J. Embryol. exp. Morph. Vol. 61, pp. 69–86, 1981
Printed in Great Britain © Company of Biologists Limited 1981

Developmental analysis of the homoeotic mutation *bithoraxoid* of *Drosophila melanogaster*

By STEPHEN KERRIDGE[1] AND JAMES H. SANG[2]

From the Genetics Group, University of Sussex

SUMMARY

The homoeotic transformations caused by *bxd* are described in detail. The anterior histoblast nests of the first abdominal segment are missing, and are replaced by one or two leg discs ventrally. Mainly anterior compartment patterns are found in the ectopic, abdominal legs of adult flies. However, cell lineage analyses show that both anterior and posterior polyclones are established early in the development of these ectopic legs, but the posterior polyclone is smaller. Cells of the anterior polyclone may regulate later in development to adjust for this and form pattern elements normally derived from the posterior polyclone. In addition, experiments show that bxd^+ is required by the second larval instar stage, and possibly as early as the blastoderm stage.

INTRODUCTION

The adult integument of *Drosophila melanogaster* is constructed from segmentally arranged groups of cells; the imaginal discs and histoblast nests. It has been demonstrated for several discs that development proceeds via a series of decisions which subdivide the future adult structures into regions called compartments, e.g. the wing (Garcia-Bellido, Ripoll & Morata, 1973), the haltere (Morata & Garcia-Bellido, 1976), the legs (Steiner, 1976) and the proboscis (Struhl, 1977). In each of these structures there is, in particular, an early determination event that subdivides the primordium into an anterior and a posterior polyclone; the first compartmental division (Garcia-Bellido, 1975; Crick & Lawrence, 1975).

The decisions which separate polyclones are probably under the control of specific genes. Many of the homoeotic genes of *Drosophila* are candidates for the control of these compartmentalization events (Garcia-Bellido, 1975) because they transform regions which coincide with compartments. For example,

[1] *Present address:* Centro de Biologia Molecular Facultad de Ciencias, U.A.M., Madrid-34, Spain.

[2] *Author's address:* Genetics Group, Biology Building, University of Sussex, Falmer, Brighton, Sussex BN1 9QG, U.K.

engrailed replaces elements of the posterior compartment with patterns of the anterior compartment (Morata & Lawrence, 1975), and *bithorax* and *post-bithorax* replace anterior and posterior metathoracic regions with structures of the anterior and the posterior mesothoracic compartment, respectively (Lewis, 1963; Garcia-Bellido, 1975). However, some homoeotic mutants affect structures where compartments have not been shown to exist. One of these, *bithoraxoid* (*bxd*), transforms the first abominal segment to a metathoracic segment, bearing halteres and legs (Lewis, 1963).

The available *bxd* mutants show variable penetrance and expressivity: the haltere transformation is rare, the ectopic legs vary in size and sometimes only one is found. These legs are also abnormal and contain, predominantly, pattern elements of the anterior compartment. Nevertheless, we have been able to show by clonal analysis that the initial compartment separation is established early, although there are fewer cells in the primordium than normal, possibly due to the leakiness of the mutant. A secondary consequence is that the compartment boundary may be crossed later in development as the leg regulates somewhat. Our data also suggest that *bxd* acts prior to the second larval instar, and possibly as early as blastoderm formation.

MATERIALS AND METHODS

Bithorax *mutants*. The following *bithorax* mutations were generously supplied by Professor E. B. Lewis: bxd^{51j} (a point mutation), $Df(3)Ubx^{109}$ and *Df(3)P9* (deficiencies of the *bithorax* region). For other mutants refer to Lindsley & Grell (1968).

Analysis of phenotype. The terminology used for the cuticular structures of the thorax and abdomen is that employed by previous authors (Ferris, 1950; Steiner, 1976; Schubiger, 1968; Lawrence, Green & Johnston, 1978). Abdominal histoblast nests were examined using the techniques described by Roseland & Schneiderman (1979). After fixation (in formaldehyde; ethanol; acetic acid; water; 6:16:1:30) and dissection, third instar larvae were stained with Hanson's trioxyhaematin. The number of cells in each histoblast nest of the abdominal segments was then scored.

Cell lineage analysis. Mitotic recombination was induced by X-rays or by gamma irradiation (from a ^{60}Co source) using 500R for early induction (4 ± 2 h after egg lay, A.E.L.) and 1000R for late inductions (12 ± 6, 36 ± 6, 60 ± 6 h A.E.L.) of clones. The *Minute* and twin-spot techniques were employed. The *Minutes* and marker mutants used were as follows: *multiple wing hairs* (*mwh*), *yellow* (*y*), *javelin* (*jv*), *forked* (f^{36a}), *singed* (sn^3), $M(1)o^{Sp}$ $M(3)i^{55}$. These markers and the *Minute* and twin-spot techniques have been described in recent papers (Garcia-Bellido *et al.* 1976; Morata & Ripoll, 1975; Steiner, 1976; Wieschaus & Gehring, 1976). Two techniques were employed for the *Minute* analysis. In the first, ♀ *y* wf^{36a}/*FM6*; *Df(3)P9/Dp(3)P5* were crossed to ♂ $M(1)o^{Sp}$; bxd^{51j}/

Dp(1:3)A59 (where *Dp(1:3)A59* is a duplication of $M(1)o^+$ and f^+ on the third chromosome allowing the *Minute* to be introduced from the male). Embryos or larvae were then irradiated producing $y\ w^A\ f^{36a}\ M(1)o^+$ clones in flies not carrying *Dp(1:3)A59*. In the second, $y/y;\ Dp(1:3)sc^{J4}\ y^+\ M(3)i^{55}\ bxd^{51j}/mwh\ jv\ Df(3)Ubx^{109}$ flies were irradiated producing $y\ mwh\ jv\ M(3)i^+$ clones. For the twin-spot analysis, embryos or larvae of the genotype $y\ sn^3/f^{36a};\ bxd^{51j}\ Df(3)Ubx^{109}$ were irradiated producing $y\ sn^3$ and f^{36a} twin spots. *bxd* flies with large ectopic legs were selected for scoring, and thoracic and abdominal legs were mounted and scored under the compound microscope.

