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Pattern regulation and regeneration

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Insect legs develop from small regions of the embryonic thorax. In most insects they differentiate in the embryo, forming functional larval legs, which grow and moult through larval life. In *Drosophila* the presumptive legs invaginate to form imaginal discs, which grow through larval life but only differentiate in the pupal stage. Analysis of the structures formed after amputation, grafting and wounding experiments on larval legs and on mature and immature imaginal discs suggests that the same organization of positional information and cellular behaviour is involved in the response of the developing leg to disturbance at early stages (termed 'regulation') and at later stages (termed 'regeneration').

The results suggest that developing legs form pattern in accordance with positional information specified in two dimensions within the epidermis, along polar coordinates. A continuous sequence of positional values runs around the circumference and an independent sequence runs down the leg. Two rules govern cellular behaviour after a disturbance. *The shortest intercalation rule:* interaction between cells with different positional values provokes local growth, producing cells with intermediate values (by the shortest route in the case of the circumferential values). *The distalization rule:* if intercalated cells have positional values identical to those of adjacent pre-existing cells then the new cells adopt a more distal value. These rules will produce a complete distal regenerate from a complete circumference and may produce a symmetrical regenerate from a symmetrical wound surface. This regenerate may taper (converge) or widen (diverge) and branch into two distal tips, depending on the extent of the original wound and the way in which it heals.

The polar coordinate model provides a simple and unified interpretation, in terms of only local interactions, of a wide range of experimentally produced and naturally occurring insect (and crustacean and amphibian) limbs showing regeneration of missing structures, duplication of structures, and the formation of complete, tapering or branching supernumeraries. It is not yet clear what molecular mechanisms could underlie a polar map of positional information, nor how such a map could be initially established at a particular site in the early embryo.

> *To avoid the taint of theory in morphology is impossible however much it may be wished.*
> (Bateson 1894)

Introduction

The major features of an animal's morphology are set in early embryonic development when there are relatively few cells, and distances are small. Grafting and extirpation experiments on a wide range of embryos have shown that the spatial patterns of tissues and organs are built up by interactions occurring during development, and are not derived directly from some corresponding pattern of determinants preformed in the egg. Cells acquire developmental fates according to positional information derived from interactions with their neighbours (Wolpert 1969). This process of pattern formation is usually studied by means of pattern regulation which is a change in the developmental fate of cells provoked by a change in their relative position (e.g. after a longitudinal ligature each half of some insect eggs can form a complete embryo (Sander 1971)). Regulation may occur over the entire early embryo until regions become coarsely determined to form particular structures, such as limbs. These regions can often be shown to be independent fields (Weiss 1939) within which pattern regulation can still occur. In many embryos the cells then lose their lability, become finely determined and eventually differentiate into recognizable cell types (and often form specific structures such as bristles). Many larval animals, however, can regenerate lost or damaged body parts, including limbs. The two terms 'regulation' and 'regeneration' have been used in many different ways in the literature but, in general, pattern regulation refers to a change in developmental fate of undifferentiated embryonic cells, while regeneration has been used where new structures are formed later in development after damage to a differentiated functional body part (Slack 1980).

We know a great deal about the molecular events involved in cell differentiation, but there is very little information at that level about the interactions of pattern formation that lead cells into particular paths of differentiation. In analysing complex patterns we are still searching for acceptable models with formal rules, by which we can understand and predict the behaviour of developing tissues, and which may give clues about the molecular mechanisms of pattern formation. I shall now discuss development, regulation and regeneration in insect legs, and the extent to which these can be explained by the recent polar coordinate model (French *et al.* 1976; Bryant *et al.* 1981).

The development of insect legs

The insect embryo forms from a ventral region of the blastoderm cell layer, which surrounds the egg yolk. The segmental pattern first becomes clearly visible at germ band stage and by this time regions of the embryo are determined to develop into the legs and surrounding areas of thorax (figure 1). Hemimetabolous insects such as cockroaches and crickets (and some holometabola such as beetles) form limb buds that grow and become visibly segmented. The limb epidermis secretes cuticle bearing characteristic bristles and claws, and the limb muscles differentiate. The larva hatches with functional legs, which grow and secrete a new cuticle before each larval moult, and eventually form the adult legs (after considerable changes in form in the pupal stage of the holometabolous insects). In many holometabolous insects, such as *Drosophila*, the presumptive leg cells do not form a protruding limb bud but invaginate in the late embryo to form an internal imaginal disc of less than 50 cells. The legless larva hatches and the discs grow through larval life, remaining connected by a stalk to the larval epidermis but not differentiating or forming cuticle. In the pupal stage the mature imaginal disc (comprising many thousands of cells) evaginates, secretes cuticle and forms muscles, and eventually fuses with other discs (the wing disc, etc.) to form the adult fly, as the larval epidermis dies (figure 1).

Distal regeneration

Many insects, such as cockroaches, crickets and beetles, will readily regenerate larval legs after loss or amputation at any level in the leg or even in the surrounding thorax. The amputation site is closed by a clot of dried haemolymph, and the epidermal cell layer of the stump separates locally from the overlaying cuticle and migrates in to form a continuous layer under the clot. Cell division follows, producing a regenerate folded up beneath the old cuticle of the stump (Penzlin 1963; Bullière 1972). The epidermis secretes cuticle that may differ slightly in

