Positional information around the segments 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 consisting of that section of the circumference normally separating graft and host positions, by the shorter route. This result is extended to other leg segments; the tibia and coxa (TT and CC Grafts). Grafting strips of integument from the tibia (TF Grafts) or the coxa (CF Grafts) to a corresponding position on the host femur results in simple healing. Grafting to a non-corresponding position leads to intercalation of the shorter intermediate arc of circumference, composed partly of graft-segment and partly of host-segment structures. These results show that the same continuous sequence of positional values is distributed around the circumferences of the coxa, femur and tibia. Cellular interactions along the edges of strip-grafts obey the Shortest Intercalation Rule.

At the ends of strip-grafts intercalation usually restores continuity of positional values where possible but, when a complete circumference is generated, a supernumerary distal regenerate is usually formed. This is in general agreement with the Complete Circle Rule and the exceptions are discussed.

In intercalary regeneration following the intersegmental strip-grafts, the host femur cells seem unable to intercalate beyond two positions (posterior/internal and posterior/external). These lineage restrictions operating during regeneration indicate that the cockroach leg, like the Drosophila leg disc, may consist of an anterior and a (smaller) posterior ‘compartment’.

INTRODUCTION

Developing cells divide, move and differentiate to form characteristic spatial patterns. Grafting and extirpation experiments have repeatedly shown that the cells can respond (‘regulate’) to changes in position by adjusting their developmental fate (termed ‘morphallaxis’ by Morgan (1901)) or their growth rate and fate (‘epimorphosis’). This result implies that cells do not normally develop autonomously according to instructions localized in that part of the egg from which they are descended, but rather they develop according to positional information (Wolpert, 1969) derived from interactions with their neighbours. It is important to determine whether such cellular interactions give positional

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information in one, two or three dimensions (i.e. within files, sheets or blocks of cells). In addition, each cell's positional information may be unique or may consist of several independent components, so that the field of positional information would resemble a conventional ‘map’ with each point characterized by a combination of the positional values along different axes. We have argued (French, Bryant & Bryant, 1976) that secondary embryonic fields have such a two-dimensional map with two sequences of positional values specified along polar (rather than orthogonal) co-ordinates.

Grafting experiments performed on the cockroach larval leg segment demonstrate independent sequences of positional values running down and around the leg epidermis. Bohn (1967, 1970a) and Bullière (1971) showed that grafting together different proximal-distal levels of a segment results in localized growth at the junction and the intercalary regeneration of the intermediate levels between graft and host. Similarly, when strips of femur epidermis are grafted into different circumferential positions (French, 1978) normally non-adjacent cells interact along the graft-/host junctions. Regardless of the proximal-distal levels involved, this results in growth and the intercalary regeneration of the shorter section of the femur circumference which normally separates the confronted cells. At the ends of a strip-graft, a supernumerary distal regenerate often formed if the graft, plus the intercalary regenerates formed along the edges, plus the adjacent host tissue together form a complete femur circumference.

This and much other evidence from regenerating insect and amphibian appendages suggested that secondary fields have a sequence of positional values running proximal-distal, and an independent continuous sequence of values running around the circumference. If the appendage is represented as a circle with the distal tip in the centre, position is specified by polar co-ordinates, with every point having a radial and an angular value (French et al., 1976). Interactions between cells bearing different values follow a Shortest Intercalation Rule (whereby intercalary regeneration removes discontinuities of positional value) and a Complete Circle Rule (whereby distal regeneration occurs from any complete circumference exposed by amputation or generated by grafting).

The cockroach leg consists of several segments bearing different cuticular patterns. Bohn (1970a, b, 1976b) and Bullière (1971) grafted together different levels of different leg segments and demonstrated that the proximal-distal sequence of values is repeated in each segment. In the experiments reported here I have grafted together cells from different positions around the tibia and the coxa, and have performed similar grafts between the different segments. Some preliminary results have been published (French & Bullière, 1975). The results indicate that the same circumferential sequence of positional values is present at all levels of the leg, and also suggest that the cockroach leg (like the imaginal leg disc of Drosophila) consists of two lineage compartments.
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MATERIALS AND METHODS

Laboratory stocks of the cockroach, *Blabera cranifer*, were maintained as previously described (French, 1978) and 3rd-6th instar larvae were used for grafting operations 2-4 days after molting. Operations involved removing a longitudinal strip of cuticle plus epidermis from a particular circumferential position on the host metathoracic leg, and grafting into the site a strip of similar size taken from a precise position on the donor meso- or metathoracic leg (see French, 1978 for details of the technique). Grafts were performed between tibia and tibia (Series TT), coxa and coxa (Series CC), tibia and femur (Series TF), and coxa and femur (Series CF). In some cases, the operated leg autotomized or the grafted epidermis died, but successful grafts were clearly present after the 2nd or 3rd post-operative moult, when the operated leg was removed, fixed and examined.

The coxa, femur and tibia of the normal non-regenerated *Blabera* leg are shown in Fig. 1. Following the conventions developed previously for the femur (French, 1978) epidermal cell position on the leg segment will be denoted by proximal/distal level and circumferential position (with respect to the four faces of the leg (anterior, posterior, internal and external) and, more precisely, by numbered positions $1^F-12^F$ for the femur, $1^C-12^C$ for the coxa, and $1^T-12^T$ for the tibia: see Fig. 1). The results of strip-grafts will be given in terms of the four graft/host junctions: the inner edge (the longitudinal junction nearest to the host internal face) and outer edge, and the proximal and distal ends.

RESULTS AND DISCUSSION

Pattern of cuticular structures on the leg

The metathoracic femur has several longitudinal cuticular features at particular circumferential positions. The external face bears a double row of bristles on a band of dark cuticle (positions $12^F-1^F$) and the internal face has a sparse row of long anterior bristles (position $7^F$) and a dense row of posterior bristles (position $5^F$), separated by a band of very light cuticle. These cuticular structures also occur reliably on regenerated legs (French, 1978) and can thus be used as circumferential markers.

The metathoracic or mesothoracic tibia bears numerous large spines, restricted to a double row along the internal face (positions $7^T-5^T$) and three irregular rows along the external face (positions $11^T-1^T$). Scattered bristles occur on the posterior face ($2^T-4^T$) but not on the anterior face ($8^T-10^T$). On the tibia of a regenerated leg, the spines are more numerous but are similarly restricted to internal and external faces.

The meso- or metathoracic coxa is an approximately conical segment whose cuticular features change slightly along its length. In general, there are dense bristles on the internal and anterior regions (positions $6^C-11^C$), sparse bristles
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on the external face ($1^{c}-2^{c}$) and no bristles on much of the posterior face ($3^{c}-6^{c}$). The prominent distal flange (position $10^{c}$) is a useful circumferential marker.

