Genetic and developmental characteristics of the homeotic mutation *bx*¹ of *Drosophila*

By ERNESTO SÁNCHEZ-HERRERO¹ AND GINÉS MORATA¹

From the Centro de Biología Molecular, Universidad Autónoma de Madrid

SUMMARY

The mutations at the bithorax locus produce a transformation of anterior haltere into anterior wing. The *bx*¹ allele presents unusual features when compared with other *bx* alleles. The phenotype of *bx*¹ homozygotes is temperature sensitive but only with regard to the distal and not to the proximal transformation, thus suggesting two different components in the bithorax transformation. The phenotype of *bx*¹ homozygotes is stronger than that of *bx*¹ over the deletion of the gene, suggesting a *trans* interaction of the *bx*¹ chromosomes which results in mutual partial inactivation. We show by temperature shift and clonal analysis experiments that the decision on whether to differentiate haltere or wing structures is taken at the end of the proliferation period of the mutant disc.

INTRODUCTION

The bithorax complex (*BX-C*) is a group of homeotic genes which specify the development of different thoracic and abdominal segments (Lewis, 1963, 1978). Each individual gene within the complex is necessary for the correct development of one specific compartment. One particular locus (bithorax) is required for the proper development of the metathoracic segment. Mutations in this locus produce a transformation of the anterior compartment of this segment into the homologous compartment of the mesothoracic segment. There are several alleles known at the *bx* locus and the degree of transformation they produce is characteristic of the allele. Of the alleles studied in detail (Morata & Kerridge, 1980) *bx*^{34e} and *bx*³ show little variability in the expression of the mutant phenotype. In *bx*³ practically all the anterior haltere cells are transformed towards perfect or almost perfect wing structures. In *bx*^{34e} there is a weaker transformation: almost all the anterior haltere cells are transformed, but into structures which are intermediate between haltere and wing (Morata & Kerridge, 1980). By contrast, the allele *bx*¹ presents unusual features: its phenotype is very variable and erratic even when comparing both sides of the same fly (Morata & Kerridge, 1980). Usually a *bx*¹ haltere is a mixture of haltere (untransformed) and wing (totally transformed) cells, that is the metathoracic imaginal disc contains cells in different states of determination. The number of

¹ Authors' address: Centro de Biología Molecular, CSIC, Universidad Autónoma de Madrid, Canto Blanco, Madrid 34, Spain.

cells becoming determined one way or another depends on the temperature (Morata & Kerridge, 1980) among other factors.

Because of the unusual characteristics of the allele bx^1 , we report a detailed study of the homeotic transformation it produces in different genetic combinations. We also define cell lineage relationships of wing and haltere cells in the mutant disc.

MATERIALS AND METHODS

a) *Measure of the expressivity*

In the adult cuticle the metathoracic segment is represented by a ventral appendage – the hind leg, a dorsal appendage – the haltere and a narrow band of cuticle on the body called metanotum (Fig. 1). These structures are differentiated by two imaginal discs, the third leg disc and the haltere disc. The cuticle of the mesothoracic segment consists of the second leg, the wing and the mesonotum (Fig. 1). These structures are differentiated by the second leg disc and the wing disc.

The mutations at the bithorax locus produce a transformation of the anterior compartment of the metathoracic segment towards the anterior compartment of the mesothoracic segment. Although the transformation affects also the legs, it is particularly clear in the dorsal structures because the haltere and wing are very

Fig. 1. Dorsal components of mesothoracic and metathoracic adult cuticle. The mesothoracic structures used to measure the expressivity of bx^1 transformation are: *MN*, mesothorax (or mesonotum); *Co*, costa bristles, typical of the proximal and medial wing blade; *MTR*, medial bristles of the triple row, typical of the medial-distal wing margin. Metathoracic structures: haltere appendage, *H*, and metanotum, *MT* ($\times 40$).

different in size and in the differentiation of individual elements. Therefore we have used the dorsal structures to measure the phenotypes. To quantify the transformation produced by the different combinations we have measured the number of mesothoracic and wing bristles which appeared in the dorsal metathoracic disc derivatives. We have distinguished three types of mesothoracic bristles which correspond to structures located along the proximodistal axis of the appendage: mesothoracic bristles (MN) as markers of the transformation in the proximal region, Costa bristles (Co), which mark the proximal-medial part of the wing blade, and medial triple row bristles (MTR) for the most distal transformation. A minimum of 25 appendages were used for each case. The degree of transformation for each region is expressed as the percentage of the number of the same type of bristles which are present in the normal wing disc.

