# GRADIENTS IN THE INSECT SEGMENT: THE ORIENTATION OF HAIRS IN THE MILKWEED BUG ONCOPELTUS FASCIATUS

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### INTRODUCTION

In 1940 Wigglesworth observed that, in *Rhodnius*, the bristles were aligned in the anteroposterior axis. He noted that when a square of integument was rotated through  $180^{\circ}$  and replaced, the orientation of the bristles which later were formed by the tissue was also rotated through 180 degrees. Later (1959*a*) he commented that these observations might indicate a 'cytoskeleton' in the epidermal cell which 'defines its anteroposterior axis' (p. 40).

Further information on this 'cytoskeleton' has come from the studies of Piepho and his school on the scales of *Galleria*. Piepho & Marcus (1957) noticed that, as the scales develop, the scale and socket cells become progressively oriented one to another until at the time of scale outgrowth the cells are aligned to within 30 degrees of the anteroposterior axis. However, the direction of outgrowth of the scale itself lay closer to this axis, showing a deviation of only a few degrees; it was concluded that an orienting influence acted in the first place on the formative cells of the scales, and later on the outgrowth itself. In experiments similar to Wigglesworth's, Piepho (1955*a*) rotated areas of integument in the last larval stage of *Galleria*, and found that although the central area of the graft bore scales which had the expected reversal of polarity, the scales in the vicinity of the junction between host and graft were intermediately oriented. Piepho concluded that these scales were subject to two opposing orienting influences ('*richtender Faktoren*') and that accordingly they assumed an orientation which related to the resultant of those two forces.

The surface of the abdominal segment of adult *Rhodnius* is covered with cuticular ripples which run laterally. Locke (1959) investigated the factors controlling the orientation of these ripples and found that operations in which pieces of integument were excised and then replaced in the normal orientation caused no alteration in the adult pattern. On the other hand, removal of pieces of integument of the 5th-stage larva and their replacement in the inverse orientation resulted in a striking pattern of whorled ripples in the adult. When areas of integument within one segment were transplanted to a region more anterior or posterior than their sites of origin, similar disturbances of the adult pattern resulted. However, when transplants were made between equivalent sites in two adjacent segments, they caused no alteration in the adult pattern. Experiments, in which areas of integument which were laterally separated but similarly situated on the anteroposterior axis of a segment were inter-

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changed, also had no effect on the adult pattern. A careful analysis of these and other results led Locke to the conclusion that, since only pieces of integument had been transplanted, there was a gradient of some factor which varied in the epidermal cells themselves, was responsible for the orientation of the ripples, and was repeated in each segment. He further suggested that cells which differed in position in the gradient were incompatible in some way, and, when such cells were placed together by experiment, the discontinuity between them was reflected in the altered orientation of the cuticular ripples which joined up with ripples from the same level in the axis or with themselves to form a 'discontinuity pattern'. Locke (1959) saw the 'discontinuity pattern' as 'the result of the capacity of the epithelium for uniting cells of the same level in the axis' (p. 471).

Locke hinted that there might be a common basis for his and Piepho's results, and the studies on *Oncopeltus* which follow allow for a more detailed examination of this suggestion.

### HAIR DEVELOPMENT IN ONCOPELTUS

Although the larvae possesses only sparse bristles, adult Oncopeltus are covered with a dense mat of non-innervated hairs. These hairs develop from a mother cell, which undergoes a particular series of differentiative divisions, as in the formation of the hairs of Limnophilus (Rönsch, 1954) or the scales of Lepidoptera (Stossberg, 1938). A cytological account of the development of these hairs is in preparation (Lawrence, 1966), but we may note here that during development there is a progressive orientation of the hair-forming cells, which become aligned one to another and to the anteroposterior axis (Text-fig. 1). When the hair grows out it is aligned even more precisely to this axis. This progressive development of a preferred orientation is here understood as an indication that both the formative cells and the outgrowing hairs are aligning to some unseen influence in the epidermis. The hairs can thus be regarded as polarity indicators in so far as they reveal some orientation which exists in their neighbouring epidermal cells. Like the scales of Galleria, the hairs can be used to study the 'cytoskeleton' of the epidermal cells.

