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Compartmentalization and growth of the *Drosophila* abdomen

By PETER A. LAWRENCE,[1] SHEILA M. GREEN[1] AND PAUL JOHNSTON[1]

From the MRC Laboratory of Molecular Biology, Cambridge

SUMMARY

An analysis of the cell lineage of the adult *Drosophila* abdomen is reported. Genetically marked clones are produced in the tergites in the embryo and in the sternites and pleura during larval life. The spatial disposition of the segment primordia is also studied in a series of 250 gynandromorphs. We conclude that in the embryo the segments are all separate polyclones soon after blastoderm and these are probably adjacent to each other. Gynandromorph analysis suggests that segments 2–6 develop from similarly sized cell groups but that the first tergite develops from a primordium which is equal to that of two other segments. We suggest that presumptive adult and larval cells are not separated at blastoderm. Our estimate from clonal analysis of the number of larval cells (12) which construct the sternites and pleura is equal to the number observed directly in the ventral nest of histoblasts.

INTRODUCTION

In *Drosophila* we know that major determinative events program the cells at the blastoderm stage (Illmensee, 1976; Chan & Gehring, 1971). The groups of primordial cells which will construct the anterior and posterior compartments (Garcia-Bellido, Ripoll & Morata, 1973) of the adult mesothoracic segment are defined at or shortly after the blastoderm stage (Steiner, 1976; Lawrence & Morata, 1977). These cells are probably adjacent (Lawrence & Morata, 1977), not mixing during subsequent growth presumably because they have different states of determination and different cell affinities (Morata & Lawrence, 1975; Lawrence & Morata, 1976). Our main aim in this paper is to extend our knowledge of the blastoderm – we have attempted to estimate the relative numbers and positions of those cells which generate the adult abdominal cuticle. We have tried to find out if the blastoderm is already subdivided into segmental polyclones (Crick & Lawrence, 1975) and whether they are adjacent. As detailed analyses of postembryonic growth of the tergites are already available (Garcia-Bellido & Merriam, 1971*a*; Guerra, Postlethwait, & Schneiderman, 1973), we have limited our study of postembryonic growth to the ventral abdomen – the sternites and pleura.

[1] *Author's address:* MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH, U.K.

We conclude that the primordia for the abdominal histoblasts are adjacent and that therefore cell affinity differences may keep them from intermingling. We suspect that the presumptive larval and adult cells are together in one polyclone at the blastoderm stage. The first abdominal tergite is anomalous; possibly, it develops from twice as many cells at blastoderm as any of the other segments. From the average clone size in adults, we estimate the number of cells in the ventral histoblasts of the larva. This estimate (12.5 cells) is equal to the mean number of cells observed in dissected material.

MATERIALS AND METHODS

Gynandromorphs

Gynandromorphs were made by crossing males of a suitably unstable ring stock (In(1)w^{vC}; see Lindsley & Grell (1968) for description of all genotypes used) to females homozygous for *y w f*^{36a}; 250 were collected in a preliminary study and abdominal segments 3–5 mounted. This batch, probably because screening was not meticulous enough (some inconspicuous gynandromorphs were overlooked) gave, on average, an excess of male over female tissue. Subsequently a further 250 gynandromorphs were very carefully collected and mounted so that segments 1–6 could be scored. In this batch male and female structures were equally common. Occasionally the ring chromosome is lost or involved in somatic recombination very late in development (Garcia-Bellido & Merriam, 1971*a*; Ripoll, 1972) so we ignored those few cases where only one (♂) *y w f*^{36a} bristle was present in an otherwise (♀) wild-type segment.

Clonal analysis of embryos

Males *mwh Sb*^{63b}*M*(3)w^{124}/*Ubx*^{130} were crossed to wild-type females of the Oregon R stock. The population was kept continuously in good conditions for egg laying and eggs were collected over 4 h periods to be irradiated (500R 250 kV at 15 mA, half value layer 2·3 mm copper, distance of 3 cm, rate of 530R/min). Abdomens of flies of the genotype *mwh Sb*^{63b}*M*(3)w^{124}/+ were squash-mounted dorsal side uppermost on slides for each age group (4 ± 2 and 24 ± 2 h after egg laying (AEL)), and the slides screened. We inverted the slide to inspect the ventral surface. Unirradiated controls from the same experiment were also mounted. All the slides were mixed and screened 'blind' for clones of wild-type bristles in the *Stubble Minute* background. Because of the relative insensitivity of eggs at around the blastoderm stage to X-rays (Wieschaus & Gehring, 1976*a*) we expect our 4 ± 2 h sample to be much enriched for that stage.

