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The determination of polarity in the developing insect retina

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SUMMARY

The retina in *Oncopeltus fasciatus* is a polarized structure in which all ommatidia are orientated the same way. By a series of grafting experiments we have shown that the orientation of ommatidia is partly dependent upon the orientation of the epidermis from which they develop and partly on the orientation of the host eye. Grafts which invert the dorsoventral axis of the presumptive eye epidermis do not disrupt the orientation of the nascent ommatidia. Grafts which are rotated by 90° or 180° produce altered patterns of orientation. The polarity of the epidermis is to some extent conserved in these cases. Significantly ommatidia at the graft/host border take up orientations intermediate between the extremes found in the graft and host. Small rotated grafts assume the polarity of the host retina. These results are compared with the effects of similar grafts on developing insect bristle patterns.

INTRODUCTION

The insect compound eye grows by an unusual mechanism: at the anterior edge of the enlarging eye epidermal cells are recruited by an inductive action of the eye margin (White, 1961; Hyde, 1972). Following recruitment cells first proliferate and then differentiate to form new ommatidia. In the cockroach (Hyde, 1972) and *Oncopeltus* (Green & Lawrence, 1975) grafting experiments have shown that under the influence of the advancing eye margin, ommatidia can form from cells that would not normally have made eye.

The ommatidia are polarized structures, the orientation given by the arrangement of the constituent cells. Organules in the insect abdominal segment are also polarized and their orientation appears to be determined by an underlying gradient of positional information (Lawrence, 1973). It has been supposed that similar gradients may provide the basis for specification of cells in various parts of the brain (Wolpert, 1969; Gaze, 1970). In some ways the insect retina is a model brain system. It consists of serially repeated sets of neurons and accessory cells within which the neurons are further divided into sub-sets of retinula cells.

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For the proper functioning of the compound eye it is essential that the receptor cells within ommatidia are organized with consistent polarity across the retina. This is particularly apparent in Diptera where eye function depends upon a remarkable geometrical arrangement of receptor cells (Braitenburg, 1967; Kirschfeld, 1967). In this paper, we have tested the hypothesis that the orientation of ommatidia is dependent upon the polarity of the epidermis from which they develop. Our results are not inconsistent with the presence of an underlying gradient of positional information.

Using *Oncopeltus fasciatus* we previously studied cell lineage relationships in the development of ommatidia by grafting normally orientated pieces of genetically marked head epidermis (Shelton & Lawrence, 1974). Here, we describe the results of grafting pieces of epidermis that are rotated from their normal orientation. We find that the polarity of the graft is partially conserved on incorporation; the polarity of the nascent ommatidia depending both on the orientation of the piece of transplanted tissue, and of the eye margin.

MATERIALS AND METHODS

The operations were made on newly moulted 3rd and 2nd stage larvae of *Oncopeltus fasciatus*. Small pieces of epidermis near to the eye margin of wild-type donor were cut out with fine scissors, and transplanted to a similar site on a mutant host. The host was homozygous for two mutants affecting eye colour (white body, *wb*; and red eye, *re*; Lawrence, 1970) which together give a white eye. The number of operations done, the number of survivors to the adult stage, and the number of mosaic eyes produced are given in the figure legends.

The retinae were fixed in one of two ways. Some were fixed in phosphate-buffered osmium tetroxide for 2–24 h. Others were prefixed in a mixture of paraformaldehyde-glutaraldehyde (Karnovsky, 1965), for 24 h, and then washed in buffer prior to the osmium treatment. The latter technique provided the best fixation but stained sections of eyes fixed in osmium alone gave enhanced contrast. The material was embedded in Araldite and serially sectioned at 1 μm using a Huxley Ultramicrotome. Sections were collected singly and mounted in order on glass slides. They were stained with 1 % toluidine blue in borax solution. Diagrams showing the orientations of ommatidia over large areas of retina were prepared from these series with the aid of photographs and a Zeiss drawing apparatus. In the diagrams and figures an arrow is used to indicate the antero-posterior axis of each ommatidium. It points anteriorly.

RESULTS

Symmetry of ommatidia and polarity in the normal retina

In *Oncopeltus*, retinula cells are arranged on the open rhabdomere plan and each ommatidium is symmetrical about the antero-posterior axis (Shelton &

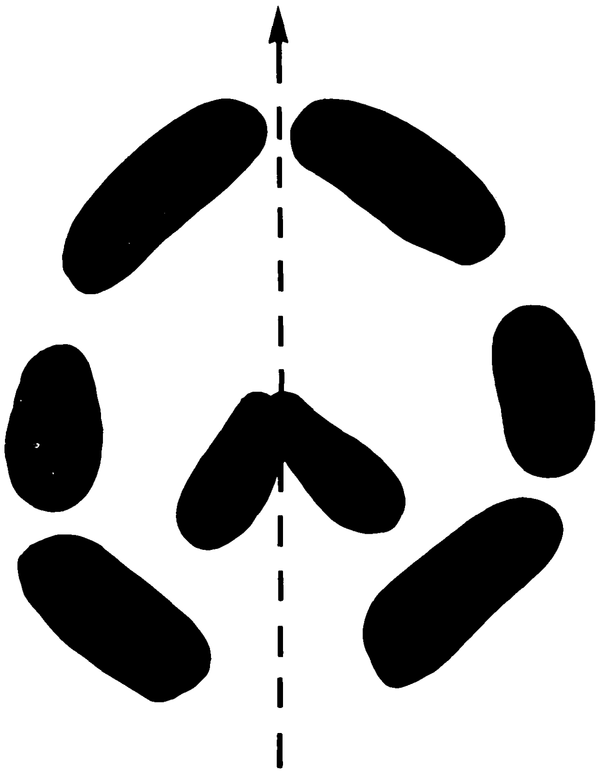

Fig. 1. The arrangement of rhabdomeres in an ommatidium. *In situ* the arrow points anteriorly.