### EXCISION OF THE INTERSEGMENTAL MEMBRANE

Locke's theory implies that an intersegmental membrane interposes between the highest and lowest portions of two adjacent segmental gradients (Text-fig. 2), and would predict that the removal of a piece of intersegmental membrane would result in considerable disturbances in the ripples. This prediction has been confirmed by experiment (Locke, 1960), and leads to the hypothesis that a similar operation would affect the orientation of the adult hairs in *Oncopeltus*. This experiment proved difficult on account of the extraordinary powers of regeneration possessed by the intersegmental membrane. Excision of even as much as 1 mm. of the membrane is soon followed by its reconstitution across the gap and only temporary changes in the orientation of the bristles occur. Shortly after extirpation of a length of intersegmental membrane, a stream of large, elongated cells migrates out from each cut end to regenerate the membrane. It would seem that the need for continuity, a property present in all epidermal cells (Wigglesworth, 1937), is manifest to a much greater extent in the cells of the intersegmental membrane. Usually, therefore, when such an experiment was performed on 3rd-stage larvae, in the adults there was a continuous intersegmental membrane and only slight abnormalities in hair orientation. However, in some 5thstage larvae, nearby to the intersegmental discontinuity, there were bristles whose



Text-fig. 1. The progressive development of a preferred orientation during the ontogeny of the hairs. A shows the orientation of the first differentiative divisions (cf. Lawrence, 1966). B illustrates two stages; one earlier (hollow circles) and one later (full circles) during the orientation of the hair-forming cells.



Text-fig. 2. Diagram to represent the normal situation of a gradient which is repeated in each segment (after Locke, 1960).

orientation was completely inverted. In one experiment only, in which sternal tissue had been transplanted, in the normal orientation, on to the hairless tergites, the intersegmental membrane failed to regenerate completely, and the small area of grafted tissue showed hairs in the inverse orientation. In this case its situation between two adjacent segments had caused a total inversion of the polarity of the transplant.

#### A NATURAL EXPERIMENT

Although insects showing a discontinuity in the intersegmental membrane have been difficult to create by experiment, such insects do occur at low frequency in the culture. These individuals lack the central portion of one intersegmental membrane, so that the general integumental surface of the sternites is continuous from one segment to the next in this region. Usually this malformation involves abdominal sternites 2 and 3, but occasionally it is found to affect the intersegmental membrane between 3 and 4 and rarely that of 1 and 2. Insects which are malformed in this way occur at



Text-fig. 3. A diagram drawn with the camera lucida from a natural defect similar to the instance illustrated in Pl. 1 A.

the frequency of about 1 in 400 of the cultured population. In the region of the intersegmental discontinuity there is an extraordinary change in the orientations of the hairs in both neighbouring sternites (Pl. 1A, Text-fig. 3). The pattern formed is so neat that the orientation of each hair must be the result of some very precise influences. The following model has been helpful to a consideration of these influences.

#### THE SAND MODEL

If a model of sand is constructed such as to represent the segmentally repeating gradient demonstrated by Locke in *Rhodnius* (Pl. 1B) and the glass plates, which represent the intersegmental membrane, opened to form a breach, sand flows through from the 'posterior' segment to the 'anterior' until it takes up a disposition pictured in Pls. 1 C and 2 A. Through the central portion of the breach the sand gradient is now aligned in the inverse direction, and more peripherally, in both segments, gradients intermediate in orientation are found. The declivity of this inversed gradient is limited by the settling properties of the sand. If lines are drawn (perpendicular to the contours)

to mark the direction of the steepest gradients, the pattern achieved is exactly that found in the aberrant insects (cf. Text-fig. 3 with Pl. 2 A). The new condition of the sand model is quite stable because the force of gravity is opposed by the friction between the sand grains.

