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A clonal analysis of segment development in *Oncopeltus* (Hemiptera)

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SUMMARY

X-irradiation of eggs and larvae of *Oncopeltus fasciatus* results in the development of clonal patches of epidermal cells of unusual pigmentation. The frequency, size and distribution of these patches is dependent on the dose and timing of irradiation. Analysis of these clones in the abdomen has shown that the presumptive epidermis becomes effectively segmented during blastoderm formation and thereafter the clones are restricted to within a segment quadrant (dorsal or ventral, left or right). There are approximately 10 presumptive epidermal cells per segment quadrant. The shape of the clones and the orientation of mitoses in larvae suggest that both early and late cells of the anterior margin of the segments divide with a preferred orientation (cleavage plane parallel to the antero-posterior axis). Elsewhere in the larval segment the mitoses are randomly oriented, and the segment grows evenly all over. The number of mitoses/cell/moult cycle is not precisely determined, but the amount of cell division is perhaps under a general probabilistic control. It is suggested that the segmental gradient may be involved in this control.

INTRODUCTION

Although segmentation of the insect embryo has been considerably studied (references in Counce, 1973) little is known about the number of cells present in the nascent segments nor about their subsequent growth. Even in *Drosophila*, where clonal analysis could, in principle, provide this information, the absence of suitable markers expressed in larval cells has precluded such a study. I have found that irradiation of eggs or larvae of the milkweed bug, *Oncopeltus fasciatus*, results in patches of differently coloured epidermal cells (Lawrence, 1971, 1973). The frequency, size and distribution of these patches is dependent on the dose and timing of the irradiation. This finding has allowed a clonal analysis of the segmentation and growth of the abdominal epidermis of *Oncopeltus*. The main conclusions are:

(i) As in *Drosophila*, cleavage is indeterminate.

(ii) The segments have independent lineages from a very early stage; this may be due to a preferred orientation of mitosis in the cells of the anterior margin.

(iii) Demarcation of segments coincides with formation of the blastoderm.

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(iv) There are approximately ten presumptive epidermal cells in each nascent segment quadrant (dorsal or ventral, left or right).

(v) The number of mitoses per cell in each moult cycle is not fixed, but may be subject to probabilistic control.

MATERIALS AND METHODS

Eggs and larvae of *Oncopeltus fasciatus*, reared at 29 ± 1·5°C, 16*L*/8D, and fed on milkweed seed, were collected over 2 h periods and irradiated with various doses of X-rays (220 kV at 15 mA, 1 mm aluminium filter, distance of 5 cm; rate of 500 R/min, R = $2{\cdot}58 \times 10^{-4}$ C/kg). The insects were usually screened as 5th-stage larvae (aged from 24–48 h from ecdysis; that is, before proliferative mitoses (Lawrence, 1968), but after feeding has smoothed out the cuticle).

The irradiated insects were heterozygous for the markers, *white body* (*wb*), *cream body* (*cb*) and *red eye* (*re*) (Lawrence, 1970*a*) because preliminary tests showed that irradiation of this genotype yielded a somewhat higher frequency of clones than irradiation of wild-type individuals.

Nature of the clones

The most useful clones are cells with altered pigmentation. In *Oncopeltus* the pteridene pigments are found in the epidermal cytoplasm and are deposited in spheroidal granules of about 1 μm in diameter. In the wild-type most of these granules are orange, and some are red. In the mutant strains homozygous for *cb* or *wb* the granules are white, due probably to the absence of erythropterin and the yellow pteridene, YP2 (Lawrence, 1970*a*). One of the commonest X-ray induced clones is white (*ca.* 20% of all clones).

Another common clone type is pink (*ca.* 25%) which seems to lack a yellow pigment. There is a series of clones which have reduced numbers of pigment granules, each clone containing cells with homogeneous pigmentation but different clones having different amounts. Some clones which are almost totally lacking in granules are found (*ca.* 8%); these are quite transparent (Fig. 9) and found exclusively in males (which are hemizygous for the *X* chromosome). Scarlet clones are quite common but these are always small and the boundaries are smooth suggesting that they do not grow normally; they were excluded from the quantitative study. Sometimes twinspots are found, the commonest (*ca.* 8%) being adjacent, similarly sized spots of dark orange and white, both clearly distinguishable from the orange background (Fig. 10). Many other different types of clones were also found. The clones always had a sharp border.

Quantitative analysis: some problems

Precise analysis of frequency and size of clones is subject to several important reservations:

(i) The screening is not objective; it is easier to detect large clones than small

ones. Because the number of clones found depends partly on how hard one looks, the screening method chosen must be sufficiently effective, without being too laborious. Under the dissecting microscope clones under 150 cells could not be reliably detected and therefore only clones above that size were scored when this screening method was used. In 1000 unirradiated insects of the same stock, only six such clones (frequency = 0·006 clones/abdomen) were found whereas after irradiation with 100 R the frequency ranged from 0·05 (6–8 h) to 0·5 (35–37 h). It did not seem worth while correcting for control clones in these groups. The diversity of appearance of individual clones, as well as their relatively low frequency, made it very unlikely that two independent clones would be scored as one.

Irradiation after 36 h of egg development produced significant numbers of clones which were too small to be reliably detected under the dissecting microscope. Unstained slides were therefore made of irradiated insects at 5th-stage larvae aged 24–48 h after ecdysis (Carnoy's fixative, cleared and mounted in Canada balsam) and screened with the compound microscope (× 10 objective, crossed polaroids). Much smaller clones could then be scored, which increased the number of clones detected in unirradiated controls (frequency = 0·46 clones/abdomen). The control clones tended to be a smaller subset of the clones found in experiments where the frequency ranged from 2 (48 h) to 7 (1st-stage larvae), so that the expected number and area of control clones was subtracted from the experimental results before the means were calculated. This correction had only a small effect on the results; for example, at 48 h it raised the mean clone size from 84 to 104 cells.

(ii) The genetic basis of clone production is not clear. It is likely that many clones result from chromosome breaks which are perhaps not as lethal to the cell in *Oncopeltus* as in an insect with more typical chromosomes. *Oncopeltus* has holokinetic chromosomes, small pieces segregating normally over many cell generations (LaChance & Degrugillier, 1969). The formation of twin spots suggests that somatic crossing over may occur, although they could also arise from non-disjunction of whole chromatids. These points warn that the clone may be initiated at a varying number of cell divisions after irradiation.

