3 and A1. We examined bx^{3} and abx^{SK} hemizygous larvae for such an effect. Both second and third instar larvae of both genotypes show clear but partial reiteration of the anterior spiracles in T3 but not in A1 (first instar larvae were not scored as they lack these distinctive structures). All hemizygous bx^{3} larvae have enlarged T3 tracheal trunks, similar to those seen in T2. Occasional individuals also show rudimentary spiracles in T3 (Figure 7 illustrates such an individual). Larval transformation is variable in abx^{SK}, with most individuals transformed only unilaterally.

Transvection tests

One distinctive feature of the BX-C is transvection (Lewis, 1954), the synapsis-dependent complementation seen in certain mutant combinations. For example, bx^{34e}/Ubx^{1} flies show little metanotal transformation. When a rearrangement is introduced onto either chromosome which disrupts pairing of the BX-C of the salivary gland polytene chromosomes, and presumably also of the chromosomes of the rest of the fly, there is a significant metanotal transformation. Lewis has tested many mutant combinations for transvection (Lewis, 1985 and personal communication) and we tested several more (Table II).

Table II. Transvection tests

Mutant vs.	Mutant				
	Ubx^{1}	*Ubx*^{992}	*bx*^{3}	*bx*^{34e}	*abx*^{1}
Ubx^{1}	–	No^{L}	Yes^{L}	Yes^{L}	Yes^{L}
Ubx^{195}	n.d.	n.d.	Yes	n.d.	Yes
bx^{34e}	Yes^{L}	Yes^{L}	No^{L}	–	Yes^{L}
bx^{3}	Yes^{L}	Yes^{L}	–	No^{L}	Yes^{L}
bx^{1}	Yes^{L}	n.d.	n.d.	n.d.	n.d.
bx^{AV}	Yes	n.d.	No	n.d.	Yes
bx^{8}	Yes	n.d.	No	n.d.	Yes
bx^{F31}	No	n.d.	No	n.d.	n.d.
bx^{4}	No	n.d.	No	n.d.	Yes
abx^{1}	Yes^{L}	Yes^{L}	Yes^{L}	Yes^{L}	–
abx^{SK}	Yes	n.d.	n.d.	n.d.	Yes/ No
abx^{CAC4}	Yes	n.d.	n.d	n.d.	No

Pairs of alleles were tested for tranvection as described in the Materials and methods. A yes indicates that the mutant phenotype of a given pair of alleles increases in severity in the presence of a rearrangement. Entries with superscript L were determined by E.B.Lewis; n.d. not done.

Transvection between gypsy-induced *bx* mutants (and also *bx*^{8}) and *Ubx* mutants results primarily in significant alteration in the degree of metanotal transformation. For example, in *bx*^{AV}/*Ubx*^{1} heterozygotes, a transvecting rearrangement results in an increase in new metanotal tissue from small patches to a band of 2/3 the width of the first abdominal tergite. Haltere size, however, increases by <25%. In contrast, transvecting rearrangements result in no noticeable change in the phenotype of two of the weaker *bx* alleles, *bx*^{4} and *bx*^{F31}. It may be that any effect on these alleles is so weak as to be obscured by the variability in the small sample we examined. Pairs of *bx* alleles do not transvect with each other. *abx* transvection is less clearcut, in part because of the variability of *abx* mutant combinations, and involves approximately equal changes in both metanotal and haltere transformation. An example of the effect is that seen in *abx*^{1}/*Ubx*^{1} heterozygotes. In the presence of a transvecting rearrangement, a patch of metanotal tissue appears where there is usually none, and average haltere size increased by ~50%. The status of transvection between *abx* alleles remains ambiguous, since two different rearrangements used to test transvection between *abx*^{SK} and *abx*^{1} yield differing results. Finally, in spite of the proximity of the *bx* and *abx* lesions to the −53 kb microexon of the *Ubx* transcript, *Ubx*^{195}, a *Ubx* point mutant localized to this microexon (M.Akam, personal communication), transvects with *bx* and *abx* to the same degree as *Ubx* mutants affecting the 5′ and 3′ exons.

Discussion

abx and *bx* are the best characterized of the recessive alleles of the BX-C. We have compared DNA lesions and the resulting phenotypes in 19 *abx* and *bx* alleles. We will consider below the mobile element insertions, focusing on those caused by the gypsy element. Next we will discuss the effects of the different deletions. We will then examine the implications of these findings for the nature of the elements affected by *abx* and *bx* mutations and present a model for the expression of the BX-C based on these conclusions.