Lawrence, 1974). This symmetry is particularly obvious in the arrangement of the rhabdomeres of the 8 retinula cells. In sections at the rhabdomere level it is possible to draw an arrow through the structure which defines this axis and indicates the anterior side of the ommatidium (Fig. 1). Examination of large areas of the eye shows that the orientation of ommatidia is consistent across the retina. It seems likely that, as in Diptera (Kirschfeld, 1967), this ordering is of functional significance.

The symmetry is also evident at the level of the two primary pigment cells. One is dorsal and the other is ventral; the junction between the cells defining the antero-posterior axis (Shelton & Lawrence, 1974). However, these cell boundaries can be difficult to see and since the arrangement of rhabdomeres is clearer we have used it as the primary indicator of ommatidial polarity.

In the following experiments we have used various graft situations to investigate the developmental mechanisms which bring about the accurate orientation of ommatidia.

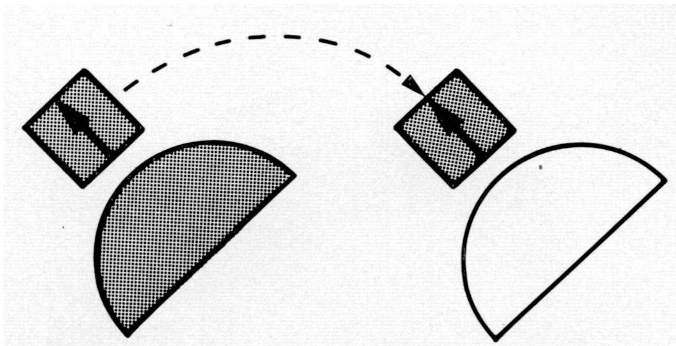

Fig. 2. Control operation; shaded is wild type, unshaded mutant tissue. (n = 34; survivors to adult = 20; no. of mosaic eyes = 7.)

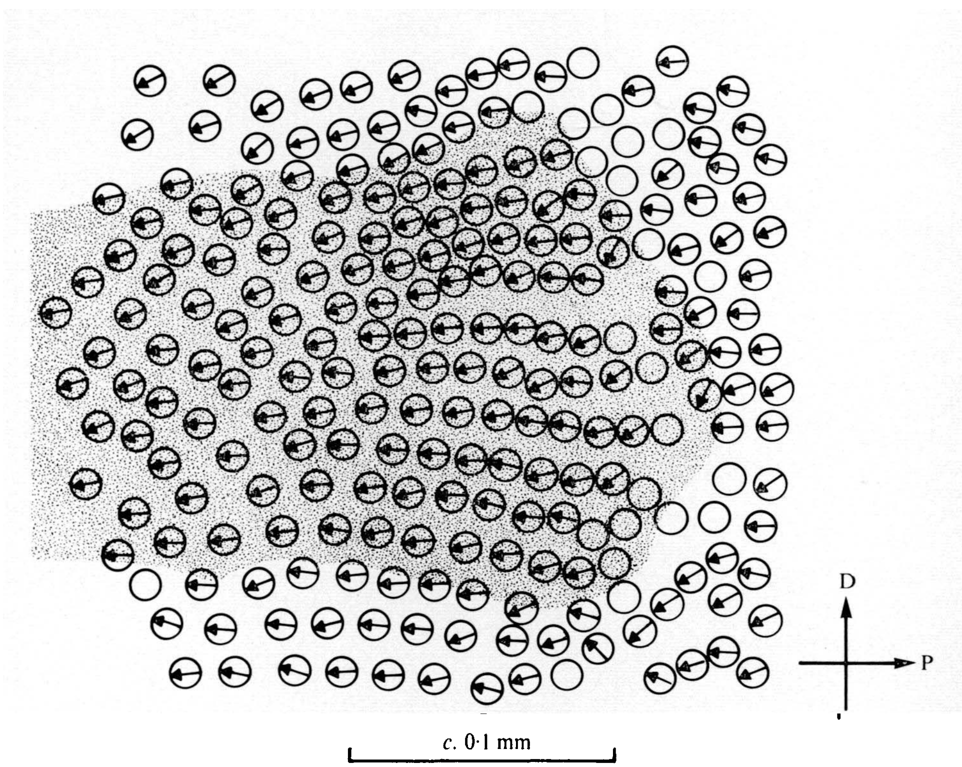

Fig. 3. The orientation of ommatidia after a control operation on the left eye. The polarity of each ommatidium is indicated by an arrow (see Fig. 1). Ommatidia without an arrow are defective. The shaded area marks the transplanted wild type tissue.

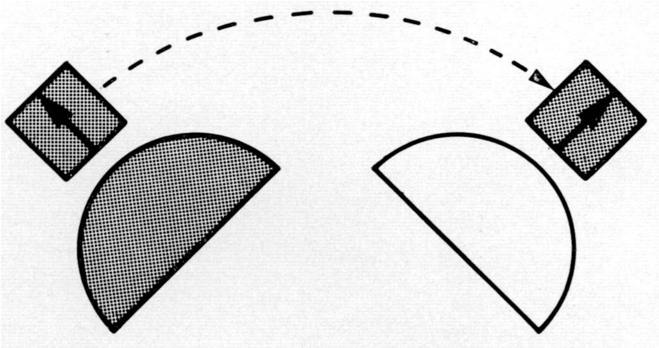

Fig. 4. Grafts from left to right eye, no rotation. (n = 7; survivors to adult = 7; no. of mosaic eyes = 6.)

Control grafts

In this series of operations a square of epidermis from close to the eye of the donor was grafted with normal orientation into a corresponding region of the host (Fig. 2). The mosaic eyes obtained have a normal profile and show damage and disruption of orientation only at the graft edge which is first recruited into the retina (Fig. 3).