Tibia/tibia (TT) and coxa/coxa (CC) strip grafts

Control grafts

Strips were removed from a number of different positions on the meso- or metathoracic tibia or coxa, and grafted into corresponding sites on the host metathoracic tibia or coxa, respectively. All successful control grafts healed with no sign of regeneration at any graft/host junction.

TT Graft 1: left internal face grafted to left anterior face

As shown in Fig. 2, a strip was grafted from the internal face of the left metathoracic tibia, into the anterior face of the host left metathoracic tibia. Along the outer junction an unrecognizable intercalary regenerate was formed, while along the inner junction an internal tibial face was intercalated. In no case were any supernumerary distal structures formed at the ends of the graft.

TT Graft 2: right posterior face grafted to left anterior face

As shown in Fig. 3, a strip was grafted from the posterior face of the right metathoracic tibia, into the anterior face of the host left tibia. Unrecognizable tissue was formed along the outer junction while along the inner junction, a tibial internal face was intercalated. At the proximal end of the graft, there was no regeneration or regeneration of a tibial apex and four-segmented tarsus and, at the distal end, a tibial apex and tarsus were regenerated.

CC Graft 1: right posterior face grafted to left anterior face

A strip was removed from the posterior face of the right mesothoracic coxa and grafted into the anterior face of the host left coxa. The coxa is a conical segment, and the results of the short strip-grafts are difficult to interpret in terms

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Fig. 1. Cuticular pattern of the metathoracic leg of Blabera craniifer. A, P, I, E, anterior, posterior, internal and external faces of the leg; p, proximal; d, distal; ff, flange at the distal end of the anterior face of the coxa; Tr, trochanter; t, tarsus. Twelve positions are marked around the circumference of the metathoracic coxa (Co) by numbers $1^{c}-12^{c}$, femur (Fe) by $1^{f}-12^{f}$, tibia (Ti) by $1^{t}-12^{t}$. The left metathoracic leg is shown by camera lucida drawings of the anterior view (i) and cross-sections at the level of mid coxa (ii), mid femur (iii) and mid tibia (iv), showing the distribution of bristles and spines. On the femur the bands of internal light cuticle (positions $5^{f}-7^{f}$) and external dark cuticle (positions $12^{f}-1^{f}$) are shown by dots and filled circles respectively, and position $5^{f}$ and $7^{f}$ bristles are distinguished by size whereas, in fact, the former are much more numerous. A ii–A iv also show lengths of segment which have been cut along the posterior face and opened out flat to show the cuticular features. The femur internal face (iii) is shown by dots and the dark cuticle of the external face by dashed lines, and the tibial spines (iv) have been removed and their bases shaded.

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Fig. 2. TT Graft 1, left tibia internal face grafted to left tibia anterior face. i, ii, schematic cross-sections showing the operation (i) and the result (ii) after two moults (M). iii, camera lucida drawing of resulting tibia cut along the posterior face and opened out flat, as in Fig. 1 iv. At both inner and outer graft/host junctions the part of the circumference normally separating the confronted edges of graft (G) and host (H) has been intercalated (R). Unrecognizable (presumably anterior face) tissue has formed along the outer junction (53/53 cases) and a recognizable internal face has been formed at the inner junction (with spines, usually in two rows, in 47/53 cases).

of the four original graft/host junctions. Only 8/22 successful grafts formed interpretable structures (see Fig. 4). In all eight cases (and also in many of the uninterpretable cases) a distal flange, a coxal apex and a broken distal regenerate were formed at both proximal and distal ends of the graft.

**Tibia/tibia and coxa/grafts: discussion**

The results of TT Grafts 1 and 2 (and other similar strip grafts involving the external and anterior faces) demonstrate that intercalary regeneration occurs between tibial cells from different circumferential positions. Evidently, tibial spines are not formed reliably on intercalary regenerates (in contrast to their formation on distal regenerates) but nonetheless the results are consistent and compatible with the Shortest Intercalation and Complete Circle Rules. Hence intercalation between $7^t$ and $4^t$ (inner junctions of both grafts) forms $7^t$, $6^t$ and $5^t$ (the internal face) and often the appropriate spines; interaction between
Fig. 3. TT Graft 2, right tibia posterior face grafted to left tibia anterior face. i–ii, abbreviations and notations as in Fig. 2. At the outer junction unrecognizable (presumably external) tissue has formed (19/19 cases) while an internal face has been intercalated along the inner junction (with spines in 6/19 cases). iii, anterior/internal view of resulting tibia showing the internal face of the intercalary regenerate (RI) formed between the grafted posterior face (GP) and the internal face of the host (HI). HA, anterior face of host tibia; S, supernumerary tibial apex and tarsus regenerated from proximal and distal graft/host junctions.

10_{1/2}^T$ and $7_{1/2}^T$ forms $10^T$, $9^T$ and $8^T$ (the anterior face), while intercalation between $10_{1/2}^T$ and $1_{1/2}^T$ forms the external face (11$^T$, 12$^T$, 1$^T$) but not the external spines. This interpretation is supported by the supernumerary distal structures formed from the ends of TT Graft 2 where host (anterior), graft (posterior) and intercalary regenerates (internal and external) form a complete circumference.
In contrast, at the ends of TT Graft 1 the host (anterior), graft (internal) and the two intercalary regenerates (anterior and internal), do not form a circumference, and no supernumeraries form.

Despite the lack of reliable coxal markers, the results of CC Graft 1 (and other similar grafts) are also consistent and compatible with the Shortest Intercalation and Complete Circle Rules. Hence intercalation between $9\frac{1}{2}$ and $3C$ forms external face (indicated by the flange of $10C$) while $8C$ and $3\frac{3}{4}$ interact to form internal structures. Similarly, supernumerary distal structures form from the circumferences generated at the ends of CC Graft 1 but not from the ends of other grafts performed between adjacent faces.

These results indicate that the tibia and the coxa, like the femur (French, 1978) have continuous sequences of positional values $(1^T-12^T, 1^C-12^C$ and $1^F-12^F$ respectively) corresponding to the positions of the epidermal cells around the circumference (see Fig. 1). Bulliere (1971) suggested that circumferential position is a general feature of leg organization, independent of leg segment type. This would imply that a common circumferential sequence of values is present at all levels of the leg. I have investigated this possibility by grafting strips of epidermis between different leg segments, thereby confronting cells of different segment type, but from corresponding or non-corresponding circumferential positions.

Tibia/femur (TF) strip-grafts

TF Graft 1: right posterior tibia grafted to right posterior femur

A strip was grafted from the posterior face of the right mesothoracic tibia, into the posterior face of the host right femur. In all 31 successful cases the graft retained its scattered bristles and darker colour but no regeneration occurred from the edges or ends of the graft.