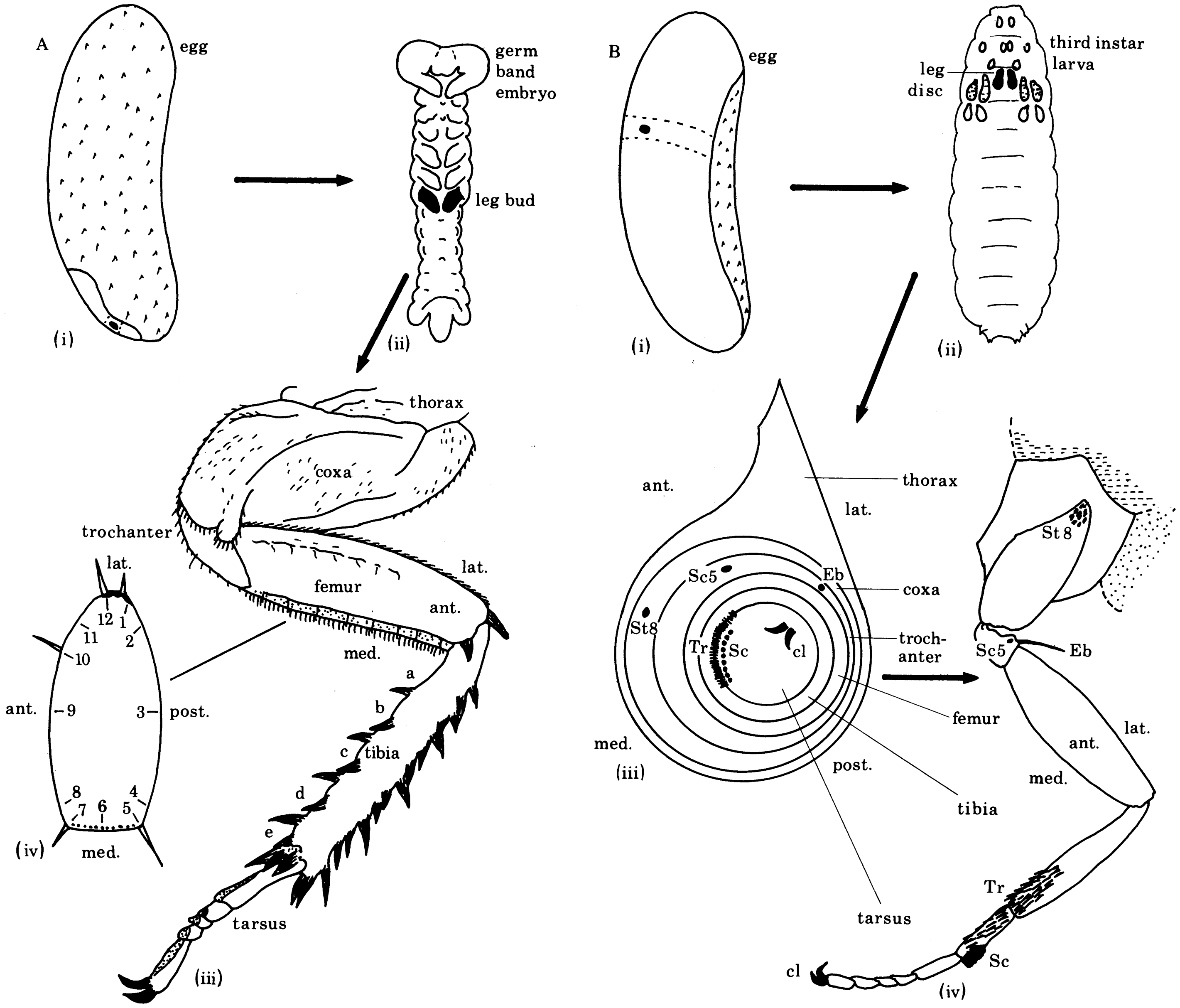

Figure 1. Leg development in the cockroach (A) and *Drosophila* (B). The insect embryo forms from the ventral region of the egg (i), while extra-embryonic membranes form from the dorsal region (stippled). The germ band stage embryo has recognisable head, thoracic and abdominal segments.

A. In hemimetabolous insects the germ band (Aii) has protruding thoracic leg buds, which grow and differentiate in the embryo to form the larval legs. The larval left metathoracic leg is shown in anterior view (Aiii) and in transverse section through the femur (Aiv). Aiii shows five proximal–distal levels (a–e) marked on the tibia; Aiv shows 12 positions (1–12) marked around the leg circumference as well as the anterior (ant.), posterior (post.), medial (med.) and lateral (lat.) faces of the leg. Aiv also shows the distribution of the rows of bristles, light cuticle (positions 5–7) and dark cuticle (12–1) on the femur.

B. In *Drosophila* the presumptive adult head and thorax tissue invaginates in the embryo to form separate imaginal discs (e.g. the mesothorax has two wing discs and two leg discs). The discs grow but do not differentiate in the larva (Bii). In the pupal stage the imaginal discs evert and differentiate, fusing to form the adult body, while the larval epidermis dies. Biv shows the adult male left prothoracic leg with some of the specific markers (St8, Sc5, etc), and Biii shows a simplified fate map of the corresponding mature imaginal disc, showing the position of the presumptive segments and cuticular markers. Anterior (ant.), posterior (post.), medial (med.) and lateral (lat.) are labelled on the leg and disc for direct comparison with the cockroach leg (this nomenclature differs from that normally used in imaginal disc literature).

pattern from the original leg (e.g. forming fewer tarsal segments and different spine patterns), and muscles form in approximately the original pattern. The regenerate freed at the next moult is therefore a fair copy of the structures normally distal to the amputation site. Even if regeneration occurs from a surface that originally faced proximally, distal structures are still formed, duplicating those present in the stump.

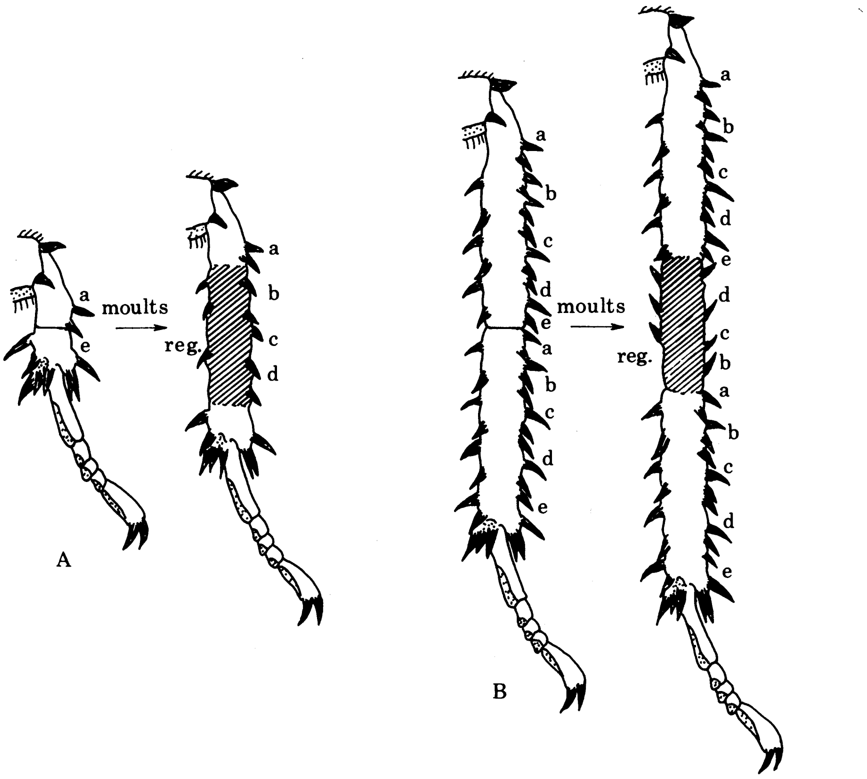

Figure 2. Proximal–distal intercalary regeneration. Interaction between proximal (a) and distal (c) levels of the cockroach tibia leads to local growth and the formation of an intercalary regenerate (reg., shaded) consisting of the intermediate levels (b, c, d).

If the mature *Drosophila* imaginal leg disc is fragmented and the pieces implanted separately into mature larval hosts, they form complementary parts of the normal pattern; hence a detailed fate map can be made, locating the presumptive leg segments and specific marker structures (figure 1Biii). The fate map forms a nest of concentric rings with distal structures in the centre and proximal thorax around the periphery of the disc. If the peripheral part of the disc is implanted into an adult fly for a few days before being reimplanted into a larval host, it grows and reforms the missing distal structures (Schubiger 1971). Isolated centres of leg discs fail to develop, but central wing disc pieces form a duplicate copy of their distal structures (Bryant 1975).

Thus distal regeneration occurs after an amputation through the larval leg or mature leg disc. Clues about this process come from experiments in which epidermal cells from different positions on the leg are grafted (or heal) together.