In the combinations of *bx¹* with the different chromosomes carrying mutations of the *BX-C*, females of the stock *y; mwh jv sbd² ss^a bx¹/TM1* were always used. Some of the *Ubx* alleles used in this work are associated with chromosomal rearrangements visible in the compound microscope (*Ubx⁴⁻³⁰*, *Ubx¹²⁻⁵*, *Ubx⁵⁻²²*, *Ubx⁶⁻²⁶*, *Ubx⁸⁰*, *Ubx¹⁰¹*, *Ubx⁸⁸²*, *Ubx¹³⁰*) and others do not show any detectable chromosomal aberrations (*Ubx⁹⁻²²*, *Ubx⁵⁻¹²*, *Ubx⁸⁻⁸*, *Ubx¹*). Some of them have been induced recently (Kerridge & Morata, 1982) and others were already known (Lindsley & Grell, 1968). The chromosome *Df(3R)P115* is deficient for all the *BX-C* genes.

b) Temperature shift experiments

Females of the stock *y; mwh jv sbd² ss^a bx¹/TM1* were crossed to males *su(Hw)² bx¹ bxd/TM1*. The egg laying period was 24 h, the adult flies being kept at 25 °C. Afterwards the parents were removed and the cultures maintained at 17 °C or 29 °C until the temperature shift. Males and females of genotype *mwh jv sbd² ss^a bx¹/su(Hw)² bx¹ bxd* were selected from each culture, prepared for microscopical analysis and the expressivity measured as described above.

c) Clonal analysis

Females of the stock *y; mwh jv sbd² ss^a bx¹/TM1* were crossed to males of the genetic constitution *y; Dpsc¹⁴ M(3)ⁱ⁵⁵ sbd² bx¹/TM1*. The offspring were irradiated with 1000R and those of genotype *y/y; mwh jv sbd² ss^a bx¹/Dpsc¹⁴ M(3)ⁱ⁵⁵ sbd² bx¹* were collected as adult flies and examined under the compound microscope. Flies of this genotype are homozygous for *bx¹* and therefore the haltere is partially transformed towards wing. In addition they have a combination of cell marker mutants to permit the detection of clones generated by mitotic recombination; after recombination in the left arm of the third chromosome, clones are produced which are homozygous for the marker *multiple wing hairs* (*mwh*, affects the number of hair processes per cuticular cell) and *javelin* (*jv*, affects the shape of bristles), lose the wildtype allele of *yellow* (*y*, removes

pigment from cuticle and bristles rendering yellow colour) and the retarding Minute allele. Thus the clones are triply marked with *y*, *mwh* and *ju*. This particular combination of cuticular markers has already been reported in several publications (Steiner, 1976; Lawrence & Morata, 1977). The Minute technique (Morata & Ripoll, 1975) has been used to increase the size of the marked clones.

The time of irradiation was 48–72 and 96–120 h after egg laying, which corresponds to second and third larval period. The cultures were maintained at 29 °C as at this temperature the expression of the *bx*¹ transformation was the strongest.

RESULTS

a) *The effect of temperature on homozygous and hemizygous bx*¹ *flies*

The phenotype of *bx*¹ homozygotes at different temperatures has already been described (Morata & Kerridge, 1980); here we confirm this description using a different *bx*¹ stock and compare it with the phenotype of the hemizygotes *bx*¹/*Df*(3R)*P115* where there is only one dose of *bx*¹ present. The results are shown in Table 1. There are two clear points emerging from these data: (1) the phenotype of homozygotes *bx*¹/*bx*¹ is stronger than that of hemizygotes *bx*¹/*Df*(3R)*P115* (Fig. 2). This has already been noticed (Morata & Kerridge, 1980) and clearly indicates that *bx*¹ is not a hypomorphic mutation; (2) in *bx*¹/*bx*¹ the amount of medial (Co) and distal (MTR) structures increases with the temperature, but in *bx*¹/*Df*(3R)*P115* does not. This suggests a difference in the mode of production of the homeotic transformation in the two genotypes (see Discussion).