Similar grafts of external tibia to external femur, and of anterior tibia to anterior femur also failed to provoke any regeneration, so either homologous positional values are similarly distributed around the femur and tibia (as implied in the labelling of Fig. 1), or the systems of positional values are distinct and the cells do not interact. To distinguish between these alternatives, I have grafted tibial strips to non-corresponding sites on the femur.

Fig. 4. CC Graft 1, right coxa posterior face grafted to left coxa anterior face. i, ii, schematic cross-sections of the operation (i) and the result (ii), showing intercalary regeneration (R) between the confronted edges of graft (G) and host (H). iiiia, camera lucida drawing of anterior/internal view of a resulting coxa showing the internal face (RI) intercalated between the grafted posterior face (GP) and the host internal face (HI); S, broken supernumerary distal structures formed at proximal and distal graft/host junctions. iiiib, anterior/external view of the resulting coxa showing the external face (RE) formed between the graft (GP) and the host external face (HE). The external and anterior faces of the apex of the coxa are characterized by articulatory membrane (A) and a cuticular flange (fl) and these structures, plus a broken supernumerary (S) are formed at both ends of the graft.
TF Graft 2: left internal tibia grafted to left anterior femur

A strip was grafted from the internal face of the left meso- or metaphothoracic tibia, into the anterior face of the host left femur. In all 86 successful cases the graft retained its double row of tibial spines, and intercalary regeneration occurred along both longitudinal edges. An intercalary regenerate consisting of unrecognizable tibia and femur tissue was formed along the outer junction, while internal tibia and femur structures were intercalated along the inner junction, as shown in Fig. 5. At both proximal and distal ends of the graft there was some intercalary regeneration in all but one case, when a tibial apex was regenerated.

TF Graft 3: left external tibia grafted to left anterior femur

A strip was grafted from the external face of the left mesothoracic tibia, into the anterior face of the host left femur. Tibial and femur tissue was intercalated along the outer junction (forming unrecognizable or external structures) and
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Fig. 6. TF Graft 3, left tibia external face grafted to left femur anterior face. i–ii, abbreviations and notations as in Fig. 5. iii, camera lucida drawing of the anterior view of a resulting femur. The femur external face of the host (HFE) and of the intercalary regenerate (RFE) formed along the outer junction is shown by bristles and dashed lines. Heavy dashed lines separate host, intercalary regenerate and the grafted tibia external face (GTE), and the limit of tibial cuticle is shown by a light continuous line. The outer junction formed a recognizable external femur face in 11/23 cases and unrecognizable tissue in 12 cases, while the inner edge formed unrecognizable tissue in all 23 cases. R, intercalary regenerate formed at distal and proximal ends of the graft; HFA, anterior face of the host femur.

along the inner junction (forming unrecognizable tissue), as shown in Fig. 6. At both proximal and distal ends of the graft there was some intercalary regeneration in all cases.

TF Graft 4: right posterior tibia grafted to left anterior femur

A strip was grafted from the posterior face of the right metathoracic tibia, into the anterior face of the host left femur. Femur and tibia tissue was inter-
calated along the inner junction (forming internal structures) and the outer junction (forming external, internal or unrecognizable structures), as shown in Fig. 7. In cases where the 'internal' pattern was formed along both inner and outer edges, intercalary regeneration of internal structures also occurred around the proximal and distal ends of the graft. In cases where the 'external' or 'unrecognizable' pattern was regenerated along the outer edge there was either no regeneration or supernumerary distal regeneration from the proximal and distal ends of the graft.

As shown in Fig. 8, the distal structures regenerated from the end of a graft consisted of a composite femur/tibia segment apex plus a four-segmented tarsus with, on the host femur side, a proximal bulge of tissue often bearing a few tibial spines.

**TF Grafts 1–4: discussion**

The results of TF Grafts 2–4 demonstrate that confrontation of tibial and femur cells from non-corresponding circumferential positions leads to intercalary regeneration of bands of tibial tissue (on the graft side) and femur tissue (on the host side). The absence of intercalation following TF Graft 1 can therefore be attributed to similar distributions of homologous positional values around the two segments (see Fig. 1) so that cells from corresponding circumferential positions have similar values (e.g. \(4^F\) and \(4^T\)). Assuming this distribution of homologous tibia and femur values, then the nature of structures intercalated between cells with different values conforms to the Shortest Intercalation Rule. Hence, for example, tibia and femur internal structures (positions 5, 6, 7) are formed between \(4^T\) and \(8^F\) (inner junctions of TF Grafts 2 and 4), and femur external structures (positions 12, 1) are formed between \(2^F\) and \(9_{\frac{1}{3}}^F\) (outer junction of TF Graft 3). Furthermore, the confrontation of approximately opposite positions (\(2^T\) and \(9^F\) in TF Graft 4) leads to intercalation of either the internal or external half circumference. Analysis of a number of different femur/femur grafts (French, 1978) showed that the confrontation between positions approximately \(3^F\) and \(9^F\) (but not between other pairs of opposite positions) also gave a 3rd category of result where graft and host healed together with no intercalation. This did not occur between \(2^T\) and \(9^F\) in TF Graft 4 so a further graft (TF 6 – see below) was performed.

It is striking that the border between the tibial and femur components of the internal face intercalated between \(4^F\) and \(8^F\), formed reliably between \(5^T\) and \(6^F\). In all cases (from 86 TF Graft 2 and 68 TF Graft 4 inner junctions) the light femur internal face cuticle (\(6^F\)) was flanked on the graft side, not by the dense row of bristles normally found at position \(5^F\), but by a band of tibia cuticle often bearing a single row of spines (\(5^T\)). Furthermore, where internal structures were intercalated between \(2^T\) and \(9^F\) (9 TF Graft 4 outer junctions) the tibia/
Fig. 7. TF Graft 4, right tibia posterior face grafted to left femur anterior face. Abbreviations and notations as in Fig. 6. Along the inner graft/host junction internal tibia and femur structures were intercalated (iiia), with the border forming reliably between positions $5^T$ and $6^F$ in all 68 cases. Along the outer junction there was intercalation of a half circumference bearing either a femur external face (iib, 26/68 cases) or internal tibia and femur structures (iiib, 26/68 cases) or no recognizable structures (29/68 cases). In the remaining four cases, ‘internal’ and ‘unrecognizable’ patterns were formed along different parts of the same junction, separated by a regenerated segment apex iii. Camera lucida drawings of the anterior/external (iiiia) and anterior/internal (iiib) views of a resulting femur showing the femur external face (RFE) intercalated along the outer junction, the internal tibia (RTI) and femur (RFI) structures intercalated along the inner junction, and the (broken) supernumerary distal regenerates (S) formed at the ends of the graft.
Fig. 8. Supernumerary structures regenerated from two distal graft/host junctions after TF Graft 4 (see Fig. 7). The regenerates consist of a segment apex bearing tibial spines (TA) on the graft side and femur articulatory membrane (FA) on the host side, and then a composite segment consisting of first tarsal segment (r') with a lateral bulge of tibial cuticle (T) bearing characteristic spines, and then the distal segments (r''-r') of a regenerated tarsus.

femur border again formed between $5^T$ and $6^F$. Further grafts (see below) were performed to confront other tibia and femur positions.