Clone size was estimated in the twin-spot analysis by counting the number of bristles in each clone. To allow for spontaneous clone induction, only clones of > 4 bristles were scored in flies irradiated at 36 ± 6 h or earlier. At 60 ± 6 h clones of > 2 bristles were scored. Average clone size was then calculated by taking the $\log^{10}$ of the number of bristles in each clone before statistical analysis. This was done to account for the exponential growth of cells.

Temperature-shift and -pulse experiments. A homozygous bxd^{51j} stock was selected for the presence of abdominal legs during one year. Temperature shifts, or temperature pulses, were made on the progeny from this stock as indicated in the text. Emerging adults were counted and scored for the presence of abdominal legs. All ectopic legs were then mounted and scored.

Ether phenocopies. Eggs collected from the cross $Df(3)P9/Dp(3)P5 \times bxd^{51j}/bxd^{51j}$ were exposed to ether vapour at the blastoderm stage, using the method of Capdevila and Garcia-Bellido (1978). Wild-type and *bxd* segregants were then scored for '*bx*' phenocopies in the metathoracic and first abdominal segments.

RESULTS

The bxd *phenotype*

The main transformation in *bxd* flies is the replacement of first abdominal pattern elements by thoracic cuticle (Lewis, 1963). In extreme *bxd* genotypes ($bxd/Df(3)P9$, $bxd/Df(3)Ubx^{109}$) the first abdominal tergite (Figure 1*b*) and sternite vestige are always missing. Less than 0·01 % of extreme *bxd* flies possess abdominal halteres dorsally (Fig. 1*c*). Unfortunately, the dorsal metanotum lacks recognizable pattern elements, and it is difficult to tell whether thoracic notum replaces the first abdominal tergite. However, the mutation *Tuft* (*Tft* 2–53.2) puts a row of bristles on the metanotum. In combination with *Tuft*, 40–50 % of $bxd/Df(3)P9$ flies produce two rows of bristles; one for the metathoracic notum proper, and a reduced one for the homoeotic metanotum (Fig. 1*a* and *b*).

The phenotype is more strongly expressed ventrally: 50–70 % of $bxd/Df(3)P9$ flies possess an ectopic leg (Fig. 1*d*). The morphology of ectopic legs varies, but in their most extreme expression they may resemble perfectly formed