Apart from more complex electrical models, the sand model is the only way such a pattern can be produced easily; it would indeed seem to describe the condition in the aberrant insect. We have, then, to imagine a system where the polarity of the cells depends on, or is, the direction of slope of a gradient. This gradient, however, has a maximum stable slope and if cells of two widely different levels are apposed, 'flow' occurs as the very stuff of the gradient moves from the higher cell to the lower cell until the maximum stable slope is produced. This 'flow' alters not only the gradient levels of each cell affected by it but also, thereby, the direction of the gradient and consequently the polarity of the cells themselves.

As the new pattern is relatively stable we must assume that, in some way, the new direction of the gradient is *maintained by the cells themselves* (see p. 616).

The precise agreement between the sand model, and the orientation of the hairs in the aberrant insects, leads to the hypothesis that the model will also be a convenient means of describing and analysing the results of Piepho and Locke.

#### COMPARISON WITH THE WORK OF PIEPHO (1955a)

Piepho removed a piece of the integument of *Galleria* and rotated it through 180 degrees before grafting it back on to the larva. Piepho's diagram of an adult after such an experiment is reproduced in Text-fig. 4. Text-fig. 5 examines the situation in a localized area as predicted by the sand-model principle. A shows the state immediately after the rotation on one side of the host-graft junction and B the situation after 'flow'. No 'flow' will occur in the middle of the edge of the transplant (level 6) and most



Text-fig. 4. Reproduction of the diagram by Piepho (1955*a*) showing the result of his rotation experiment on *Galleria*. The arrows mark the orientation of scales in the adult.

'flow' will occur distally from this centre (11 and 1). The entire pattern which is predicted by the application of such a system to the whole area of the experiment (Text-fig. 6) is exactly that obtained by Piepho. As Piepho himself remarked, the pattern is the same as the lines of force which result when a small electrical field is placed in a large, inversely oriented field. Barbier (1965) has also rotated pieces of



Text-fig. 5. Diagram to illustrate the application of the sand-model principle to Piepho's rotation experiment. A. Before 'flow'. B. After 'flow'. Horizontal arrows cover the area affected by 'flow'.



Text-fig. 6. Pattern derived from application of the sand-model principle to Piepho's rotation experiment.

cuticle of *Galleria* larvae, and reports that the scales of the adult have the expected inverse orientation but does not mention whether the scales near the host-graft junction are intermediately oriented.

Attempts to repeat Piepho's experiment on Oncopeltus have succeeded only partially, but exactly the same disposition of hairs that Piepho achieved with scales has been found in an adult Oncopeltus, on one side of the transplanted integument after an

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operation performed on a 3rd-stage larva. The situation on the other side of the graft was confused by migration of the intersegmental membrane. Nevertheless, the pattern was so precisely reproduced on the one side, that there can be no doubt that similar orienting influences control the scales of *Galleria* and the hairs of *Oncopeltus*.

### COMPARISON WITH THE RESULTS OF LOCKE (1959, 1960)

Locke has clearly shown that there is a gradient within the segment responsible for the ripple pattern of adult *Rhodnius*. The experimental juxtaposition of cells of different levels in the gradient results in considerable deformations of the ripple pattern.

The ripples of *Rhodnius* are cuticular structures whose width is more than two cells; each cell does not therefore produce any particular part of the ripple. The ripple pattern could be formed as a result of mechanical stress in the double-layered cuticle. Locke's experiments show, however, that the orientation of ripples does depend on the cells themselves, even though the form of each ripple would seem to result from the mechanical properties of the cuticle. He was inclined to regard the ripples as indicating a continuity in the cells which underly each ripple, and suggested then that observed deviations of ripples might even reflect the migration of the underlying cells. The hypothesis of cell migration has now been abandoned (Locke, personal communication) and a close study of the ripples themselves leads to a different hypothesis. The ripples are not autonomous structures and do not extend indefinitely without interruptions; rather they are simply oriented folding of the epicuticle. As it is clear that this orientation is under the influence of the epidermal cells, we may adopt the hypothesis that the ripples are polarity indicators. Seen from this viewpoint, a circular whorl of ripples implies that the polarity of each of the underlying cells is aligned in relation to that circle. If the ripples of Rhodnius indicate polarity in the same way as do the hairs of Oncopeltus, their orientation after any transplantation experiment should be predictable by the sand model.

