Intercalary regeneration around the circumference of the cockroach leg

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SUMMARY

Epidermal cells from different circumferential positions around the femur of Blabera craniifer can interact to form an intercalary regenerate. Removal of a longitudinal strip of integument (cuticle plus epidermis) from any position around the circumference leads to the cut edges healing, localized growth and intercalary regeneration of the missing section of the circumference, so that the resulting femur is approximately normal in size and pattern of cuticular structures. Grafting a longitudinal strip of femur integument into a different circumferential position on the host femur confronts epidermal cells from different positions along both the inner and outer longitudinal graft/host junctions. In numerous different situations this results in local growth and intercalary regeneration of that section of the circumference normally separating graft and host positions, by the shorter route around the circumference. Confrontation of opposite positions results in the intercalation of either of the intervening half circumferences. In one opposite confrontation, between mid-anterior and mid-posterior, there was also a third result where graft and host healed together, provoking no intercalary regeneration. Grafts made with reversed proximal/distal polarity show that a confrontation between different circumferential positions gives the same result, regardless of the proximal/distal levels involved, hence circumferential position is an independent aspect of position on the femur.

These results strongly suggest that epidermal position is not specified with respect to two transverse axes running through the epidermis and internal tissue of the leg, but that there is a continuous circular sequence of positional values running around the circumference, in the epidermis. This is analogous to but independent of the sequence previously shown by Bohn (1967) and Bullière (1971) to run proximal/distal along a leg segment. Hence epidermal position on the femur is specified in two dimensions and can be represented in terms of the French, Bryant & Bryant (1976) polar co-ordinate model. Interactions along the edges of the strip-grafts conform to the Shortest Intercalation Rule (French et al. 1976).

At the proximal and distal ends of strip-grafts intercalation restores normal sequences of positional values where possible. However, where the graft, together with the intercalary regenerates formed at the longitudinal graft/host junctions and the adjacent host tissue formed a complete sequence of circular values, then a supernumerary distal regenerate was formed, in agreement with the Complete Circle Rule of French et al. (1976).

The problem of generating a continuous circular sequence of positional values by one or more circumferential gradients, is briefly discussed.

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INTRODUCTION

Hans Driesch (1908) first stressed the importance of a cell's position in deciding its developmental fate, and more recently these ideas have been extended by Wolpert (1969, 1971) into the concept of positional information. He suggested that a system of cellular interactions specifies a 'map' of positional values across a domain of cells which, at this stage, can regulate by systematically changing the developmental fates of cells in response to removal, addition or translocation of tissue. Many embryos subsequently lose this ability to regulate as the cells become determined, irrevocably interpreting their current positional values and differentiating accordingly. However, other animals (e.g. cockroaches) can regenerate lost or damaged appendages in larval life. The cellular interactions underlying pattern regulation in these regenerating systems should shed light on the initial formation of pattern in the early embryo.

Many studies have been made of pattern regulation in insect postembryonic epidermis, and favourite experimental systems have been the imaginal discs of Drosophila (Bryant, 1974, 1975), the abdominal tergites and sternites of Hemiptera (Lawrence, 1970; Lawrence, Crick & Munro, 1972), and cockroach legs.

Cockroach pro-, meso- and metathoracic legs differ only slightly in the details of their cuticular pattern, and all regenerate from any amputation level distal to the thoracic sternite (Bohn, 1974). Bullière (1971) has suggested that, during leg regeneration, epidermal cells make the various cuticular structures in response to four independent aspects of position: metameric segment (pro-, meso- or metathoracic), leg segment (coxa, femur, etc.), proximal/distal level within a segment, and circumferential position.

Metameric segmentation is labile in very early insect embryos (Sander, 1976) but is determined long before hatching, so that larval cockroach leg tissues grafted onto a different leg will regenerate structures characteristic of the segment of origin (Bohn, 1965a; Bullière, 1970). Leg segment type and proximal/distal level are clearly not determined within the epidermis since cells at any amputation site can divide and form all the more distal structures. Bullière (1972, 1973) suggested that circumferential position is determined so that, during distal regeneration from an amputation site or supernumerary regeneration from a graft/host junction, cells will give rise to linear files of progeny of more and more distal levels but the same circumferential position. However, using interspecies grafts, Bohn (1972b) showed that this is not the case since, during regeneration, a quarter of the circumference of the tibia could give rise to a variable portion of the tarsal circumference.

Bohn (1967, 1970) and Bullière (1971) have studied pattern regulation in the proximal/distal axis of the leg segment. They found that confrontation of corresponding levels of host and graft segments resulted in simple healing, while confrontation between different levels resulted in localized growth at the
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junction and the intercalary regeneration of the levels normally intermediate between the host and graft levels. This indicates that the cells at different proximal/distal levels have different positional values, and that their interaction leads to local stimulation of growth and intercalation of intermediate values within the new tissue.

In the experiments reported here I have confronted epidermal cells from different positions on the circumference of the cockroach leg. Intercalary regeneration occurs around the circumference, indicating that cells at different positions have different positional values in a circumferential sequence which is analogous to but independent of the proximal/distal sequence. This leads to a two-dimensional representation of the epidermis of a cockroach leg segment, and two simple rules for cellular interaction within it. Some of the preliminary results have been published (French & Bullière, 1975) and this work has contributed to the recent formulation of a general polar co-ordinate model of pattern regulation, which accounts for much of the data on regeneration in insect and amphibian appendages (French et al. 1976; Bryant, Bryant & French, 1977; French, 1976; Bryant & Iten, 1976).

MATERIALS AND METHODS

Larvae of the cockroach Blabera craniifer were kept at 25–27 °C, fed on laboratory rat pellets and provided with moist cotton wool. 3rd–6th instar larvae were taken from mass cultures 2–4 days after moulting (an instar lasts approximately one month in these conditions) and anaesthetized with CO₂. Operations were done under the dissection microscope, using fine forceps and knives made from razor-blade fragments, and operated animals were kept in plastic sandwich boxes in groups of 20–30 until the second post-operative moult when the operated leg was removed, fixed in 70% ethanol and examined. All experiments were performed on the femur since it has more cuticular markers at particular circumferential positions than the other leg segments.

