Cell lineage and cell determination in the developing compound eye of the cockroach, *Periplaneta americana*

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SUMMARY

By grafting between eye colour mutants of the cockroach *Periplaneta americana* we have investigated (i) the hypothesis that cells within an ommatidium of the fused rhabdom type are clonally derived from a single mother cell and (ii) we have tested the suggestion that cells from non-prospective eye epidermis can form ommatidia when grafted next to eye tissue. Mosaic eyes containing cells of the two genotypes contain ommatidia with unpredictable combinations of the two sorts of cells at the host/graft border. This finding is inconsistent with the first hypothesis. Using grafts of prothoracic epidermis and head epidermis from non-prospective eye regions we have shown that only cells from the prospective eye region can form ommatidia. Possible ways that eye cells could be determined are discussed in the light of these findings.

INTRODUCTION

In this paper we have tested the hypothesis that cells within ommatidia of the insect compound eye are determined by a lineage mechanism (Bernard, 1937; Kühn, 1965). Secondly we have examined the claim that epidermal cells which are not prospective eye cells can be transformed into ommatidia (Hyde, 1972).

Examination of histological sections of the ant, *Formicina flava*, led Bernard (1937) to propose that the cells in a single ommatidium are descended from a single mother cell. Doubts concerning the validity of this claim came from two studies on insects with open rhabdom ommatidia. In this sort of ommatidium the eight retinula cells have individual rhabdoms with separate visual axes and this is reflected in a complex pattern of distribution of their axon terminals in the optic lobe (Braitenberg, 1967; Kirschfeld, 1967; Ioannides & Horridge, 1975; Meinertzhagen, 1976). Genetically mosaic eyes were generated either by grafting between eye colour mutants of *Oncopeltus fasciatus* (Shelton & Lawrence, 1974) or by X-irradiation of *Drosophila melanogaster* (Hanson, Ready & Benzer, 1972; Ready, 1973; Benzer, 1973; Ready, Hanson & Benzer, 1976). In each study the mosaic eyes consisted of red and white areas. According

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to the cell lineage mechanism, at the borders between the red and white parts of the eye no ommatidium should contain both types of cell. However, ommatidia at the borders were found to contain mixtures of cells in many different and unpredictable combinations.

Bernard's original (1937) study concerned ommatidia of the fused rhabdom type in which the eight retinula cells each contributes a segment to a central fused rhabdom and consequently share the same visual axis. Their axons all project in a simple pattern to one locus in the optic lobe (Horridge & Meinertzhagen, 1970; Meinertzhagen, 1976). In the present study we have grafted between eye colour mutants of *Periplaneta americana* to study cell lineage in a fused rhabdom eye. Our results agree with the work on *Oncopeltus* and *Drosophila* and we discuss the implications of these results for mechanisms of cell determination within ommatidia.

The second problem concerns the nature of the prospective eye region. This is the area of head epidermis which will eventually form the eye. Ommatidium formation proceeds across this region in a wave spreading out from its posterior margin (extensive literature reviewed in Meinertzhagen, 1973). Cells in the prospective eye region do not form ommatidia unless they are adjacent to cells which are already differentiated or differentiating into eye tissue (Wolsky, 1949, 1956; White, 1961, 1963; Hyde, 1972; Anderson, 1976). It has been suggested that this transformation of head epidermal cells (here termed recruitment) might depend more on their proximity to an inductive eye margin than on their own ancestry (Green & Lawrence, 1975). This view is supported by the claim that epidermal cells from the prothorax of *Periplaneta americana* can form ommatidia if they are grafted near to the advancing eye margin or next to fully differentiated ommatidia (Hyde, 1972). In view of the importance of this claim we felt bound to repeat and extend her experiments also using *Periplaneta americana*. We find that cells not of the prospective eye region are not recruited and we discuss the relevance of this finding to our understanding of the role of the eye margin in eye development.

**MATERIALS AND METHODS**

Stocks of *Periplaneta americana* were kept at 34 °C and fed commercial rat food and water. 4th–6th instar larvae were selected for operation 1–2 days after a moult. The animals were anaesthetized either with CO$_2$ followed by 5 min drowning in water or by cooling in small glass vials placed on ice. Operations were performed using slivers of razor blade in metal holders and fine watchmakers’ forceps. The grafts were held in place with a coating of insect wax (Krogh & Weis-Fogh, 1951).