(iii) Almost certainly some clone types would not grow as well as wild-type cells and, therefore, lead to underestimation of the growth potential of cells at the time of irradiation.

(iv) Instability of the clones could cause considerable errors, but most common clone types often formed coherent patches of thousands of similar cells. In these patches, there were never smaller inset groups of cells of wild-type or other clone type, suggesting, for the most part, that the clone is stable.

(v) There is the possibility that the X-rays may kill a significant fraction of the cells at the time of irradiation, thus increasing the number of cell divisions subsequently made by the surviving population. Partly for this reason an unusually low dose of X-rays (100 R) was used. 250 R killed 50 % of cleaving eggs,

but later stages could tolerate 500–1000 R. In larvae, very little damage could be seen after irradiation with 100 R, but after 500 R dying cells were found, 1000 R causing very considerable damage which could be seen two moult cycles from irradiation. 2000 R was lethal, although some individuals survived two or even three moult cycles.

Because of these difficulties the results can only be regarded as estimates, and little attention should be paid to the precise values of any calculated mean.

Measurement of the cell number

The numbers of cells in the smaller clones (produced by irradiation of larvae) were counted directly, but the cell number of the larger clones (irradiation of eggs) was estimated. To do this the outline of the clone was drawn with a Zeiss drawing apparatus on the compound microscope, and the total area obtained from the weight of the paper. The average cell density was measured by finding the mean area covered by randomly chosen groups of 100 cells in 20 insects fixed at the time of screening. For growth of the larval abdomen, ten partially fed individuals of each instar were drawn to scale and the area and cell number of the abdomen estimated as above. Measurements showed the mean cell density was the same in the different larval stages. For simplicity the abdomen was considered to be of six segments, the fused first and second embryonic segment (Bonhag & Wick, 1953) treated as one, and the terminal seventh and eighth segments were ignored.

Statistics

Because the cell number increases exponentially, a plot of log cell number of clones resulting from a particular time of irradiation gave a normal distribution. Means and standard errors were, therefore, calculated on the log cell numbers.

Staining of eggs

Following Sander (1971, and personal communication) the eggs were fixed in acetic acid:formalin:ethanol:water (1:6:16:30) for 30 min at 60 °C, punctured, and after prolonged washing in 70 % ethanol and distilled water were stained in thionin dissolved in 95 % ethanol for 30 min. The eggs were dehydrated, cleared in cedar oil and mounted in Canada balsam. The embryos were examined under bright field and Nomarski optics.

RESULTS

Batches of eggs were collected every 2 h and irradiated after different intervals. Fifth-stage larvae were screened and clone size and cell number recorded (Fig. 1). Any one clone was often separated into patches, their distribution was noted and whether they were restricted to one segment quadrant or extended to two or more.

Table 1. *Classification of clones* not *restricted to one quadrant of the abdomen*

	Age at irradiation (hours)						
	0–6	6–8	8–11	10–14	14–18	18–24	Total
Class I (3 or all 4 quadrants of abdomen)	3	3	8	1	0	0	15
Class II (2 dorsal *or* 2 ventral quadrants)	2	2	1	0	0	0	5
Class III (2 left *or* 2 right quadrants)	2	14	11	9	5	4	45
Class IV (other combinations of 2 quadrants)	0	0	0	1	0	0	1

Early irradiation

Egg batches aged between 0 and 24 h gave a low frequency of clones (*ca.* 0·05–0·1 clones/abdomen). The lethal dose for this group was lowest (250 R killed about 50 % of the eggs), and the largest clone found covered some 20 % of the abdomen surface. For classification of the clones the abdomen was subdivided into four quadrants (dorsal or ventral, left or right) and those clones which extended into more than one quadrant classed into four groups: those found in three or four quadrants (class I), those restricted to either dorsal or ventral halves (class II), or to left and right halves (class III), those restricted to two other quadrants (e.g. dorsal right and ventral left) (class IV) cf. Sturtevant, 1929). The results are shown in Table 1.

Class II, III and IV are of most interest, since each includes comparable areas of abdomen (half in each case). If the orientation of the first division and subsequent segregation of nuclei were random one would expect equal number of clones in each of these three classes. The over-representation of either class II or III would show that clonally derived patches tend to stay together, and that there is not random mixing of the early nuclei. The preponderance of class III shows that the dorsal and ventral quadrants of one side are more closely related by lineage than, for example, the left and right sides of the dorsum. This suggests the separation of lineages between left and right abdominal epidermis occurs earlier, or is more effective than that between dorsal and ventral.

Fig. 1 describes the relationship between cell number of the clones (when screened in 5th-stage larvae) and the time of irradiation with 100 R. The average clone size drops from some 4000 to 30 cells following later and later irradiations during egg development. The steepest slope (highest rate of cell division) occurs during the first 12 h of development, then there follows a slower rate of decline, with a long period (*ca.* 60–96 h) when the cell number remains constant.