Different mobile elements act in different ways

70% of the *bx* insertions are of the gypsy element, a set coincident with the mutations suppressible by *su(Hw)*. This confirms gypsy prevalence among spontaneous mutations of the BX-C (Bender *et al.*, 1983a; Karch *et al.*, 1985), unlike other loci such as *white* (Zachar and Bingham, 1982) or *rosy* (Coté *et al.*, 1985). The mobile elements most common in spontaneous mutants, B104 and copia, are not associated with any BX-C mutations. While it is possible that local or regional target specificity is the cause of this bias, we suspect otherwise. Many mobile element insertions in the BX-C may be phenotypically silent, like the Doc element and the gypsy LTRs in the two complete revertants. Gypsy elements may act at a greater distance, by a unique mechanism, or with a particular tissue specificity, making them better mutagens of the BX-C.

Different mobile elements have quite different effects. The elements in *bx*^{8} and *bx*^{1}, inserted within several hundred base pairs of each other, cause mutations at the two ends of the phenotypic severity scale. Both differ significantly in severity from the nearest gypsy insertions. *bx*^{1} is variable in phenotype while *bx*^{8} is not. Different mobile elements also have discordant effects on various parts of the fly. For example, while *bx*^{F31} and *bx*^{G} have similar haltere and notal transformation, the effect of *bx*^{G} on the hypopleura and leg is stronger than that of *bx*^{F31}.

Evaluating models of gypsy action

The five mutations with gypsy inserted in the left to right transcriptional orientation show a monotonic progression of phenotype. The leftmost insertion (*bx*^{G}) is nearly wild type, and the phenotype increases gradually, such that the rightmost insertion (*bx*^{83Kb}) is the most severe. A simple model is that the insertions affect something to the right of −55 kb, proximity to which determines the severity of transformation. The two mutations with gypsy insertions in the opposite transcriptional orientation complicate this picture. *bx*^{AV} and *bx*^{83Ka} are more severe than predicted by their positions, though once again the rightmost insertion is most severe. The simplest suggestion to explain this difference is that a sequence near the 5′ end of the gypsy affects something to the right of −55 kb. Thus a gypsy element in the right to left orientation, with its 5′ end 7 kb (the length of the element) further to the right, would be more severe than an element in the same position in the opposite orientation. *bx*^{AV} and *bx*^{83Ka} also have a more severe effect on the notum relative to the haltere, compared to the other gypsy-induced *bx* alleles. Phenotypic gradation is also observed with the *bxd* gypsies (Bender *et al.*, 1983a and unpublished results).

In the light of this correlation we can begin to evaluate models for gypsy interference with BX-C function. Insertional interruption of coding sequence has been ruled out (Bender *et al.*, 1983a). A second model postulates tissue-specific termination of BX-C transcription within the gypsy element, mediated by the *su(Hw)* gene product, analogous to that suggested to occur in the copia element-induced allele *white apricot* (*w*^{a}; Levis *et al.*, 1984). This model has several difficulties. Rearrangement breaks, which cause unconditional termination, result in a *Ubx* rather than a *bx* phenotype (Bender *et al.*, 1983a). Second, this model requires that gypsy contain a bidirectional terminator or two terminators in opposite orientations, both responsive to *su(Hw)*. Finally, it seems unlikely that, if all seven gypsies are within the intron of a *bx* or *Ubx* product, termination of the transcript at different points in this intron would result in strikingly different phenotypes.

In the vicinity of −55 kb there is only one known element with which gypsies could interfere. This is an exon of some *Ubx* mRNAs, found at position −53 kb (Figure 1; R.Saint and M.Goldschmidt-Clermont, personal communication: M.O'Connor, personal communication). Gypsy elements might interfere

with splicing of certain compartment-specific *Ubx* products to a degree that depends on proximity to the exon−intron boundary, producing the subset of the *Ubx* phenotype that *bx* represents. This leaves numerous unanswered questions, however, such as why analogous sets of insertions upstream or downstream of the other exon−intron junctions of *Ubx* are not found. We feel that an effect of gypsy elements on splicing is unlikely, as numerous *bxd* mutations, which map some distance upstream and thus outside of the *Ubx* transcription unit, result in similar mutant effects, though in different segments.