Inversion of the dorso-ventral axis only

Inversion of the dorso-ventral axis is achieved by grafting a square of epidermis from in front of a left eye to a corresponding location in front of a host's right eye (Fig. 4). These grafts take well, give eyes of normal profile and show disruption and damage mainly near the cut edge (Figs. 5 and 11). They are thus in all respects similar to control experiments. This suggests that the underlying influence which governs antero-posterior polarity does not vary in the dorso-ventral axis.

90° rotations

We grafted a square of epidermis to a host eye of the same side and rotated the square by 90° (Fig. 6). These grafts are not as successful as Left–Right transplantations, and often the mosaic eyes produced are abnormally shaped and may have an uneven surface, with frequently a small tower of tightly packed ommatidia that rises above the normal surface of the eye. In this tower the ommatidia are usually abnormal, and may extend central to the basement membrane. The orientation of the ommatidia, provided the graft is above a certain size, is invariably altered with large fields of similarly oriented ommatidia pointing in abnormal directions. The orientation of the graft ommatidia does not make any simple pattern, but sometimes they express the original orientation of the grafted piece. A good example of this is shown in Figs. 7 and 12. What is important is that the ommatidia are consistently orientated over a large area with gradual rather than abrupt changes of polarity. In the case of

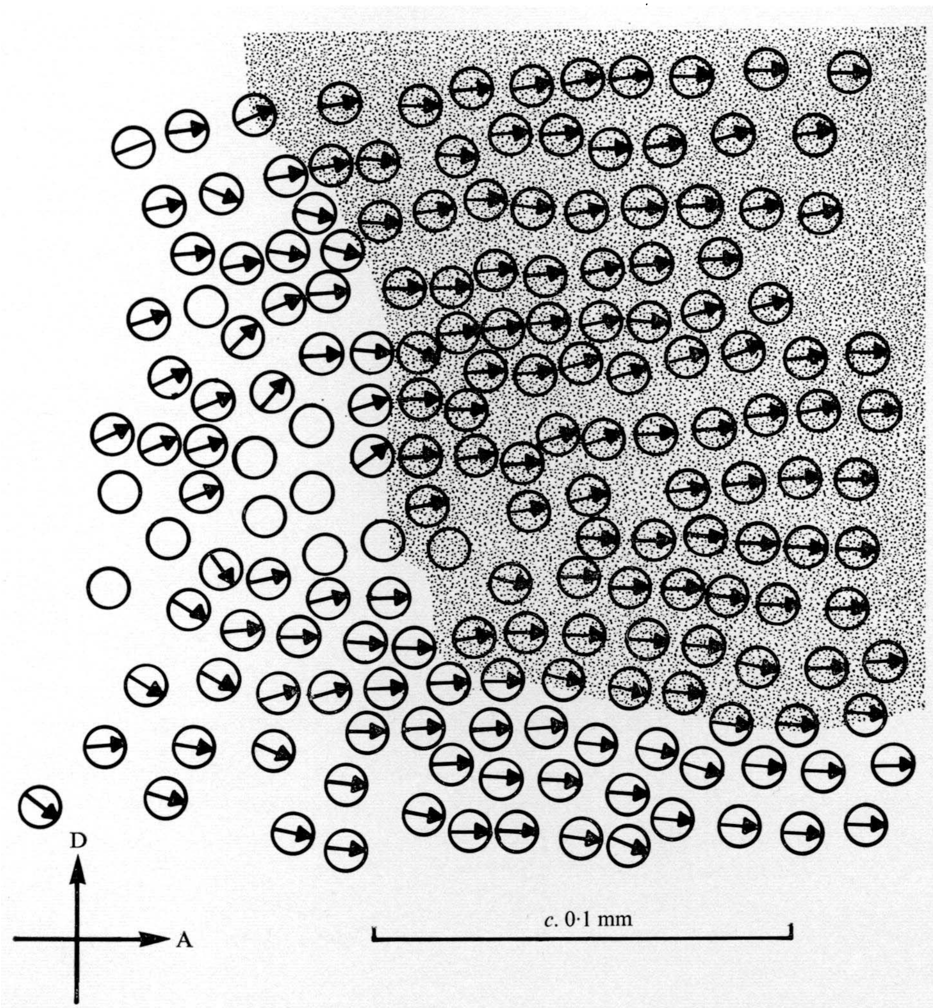

Fig. 5. The orientation of ommatidia after a graft from left to right eye.

the ventral part of Fig. 7 there is an S-shaped pattern, which crosses the host-graft border, suggesting interaction between host and graft. In the dorsal part of the eye, both host and graft ommatidia have rotated so that both point dorsally.

In some cases the interaction across the host-graft border is more limited. Fig. 8 shows the result of a 90° clockwise rotation on a right eye where the single ommatidium spanning the border has an orientation precisely intermediate between that of host and graft.

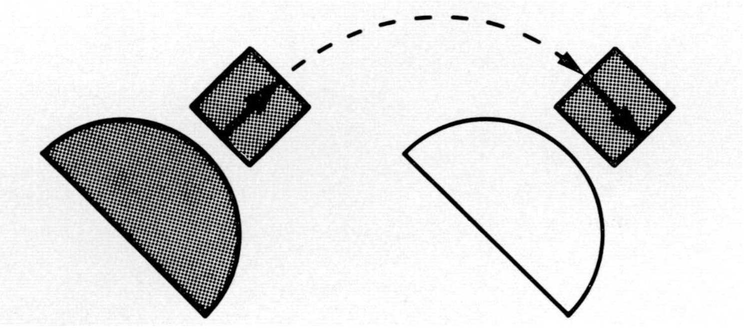

Fig. 6. 90° rotation experiment; this diagram shows a right eye 90° clockwise rotation. (n = 66; survivors to adult = 50; no. of mosaic eyes = 12.)