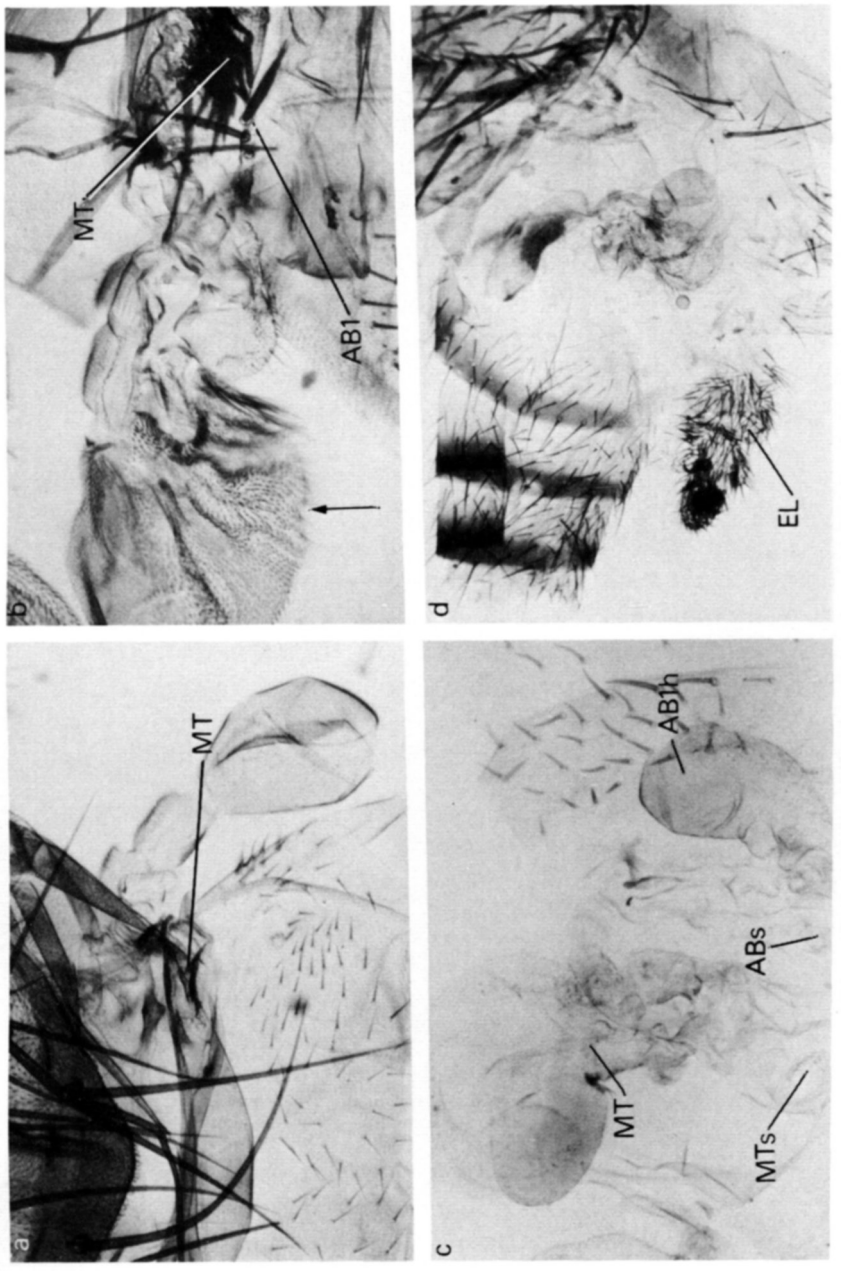

Fig. 1. (*a*) Wild-type metathoracic and first abdominal segments. This fly is also heterozygous for *Tft* which puts a row of large bristles on the metanotum (MT) (× 50). (*b*) $Df(3)P9/bxd^{6\dot{1}1}$ fly also with *Tft* showing a dense row of bristles on the metanotum (MT) and three bristles on the homoeotic first abdominal segment (AB1) (arrow). Note that the first abdominal segment is missing and that the posterior region of the haltere is replaced by wing structures (arrow) (× 50). (*c*) Extreme *bxd* phenotype (× 50) showing double haltere. The genotype is $bxd^{6\dot{1}1}/Df(3)Ubx^{109}$ and also f^{36a}/f^{36a}. Note both the metathoracic (MT) and ectopic halteres (AB1h) show posterior wing structures. *MTs*. – metathoracic spiracle. *ABs*. – homoeotic first abdominal spiracle. (*d*) Extreme $Df(3)Ubx^{109}/bxd^{6\dot{1}1}$ fly showing an ectopic leg (EL) with fused segments and uneverted segments (× 50).

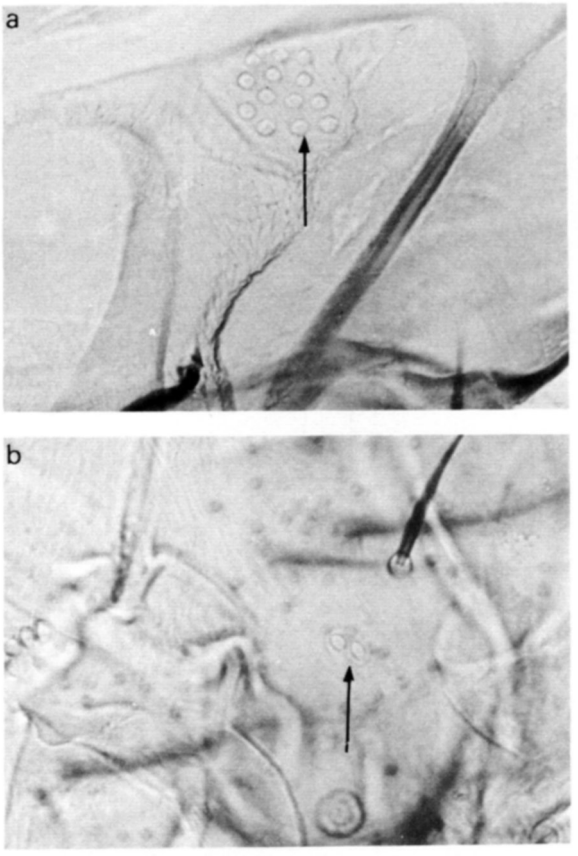

Fig. 2. (*a*) Proximal region of the coxa of an ectopic leg from $Df(3)P9/bxd^{51j}$ fly (× 200) showing multiplication of units of the *St8* group (arrow). (*b*) *Sc3* group (arrow) of the posterior compartment of the trochanter of an ectopic leg from $Df(3)P9/bxd^{51j}$ fly. Note that only two sensilla campaniformia are present (deficiency of units) (× 400).

legs. Most frequently, ectopic legs are small and exhibit segmental fusion, or they can be uneverted inside the abdomen of the fly.

In addition, *bxd* replaces posterior metathoracic structures with posterior mesothoracic elements. This may reflect a polarity effect of *bxd* on the adjacent *postbithorax* locus (Lewis, 1963).

Pattern in ectopic legs

Microscopical examination of the ectopic legs of *bxd* flies consistently shows pattern abnormalities, as illustrated by the numbers of sensilla groups (Table 1). Sensilla characteristic of the anterior compartment are found more frequently than elements of the posterior compartment. In addition, sensilla of the anterior compartment frequently exhibit multiplication of units (Fig. 2*a*). By contrast,

Table 1. *Frequency of sensilla groups in the anterior and posterior compartment of ectopic legs of* bxd^{511}/Df(3)Ubx^{109}

	Anterior compartment sensilla groups					Posterior compartment sensilla groups				
	St8	*2GSt*	*Sc5*	*St1*	*Sc1*	*St4*	*St5*	*Sc3*	*Sc8*	*Sc11*
Percentage occurrence in 127 ectopic legs	90	95	89	86	86	4	89	20	9	82
% with deficiency of units	0	0	0	—	—	0	77	100	100	5
% with multiplication of units	54	63	65	5	3	0	0	0	0	0

Sc = sensilla campaniformia; *St* = sensilla trichodea. *St1* and *Sc1* deficiencies results in their absence. Deficiency of units means the sensilla group, when present, has fewer sensilla than expected. Thus *St8* may have five sensilla instead of eight. Multiplication of units means that the sensilla group has more sensilla than in thoracic legs. Thus *St8* may have 12 units in some cases.

Table 2. *Comparisons of numbers of cells in the histoblast nests of the first and second abdominal segments in wild type and* bxd

	Dorsal anterior	Dorsal posterior	Ventral	N
AB1				
Wildtype	14±3	7±2	15±3	18
bxd/Df Ubx^{109}	3±1	6±2	1±1	28
AB2				
Wildtype	17±3	6±2	15±3	18
bxd/Df Ubx^{109}	14±3	5±1	15±3	28

N = number of hemisegments scored. Standard deviations of scores are given.

posterior compartment sensilla exhibit a deficiency of units (Fig. 2*b*). These compartment specific abnormalities are also seen in the bristle patterns of the ectopic legs.