Parkhurst and Corces (1985) proposed a transcriptional interference model for gypsy action, analogous to the effect of Ty elements in yeast (Silverman and Fink, 1984). They have shown that a gypsy insertion near or in the *forked* gene reduces the level of stable mRNA without resulting in shortened transcripts like those seen in w^a. The only known transcription unit in the *bx* region is that of *Ubx*, whose 5′ end is at −30 kb, 25 kb upstream of the nearest *bx* gypsy. Effects on an element at the 5′ end of *Ubx* would be at a remarkable distance; it is odd that insertions closer to its 5′ end are not found. It is also difficult to explain why the *bx* and *bxd* gypsies, approximately equal in distance from the 5′ end of *Ubx* and indistinguishable in structure, affect quite different segments. It is possible that there are as yet uncharacterized BX-C products with a promoter near −55 kb. This would still require that gypsy act bidirectionally at a distance of >9 kb.

Parkhurst and Corces postulate that gypsy-induced mutations occur in genes expressed at a time and place in which gypsy is expressed. They demonstrate that gypsy expression is maximal during mid-pupation. While many suppressible genes are expressed maximally during this time, gypsy-induced mutations of the BX-C affect a variety of tissues and times. The adult phenotypes are in part the result of events in mid-pupation. bxd^1, however, affects the embryonic and larval cuticle (Lewis, 1978) and the larval nervous system (Ghysen *et al.*, 1985). Likewise, bx^3 affects the larval T3 imaginal discs (Lewis, 1963) and the larval tracheal system (Figure 7).

Deletions − their similarities and differences

All four deletions we examined are quite variable in phenotype, in contrast to most of the insertions. This variability is not only manifested in differences between flies. Individuals often have one side extremely transformed and the other side overlapping wild type (Figure 6a). Less often, there is extreme transformation of one structure (such as the metanotum) while a neighboring structure (such as the haltere) is only slightly affected. In $bx^{34e\text{-}prv}$, neighboring groups of cells within a structure can differ radically in their degree of transformation (Figure 6b). It is mysterious why variability is a feature of deletions, which in a simplistic view should cause consistent, complete loss of function.

The differences in both phenotype and DNA lesion between *abx* and *bx* leads one to question whether *bx* and *abx* mutations affect different genetic elements, or affect the same element in different ways. The phenotypic distinction between the *abx* deletions and the gypsy-induced *bx* mutations is clear and unequivocal, but these classes differ both in the location and nature of their DNA lesions. $bx^{34e\text{-}prv}$ is thus critical to the question. It results from a deletion, like the *abx* mutations, but maps to their right, in the midst of the *bx* mutations. Phenotypically, it has characteristics of both mutant classes. Like *abx*, it is quite variable in phenotype. Unlike *abx*, however, it has no preferential effect on the proximal wing or anterior-most notum. It has little or no effect on the second leg, and affects only weakly the hypopleura and anterior third leg. In these characteristics it resembles a weak *bx* rather than an *abx*. On balance, this deletion behaves more like a *bx* allele, supporting the presence of at least two genetic units in the *abx/bx* region. This conclusion is also supported by the lack of transvection between *abx* and *bx* alleles and by their partial complementation. It may be that the *abx* genetic element is more readily affected by deletions, while the *bx* element is more sensitive to gypsy insertions.

$bx^{34e\text{-}prv}$ deletion also reveals something about gypsy element action. While quite variable in phenotype, most revertant flies are closer to wild type than bx^{34e}. Thus it is more disruptive to insert a gypsy element at −63.5 kb than to delete the 10 kb of DNA around the site of its insertion. In fact, this deletion removes the site of insertion of five of the seven gypsy elements, but it is much less severe than most of them. This supports the idea that gypsy elements act at a distance from their site of insertion, in this case probably >5 kb.

Despite their similar variability and qualitative phenotype, the three *abx* deletions differ markedly in severity. If all deleted a single element, all should have the same amorphic phenotype. That they vary in a continuous fashion as more DNA is deleted is similar to the situation in the *bxd* region. *bxd* rearrangement breaks are successively more severe the closer the break is to the 5′ end of *Ubx* (Bender *et al.*, 1985). Both results could be explained by the presence of multiple elements within the respective regions. Caution must be used in interpreting abx^{CAC4}, however, as its deletion is accompanied by a Hobo insertion. One explanation for the phenotypically graded effects of gypsy elements in the *bx* region is the presence of multiple elements in that region as well, with the severity of an insertion depending on the number of elements affected.

