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Phil. Trans. R. Soc. Lond. B 1981 **295**, 595-599
doi: 10.1098/rstb.1981.0162

Phil. Trans. R. Soc. Lond. B **295**, 595–599 (1981)
Printed in Great Britain

The regeneration of segment boundaries

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The segment borders in the abdomen of *Oncopeltus* (Hemiptera) are both elements in the integumental pattern and compartment boundaries. When regions of the cuticle and epidermis are extirpated, and the free edges joined together, intercalation occurs. This intercalation always takes the shortest available route along the 'segmental wavelength', and can result in the formation of an adventitious segment boundary.

Introduction

One traditional approach to the vexing problem of pattern formation is to study the regeneration of parts of animals. In insects, the limbs and the abdominal epidermis are convenient material, and the pattern of regeneration is partly understood. Less is known about the cell lineage of the process. For example, in insect legs there are lineage restrictions that operate during normal development to subdivide the limb into two precisely defined areas or *compartments* (Garcia-Bellido *et al.* 1973; Steiner 1976; Lawrence *et al.* 1979). Although rules governing the regeneration of replacement or supernumerary legs have been described (Bohn 1965, 1970, 1971; French *et al.* 1976), the detailed fate of the original compartments has not; however, this is now under study (French, this symposium).

We also know more about pattern and polarity in the abdominal epidermis than we do about cell lineage (reviewed in Lawrence 1973*a*). Each segment of the epidermis is a compartment (Lawrence 1973*b*) and here we describe some extirpation experiments where we pay attention to the pattern of regeneration, to cell lineage, and to the compartment boundaries. One experiment leads to the regeneration of a compartment border after extirpation, another to elision of a segment, and another to the formation of an adventitious compartment boundary. The experiments are described in detail elsewhere (Wright 1979; Wright & Lawrence 1981 *a*, *b*).

Our experimental material is the epidermis of the hemipteran *Oncopeltus*. A clonal analysis has shown that the epidermis is subdivided into segmental compartments (Lawrence 1971, 1973*b*, 1981). The compartment, or segment, boundary is at a precise place in the pattern of the integument, and is defined by the behaviour of clones of cells. After a very early stage in development, marked cells produce patches of descendants that are always on one side of the boundary; although they frequently define it, they never cross it. The boundary is very straight and coincides with a groove in the outer surface of the cuticle, a ridge on the inner surface and an abrupt change in the shape and colour of the epidermal cells (Lawrence & Green 1975; Wright 1979; Wright & Lawrence 1981*a*). The segment boundary is thus both a lineage restriction and an element in the pattern of the integument.

Results

Experiment 1: Extirpation of a compartment boundary

The entire segment boundary is removed by cutting out a strip of cuticle and epidermis and bringing the ends together (figure 1). This abuts cells that are normally far apart and is followed by wound healing and augmented cell division in the area. When the insect moults to the next instar we find a regenerated boundary with all the characters of a normal boundary. Experiments with genetically marked cells show that the regenerated boundary forms precisely at the interface between the cells originating from the two different segments: not a single cell strays across the line (Wright & Lawrence 1981 *b*).

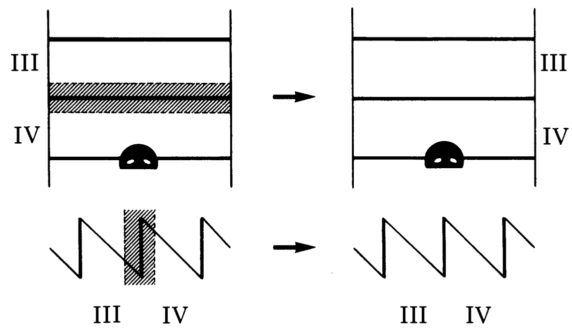

FIGURE 1. Experiment 1. The dorsal segments III and IV of *Oncopeltus* are indicated, the black dot being the scent gland. Below, the repeating segmental gradient is drawn. In all sketches the thick lines represent segment boundaries and the hatched areas, the regions that were extirpated. The experiment is described on the left, and the result, after one or two moults, on the right (for details see Wright & Lawrence (1981 *a*, *b*)).

Experiment 2: Extirpation of an entire segment

A strip extending from the middle of one segment to the middle of the next is removed, and the edges brought together as before (figure 2). Apart from a little wound healing there is no growth and, after the moult, there is a mosaic segment that is stable.

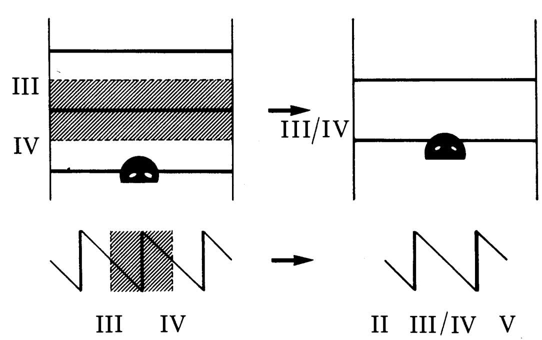

FIGURE 2. Experiment 2. See legend to figure 1.

Experiment 3: Extirpation of a small part of the segment

A small part of the segment is excised and the edges brought together as before (figure 3). There is regeneration and after the moult the segment returns towards its normal length. No boundary forms.