Clonal analysis of larvae

For analysis of the growth of sternites and pleura it was necessary to mark both bristles and trichomes; for this we crossed males *Dpsc*^{J4} *flr*/*TM*1 to females *y f*^{36a}; *mwh. flare* (*flr*, 3–39) is a new cell marker mutant which affects

both trichomes and bristles (Garcia-Bellido & Dapena, 1974). Recombination in the IIIL proximal to *flr* gives *y*; *mwh* and *flr* spots in twin. Recombination distal to *flr* gives single *y*; *mwh* spots. The bristles are marked by *yellow*, and *mwh* marks *all* the cell surface area of the pleura and sternites. Larvae were irradiated at different times before puparium formation (BPF). The newly formed puparia were collected at different intervals after irradiation of larvae. Puparia were irradiated at 1 ± 1, 3 ± 1, 5 ± 1 and 7 ± 1 h after puparium formation (APF). In order to extend the cuticle for examination we injected the abdomens under pressure with Carnoy's fixative and slit down the mid-dorsal line, then opened up the abdomens and mounted them flat. This allowed the accurate drawing of clones with a drawing apparatus attached to a compound microscope. Their area was estimated by weighing planimetry paper. The *flr* territories provided little additional information as they were often rather small, and the cuticle looked damaged; they did serve to indicate the general fate of the sister cell.

Whole mounts of larvae were made by Pearson's method (1972). Larvae were decapitated and the internal organs pulled out with forceps. The headless larvae were then slipped over a pipette, held with forceps and inflated with Ringer's followed by Carnoy's fixative. The inflated skins were then kept in Carnoy's for 7–10 min, cut down the ventral midline with scissors and pinned out. The muscles were removed with fine needles to reveal the nests of histoblasts. The preparations were then stained with Hansen's trioxyhaematein, cleaned and mounted on slides.

RESULTS

Anatomy of the abdominal segments

Previous descriptions of the anatomy (Ferris, 1950) have not included a feature which is shown in the dissected abdomens illustrated in Fig. 1. Each sternite bears two sensilla characteristically placed in each segment (Wheeler, 1960). In both sexes these sensilla are present on sternites 1–7. In some females one or two sensilla have been noted posteriorly to the seventh sternite, indicating some development of the eighth abdominal segment. Thus although the ventral segments 6 and 7 have been described as absent in males it might be more accurate to say they lack bristles. An important feature of the tergites is that when the abdomen is expanded before mounting, only about half the area is covered with bristles (Fig. 2).

Gynandromorph analysis

Most of the ensuing data is taken from the second set of 250 carefully searched gynandromorphs in which there were on average equal amounts of male and female tissue (for example, the second segment was entirely male in 227 cases, entirely female in 207 cases and mosaic in 66 cases).