As the ripples in *Rhodnius* run transversely, they should be aligned parallel to the contours of the sand model, that is perpendicular to the lines of steepest gradient. Accordingly we should be able to predict the pattern resulting from a rotation of a piece of integument through 180 degrees by simply drawing contour lines in the hair-orientation diagram (Text-fig. 6; dotted lines). The result is indeed that found by Locke; a double system of whorls with deflected ripples anterior and posterior. By means of the sand model we are now in a position to predict not only whether there will be a disturbance in the pattern produced by a particular transplantation experiment, but also the nature of the resultant pattern itself.

We may consider one other such experiment by way of example. Locke interchanged anterior and posterior pieces of integument in one segment of the 5th-stage larva (Text-fig. 7a and b). This operation resulted in asymmetric 'discontinuity patterns' in the adult. Greater disturbance was noted on the intersegmental sides of the grafted areas than on the central sides. When the sand-model system is applied to this experiment, we find it predicts the observed result (without recourse to an additional hypothesis) as a consequence of the property of flow. Consider, first, the sides of the transplanted squares nearest the intersegmental membrane; here, although the initial difference is 5 units, as soon as the sand begins to flow the difference becomes

effectively greater; the result being that the area affected by flow, and thereby the area in which the gradient values change to produce deflected ripples, is large. On the other hand, at the sides of the squares of the transplanted integument nearest the centre, initial sand flow effectively *reduces* the original difference of 5 units and the flow is damped. The result is that the area affected by flow and the region bearing deflected ripples is small. (Text-fig. 8).



Text-fig. 7. Locke's experiment. A. Anterior square (a) and a posterior square (b) interchanged. B. The resulting pattern in the adult (after Locke, 1959).



Text-fig. 8. A diagram to show the situation prior to 'flow' after Locke's experiment. The large arrows demarcate the transplants, and the small the expected directions of 'flow'.

#### FURTHER PROPERTIES OF THE ORIENTING PROCESS

(i) Wounding. In a study of the regeneration of sensilla after wounding in *Rhodnius*, Wigglesworth (1940) noted that those bristles which were central in the burnt area sometimes had an irregular orientation. This observation has been confirmed in *Oncopeltus*. Very severe burns in the 3rd-stage larva still result in a bald patch which is located in the middle of the damaged area in the adult. In the immediate vicinity of the bald patch the hairs have an irregular orientation, while more peripheral regions

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bear hairs with the normal alignment. Observations on the 5th-stage larva reveal that this orientation spreads centripetally, for, in this earlier instar, unoriented bristles occur more peripherally around a larger bald patch. Wigglesworth (1959*a*) thought that migrating cells retained their polarities and was more inclined to regard the lack of preferred orientation in the central bristles as 'the result of the rotation or disorientation of epidermal cells in the confused zone where two epidermal sheets are re-establishing continuity' (p. 40). However, since observations suggest that cellular migration ceases prior to the first ecdysis after wounding, and bristle formation does not occur until the second moult, this interpretation would now seem to be of more doubtful validity. Indeed, observations on the migrating cells reveal them to be extending pseudopodia and migrating in an amoeboid fashion across the area of dead cells; it seems likely that they soon lose any polarity relative to the axis of the animal. This interpretation is supported by some recent observations of Locke (1966) who has evidence that cells retain their polarity only temporarily when they migrate into a burnt area. When a burn is made just before the deposition of the epicuticle, the pattern which results shows the normally transverse ripples deflected to point to the centre of the burnt region. Burns made slightly earlier have no effect on the adult pattern as, in this case, the polarity of the cells has had time to be redetermined prior to deposition of the epicuticle.