180° rotations

These operations are carried out in a similar way to the 90° rotations (Fig. 9). The success rate is very low and as with 90° rotations the eye surface may be uneven. In some cases ommatidia at the host/graft border are opposed (Fig. 13) but more usually the polarity in both host and graft is changed. Consequently, we find large areas of similarly orientated ommatidia whose polarity is inconsistent with the original orientation of both host and graft. Often there is evidence for graded interaction across the host-graft border (Figs. 10 and 14). Figure 10 shows such a case where the original orientation of the graft has undergone considerable alteration as a result of the operation and interaction with the host.

Small rotated grafts

It is difficult to control the amount of tissue that becomes incorporated into the eye. Sometimes the grafts are only partially successful, and in these cases only small regions of wild-type tissue may become incorporated. Often the original orientation of the graft becomes lost, and the marked cells become part of normally orientated ommatidia. Two such cases, both of which were rotated 180° at the time of grafting, are illustrated in Figs. 15 and 16. In one the grafted cells survive only as an isolated strip and are restricted to about 15 ommatidia, in another the grafted piece is larger, but has totally adopted the host's polarity.

Orientation within an ommatidium

The ommatidium is a bilayered structure with the primary pigment cells and cone cells at the periphery, and the more central retinula cells. It is remarkable that, for an intact ommatidium, even when it is in a field of ommatidia with diverse orientation, it is always possible to monitor its polarity; the arrangement of rhabdomeres is precise and internally consistent. Are the two layers of the organ always orientated in the same way? In the light microscope it is normally

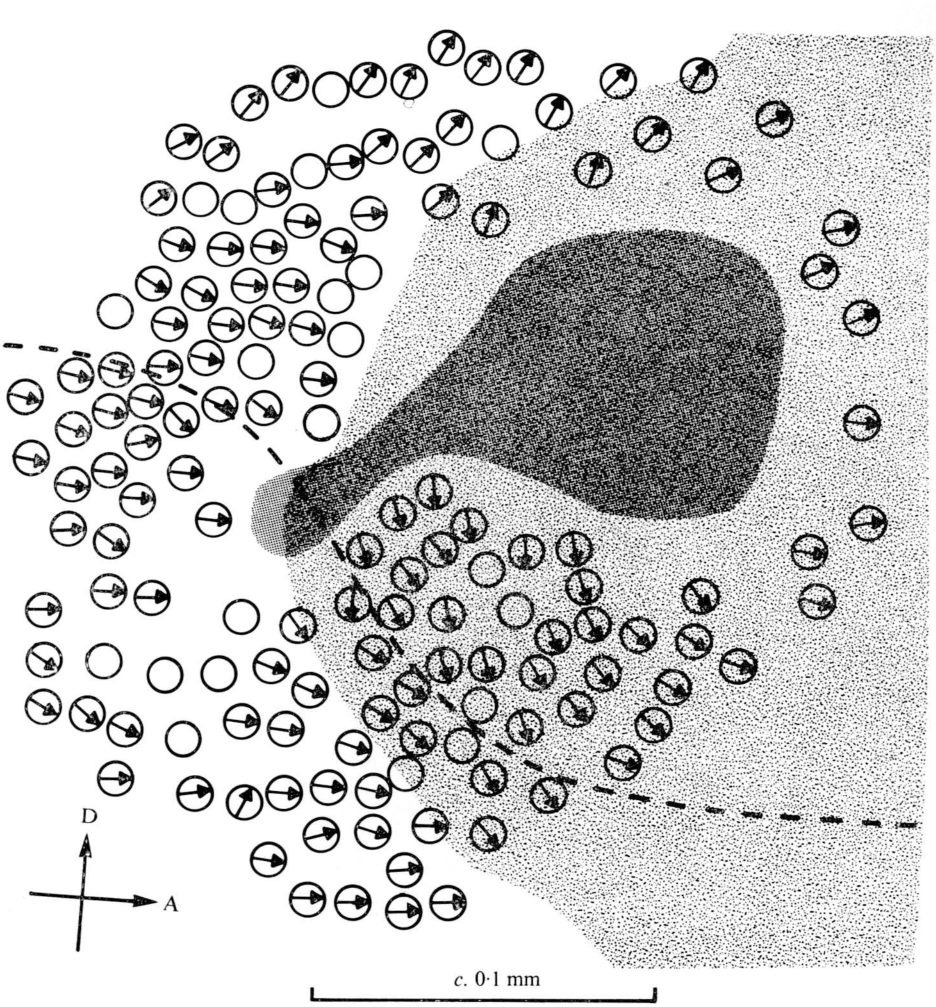

Fig. 7. The orientation of ommatidia after a rotation on a right eye of 90° clockwise. The lightly shaded area is wild type tissue, the darkly shaded area is a region of severe damage. The S-shaped curve is indicated by a dotted line.

difficult to see the positions of dorsal and ventral pigment cells, but when one cell is wild type, and the other mutant, the junction is obvious (Fig. 14, *M*). By examination of serial sections we found that sometimes the primary cells and the rhabdomeres of a single ommatidium were out of agreement by the maximum detectable (90°). This shows that the arrangement of the two layers of component cells need not be coordinated.

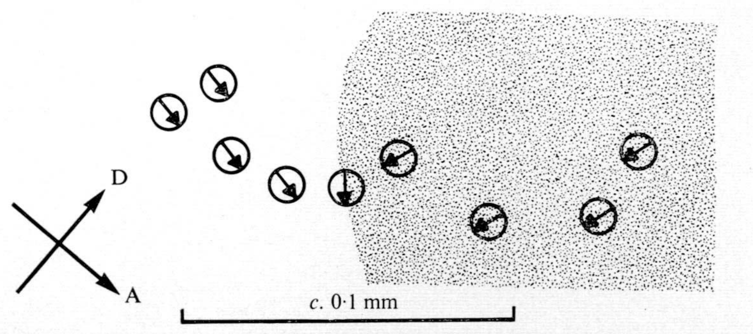

Fig. 8. Detail of an eye after 90° clockwise rotation to show local interaction. Only the mosaic ommatidium has an orientation intermediate between host and graft (shaded).