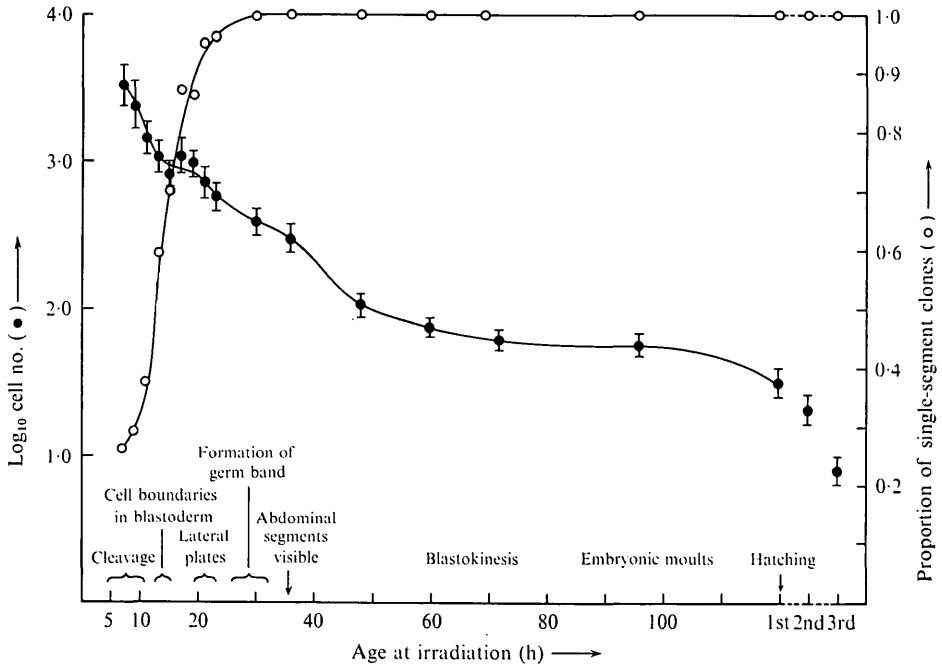


Fig. 1. Variation of log cell number in clones (●), and proportion of clones confined to a single segment quadrant (○) with age at irradiation (100R). Vertical lines demarcate ± S.E.M. Information on development from own observations and Dorn (1972).

Demarcation of segment boundaries

(i) *Clonal analysis.* Clones were often broken up into patches (Figs. 2, 3, 8) and even after the earliest irradiation a segment quadrant always contained patches of both wild-type and the clone colour. In no case did a clonal patch include a whole segment quadrant. This shows that there is: (i) cell mixing after clonal initiation during cleavage, (ii) a group of cells, not closely related by lineage and not themselves constituting a clone, form the primordium of the segment quadrant.

After some 10 h of egg development even quite large clones began to be confined to a single segment quadrant, the largest such clone found covering 20% of the quadrant. They frequently had a remarkably straight edge near the intersegmental border (Fig. 3*b*, *e*) and at the lateral margin of the abdomen. They rarely crossed the midline, although the edge of clones near the midline was not particularly straight (Fig. 3*a*). By 30 h irradiation produced only clones that were confined to a single segment quadrant. At 24 h these single segment clones covered an average of 5% of the quadrant, and were often split up into patches (Fig. 3*d*). It is clear from a comparison of the curves on Fig. 1 that the rapid rise in the proportion of single segment clones (between 11 and 23 h) cannot be explained simply by the small decrease in average clone size during the same

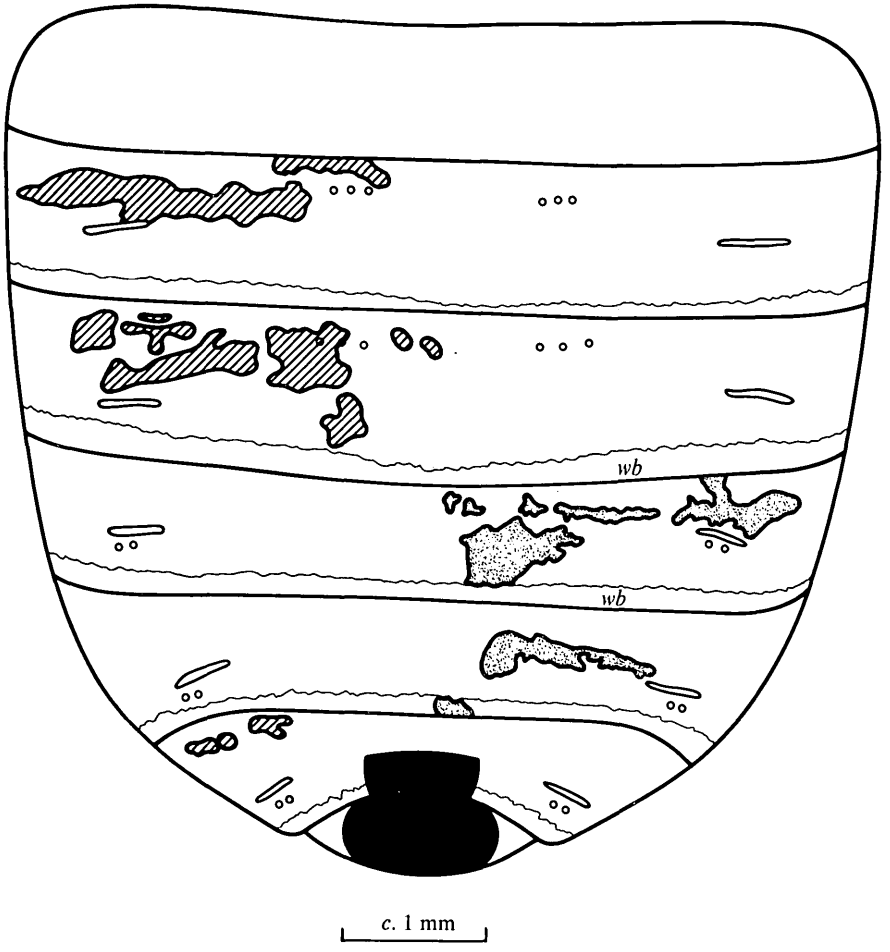


Fig. 2. Composite drawing of ventral abdominal epidermis showing two clones, one confined to each side of the abdomen. The clones were produced by irradiation between 6 and 12 h. *wb* = white band.

period. An independent process must be occurring to separate the lineage of the different segment quadrants, and the straight edges of the clones where they meet the intersegmental border (independently marked by a colour change) confirms this. In larvae the anterior margins of the segments consist of a zone of more or less spindle-shaped cells which divide with the cleavage plane parallel to the antero-posterior axis, thus effectively maintaining the discontinuity between the segments (Lawrence, 1973). These margin cells are even more striking in 1st-stage larvae, where the rest of the segment consists of fewer cells. Although the banded colour pattern, characteristic of the later larval stages, is absent in the newly hatched larva, the segment margins are marked by delicate grooves in the surface of the cuticle. Along the line of these grooves the cells are