Intercalary regeneration

When the distal level of the tibia of the cockroach leg is grafted onto the proximal level of the host tibia, local growth occurs at the junction and the intervening mid-tibia is formed by intercalary regeneration (Bohn 1970; Bullière 1971). The resulting tibia is approximately normal in size and pattern (figure 2A). Grafting proximal tibia onto the distal level of the host also results in intercalary regeneration of intermediate levels, but in reversed orientation so that the resulting leg, although very abnormal in size and pattern, has no positional discontinuities between adjacent cells (figure 2B). Since the regenerate is formed from both graft and host (Bohn 1971), cells can form more proximal or more distal structures during intercalation. Grafting between different leg segments (e.g. tibia and femur) shows that the proximal–distal organization of the tibia is repeated in each leg segment (Bohn 1970; Bullière 1971).

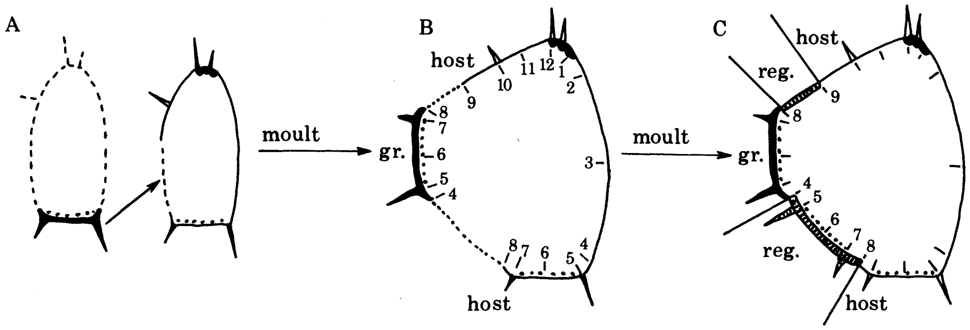

Figure 3. Circumferential intercalary regeneration. Schematic cross sections, through the cockroach femur showing the graft of medial face of the left femur into the anterior face of the host left femur (A), the result after one moult (B) with a band of new tissue (dotted) separating graft (gr.) and host, and the final result after two moults (C). The recognizable intercalary regenerate (reg.) consists of tissue normally lying between the confronted positions by the shortest route around the circumference. Hence between positions 8 and 9 (see figure 1Aiv) a small band has formed, while between positions 4 and 8 a new medial face (positions 5, 6, 7) has been intercalated.

Intercalary regeneration can also occur around the circumference of the insect leg (French 1978). When strips of femur cuticle plus epidermis are moved around the circumference and grafted into a different position, normally non-adjacent cells interact along the graft–host junctions. One moult after the operation, the original graft and host tissue retain their differentiated markers (e.g. rows of bristles) but are separated by an intercalary regenerate that usually bears few recognizable structures (figure 3). New bristle rows and other markers appear at the second moult and show that the intervening sections of circumference are formed within the new tissue, resulting in an abnormal but continuous pattern, which grows with the rest of the leg during succeeding larval instars. If the circumference is labelled as in figure 1Aiv, then intercalation reliably occurs by the shortest route so that interaction between cells from positions 4 and 8 generates tissue characteristic of positions 5, 6, 7 (rather than 3, 2, 1, 12, 11, 10, 9). This rule is obeyed at all positions around the circumference, and confrontation between opposite positions (e.g. 10 and 4) can lead to intercalation of either intervening half circumference. Grafting between different leg segments shows that the circumferential organization of the femur is repeated in other leg segments (French 1980). During intercalation after these grafts, the distinct host- and graft-derived cells can form structures lying in either direction around the circumference but seem unable to cross two anterior–posterior restriction lines (French 1980, 1981).

Intercalary regeneration can also occur in fragments of mature *Drosophila* imaginal disc, provided that they are given a period for growth before undergoing metamorphosis. If the wing disc is cut into $\frac{1}{4}$ and $\frac{3}{4}$ sectors, regeneration of the $\frac{3}{4}$ fragment completes the pattern while the $\frac{1}{4}$ fragment duplicates the structures in that region of the fate map (Haynie & Bryant 1976).

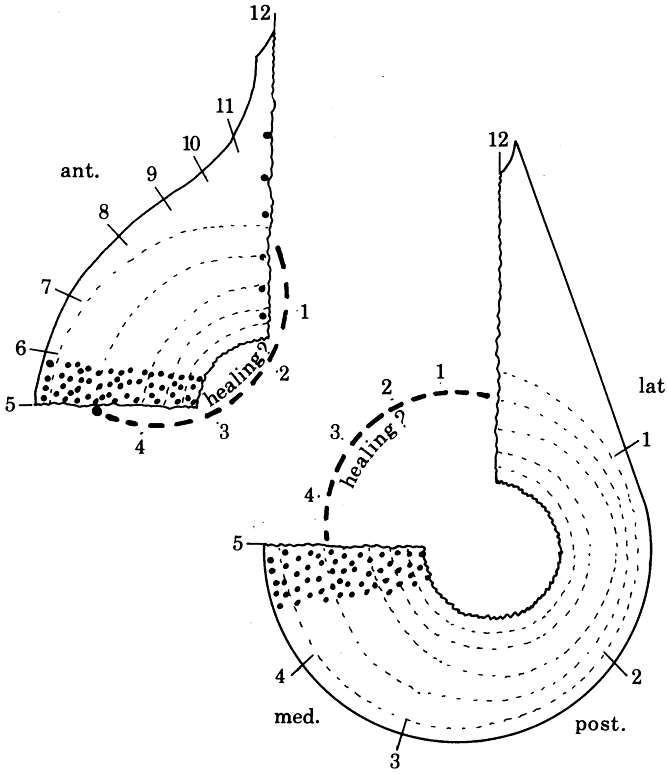

FIGURE 4. Regeneration in the *Drosophila* prothoracic leg disc. An anterior quadrant of the leg disc (see figure 1Biii) can grow and regenerate the missing structures, while the complementary $\frac{3}{4}$ fragment forms the same set of extra structures, thereby duplicating its pattern. Growth occurs predominantly from one edge of the fragments (large dots) and it is not clear whether the cut edges heal together (heavy broken line), as in similar wing disc fragments. If 12 unequally spaced circumferential positions are assigned to the disc (1–12), then the new structures represent the shortest route between the positions of the two cut edges. This spacing of positions is also compatible with the behaviour of other fragments. If the central (tarsal) region is removed from the fragments, the anterior quadrant regenerates the tarsus, and the duplicating $\frac{3}{4}$ fragment often forms duplicated tarsal structures (Schubiger & Schubiger 1978).