The *frequency of mosaicism* of a region is a measure of the number of blasto-

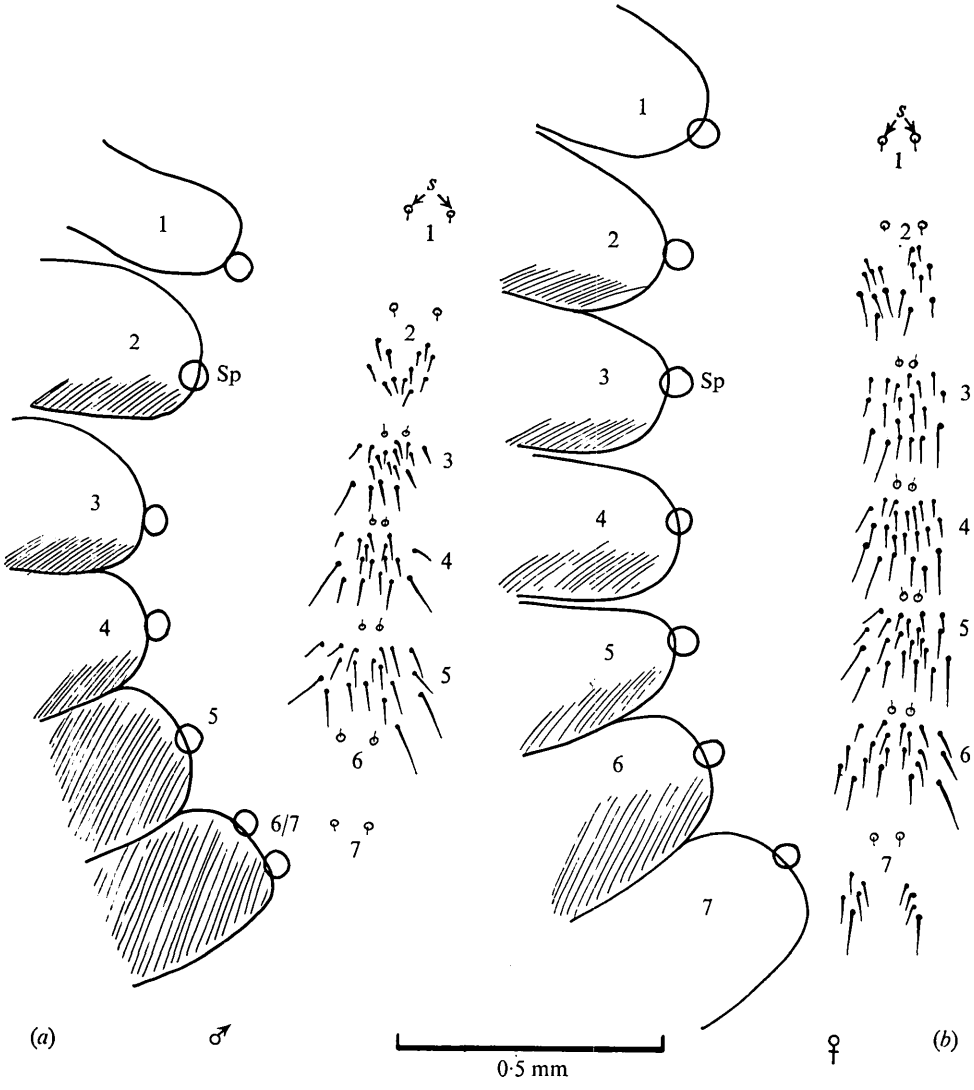

Fig. 1. Scale drawings of part of expanded male (*a*) and female (*b*) cuticles, which have been cut down the mid-dorsal line and opened out. Sternites and pleura are marked for the different segments. Note the position of the characteristic sensilla (*s*) which mark the anterior region of each sternite.

derm cells in the primordia which give rise to it (Hotta & Benzer, 1972). This is because the more cells in the primordium at blastoderm the greater the probability that the male/female borderline in gynandromorphs will pass through it. For tergites 2–6 inclusive it was identical at 13 % (the figures were 66, 64, 69, 61 and 59 cases out of 500 sides for tergites 2–6 respectively; these figures are not significantly different from each other). However the frequency of mosaicism for tergite 1 was significantly higher ($P \simeq 0{\cdot}001$), being 21 % (106 cases from