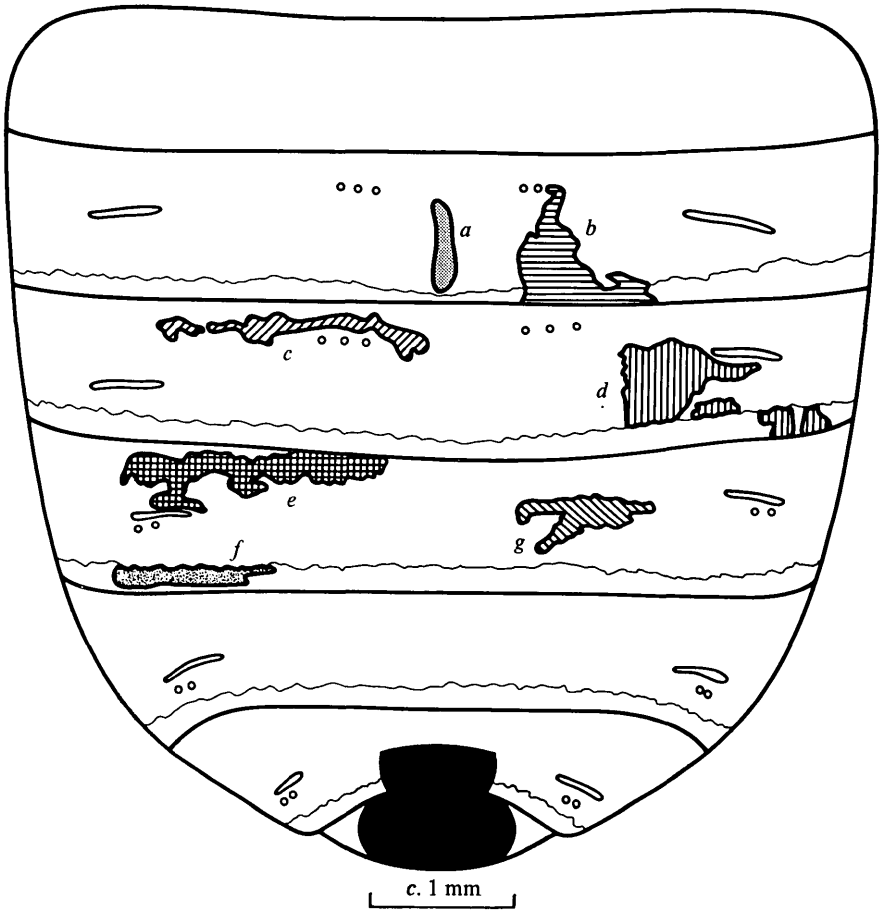


Fig. 3. Composite drawing of ventral abdominal epidermis showing seven clones (*a–g*), each confined to a single segment quadrant. Results of irradiation between 14–20 h.

elongated transversely. It seems possible that it is a similar change in cell shape (and consequent preferred orientation of mitoses) that segregates the nascent segments in the egg.

The results of irradiation during the formation of effective segment borders (*ca.* 10–20 h) allow an estimate of the cell number in the nascent segment quadrants. When these are effective with respect to about 50 % of the clones formed (irradiation at 13 ± 1 h) the average clone size resulting in the 5th-stage is 1060 ± 130 cells; that is, approximately 10 % of the whole segment quadrant (average segment quadrant in the 5th-stage larva has 10500 cells (Fig. 6)). I make the following assumptions (cf. Bryant & Schneiderman, 1969):

(i) Every primordial cell is likely to form the same proportion of the entire segment.

(ii) The X-rays do not kill a significant proportion of the cells.

(iii) The cells of altered phenotype grow as well as normal cells.

(iv) The initiation of altered phenotype follows immediately after irradiation.

One can estimate that there are 10500/1060, i.e. approximately ten presumptive epidermal cells per segment quadrant at the mean time of formation of the boundaries between them.

The shape of clones might be expected to contain information about the orientation of mitoses during this critical period. In order to increase the probability that the clones examined originated from a single cell present at the time of segment definition, single-segment clones that consisted of about 1000 cells (*ca.* 10% of the whole segment) were studied. Seventy-six such clones were found amongst those produced by irradiation between 10–24 h of egg development. These clones were roughly classified into those that were confined to the anterior half of the segment (23/76), those confined to the posterior half (19/76), and those extending to both halves (34/76). Although many clones were sufficiently long to have crossed a segment, no clone included both anterior and posterior margin. All clones in the anterior half (e.g. Figs. 3*c*, *e*, 8) were extremely elongated medio-laterally (mean ratio of medio-lateral extent to antero-posterior extent = $5{\cdot}6 \pm 0{\cdot}5$). One possible explanation is that they arose from successive divisions whose cleavage planes were oriented in the antero-posterior axis. Posterior clones were not so elongated although their medio-lateral extent was also greater than the antero-posterior (ratio = $2{\cdot}5 \pm 0{\cdot}3$). Clones in the middle of the segment were variously shaped although they too often extended further in the medio-lateral axis (ratio = $2{\cdot}3 \pm 0{\cdot}2$). The only exception of this general feature of the clones was those near the mid-dorsal or mid-ventral line which were often small and longer in their antero-posterior extent (Fig. 3*a*).

These observations are consistent with two conclusions: (i) the anterior margin and posterior margin cells of the nascent segment have an independent lineage, at least from 10 h (ii) early divisions of all cells tend to be with the cleavage planes oriented in the antero-posterior axis, and this is particularly true of the anterior margin cells. It is therefore possible that the situation observed in larvae (where the anterior margin cells divide with a preferred orientation) obtains in the embryo when the segments are forming.

Frequently the clones stop fairly sharply along the white band found alongside the posterior margin of the segment. A very suggestive example is shown in Fig. 3*d*, where the clone is subdivided into two distinct parts, one confined within the band and one stopping short where it meets the band. The line between the band and the rest of the segment is not particularly straight and is not accompanied by any change in cell shape; nevertheless, these clones raise the possibility that there is a further subdivision of lineage within the segment, the white band possibly arising from the transversely elongated cells near to the posterior margin of the segment in 1st-stage larvae (Fig. 4).