We used as cell markers the independent autosomal recessive mutations *pearl* (white-eyed) and *lavender* (purple-eyed) (Ross, Cochran & Smyth, 1964). *lavender* and wild-type (black-eyed) are expressed autonomously in a mosaic but
when ommatidia of either genotype are adjacent to *pearl* ommatidia, wild-type pigments are synthesized in the *pearl* tissue (Hyde, 1972). To avoid possible difficulties in interpretation all grafting operations initially were performed between *lavender* and wild-type animals with some additional control grafts using *pearl* donors and *lavender* hosts.

Small grafts of epidermis were taken from (a) the prothorax, (b) the head posterior to the eye, (c) the vertex far from the eye, or (d) the vertex close to the eye, of donor animals. The grafts were placed close to the dorsal eye margin of host animals (Fig. 1). The last series was used to provide the genetic mosaics for the analysis of cell lineage and also served as a control for the other series. Series *a* grafts were taken from a body segment which clearly does not normally form eye tissue and series *b* grafts, although they were from the head, were from a non-prospective eye region, since the cockroach eye does not grow along its posterior margin (Anderson, 1976). Series *c* grafts were inverted in either the anteroposterior or the dorsoventral axis with respect to the host.

Mosaic retinae were fixed in a paraformaldehyde/glutaraldehyde mixture (Karnovsky, 1965) for 4 h, post-fixed in phosphate-buffered 1% osmium tetroxide for 12 h and embedded in Araldite after dehydration by standard methods. Material was sectioned using a Huxley Ultramicrotome and a Dupont
diamond knife. 1 \( \mu \text{m} \) sections were collected serially. The sections for light microscopy were stained in 1 % toluidine blue in borax solution and photographed using Zeiss oil immersion objectives. Electron microscope sections were cut at 12 \( \mu \text{m} \) intervals in the series and collected on Formvar films mounted on stainless-steel rings. They were transferred to slot grids (Sjöstrand, 1967) and stained in the usual way with lead citrate and uranyl acetate. They were examined using an AEI-802 electron microscope.

The genotypes of cells in the vicinity of the host/graft borders of two mosaic retinae were displayed in schematic maps. For most of the host/graft borders ommatidia were arranged with hexagonal packing. However, there are sometimes small deviations from this strictly regular pattern such as the dislocation of ommatidial rows or the interpolation of extra rows of ommatidia (Butler, 1973a). There were no major irregularities in ommatidial packing along the host/graft border in either mosaic retina. Nevertheless the maps are schematic only and do not show small irregularities in the packing. We used Braitenberg’s (1967) convention for naming the \( x, y \) and \( z \) axes of the retina to help the reader
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RESULTS

The organization of the retina and the identification of cells

The ommatidia of *Periplaneta americana* have been described previously at the ultrastructural level by Butler (1973b). Our observations agree with his description. The four classes of cells found in each ommatidium are as follows: the four crystalline cone cells which, in conjunction with the cuticular cornea secreted at the ommatidial surface, form the dioptric apparatus and direct light onto the underlying receptor cells; eight retinula cells forming the photoreceptive...
layer; two primary pigment cells and a variable number of accessory pigment cells which regulate the passage of light into and between ommatidia.

Most of the cells are arranged within the ommatidium in a highly characteristic pattern which is identical in all ommatidia throughout the retina. Such precision of cell arrangement allows these cells to be uniquely identified. The cone cells (C₁–C₄) are structurally indistinguishable but are always arranged in a predictable pattern (Fig. 2). The primary pigment cells (P₁ and P₂) are identical in structure but always occupy a posterior and anterior position respectively (Fig. 2). The retinula cells are of similar appearance to each other but of unequal size and spectral sensitivity (Butler, 1971). The rhabdom contributions of some of the cells extend more distally than others. Using these criteria individual retinula cells can be identified (Butler, 1973b). The accessory pigment cells are not individually distinguishable and are unpredictable both in number and in their anatomical relationship with other cells in the ommatidium.

Thus all the cells of the ommatidium except the accessory pigment cells can be identified individually on the basis of their structure or position.

Identification of cell genotype

The genotypes of individual cells could be identified only by the relative number and size of their pigment granules. The pigment appears black in transmitted light but during the preparation of thin sections for electron microscopy it tends to shatter or fall out of the sections leaving circular holes (Figs. 6, 7, 9 and 10). All cone cells lack pigment and therefore their genotypes could not be determined. The genotypes of all other cells could be scored.