We may thus regard the lack of orientation in the central bristles of *Oncopeltus* as indicating a lack of polarity in the underlying cells, a polarity which therefore spreads centripetally during the succeeding moults after a severe burn.

This observation supports the generalisation that wounded cells suffer a temporary loss of certain characters, such as the power to form cuticle of a particular kind, to melanise this cuticle, or to differentiate hairs or bristles. In this case, unlike the others, the recovery after wounding is regulative: the polarity is affected by that of the neighbouring cells.

(ii) The intersegmental membranes. Piepho's experiments (1955b) revealed that the intersegmental membrane played some active part in the polarity of the segment. Whenever pieces of the anterior or the posterior fringe of the intersegmental membrane were implanted in fully grown caterpillars, the nearby scales in the adult oriented away from them or towards them, respectively. These observations have been confirmed on Oncopeltus. It might, on these observations alone, be tempting to suggest that the intersegmental membranes are responsible for maintaining the gradient. However, Locke's experiments indicate that cells in the centre of a reversed piece can retain an orientation inappropriate to the position of the intersegmental membranes over many moults.

The function of the intersegmental membranes in the system is still unclear and we are still in the same position as Piepho (1955b) who pointed out that, although his experiments indicated that the scales might be oriented exclusively by the intersegmental membranes, it was quite possible that the orienting factors were also present in the main area of the segment.

(iii) Growth. A further important property of the system is that it is self-amplifying; the area of the segment increases many times during post-embryonic development. The mitotic spindles of the cell divisions in the epidermis show no special orientation, and yet the daughter cells develop the appropriate polarity.

(iv) Timing. In the sand model, when cells of two different gradient values are apposed, there are two counteracting influences which determine the final position after flow. One is the force of gravity, and the other is the friction between the sand grains. In the model, although the whole process of flow occurs rapidly, we may note that the sand will fall faster immediately after the opening of the glass plates, and will move more slowly as an 'equilibrium' is approached. There are several indications that the dynamics of the system in the insect are similar, in that an equilibrium is being attained. Locke noted that the ripple pattern in the adult Rhodnius was more circular the earlier the larval stage on which he had performed the rotation experiment. From the viewpoint adopted here, this means that 'flow' extends gradually farther and farther from the host-graft junction. This would suggest that the attainment of an equilibrium is a very slow process, which may take several moults. The same sort of observation has been made on Oncopeltus. When pieces of integument were transplanted in some unusual orientation in the 3rd-stage larva, the area in which there was an alteration in the orientation of the bristles of the host and graft near the junction increased in each moult. It is not known whether the process of 'flow' continues with time or whether it can only progress during a particular phase in each moult cycle.

### THE SAND MODEL: A CHEMICAL SYSTEM?

It is tempting but difficult to adapt the sand model to a chemical system which has all the observed properties. If the gradient is to be of some substance (and there is still no evidence that this is so), then the actual equivalent of the force of gravity is likely to be diffusion. Of more critical importance, however, is the equivalent of the friction between the sand grains, which limits the extent of this diffusion, so that an equilibrium is reached. During the post-embryonic development of the three experimental insects so far studied, local inverted gradients can persist over several moults. Yet the recovery of polarity in a larger wounded area may be completed in two moults. This maintenance of a gradient by the cells themselves suggests that they must hold the local gradient at a maximum stable slope by generating some resistance to the diffusion of the substance—a force equivalent to the friction between the sand grains. This resistance could result from the active transport of the substance in *the opposite* direction to the gradient. The evidence at hand is not adequate to determine whether such active transport is a necessary hypothesis. However, the suggestions that the system is not only self-amplifying, but that a gradient is reconstituted in wounded cells leads me to favour the concept that the cells themselves produce the substance and that this is actively pumped against the concentration gradient. If this speculation proves acceptable, the direction of pumpingwould be an active property of the cellsa polarity which is determined by the concentration gradient, and which also maintains that concentration gradient. We would thus have the curious situation in which the gradient and the polarity of the cells were interdependent; if the gradient were changed, this would affect the polarity, which in turn would maintain the gradient in its new orientation. In order to respond to the direction of the gradient the cells would have to be sensitive to differences of concentration across their own boundaries. This would seem to be a rigorous requirement, but that cells can perceive a very small gradient (in this case, oxygen tension) can be deduced from the observations of Wigglesworth