In terms of general pattern, the abnormal homoeotic structures have been considered equivalent to a transformed metathoracic segment, i.e. both anterior metathorax (*AMT*) and posterior mesothorax (*PMS*) (Lewis, 1963). This is true for the rare ectopic halteres, and of the ectopic legs. However, in ectopic legs structures characteristic of the metathoracic leg are found in the posterior compartment such as the lack of bristles close to *Sc3*, the presence of bristle patterns distal to *Scl*, and the transverse row bristles. This is possibly due to a weak polarity effect of *bxd* on *pbx* in the ectopic leg.

Examination of histoblasts in bxd

The morphological features of the histoblast nests which give rise to the abdominal segments have been described by Madhavan & Schneiderman (1978). Here we examine these nests in the first two abdominal segments to see if they are normal or not. The preparations also allow us to identify imaginal discs in these segments.

Numbers of cells in the dorsal anterior and ventral nests of the first abdominal segment are reduced in *bxd*^{51j}/*Df(3)Ubx*^{109} larvae (Table 2), and the same is true for all *bxd* genotypes. Generally, extreme *bxd* genotypes lack histoblasts altogether in these larger nests which are situated anteriorly in each larval segment. Larval epidermal cells fill in the positions of the missing histoblast cells. Unlike the large nests, the posterior dorsal nest in the first abdominal segment is present in all *bxd* genotypes (Fig. 3*b*), including extreme genotypes.

In addition, especially in extreme genotypes, we have observed vesicles that are reminiscent of imaginal discs in the first abdominal segment. However, these have been seen only ventrally, in a midventral location (Fig. 3*b*). Using *in vivo* transplantation (Hadorn, 1965), these discs have been shown to be leg

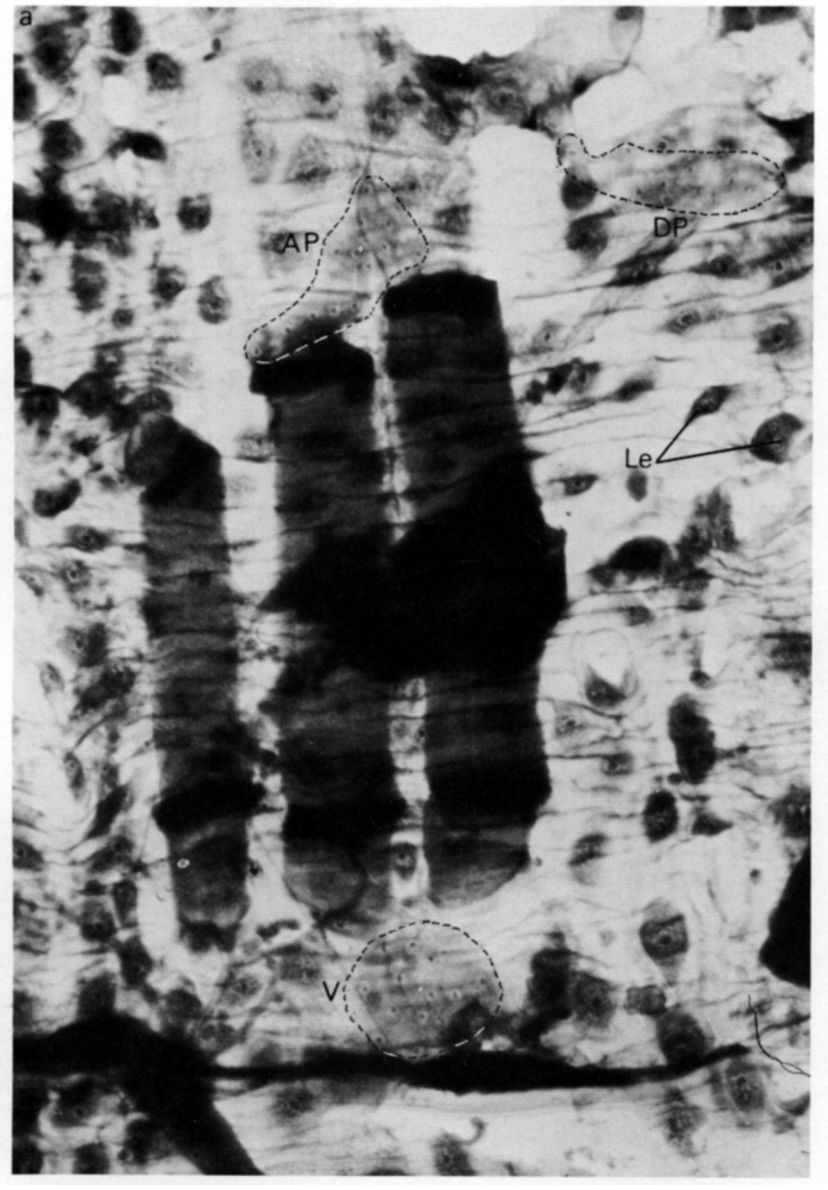

Fig. 3*a*

discs on the basis of bristle and sensilla group patterns on the metamorphosed discs. As in ectopic legs, implants predominantly exhibit patterns of the anterior compartment of the leg together with the pattern abnormalities described for ectopic legs *in situ* (data not shown).