Strip-removal experiments

A narrow longitudinal strip of integument (cuticle plus epidermis) was removed from a precise circumferential position along the entire (or almost entire) length of the metathoracic femur. This was done by making superficial cuts with a razor-blade knife, freeing any muscle attachments and lifting off the integument. Often the leg dried up and was autotomized but in successful cases haemolymph dried to seal the wound.

Strip-graft experiments

A rectangle of integument was removed from a precise circumferential position on the host metathoracic femur, and into the site was grafted a similar-sized rectangle of integument taken from a particular circumferential position
Fig. 1. Cuticular pattern of the normal (A, B, C), and regenerated (D) metathoracic femur. A, P, I, E, – anterior, posterior, internal and external faces of the femur; p – proximal; d – distal. In B, C, and D twelve positions are marked around the circumference by numbers 1–12/0. (A) Normal left metathoracic femur, anterior view. F – femur; Tr – trochanter; Ti – tibia. (B) Schematic cross-section at the level of mid-femur showing the distribution of circumferential positions, the double row of bristles at position 12/0–1, and the single rows at 10, 7 and 5. The difference between position 5 and position 7 is indicated by size of bristle whereas, in fact, position-5 bristles are much more numerous. The bands of light cuticle on the internal face (positions 5–7) and dark cuticle on the external face (positions 12/0–1) are shown by dots and filled circles respectively. (C) and (D) Proximal/distal lengths of normal (C) and regenerate (D) femur cut along the posterior face and opened out flat to show the longitudinal rows of bristles and bands of cuticle.
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on the donor meso- or metathoracic femur of the same animal. Grafts were secured by dried haemolymph. Some operated legs were autotomized and in some cases the grafted epidermis died, but in successful cases the grafted tissue was clearly present after the 2nd postoperative moult.

RESULTS AND DISCUSSION

Cuticular pattern of the normal femur

The femur of the normal non-regenerated Blabera metathoracic leg is shown in Fig. 1 A–C, and the mesothoracic femur (used as donor in some graft combinations) shows essentially the same cuticular features. Position on the femur will be given in terms of proximal/distal level and circumferential position with respect to the four faces (anterior, posterior, internal and external) and, more precisely, numbers 1–12/0 which denote particular positions around the circumference (Fig. 1 B). The femur bears a number of longitudinal cuticular features at characteristic circumferential positions. On the anterior face at position 10 is a single row of bristles set in a more-or-less continuous cuticular ridge. The internal face between positions 7 and 5 is covered by very light cuticle almost free of bristles and flanked by two ridges, the anterior one (position 7) bearing a sparse row of long bristles and the posterior one (position 5) bearing a single row of much more numerous bristles. The external face (positions 12/0–1) has two rows of bristles on a band of dark cuticle.

Cuticular pattern of the regenerated femur

Twenty metathoracic legs were amputated between the trochanter and femur, and the femurs of the resulting regenerates were examined two moults later. Figure 1 D shows that the normal circumferential markers are present on the regenerated femur, with some minor differences such as a less pronounced cuticular ridge at position 10, much less regular boundaries to the internal face (positions 7–5), and less regular rows of bristles at positions 5 and 12/0–1. The normal cuticular features occur reliably on the regenerated legs so they can be used as circumferential markers for regenerated tissue.

Strip-Removal experiments

In order to investigate the possibility of circumferential intercalary regeneration of missing regions of epidermis, I performed a number of different strip-removal experiments.

(a) Removal of epidermis between positions 4 and 6 (Fig. 2 A)

In all 22 successful cases the resulting femur had an almost normal circumferential pattern: the internal face (position 7–5) was of normal size with a rather irregular posterior boundary (position 5) bearing numerous bristles in an irregular row.
Fig. 2. Strip-removal experiments. Removal of strips of integument between positions 4 and 6 (A), positions 6 and 8 (B) or positions 11 and 2 (C). i, ii, iii – schematic cross sections of femur (as in Fig. 1B). Removal of a section of circumference (i) results in the cut edges healing together (ii) and, after 2 moults (M), the intercalary regeneration of the missing section (iii). The regenerate (R) is shown by dashed lines. iv – Camera lucida drawing of the resulting femur cut along the posterior face and opened out flat (as in Fig. 1C, D). The light cuticle of the internal face is shown by dots and the dark band along the external face is shown by dashed lines.
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(b) Removal of epidermis between positions 6 and 8 (Fig. 2B)

In 25/26 successful cases the resulting femur had an internal face (positions 7-5) of normal size with a slightly irregular anterior boundary (position 7) bearing the normal sparse row of bristles.

(c) Removal of epidermis between positions 11 and 2 (Fig. 2C)

In 40/43 successful cases the resulting femur had a fairly normal set of external structures (positions 12/0-1) with a band of dark cuticle bearing a large number of bristles most of which were in a single row rather than the normal two rows. The other three cases had the dark band, but only a few bristles were present.

(d) Other strip-removals

Removal of strips from other circumferential positions resulted in a femur of normal size with normal pattern, except that the bristles and ridge normally present at position 10 were only poorly regenerated if they had been removed.

Strip-removals: Discussion

Removal of a strip of epidermis from any position around the circumference results in the cut edges healing together, cell proliferation and intercalary regeneration of the missing section of the circumference, so that the resulting femur is approximately normal in size and circumferential pattern.

Strip-Graft experiments

Healing after removal of a strip of epidermis necessarily confronts cells which are normally only a short distance apart around the circumference and intercalary regeneration results in the re-establishment of the normal pattern. In order to investigate this intercalary regeneration by confronting cells from more disparate positions, I have done a large number of different strip-graft experiments. In all grafts in this section graft and host were taken from the same proximal/distal level so that no proximal/distal discontinuities were created between adjacent host and graft cells. Each graft created four graft/host junctions: inner (the longitudinal edge nearest to the host internal face), outer, proximal and distal.

Control experiments involved removing strips from a number of different positions on meso- or metathoracic donor femurs and grafting them to corresponding sites on the host metathoracic femur. All control grafts healed in with no regeneration at any of the graft/host junctions.