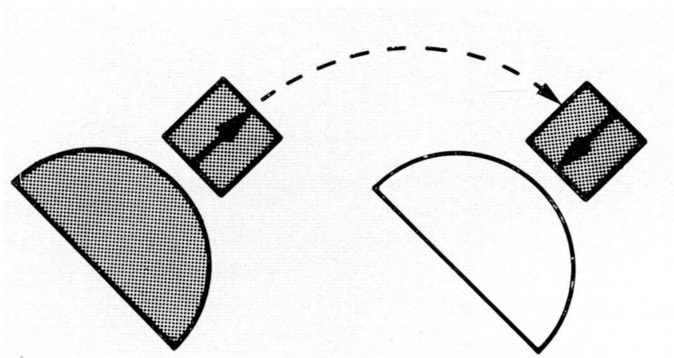

Fig. 9. 180° rotation; this diagram shows an experiment on the right eye. (n = 121; survivors to adult = 82; no. of mosaic eyes = 14.)

DISCUSSION

The development of the compound eye and its central neuronal connections is now under intensive study (Meinertzhagen, 1973, 1974). One particular feature seems to be a front which spreads across the eye from posterior to anterior (White, 1961, 1963). In *Drosophila*, after the front has passed, the ommatidia are no longer sensitive to the rough effect produced by X-irradiation (Becker, 1957), or to damage produced at the restrictive temperature in *shibire*^{ts} flies (Poodry, Hall & Suzuki, 1973). This front therefore seems to be critical to ommatidial differentiation. In hemimetabolic insects a similar front spreads out from the eye during larval growth, recruiting epidermal cells as it proceeds (Hyde, 1972; Shelton & Lawrence, 1974). An early stage in ommatidial differentiation is the formation of clusters (Meinertzhagen, 1974), the cells entering any particular cluster being not related by lineage in any simple way (Benzer, 1973; Shelton & Lawrence, 1974).

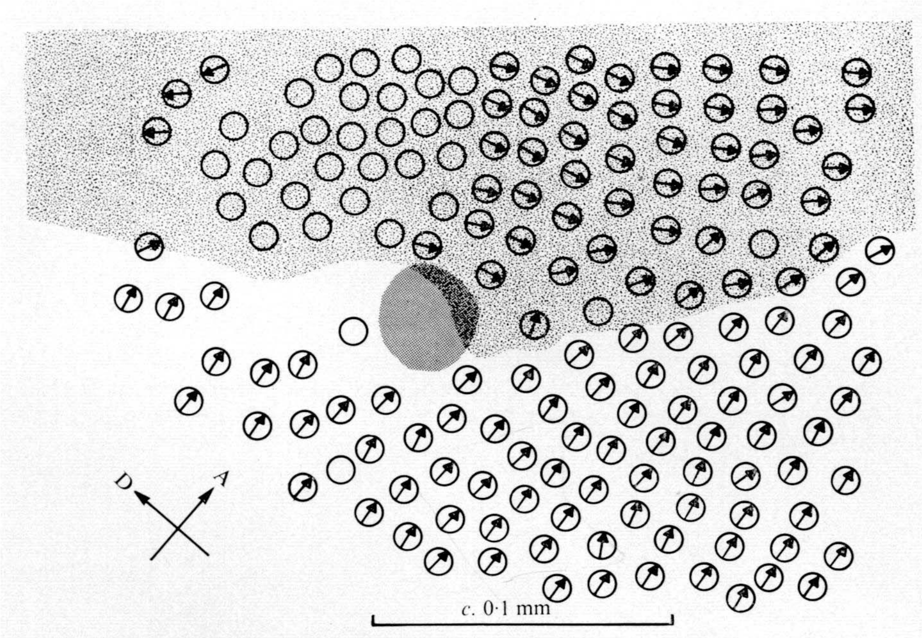

Fig. 10. Orientation of ommatidia after rotation of graft 180°, the arrow indicates orientation of each ommatidium (see Fig. 1), ommatidia without arrows are defective: light shading, wild-type graft; dark shading, damaged area.

We know almost nothing about the 'organizational front', but present experiments show that the polarity of the host epidermal cells can be partially conserved as they are recruited into eye. Therefore, the organization of the head epidermis is important in determining the polarity of retinal structures, but much also depends upon the advancing front. For instance, in a cockroach, Hyde (1972) claims that prothoracic cells can become eye if grafted near to a growing anterior margin. In *Oncopeltus*, while no success was achieved with thoracic cells, apparently normal ommatidia can be formed by cells from the