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(1959b) on tracheolar migration in *Rhodnius*. Wigglesworth showed that the epidermal cells respond to oxygen deficiency by sending out filaments of cytoplasm which attach themselves to the tracheole and then contract. Wigglesworth's drawings indicate that these filaments grow out only in the direction of the nearest tracheole.

Stumpf (1965 a, b; 1966) has recently and independently developed a theory which is basically similar to that propounded here. She considers the gradient to be a concentration gradient of a diffusible substance, and she does not, in these current publications, examine the maintenance or origin of this gradient. The model of the diffusible substance is perhaps not as complete as the sand model since it fails to emphasize the 'force' which limits the extent of the diffusion, and therefore omits to consider the part played by the epidermal cells themselves, or to provide a ready explanation for the observed maintenance of local, inverted gradients.

#### THE GRADIENT: WIDER IMPLICATIONS

In individuals which show an interruption in the intersegmental membrane a pigment spot is found in the central area where the hair orientation is reversed. This effect on the pattern of the insect suggests that the gradients may have a more fundamental function than orienting the hairs, scales, and ripples. Wigglesworth noted (1940) that transplanted tissues of *Rhodnius* always developed according to their prospective fates when the host underwent metamorphosis. These observations were confirmed in Ephestia by Yosii (1944), but Marcus (1962) made the striking observation on Galleria that in certain circumstances transplanted cells would produce cuticle and scale types different from their prospective fates. Marcus transplanted small areas of integument from place to place within the segment and found that while the central area of the graft usually developed, after metamorphosis, into the expected integumental type, peripheral cells of the transplanted tissue and neighbouring cells of the host tissue could produce different integumental types. Marcus described a 'new segment axis' in the integument which was arranged radially from the centre of the graft, and along this axis cuticular types followed each other in the same sequence as found in the organism.

We may observe that, with the additional hypothesis that the level of a cell within the gradient determines the type of cuticle it will produce after metamorphosis, these curious results of Marcus are in accord with the sand-model principle in the following way: transplanting an anterior piece of integument (say level 9) into the median region (say 5) will produce, after 'flow,' cells of host and graft of positions 6, 7 and 8; and these cells 'redetermined' by their new gradient levels will produce integumental characters equivalent to 6, 7 and 8. Although Marcus' results suggested to the author (Lawrence, 1965) that a particular level of the gradient might determine the pattern, there was no other experimental evidence for the hypothesis. Quite independently Stumpf (1965 c) has discovered that in *Galleria* the absolute 'level' in the gradient may be associated with a particular structure in the surface of the segment. She found that if this level is experimentally produced elsewhere in the segment, the appropriate structure is formed in that place.

Marcus noted that the area in which scale orientations were altered was larger than that in which new integumental characters were formed. The scale orientation was related only approximately to the 'new segment axis'. This observation can be seen to be in accord with the sand-model principle, if the timing of determination of these integumental characters is such that the event occurs before completion of 'flow'; we have already noted that the latter seems to be a slow process. As 'flow' continues the 'topography' of the area changes and the scales orient to gradients which are then differently aligned. This hypothesis could be tested on *Galleria* by performing the operation on an earlier larval stage; in this case the areas of altered scale orientation and new cuticular types should be more co-extensive, and the orientation of scales should be nearer to the 'new segment axis'.