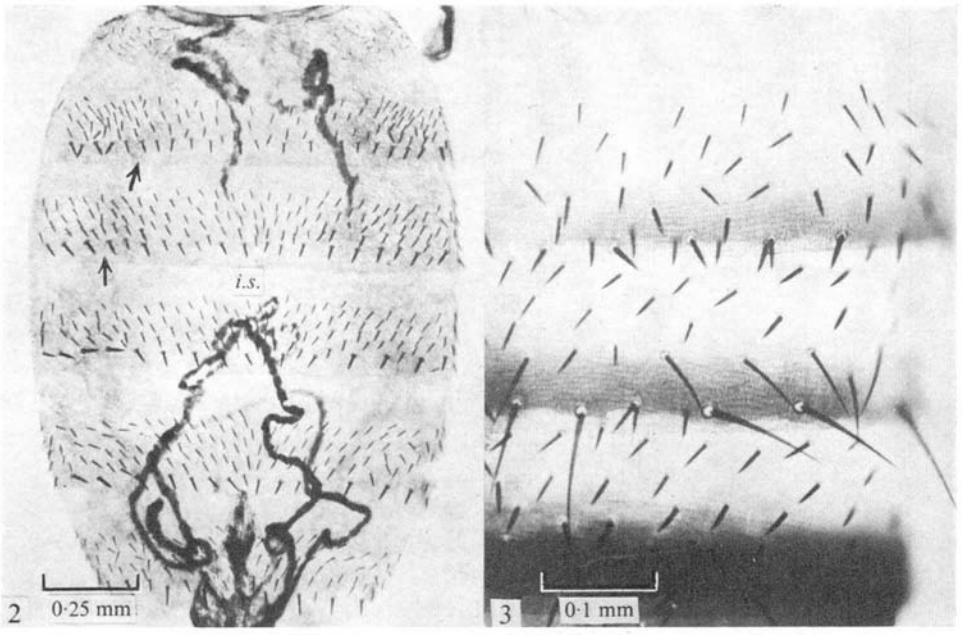

Fig. 2. A dorsal view of an expanded female abdomen of the genotype *mwh* Sb^{63b} $M(3)w^{124}/+$. Note the large regions of intersegmental territory (*i.s.*) covered with trichomes which intervene between the tergites. These regions still contain folded and unexpanded parts (arrows). In other preparations these regions are sometimes completely expanded – then one can see bands which completely lack trichomes and show small indentations: these probably are the true intersegmental boundaries.

Fig. 3. Region of tergites of the genotype *mwh* Sb^{63b} $M(3)w^{124}/+$ to show a Sb^{+} $M(3)w^{+}$ clone of 11 bristles in the 3rd tergite. Bright field.

500). This frequency was equal to that for two adjacent tergites taken together (e.g. 3+4, 110/500; 4+5, 98/500; 5+6, 100/500; mean = 21 %).

The *frequency of separation* is a measure of the proximity of two groups of cells at blastoderm (Wieschaus & Gehring, 1976*b*; Lawrence & Morata, 1977) and is the number of cases where two compartments in the adult are of *entirely* different sex. For adjacent tergites of the same side such cases are rare; between tergites 1 and 2 on the same side there were 3 cases out of 500; tergites 2–3, 4 cases from 500; 3–4, 10 from 1000; 4–5, 9 from 1000; and 5–6, 4 from 500. The frequency of separation between left and right was much higher, although it did not reach that expected purely by random assortment – the mean observed was 21 %, the mean expected due to chance was 37 %.

The *frequency of coincident mosaicism* (Lawrence & Morata, 1977) is the proportion of gynandromorphs where the two regions being considered each contain both male and female tissue. If the borderline between male and female regions on the blastoderm goes through one primordium it is also likely to pass

through an adjacent one. Thus the frequency of coincident mosaicism is a measure of the closeness of two primordia and should be compared to the frequency of coincident mosaicism expected between two remote primordia (which will simply be the product of the average frequency of mosaicism for the two primordia). In adjacent tergites the frequency of coincident mosaicism is consistent, suggesting that the segment primordia are packed regularly (tergites 2–3, 25 cases of coincident mosaicism out of 500; 3–4, 23/500; 4–5, 31/500; 5–6, 22/500; mean = 5·0%). This figure is higher than it would be if due to chance factors (the expected is equal to the square of the frequency of mosaicism, or $0{\cdot}13^2 = 1{\cdot}7\%$). The observed frequency of coincident mosaicism for tergites two segments apart is 3·2% (tergites 2–4, 14 cases of coincident mosaicism from 500 sides; 3–5, 12/500; 4–6, 22/500; mean = 3·2%). For three segments apart the frequency of coincident mosaicism is 2·2% (2–5, 11/500; 3–6, 11/500), which is not significantly different from the expected frequency of coincident mosaicism due to chance.