b) *Phenotype of bx*¹ *over different Ubx mutations*

The data on the transformation shown by *bx*¹ over different *Ubx* alleles are

Table 1. *Expressivity of the homeotic transformation shown by homozygotes (bx*¹/*bx*¹) and hemizygotes (bx¹/*Df*(3R)*P115*) at different temperatures

	t (°C)	MN	Co	MTR
<i>bx</i> ¹ / <i>DfP115</i>	17	14(16 ± 18)	3(2 ± 3)	3(3 ± 3)
	25	7(8 ± 13)	3(2 ± 3)	6(5 ± 6)
	29	6(7 ± 16)	3(2 ± 3)	7(6 ± 6)
<i>bx</i> ¹ / <i>bx</i> ¹	17	29(34 ± 20)	3(2 ± 5)	0.0
	25	39(45 ± 22)	19(11 ± 8)	19(16 ± 13)
	29	44(51 ± 25)	43(25 ± 8)	49(42 ± 20)

The numbers express the amount of mesothoracic bristles appearing in the metathorax and are given as a percentage of the normal amount of mesothoracic bristles. Actual numbers and standard deviations in parenthesis. *MN*, notum bristles in the metanotum; *Co*, costa bristles in the proximal haltere; *MTR*, medial triple row bristles in the distal haltere.

Fig. 2. Typical examples of the homeotic transformation shown by (A) bx^l homozygotes and (B) $bx^l/DfP115$. Note that the amount of mesothoracic tissue (arrows) is greater in (A) than in (B). ($\times 100$).

shown in Table 2. In these experiments flies were cultured at 25°C. The reason for using *Ubx* mutations is that they always fail to complement the mutations *bx* (and *pbx* and *bxd*, see Lewis, 1963, 1978 for a description of these genes and their interactions), that is, the loss of *Ubx*⁺ function inactivates the *bx*⁺ gene located nearby. However this inactivation is not total in all cases and there are both weak and strong *Ubx* alleles (Kerridge & Morata, 1982). From the analysis of Table 2

Table 2. *Expressivity of the homeotic transformation shown by bx^1 in combination with different Ubx alleles*

	<i>MN</i>	<i>Co</i>	<i>MTR</i>
(A) bx^1/Ubx^1	0.0	0.0	0.5(0.5 ± 1)
bx^1/Ubx^{9-22*}	0.0	0.0	0.4(0.4 ± 1)
bx^1/Ubx^{5-12}	29(33 ± 21)	9(5 ± 4)	6(5 ± 6)
bx^1/Ubx^{8-8}	24(27 ± 21)	10(6 ± 4)	6(5 ± 5)
(B) bx^1/Ubx^{4-30}	5(6 ± 10)	3(2 ± 2)	2(2 ± 3)
bx^1/Ubx^{12-5}	5(6 ± 11)	7(4 ± 6)	1(1 ± 1)
bx^1/Ubx^{101}	6(7 ± 12)	3(2 ± 8)	2(2 ± 4)
bx^1/Ubx^{130}	6(7 ± 11)	2(1 ± 3)	6(5 ± 5)
bx^1/Ubx^{882}	16(18 ± 23)	7(4 ± 4)	3(3 ± 3)
bx^1/Ubx^{5-22}	20(23 ± 20)	14(8 ± 6)	12(10 ± 7)
bx^1/Ubx^{80}	26(30 ± 22)	10(6 ± 8)	6(5 ± 2)
bx^1/Ubx^{6-26*}	60(69 ± 25)	31(18 ± 10)	27(23 ± 24)
$bx^1/DfP115$	7(8 ± 13)	3(2 ± 3)	6(5 ± 6)
bx^1/bx^1	39(45 ± 21)	19(11 ± 8)	19(16 ± 13)

The series A) includes *Ubx* mutations not associated and series B) associated with visible chromosomal aberrations. The cultures were kept at 25 °C. Those combinations marked with a (*) are taken from Morata & Kerridge 1980. Data presented as in Table 1.

the following points can be made: (1) the expressivity within any given genotype is very variable as shown by the high values of standard deviations; (2) the expressivity is also very variable when different combinations are compared, ranging from near complementation in bx^1/Ubx^1 or bx^1/Ubx^{9-22} to a strong transformation in bx^1/Ubx^{6-26} ; (3) in general the transformation is stronger in the proximal than in the distal structures; (4) the degree of transformation produced by bx^1 over those *Ubx* chromosomes bearing cytological aberrations in the *BX-C* region (series B, Table 2) is not different from that of cytologically normal alleles (series A, Table 2).