Graft 1: left internal face grafted to left anterior face

As shown in Fig. 3, a strip comprising positions 8–4 was removed from the left mesothoracic femur and grafted into a site lying between positions 9 and 8
Fig. 3. Strip- graft 1, left internal face grafted to left anterior face. i, ii - Schematic cross sections showing the operation (i) and the result (ii) after two moult s (M). iii - Camera lucida drawing of resulting femur cut along the posterior face and opened out flat. At both inner and outer graft/host junctions the part of the circumference normally present between the confronted edges of graft (G) and host (H) has been intercalated (R). iv - Anterior/internal view of the resulting femur showing the internal face of the intercalary regenerate (RI) formed between the grafted internal face (GI) and the internal face of the host (HI). Intercalary regeneration (R) has also occurred at the proximal and distal graft/host junctions, joining the graft internal face to the internal face regenerated along the inner graft/host junction.
Fig. 4. Strip-graft 2, right internal face grafted to right posterior face. Abbreviations and notations as in Fig. 3 except that iii shows a camera lucida drawing of the resulting femur cut along the anterior face and opened out flat. At both inner and outer graft/host junctions the circumferential positions normally lying between graft (G) and host (H) have been intercalated (R), resulting in a recognizable internal face being formed at the inner junction.

on the host left metathoracic femur. Along the outer junction position-9 host cells confronted position-8 graft cells and, in all 89 successful cases, this resulted in the intercalary regeneration of tissue bearing no recognizable structures. Along the inner junction position-8 host cells confronted position-4 graft cells and, in all 89 cases, there was intercalary regeneration of a new internal face in mirror-image relationship to the host and graft internal faces. In 80/89 cases the intercalated internal face was well separated from the graft and host internal faces, hence the tissue intercalated between the confronted positions 8 and 4 corresponds to the intervening positions 7, 6 and 5 (Fig. 3ii, iii). In the other 9 cases the intercalated face was partially fused to the graft or host internal face.

In all 89 cases tissue was intercalated at the proximal and distal junctions in such a way that the regenerated internal face was joined around to the graft internal face (Fig. 3iv).
Graft 2: right internal face grafted to right posterior face

As shown in Fig. 4 a strip comprising positions 4–8 was removed from the right mesothoracic femur and grafted between positions 3 and 4 on the host right femur. The outer junction confronted position-3 host cells and position-4 graft cells and, in all 22 successful cases, there was intercalation of tissue bearing no recognizable structures. The inner junction confronted position-4 host and position-8 graft cells and, as in the reciprocal confrontation of Graft 1, this resulted in all cases in the intercalary regeneration of an internal face (positions 5, 6, 7), as shown in Fig. 4ii, iii.

In all 22 cases intercalary regeneration at proximal and distal junctions joined the regenerated internal face around to the graft internal face.

Graft 3: left external face grafted to left anterior face

As shown in Fig. 5, a strip comprising positions 2–11 was removed from the left mesothoracic femur and grafted between positions 8 and 8 on the host left femur. Along the outer junction, confrontation of position-8 host cells and position-2 graft cells resulted in intercalary regeneration in all 26 successful cases. In 20/26 cases the intercalated tissue clearly bore the dark band and bristles of an external face (positions 12/0–1) and in the other six cases there
Fig. 6. Strip-graft 4, right posterior face grafted to left anterior face. Abbreviations and notations as in Fig. 3. iv - Anterior view of the resulting femur showing the row of external hairs (RE) intercalated along the outer junction between host and graft (G), the internal face (RI) intercalated at the inner junction between host (H) and graft (G), and supernumerary distal structures (S) regenerated from proximal and distal graft/host junctions.

were no recognizable structures. The inner junction confronted position-8 host cells and position-11 graft cells and, in all cases, this resulted in intercalation of tissue bearing no recognizable structures.

In all 20 cases the regenerated and graft external faces were joined by intercalation at proximal and distal junctions.
Graft 4: right posterior face grafted to left anterior face

As shown in Fig. 6, a large strip comprising positions 2-3 was removed from the right mesothoracic femur and grafted between positions 10 and 8 on the host left femur. Along the outer junction position-10 host and position-2 graft cells were confronted and, in all 20 successful cases, this resulted in intercalary regeneration (Fig. 6ii, iii). In 3/20 cases there were no recognizable structures but in the remaining 17 cases the regenerate bore the characteristic dark band and bristles of the external face (positions 12/0-1). The inner junction confronted host position-8 cells and graft position-3 cells and, in all 20 cases, an internal face (positions 7, 6, 5) was intercalated. In 3/20 cases the regenerated internal face was either partly fused to the host internal face or recognizable for only part of the length of the junction.

At the distal junction there was no regeneration in 2/20 cases but, in the other 18 cases, a supernumerary set of distal structures was regenerated (Fig. 6 iv). Five of these were complete, consisting of femur apex, tibia, tarsus and claws, but the others were broken at the femur apex or in the tibia. At the proximal junction there was no regeneration in 8/20 cases and regeneration of a supernumerary leg in the other 12 cases, one of them complete from femur apex to claws.

Graft 5: right posterior face grafted to left anterior face (internal half)

As shown in Fig. 7 a strip comprising positions 2-4 was removed from the right metathoracic femur and grafted between positions 9 and 8 on the host left femur. The inner junction confronted position-8 host cells and position-4 graft cells and, in all 42 successful cases, this resulted in the intercalation of an internal face (positions 5, 6, 7). The outer junction confronted position-9 host cells with position-2 graft cells and gave very variable results (Fig. 7ii, iii). In 32/42 cases the 9/2 confrontation resulted in the intercalation of a recognizable external face along at least part of the junction (Fig. 7iiib, iiiib). In 7/42 cases an internal face was intercalated along part of the junction (Fig. 7iiic, iiic) while in 17/42 cases no intercalary regeneration occurred and the disparate host and graft cells simply healed together (Fig. 7iia, iiia). In 18/42 cases the confrontation gave different results at different points along the length of the same junction.