All three *abx* mutations do delete one distinctive DNA sequence, the M or opa repeat. This repetitive sequence is shared by several homeotic and segmentation genes, along with many others (Regulski *et al.*, 1985; Wharton *et al.*, 1985), but its function remains unclear. A copy is found in the left half of the BX-C at position −77.5 kb (Regulski *et al.*, 1985). In *Deformed* (Regulski *et al.*, 1985) and *engrailed* (Poole *et al.*, 1985) the M repeat forms a long stretch of glutamine codons in the same reading frame as the homeobox. In contrast, the M repeat deleted by *abx* is not part of the known homeobox-containing *Ubx* mRNAs. Instead, it consists of several small clusters of M repeat-like sequence, in different frames and on both DNA strands (Gray, 1985).

Spatial regulation of Ubx

Several lines of evidence now implicate *abx* and *bx* as mutations affecting spatial regulation of the *Ubx* transcription unit. We will examine these in turn, and then offer a model for BX-C function. It has long been apparent that the previously characterized *abx* and *bx* lesions are distinct in position from the *Ubx* mutations but nonetheless fail to complement with them. The parts of the fly that *abx* and *bx* affect are only a subset of those affected by *Ubx*. *abx* and *bx* are not merely weak *Ubx* mutations, but rather seem to affect *Ubx* in specific spatial domains.

abx and *bx* mutations differ from *Ubx* mutations both in the nature and location of their lesions. Many of the mutations in the three homeobox-associated genetic units in the BX-C, *Ubx*, *iab-2* and *iab-7*, are point mutations which are usually recessive lethal (Karch *et al.*, 1985; E.B.Lewis, personal communication; Sanchez-Herrero *et al.*, 1985; Tiong *et al.*, 1985). In contrast, of >50 alleles of the non-homeobox-associated genetic units of the BX-C previously examined, no point mutants were found

(Bender *et al.*, 1983a; Karch *et al.*, 1985). This is extended by our results; 18 of 19 mutations result from deletion or mobile element insertion. bx^4, if it is a point mutant and not a small rearrangement missed in our analysis or a modifier mapping outside the complex, would be an exception. Regardless, it is clear that the target size for point mutants in the non-homeobox-associated genetic units is very small, and judging from bx^4, point changes in them have at most a weak effect. *bx*, *abx*, and the others seem to represent targets, such as regulatory sequences or coding sequence for untranslated RNAs, which are less susceptible to point mutation.

bx lesions spread over 19 kb and *abx* lesions over 16 kb (Figure 3). While this span is larger than many *Drosophila* genes, the lesions cover only a small part of the *Ubx* transcription unit within which they are contained (Figure 1). Such clustering is surprising. No *abx* or *bx* mutant lesions fall between −55 and −30 kb, or between −100 and −80 kb. This clustering suggests that *abx* and *bx* affect a set of *cis*-acting regulatory sequences, located in the *abx* or *bx* region, necessary only in particular cells. Alternately, they could affect cell-specific *Ubx* products with a unique requirement for the *abx* or *bx* region. This spatial effect was graphically demonstrated by White and Wilcox (1985b) and Cabrera *et al.* (1985), who assayed the effects of bx^{34e}, bx^3 and abx^{SK} on the expression of *Ubx* protein. These mutants reduce or abolish the expression of *Ubx* protein in particular parts of the developing fly, in a fashion consistent with their phenotypic effects.

The mutant phenotypes give a clue as to the pattern of these spatial effects. Recently it has been proposed that many homeotic transformations involve not segments, but parasegments (Martinez-Arias and Lawrence, 1985), adjacent posterior−anterior compartments. *Ubx* would thus affect most severely the region between T2p and A1a, parasegments 5 and 6. *abx* and *bx* transformations both support and dissent from the parasegmental model of developmental control. The *ppx* transformation seen in *abx* and *bx* mutants (Casanova *et al.*, 1985 and these results) fits into the parasegmental model of *Ubx*. Paradoxically, however, while transformation of posterior T1 leg and humerus is seen, postnotum and posterior wing remain, demonstrating a differential effect of these mutants on regions within a compartment. Likewise, the strongest *bx* mutants cause a slight notal reduction, and in some alleles a reduction of the wings (both part of T2a) but show no effect on postnotum (in T2p). In addition, Casanova *et al.* (1985) have shown that *abx* is active in the posterior T3 leg, as well as in the posterior T2 leg. The difference between *abx* and *bx* as to which structures of the haltere and notum they most affect also follows no clear compartmental boundary. Thus the effects of neither *abx* or *bx* coincide well with a single parasegment or compartment. *bxd* and *pbx* mutations show similar overlap in their effects on T3p and A1a (Bender *et al.*, 1983a, 1985 and unpublished results).