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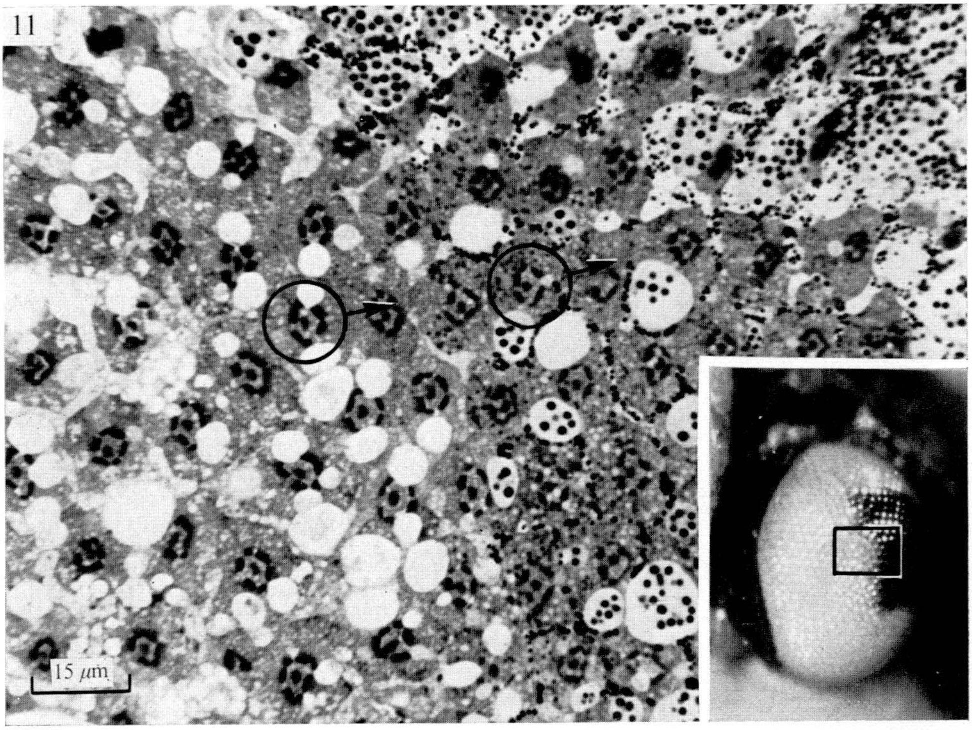
Figs. 11–16. The insets show as accurately as possible the region and the orientation of the eye from which the section was cut. In all insets anterior is to the right and dorsal is to the top.

FIGURES 11 AND 12

Fig. 11. A section through a mosaic eye at the level of the rhabdomeres. The mosaic is the result of a left/right graft which inverted the dorso-ventral axis. The significant finding is that after this class of operation the orientation of ommatidia is the same in both host and graft.
Fig. 12. This plate shows part of the graft/host border after a 90° clockwise rotation of presumptive eye epidermis. Orientation of ommatidia is significantly different on either side of the graft border. There is a clockwise rotation of ommatidia in the graft.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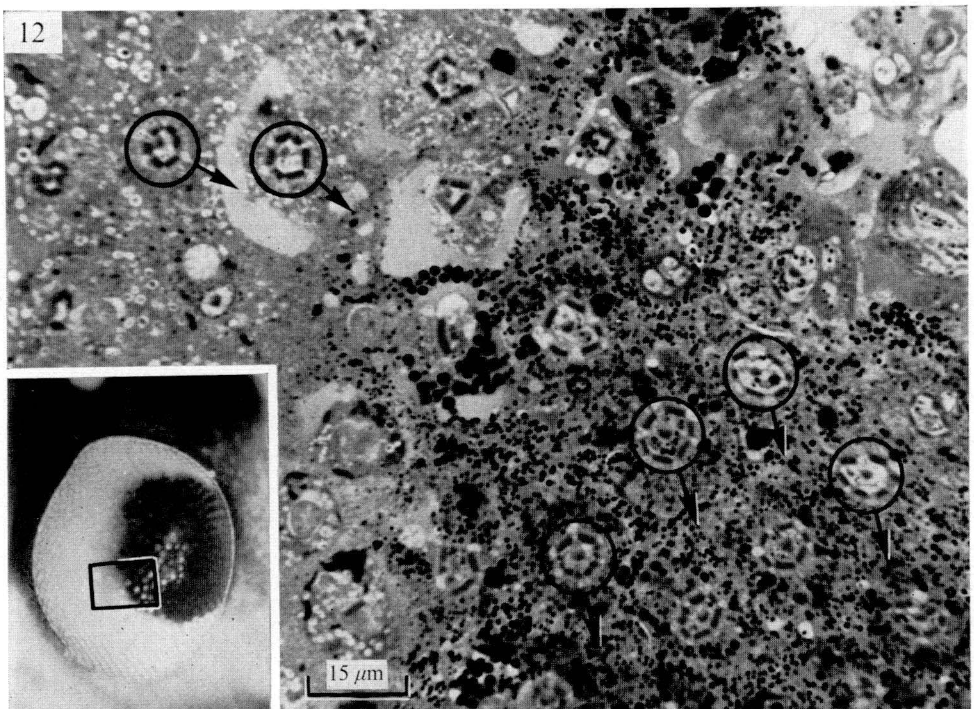
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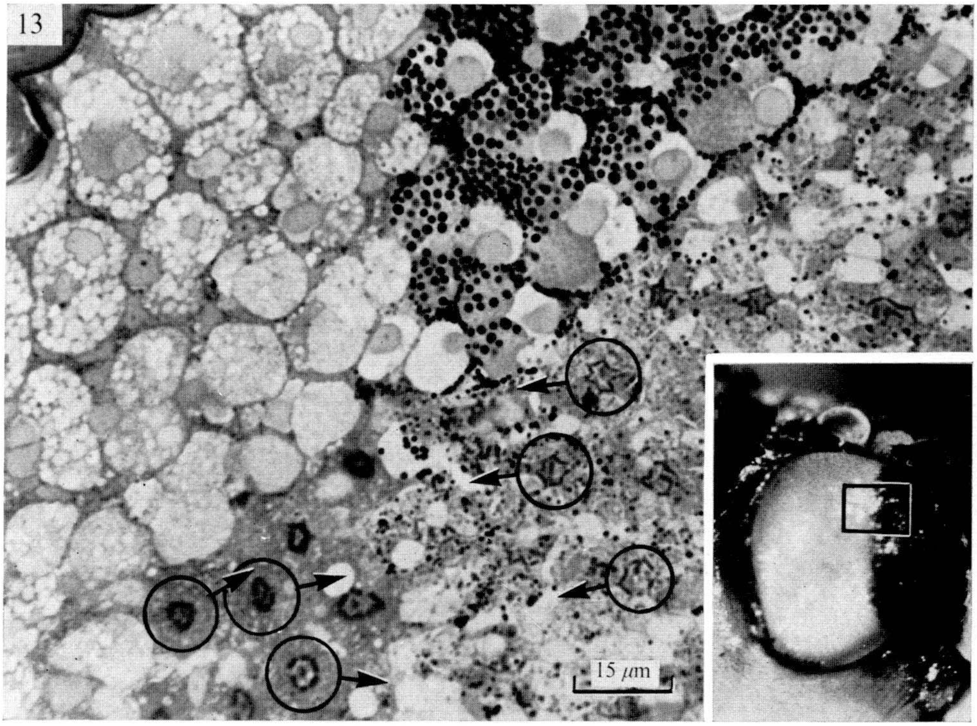
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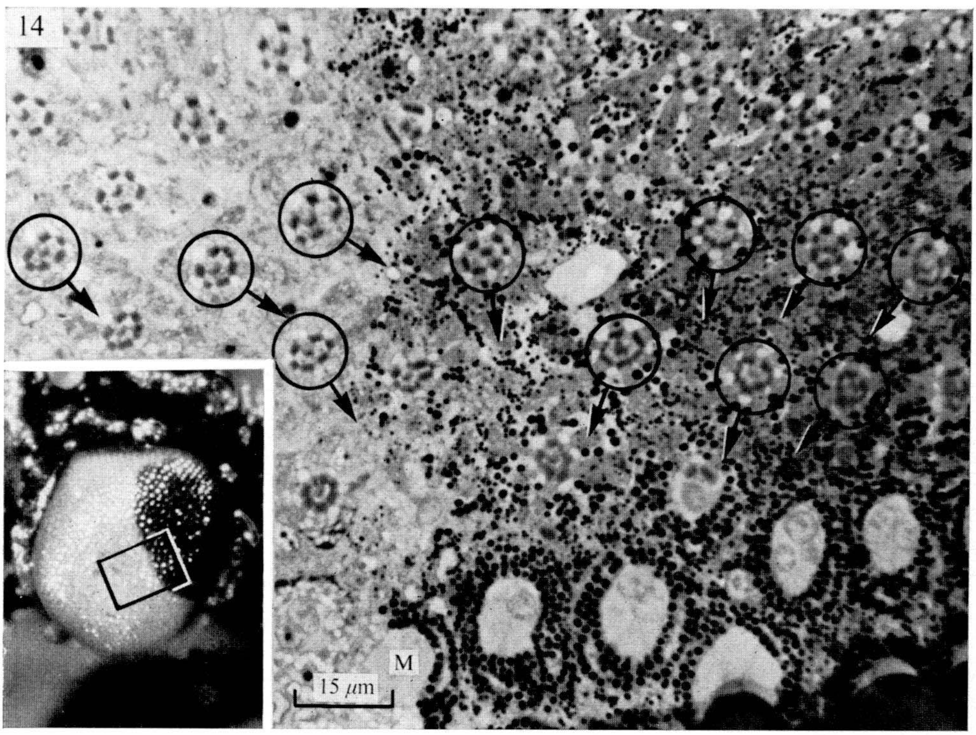