The *sturt* distance also measures relative positions of the primordial cells generating specific landmarks (Garcia-Bellido & Merriam, 1969; Hotta & Benzer, 1972). In gynandromorphs, the further apart two primordia are situated on the blastoderm, the more often will they be of different sex. When two landmarks are of different sex in 1% of the gynandromorphs they are described as being separated by one sturt (Hotta & Benzer, 1972). We have measured this distance across the intersegmental border in the ventral sternites and in the tergites. In the sternites the mean distance between the posterior sternite bristle (on one side) and the most anterior one in the next segment (on the same side) is 6 sturts (3–4, 32/500 cases where bristles are of opposite sex; 4–5, 29/500). For the tergites the mean sturt distance between the nearest bristles in the different segments (measured at the dorsal midline) is 9 sturts (tergites 1–2, 42/500; 2–3 49/500; 3–4, 44/500; 4–5, 47/500 and 5–6, 42/500). This measurement consists of the true intersegmental distance plus a contribution by a region of the tergites (nearly half the width) which does not bear bristles. As the distances within the tergites from the extreme anterior to the extreme posterior bristle average about 3·5 sturts (1, 21/500; 2, 14/500; 3, 22/500; 4, 17/500; 5, 21/500 and 6 14/500) a reasonable estimate of this contribution would be 3 sturts. Thus the true intersegmental distance across the segment boundary from posterior tergite cells to anterior ones of the next segment would be some 6 sturts.

Clonal analysis of the embryo

The aim of this study was to estimate how much the presumptive adult abdominal cells grow during the embryonic period and to determine whether the segmental polyclones are defined at blastoderm. Unirradiated flies show a high frequency of clones in the abdomen (Garcia-Bellido & Merriam, 1971*a*) so that special care had to be taken to correct for these spontaneous clones. 1000 abdomens irradiated at 4 ± 2 h AEL, 500 at 24 ± 2 and 500 unirradiated

Table 1. *The size and frequency of* Sb^+ M(3)w^+ *clones produced in* mwh Sb^{63b} M(3) $w^{124}/+$ *flies by irradiation with 500R at 4 h and 24 h AEL.*

The experimental series were corrected for spontaneous clones by subtracting the clone distribution found in unirradiated flies of the same cross.

	No. of abdomens	No. of clones	Frequency clones/abdomen	Log mean size	Log S.E.M.	No. of bristles per clone
Controls	500	51	0·10	0·46	0·04	2·9
4 ± 2 h (corrected)	1000	58	0·06	0·59	0·06	3·9
24 ± 2 h (corrected)	500	114	0·23	0·45	0·03	2·8

controls were scored 'blind' for clones. Histograms were prepared and the control clones subtracted from the experimental ones. The $Sb^+M(3)w^+$ clones were very conspicuous (Fig. 3), so that screening was rapid and reliable. Unfortunately these markers cannot be used for the first abdominal segment, the phenotype is unclear there. The frequency and sizes of the clones are shown in Table 1.

There are two main conclusions from Table 1: (1) the corrected clone frequency at 24 h AEL is some four times that at 4 h, (2) the clones are significantly larger at 4 h than at 24 h AEL.

In the 4 h series there were 10 half abdomens (left or right) that had two or more clones. Of these, 6 clone pairs were in adjacent segments. This is similar to that expected by chance (4/10 pairwise combinations from segments 2–6 will be in adjacent segments). There is thus no indication that clones can cross from segment to segment.

In the first 500 abdomens irradiated at 4 ± 2 h AEL, clones on the sternites were also searched for and 11 were found. None of these coincided with clones on the tergite of same side and segment.