Marcus compared his results with the double gradient system envisaged by Hörstadius (1939) which determined the fate of the cells in the embryo of a sea urchin and pointed out how in the insect, unlike the echinoid, there was already a 'labile determination' which interacted with the 'labile determination' of the neighbouring cells. His concept of a 'labile determination' is precisely in accord with the sand-model system.

Marcus' results also bear comparison with the work of Yamada (1940) on the developing mesoderm of the newt. Yamada transplanted pieces of presumptive notochord into the presumptive blood island, and found that the two tissues interacted in such a way that pronephros, a tissue normally present between the notochord and the blood island, was formed. Dalcq & Pasteels (1938) pointed out how one could regard these results as indicating a diffusion gradient, 'Il sa'git vraisemblablement d'une diffusion et la stabilisation se fait sur une valeur moyenne, qui est celle de pronephros' (p. 294).

There is also an indication that the gradient system may be responsible for the integrity of the segment itself. Another kind of natural defect has been found in the culture in which the central area of the 3rd sternite has 'disappeared' (Pl. 2C). The tergites and lateral margins of the segments are perfectly normal. The location of this defect suggests that it is the same kind as the other natural defects we have been considering. In this case we can imagine that the interruption of the intersegmental membrane was so large, or so early, as to allow the gradient system of segment 2 to engulf that of segment 3 completely. The two adjacent gradient systems would become as one in this region. These comments on the observed defect are pure guesswork but if they were correct these gradient phenomena, which have expression in the postembryonic insect, may also underly its embryonic development. Indeed, Spiegelman (1945) has demonstrated that gradients and fields are part of the same concept, and one can thus imagine the intersegmental membrane as normally separating two self-regulating fields.

In the insect, with its hairs or scales that indicate the direction of local slopes in the hypothetical gradient, we may have an experimental system that will allow a new understanding of these fundamental features of development which have intrigued embryologists since the classical paper of Boveri (1901).

#### SUMMARY

1. During metamorphosis in *Oncopeltus* the hair-forming cells orient to some unseen influence in the epidermis. The hairs can thus be used as indicators of the polarity of this influence.

2. Excision of the intersegmental membrane can cause a total inversion of the direction of hair outgrowth in the nearby region.

3. Individual insects, each with a large gap in the intersegmental membrane and an associated pattern of hairs which are unusually oriented, occur occasionally in the culture.

4. A study of these defects, and of Locke's observations on the segmental gradient of Rhodnius, leads to the development of a sand model which can be applied not only to the experiments of Locke on the ripple pattern of Rhodnius, but also to those of Piepho on the orientation of scales in Galleria.

5. The sand model helps in a description of the hypothetical chemical basis of the gradient in the insect segment.

6. The relationship between the gradient and pattern is briefly discussed.

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#### EXPLANATION OF PLATES

#### PLATE I

A. The natural defect. This picture shows two adjacent sternites and the intervening intersegmental membrane. The intersegmental membrane (i.m.) is interrupted in the centre of the picture and associated with this there is an area of hairs whose orientation is inverted. The picture was taken under phase contrast and then printed in the negative.

B. The sand model 1. This side view of the sand model shows the two sand gradients in position and the glass plates (g.) close together.

C. The sand model 2. Sand has now flowed through the gap created by opening the glass plates, setting up a new gradient system in the inverse orientation.

#### PLATE 2

2A. The sand model 3. The same situation as in Pl. 1C, but the picture is taken from above the model. The direction of steepest gradients are indicated by arrows. This photograph should be compared with Text-fig. 3.

2B. The ventral surface of the normal female abdomen.

**2C.** The aberrant individual with loss of the central part of the 3rd sternite. The lateral margins are normal. Notice the large interruption in the intersegmental membrane between sternites 2 and 3.

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