14
M
15 μm

adult head epidermis which would not have made eye *in situ* (Green & Lawrence, 1975). These experiments show that the presumptive eye territory is not determined, and that the process is one of recruitment of uncommitted cells by the advancing eye margin.

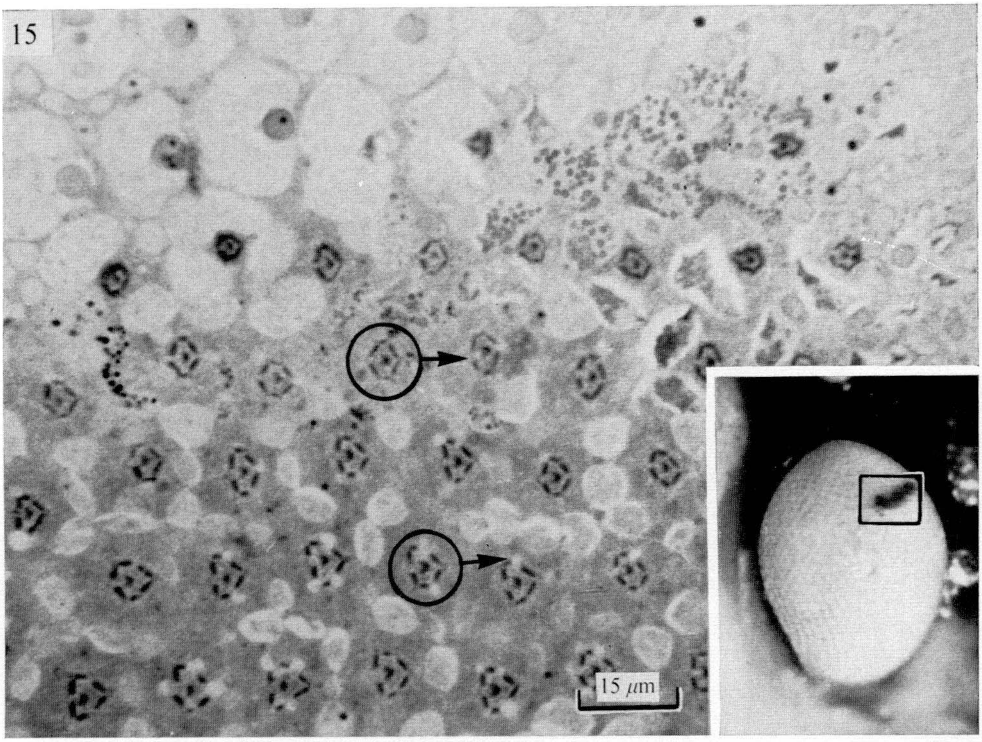
Similar situations have been reported in other systems. An organizational front spreads out bilaterally from the midline in chick dermis, and is responsible for establishing the spaced feather primordia, apparently by local aggregation of dermal cells (Sengel, 1974). In certain experimental situations recruitment can occur in the insect abdomen; for example, if genetically marked cells from the segment margin are grafted to the middle of the segment of *Galleria*, they will transform some of the host cells into margin cells (Marcus, 1963; Lawrence, 1973). The eye margin may therefore share some properties with the abdominal segment margin, and in other respects our rotation experiments in the eye can be compared with similar experiments on the abdomen: Those experiments in which pieces of epidermis from near to the left eye were placed near to the right eye (only inverting the dorso-ventral axis) gave results indistinguishable from controls. For the criteria we have used the dorso-ventral axis is therefore immaterial. Likewise in the abdominal epidermis only one axis (the antero-posterior) is important in the control of orientation of integumental structures (Lawrence, 1973). As in the eye, unrotated grafts exchanged between left and right side (without displacement in the antero-posterior axis) give the same result as simply removing a piece of epidermis and replacing it in the same site. What happens to the connexions between the ommatidia and the lamina where the position on the dorso-ventral axis is critical, is an open and interesting question. In the eye, as in the abdomen, small grafts lose their original polarity and become incorporated into the host pattern.