We have tested for the *Ubx* alleles whether there is an effect of the temperature on the transformation of distal structures. The results indicate that some combinations with *Ubx* are affected but others are not (Table 3). In the first part of Table 3 are shown the *Ubx* alleles which in combination with bx^1 do not show an effect of temperature and in the second part those which are affected. The increase is not due to the effect of the temperature in the *Ubx* alleles as the phenotype of *Ubx*/+ does not change with temperature. It is of interest (see Discussion) that the *Ubx* alleles (with the exception of Ubx^{882}) that show an increase in expressivity with temperature are those that at 25 °C show thoracic transformation comparable to that of bx^1 homozygotes. The thoracic transformation was not in general affected by the temperature; only in the combinations $bx^1/Df(3R)P115$, bx^1/Ubx^{101} and bx^1/Ubx^{882} there is a slight increase as lowering the temperature which is probably not significant.

Table 3. *Effect of the temperature on the expressivity of bx^1 in combination with different Ubx alleles*

	t (°C)	MN	Co	MTR
Part 1				
bx^1/Ubx^{4-30}	17	3(4 ± 7)	2(1 ± 1)	3(3 ± 3)
	25	5(6 ± 11)	3(2 ± 2)	2(2 ± 3)
	29	3(4 ± 11)	3(2 ± 4)	2(2 ± 5)
bx^1/Ubx^{130}	17	11(13 ± 18)	2(1 ± 2)	2(2 ± 2)
	25	6(7 ± 11)	2(1 ± 3)	6(5 ± 5)
	29	8(10 ± 19)	8(5 ± 7)	6(5 ± 8)
bx^1/Ubx^{12-5}	17	2(2 ± 5)	2(1 ± 1)	1(1 ± 1)
	25	5(6 ± 11)	7(4 ± 6)	1(1 ± 1)
	29	5(6 ± 13)	7(4 ± 4)	1(1 ± 3)
bx^1/Ubx^1	17	0.0	0.0	0.5(0.5 ± 1)
	25	0.0	0.0	0.5(0.5 ± 1)
	29	0.0	0.0	0.0
bx^1/Ubx^{101}	17	11(13 ± 16)	2(1 ± 1)	1(1 ± 1)
	25	6(7 ± 12)	3(2 ± 8)	2(2 ± 4)
	29	3(4 ± 9)	3(2 ± 3)	2(2 ± 3)
bx^1/Ubx^{882}	17	21(24 ± 23)	12(7 ± 3)	5(4 ± 2)
	25	16(18 ± 23)	7(4 ± 4)	3(3 ± 3)
	29	10(12 ± 21)	14(8 ± 6)	9(8 ± 9)
Part 2				
bx^1/Ubx^{5-12}	17	23(26 ± 23)	2(1 ± 1)	0.2 ± 1
	25	29(33 ± 21)	9(5 ± 4)	6(5 ± 6)
	29	25(28 ± 27)	12(7 ± 8)	11(10 ± 12)
bx^1/Ubx^{8-8}	17	20(22 ± 20)	3(2 ± 2)	3(3 ± 3)
	25	24(27 ± 21)	10(6 ± 4)	6(5 ± 5)
	29	14(16 ± 22)	15(9 ± 7)	13(11 ± 11)
bx^1/Ubx^{5-22}	17	18(21 ± 20)	7(4 ± 4)	5(4 ± 3)
	25	20(23 ± 20)	14(8 ± 6)	12(10 ± 7)
	29	16(18 ± 19)	19(11 ± 9)	22(19 ± 19)
bx^1/Ubx^{80}	17	26(30 ± 22)	5(3 ± 2)	4(4 ± 3)
	25	26(30 ± 22)	10(6 ± 8)	6(5 ± 2)
	29	25(29 ± 23)	26(15 ± 9)	25(22 ± 18)
bx^1/Ubx^{6-26}	17	40(57 ± 19)	19(11 ± 7)	9(8 ± 9)
	25	60(69 ± 25)	31(18 ± 9)	27(23 ± 14)
	29	45(51 ± 24)	32(19 ± 11)	34(30 ± 20)

Part 1 include those *Ubx* alleles which are not affected by temperature and part 2 those which are. Data presented as in Tables 1 and 2.