Fig. 3 *b*

Fig. 3. Larval epidermis from third instar larvae stained with Hanson's trioxyhaematin showing first abdominal histoblast nests of (*a*) wild-type and (*b*) bxd^{51j}/ *Df(3)P9* larvae (× 100). The histoblasts are small diploid cells distinct from the large polytenised larval epidermal cells (LE). In (*a*) note the three groups of nests; anterior dorsal (DA), posterior dorsal (DP), and ventral (V) nests situated close to dorsoventral muscles (m). In (*b*) note the reduction in the number of cells in the anterior dorsal and the absence of cells in the ventral nests, and the presence of the smaller posterior dorsal nest. A disc is present ventrally (*d*).

Table 3. *The numbers of* Minute$^+$ *clones found in the third leg (control) and in ectopic legs of* bxd^{51j}/bxd$^-$ *flies irradiated at different ages*

Irradiation time, in *Minute* time	N	3rd leg		N	4th leg		Anterior/ Posterior
		Anterior	Posterior		Anterior	Posterior	
4±2 h A.E.L.	466	7	6	254	7	4	1
12±6	467	5	2	349	4	1	2
36±6	631	26	17	409	19	4	0
60±6	450	32	16	283	16	7	2
Total	2014	70 (3·5%)	41 (2·0%)	1295	43 (3·3%)	16 (1·2%)	5

Clones induced at 4 h were *y f*^{37a} *M(1)o*$^+$, whereas those at later stages were *y mwh M(3)i*$^+$. The six clones which only just crossed from the anterior to the posterior compartment are included in the anterior class since they mainly marked patterns of this compartment. The five clones clearly crossing this boundary is significantly greater than would be expected if they resulted from double inductions (expected number: $0{\cdot}033 \times 0{\cdot}012 \times 1295 = 0{\cdot}52$; $P < 0{\cdot}01$ by χ^2 test); no double inductions were found in the controls.

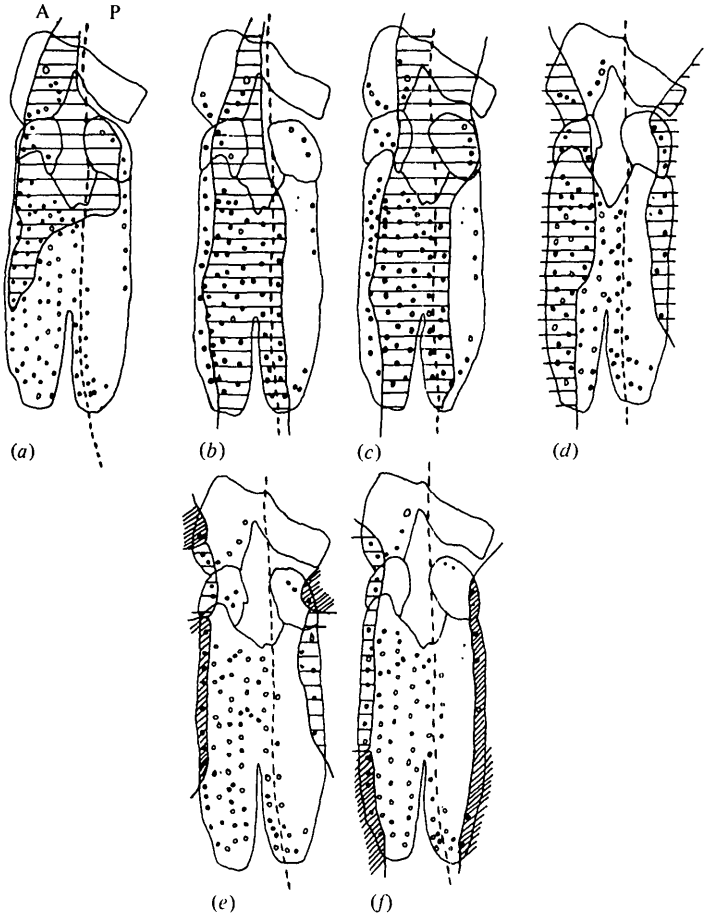

Fig. 4. Some examples of clones violating the anterior–posterior compartment boundary in ectopic legs of *bxd* flies. (*a*, *b*) (induced at 12 ± 6 h A.E.L.), (*c*, *d*) (induced at 60 ± 6 h A.E.L.) are *Minute*$^{+}$ clones. (*e*) (induced at 4 ± 2 h) and (*f*) (induced at 36 ± 6 h) are *y sn*^{3} (horizontal shading) and f^{36a} (diagonal shading) twin spots. The diagrams are modified from Steiner (1976) to show the bristle patterns in the ectopic legs where the clones were found. The dotted lines represent the boundary separating anterior (A) and posterior (P) compartments.

Cell lineage analysis

The morphological features of the *bxd* mutant present a problem. The mutant produces mainly anterior leg structures in place of the first abdominal segment. One interpretation of this is that *bxd* causes a switch in the determination of the primordial cells of the first abdominal segment to an anterior thoracic polyclone, subsequently regenerating the posterior polyclone from these anterior cells. Here we have used clonal analysis to see whether or not compartments are set up in ectopic legs as they are in normal legs (Steiner, 1976). If the regeneration hypothesis is correct, there should be no compartment boundary and clones should mark elements of both the anterior and posterior compartments.

Table 4. *Clone size, frequency and estimated number of primordial cells in ectopic and metathoracic legs*

	Age at irradiation (h)	Total legs examined	Frequency of clones		Mean clone size (± S.D.) in bristles		Estimated cell number in primordium
			A	P	A	P	
Metathoracic leg (control)	4±2	1010	0·02	0·01	23±2 (19)	20±3 (15)	7
	12±6	537	0·04	0·02	17±2 (25)	22±2 (12)	8
	36±6	554	0·06	0·04	18±2 (36)	17±3 (25)	9
	60±6	197	0·16	0·06	6±2	7±1	24
Ectopic leg (experimental)	4±2	735	0·03	0·01	29±2 (21)	36±3 (6)	4
	12±6	348	0·04	0·01	28±2 (16)	35±3 (7)	4
	36±6	356	0·07	0·02	22±2 (27)	38±2 (7)	4
	60±6	142	0·10	0·02	6±2 (15)	14±3 (4)	12

Clone sizes were estimated by counting the number of bristles in the clones (excluding tarsal segments). Numbers in brackets indicate the number of spots from which clone sizes were calculated. Estimates of primordial cell number were made from counts of 308 bristles in metathoracic legs and 240 bristles in ectopic legs (excluding tarsal segments). These are the mean bristle numbers found in legs containing clones. A is the anterior, and P the posterior compartment.