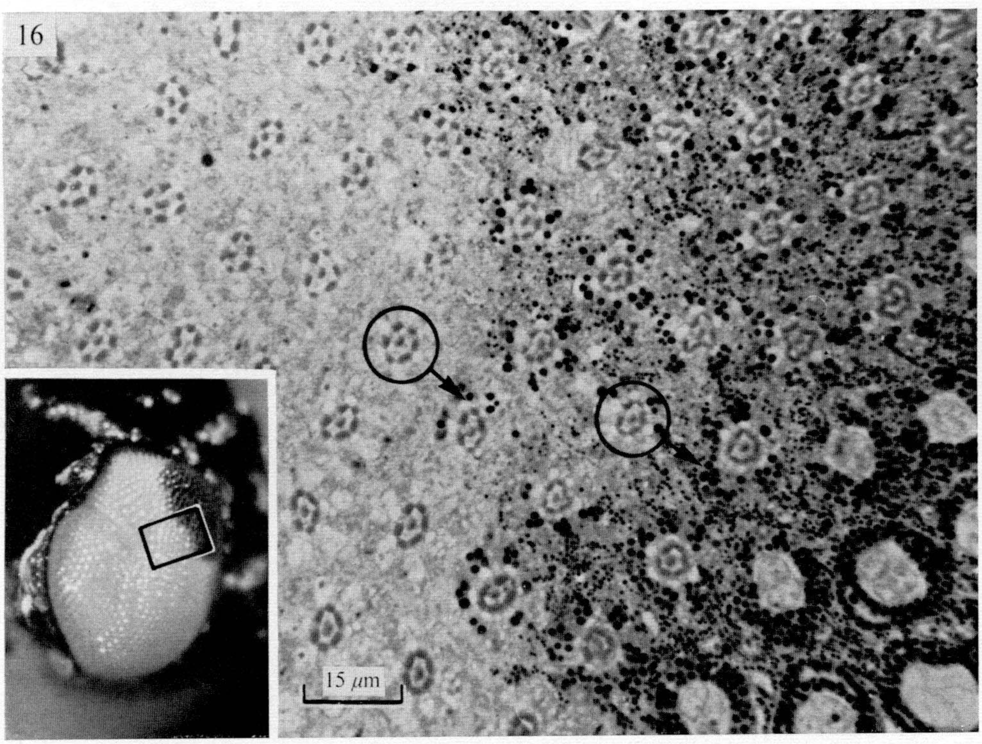
For large grafts rotated through 90° or 180° the patterns of ommatidia can be compared with some difficulty to those formed by abdominal scales of moths, or ripples of *Rhodnius*, after similar operations (for references see Lawrence, 1973). The eye results are complicated by damage near the host-graft border and disorganization associated with the towers of ommatidia that frequently form. The possibility of graft rotation must be also remembered (Bohn, 1974;

FIGURES 13 AND 14

Fig. 13. This section shows opposed orientation of ommatidia after a 180° rotation of the donor epidermis.

Fig. 14. This plate shows the sort of interaction usually found at the graft/host border after rotation experiments. Here the graft had been rotated by 180° but in this field the final orientation of ommatidia in the graft deviates from the host by considerably less. In addition, the orientation of ommatidia at the border is intermediate between the extremes found in host and graft. The section also shows an ommatidium which is mosaic for primary pigment cells (*M*): the primary pigment cell to the left is of the mutant genotype and the right-hand one is wild type. The junction between them normally defines the antero-posterior axis.





Figs. 15 and 16. In small grafts the original orientation of the donor tissue is lost. In both of these cases the graft was rotated by 180° but the orientation of ommatidia is normal.

Lawrence, 1974; Nübler-Jung, 1974). Nevertheless, the most important feature is interaction between fields of differently orientated ommatidia which leads to intermediate orientation at the border between them. These observations, true of both abdomen and eye, emphasize the supracellular nature of those influences which orientate developing structures. In the abdomen the segmental gradient shares properties with a concentration gradient of a diffusible substance, the local steepest slope giving the polarity (Lawrence, 1966*a*). This polarity can be expressed intracellularly as for example the orientated assembly of microtubules in bristle outgrowth (Lawrence, 1966*b*) or, at a multicellular level, as the progressive arrangement of the three component cells of a hair into an oriented line (Lawrence, 1966*b*). A similar arrangement of cells by small movements in response to a supracellular gradient could give the developing ommatidium its polarity – although in that case, unlike the hair, the component cells are not related by cell lineage. Because, for technical reasons, we have been unable to transpose small grafts up and down the antero-posterior axis, we cannot claim that these results provide more evidence for gradients of positional information. Nevertheless, it is possible that a gradient, similar to that found in the insect abdomen, controls the polarity of the ommatidia and provides the basis for specifying the connexions that retinula cells make with the underlying lamina.

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