Since this is true of any $\frac{3}{4}$ and $\frac{1}{4}$ fragments the direction of regeneration cannot be decided autonomously at one cut edge; it must result from the size of the fragment or an interaction between the cut edges. Indeed, these fragments do heal their two edges together (Reinhardt *et al.* 1977) and there is radioautographic evidence of cell division at that site (Dale & Bownes 1980). Healing confronts cells from different circumferential positions and the new structures formed separate those positions by the shortest route (completing the $\frac{3}{4}$ and duplicating the $\frac{1}{4}$ fragment). There is direct evidence that different regions of wing disc can interact. If small fragments (which would each duplicate their partial patterns) are taken from opposite sides of the disc, mixed (accurate grafting is not possible) and implanted, structures of the intervening disc regions are regenerated (Haynie & Bryant 1976).

If the imaginal leg disc is divided into $\frac{1}{4}$ and $\frac{3}{4}$ sectors, the anterior quadrant (referred to as upper medial or UM in the disc literature) can regenerate the rest of the pattern (figure 4), while the complementary $\frac{3}{4}$ piece duplicates (Schubiger 1971; Strub 1977). Other $\frac{1}{4}$ pieces duplicate while other $\frac{3}{4}$ sectors (which will contain the anterior quadrant) complete the pattern. Clonal analysis shows that the new structures are produced successively by growth, predominantly from one cut edge of a fragment, and that the normal anterior–posterior compartment border can be crossed early in this process (Abbott *et al.* 1981). Although it is not clear that these fragments do heal their edges together (G. Schubiger, personal communication), some interaction must occur, and intercalary regeneration has been demonstrated between mixed leg disc fragments (Strub 1979).

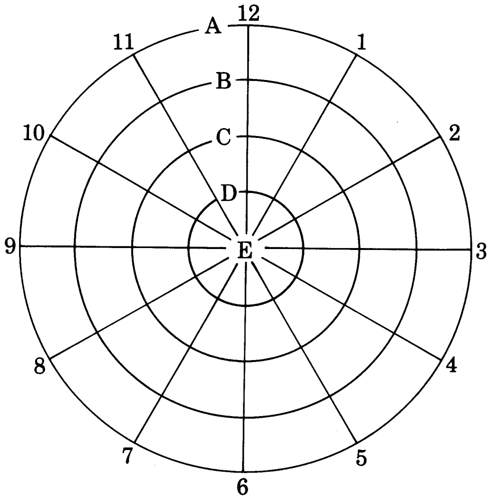

Figure 5. Positional information specified along polar coordinates in the epidermis of the insect limb. Thoracic structures form around the periphery of the field and distal structures (claws) form in the centre. Cells have information with respect to position on a radius (A–E) and on a circumference (1–12). There is no discontinuity between 12 and 1 so a continuous sequence of positional values runs around the limb.

The polar coordinate model

We proposed (French *et al.* 1976) that fields, such as insect larval legs or imaginal discs, have a map of stable positional values specified along polar coordinates in two dimensions (i.e. within the epidermis in insects). The distal tip of the leg is at the centre of the field, which therefore roughly represents an end-on view of the larval leg and the fate map of the imaginal disc (figure 5). Cells will have a radial positional value in a sequence (A–E) running proximal–distal and a circular value in another sequence (1–12) running around the leg. Of course, the notation of 12 circular and 5 radial values is completely arbitrary: there is likely to be a far larger number of different values.

Shortest route intercalation

When cells from two normally non-adjacent positions interact after grafting or wound healing, the discontinuity stimulates local growth and the intercalation of cells with the intermediate positional values, as shown for the radial sequence in figure 2. Circumferential intercalation occurs by the shortest route around the sequence of values, which is continuous from 11, through 12, to 1, as shown in figure 3.

Positional values will not necessarily be evenly spaced on the actual cell sheet, and the spacing of circumferential values can be deduced from the routes by which intercalation occurs in the various graft combinations or fragments. Values seem to be fairly evenly spaced around the cocroach leg (figures 2 and 3), but the *Drosophila* leg disc seems to have a very uneven spacing (French *et al.* 1976; Strub 1977) with over half the values clustered in the anterior quadrant (which completes the pattern during regeneration) and the others distributed around the remaining $\frac{3}{4}$ of the disc (which duplicates its partial pattern), as shown in figure 4.

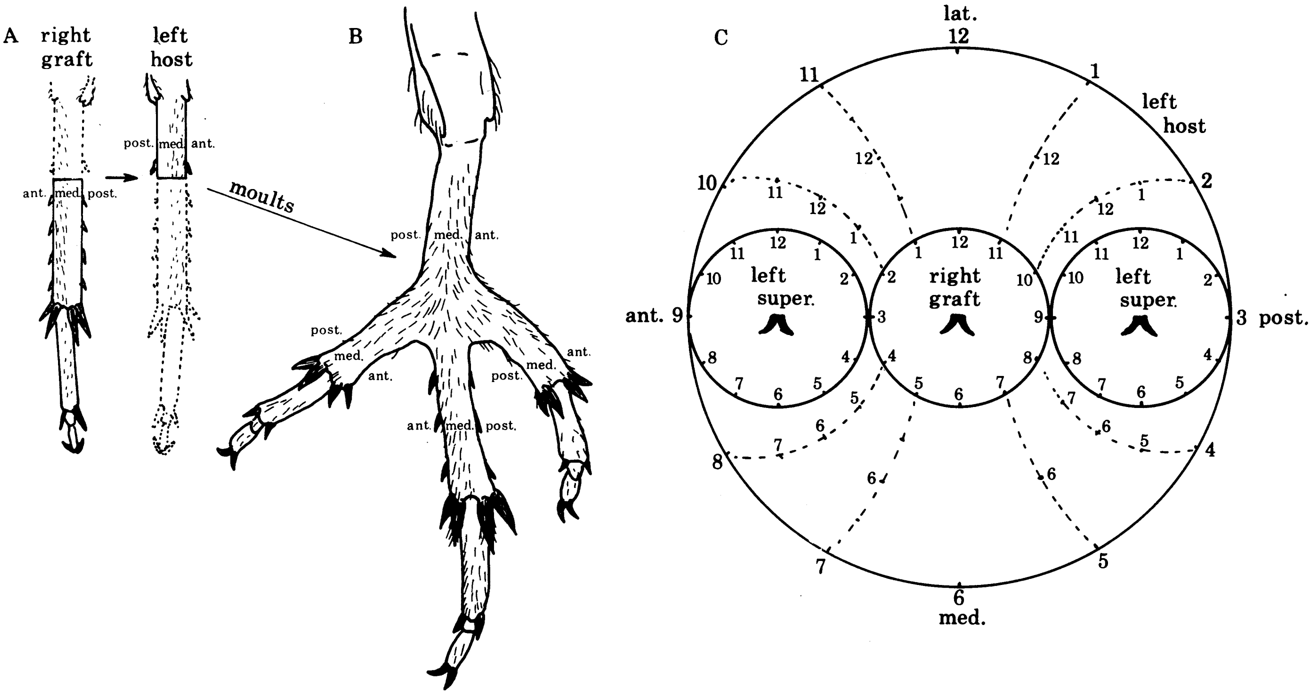

FIGURE 6. Regeneration of supernumerary cricket legs.

(A) Diagram of graft at proximal tibia level between left and right metathoracic legs. The medial–lateral (med.–lat.) axes are in continuity, but the anterior–posterior (ant.–post.) axis of the graft is reversed relative to the host.