Clonal analysis of the larva and pupa

Two detailed studies of the clonal growth of the abdominal tergites during the larval and pupal period have been previously reported (Garcia-Bellido & Merriam, 1971*a*; Guerra *et al.* 1973). We therefore concentrated on the sternites and pleura (Fig. 1) which, because all the cells can be marked, have an advantage over the tergites. Aged larvae and pupae were irradiated with 1000R and the adult abdomens mounted flat and screened for *multiple wing hairs* (*mwh*) clones and twin spots (*y; mwh* and *flr*). Both these markers showed on the pleura and sternites and *mwh* was very clear (Fig. 4). Fig. 5 shows the mean clone size with

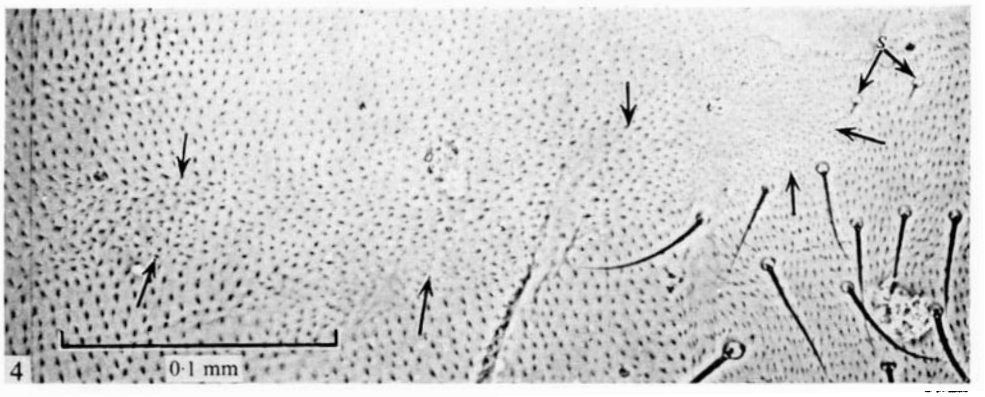

Fig. 4. Region of sternite and pleura to show *mwh* clone. Arrows mark the boundary of the clone. The two anterior sensillae are visible (*s*). Phase contrast.

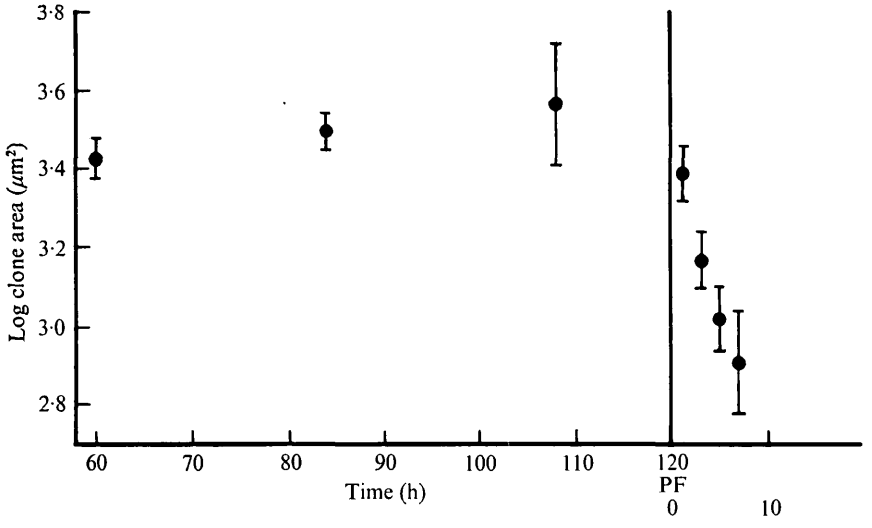

Fig. 5. Log mean clone size during larval and early pupal growth. Bars show S.E.M. Hours are after egg laying, PF marks the moment of puparium formation. The mean area for one lateral half of a ventral adult segment is 80000 μm^2.

age in respect of puparium formation. Clearly there is no change during the larval period, which implies no growth. At around pupariation the clones rapidly become smaller, giving a slope indicating a mean cell cycle time of 3·2 h. The mean clone size (*mwh*) during the larval period is 1/25 (n = 148) of the mean area of a sternite plus pleura on half of one side which, allowing for the division required to segregate the marked cell, indicates 12·5 cells for the primordium.

Histological preparations showed similar numbers of ventral histoblasts in each segment and from a sample of two mounted abdomens the mean number of cells in 13 ventral nests was 11·5.