In lavender eye tissue, the primary pigment cells are unpigmented (Fig. 2), the accessory pigment cells are unpigmented and the retinula cells have sparse pigmentation with a few small grains (0.2–0.45 μm in diameter in the cytoplasm adjacent to the rhabdom (Figs. 6, 7, 9 and 10). In wild-type tissue, the primary pigment cells are tightly packed with large pigment grains (0.3–1.7 μm in diameter) (Fig. 2), the accessory pigment cells contain relatively fewer grains (0.45–1.4 μm in diameter) and the retinula cells contain many grains.

Figures 5–7

Sections through the PLM retina. Arrows indicate the Y axis. The sections illustrate the appearance of rhabdoms and retinula cells at their more distal levels.

Fig. 5. 1 μm section through part of the border between host lavender tissue (to the right) and graft pearl tissue (to the left). The pearl tissue has a wild-type pigmentation pattern. Two ommatidia, numbered 8 and 34, are shown at EM level in Figs. 6 and 7.

Fig. 6. Electron micrograph of ommatidium 8. One retinula cell, R₇, is pearl; the other retinula cells are lavender. Arrowheads indicate wild-type pigment.

Fig. 7. Electron micrograph of ommatidium 34 which contains 2 pearl retinula cells, R₂ and R₇; the other retinula cells are lavender. Arrowheads indicate wild-type pigment.
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(0.45–1.1 μm in diameter) (Figs. 8–10). Pearl eye tissue in a lavender background appears black (Hyde, 1972) and has the same pattern of pigmentation as wild-type tissue although the granules are less densely packed.

Lineage relations of retinula and primary pigment cells

Three mosaic eyes were obtained from about 50 control operations (series d). One had a lavender patch in a wild-type host and two had pearl patches synthesizing wild-type pigments incorporated into lavender hosts. One pearl/lavender mosaic (PLM; Fig. 3) and the lavender/wild-type mosaic (LWTM; Fig. 4) were chosen for detailed analysis of cell lineage. Typical sections used for the analysis are shown in Figs. 5–10.

Only the retinula cells and primary pigment cells could be uniquely identified with respect to both cell numbering and genotype, and these were recorded in 98 ommatidia at the host/graft border of the PLM eye (Fig. 11) and in 89 ommatidia of the LWTM eye (Fig. 12). The PLM eye contained 32 ommatidia with both pearl and lavender cells and the LWTM eye contained 24 ommatidia with both lavender and wild-type cells. These results show that each ommatidium is not clonally derived from a single precursor cell.

In both eyes it is clear that ommatidia containing both types of cell do so in a great variety of unpredictable combinations (Figs. 11 and 12). A more detailed analysis reveals that many other possible patterns of lineage relationship between cells are also ruled out. Firstly, there is no consistent correlation between the genotypes of the primary pigment cells and the retinula cells. In ommatidia 12, 30 and 46 of the PLM eye, primary pigment cell P1 is pearl while P2 and the retinula cells R1–R8 are all lavender. In ommatidia 37, 55, 71, 77 and 78 of the PLM eye, P2 is lavender while the other scorable cells are pearl (Fig. 11). Thus P1 and P2 may be derived independently of each other and of the retinula cells. Secondly, each retinula cell may be derived independently of the other retinula cells in the same ommatidium. This is particularly clear where a single retinula cell has a different genotype from the other seven in its ommatidium: R3 in 52

Figures 8–10

Sections through the LWTM retina. Arrows indicate the Z axis. The sections illustrate the appearance of rhabdoms and retinula cells at their more proximal levels.

Fig. 8. 1 μm section through part of the host/graft border. Host wild-type tissue is to the left and graft lavender tissue to the right.

Fig. 9. Electron micrograph of ommatidium 49 indicated in Fig. 8. Two retinula cells are wild-type (R4 and R6) and six are lavender (R1, R2, R3, R5, R6, R7 and R8). Accessory pigment cells of wild-type (wt) and lavender (l) genotype are present.

Fig. 10. Electron micrograph of ommatidium 47 shown in Fig. 9. Four retinula cells are wild-type (R1, R2, R3 and R4). The remaining retinula cells (R5, R6, R8 and R7) are lavender. l, lavender accessory pigment cells; wt, wild-type accessory pigment cells.
Fig. 11. Schematic diagram to show the genotypes of the primary pigment cells (P₁ and P₉) and the retinula cells (R₁–R₈) in 98 ommatidia along the host/graft border of the PLM retina. Black areas are *pearl*; white areas are *lavender*. D, dorsal; V, ventral.