Since ectopic legs have abnormal patterns, it is difficult to allocate these to the anterior or posterior compartment. To minimise this difficulty we scored clones only in large ectopic legs and then only in the proximal leg segments (trochanter and femur) where anterior and posterior patterns can be clearly distinguished. Thus, in the femur, only those legs with five distinct bristle rows, typical of the anterior compartment, and two distinct bristle rows of the posterior compartment (see Figure 6 in Steiner, 1976) were scored.

Minute *analysis. Minute*$^+$ clones were scored in ectopic (experimental) and metathoracic (control) legs of *bxd* flies. Leg clones in the controls respected the anterior–posterior compartment boundary, as already described by Steiner (1976).

The majority of clones in ectopic legs (Table 3) was restricted to elements of the anterior or of the posterior compartment (37 anterior and 16 posterior clones). This indicates that the compartment boundary is established in ectopic legs as early in development as in thoracic legs. Contrary to this finding, an additional 11 anterior clones crossed the compartment boundary, although only two or three bristles of the posterior compartment of the trochanter were marked in six of these cases. The remaining five clones clearly violated the compartment-boundary-marking elements in the posterior compartment of the femur (Table 3 and Fig. 4*a*, *b*, *c*, *d*). This number is significantly greater than expected from double inductions (Table 3). Since the clones marked a larger region of the anterior compartment but only a few bristles in the posterior compartment, it is likely that this is a late event (see Discussion). It cannot be due to regeneration of the entire posterior compartment from the anterior polyclone.

Twin-spot analysis. Here we induced and scored twin spots and single spots in metathoracic and ectopic legs (see Materials and Methods). The purpose was to analyse the proliferation dynamics, and see if violation of the compartment boundary could be detected in cells dividing at the normal rate in ectopic legs.

All clones in metathoracic legs marked only those patterns of the anterior or of the posterior compartment. Of the clones induced in ectopic legs at 36 h A.E.L. or earlier (Table 4), 39 anterior and 16 posterior clones marked bristle rows adjacent to the compartment boundary of the femur. Seven of these, mainly anterior clones, marked two or three bristles of the trochanter which are usually derived from the posterior polyclone. Three further clones clearly crossed the compartment boundary in the femur (Fig. 4*e*, *f*). These results show that cells growing at the normal rate can cross the anterior–posterior compartment boundary.

We also measured clone sizes in control and experimental legs by counting the number of bristles marked by clones induced at different stages of development. The results show (Table 4) that clone sizes are significantly larger ($P < 0{\cdot}01$) in ectopic than in metathoracic legs. Since mean clone sizes in

Table 5. *The effects of 12 h temperature pulses on the size of the ectopic legs of* bxd^{51j} *flies*

Time of pulse (h A.E.L.)	Development 28°: 19° pulse	Development 19°: 28° pulse
Controls (no pulse)	25 (203)	60 (197)
0–12	52 (201)	19 (201)
12–24	22 (213)	53 (193)
24–36	29 (203)	55 (190)
36–48	22 (250)	60 (206)

Number of flies scored in parentheses. The figures are for the percentage of flies with large (>30 bristles) legs.

ectopic legs were about twice that of controls (Table 4), cells in ectopic leg primordia must undergo an extra division, indicating a size regulation within the ectopic leg polyclones.

Differences in clone size also reflect the number of cells within a primordium when expressed as a fraction of the total number of cells (Bryant & Schneiderman, 1969). These fractions are inversely related to the number of cells at the time of irradiation. Because we used single spots which represent only half the progeny of the recombination event, the real clone size would be twice as large. In addition, we have to take account of the smaller number of bristles in ectopic legs (Table 4). The results show that the ectopic leg derives from about half the number of cells found in normal leg primordia. The estimates of progenitor cell numbers are probably too low since we are counting only bristles and there will be some inaccuracy due to differences of bristle density in different regions of the legs.

Clone frequencies also give estimates of the relative numbers of cells giving rise to a structure. However, we found no consistent difference in the clone frequencies between the control and experimental series. However, clone size data give a clearer estimate of the relative primordial-cell numbers since frequency estimates rely on the equal sensitivity of the cells to mitotic recombination in the two structures.

Clone sizes in both ectopic and control legs were significantly smaller at 60 ± 6 h A.E.L. than at 36 ± 6 h A.E.L. This suggests that cells in both series enter the main proliferation phase at around the same time. Furthermore, *bxd*$^{+}$ must be active during this stage (the second larval instar) since abdominal cells do not divide during larval stages (Garcia-Bellido & Merriam, 1971).

Time of bxd *gene activity*

The cell lineage analysis shows that *bxd* is active in imaginal leg primordia by the second larval stage. The experiments described below were designed to

Table 6. *The numbers of* bx *phenocopy spots in* bxd^{51j}/Df(3)P9 *flies after ether treatment at blastoderm stage*

	No. of hemisegments	No. of phenocopies
Dorsal metathorax	220	44
Ventral metathorax	220	36
Ventral first abdominal	139	4

Dorsal phenocopies include wing and mesonotum structures in the metathorax; ventral phenocopies include the sternopleural and apical bristles of the mesothoracic leg. Only sternopleural phenocopies were seen in *bxd* ectopic legs.

see if *bxd* has an earlier function in the determination of the first abdominal segment.