**TF Graft 5: left internal/posterior tibia grafted to left anterior femur**

A strip comprising half of the internal and half of the posterior face was grafted from the left mesothoracic tibia into the anterior face of the host left femur. Intercalary regeneration of tibial and femur internal structures occurred along both longitudinal edges of the graft, as shown in Fig. 9. At proximal and distal ends of the graft, intercalary regeneration usually joined the internal structures formed along both edges but, in the remaining cases, there was no regeneration or intercalary regeneration plus the formation of more distal structures (Fig. 9iii).

**TF Graft 6: right posterior tibia grafted to left anterior/external femur**

A strip was grafted from posterior right metathoracic tibia into an anterior/external position on the host left femur. Intercalation of a femur external face occurred along the outer edge, while at the inner edge there was either no intercalation or formation of tibial and femur internal, external or unrecognizable structures, as shown in Fig. 10. At proximal and distal ends of the graft, there was either no regeneration (usually associated with external or unrecognizable
Fig. 9. TF Graft 5, left tibia internal/posterior face grafted to left femur anterior face. Abbreviations and notations as in Fig. 6. Along the inner graft/host junction internal tibia and femur structures were intercalated, with the border forming between $5^T$ and $6^T$ in all 29 cases. Along the outer junction internal tibia and femur structures were intercalated in 24/29 cases, with unrecognizable tissue forming in four cases and external structures forming in one case. iii, anterior view of two resulting femurs showing intercalary regeneration of internal tibia ($RTI$) and femur ($RFI$) along the longitudinal edges of the graft ($G$). At the ends of graft usually intercalary regeneration joined the two sets of internal structures (iii a, distal end) but sometimes there was no regeneration (iii a, proximal end) or intercalary regeneration plus the formation of a segment apex (iii b) with distal femur spines ($sp$) and articulatory membrane ($FA$).
Fig. 10. TF Graft 6, right tibia posterior face grafted to left femur anterior/external face. Abbreviations and notations as in Fig. 6. Along the outer graft/host junction the intercalary regenerate bore a recognizable femur external face in 49/57 cases (iiia), but at the inner junction there were four classes of result: iiia, no intercalation (NR) between the confronted graft (G) and host (H) positions in 11/57 cases; iiib, intercalation of a half circumference bearing tibial and femur internal structures in one case; iicc, intercalation of the half circumference bearing a femur external face in 6/57 cases; and intercalation of unrecognizable tissue in one case. In the remaining 38 cases different results occurred at different points along the same junction. iii, anterior view of a resulting femur showing the external structure (RFE) formed along the outer junction, intercalated internal structures (RTI, RFI) and absence of intercalation (NR) at different points along the inner junction, and a broken supernumerary (S) formed at the distal end of the graft (G).

intercalation along the outer edge and no intercalation along the inner edge), intercalary regeneration joining external structures formed along both edges, or distal regeneration of an apex or a complete supernumerary (associated with
Fig. 11. TF Graft 7, left tibia anterior face grafted to right femur posterior face. Abbreviations and notations as in Fig. 6. iv, posterior view of a resulting femur showing the unrecognizable intercalary regenerate (RE) formed along the outer junction in 41/45 cases (internal structures were formed in four cases), the internal tibial and femur structures (RI) intercalated along the inner junction in 28/45 cases (unrecognizable tissue was formed in 17 cases) and the (broken) supernumerary regenerate (S) formed at the distal end of the graft.

external or unrecognizable intercalation along the outer edge and internal or no intercalation along the inner edge).

**TF Graft 7: left anterior tibia grafted to right posterior femur**

A strip was grafted from the anterior face of the left metathoracic tibia into the posterior face of the host right femur. An internal or unrecognizable intercalary regenerate of both tibial and femur tissue was formed along both longitudinal edges, as shown in Figure 11. Where recognizable femur ‘internal’ structures had been regenerated along both longitudinal graft/host junctions, intercalary regeneration joined them around the ends of the graft. Where the outer edge bore an ‘unrecognizable’ regenerate while the internal edge had
regenerated ‘internal’ structures, supernumerary distal structures were usually formed at the ends of the graft, but when both edges bore ‘unrecognizable’ regenerates usually no distal structures formed.

TF Grafts 5–7: discussion

The results of TF Grafts 1–4 showed that confrontations between tibia and femur cells obey the Shortest Intercalation Rule and the results of TF Grafts 5–7 support this conclusion. Hence external structures are clearly formed between 11\(F\) and 2\(T\) (TF Graft 6), while internal structures are formed between 9\(F\) and 6\(T\), 8\(F\) and 3\(T\) (TF Graft 5), and between 3\(\frac{1}{2}F\) and 8\(T\) (TF Graft 7). In addition, the confrontation between the more-or-less opposite positions 9\(F\) and 4\(T\) (TF Graft 6) could intercalate either of the intervening half circumferences, or neither – the ‘null’ result previously found in similar femur-femur confrontations (French, 1978). As in the femur grafts, other confrontations between opposite positions do not give the null result (the 9\(F\)/2\(F\) confrontation of TF Graft 4: unpublished 8\(F\)/2\(T\) confrontation giving 20/37 external, 2 unrecognizable and 15/37 internal intercalations).

The results of TF Grafts 1–6 demonstrate that the ends of tibia-femur grafts usually obey the Complete Circle Rule. Supernumerary distal regenerates usually form when a complete circumference is generated by graft, host and recognizable intercalary regenerates. When the same structures are intercalated along both edges, intercalary regeneration joins them around the ends. If the Complete Circle Rule also applies to the results of TF Graft 7, it suggests that the ‘unrecognizable’ intercalary regenerate (17 inner and 41 outer junctions) consists of an external part-circumference, occasionally bearing tibial spines, but never bearing femur external structures. Hence the 2\(\frac{1}{2}F\)/10\(T\) outer junction usually intercalates external structures, as predicted by the Shortest Intercalation Rule.