(B) Medial view of resulting leg showing a supernumerary set of distal parts with host handedness formed at each point of maximum discontinuity between host and graft.

(C) Polar coordinate model interpretation of supernumerary formation. Schematic distal view showing host circumference as the outer circle, the graft circumference as the inner circle and between these the positions formed by shortest route intercalation between host and graft cells confronted at the juction. A complete circumference is formed at anterior and posterior positions and from each a supernumerary (super.) will regenerate.

Distalization

After amputation across the proximal–distal axis of larval legs and imaginal discs, regeneration produces cells with more distal positional values. We originally proposed a 'complete circle' rule whereby growth and formation of all more distal parts occurs wherever a complete circumference is exposed by amputation or generated by grafting, wound healing or intercalation. This rule provided an explanation of the two supernumerary legs regenerated by cockroaches (and many other insects) when a larval leg is amputated and grafted onto the contralateral stump (French *et al.* 1976). Intercalary regeneration between confronted graft and host cells will generate two complete circumferences at the junction, and these will each form an extra copy of the distal parts (figure 6).

The 'complete circle' rule had the corollary that, without a complete circumference, no distal regeneration should occur. However, there have been several recent demonstrations that incomplete circumferences can form partial distal regenerates (Bryant *et al.* 1981). In the cockroach leg a surgically constructed 'double-half' amputation surface sometimes fails to regenerate

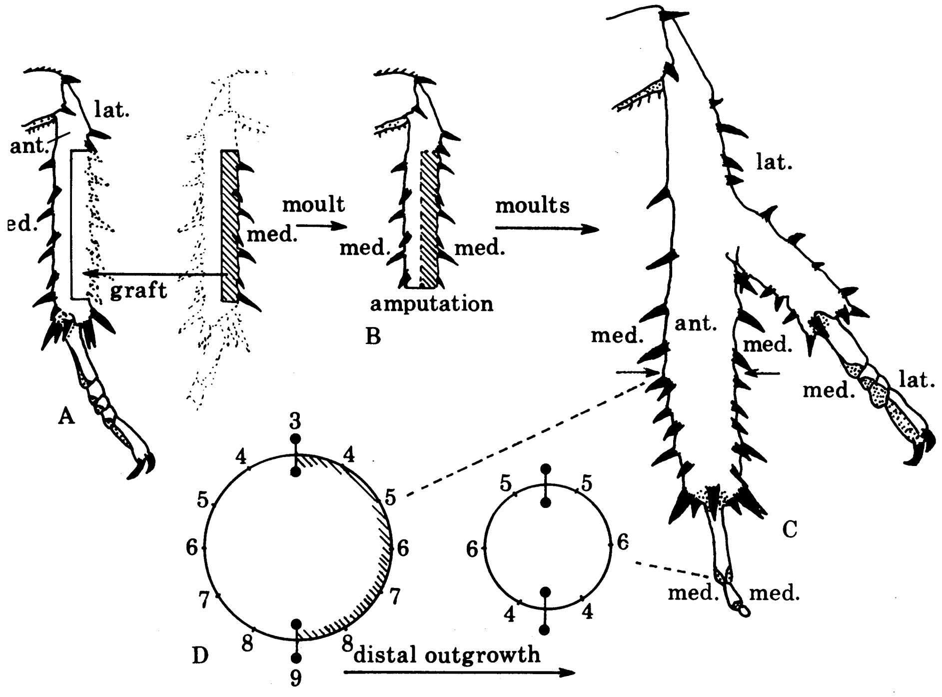

FIGURE 7. Regeneration from a double-medial amputation surface in the cockroach tibia. Grafting the medial half of a left tibia (hatched) in place of the lateral half of a right tibia (A) and then amputating through the graft (B) creates a complete circumference at the proximal end of the graft, but a symmetrical double-medial amputation site. C shows the result after two moults with the proximal confrontation regenerating a normal complete tarsus, while the amputation site (arrows) regenerates a symmetrical, distally incomplete tarsus. D shows the circumference at the amputation site, with two medial halves separated by two lines of symmetry where positional values are progressively lost during distalization.

but often forms a tapering symmetrical regenerate (Bohn 1965), as shown in figure 7. Similar experiments have been performed in *Drosophila* wing (Karlsson 1980) and leg (Schubiger & Schubiger 1978) discs, showing that proximal fragments that duplicate (and hence are assumed to contain less than half the circumferential values) can nevertheless undergo various degrees of distal outgrowth (see figure 4).

These results can be understood if distal outgrowth results from local interactions between cells with different circumferential values as they come together during healing at an amputation site (figure 8A). Normally such an interaction would lead to the intercalation of cells with positional values intermediate between those of the confronted cells. However, if these values are already present on adjacent cells, we propose that the new cells adopt a more distal radial value (Bryant *et al.* 1981). In this way almost any healing pattern will provoke intercalation of a new circle of cells with more distal values, and repeated rounds of intercalation and distalization will produce a complete regenerate.

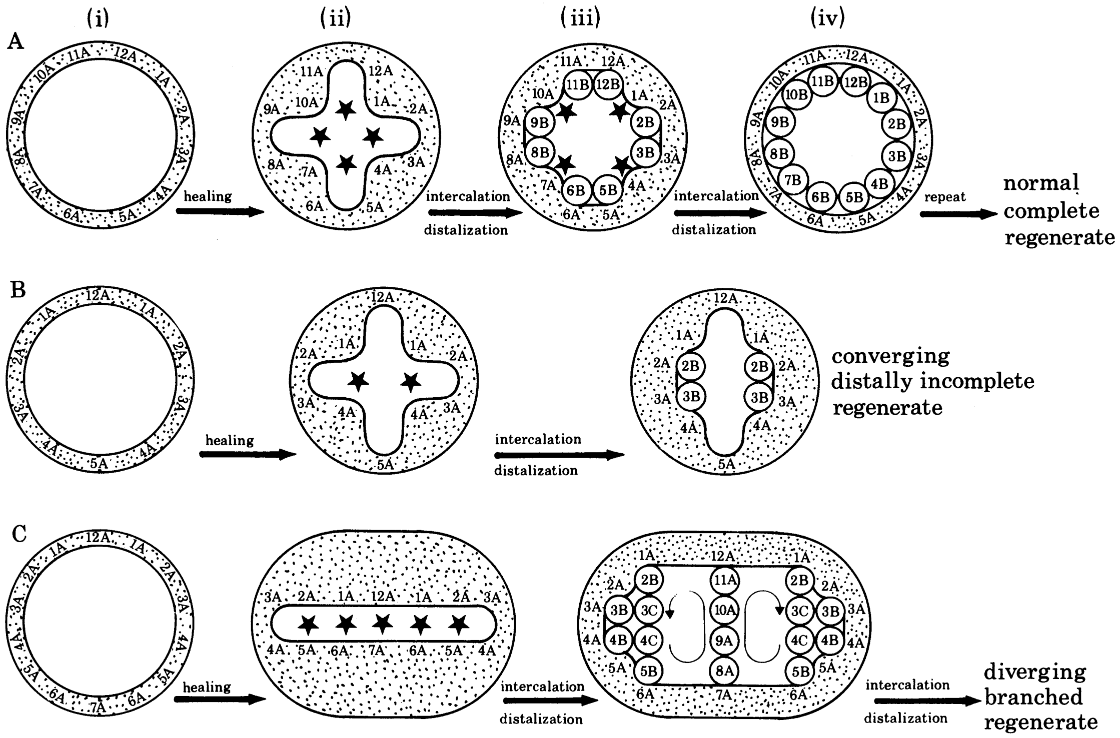

FIGURE 8. Regeneration from asymmetrical and symmetrical wound surfaces. The proximal tissue remaining after removal of distal levels B, C, D and E is stippled, and the wound edge is outlined by the inner circle in (i).