After the segments have formed there is a rapid period of growth until about 60 h after egg-laying. At this time, by the same method of estimation (mean

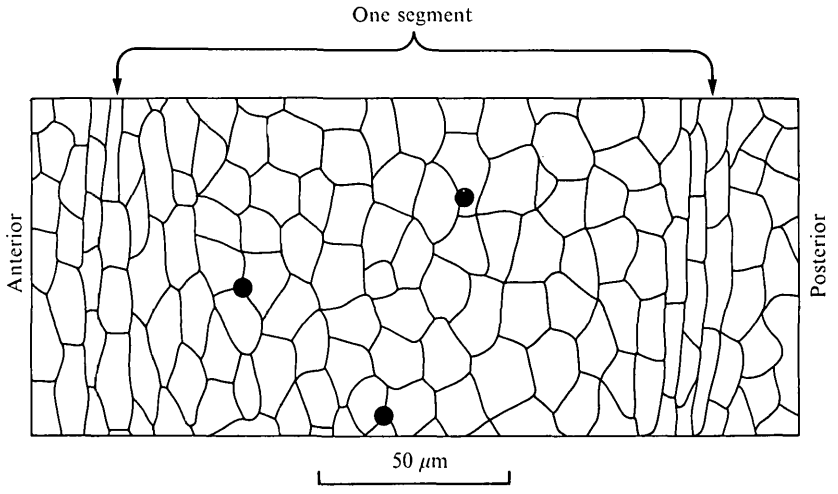


Fig. 4. Camera lucida drawing of the epidermal cell shapes in a dorsal abdominal segment in a 1st-stage larva. The approximate segment boundaries are indicated. The black dots are bristle insertions. Note the elongated cells near the segment boundaries.

clone size = 76) there are 10500/76, i.e. about 140 cells in each segment quadrant. There is relatively little growth in the abdominal epidermis between 60 h and hatching at about 120 h. The newly emerged 1st-stage larva has about 330 cells (10500/32) per segment quadrant according to estimate by clonal analysis and about 400 according to direct measurement from the area of the abdomen (Fig. 6).

(ii) *Direct observations on eggs.* The events of development were followed in whole mounts of eggs. At 11 h from egg-laying pairs of nuclei appear at the surface of the egg. The first signs of cell boundaries can be seen under Nomarski optics at 14 h, and at 16 h the blastoderm includes clear cell boundaries. Cell divisions continue in the blastoderm, and by 24 h this layer is visibly thickened ventro-laterally, due to the formation of lateral plates (*Seitenplatten*, Seidel, 1924). Movement of the blastoderm begins at about 26 h, and by 32 h the germ band extends to the anterior pole. By 36 h the thoracic segments are distinct. Examining sections of eggs cultured in conditions giving a comparable rate of development, Dorn (1972) detected abdominal segmentation at 34 h. It is clear that the acquisition of independent segment lineages in the presumptive epidermis coincides approximately with the development of cell boundaries in the blastoderm and *precedes formation of the germ band and any outward signs of segmentation.*

Irradiation of larvae

Clones were induced by irradiating larvae within a few hours of ecdysis. Because of possible ambiguity and bias in scoring small clones, larvae were fixed and scored 'blind'. The insects were fixed at different numbers of moult cycles

Table 2. *Clone size following irradiation of newly moulted larvae*

Dose	Stage irradiated	Stage screened	No. of clones	Log mean no. of cells ± S.E.M.	Mean no. of cells	'Expected' from growth of abdomen
500R	3rds	5ths	97	0·92 ± 0·02	8	5
	2nds	5ths	29	1·36 ± 0·04	23	12
	1sts	5ths	41	1·54 ± 0·04	35	27
100R	3rds	5ths	31	0·89 ± 0·04	8	5
	2nds	5ths	30	1·31 ± 0·05	20	12
	1sts	5ths	47	1·50 ± 0·05	32	27
			Twinspots			
500R	1sts	5ths	29	1·29* ± 0·05	19	
				1·30† ± 0·06	20	
				1·61‡ ± 0·05	41	

* White halves of white/dark orange twinspots.
† Dark orange halves of white/dark orange twinspots.
‡ Both halves of twinspot.

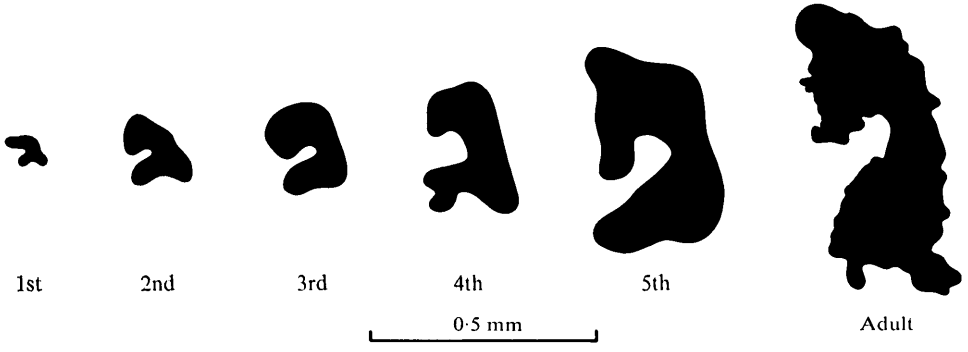


Fig. 5. The progressive development of an individual clone, drawn to scale in each larval instar. The drawings were not made at precisely equivalent stages in each moult cycle.

after irradiation. Larvae irradiated as 4th stages gave very few clear clones when examined in the 5th stage, although some were found. The appearance of clones could well depend on the dilution out of pre-existing pigmentation, and more consistent results were obtained when two or more moult cycles intervened between irradiation and examination (Fig. 11). The results are summarized in Table 2.

Some individual clones were followed during larval growth, being drawn to scale from the living insect. The development of one such clone is shown in Fig. 5. The shape of the clone changes only gradually as it grows in size, suggesting there is little cell migration during growth of the epidermis in *Oncopeltus*. In a

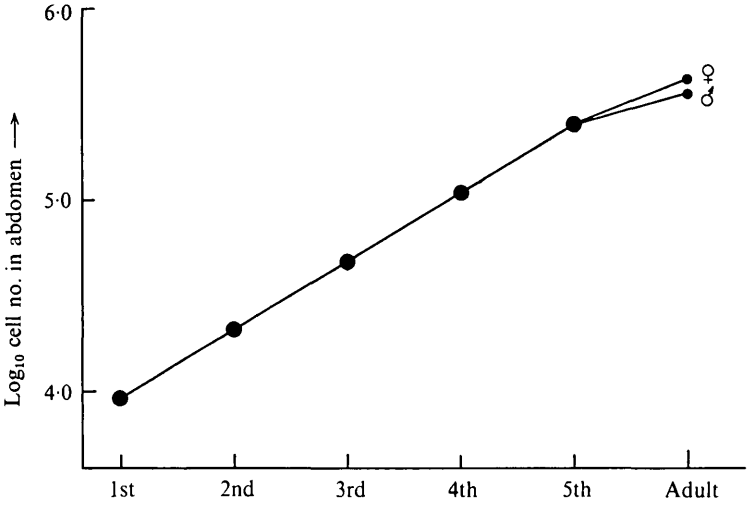


Fig. 6. Estimated number of cells in the larval abdomen at each stage. Over the four larval moult cycles the average slope = log 0·357. Standard errors are included within the points. To estimate the number of cells in an average quadrant the total number of cells in the abdomen is divided by 24 (number of segment quadrants, see Methods).