c) Cell determination in bx^1 dorsal metathorax

Two types of cells coexist in a bx^1 haltere; those that differentiate wing (mutant) structures and those that differentiate haltere (wildtype) structures. In

general, there is a clear distinction between the two types of differentiation which usually form coherent patches. Very frequently, in flies grown at 25 °C and 29 °C vesicles of wing or haltere trichomes can be observed inside the appendage (Fig. 3A, B). These are probably the result of sorting out due to specific cell affinities

Fig. 3. *bx*¹ halteres frequently present vesicles of wing or haltere tissue segregated inside the appendage. (A) vesicle of wing trichomes (arrow) in an appendage made mainly by haltere trichomes. (B) vesicle of haltere trichomes (arrow) in a territory made of wing trichomes. ($\times 400$).

of wing and haltere determined cells (Morata & Garcia-Bellido, 1976). Since the two types of cells proliferate jointly during development and derive from the same promordium, it would be of interest to find the lineage relationship between them. In particular, one would like to know the moment when the cells become determined to differentiate one type of structure or another. It is worth noting that in normal development presumptive haltere and wing cells are developmentally and positionally segregated from the blastoderm period.

To identify the period of determination in the mutant bx^1 cells we have performed two series of experiments: (1) Temperature shift and (2) Clonal analysis.

1) Temperature shift experiments

Because at 29°C bx^1 differentiate a large amount of wing structures (Table 1) but very little or none at 17°C, we set up an experiment to establish the phenocritical period of the wing transformation. We measured the number of MTR bristles (indicative of wing transformation) appearing in flies that underwent a temperature shift as embryos or larvae: The phenocritical period (Fig. 4) appears to be during the third larval stage, in agreement with the results obtained

Fig. 4. Temperature shift experiment. Solid dots represent the values of expressivity for the distal transformation of bx^1 homozygotes transferred as larvae from 17 to 29°C. Open dots show the expressivity after the reciprocal change 29 to 17°C. The transformation is measured as the number of medial triple row (MTR) bristles appearing in the appendage.

Table 4. *Results of the clonal analysis experiment*

Age of irradiation in hours after egg laying	Number of halteres examined	Number of clones found	Number of clones extending to haltere and wing territories
48–72	448	13	7
96–120	1140	59	4

Larvae of genotype *y; mwh jv sbd² ss² bx¹/Dpsc¹⁴ M(3)F⁵⁵ sbd² bx¹* were irradiated (1000 R) and screened as adults for *y; mwh jv* clones.

for other *bx* mutations (Kaufman *et al.* 1973). A similar measurement performed for Costa bristles (data not shown) yielded the same result. This indicates that in the mutant *bx¹* disc the final decision on whether differentiate wing or haltere structures is taken near the end of the larval life.

2) Clonal analysis

Clones marked with *mwh* were generated at 48–72 h and 96–120 h of development. This corresponds to the second instar and early third instar in Minute larvae grown at 29°C. The aim of the clonal analysis was to define the lineage relationship of haltere and wing structures. In particular we were interested on whether marked clones could extend to both territories or whether there was a lineage restriction between them. The results are shown in Table 4. Although the majority of clones are confined to one type of cells, several extend to both territories, clearly indicating that the mother cell of those was not determined for either type of structure (Fig. 5). Few clones are expected to extend to both

Fig. 5. *mwh* clone including wing and haltere trichomes. The limit of the clone is marked by the dotted line. ($\times 300$).

Fig. 6. Example of a bx^1 appendage (A) showing many more haltere elements, trichomae and sensilla than a wildtype haltere (B). Note the multiplication (arrows) of sensilla trichoidea. ($\times 170$).

structures because clone size is small compared to that of wing and haltere territories.

DISCUSSION

Genetic characteristics of bx^1

The mutation bx^1 produces a partial transformation of the anterior compartment of the haltere into that of the anterior wing. Similar homeotic transformations are produced by other mutations in the bx locus. Since the entire deletion

of the gene produces a complete transformation, as seen in the phenotype of embryos deficient for the whole complex (Lewis, 1978), it indicates that the different *bx* alleles represent partial loss of *bx*⁺ function. However, the allele *bx*¹ shows some genetic features which make it different from the other two alleles, which are considered to be typical hypomorphs (Morata & Kerridge, 1980). The *bx*¹ homozygotes show a transformation stronger than that produced by the hemizygotes (*bx*¹/*Df*(3*R*)*P115*) although *bx*¹ is totally recessive; one dose of *bx*⁺ present in *Dp*(3;3)*P115* is sufficient to cover the mutant phenotype of both the homozygotes and hemizygotes. The phenotype of the homozygotes is affected by temperature in a particular manner; the transformation of the medial and distal regions is affected by temperature but that of the proximal regions is not (Table 1).