At the distal junction there was no regeneration in 5/42 cases but, in the other 37 cases, a distal regenerate was present and 12 of these were complete supernumerary structures consisting of femur apex, tibia, tarsus, and claws. At the proximal junction there was no regeneration in 19/42 cases, a distal regenerate in 17/42 cases and, in the other six cases, there was intercalary regeneration of internal face tissue joining internal faces which had been intercalated along the inner and proximal end of the outer junction.
Fig. 7. Strip-graft, 5, right posterior face grafted to left anterior face (internal half). Abbreviations and notations as in Fig. 3. Along the inner graft/host junction an internal face was intercalated (ii and iii) but at the outer junction there were three classes of results: iia, iia – No intercalation (NR) between confronted graft (G) and host (H) positions. iib, iii b – Intercalation of the half circumference bearing an external face. iic, iic – Intercalation of the half circumference bearing an internal face.
Strip grafts 1–5: discussion

Grafting together cells from corresponding circumferential positions results in healing, but confrontations of cells from different positions results in intercalation of tissue. Only circumferential positions 12/0–1, 5, 6 and 7 bear recognizable and reliably regenerated cuticular markers but these, in conjunction with the amount of unrecognizable tissue regenerated in the various situations, demonstrate that the intercalated tissue consists of that section of the circumference which normally separates host and graft positions, as measured by the shorter route around the circumference. For example, the host-8/graft-4 interaction intercalates positions 7, 6, 5 and not the alternative much longer set of intermediates 9, 10, 11, 12/0, 1, 2, 3 (inner junction of Graft 1), as does the reciprocal host-4/graft-8 interaction (inner junction of Graft 2). Strip grafts 1–4 have produced no confrontation where this ‘shortest intercalation’ rule is not obeyed and, at the outer junction of Graft 5, confrontation between approximately opposite positions produced the predictable ‘flip-flop’ with two classes of result. In most cases the 9\(\frac{3}{4}\)/2\(\frac{1}{4}\)-interaction intercalated the half circumference comprising positions 10, 11, 12/0, 1, 2 while in some cases the alternative half-circumference 9, 8, 7, 6, 5, 4, 3 was regenerated. There was also the unexpected third class of result where host and graft healed together, provoking no intercalary regeneration. This ‘null’ result could be associated with any flip-flop situation, with any interaction involving either the position-9 region or the position-3 region, or it could be associated only with an approximately 9/3 flip/flop confrontation. Grafts 6–8 were performed to explore these possibilities.

Graft 6: right posterior face (internal half) grafted to left anterior face (external half)

As shown in Fig. 8, a strip comprising positions 3–4 was removed from the right metathoracic femur and grafted between positions 11 and 9 on the host left femur. The outer junction confronted host position 11 and graft position 3 and, in all 31 successful cases, there was intercalary regeneration of an external face (position 12/0–1). In 25 of these the characteristic dark band and bristles were both present. Along the inner junction host position-9 cells confronted graft position-4 cells and, as shown in Fig. 8ii, iii, there were three classes of result. Along at least part of the junction a clear internal face (position 5, 6, 7) was intercalated in 23/31 cases (Fig. 8ii b, iii b) the dark band and bristles of the external face (positions 12/0–1) were intercalated in six cases (Fig. 8ii c, iii c) and in 26/31 cases there was no intercalation (Fig. 8ii a, iii a, iv). Different results occurred at different points along the same junction in 22 cases (Fig. 8v).

At the distal junction there was a distal regenerate in 23 cases, no regeneration in four cases and, in the remaining four cases, there was intercalation of an external face joining external faces which had been intercalated along the inner
Fig. 8. Strip graft 6, right posterior face (internal half) grafted to left anterior face (external half). Abbreviations and notations as in Fig. 3. Along the outer graft/host junction an external face was intercalated (ii, iii) but at the inner junction there were three classes of result: iia, iii a – No intercalation (NR) between confronted graft (G) and host (H) positions. iib, iiib – Intercalation of the half circumference bearing an internal face. iic, iiic – Intercalation of the half circumference bearing an external face. iv – Anterior view of femur with an external face (RE) intercalated along the outer junction between graft (G) and host (H), and no intercalation (NR) along the inner junction. v – Internal view of femur showing the different results of confrontation along one inner junction. RE, intercalated external face; NR, no intercalation; RI intercalated internal face. S, supernumerary distal structures (broken off) regenerated at the distal junction. H, host; HI, host internal face; Ti, cut end of host tibia.
Fig. 9. Strip-graft 7, left posterior face (external half) grafted to left anterior face. Abreviations and notations as in Fig. 3. Along the inner graft/host junction there was intercalation of either the half circumference bearing an external face (iia) or the other half circumference bearing an internal face (iid). Along the outer graft/host junction there were three classes of result: iia – No intercalation (NR) between confronted graft (G) and host (H). ii b – Intercalation of the half circumference bearing an internal face. ii c – Intercalation of the half circumference bearing an external face.

and outer junctions. At the proximal junction the same three results occurred in 4, 22 and 5 cases respectively.

**Graft 7: left posterior face (external half) grafted to left anterior face**

As shown in Fig. 9, a strip comprising positions $1\frac{1}{2} - 3\frac{1}{2}$ was removed from the left mesothoracic femur and grafted between positions 8 and $9\frac{1}{2}$ on the host left femur. Along the inner junction host position 8 and graft position $1\frac{1}{2}$ were confronted and, in all 40 successful cases, this resulted in intercalary regeneration. In one case the intercalated tissue was unrecognizable, in 30 cases it bore an external face and in six cases an internal face. In the other three cases the confrontation resulted in intercalation of external and internal faces at different points along the same junction. Along the outer junction host position $9\frac{1}{2}$ and graft position $3\frac{1}{2}$ were confronted and, as in the similar confrontations of grafts 5 and 6, there were three classes of result. Along at least part of the junction an internal face was intercalated in 6 cases, an external face in 33 cases, and no intercalation occurred in 15 cases.
Fig. 10. Strip-graft 8, right anterior face grafted to right posterior face (internal half). Abbreviations and notations as in Fig. 3. Along the inner graft/host junction there was intercalation of either the half circumference bearing an external face (iiA) or the other half circumference bearing an internal face (iID). Along the outer graft/host junction there were three classes of result: iiA – No intercalation (NR) between confronted graft (G) and host (H). iiB – Intercalation of the half circumference bearing an internal face. iiC – Intercalation of the half circumference bearing an external face.

At the proximal junction there was no regeneration in 14/40 cases, and, in the other 26 cases, an intercalated external face joined external faces which had been intercalated at the proximal end of the inner and outer junctions. At the distal end the same two results occurred in 3 and 26 cases respectively and, in the remaining 11 cases, there was distal regeneration of a supernumerary leg. Nine out of eleven of these were associated with the intercalation of a clear external face along one of the longitudinal junctions and an internal face along the other one.