Temperature shift and temperature-pulse experiments. A line of *bxd* homozygotes was selected for the presence of abdominal legs over a period of one year. After selection, between 50–60 % of flies possessed one or two ectopic legs under optimal culture conditions.

A series of shift-up and shift-down experiments showed that the size of *bxd*^{51j} ectopic legs had a temperature-sensitive phase (T.S.P.) during embryogenesis (data not shown). Therefore we decided to analyse the phase more accurately using temperature pulses. The results (Table 5) show that the T.S.P. is most pronounced during the first 12 h of development.

bx *phenocopies in* bxd *flies.* Exposure of *Drosophila* blastoderm-staged embryos to ether vapour causes phenocopies of the *bithorax* (*bx*) mutation in a proportion of the emerging adults (Gloor, 1947). There are many interpretations of this result; for example, ether may affect the expression of the *bx*$^+$ gene, or the cortical signals which activate the *bx*$^+$ gene, or it may cause phenocopying in a disruptive way. However this may be, the ether action is restricted to a very precise time, at blastoderm. If the cells of the presumptive first abdominal segment of *bxd* individuals are determined to a metathoracic pathway of development at the blastoderm stage, it should be possible to induce *bx* phenocopies in the first abdominal segment of *bxd* flies, which may thus be indicative of when *bxd* is activated.

Blastoderm-staged eggs (2–3 h old) from the cross *Df(3)Ubx*^{109}*/TM1* × *bxd*^{51j}*/ bxd*^{51j}, were exposed to ether vapour. Emerging adults were then scored for the presence of wing mesonotum and second leg structures in the metathoracic and first abdominal segments (Table 6). Phenocopies of *bx* were found in the metathoracic segment, as shown previously (Bownes & Seiler, 1977; Capdevila & Garcia-Bellido, 1978). We also found mesothoracic structures in the ectopic legs of *bxd* flies (Table 6) showing that ether disrupts not only metathoracic determination in the normal metathoracic primordium but also

in the homoeotic *bxd* first abdominal segment. The *bxd* mutation must therefore cause a metathoracic determinative event in the homoeotic abdomen at the same time as, or earlier than, the equivalent event in the metathorax.

DISCUSSION

Compartments and bxd. The fact that patterns in ectopic legs of *bxd* flies are predominantly those of the anterior compartment led us to propose, as a working hypothesis, that these legs arose exclusively from the anterior polyclone. In that event, the incomplete posterior compartment would be regenerated from the anterior cells and clones should violate the compartment boundary. Examination of *Minute*$^+$ clones in ectopic legs shows that the anterior and posterior polyclones are established very early in development. Thus cells are set aside to form the posterior compartment by this stage. However, some clones were found which transgressed the anterior–posterior compartment boundary. Due to the *Minute*$^+$ growth advantage, such a clone would be expected to fill the posterior compartment if it arose early in leg development, but none of the *Minute*$^+$ clones crossing the boundary fill a large portion of the posterior compartment. Crossing of clones from the anterior to the posterior leg compartment must therefore be a late event during ectopic leg morphogenesis.

The question arising therefore is why do some patterns show a lack of provenance from the posterior polyclone? The most likely cause is the leaky nature of available *bxd* alleles. At differentiation, patterns typical of the anterior compartment are found more frequently than those of the posterior compartment and are always more complete (Table 1). The deficiencies of cells must be due to the relatively smaller number of initial cells determined for ectopic leg-disc development (Table 4). Clone size is greater, and this deficiency is therefore greater in the posterior compartment (Table 4), and is reflected in its shortage of typical structures. This disparity between the numbers of cells in the two compartments may cause subsequent regulation during development. Hence, mainly anterior clones (six *Minute*$^+$ and seven twin clones) span the compartment boundary to mark one or two bristles of the posterior polyclone, and in other cases violation of the boundary may indicate a greater deficiency of posterior compartment cells and greater regulation. The maintenance of relative cell numbers between the anterior and posterior compartments may be important for normal leg development.

There are two possible explanations of the observed regulation. First, some posterior cells may arise by regeneration from anterior cells. In this case, the regenerated cells would lose their anterior commitment and then follow the posterior pathway. This has been shown to occur in the wing disc, after experimental intervention (Szabad, Simpson & Nöthiger, 1979). A second possibility is that crossing reflects a shift in the compartment boundary

upsetting the normal provenance of some posterior patterns. Such variants in the origin of pattern elements have already been noted during the normal development of the tarsus (Lawrence, Struhl & Morata, 1979) and of the antenna (Morata & Lawrence, 1979).

Function of bxd. Our results show that *bxd* larvae are deficient in histoblast nests in the first abdominal segment. Their absence is correlated with the development of vesicles resembling imaginal discs which differentiate as legs when cultured *in vivo*. With respect to imaginal cells, bxd^+ must therefore be concerned with the proper organisation and determination of histoblasts in this segment and not in more posterior abdominal segments.

Analysis of cell proliferation in the ectopic leg primordia show that cells enter the main proliferation phase at about the same time as in thoracic legs. This period corresponds with the second larval stage and is consistent with the direct observation of mitoses in normal leg discs (Madhavan & Schneiderman, 1978), showing that *bxd* must be active at least by this stage since the histoblasts do not normally divide during larval stages (Garcia-Bellido & Merriam, 1971).