(A) The normal complete circumference will begin to heal over the wound surface and will confront cells of differential value (ii). Shortest route intercalation (stars) produces new cells with circumferential positional values identical to those of adjacent stump cells, so by the distalization rule they adopt a more distal radial value (iii), becoming 2B, 3B, etc Subsequent intercalation completes the B level (iv) and the process is repeated until all distal structures are formed. The result is almost independent of variation in the directions of wound healing.

(B) The result from a symmetrical stump (as in figure 7) depends on the direction of wound healing. If healing occurs along the plane of symmetry, confrontations will be between like cells (1A and 1A, 2A and 2A, etc.) and no intercalation will be provoked. If the edges heal in fairly regularly (as shown), intercalation and distalization will occur at some positions but values will be lost at the lines of symmetry, so the symmetrical regenerate will converge and (in this case) will be distally incomplete. Healing across the plane of symmetry will confront 12A with 5A and give a rather more complete converging regenerate.

(C) If the stump consists of symmetrical copies of more than half the values, the results will be as in (B) except that healing across the plane of symmetry will lead to the intercalation of missing values in the midline, forming a diverging, branched, distally complete regenerate.

The extent of distalization from a double-half circumference (as in figure 7A) will depend critically on the direction of wound healing. As shown in figure 8B, fairly regular healing will produce a tapered, converging, distally incomplete outgrowth, which will lose values in the plane of symmetry. If healing occurs directly along the plane of symmetry, no intercalation or outgrowth will be provoked. If the wound consists of two copies of at least half the circumference, healing across the plane of symmetry will result in the intercalation of missing values (figure 8C). The outgrowth will diverge, gaining values in the plane of symmetry and branching into two mirror-image distally complete regenerates.

The modified polar coordinate model, with the shortest intercalation and distalization rules, relates distal outgrowth closely to intercalary regeneration and depends only on local interactions between adjacent cells. It provides an explanation not only for the complete supernumerary legs regenerated after contralateral grafts (figure 6), but also for the regeneration of a wide range of abnormal partial or branched supernumeraries from damaged larval legs and imaginal discs (Bryant *et al.* 1981).

REGENERATION OF PARTIAL SUPERNUMERARY LEGS

Removal of a deep V-shaped notch from the side of the leg of a cockroach or cricket creates a wound surface consisting of two symmetrical copies of part of the circumference. These wounds usually heal, replacing the damaged area, but sometimes they regenerate symmetrical, converging, distally incomplete outgrowths or (rarely) symmetrical, diverging, distally complete

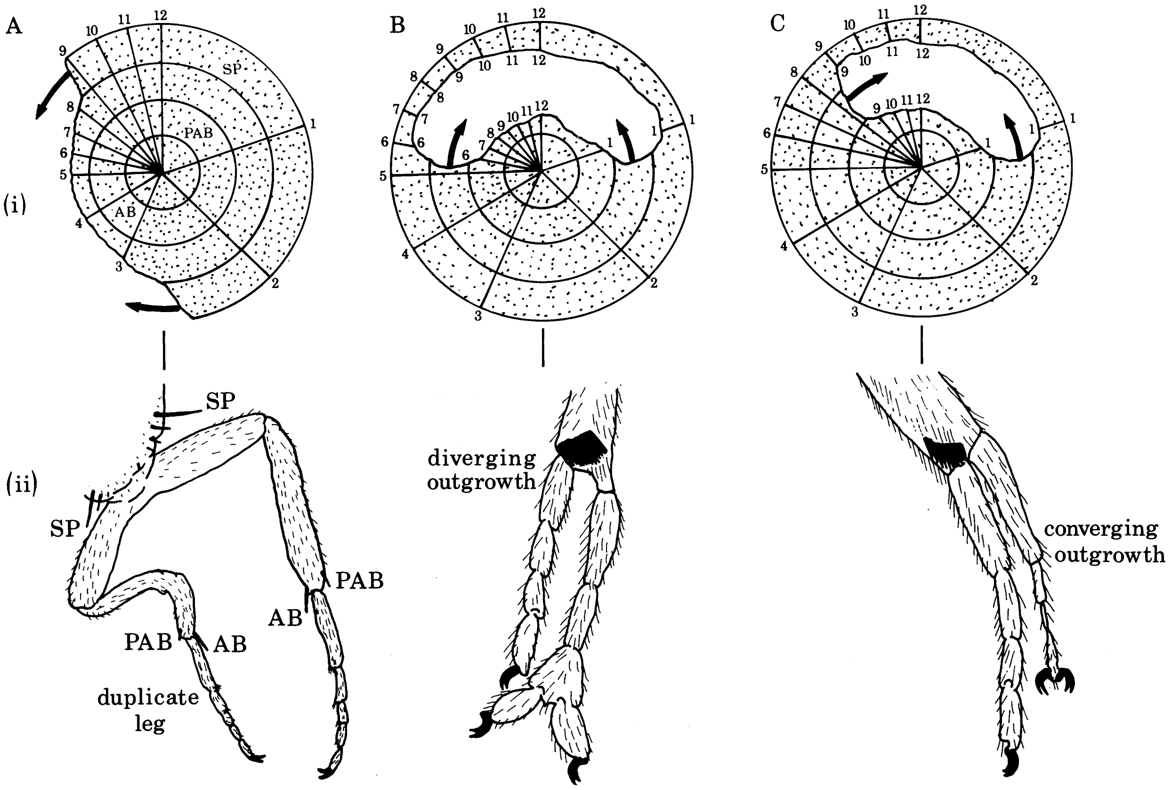

FIGURE 9. Leg abnormalities following temperature treatment of *Drosophila 726* larvae. Patches of cell death may leave the imaginal discs (i) with large wounds, which may heal (arrows) to give intercalation and distalization, forming supernumerary leg structures (ii). The discs (adapted from Girton 1981) are shown with unequal distribution of positional values (see figure 4) and the legs (ii) are sketched from Girton & Russell (1980, fig. 1) and Girton (1981, fig. 2).

(A) A peripheral anterior–medial wound could heal and intercalate to give an extra complete circumference and hence a duplicate leg in mirror symmetry to the original leg. At proximal levels the lateral structures (e.g. SP bristles) will be duplicated and the medial ones will be missing while, distal to the site of the wound, both lateral (PAB) and medial (AB) structures will be present on both legs.