time-lapse film made for me by Dr Clarke Slater, and filmed over 48 h *in vivo*, we followed the growth of a small clone. Any change of shape seemed to result from the dividing cells pushing on their neighbours, the sheet of epidermal cells apparently remaining intact throughout growth.

The growth of the larval abdomen was also measured directly and the cell number estimated (Fig. 6). There is a remarkably constant growth rate and the segments change shape very little during larval development. These two phenomena may depend partly on inflation of the abdomen during feeding and random orientation of mitoses over most of the epidermis. Fig. 7 summarizes the orientation of mitoses observed in larvae which, apart from the extreme anterior margin, show no preferred alignment.

The clonal data can be compared with the basic growth pattern in larvae. (The mean cell number increases by a log factor of 0·357 per moult cycle = × 2·3). Starting with one irradiated cell, after two moults the mean log cell number of the clone would be expected to be 0·714 (=5), after three moults 1·071 (= 12) and after four 1·428 (= 27). A comparison between these figures and the results (Table 2) shows moderate agreement particularly over the three-moult period. The discrepancies may be due to the ease with which the smallest clones can be over-looked, and to some reduction of the initial cell population by the irradiation. Most of the common clones appeared to be initiated by the action of the X-rays on single cells which hand on the new phenotype to both daughters.

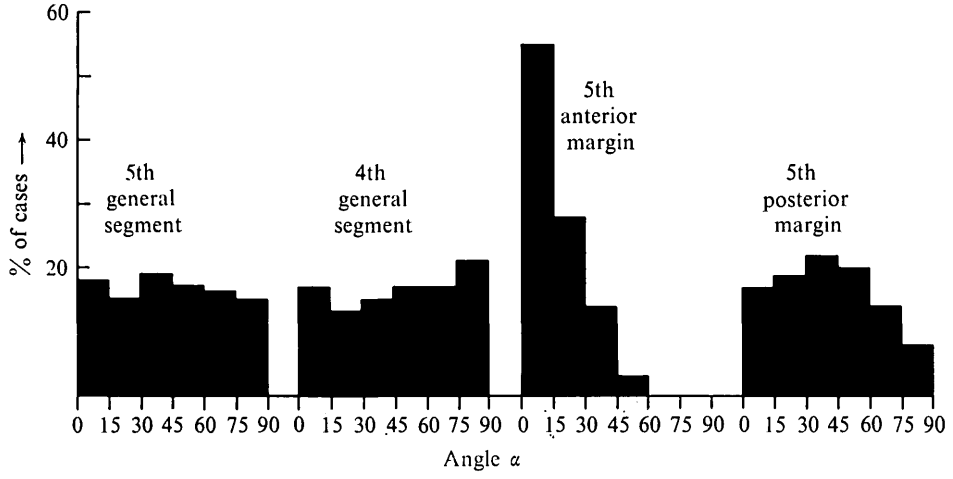


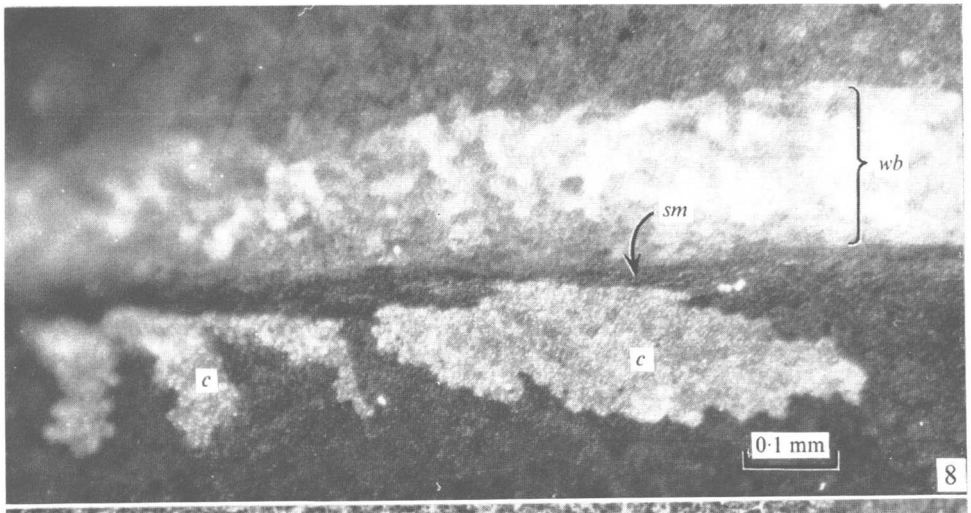
Fig. 7. Histograms showing orientation of mitoses. Angle α is that made between the cleavage plane and the antero-posterior axis.

Twin spots

Dark orange/white twin spots constituted about 8% of all clones (Fig. 10). All twin spots found in 5th-stage larvae that had been irradiated with 500 R as 1st-stage larvae were studied for analysis. The mean size of each spot is about half the size of the other types of clone produced by irradiation at the same stage (Table 2) suggesting that in this case, unlike the situation outlined above, each daughter of the affected cells is different from the other, as well as from the parent. It was found that, on average, both patches grew equally, there being no significant difference between the means of cell number for the two sister clones. In some individuals, however, the white patch could contain more cells than the orange patch, or vice versa; for example, in one twin spot there were 35 white cells to 13 orange, and in another 24 white to 40 orange. Likewise, following irradiation of 1st or 2nd-stage larvae, even within the same individual, clones of the same type can vary considerably in size. These observations suggest that the control of growth through cell division and cell death is not digital, and is achieved instead by control of the average probability of mitosis.