Indirect evidence of ether phenocopies and temperature pulses suggests, but does not prove, that bxd^+ may be required as early as the blastoderm stage of development. Ether is taken to interfere with the metathoracic determination by affecting bx^+ gene expression (Gloor, 1947; Capdevila & Garcia-Bellido, 1978). If so, this event must also be affected in the first abdominal segment of *bxd* embryos. It follows that bx^+ must also be active in the primordia of ectopic leg discs at blastoderm, and possibly that *bxd* is required at this stage. Morata and Garcia-Bellido (1976) have already shown that bxd^+ is active during the later larval stages so bxd^+ must function not only during primary determination, but also subsequently.

We wish to thank Drs A. Garcia-Bellido, E. B. Lewis, P. A. Lawrence, G. Morata, M. J. Pearson, C. R. Roseland, and J. R. S. Whittle for their help during the course of this work. The research was supported by an S.R.C. studentship to S.K.

REFERENCES

BOWNES, M. & SEILER, M. (1977). Developmental effects of exposing *Drosophila* embryos to ether vapour. *J. exp. Zool.* **199**, 9–24.

BRYANT, P. J. & SCHNEIDERMAN, H. A. (1969). Cell lineage, growth and determination in the imaginal leg discs of *Drosophila melanogaster*. *Devl Biol.* **20**, 263–290.

CAPDEVILA, M. P. & GARCIA-BELLIDO, A. (1978). Phenocopies of *Bithorax* mutants. Genetic and developmental analyses. *Wilhelm Roux's Arch. devl Biol.* **185**, 105–126.

CRICK, F. H. C. & LAWRENCE, P. A. (1975). Compartments and polyclones in insect development. *Science* **189**, 340–347.

FERRIS, G. F. (1950). External morphology of the adult. In *Biology of* Drosophila (ed. M. Demerec), pp. 268–418. New York: Wiley and Sons.

GARCIA-BELLIDO, A. (1975). Genetic control of wing disc development in *Drosophila*. In *Cell Patterning* **29**. Ciba Symposium. Elsevier, Amsterdam.

GARCIA-BELLIDO, A. & MERRIAM, J. R. (1971). Clonal parameters of tergite development in *Drosophila*. *Devl Biol.* **26**, 264–276.

GARCIA-BELLIDO, A., RIPOLL, P. & MORATA, G. (1973). Developmental compartmentalisation of the wing disc of *Drosophila*. *Nature New Biol.* **245**, 251–253.

GARCIA-BELLIDO, A., RIPOLL, P. & MORATA, G. (1976). Developmental segregation in the dorsal mesothoracic disc of *Drosophila*. *Devl Biol.* **48**, 132–149.

GLOOR, H. (1947). Phänokopie-Versuche mit Aether an *Drosophila*. *Rev. Suisse Zool.* **54**, 637–712.

HADORN, E. (1965). Problems of determination and transdetermination. In *Genetic Control of Differentiation. Brookhaven Symp. Biol.* **18**, 148–161.

LAWRENCE, P. A., GREEN, S. & JOHNSTON, P. (1978). Compartmentalisation and growth of the *Drosophila* abdomen. *J. Embryol. exp. Morph.* **43**, 233–245.

LAWRENCE, P. A., STRUHL, G. & MORATA, G. (1979). Bristle patterns and compartment boundaries in the tarsi of *Drosophila*. *J. Embryol. exp. Morph.* **51**, 195–208.

LEWIS, E. B. (1963). Genes and developmental pathways. *Am. Zoologist* **3**, 33–56.

LINDSLEY, D. L. & GRELL, E. L. (1968). Genetic variations of *Drosophila melanogaster*. *Carnegie Inst. Wash. Publ.* No. **627**.

MADHAVAN, M. M. & SCHNEIDERMAN, H. A. (1978). Histological analysis of the dynamics of growth of imaginal discs and histoblast nests during larval development of *Drosophila melanogaster*. *Wilhelm Roux's Arch. devl Biol.* **183**, 269–305.

MORATA, G. & LAWRENCE, P. A. (1975). Control of compartment development by the *engrailed* gene in *Drosophila*. *Nature New Biol.* **255**, 5510, 614–617.

MORATA, G. & LAWRENCE, P. A. (1979). Development of the eye-antenna disc of *Drosophila*. *Devl Biol.* **70**, 355–371.

MORATA, G. & RIPOLL, P. (1975). *Minutes*: mutants autonomously affecting cell division rate in *Drosophila*. *Devl Biol.* **42**, 211–221.

MORATA, G. & GARCIA-BELLIDO, A. (1976). Development analysis of some mutants of the *bithorax* system of *Drosophila*. *Wilhelm Roux's Arch. devl Biol.* **179**, 125–143.

ROSELAND, C. R. & SCHNEIDERMAN, H. A. (1979). Regulation and metamorphosis of the abdominal histoblasts of *Drosophila melanogaster*. *Wilhelm Roux's Arch. devl Biol.* **186**, 235–265.

SCHUBIGER, G. (1968). Anlageplan, Determinationszustand, und Transdeterminationsleistungen der annlichen Vorderbeinscheibe von *Drosophila melanogaster*. *Wilhelm Roux's Arch. devl Biol.* **160**, 9–40.

STEINER, E. (1976). Establishment of compartments in the developing leg imaginal discs of *Drosophila melanogaster*. *Wilhelm Roux's Arch. devl Biol.* **180**, 9–30.

STRUHL, G. (1977). Developmental compartments in the proboscis of *Drosophila*. *Nature New Biol.* **270**, 723–725.

SZABAD, J., SIMPSON, P. & NÖTHIGER, R. (1979). Regeneration and compartments in *Drosophila*. *J. Embryol. exp. Morph.* **49**, 229–241.

WIESCHAUS, E. & GEHRING, W. J. (1976). Clonal analysis of primordial disc cells in the early embryo of *Drosophila melanogaster*. *Devl Biol.* **50**, 249–263.

(Required 11 April 1980, revised 10 September 1980)