The combinations of *bx*¹ with the *Ubx* mutations may be, according to their phenotype, subdivided into two classes (Table 3): (1) Those that produce a very weak transformation similar or even weaker than that of *bx*¹/*Df*(3*R*)*P115*. These are the combinations with *Ubx*⁴⁻³⁰, *Ubx*¹²⁻⁵, *Ubx*¹⁰¹, *Ubx*¹, *Ubx*⁹⁻²² and *Ubx*¹³⁰. Their phenotype is not enhanced by the temperature (Table 3). (2) Those that show a phenotype similar to *bx*¹ homozygotes. These are the combinations with *Ubx*⁵⁻²², *Ubx*⁸⁻⁸, *Ubx*⁸⁰, *Ubx*⁶⁻²⁶ and *Ubx*⁵⁻¹². Their phenotype is enhanced by the temperature to a variable degree, but only the distal component is affected (Table 3). Again this is similar to what is observed for *bx*¹ homozygotes. *Ubx*⁸⁸² is unique in that it shows some proximal transformation but is not affected by the temperature. It has been brought to our attention (M. Akam, personal communication) that the *Ubx* alleles included in our class 1 and *Ubx*⁸⁸² map in the DNA to the left of *bx*¹ while those of class 2 map to the right (*Ubx*⁸⁰ and *Ubx*¹⁰¹ have not yet been mapped). This observation might suggest a functional diversity within the *Ubx* locus.

These results again point to a distinction between *bx*³ and *bx*^{34e} on one hand and *bx*¹ on the other. The two former alleles do always show a clear mutant phenotype over any *Ubx* allele tested to date (Kerridge & Morata, 1982).

It is worth noticing that the strength of the transformation of *bx*¹ with the *Ubx* alleles seems to be unrelated to the presence of chromosomal rearrangements in the complex; *Ubx*¹³⁰ and *Ubx*¹⁰¹ have breakpoints (Lindsley & Grell, 1968) within the complex and have very little effect over *bx*¹ while *Ubx*⁸⁻⁸, which is not associated with a visible breakpoint (Kerridge & Morata, 1982), shows a stronger phenotype. Neither is the strength of the transformation related to that of other *bx* alleles: *Ubx*¹³⁰ and *Ubx*¹⁰¹ give very strong combinations with *bx*³ and *bx*^{34e} but weak with *bx*¹.

Thus, it appears that the bithorax transformation contains two components, one proximal and one distal. In the case of *bx*¹ the distal component is temperature sensitive while the proximal one is not, in other cases such as *bx*³ the two components are equally expressed with temperature. At low temperature combinations like *bx*³/*bx*¹ show proximal transformation almost exclusively (Morata & Kerridge, 1980).

The mode of action of bx^1

The observation that $bx^1/Df(3R)P115$ shows a phenotype that approaches wildtype, and little different from $bx^+/Df(3R)P115$, indicates that the loss of bx^+ function in the bx^1 chromosome is not very great. In $bx^1/Df(3R)P115$ flies the only source of bx^+ function is the bx^1 chromosome and yet the flies are almost normal. However, the presence of two doses of bx^1 produces a marked reduction in bx^+ function. It follows that the bx^1 phenotype is produced by the interaction of the two bx^1 chromosomes, each of them contributing to inactivate part of the bx^+ function remaining in the other. This 'trans' interaction appears to be affected by temperature. It is not therefore surprising that the expressivity of the hemizygotes does not respond to temperature (as there is only one bx^1 gene present). This interpretation receives support from the results shown in Table 3 where the effect of temperature of bx^1 with different *Ubx* alleles is shown; in those cases that at 25° there is a proximal transformation, (the ' bx^1/bx^1 ' phenotype) there is an increase in expression with temperature (with the exception of *Ubx*⁸⁸²). The *Ubx* alleles that have little transformation at 25° (the ' $bx^1/Df(3R)P115$ ' phenotype) show no increase. Presumably in the former there is a chromosomal interaction and in the latter there is not.