In 154 cases from TF Grafts 2 and 4, the border between the tibial and femur components of an ‘internal’ regenerate intercalated between 4\(T\) and 8\(F\), formed between positions 5\(F\) and 6\(F\). In TF Grafts 5 and 6, ‘internal’ tissue was intercalated between 3\(T\) and 8\(F\) and between 4\(T\) and 9\(F\) respectively but, in all 56 cases, the border again formed between 5\(T\) and 6\(F\). Unpublished confrontations between 2\(T\) and 8\(F\) (see above) gave the same result in all 15 cases. Reciprocally, when an internal regenerate is formed between anterior tibia and posterior femur, as in the 3\(\frac{1}{2}F\)/8\(T\) confrontation of Graft 7, position-5 structures are of femur type while adjacent tissue is tibial, occasionally bearing spines and presumably corresponding to 6\(T\) and 7\(T\). This is also true for internal regenerates formed between 4\(F\) and 10\(T\) (unpublished – 31 cases) and between 2\(\frac{1}{2}F\) and 8\(T\) (unpublished 31 cases). Tibial internal spines are not formed reliably, so interpretation depends upon which femur structures are present or absent. Nonetheless, it seems that the border between tibia and femur components of an internal intercalary regenerate always forms between circumferential positions 5 and 6, whatever the confrontation.
When external structures are intercalated between anterior femur and posterior tibia, a recognizable femur external face \((12^F-1^F)\) is often formed (the \(2^T/9^F\) confrontation of TF Graft 3, 11/23 cases; \(2^T/9^F\) of TF Graft 4, 26/59 cases; \(2^T/11^F\) of TF Graft 6, 49/57 cases; unpublished \(4^T/11^F\), 42/42 cases). However, when intercalation between posterior femur and anterior tibia produces tissue which is clearly not internal and which can be interpreted (on the basis of supernumerary structures formed from the ends of the graft) as external, then a femur external face is never formed (the \(10^T/2^F\) confrontation of TF Graft 7, 41 cases; \(8^T/3^F\) of TF Graft 7, 17 cases). This is difficult to interpret since external structures are not always formed on femur-femur external regenerates (e.g. only 20/26 cases following the \(2^F/8^F\) confrontation of Graft 3 of French (1978)). Nonetheless, the results indicate that the border between femur and tibia components of an external regenerate may reliably form posterior to position 1. Hence femur external face bristles \((12^F-1^F)\) are never formed from an anterior tibia/posterior femur confrontation, but are usually formed following an anterior femur/posterior tibia interaction.

This interpretation of the composition of intercalary regenerates also applies to those formed between femur and coxa (see below).

**Coxa/femur (CF) strip-grafts**

**CF Graft 1: right posterior coxa grafted to right posterior femur**

A strip was grafted from the posterior face of the right mesothoracic coxa, into the posterior face of the host right femur. In all 59 successful cases, the graft retained its appearance, but no regeneration occurred from the graft/host junctions. Similar grafts of anterior coxa to anterior femur also failed to provoke appreciable intercalary regeneration.

**CF Graft 2: left external coxa to left anterior femur**

A strip was grafted from the external face of the left mesothoracic coxa, into the anterior face of the host left femur. Intercalary regeneration of a femur external face occurred along both longitudinal edges of the graft and around the proximal and distal ends, as shown in Fig. 12.

**CF Graft 3: right posterior coxa grafted to the left anterior femur**

A strip was grafted from the posterior face of the right metathoracic coxa, into the anterior face of the host left femur. Intercalary regeneration of coxa and femur tissue occurred along both edges of the graft, forming internal structures along the inner edge and either external, internal or unrecognizable structures along the outer edge, as shown in Fig. 13. Where ‘internal’ structures were regenerated along both longitudinal edges, intercalary regeneration joined them around the ends of the graft (often with formation of a rudimentary apex at the distal end – Fig. 13v). Where ‘internal’ structures were formed along the inner edge with an ‘external’ or ‘unrecognizable’ regenerate intercalated along the
Fig. 12. CF Graft 2, left coxa external face grafted to left femur anterior face. Abbreviations and notations as in Fig. 6. An intercalary regenerate (RF) bearing a femur external face is formed along outer (23/23 cases) and inner (22/23 cases) graft/host junctions.

Fig. 13. CF Graft 3, right coxa posterior face grafted to left femur anterior face. i–iii, abbreviations and notations as in Fig. 12. Along both graft/host junctions an intercalary regenerate was formed consisting of coxa (RC) and femur (RF). The inner regenerate bore a partial femur internal face (ii a) with the border between femur and coxa components (shown by a double line) forming reliably between positions 5C and 6F in all 38 cases. The outer regenerate usually (31/38 cases) bore a femur external face (ii a) but sometimes consisted of internal structures (ii b) with, again, the border forming between positions 5C and 6F. iii, camera lucida drawings of the external (iii a) and internal (iii b) views of a resulting femur, showing external structures (RE) formed along the outer junction, the internal structures (RI) intercalated at the inner junction and the (broken) supernumerary distal regenerates (S) formed at the ends of the graft. iv, supernumerary regenerate formed from a distal graft/host junction. The composite coxa/femur segment apex has femur articulatory membrane (FA) and femur distal spines (sp), and bears a composite segment consisting of tibia (T) with a lateral bulge (X), and then a tarsus (t1–t4). v, intercalary regenerate formed around the distal end of a graft (G), joining internal structures (RI) formed along both longitudinal edges of the graft. There has also been formation of the articulatory membrane (FA) and spines (sp) found on the distal apex of the femur.
outer edge, the proximal and distal ends usually formed supernumerary distal structures. These were often broken off but complete regenerates consisted of a composite coxa/femur segment apex, an unrecognizable bulge on the graft side and then a tibia and tarsus (Figure 13iv).

**CF Graft 4: left anterior coxa grafted to right posterior femur**

A strip was grafted from the anterior face of the left mesothoracic coxa, into the posterior face of the host right femur. Intercalary regeneration of coxa and femur tissue occurred along both edges of the graft, forming internal structures along the inner edge and usually forming unrecognizable structures along the outer edge, as shown in Fig. 14. At proximal and distal ends of the graft there was usually regeneration of a composite apex with the characteristic external coxal flange on the graft side (see Fig. 14), bearing a rudimentary or broken distal regenerate.

**CF Grafts: discussion**

The results of CF Graft 1 (and other unpublished grafts) show that confrontation of cells from similar circumferential positions on femur and coxa does not lead to intercalation. Results of CF Grafts 2-4 (and many others) show that other confrontations lead to intercalation of bands of coxa (on the graft side) and femur (on the host side). If homologous positional values are similarly distributed around the coxa and femur (see Fig. 1), then the nature of the intercalary regenerates generally conforms to the Shortest Intercalation Rule. Hence internal structures form between $2^C$ and $8^F$ (CF Graft 3) while external structures form between $2^F$ and $10^F$ (CF Graft 2).