The pattern of growth

A comparison between smaller clones allowed study of the growth rate of different regions of the segment. Clones produced by irradiation from 96 h until 1st-stage larvae occupied only small areas of the segment so that the mean size of clones found in different regions could be compared. No significant differences between anterior clones (log cell no. = $1{\cdot}56 \pm 0{\cdot}05$), median clones ($1{\cdot}50 \pm 0{\cdot}04$) and posterior clones ($1{\cdot}58 \pm 0{\cdot}06$) were found. Nor was there any significant difference between the mean sizes of lateral and central clones. These results



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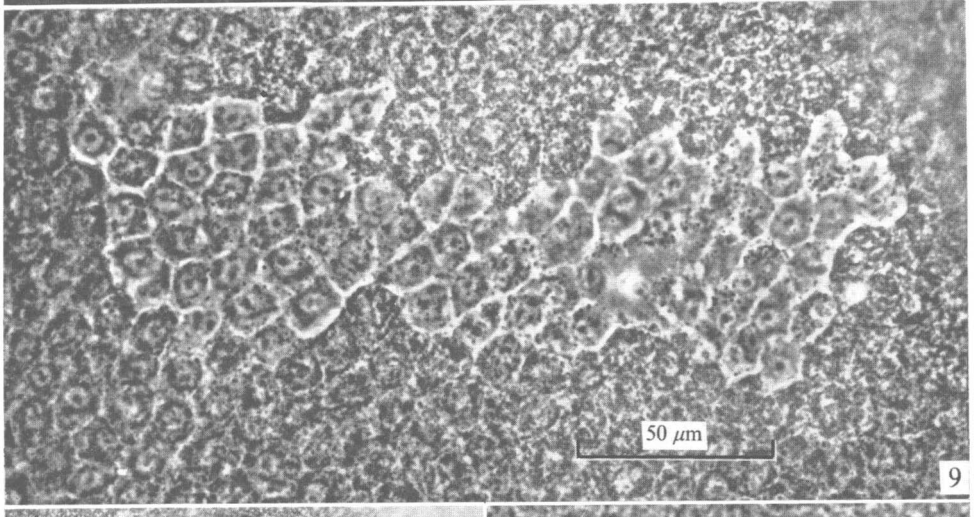

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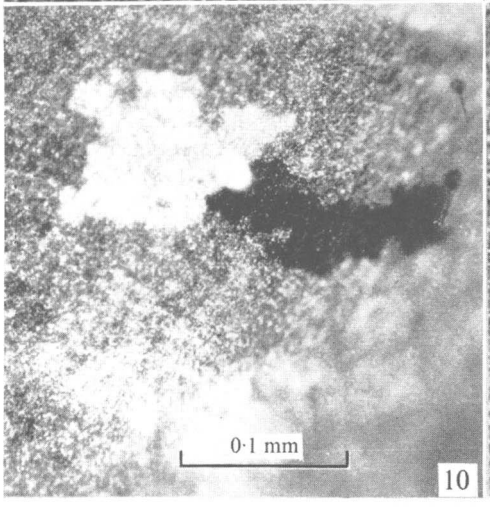

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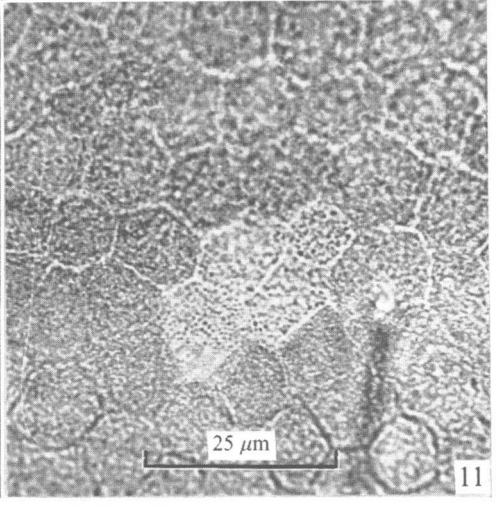

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suggest that at least from 96 h of egg development, the segments grow evenly all over.

It is noteworthy that apart from the white band there were no local subdivisions of cell lineage within the segment. Even the cells at the extreme anterior margin do not have an independent lineage; clones produced following irradiation of even 2nd-stage larva frequently include both elongated cells at this margin, and isodiametric cells in the segment proper.

DISCUSSION

The method

The various types of clone probably result from different kinds of chromosome damage. In spite of this the data are consistent, and the following points increase confidence in the results. (i) In the 1st-stage larva the estimate of cell number in the abdomen was made by two independent methods: clonal analysis and cell counting. These two estimates were reasonably close (330 and 400 cells/segment quadrant respectively). (ii) No significant differences were found between the average sizes of clones of several different colours, when produced by irradiation at the same age. (iii) Each spot in a twin-spot was approximately half the size of normal clones induced at the same time, which suggested that normally the X-rays affect a cell and that the altered phenotype is expressed by both daughter cell lines. This is consistent with the results of irradiating newly moulted larvae, where the epidermis is essentially a population of cells entirely in the G1 phase of the cell cycle, DNA synthesis in each cell not beginning until shortly before its mitosis (Lawrence, 1968). Here there is moderate agreement between the expected cell number in a clone deduced from observed growth of the larval abdomen, and the observed cell number (p. 691, Table 2).

Cleavage

X-ray induced clones initiated during cleavage have not been obtained in *Drosophila* (Bryant & Schneiderman, 1969). This is because of the high sensitivity of such eggs to irradiation.The direct observation on the eggs of *Oncopeltus*,

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FIGURES 8–11

Unstained whole mounts of 5th stage epidermis.

Fig. 8. Iridescent white clone (*c*) confined to one segment quadrant produced by irradiation of 14–16 egg. The white band (*wb*) is found along the posterior part of the segment and stops at the segment margin (*sm*).

Fig. 9. Transparent clone produced by irradiation of 1st-stage larva. Phase contrast.

Fig. 10. Twin spot of white and dark orange spots produced by irradiation of 29–31 h egg. Polarized light.