(B) The edges of a large internal anterior–lateral wound form symmetrical copies of more than half of the circumference. Healing can result in intercalation of missing values (see figure 8C) and the formation of a diverging branched distally complete outgrowth. In this case the most medial distal tip will be the original one (see Girton 1981).

(C) The edges of a smaller internal anterior–lateral wound form symmetrical copies of less than half the circumference and can therefore only form a converging outgrowth (see figure 8B), which may be distally complete or incomplete (Girton 1981).

structures (Bohn 1965). Similar results are obtained after surgically damaging immature *Drosophila* leg discs *in situ* (Bryant 1971).

Partial duplications and triplications of *Drosophila* legs have recently been studied in considerable detail by the use of the temperature-sensitive cell-lethal mutation *l(1)ts-726*. If mutant larvae are kept at high temperature for a short time, local patches of cell death occur in the imaginal discs and dramatic abnormalities appear in the adult legs (Russell 1974). After temperature treatment of early larvae, partial leg duplications often occur. The proximal leg consists of two symmetrical copies of the lateral and posterior regions (with the complementary medial and anterior parts missing) while the distal part diverges and branches into mirror-image distal tips (Russell *et al.* 1977; Postlethwaite 1978). Clonal analysis (Girton & Russell 1980) suggests that these legs result from cell death at the edge of the disc, intercalary regeneration and distal outgrowth from a complete circumference, as shown in figure 9A.

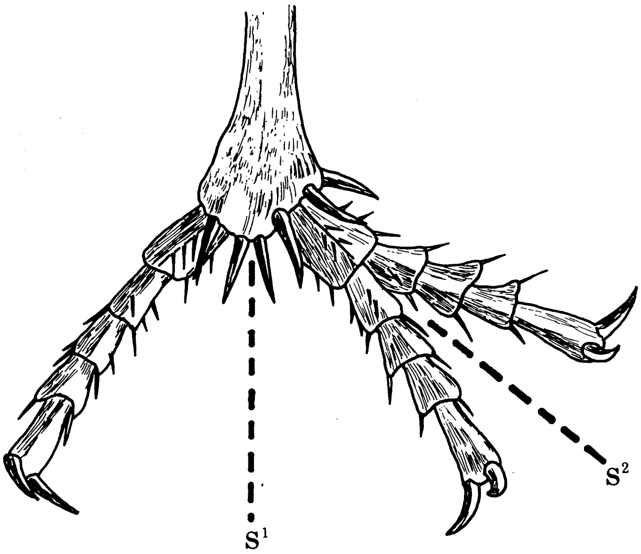

FIGURE 10. Naturally occurring triplication in a beetle leg (redrawn from Bateson 1894, fig. 156). Bateson noted that the three distal structures lay in one plane and there were planes of mirror symmetry between the original and the nearer supernumerary leg (S^1) and between the two supernumeraries (S^2).

After temperature treatment of *726* larvae in early 3rd larval instar, however, the most frequent pattern abnormality is a partial triplication in which a leg of normal structure bears a symmetrical outgrowth. Outgrowths either converge, losing bristle rows in the plane of symmetry and often tapering out before the distal tip, or diverge, gaining bristle rows in the plane of symmetry and branching into two complete mirror-imaged distal tips (Girton 1981). These triplications probably result from a zone of cell death within the imaginal disc, creating a wound bounded by symmetrical sets of circumferential positional values. Whether distalization occurs and whether a symmetrical regenerate converges or diverges will depend on the proportion of circumference present around the edge of the wound, and on the direction of wound healing, as shown in figure 9B, C.

Bateson (1894) described many naturally occurring insect legs with symmetrical supernumerary outgrowths. In many cases a supernumerary that appears incomplete at its base diverges to give two complete circumferences, which remain fused or branch into two separate distal tips (figure 10). The symmetry relations of these structures (known as 'Bateson's rule') are the same as those of the experimentally produced partial triplications (figure 9), so it is likely that these legs arose by regeneration after accidental damage to the developing legs during embryonic or larval life (Przibram 1931).

Models of limb regeneration

The two rules of the polar coordinate model allow us to understand the diverse data on insect leg regeneration, the supernumerary limbs found in Crustacea after grafting (Mittenthal 1981) or accidental damage (Shelton *et al.* 1981) and a wide range of results from regenerating amphibian limbs (discussed in Bryant *et al.* 1981). I shall now discuss some limitations of the model and some alternative ideas.

As mentioned above, grafting between different cockroach leg segments indicates that the circumferential organization (values 1–12) is present at all levels in the leg, but that the proximal–distal organization (values A–E) is repeated in each segment. There must be other information that distinguishes the segments, and the model does not explain how distalization does more than complete a segment.

The model does not illuminate the relation between pattern and lineage. From blastoderm stage, *Drosophila* leg discs develop as clonally separate anterior and posterior compartments (Steiner 1976) but the borders can be crossed in the early stages of regeneration (Abbott *et al.* 1981). Similarly, intercalation in cockroach and cricket larval legs suggests a single continuous sequence of values (1–12) but there are two lineage discontinuities (probably equivalent to compartment borders), which apparently cannot be crossed (French 1981).

The polar coordinate model requires only local interactions between cells and their nearest neighbours, so it suggests that positional information may be a property of the cell surface rather than the intracellular concentration of a conventional 'diffusible morphogen'. The model has not, however, suggested any satisfactory mechanism of coding circumferential position without a boundary region having special properties. A circumferential 'gradient', for example, would have a discontinuity where maximum and minimum values are adjacent, and there is no experimental evidence for such a special region (see French 1978). The mechanism must be capable of averaging across a discontinuity but remaining stable at a position of local symmetry (e.g. at values 4 and 8 in figure 3). Another problem concerns the central (distal) point of the field, where all circumferential values are represented (Winfree 1980).

The polar coordinate model is two-dimensional, with position specified in the insect epidermis (figure 11A). During distal regeneration, however, internal muscle patterns as well as surface cuticular patterns are formed. There is some evidence (see Williams & Caveny 1980) that insect muscles form by interaction with their epidermal surface attachment sites and invaginated apodemes, so three-dimensional pattern could be directed by cues from the epidermis. The problem is much greater in considering the formation of internal patterns of dermis, muscle and cartilage in the amphibian limb, and Stocum (1980) has proposed an additional surface–core axis of positional information.