**Graft 8: right anterior face grafted to right posterior face (internal half)**

As shown in Fig. 10, a strip comprising positions 8f-10 was removed from the right mesothoracic femur and grafted between positions 3 and 4 on the host femur. Along the inner junction host position 4 and graft position 10 were confronted and, in all 13 successful cases, intercalary regeneration resulted. In two cases the intercalated tissue was unrecognizable, in four cases it bore an internal face, in six cases an external face, and in one case external and internal faces were formed in different regions of the junction. Along the outer junction
host position 3 and graft position 8/3 were confronted and, along at least part of the junction, an internal face was intercalated in six cases, an external face in six cases, and no intercalation occurred along at least part of 8/13 junctions.

At the proximal junction there was no regeneration in eight cases and, in the other five cases, intercalary regeneration of either internal or external faces joining corresponding faces which had been intercalated along both longitudinal junctions. At the distal junction there was no regeneration in four cases, intercalary regeneration in five cases and, in the remaining four cases, distal regeneration of supernumerary leg structures.

Strip grafts 1-8: discussion

Grafts 6-8 each included one confrontation between positions approximately 9 and 3, and all produced the three categories of result: intercalation of the intervening internal half-circumference, intercalation of the alternative external half-circumference, and no intercalation at all. This confirms the result obtained from Graft 5, and shows that it occurs both between host 9/graft 3 and host 3/graft 9.

Grafts 7 and 8 produced two confrontations (8/1½ and 4/10 respectively) which were 'flip-flop' situations in which either intervening half circumference could be intercalated, but which did not result in any cases where intercalation did not occur. This strongly suggests that a flip-flop by itself does not produce the null result, but that it results in some way from positions approximately 9 and/or 3. The outer junction of Graft 6 confronted 11/3 and gave no null results, while the outer junction of Graft 3 confronted 8½/2 and also gave no null results, hence neither the position-9 nor -3 region alone is sufficient. It seems that failure to intercalate can occur only at the flip-flop confrontation between the position-9 region and the -3 region.

Strip grafts with reversed proximal/distal orientation

In the preceding strip-removal and strip-graft experiments the confrontation at each point along the junction was between cells of different circumferential position but the same proximal/distal level. In flip-flop situations different points along the junction may intercalate in different directions, but in all other situations the same intercalation occurs at all points along the junction. This strongly supports the assumption that cells at a given circumferential position have the same positional value along the entire length of the femur, e.g. that the bristle row at the anterior edge of the internal face is the interpretation of positional value 7 along its entire length.

However it is possible, for example, that positional values could spiral down the femur and be interpreted differently at different proximal/distal levels. To show directly that circumferential positional value is indeed independent of proximal/distal level I have grafted together cells from different positions and
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different levels, and compared the results with the same circumferential confrontation between cells of the same proximal/distal level. Grafts were made with reversed proximal/distal orientation so that the same circumferential confrontation occurred all along the junction but between proximal host/distal graft level cells, mid host/mid graft level, and distal host/proximal graft level cells.

Control Graft, proximal/distal reversed

A strip was removed from the anterior face (internal half) of the left metathoracic femur and grafted proximal/distal reversed to the same position on the right metathoracic femur, creating a 9/9 confrontation along the outer junction and an 8/8 confrontation along the inner junction. In all 30 successful cases there was no intercalation of new circumferential markers. No growth occurred in the mid regions of the junctions, but there was growth around both ends (28/30) or one end of the graft.

Graft 1, proximal/distal reversed

The internal face of the right mesothoracic femur was grafted proximal/distal reversed into the anterior face of the left metathoracic femur, creating a 9/8 confrontation along the outer edge and an 8/4 confrontation along the inner edge, as in Strip Graft 1. There was more extensive growth around the ends of the graft than in the mid region but, in all 24 successful cases, tissue bearing no recognizable markers was intercalated along the outer edge and a new internal face was intercalated along the entire length of the inner junction (Fig. 11 A).

Graft 4, proximal/distal reversed

The posterior face of the left mesothoracic femur was grafted proximal/distal reversed into the anterior face of the left metathoracic femur, creating a 10/2½ confrontation along the outer edge and an 8/4 confrontation along the inner edge, approximately as in Graft 4. There was more extensive growth around the ends of the graft than in the mid region but, in all 12 successful cases, an internal face was intercalated along the inner junction (Fig. 11 Bi) and either an external face (10/12) or unrecognizable tissue was intercalated along the outer junction. In all 12 cases there was distal regeneration of a supernumerary leg from proximal and distal junctions.

Reversed strip grafts: discussion

It is expected that proximal/distal reversal of a strip graft will produce extensive growth around the ends of the graft, since Bohn (1967, 1970) and Bullière (1971) have shown that grafting together cells from proximal and distal levels of a leg segment provokes growth and the intercalation of intervening levels. Nonetheless with respect to intercalation of circumferential markers, a confrontation between different positions gives the same result regardless of the
Fig. 11. Intercalation provoked by strip-grafts with reversed proximal/distal orientation. Camera lucida drawings of results two moults after strip-grafts with reversed proximal/distal orientation. As in camera lucida drawings of Figs. 2–8, the internal faces of the host (HI), graft (GI) and intercalary regenerate (RI) are dotted, and the external faces of host (HE) and regenerate (RE) are shown by dashed lines. Heavy dashed lines separate host, regenerate and graft (G). HTi, cut end of host tibia. A – Anterior/internal view of femur after Graft 1 proximal/distal reversed. Extensive growth has occurred around the ends of the graft and an internal face (RI) has been intercalated along the inner junction. B – External (i) and internal (ii) views of femur after Graft 4 proximal/distal reversed. An external face (RE) has been intercalated along the outer junction (i) and an internal face (RI) has been formed along the inner junction (ii). Supernumerary distal regenerates (S) have formed at proximal and distal ends of the graft.
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proximal/distal levels involved, hence circumferential position is an independent aspect of position on the femur.