Developmental effect of bx^1

In the cases of good expression of bx^1 transformation, the mutant haltere disc differentiates a mixture of wing and haltere cells, usually the cells showing a perfect differentiation of one or the other type (Fig. 5). Our experiments were aimed to know when the cells of the mutant disc become committed to one type of structure or another. The two experiments that we have done, temperature shift and clonal analysis, point to the same conclusion. The cells become committed at the end of the proliferation period of the disc. This is similar to the situation in *Contrabithorax* (Morata, 1975), which also produces a mixture of wing and haltere cells. In the case of *Contrabithorax* it was shown that the proliferation rate of the cells did not affect the final differentiation.

In our view the cells of the mutant disc do not have a fixed commitment and during proliferation this can change from one cell division to the next perhaps depending on the level of bx^+ function in each cell. It has been shown that the cells can change their state of determination until the end of the proliferation period if the wild-type alleles of homeotic genes are eliminated: this is the case of *bithorax* (Morata & Garcia-Bellido, 1976), *engrailed* (Garcia-Bellido & Santamaria, 1972; Morata & Lawrence, 1975), *spineless-aristapedia* (Postlethwait & Girton, 1974). Under this hypothesis is not surprising to find some cases of bx^1 halteres where even though all the cells differentiate haltere trichomes, the number of them is much greater than in a normal haltere. Clearly, in the appendage shown in Fig. 6 the disc has proliferated as a wing disc but finally nearly all the cells differentiated haltere (except a few which differentiate wing bristles).

This work was supported, in part, by a grant from the Fondo de Investigaciones Sanitarias to E. S-H.

REFERENCES

- GARCIA-BELLIDO, A. & SANTAMARIA, P. (1972). Developmental analysis of the wing disk in the mutant *engrailed* of *Drosophila*. *Genetics* **72**, 87–107.
- KAUFMAN, T. C., TASAKA, S. E. & SUZUKI, D. T. (1973). The interactions of two complex loci, *zeste* and *bithorax* in *Drosophila melanogaster*. *Genetics* **75**, 299–321.
- KERRIDGE, S. & MORATA, G. (1982). Developmental effects of some newly induced *Ultrabithorax* alleles. *J. Embryol. exp. Morph.* **68**, 211–234.
- LAWRENCE, P. A. & MORATA, G. (1977). The early development of mesothoracic compartments in *Drosophila*. *Devl Biol.* **56**, 40–51.
- LEWIS, E. B. (1963). Genes and developmental pathways. *Amer. Zool.* **3**, 33–56.
- LEWIS, E. B. (1978). A gene complex controlling segmentation in *Drosophila*. *Nature* **276**, 565–570.
- LINDSLEY, D. L. & GRELL, E. H. (1968). Genetics variations of *Drosophila melanogaster*. *Carnegie Inst. Wash. Publ.* **627**.
- MORATA, G. (1975). Analysis of gene expression during development in the homeotic mutant *Contrabithorax* of *Drosophila melanogaster*. *J. Embryol. exp. Morph.* **34**, 19–31.
- MORATA, G. & GARCIA-BELLIDO, A. (1976). Developmental analysis of some mutants of the bithorax system of *Drosophila*. *Wilhelm Roux' Arch. devl Biol.* **179**, 125–143.
- MORATA, G. & KERRIDGE, S. (1980). An analysis of the expressivity of some bithorax transformations. In *Development and Neurobiology of Drosophila* (ed. O. Sidiqqi, P. Babu, L. Hall, J. Hall), pp. 141–154. London: Plenum Press.
- MORATA, G. & LAWRENCE, P. A. (1975). Control of compartment development by the *engrailed* gene of *Drosophila*. *Nature* **255**, 614–617.
- MORATA, G. & RIPOLL, P. (1975). Minutes: Mutants of *Drosophila* autonomously affecting cell division rate. *Devl Biol.* **42**, 211–221.
- POSTLETHWAIT, J. H. & GIRTON, J. R. (1974). Development in genetic mosaics of *aristapedia*, a homeotic mutant of *Drosophila melanogaster*. *Genetics* **76**, 767–774.
- STEINER, E. (1976). Establishment of compartments in the developing leg imaginal discs of *Drosophila melanogaster*. *Wilhelm Roux' Arch. devl Biol.* **180**, 9–30.

(Accepted 16 March 1983)