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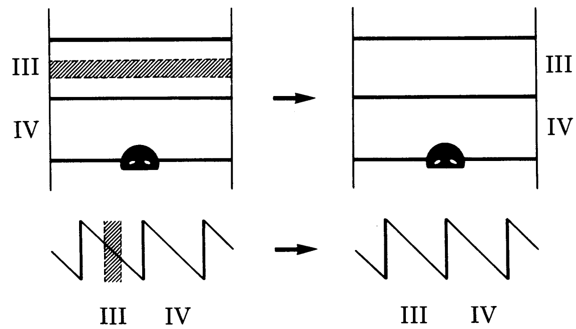

FIGURE 3. Experiment 3. See legend to figure 1.

Experiment 4: Extirpation of a large part of the segment

When a large amount of the segment is extirpated (figure 4) an ectopic boundary is formed, and this has all the properties of a normal segment boundary, including that of a lineage restriction. It is clear from the polarity of the cuticle nearby and the colour of the cells on one side that the antero-posterior orientation of this boundary is reversed (Wright & Lawrence 1981 *a*).

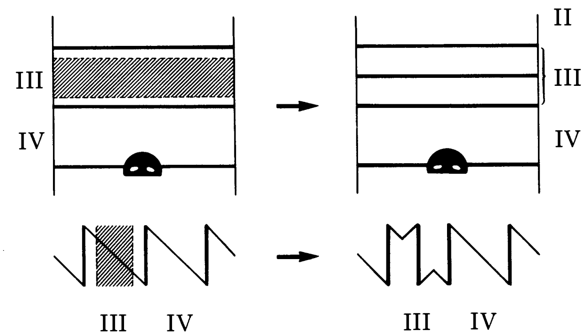

FIGURE 4. Experiment 4. See legend to figure 1.

DISCUSSION

In his studies on proximo-distal regeneration of the cockroach limb, Bohn (1970) has shown that, when cells from different positions in the long axis of the tibia are brought together, an intercalary regenerate forms between them and the missing interval is replaced. When pieces of leg from different places on the circumference are apposed, intercalation occurs and in principle it could go two ways (figure 5). French *et al.* (1976) have shown that, in practice, the shorter of the two routes is taken. Our four experiments can find a similar explanation, if certain assumptions are made. Grafting experiments show that each abdominal segment is homologous to the next (Locke 1959), and therefore the repeated pattern of segments and segment boundaries can be viewed as having a reiterated wave-like form. The route between any two points, taken during intercalary regeneration, will be along the wave, and the shortest route will be the smallest fraction of the wavelength in either direction. Sometimes intercalation will be within the segment as in experiment 3, sometimes it will include intercalation of segment boundary as in experiments 1 and 4. In experiment 4 the intercalation will be expected to produce an ectopic boundary in opposite orientation, as is observed

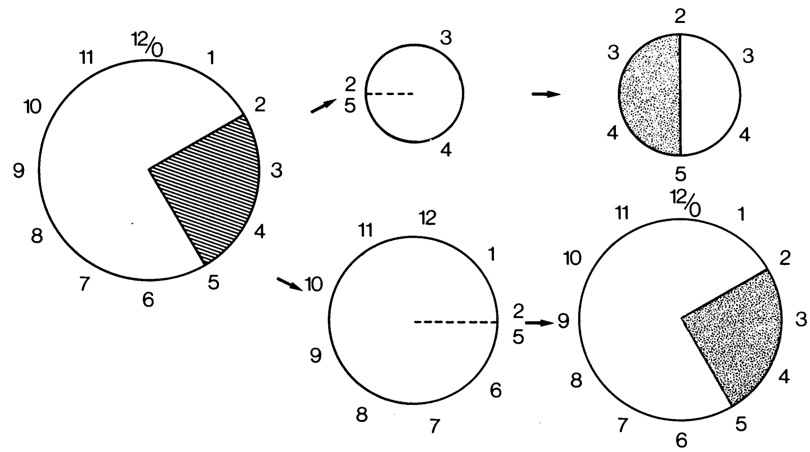

FIGURE 5. Diagram to indicate the shortest intercalation rule of French *et al.* (1976). The pattern (e.g. cockroach limb, imaginal disc of *Drosophila*) is indicated as a clock face, and in the experiment it is divided into two pieces, one larger and one smaller (hatched). As each piece heals, this confronts cells from the original locations 2 and 5. In principle, intercalation between 2 and 5 could go via 3 or via 6, 9 and 12/0. In practice, intercalation (shaded) always takes the shorter of the two possible routes, i.e. via 3. This leads to regeneration by the larger piece and duplication by the smaller.

(figure 4). In experiment 2 no intercalation is expected, because even though the confronting cells are in different segments, they are in 'phase'. No intercalation is observed.

The polarity of cuticular elements, and the pattern of the structures formed, is controlled by a segmental gradient (Locke 1959; reviewed in Lawrence 1973*a*). For example, Stumpf studied a narrow ridge of cuticle that formed at a particular level in the gradient. After grafting experiments, the ridge formed exactly where the gradient model predicted – as if it were running along a contour in the gradient landscape (Stumpf 1968; Lawrence *et al.* 1972). In our model, the gradient represents a section of the wavelength of the entire segment; the compartment boundary, like the ridge studied by Stumpf, should form at a particular phase angle in that wavelength. An experiment of Nübler-Jung (1979, fig. 12) can be used to test this idea; she rotated (by 180°) a square of integument of the bug *Dysdercus* that included the segment boundary parallel and close to one edge of the square. After moulting the result was a deflected boundary, continuous with the existing border, plus an isolated ellipse of boundary: exactly the form of the ridge described by Stumpf (1968). We may conclude that although a segment boundary is a special feature of the reiterated segmental pattern and defines a lineage restriction, it is nevertheless subject to the same rules as any other element in that pattern.

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