Fig. 12. Schematic diagram to show the genotypes of the primary pigment cells (P₁ and P₉) and retinula cells (R₁–R₈) in 89 ommatidia at the host/graft border of the LWTM retina. Black areas are *lavender*, white areas are wild-type. D, dorsal; V, ventral.
and 56 of LWTM; R₂ in 72 of PLM; R₃ in 21 of PLM; R₆ in 74 of PLM; R₇ in 8 and 43 of PLM; R₈ in 44 and 45 of LWTM (Figs. 11 and 12). Retinula cells R₄ and R₅ can also be derived independently of the others. This is shown in the following argument. In ommatidium 38 of PLM, R₂, R₃ and R₄ are pearl while R₁, R₅ R₆, R₇ and R₈ are lavender (Fig. 11). This shows that R₄ may be clonally related to R₂ and R₃ but not to R₁, R₅, R₆, R₇ and R₈. However, in ommatidium 58 of PLM, R₄ is pearl while R₂ and R₃ are lavender. Therefore R₄ may also be derived independently of R₂ and R₃. Using a similar argument and considering ommatidia 35 and 36 of PLM, it is possible to show that R₅ is not necessarily related by lineage to any of its seven retinula cell neighbours.

**Lineage relations of accessory pigment cells**

Each ommatidium is surrounded by a sheath of accessory pigment cells and unlike other classes of cell they cannot be recognized individually. By examining serial 1 μm sections the numbers of accessory pigment cell nuclei associated with ten ommatidia were counted. For each ommatidium there are between 12 and 28 cells. In the absence of any system for accurately identifying individual accessory pigment cells, no detailed statement can be made concerning their lineage relations with surrounding cells. However, from electron micrographs of the LWTM retina we can say that the genotypes of accessory pigment cells frequently differ from those of adjacent accessory pigment cells, retinula cells and primary pigment cells. Examples of four ommatidia with adjacent accessory pigment cells of both genotypes are illustrated in Fig. 13.

**Other cellular constituents of the retina – interommatidial bristles and tracheoblasts**

In addition to the cells which form ommatidia there are also cells which form corneal bristles and there are cells associated with tracheoles. The occurrence of neither type of cell affects our arguments concerning cell determination of cells within ommatidia. The corneal bristles are randomly placed and they are extremely few in number (Butler, 1973a). Over large areas of the corneal surface there are no bristles (Shelton, unpublished observations). The retina is well supplied with tracheoles (Smith, 1968). From electron micrographs we estimate one per ommatidium. However, the tracheoblasts which form the terminal branches of the tracheal system are extra-retinal in origin: the retina is derived from a sheet of epidermis and this receives its tracheoles from external sources (see Miller, 1964).

**Cell mixing at the host/graft border**

An important observation from the analysis of both grafts is the relatively small spread of the graft cells into the host tissue (Figs. 11 and 12). For much of the border only a single row of ommatidia contains both types of cell.
Fig. 13. Drawings from electron micrographs of the LWTM retina showing four mosaic ommatidia with adjacent accessory pigment cells. The retinula cells are numbered R₁–R₈. Shading indicates lavender genotype, stippling indicates wild-type. There is no consistent relationship between the genotype of an accessory pigment cell and the genotypes of its neighbours.

**Fate of the epidermal grafts**

Of approximately 400 operated animals of the series a–d, about half survived to adults. The grafts could be recognized by their colour or cuticle type or by a slight bulge around their outline (which tended to become rounded in succeeding instars) where host and graft had not healed smoothly together. The majority result was that during the six to eight instars following the operation the host eye margin did not reach the graft. Indeed this was the case for all of the 34 adults with dorsoventrally inverted grafts in the series c.

In the 22 cases where the host eye margin did reach the graft, two distinct types of result were obtained. The three control grafts were successfully trans-
Heads of adult cockroaches following grafts, during the 6th instar, of prothoracic epidermis (Fig. 14), posterior head epidermis (Fig. 15) or vertex epidermis from close to the midline of the head (Fig. 16) to a position above the dorsal edge of the retina. The host retina (hr) has failed to recruit the grafted epidermis (ge).