It is also clear that *Ubx* action is not confined to parasegments 5 and 6. Homozygous *Ubx* embryos have partial transformation of abdominal as well as thoracic segments, including effects on the tracheal trunks, the ventral pits and Keilin's organs. *bxd* is clearly involved in the regulation of *Ubx* in the abdominal as well as thoracic segments (Lewis, 1978). *bx*, *abx* and *bxd* mutations thus seem to effect regulation in a more complex pattern than is provided by either single segments or parasegments.

A model for BX-C function and its disruption by gypsy

This study and that of *bxd* (Bender *et al.*, 1985), which focus on specific subregions of the BX-C, suggest that complexity of spatial control is extremely fine-grained. This level of control is illustrated in results obtained when examining expression of *Ubx* RNA (Akam and Martinez-Arias, 1985) or protein (White and Wilcox, 1985a) in embryos and imaginal discs. Both *Ubx* RNA and protein differ in abundance between different cells within the same compartment. Mutants may affect this cell-by-cell regulation, resulting in effects on units which coincide with neither segments or parasegments. In fact, direct examination of *Ubx* protein expression in imaginal discs of abx^{SK} homozygotes (White and Wilcox, 1985b) shows a rather complex pattern of reduction which follows the compartment boundaries in general but not in detail.

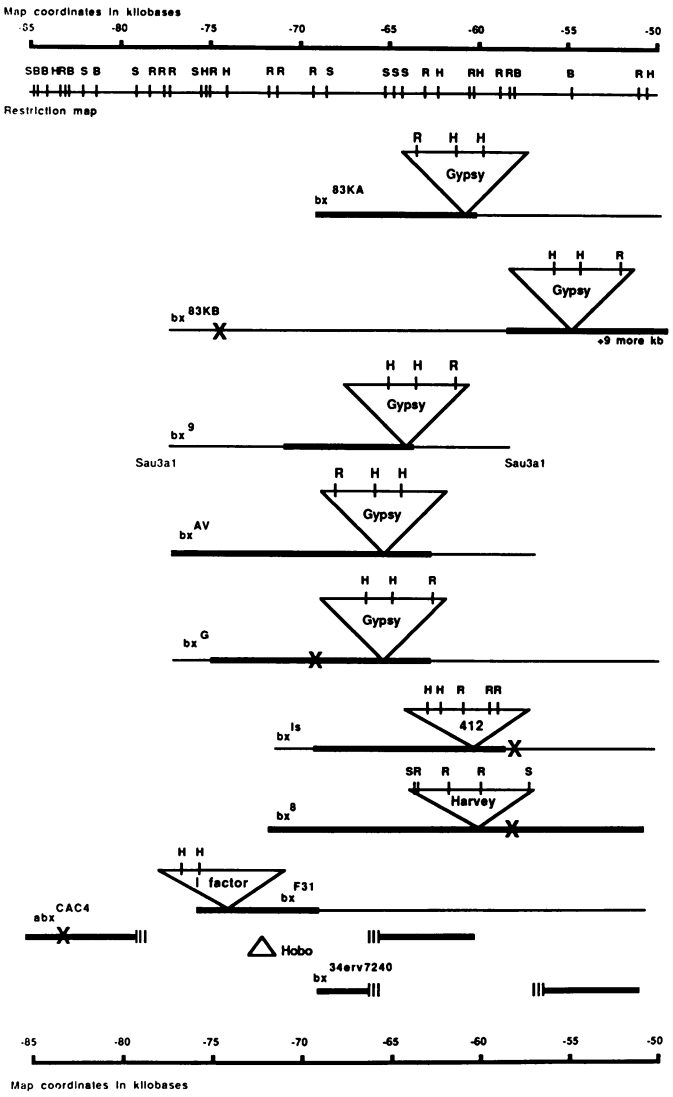

Fig. 8. Maps of clones from mutant libraries. Above and below is an expanded part of the molecular map shown in Figure 1. The associated restriction map (Bender *et al.*, 1983), showing sites for the restriction enzymes *Eco*RI (E), *Bam*HI (B), *Hin*dIII (H) and *Sal*I (S), is displayed above. Restriction maps of the inserts found in phage isolated from libraries of mutant DNA are outlined below the map. For each mutant, the thin line indicates the extent of clones mapped only with the enzyme *Eco*RI. The heavier line indicates the cloned region mapped with all four restriction enzymes. An X shows an *Eco*RI site missing due to polymorphism. Mobile element insertions are shown as triangles, with the horizontal side showing the size and restriction map of the insertion. The name of the mobile element is indicated. Deletions are shown as gaps in the line with vertical bars at the endpoints indicating the limits of uncertainty. All clones end in *Eco*RI sites, except the bx^9 clones, which end in *Sau*3AI sites.