Fig. 11. Four-cell clone produced by irradiation of newly moulted 3rd-stage larva. Bright field.

as well as the large extent of clones resulting from irradiation between 6 and 12 h, shows that these clones are produced during cleavage. The distribution of clonal patches in *Oncopeltus* larvae is very similar to that found in adult *Drosophila* gynandromorphs and similar conclusions apply: 'There is no definite pattern among the mosaics, which can only mean that the cleavage nuclei are distributed differently in different embryos. It follows that there are no potential differences among the nuclei before they migrate to the surface of the egg' (Sturtevant, 1929, p. 352).

The more effective separation between left and right, than between dorsal and ventral, was found in both *Oncopeltus* and *Drosophila* abdomen. This is probably due to the bilateral organization of the presumptive germ band where the two halves of the embryo are separated into left and right lateral plates (Seidel, 1924), with cells not destined to form epidermis in between, and to the complete separation of dorsal left and dorsal right prior to dorsal closure.

Demarcation of segments

Previous studies on the development of *Oncopeltus* (Butt, 1949; Dorn, 1972) have reported that the segments can be seen soon after the formation of the germ band. Examining sections of the germ band, Dorn detected thoracic segments at 27 h. From clonal analysis it seems that the segments have independent lineages before the formation of the germ band. Effective boundaries develop between the segments during the period 10–24 h – that is, when cell boundaries are developing in the blastoderm, and when the rate of cell division is relatively low (Fig. 1). During this period clones extending both dorsally and ventrally on one side decrease in frequency, and this is accompanied by a rapid increase in the proportion of clones confined to a single segment quadrant. The mean clone size produced by irradiation at 11 ± 1 h is only about twice that following irradiation at 23 ± 1 h, so that on average only one cell division occurs during this period. Clearly an additional factor is restricting the prospective fate of the cells. I examined the shape of clones in larvae and the pattern of cell divisions near the segment margins (Lawrence, 1973). In larvae the boundaries consist of a line of elongated cells at the anterior margin of each segment which divide with cleavage planes parallel to the antero-posterior axis.

There is some evidence that the same kind of boundary forms between the nascent segment quadrants. At the time these boundaries are 50% completed (13 ± 1 h) there are only about ten presumptive epidermal cells per segment quadrant, so that the boundaries can only consist of an even smaller number of cells. Observations on those single segment clones, which contain about 1000 cells and develop in insects irradiated between 10 and 24 h, give some clues as to the orientation of these primordial mitoses. In particular the tendency of clones in the anterior part of the segment to be streaked out along the mediolateral axis, would suggest that the cleavage planes of the anterior cells were in the antero-posterior axis. Alternatively, the shape of these clones might depend

on excessive stretching of the anterior region of the segment during dorsal closure (Sander, personal communication). In either case, the formation of effective boundaries is one of the earliest expressions of segmentation, and ensures spatial separation of the lineages of different segments. One can see the importance of this as each segment has a unique prospective fate. If individual cells are determined to a particular segment at an early stage, and that determined state is propagated through cell heredity, the cell lines belonging to different segments must be segregated.

The role of the posterior white band is not clear. From an early stage it seems to have an independent lineage, and the segment can therefore be regarded as consisting of two unequal parts. This observation is reminiscent of observations by Lewis (1963) that larger anterior or smaller posterior regions of the segment can be independently transformed by alleles of bithorax.

Experimental analyses of segmentation also point to early determination. In *Euscelis* the segmental pattern is controlled by a gradient or gradients produced by dynamic interaction between an anterior and a posterior centre (Sander, 1960, 1971). During cleavage the segmental pattern is very labile and can be altered by transposition of the posterior centre, and/or isolation of parts of the egg by ligature. In the chronomid, *Smittia*, the segment pattern can be radically altered by U.V. irradiation of the anterior pole region. This can only be done prior to blastoderm formation (Kalthoff, 1971). In many insects complete determination of the segmental pattern occurs at about the blastoderm stage.

In *Oncopeltus* the clones alter only the pigmentation of the epidermis, and information about development is therefore restricted to the integument. Nor do the results reveal the arrangement of the presumptive epidermal cells in the blastoderm: possibly the epidermal cells of the different segments could be separated by cells destined to develop into other organs. There are certainly other cells included in the segment primordia: apart from ectodermally derived organs such as the nervous system, tracheal system and many glands (Dorn, 1972) it is possible that presumptive mesoderm and endodermal structures are incorporated in the segmental organization of the blastoderm. Clearly therefore the nascent segment quadrants will contain more than the ten cells thought to contribute to the epidermis. Nevertheless, Wolpert (1969) has pointed out that pattern formation occurs in small numbers of cells, and this study shows that *Oncopeltus* is no exception.

Larval growth

The description of segmental growth in the abdomen of larvae shows:

(i) Each segment has an independent lineage.

(ii) There is almost no change in segment shape.

(iii) There is a constant proportional increase in size per moult cycle. This is an example of a phenomenon which is typical of insect growth (Dyar's rule; see Wigglesworth, 1972, p. 61).

(iv) The mitoses (except at the anterior boundary) are oriented at random.

(v) The epidermis remains as a coherent sheet during growth.

(vi) All parts of the segment grow at about the same rate.

(vii) The number of mitoses per cell per moult does not appear to be controlled precisely, although the orderly growth of the segments points to a general probabilistic control of mitoses. There is some evidence that the segmental gradient is involved in this control of growth (Locke, 1959). Bohn (1967, 1971) has transplanted pieces of leg segments of *Leucophaea* and demonstrated that a gradient discontinuity acts as a stimulus for regenerative growth, which continues until the discontinuity is replaced by a slope of normal steepness. In *Oncopeltus* when two adjacent segment halves become fused as a result of an interruption in the intersegmental membrane, the amount of growth is half the normal, and the fused segment is only as wide as one normal segment (Lawrence, 1970*b*). These and other results (Lawrence, Crick & Munro, 1972) are consistent with the anterior and posterior margin remaining fixed (in gradient height) during the whole process of growth. In this case the steepness of a linear gradient at any point provides *in principle* a local measure of the axial length of the segment. Such information is essential if the triggering of cell division is a cellular event but the probability of that event has to be subject to intercellular control.

In spite of the importance of the margins as 'organizers' of the segment (Lawrence, 1970*b*) they do not have a discrete lineage. Cells from the extreme margin and from within the segment can have a common origin, suggesting that margin cells acquire their special properties because of their position.

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