The results of CF Grafts 1–3 show that the ends of coxa strip-grafts usually obey the Complete Circle Rule with supernumerary distal regenerates forming where a complete circumference is generated around the end of a graft (CF Graft 3). Applying this rule to CF Graft 4 suggests that the regenerate formed along the outer edge of the graft (between $2^F$ and $9^C$) is external (as predicted by the Shortest Intercalation Rule).

The interaction between cells from approximately opposite positions ($3^C$ and $9\frac{1}{2}^F$ in CF Graft 2) can lead to intercalation of either intervening half circumference. A few results from unpublished experiments suggest that the $3^C/9^F$ confrontation can also give rise to the 'null' result of no intercalation, as in corresponding femur-femur (French, 1978) and tibia-femur grafts (TF Graft 6).

When an *internal* intercalary regenerate is formed between anterior femur and posterior coxa, the border between femur and coxa components forms so that femur sparse bristle row ($7^F$) and light cuticle ($6^F$) are formed, but not the dense bristle row ($5^F$). This has been shown for the following confrontations: $8^F/3^C$ (CF Graft 3, 38 cases; unpublished, 42/43 cases); $9\frac{1}{2}^F/3^C$ (CF Graft 3, 4 cases); $9\frac{1}{2}^F/4^C$ (unpublished, 20 cases), $8^F/5^C$ (unpublished, 26 cases), $9\frac{1}{2}^F/4^C$ (unpub-
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Fig. 14. CF Graft 4, left coxa anterior face grafted to right femur posterior face. Abbreviations and notations as in Fig. 13. Along the outer junction an unrecognizable intercalary regenerate (R) was formed in 20/23 cases (internal structures were formed in three cases) while, along the inner junction, there was formation of coxa (RC) and femur (RF) bearing a partial femur internal face in all 23 cases. iv, posterior/external (iva) and posterior/internal (ivb) views of a resulting femur, showing the unrecognizable outer regenerate (R), the internal inner regenerate (RI) and the (broken) supernumerary regenerates (S). fi, flange found at an anterior/external position on the apex of the coxa.

lished, 20 cases). Thus in only 1/156 internal regenerates were the $5^F$ bristles formed. Similarly, an internal intercalary regenerate formed between posterior femur and anterior coxa has the dense femur bristles of position $5^F$ and usually a
little light cuticle, but not the sparse bristles of position $7^F$. This has been shown for the following confrontations: $3^F/8^C$ (CF Graft 4, 22 cases), $2^F/9^C$ (unpublished, 16 cases), As in the tibia/femur grafts, interpretation depends solely on the presence or absence of femur structures but, again, it seems that the border between the two components of an internal intercalary regenerate lies between circumferential positions 5 and 6.

An external intercalary regenerate formed between anterior femur and posterior coxa usually bears a recognizable femur external face ($12^F-1^F$), as shown for confrontations $10^F/2^C$ and $8^F/1^C$ (CF Graft 2), $9^F/3^C$ (CF Graft 3) and for $10^F/1^C$ and $8^F/2^C$ (unpublished). However, when intercalation between posterior femur and anterior coxa produces tissue which can be interpreted as external (on the basis of supernumerary structures formed from the ends of the graft), then a femur external face is never formed. This has been shown for confrontation $2^F/9^C$ (CF Graft 4, 11 cases) and $3^F/9^C$ (unpublished, 17 cases). As explained above for the femur-tibia grafts, interpretation is difficult, but these results are consistent with the border between the components of an external intercalary regenerate always forming posterior to position 1.

**DISCUSSION**

Intercalary regeneration occurs at graft/host junctions between femur epidermal cells from different circumferential positions (French, 1978). The new tissue forms cuticular structures characteristic of the shorter arc of circumference normally separating the graft and host cells (the Shortest Intercalation Rule of French et al. 1976). These results suggested that the femur epidermal cells carry rather stable positional values which form a continuous (and probably fairly equally spaced) sequence around the circumference ($1^F-12^F$, Fig. 1). Experimentally produced discontinuities in the sequence result in local growth and intercalation of the shorter set of intermediate positional values, re-establishing continuity.

Analysis of the results of strip-grafts involving the other leg segments is more difficult, because there are fewer reliable cuticular markers. The results of grafts at tibial (TT Grafts) and coxal (CC Grafts) levels indicate that the epidermis of these segments also has circumferential positional values and obeys the rule of intercalation. Furthermore, interaction between cells from different circumferential positions on different segments (TF Grafts and CF Grafts) leads to intercalation of the shorter intermediate arc of circumference, composed partly of graft-segment and partly of host-segment structures. These results show that the same set of positional values is evenly distributed around the femur, the tibia and (rather less evenly) around the coxa (Fig. 1).

As Bullièrè (1971) suggested, circumferential value (or 'génératrice') is an aspect of positional information independent of proximal-distal level within a segment (French, 1978), of leg segment type, and also of metameric segment
Fig. 15. Positional values on the epidermis of the cockroach leg. (A) The epidermal cells of the coxa (Co), femur (Fe) and tibia (Ti) have positional values in a circumferential sequence (1–12) running around the leg. Lines of equal circumferential value (‘génératrices’, see Bullière, 1971) are shown by heavy dashed lines, and by dotted lines in the trochanter (Tr) and tarsus (t) where circumferential positional values have not been demonstrated. Epidermal cells of each segment (except perhaps the trochanter) have also been shown (Bohn, 1970a, b; Bullière, 1971) to have positional values in a proximal distal segmental sequence (A–E). I, articulation between segments (see text). (B) epidermis of the femur and part of the tibia represented in two dimensions with proximal levels around the edge and distal levels in the centre. Positional information is specified in terms of polar co-ordinates, each cell having an angular value (1–12) and a radial value in a sequence (A–E) which is repeated in each leg segment. Very heavy dashed lines mark the proposed borders between anterior and posterior compartments of the leg.

(type (since grafts from pro-, meso- and metathoracic legs behave similarly). Hence, positional value 7 leads tibial epidermis to form large multi-cellular spines, meso- or metathoracic femur to make a sparse row of long bristles, and coxa to make scattered short bristles. The tarsus and trochanter are small and not suitable for strip-grafts, but it is likely that these segments also carry the same sequence of circumferential values.)
Bohn (1967, 1970a) and Bullière (1971) found that interaction between different proximal-distal levels of the tibia resulted in intercalary regeneration of levels normally intermediate between graft and host, indicating that cells at different levels have different positional values. Grafts between different leg segments (Bohn, 1970a, b, 1976b; Bullière, 1971) showed that the sequence of values is repeated in each segment. Hence grafting between mid-femur and mid-tibia results in healing, while grafting between proximal tibia and distal femur results in an unsegmented intercalary regenerate half a segment in length (as does grafting between proximal tibia and distal tibia). The epidermis of the leg thus corresponds to the surface of a cylinder with positional information specified independently in two dimensions: in a continuous sequence (1–12) around the circumference, and in repeating proximal-distal segmental units (A–E) down the axis (Fig. 15).