The clones varied widely in shape, size and position; they frequently crossed from sternites to pleura, and sometimes they were split up into separate patches. There was no evidence for any clonal restriction lines within the sternites/pleura. Because the ventral and dorsal histoblast nests are separated one might expect them each to construct specified areas of the adult. Their progeny would then confront each other along a defined compartment border. The results were inconclusive; most clones remaining confined to the tergites or to the sternites/pleura. However, a few clones extended from the tergites onto a region of the pleura near the spiracle, but none went further. Likewise, several clones which were mainly in the pleura extended to beyond the region of the spiracle to reach the edge of the tergites. These observations suggest that the two nests of histoblasts may overlap slightly in prospective fate. In a further series of experiments, *mwh* clones that were also *Minute*(3)i^+ (Morata & Ripoll, 1975) were made (n = 57); but although these were somewhat larger, the largest being about 1/4 of the area of sternite plus pleura, no clones extending from the tergites far into the pleura were found.

DISCUSSION

We have described a clonal and gynandromorph analysis of the *Drosophila* adult abdomen during embryonic, larval and pupal growth. The results confirm and supplement earlier studies on the growth of the adult tergites during the larval and pupal period (Garcia-Bellido & Merriam, 1971*a*; Guerra *et al.* 1973; Madhaven & Schneiderman, 1977) as well as studies of the embryonic growth of *Drosophila* segments (Wieschaus, 1974; Madhavan & Schneiderman, 1977) and *Oncopeltus* segments (Lawrence, 1973). We discuss our main results in turn:

(1) *Segmentation*

The abdominal tergites (2–6) all begin existence with the same number of cells at blastoderm, and this number is approximately half that forming the primordium of the first abdominal tergite. The reasons for these conclusions are simply that the frequencies of mosaicism for each of the tergites 2–6 are identical at 13%, and that the frequency of mosaicism for the first abdominal tergite (21%) is equal to that for any other two adjacent abdominal tergites taken together.

The sturt distance between adjacent bristles in the sternites of neighbouring segments average about 6. In the tergites the distance across the intersegmental boundary is also about 6 sturts. This means that neighbouring segments may arise from adjacent cells at blastoderm: the distance is similar to that measured between adjacent wing cells in the anterior and posterior compartments (about 7 sturts), where it was concluded that the polyclones are probably adjacent (Lawrence & Morata, 1977). Using a different argument Wieschaus (in preparation) has also concluded that about 5·5 sturts could be expected to separate landmarks arising from adjacent blastoderm cells that are themselves in separate compartments. Further, the distance between the closest wing and leg

landmarks is 6·2 sturts (Wieschaus & Gehring, 1976*b*) and yet cells marked at around the blastoderm stage can produce progeny in wing and leg (Wieschaus & Gehring, 1976*a*; Steiner, 1976; Lawrence & Morata, 1977) – evidence that prospective wing and leg cells are so close they sometimes overlap at blastoderm. Moreover the frequency of separation (Wieschaus & Gehring, 1976*b*; Lawrence & Morata, 1977) between adjacent tergites (total = 30/3500 = 0·9%) is very low, being about that observed for the wing and leg (5/600 = 0·8%, Wieschaus & Gehring, 1976*b*) and the anterior and posterior wing (8/1200 = 0·7%, Lawrence & Morata, 1977 plus unpublished results). These figures also show that the tergite primordia are very close together and probably adjacent at blastoderm.

In spite of this, clones produced at 4 ± 2 h AEL do not cross from segment to segment; marked bristles in two adjacent segments are not found more often than expected by chance. There were about 58 clones actually induced at 4 h and if a substantial fraction of these had extended between two segments this would have been observed. A frequency of crossing of, say, 5% would not have been noticed. In order to show marked bristles in two segments, an X-irradiated blastoderm cell must divide once to produce a marked cell, and that cell must divide again to produce daughter cells which can then enter separate segments. Our results therefore show that by or before two cell divisions from 4 ± 2 h AEL the segmentation is complete. Probably there are mechanisms, such as differences in cell affinity, which keep the adjacent cells from mingling across the inter-segmental boundaries (as has been proposed for the anteroposterior boundary within the *Drosophila* wing primordium (Morata & Lawrence, 1975)). These differences do not exist between the primordia for the wing and the leg – and clones cross between them (Wieschaus & Gehring, 1976*a*) even though the intervening sturt distance is the same. In hemimetabolic insects segmentation of the abdomen also occurs very early, its immediate effect being to stop cells intermingling across the borders (Lawrence, 1973).