DISCUSSION

A previously neglected report of mosaic ommatidia in the silkworm, *Bombyx mori*, first suggested that an ommatidium was not derived from a single mother cell (Yagi & Koyama, 1963). This has been established beyond doubt for open rhabdom ommatidia in holometabolous insects (Hanson et al. 1972; Ready, 1973; Benzer, 1973; Ready et al. 1976) and hemimetabolous insects (Shelton & Lawrence, 1974) and for fused rhabdom ommatidia in holometabolous insects (Yagi & Koyama, 1963) and now in this present study, in hemimetabolous insects.

The presence of mosaic ommatidia alone does not exclude the possibility that some classes of cells within ommatidia might always be related by lineage. In mosaic retinae of *Bombyx* it was established that the pigment cells within one
ommatidium did not always relate by lineage (Yagi & Koyama, 1963). Their material was of low resolution and no further comment was possible then. In work on Oncopeltus the lineages of only six of the eight retinula cells and the two primary pigment cells were considered (Shelton & Lawrence, 1974) and they were found not to be necessarily related. In the present study on Periplaneta we have established that none of the retinula cells and primary pigment cells need be clonally related and there is no obvious lineage relationship between particular retinula cells or primary pigment cells and adjacent accessory pigment cells. In Drosophila where the accessory pigment cells are highly ordered and fixed in number, it can be shown that all cells except the four cone cells and the four corneal bristle cells (for which no suitable genetic marker was available) are associated in one ommatidium irrespective of lineage (Ready et al. 1976).

To attempt to reconcile these findings with the idea that particular cell lineages generate only certain cell types, requires the additional postulate of extensive and highly ordered migration of cells to their final locations. This possibility cannot be ruled out although it seems unlikely because in our mosaics and those of Ready et al. (1976) there is little cell mixing at the host/graft borders; for much of the borders, although not all, there is only a single row of ommatidia containing cells of both genotypes. In order to exclude all possible cell lineage models using data from genetic mosaics it is also necessary to know that all classes of cell are the products of cell divisions subsequent to the formation of the mosaic by grafting or X-irradiation. Cell proliferation follows recruitment in the post-embryonic growth of the retina (see Meinertzhagen, 1973; Shelton, 1976) and it is quite likely that the division phases give rise to all classes of cell. In embryonic development of the retina in Ephestia kuhniella there are two waves of mitosis which pass across the eye anlage before ommatidium formation. Most or all cells divide as each wave passes and using [3H]thymidine it can be shown that all cell classes are labelled. In spite of many descriptions of eye development there is no evidence for patterned cell death in the retina; programmed cell death could also affect our interpretation.

It has been suggested that the cells of the prospective eye region might not differ from other head epidermal cells and that the process of eye development begins with the recruitment of uncommitted cells by the advancing eye margin (Lawrence & Shelton, 1975; Green & Lawrence, 1975). The role of the eye margin in this case would be an instructional one involving the passage of information concerning the nature of the future developmental programme to be followed by the recruited cells.

The alternative view proposed here is that cells within the prospective eye region differ from other epidermal cells in being competent to form eye tissue and the role of the eye margin is to trigger the sequence of events leading to ommatidium differentiation.

The available evidence for each of these views is worth considering in detail. Hyde (1972) transplanted wild-type epidermis from the prothorax of Periplaneta
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Americanica to a position dorsal to the eye of a pearl nymph. After several molts dark pigments were observed in the dorsal part of the eye. She concluded that the wild-type prothoracic cells had been recruited into ommatidia. However, it is possible that the wild-type pigments were entirely confined to the host pearl ommatidia and were produced in response to an interaction between the grafted wild-type epidermis and the host pearl ommatidia. Alternatively, in the one case illustrated (Hyde, 1972; Fig. 14) the amount of pigment is so small with no clear graft boundary that it could result from melanization after damage during the operation (see Briggs, 1964). We were unable to repeat these results in our experiments using the lavender/wild-type combination of animals. All seven animals with successful prothoracic grafts (series a) showed the same result: the prothoracic epidermis was not recruited.

Green & Lawrence (1975) attempted to test the ability of prothoracic epidermis to be recruited in Oncopeltus fasciatus by grafting between eye colour mutants but the prothoracic grafts were either rejected or sorted out from the head epidermis so that they did not come into contact with the eye margin.