GENERAL DISCUSSION

The strip-removal and strip-graft experiments clearly demonstrate that circumferential position is not rigidly determined within the epidermal cells of the cockroach leg. Cells from one position, or their progeny, may form structures characteristic of a different region of the circumference, as was originally shown by Bohn (1972a). The experimental results also show that the structures made by the cells depend upon the nature of their epidermal neighbours, since it is only when cells are confronted by normally non-adjacent cells (after the healing of a strip-removal or at the junction of a strip-graft) that new structures are made, and the new structures are those which normally lie on the circumference in the positions between the confronted cells.

Bohn (1972a) suggested that epidermal position is specified with respect to two transverse axes (or sets of positional values) running anterior-posterior and internal-external through the epidermis and internal tissues of the leg. Circumferential intercalation is more easily interpreted as resulting from cells at different physical positions on the circumference (numbered 0–12 in Figs 1–10) having different positional values in a corresponding epidermal circular sequence 0–12. Distinguishing between twelve different positions is arbitrary and convenient; there may be a much larger number of positional values around the circular sequence. Interaction between normally non-adjacent positional values results in growth and the intercalation of all the intermediate positional values. Since the sequence of positional values is circular there will always be 2 sets of intermediate values for any confrontation, and intercalary regeneration occurs by the shorter route. This is the Shortest Intercalation Rule (French, et al. 1976). For example the 10½/2½ confrontation at the outer junction of Graft 4 results in the intercalation of positional values 11, 12/0, 1, 2 rather than 10, 9, 8, 7, 6, 5, 4, 3. This example also illustrates that the circular sequence is continuous and the notation 12/0 does not imply a boundary or discontinuity having unique properties, but results simply from putting numbers around a circle.

It seems that the direction of intercalation depends only on the positional values of the epidermal cells actually at the junction. The host-8/graft-4 epidermal cell confrontation at the inner junction of Graft 1 overlies anterior muscle while the host-4/graft-8 confrontation at the inner junction of Graft 2 overlies posterior muscle, but both intercalate an internal face. Similarly, epidermal cells away from the junction do not appear to affect intercalation since both the above grafts confronted 6, 7, 8/4, 5, 6, while the inner junction of Graft 5 confronted 6, 7, 8/4, 3 and the latter also intercalated an internal face.
The experimental results give some indication of the relationship between cell division and intercalary regeneration. Consider the intercalary regeneration occurring in the strip-removal experiments. Pattern regulation could occur by 'morphallaxis' (Morgan 1901); after the cut edges join together most of the cells could change their positional values such that the entire circular sequence is re-established within the small ring of epidermis. Subsequently cell division throughout the femur could be greater than that normally occurring during the instar and result in the femur attaining normal size. Alternatively the healing together of cells with different positional values could stimulate local cell proliferation at the junction, and intervening positional values could be formed within the new tissue, a process termed 'epimorphosis' (Morgan 1901). The results suggest that intercalary regeneration occurs by epimorphosis, as in the case of proximal/distal intercalary regeneration (Bohn, 1970). The cuticular ridges and rows of bristles have a less regular appearance on a regenerated femur than on a normal femur and, after strip-grafts and strip-removals, newly-intercalated structures have the appearance of regenerates while structures originally present on the graft or host retain their normal non-regenerated appearance. These results indicate that confrontation of normally non-adjacent positional values stimulates division of cells at the junction until cells with all the intermediate positional values have been produced: then growth ceases. The position and timing of cell division during intercalary regeneration is currently being studied.

These experiments do not show whether both graft and host cells divide at the junction to contribute to the intercalary regenerate, since there is no way of distinguishing host-derived from graft-derived tissue. I have performed other strip-grafts between the metathoracic and structurally distinct prothoracic femur, and between the femur and other leg segments (French, in preparation). The results suggest that the intercalary regenerate is derived from both host and graft cells, as is the case in proximal-distal intercalation (Bohn, 1971; Bullière, 1971). From the pigmentation patterns of lateral regenerates produced by grafting between cuticle colour mutants of Blattella, I suggested (French, 1976) that there might be clonal restrictions on the circumferential position which a cell or its progeny could form. The leg may consist of at least two lineage 'compartments' (Garcia-Bellido, Ripoll & Morata, 1973; Crick & Lawrence, 1975) which are respected during regeneration: this is supported by the results of the intersegment strip-grafts.

All the results considered here have been obtained two or, rarely, three moults after the experimental confrontation of different positional values. Intercalary regeneration has inserted the intermediate values between host and graft so there are no discontinuities, but the resulting sequence of values is often far from normal. For example, after Graft 1 the anterior side of the femur has the following values: 0/12, 11, 10, 9, 8, 7, 6, 5, 4, 5, 6, 7, 8, 7, 6. Cells with values 4 and 8 which were originally at the inner graft junction now have neighbours
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Fig. 12. Possible spacings of circular positional values around the cockroach leg epidermis. The pattern of intercalation shows that opposite positional values in the sequence are approximately 180° apart on the leg, hence the spacing of values may be regular (i) or have a number of opposite clusterings (ii) but cannot have a single region of clustering (iii) as suggested by French, Bryant and Bryant (1976) for the Drosophila leg imaginal disc.

5,5 and 7,7 respectively, instead of the normal 5,3 and 9,7. This configuration is stable and does not tend to revert to the normal single circular sequence of values during subsequent instars (French, in preparation).

It follows from the shortest intercalation rule that a confrontation between positional values 180° apart in the circular sequence will produce a ‘flip-flop’ with intercalation able to occur in either direction. The strip-grafts discovered three pairs of circumferential positions (9/3, 10/4, and 8/1½) which, when confronted, can intercalate either of the intermediate half-circumferences, and which therefore correspond to opposite positional values. Opposite values in the circular sequence occur more-or-less 180° apart around the circumference, hence values are probably fairly evenly spaced (as in Fig. 12i): there cannot be one region where values are clustered together (Fig. 12ii) although there could be a number of pairs of clusterings opposite each other (Fig. 12iii). The numbering of physical positions 0–12 on Figs. 1–10 was chosen on this basis and is slightly changed from that used in preliminary reports of these results (French & Bullière, 1975; French, 1976; French et al. 1976).

Flip-flop results were obtained from 9½/2½, 9½/3½, 8½/3, 9/4 and 10/4 confrontations. The intercalation system may be deterministic with a given confrontation always intercalating one way while a confrontation between cells in slightly different positions will always intercalate the other way. In this case the apparent flip-flop would be due to slight inaccuracies in the position of the cuts or a variable amount of cell death at the cut edges. Alternatively, a range of similar confrontations between approximately opposite positional values may all be able to intercalate in either direction.