The distinct phenotypes of the different *abx* deletions, and their qualitative differences from the phenotypes of the insertion and deletion-caused *bx* mutations suggest that two or more genetic elements are located between −80 and −55 kb. Bender *et al.* (1985) found a similar array of genetic elements in the *bxd* region, which together control the differentiation of T3p and Ala. While these elements may encode RNA or protein products which regulate the transcription or splicing of particular *Ubx* products, we favor a model in which all of these mutations affect cell-specific regulatory sequences involved in the expression of *Ubx*. The *abx* and *bx* regions may contain *cis*-acting elements which activate and regulate transcription of *Ubx* in different cells in T2p and T3a, and which remain active in the analogous cells of the more posterior segments. Regulatory elements in the *bxd/pbx* region, acting in concert with the *abx* and *bx* elements, would modify *Ubx* expression in T3p and A1a, as well in the more posterior segments. As one proceeds further back, *Ubx* expression is further modified by the *iab-2* and *iab-7* products (Struhl and White, 1985) and their respective sets of regulatory elements. Gypsy elements may act by interfering with these regulatory sequences. Their phenotypic gradation with position may reflect either a distance dependence of this interference, or may result from the different insertions affecting different numbers of elements in the *bx* region.

Materials and methods

Fly strains and assay of mutant phenotype

All crosses were done at 18°C, except the transvection tests, which were done at 25°C. Flies were examined and scored for transformation under a dissecting microscope, while anesthetized by CO_2. Haltere size was scored relative to eye size of the same fly, and then converted to percentage of wing size using the estimate that the eye is 15% the size of the wing. Legs were scored for *ppx* transformation by looking for characteristic T1 bristles on the T2 femur; if two or more such bristles were seen, the leg was considered transformed. Transformation of T3 legs was assayed by looking for pre-apical and apical bristles.

Some of the mutants used are catalogued by Lindsley and Grell (1968). Most stocks were provided by E.B.Lewis. bx^{83Ka} and bx^{83Kb} were provided by D.Kuhn, bx^{F31} and abx^{CAC4} by P.Adler and bx^{1s} by G.Ising. The origin of each of the *bx* and *abx* alleles is described in Table I. The rearrangements used in transvection tests were *In(3LR)P30* p^p *cu Sb* Ubx^1, *T(2;3) R-2 sbd* bx^3 and *T(2;3) P70* abx^1, all provided by E.B.Lewis. Transvection between abx^{SK} and abx^1 was also tested using the rearrangement *In(3LR)P30* abx^1.

Genomic Southerns and construction of recombinant libraries

Purification of fly DNA and genomic Southerns were carried out as described by Bender *et al.* (1983b). Most recombinant libraries were constructed by ligating *Eco*RI partial digests of fly DNA into *Eco*RI-digested and purified arms of the λ vectors Sep 6 (Davis *et al.*, 1980) or EMBL 4 (Frischauf *et al.*, 1983). The library of the mutant bx^9 and one of the libraries constructed from DNA of the mutant bx^{F31} were constructed by ligating partial *Sau*3A1 digests of fly DNA with *Bam*HI-digested and purified EMBL 4 arms. Phage were packaged *in vitro* (Hohn, 1979) and plaques screened for homology to the appropriate region of the bithorax complex (Benton and Davis, 1977; Bender *et al.*, 1983a). Figure 8 shows the restriction maps of the recombinant λ phage isolated from mutant libraries. Growth of phage, restriction mapping, and heteroduplex analysis were as described by Bender *et al.* (1983b). Cloned I factor DNA was obtained from D.Finnegan, and cloned Hobo element DNA was obtained from S.Beckendorf.

Mounting larvae and cuticular structures

Larvae were obtained from crosses of $Df(Ubx)^{109}$/*Stubble Tubby* females to bx^3/*Stubble Tubby* or Canton S males. Non-*Tubby* larvae were rinsed in distilled water, and mounted in Zeiss W-15 mounting medium. Adult flies were stored and dissected in ethanol:glycerol (3:1). Wings, halteres and legs were then washed in *n*-propanol, and mounted under coverslips with Euparol 'Vert' (BDH Chemicals).

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