It is not clear what happens at the intersegmental articulations (I, see Fig. 15). The repeating proximal-distal sequence may be discontinuous, with the distal level (E) of one segment separated from the maximally different proximal level (A) of the next segment by neutral tissue (I). In this case, interaction between very proximal and very distal will always intercalate the mid-segment (BCD). Alternatively, the repeating sequence may be continuous, with very distal differing by only one unit from very proximal (just as 12 only differs by one unit from 1 or from 11 in the circular sequence). In this case the joint and the invaginated apodemes could contain a large part of the sequence, so that the shortest route between the distal and proximal levels used in grafting (A and E) would usually involve the mid-segment (BCD) rather than the joint region (I). This second possibility is suggested by the occasional formation of an articulation in grafts between very proximal and very distal tibia (French, 1976a, Fig. 5), and by analogy with the abdominal segment epidermis of Hemiptera. Numerous grafting experiments (reviewed in Lawrence, 1973a) have suggested that each abdominal segment contains a linear anterior-posterior sequence (a 'gradient') of positional values with a discontinuity at each intersegmental border. Within the segment, intermediate values are created at graft/host borders, possibly by epimorphic intercalary regeneration (Nübler-Jung, 1977) similar to that occurring in leg epidermis. However, confronting very anterior and very posterior levels (by removing the border or by grafting) leads to regeneration of the intersegment border rather than the middle regions of the segment (Wright, 1979). This suggests that the anterior-posterior sequence is continuous and intercalation occurs by the shorter of the two possible routes.

Bohn (1974a, b, c) has performed an extensive series of extirpation and grafting experiments on the thoracic region surrounding the base of the cockroach leg, and interprets his results in terms of several transverse bands having qualitatively different properties (of inducing formation of a leg or sclerites). The results suggest, however, that the circumferential positional values of the leg extend proximally from the coxa onto the surrounding region of thorax...
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(Fig. 16, see French et al. 1976). Indeed, Bohn (1974c) argues that the leg base is somewhat similar to the free appendage since a strip of anterior coxa (but not posterior coxa) can provoke regeneration of a supernumerary leg when grafted to the membrane posterior to the leg. He points out that a confrontation between internal and external faces of the leg results in supernumerary distal regeneration, and the present interpretation of the leg base predicts that this will also occur following confrontation of thoracic epidermis lateral and medial to the leg base. This experiment is difficult to perform on the cockroach thorax, but supernumerary legs do result from confrontation of anterior and posterior, and of lateral and medial thoracic epidermis in Tenebrio larvae (unpublished results).

In general, the results of the present strip-graft experiments, like those of the femur strip-grafts (French, 1978), support the Complete Circle Rule (French et al. 1976). A supernumerary segment apex and the more distal segments usually form when a complete circumference is generated around the end of a graft (e.g. Fig. 7iiii) while, in the absence of a circumference, intercalary regeneration usually occurs around the end of the graft, removing discontinuities in positional value (e.g. Fig. 9iiia). Following an intersegment graft (TF and CF Grafts) the regenerated segment apex is chimaeric and the free regenerate is composed of segments distal to the host (on the host side) and those distal to the graft (on the graft side). It seems that graft- and host-derived parts of the complete circumference each form all levels distal to their origin (Figs. 8, 13iv).

There are some exceptions to the Complete Circle Rule, however. Often distal regeneration does not occur from an apparently complete circumference (especially at the proximal end of a graft – e.g. Fig. 10). More interestingly, in some cases (particularly at the distal end of a graft) where a complete circumference is not formed, nonetheless a rudimentary segment apex is regenerated (Figs. 9iiib, 13v). Similarly, when two external or two internal half-tibiae are grafted together, creating a symmetrical ‘double-half’ amputation surface, usually no distal regeneration occurs, but sometimes a bilaterally symmetrical, distally incomplete regenerate is formed (Bohn, 1965; Bryant, French & Bryant, in preparation). Similarly, bilaterally symmetrical (often distally incomplete) regenerates may form from amphibian ‘double-half’ amputation surfaces (Bryant, 1976; Bryant & Baca, 1978; Stocum, 1978; Tank, 1978; Tank & Holder, 1978; Slack & Savage, 1978a, b). Although there clearly is a relationship between the completeness of the circumference and the occurrence and extent of distal regeneration, it does not seem to be an all-or-none requirement as implied by the Complete Circle Rule.

Distal regeneration may depend on successive rounds of intercalary regeneration which occur as different circumferential regions are confronted during healing (Bryant & Baca, 1978). If the new cells take up progressively more distal values, a complete circumference will continue to generate rings of more distal tissue, but depending on the mode of healing and the number of values present, a double-half circumference may provoke a few rounds of intercalation (and
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hence a partial regenerate from the amputation surface or at the end of the strip-graft) before healing over with no discontinuities between neighbouring cells (see Bryant et al. in preparation).

There are many similarities between the regeneration behaviour of hemimetabolous insect legs and Drosophila imaginal leg discs (French et al. 1976; Bryant et al. in preparation). Epidermal cell position is specified along polar co-ordinates and the interaction which occurs between different cells after grafting or after removal of tissue, leads to intercalation of new cells with intermediate positional values. From the results of mixing fragments from different proximal-distal levels of the evaginated leg disc of Drosophila, Strub (1979) concludes that radial positional values occur as repeating segmental units, as in the cockroach leg. Haynie & Schubiger (1979) reach a similar conclusion for the wing disc.

The results of the present strip-grafts suggest another similarity between cockroach and Drosophila leg development: the subdivision of the leg into anterior and posterior cell lineage compartments. From blastoderm stage, the Drosophila presumptive leg disc consists of two clonally distinct populations of cells which will develop into the anterior and posterior compartments of the leg, with the borders running down the internal and external faces of the leg in precise locations (Steiner, 1976; Lawrence & Morata, 1977). In normal development, clones never cross the borders and will not do so even if the rest of the