While there are other explanations, one simplifying hypothesis is that the blastoderm of *Drosophila* might be subdivided into a number of identical polyclonal units (in accordance with the presumed metameric origin of insects) which are combined in pairs to construct the three thoracic and first abdominal segments. Single units making the abdominal segments 2–9. This would give, say, 16 units for the thorax and abdomen. There are about 66 cells in 2/3 of the long axis of a blastoderm stage *Drosophila* egg (see pictures in Turner & Mahowald, 1976) (the remaining third would be for the mouth parts and head) so there would be a band of cells about four wide for each polyclonal unit.

At hatching, the adult histoblasts are not adjacent; they are at the centre of the segments, and are surrounded by exclusively larval cells. This apparent paradox can simply be resolved by the hypothesis that adult and larval cells are part of the same polyclone at blastoderm. Only during later development would the adult and larval epidermal cells become segregated into separate polyclones.

This view, because of the later evolution of holometabolic from the hemimetabolic insects, is compatible with phylogeny.

(2) *Embryonic growth*

Our clonal analysis of the 4 ± 2 and 24 ± 2 h AEL can be compared with the more accurately timed results of Wieschaus (1974). We both find that the clones at the early time are significantly larger than at late times. We can presume this is due to intervening cell division. Moreover we also find an increase in clone frequency at later irradiations. Wieschaus reports a doubling of clone frequency, and a halving of size between $3{\cdot}2 \pm 0{\cdot}5$ AEL and $10{\cdot}5 \pm 1{\cdot}0$ h AEL. We find that the frequency quadruples and that the clone size only drops by some 30% between 4 and 24 h. Part of the explanation for these results must be an increase in X-ray sensitivity during embryogenesis – the sensitivity of imaginal cells to X-rays does change during development (Garcia-Bellido & Merriam, 1971*b*).

(3) *Clonal analysis of the larva*

Our analysis of the ventral abdomen (sternites and pleura) of the adult, showed that there was no growth during the larval period, that cell divisions begin around pupariation and are very rapid, with a mean cell cycle time of 3·2 h. The dorsal histoblasts behave similarly (Garcia-Bellido & Merriam, 1971*a*; Guerra *et al.* 1973). Estimates of the area of the clones showed that, on average, they were 1/25 of the sternites and pleura of one segment. This suggested there should be 12·5 ventral histoblasts, a figure close to that observed directly. Our small sample of counts gave a figure of about 11, the more accurate counts of Madhavan & Schneiderman (1977) 12–13. In the case of the tergites, estimates from clonal analysis (8, Garcia-Bellido & Merriam, 1971*a*; 11, Guerra *et al.* 1973) were about half that actually observed in the two dorsal nests of histoblasts (Madhavan & Schneiderman, 1977). However, calculations assumed that the entire dorsal abdomen is covered with bristles, which is not the case (Fig. 1). Expanded abdomens show the area to be about 1/2. Allowing for this the estimates from clonal analysis approach closely the direct counts, which permit us to suggest that both dorsal and ventral histoblasts form the cuticle and little, if anything, else of the adult.

Our failure to find a precise clonal restriction line separating dorsal from ventral abdomen may just be due to a lack of landmarks – or there may be slight variation in contributions of dorsal and ventral histoblasts to the developing adult abdomen. Certainly there is no extensive mixing in the region where they meet in the neighbourhood of the spiracle. In *Calliphora* Pearson (1977) has shown by transplantation experiments that the dorsal and ventral nests are differently determined. In *Oncopeltus* the dorsal and ventral polyclones are separate at about the same time as segmentation (Lawrence, 1973) and their progeny meet at a defined line along the lateral border of the abdomen (Wright, unpublished results). It seems most likely, therefore, that the dorsal and ventral

histoblasts of *Drosophila* are separately determined and meet at a precise border line.

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