No circumferential positions directly violate the shortest intercalation rule, but approximate positions 9 and 3 do behave atypically in that, when confronted, they sometimes fail to intercalate. This striking null result is obtained in no other situation and may eventually give some clues to the nature of the cellular
Fig. 13. Intercalary and supernumerary regeneration at the junctions of strip-grafts. Schematic anterior view of the host femur (H) bearing a strip-graft (G) and tissue (R) intercalated at the graft/host boundaries. Dashed lines connect points of equal positional values in the circular sequence 0–12, and graft, host and regenerate are separated by heavy lines with dots. The internal face is shown by dots between the row of numerous bristles (position 5) and the row of sparse bristles (position 7). The external face is shown by a single row of distally-pointing bristles (Position 12/0). (i) Distal end of Strip-Graft 1. Shortest intercalation between the cells at the distal end of the graft and the adjacent host cells forms an internal face joined around to the grafted internal face, restoring normal sequences of positional values (see Fig. 3 iv). (ii) Distal end of Strip-Graft 4. Shortest intercalation at the inner and outer graft/host junctions leads to the formation of a complete sequence of circular values at the end of the graft and a supernumerary leg (S) regenerates at this point (see Fig. 6 iv). (iii) Complete Strip-Graft 5. Shortest intercalation along the inner graft/host junction forms an internal face while intercalation along the outer junction forms either the internal half-circumference (proximally) or the external half circumference (distally). This results in the formation of a complete circumference and a corresponding supernumerary leg at the distal end of the graft (S) and half way along the outer junction (S'). Shortest intercalation at the proximal junction restores normal sequences of positional values. (iv) Distal end of Strip-Graft 7. Intercalation of an internal half circumference along the inner junction and an external half circumference along the outer junction leads to the formation of a circumference comprising all values plus two extra copies of values 2–3. An apparently normal supernumerary leg (S) regenerates.
interactions involved in intercalation. Again, there may be a precise confrontation which would never intercalate, or the absence of intercalation may be one of the results possible from a range of confrontations around 9/3.

The results of the strip-graft experiments clearly show that certain structures are quite reliably formed at the distal and proximal junctions in the various graft combinations. In Grafts 1–3 an internal (Graft 1 and 2) or external face (Graft 3) was used as a graft and intercalation resulted in another copy of the face being regenerated in mirror image along the inner (Grafts 1 and 2) or outer junction (Graft 3) in a total of 131/137 cases. In all these cases the grafted and intercalated faces were joined around the proximal and distal ends of the graft. Fig. 13i shows how this results simply from shortest intercalation between cells at the ends of the graft and adjacent host cells.

In Graft 4, intercalation usually produced an external face along the outer and an internal face along the inner junction. Fig. 13ii shows that the intercalary regenerate, the graft and adjacent host tissue together form a single complete circumference. Supernumerary leg regeneration occurred from these circumferences at the proximal (12/20) and distal (18/20) ends of the graft.

In Graft 5 regeneration of an internal face at the proximal ends of both longitudinal junctions occurred in six cases, always associated with intercalary regeneration of an internal face around the end of the graft. Regeneration of an external face at the outer junction and an internal face at the inner junction occurred in 25 cases at the distal end and 30 cases at the proximal end, and supernumerary regeneration from the resulting complete circumference occurred in 24 and 11 cases respectively. In addition, when internal and external faces were formed at different points along the outer junction, they were either separated by a zone in which intercalation did not occur (two cases) or, as shown in Fig. 13iii, by a supernumerary regenerate (two cases). These results, in conjunction with those of Bohn (1965a) suggest that distal regeneration occurs when cells with a complete circular sequence of values are exposed at an amputation site or generated by intercalation: the Complete Circle Rule of French et al. (1976).

As mentioned in the Results section, in 14 cases from Graft 7 an external face was intercalated at the distal end of one longitudinal junction and an internal face at the distal end of the other junction. 11 of these regenerated supernumerary distal structures from the distal junction. As shown in Fig. 13iv intercalary regenerates, graft and adjacent host tissue constitute a circumference with multiple copies of some values. Four of the regenerates were distally complete to claws and seemed to have a normal circumferential pattern suggesting that, during distal regeneration, duplicate copies of positional values may be eliminated from the circular sequence.

The results of proximal-distal reversed strip-grafts show that a given circumferential position has the same positional value in the circular sequence (0–12) regardless of its level on the femur. The intercalary regeneration experiments of
Bohn (1967, 1970) and Bullière (1971) show that there is a proximal-distal sequence of positional values (labelled A–E in Fig. 14) corresponding to physical levels down the segment. The cuticular structures formed by the epidermal cells and their behaviour in a wide variety of graft combinations depend on their positional values in the two sequences. The femur epidermis corresponds to the surface of a cylinder (Bullière, 1971, 1973), as in Fig. 14i, and it can be represented in terms of the French et al. (1976) general model for epimorphic pattern regulation, with proximal levels (A) around the circumference of a disc and distal levels (E) in the centre, as in Fig. 14ii. Then positional information is specified in two dimensions in terms of polar co-ordinates: radial (A–E) and angular (0–12) position. This representation and the shortest intercalation and complete circle rules for pattern regulation are derived from the results of the experiments reported here and also from much of the literature on pattern regulation in cockroach and other insect legs, amphibian limbs and Drosophila imaginal discs (reviewed in French et al. 1976).