Fig. 16. The base of the cockroach leg. A, diagram of the bases of the left meso- (MS) and metathoracic (MT) legs showing the ‘géneratrices’ (see Fig. 15) spreading back from the coxa (Co) onto the sclerites anterior to the leg (Tu, trochantin; Pr praecoxa) and the unsclerotized membrane (Pm) posterior to the leg. U, unsclerotized membrane lying between the posterior margin of one leg field and the anterior margin of the next. The mesothoracic leg is shown removed at the base and the metathoracic leg is shown amputated in mid-coxa. SS, sternal sclerite; Sp, spiracle; Ab, abdomen. B, interpretation of Bohn’s (1974a) extirpation experiments. The extirpated region is shown by horizontal dashed lines and the suggested mode of healing of the parts of the leg field(s) is indicated by large arrows. Structures regenerated after several moults (M) are shown by dots and ringed positional values. Bi, after removal of the leg plus much of the anterior sclerite (Expt. A4 of Bohn, 1974a), the leg and sclerite region regenerates. This would be expected if the remaining regions of the field healed together, intercalated by the shortest route and regenerated from the complete circumference. The regenerated leg (R) is shown removed at the base. Bii, after removal of the leg plus a large area of posterior membrane (Expt. B3 and Fig. 7 of Bohn, 1974a), the remaining anterior sclerites are duplicated, as expected if the anterior part of the field healed its cut edges and intercalated by the shortest route. Biii, after removal of a large band of membrane and part of the metathoracic sclerites (Expt. C6 and Fig. 13 of Bohn, 1974a) the remaining sclerite is duplicated and a supernumerary leg (S) is regenerated, in reversed A/P orientation. This would occur if the posterior region of the mesothoracic field healed to the anterior metathoracic field and intercalated by the shortest route, forming an extra complete circumference for distal regeneration. The mesothoracic and supernumerary legs are shown folded anteriorly and all legs are shown amputated in mid-coxa.
disc is growing much more slowly (although they will almost fill the compartment in which they arise and define its borders for a great distance). It has not yet been possible to demonstrate compartments in the embryonic development of hemimetabolous insect legs, but the lineage restriction observed during circumferential intercalary regeneration suggests that the larval cockroach leg is composed of anterior and posterior compartments.

Many of the TF and CF Grafts involve intercalation between cells from anterior and posterior faces of the leg. In different confrontations with tibia or coxa cells, anterior femur cells can form those internal structures anterior to position approximately $5 \frac{1}{2} F$, and can often form external structures of position $12F-1F$. Reciprocally, posterior femur cells can form internal structures posterior to position $5 \frac{1}{2} F$ and cannot form structures $12F-1F$. This conclusion rests on the assumption that cells continue to make structures of their leg segment of origin while undergoing intercalary regeneration, so that the cellular origin of an intercalary regenerate can be deduced from its structure. From the nature of proximal-distal intercalary regeneration between different levels of different leg segments, Bullière (1971) suggests that cells originating from the more distal segment change their character to that of the proximal segment, so that the intercalary regenerate is of dual origin but of proximal segment type. A range of similar experiments was performed by Bohn (1970a, b, 1971, 1976b), using grafts between different legs, different leg segments and differently pigmented species, and the results give no indication of such a change of leg segment type. The circumferential intercalary regenerate is clearly not wholly of proximal segment type since tibia and femur structures form from TF grafts, and femur and coxa structures from CF grafts. It is reasonable to assume that the dual structure accurately reflects dual origin, and this conclusion is supported by the results of strip-grafts between the pro- and metathoracic femur (unpublished).

If the structure of the intercalary regenerates accurately reflects their origin, then the borders between graft- and host-derived tissue (which form at position $5 \frac{1}{2}$ for internal regenerates, or posterior to position 1 for external regenerates) may correspond to compartment borders set up between anterior and posterior compartments of the femur (and presumably the rest of the leg) during embryogenesis. The lineage restriction during regeneration may not be very precise, however, since the shape of the border between graft- and host-derived tissue and its distance from the nearest femur marker (bristle row $5F$ or $7F$) may vary from specimen to specimen.

Normal cell lineage restrictions are observed during regeneration in abdominal segments of Oncopeltus. Compartment borders form between adjacent abdominal segments at blastoderm stage, and subsequently cells do not cross during normal embryonic and larval growth (Lawrence, 1973b). When the larval intersegmental border is regenerated after grafting or extirpation experiments (see above), the new border forms precisely between the previously anterior and posterior cells (Wright, 1979). In this respect, however, the hemimetabolous
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insect abdomen seems to differ from *Drosophila* imaginal discs where compartment borders can be crossed during regeneration. This has been shown to occur during intercalary regeneration between mixed fragments of the wing disc (Haynie & Bryant, 1976; Adler & Bryant, 1977). A recent study of the regeneration of wounded wing discs *in situ* (Szabad, Simpson & Nöthiger, 1979) suggests that cells may cross to form structures (and presumably take on positional values) of the other compartment only in the early stages of intercalation. Regeneration across a compartment border is most clearly demonstrated in the imaginal leg disc where a quadrant which is completely within the anterior compartment (Steiner, 1976) can regenerate all leg structures, including those of the posterior compartment (Schubiger, 1971; Schubiger & Schubiger, 1978).

When a cockroach leg is grafted, at tibial or coxal level, onto the contralateral stump, one of the transverse axes of the graft (e.g. anterior-posterior) is necessarily reversed relative to the stump, and two supernumerary sets of distal structures form from the junction (Bohn, 1965, 1972a; Bullière, 1970). The supernumeraries form at positions of maximum incongruity (e.g. anterior and posterior) and we have argued (French, 1976b; French *et al.* 1976) that they form from complete circumferences generated at these positions by intercalation between graft and host cells confronted at the junction. A circumference generated in this way (or at the end of a strip-graft) would be expected to be of dual origin with the borders between graft- and host-derived parts corresponding to one or both postulated compartment borders, depending on the exact nature of the graft. Many of Bohn's (1972a, b, 1974a, b, c, 1976a) dual origin supernumerary legs formed after *Leucophaea/Gromphadorhina* grafts, do have longitudinal borders in approximately mid internal and/or external/posterior positions (e.g. Fig. 6 of Bohn, 1972a; Fig. 10 vi of Bohn, 1976a).

The distribution of positional values around the cockroach femur was derived from the results of previous femur strip-grafts (French, 1978) and has been assumed throughout this work (e.g. Fig. 1). If this distribution and also the positions of compartment borders are correct, then the anterior compartment of the cockroach leg contains over half of the circumferential values (Fig. 15B). Hence interaction between anterior compartment positions 1 and 6 may either violate the Shortest Intercalation Rule (to form positions 12, 11, 10, 9, 8, 7) or cross the border to form the posterior compartment (positions 2, 3, 4, 5).

The present experiments show that the circumferential sequence of positional values extends from the cockroach femur to the other leg segments, and indicate that the leg may consist of two lineage compartments. In the larval legs of hemimetabolous insects and the imaginal discs of larval *Drosophila*, epidermal positional information is arranged as a two-dimensional polar co-ordinate 'map', and the creation of abnormal cell interactions leads to growth, forming cells with new positional values. Further work is needed on both systems to reveal the relationships between the embryogenesis and regeneration of the
appendages, and between intercalary regeneration, compartments and distal regeneration.

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