We also found that epidermis posterior to the eye (series d) and antero-posteriorly inverted vertex epidermis from well above the eye (series c) were not recruited. A plausible explanation of these results is that the prospective eye region remaining in the later instars is only a narrow band. Control grafts (series d) were taken from very close to the eye margin but the series c grafts were taken from a more dorsal position. Therefore control grafts contain cells from the prospective eye region and can be recruited, while grafts from dorsal vertex, prothorax or posterior head are not part of the prospective eye region and these epidermal cells cannot be recruited.

Similar results have been reported in an elegant study on the dragonfly, Aeschna cyanea (Mouze, 1975). The dragonfly eye is capable of regeneration after ablation of the region containing the differentiating ommatidia (the growth zone) by the formation of a new growth zone at the junction between differentiated ommatidia and vertex epidermis. Mouze (1975) found that a new growth zone was not formed if differentiated ommatidia were put in contact with epidermis from the abdominal tergites, abdominal sternites, the occiput or even the vertex a small distance from the eye. Additional evidence is provided by work on a holometabolous insect, the mosquito, Aedes aegypti (White, 1961). White showed that the epidermis from the prospective eye region when placed in contact with the optic rudiment of an early fourth instar larva, differentiated into eye tissue. However, if epidermis from posterior to the eye or from a position some distance anterior to the eye was grafted next to the optic rudiment, the graft did not differentiate into eye tissue.

Green & Lawrence (1975) found that head epidermis taken from adjacent to the eye of an adult Oncopeltus was recruited when grafted next to a growing larval eye. The area of larval head epidermis which is competent to form eye can therefore be larger than that which actually does form eye.
The evidence therefore favours the view that competence to form eye is restricted to an area in front of the growing edge of the larval eye and is not a general property of larval epidermis.

Little is known about the determination of the individual cells within an ommatidium during the subsequent stages of eye development. The most detailed analysis of development during these stages is a study on *Drosophila melanogaster* (Ready, 1975; Ready *et al.* 1976): the first detectable stage of ommatidium production is the division of epithelial cells from the prospective eye region. From the resulting pool of cells, groups are formed; at the centre of each one is a characteristic arrangement of five cells which do not divide further and normally become retinula cells R$_2$, R$_3$, R$_4$, R$_5$ and R$_8$ (numbering convention as in Dietrich, 1909). Further division of the cells surrounding the central core provides the remaining cells necessary to complete the ommatidium, including retinula cells R$_1$, R$_6$ and R$_7$. The completed ommatidial precursors have an arrangement typical of the final ommatidium. Two recent studies on *Drosophila* confirm these findings (Hofbauer & Campos-Ortega, 1976; Campos-Ortega & Gateff, 1976).

Two phases of cell division have also been observed in the developing eye of the flour moth, *Ephesia kuhniella*, and the cells formed in the second phase of division are probably of more than one type (Egelhaaf, Berndt & Küthe, 1975).

Hence, cells are contributed to an ommatidium in two distinct phases. The significance of this for the determination of cell type within an ommatidium is at present obscure.

By what mechanism could the pattern of the ommatidium be generated? It has been suggested that the leading edge of the retina may serve as a template on which new elements are incorporated (Ready *et al.* 1976). However, other work has shown that the eye margin does not itself spatially organize the developing ommatidia; rotation of parts of the prospective eye region results in the formation of ommatidia whose orientation conforms not to that of the previously formed ('template') ommatidia but to the original polarity of the epidermis from which they were formed (Lawrence & Shelton, 1975). It seems likely that close proximity to the eye margin triggers epidermal cells from the prospective eye region to proliferate and segregate into clusters, maintaining their polarity but that it is specification of position within the clusters, rather than a cell lineage and cell migration mechanism, which leads to the determination and differentiation of the various ommatidial components in their characteristic pattern.

We are indebted to Dr Mary Ross, Virginia State Polytechnic, for supplying the mutants without which the study could not have been conducted. Mr C. Townsend provided valuable technical assistance during the early stages of the investigation. Figures 13 and 14 were prepared by Mrs Diana Cole and Mr I. Ridell prepared Fig. 15. We thank the University of Leicester for a Research Scholarship to H. Anderson and the Science Research Council for a grant to P. M. J. Shelton and a Studentship to S. Eley. The referees comments were useful in preparing the final version of the manuscript.
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(Received 1 December 1976, revised 15 December 1976)