Some strip grafts have been performed by Bohn (1972b) on the tibia of the cockroach leg. He did not mention the occurrence of any intercalary regeneration along the longitudinal edges of the graft, but showed that control grafts (posterior to posterior, and external to external) healed in, while grafts to the opposite face (external to internal) resulted in regeneration of supernumerary distal structures at the proximal and distal graft/host junctions, as in Graft 4 (Figs. 6 and 13ii). Similarly, Bart (1971a, b) performed strip grafts on the coxa of the stick insect, Carausius morosus, mentioned no intercalary regeneration but found that control grafts healed in, while grafts between opposite faces (anterior and posterior, external and internal) resulted in supernumerary regeneration from the distal graft/host junction.
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Fig. 15. Supernumerary regeneration resulting from a skin strip-graft on the amphibian limb. Schematic composite representation of some of the experiments of Lheureux (1972, 1977). A posterior strip of epidermis plus dermis (G) is grafted to an anterior position on the contralateral limb. The leg is amputated through the distal graft/host junction (d) and a nerve (N) is deflected to emerge at the proximal graft/host junction (p). In fact, the nerve-deflection experiments (Lheureux, 1977) were done on separate animals and involved grafts of entire skin cuffs rather than strips but the interaction occurred locally around the end of the nerve and would presumably occur following a strip-graft. The limb comprises central cartilage (Ca) and peripheral epidermis (Ep) which seem not to have the stable positional values (0-12) which are present in the rings of dermis (De) and muscle (Mu). At the distal amputation site (d) interaction between grafted dermis and adjacent host dermis and muscle intercalates positional values (1, 12, 11 and 5, 6, 7) and forms a supernumerary complete circle of values (shown by large dots) which regenerates supernumerary distal structures (S) on the grafted side of the limb and in mirror-image symmetry to the normal regenerate formed from the host complete circle of dermal and muscle positional values. At the proximal junction (p), around the deflected nerve (N), intercalation between grafted dermis and adjacent host dermis and muscle forms a complete circle of values and hence a supernumerary regenerate (S'). Along the longitudinal edges of the graft there is no evidence of intercalation (NR), although there are no dermal markers and the intercalary regenerate would not be obvious.

Grafts of strips and cuffs of skin (epidermis plus dermis) have been performed on the limbs of newts (Lheureux, 1972, 1975a, 1975b, 1977) and axolotls (Carlson, 1974, 1975). There is no indication of intercalary regeneration along the longitudinal edges of these grafts, and regeneration can only occur at the proximal or distal graft/host junction after amputation at that level (Lheureux, 1972; Carlson, 1974) or deflection of a nerve to the junction (Lheureux, 1977). Control grafts produce no supernumerary structures, but grafts between anterior and posterior (in newts and axolotls) and between dorsal and ventral (in newts
Fig. 16. Gradient models for circumferential positional values. (i) A single gradient with values 12 to 0 running around the leg circumference would give every epidermal cell a unique positional value, but would create discontinuity at the boundary region. (ii) A double gradient would involve no discontinuities but each position would only be uniquely labelled if there were another variable distinguishing the two halves (labelled A and P). (iii) Two double gradients with values 12 to 0 and 12' to 0' orientated at 90° to each other would uniquely label each position.

but apparently not axolotls (Carlson, 1974, 1975)) produce supernumerary regenerates. From grafting (Lheureux, 1972, 1977; Carlson, 1975) and irradiation experiments (Lheureux, 1975b; Carlson, 1974) it seems that positional values exist in the rings of dermis and muscle but not in overlying epidermis or central bone or cartilage (Bryant, 1978). Hence the supernumerary structures resulting from strip grafts to the opposite face can be understood in terms of regeneration from a complete circle of positional values formed by intercalation between the graft dermis and adjacent host dermis (or underlying host muscle) around the end of the deflected nerve, or between graft dermis and underlying host muscle at an amputation site (Fig. 15).

At present there is no indication that cockroach leg epidermis can interact with any positional values present in the muscle, and it is possible that the muscle pattern may be derived from the values of the overlying epidermis. This is currently being investigated.

The strip-removal experiments (Fig. 2) are directly analogous to the removal of 90° sectors from the Drosophila wing disc by Haynie & Bryant (1976).
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When the $\frac{2}{3}$ or $\frac{1}{3}$ disc fragments are injected into larval hosts the cut edges heal together (Reinhardt, Hodgkin & Bryant, 1977) and, at metamorphosis each of the four 90° sectors differentiated duplicate copies of their presumptive structures while the corresponding 270° sectors regenerated the extirpated structures. This corresponds to shortest intercalation between the different circular sequence values confronted by the healing of the cut edges (Haynie & Bryant, 1976), exactly as in the cockroach strip removals.

Since Boveri (1901) and Morgan (1901), most developmental biologists have considered the specification of position in terms of the local level of a morphogenetic gradient since this accounts elegantly for the field properties of continuity, progressive change of state with physical distance, and ability to average between the states of neighbouring cells (Cooke, 1975). Bullière (1971) however, has argued that proximal-distal level on the cockroach leg segment could be specified by a sequence of qualitatively different molecules on the surfaces of the cells at different levels. It is difficult to apply this model in detail to intercalary regeneration and Bohn (1976) suggests that 'the simplest model which fits all experimental findings is that of a quantitatively graded, i.e. gradient-like distribution of only one positional marker. Every level of a segment is characterized by an appropriate level of the gradient...'. A gradient model can easily be applied to the linear proximal/distal sequence of positional values ($A$–$E$), but the circular sequence (0–12) of values is a closed system with no discontinuities. Zero equals 12 and positions 11 and 1 are only two positional values apart. Clearly a single gradient running around the circumference of the leg (Fig. 16i) would not lead to intercalation by the shortest route since there would be a discontinuous ‘boundary’ region where maximum and minimum values are adjacent and intercalation between two positions would always take the other route around the circumference, forming the intervening gradient values. A bilaterally symmetrical double-gradient could be joined around the circle with no discontinuities (Fig. 16ii) but such a model would require some other variable to distinguish between the half-circumferences with symmetrical sets of values, and some rule for re-forming the maximum or minimum values during intercalation between cells from different half-circumferences (Wolpert, personal communication). Goodwin (1977) has developed a model involving two independent double gradients running around the circumference from posterior to anterior and from external to internal (Fig. 16iii). Each position will then be specified by a unique pair of gradient values and Goodwin suggests rules by which confronted cells would average their positional values or diverge to form a gradient maximum or minimum. The model in its present form can only explain shortest intercalation in some of the graft/host confrontations.

The formal polar co-ordinate model of French et al. (1976) was based partly on circumferential intercalary regeneration in cockroach legs and Drosophila imaginal discs. It describes the two-dimensional 'map' of positional information, and gives two rules for cellular interaction. Much more work is now necessary.
to formulate and test possible mechanisms by which the positional values may be initially specified during embryogenesis and subsequently intercalated during regeneration.

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