Several authors (Winfree 1980; Lewis 1981; Mittenthal 1981) have suggested that many of the data reviewed here and by French *et al.* (1976) can be explained without resorting to a specific coordinate system. If the insect epidermis has a two-dimensional map that re-establishes *continuity* after a disturbance, then the central region (the distal tip) will be reformed after amputation, whatever the coordinate system (figure 11D). Similarly, two extra central regions will

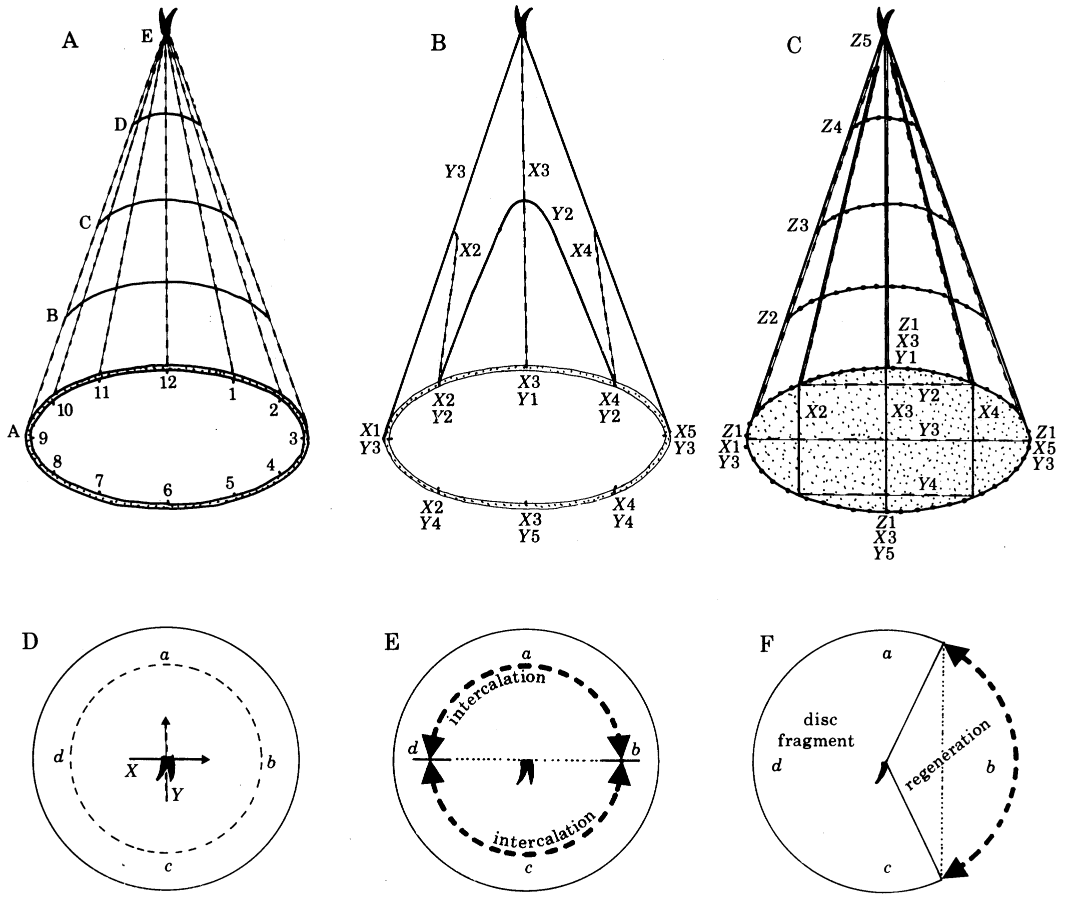

FIGURE 11. Positional information maps and intercalary regeneration. (A–C) The insect limb is represented as a cone with the proximal region at the base and the distal tip at the apex. Positional information may be specified in two dimensions in the surface epidermal layer (stippled in A and B) or three dimensions through the limb (C).

(A) The polar coordinate model (French *et al.* 1976) postulates independent axes running around (1–12) and down (A–E) the limb (see figure 5).

(B) The two-dimensional Cartesian model (Kauffman 1981) postulates axes (X and Y) running from one edge of the field, over the centre, to the opposite edge. This is much easier to visualize on a flat map of the epidermis (D).

(C) The three-dimensional Cartesian model (Slack 1980) postulates independent axes running across (X and Y) and down (Z) the limb.

(D–F) The limb is represented in two dimensions with the distal tip in the centre (as in figure 5).

(D) Two Cartesian axes (X and Y) are indicated by arrows. After amputation of the distal region, the wound surface (dashed) forms a perimeter *a b c d* and any system that re-establishes continuity of the map will replace the central tissue.

(E) By means of a strip graft, oppositing positions *d* and *b* can be confronted along a junction (shown as the two separate cut surfaces). Structures formed comprise arcs (heavy dashed lines) passing through *a* or through *c*, rather than a straight path (dotted) through the claws. Similarly, a regenerating sector of an imaginal disc (F) forms all missing parts (heavy dashed arc) including *b*, rather than just those predicted by averaging along Cartesian coordinates (dotted line).

form after a contralateral graft (Lewis 1981). However, this criterion of overall map continuity does not yield definite predictions from many grafting experiments (Lewis 1981). In some cases the results themselves are variable (e.g. the regeneration from double-half amputation sites, figure 7), but many other experiments give reliable results that do suggest a polar coordinate system. When cells from opposite positions on the cockroach leg are confronted at the edge of a strip graft (see figure 3), the structures formed at this juntion (French 1978) correspond to *arcs* in the epidermal field (figure 11E). This suggests that angle is an independent parameter of position and hence that the map is polar. If the epidermal map were based on two Cartesian axes (figure 11B), as suggested by Kauffman (1981), averaging between opposite positions would produce the structures (including claws) lying on a straight line passing through the distal tip.

Kauffman (1981) points out that, while one coordinate system can be transformed mathematically to another, each requires a different cellular property to explain regeneration. Hence the polar coordinate model requires distalization in addition to intercalation to explain distal regeneration, since the new structures are in no sense 'between' the old proximal ones. Distal regeneration follows from intercalation on a two-dimensional Cartesian model (figure 11D), but this model requires a separate rule for forming extreme values on the perimeter (figure 11E, F). Slack (1980) has proposed a three-dimensional Cartesian model (figure 11C) that explains many of the data from cockroach and amphibian legs, but the model is difficult to apply to imaginal discs, and requires separate rules both for distalization and the formation of surface values.

The polar coordinate model considers only the results of *interactions* between different cells. Cells may also systematically change their positional values while growing as a free edge (Shelton *et al.* 1981). This has been demonstrated experimentally in the insect abdomen (Wright 1979, 1981), and it may be occurring when small disc fragments (which usually duplicate by the shortest intercalation rule) occasionally regenerate missing proximal structures (see Kauffman 1981).

It is clear that there are some problems with the polar coordinate model, but it does account in a simple and unified way for almost all available data on regeneration in arthropods and amphibians (Bryant *et al.* 1981).

Limb regeneration and pattern regulation

Pattern regulation and regeneration are both changes in developmental fate after a disturbance, but they are often regarded as different processes. Regulation is held to be a property of small regions of undifferentiated embryonic cells, and to involve the sorts of interactions by which patterns are first formed. Regeneration, however, occurs in large appendages at much later developmental stages and is accompanied by dedifferentiation, growth and redifferentiation. It has often been regarded as a special property of those body regions prone to injury, selected for its adaptive value and perhaps involving very different mechanisms from those of normal development (see the discussion in Morgan (1901)).

There are great similarities in the results of grafting and extirpation operations on differentiated larval insect legs, mature but undifferentiated *Drosophila* leg discs, immature discs, and even early embryonic leg fields in *Drosophila* (Bownes 1975). From the time when it consists of only some 20 cells, the developing limb appears to retain the same basic organization of positional information and the same regulative responses, suggesting that there may be no fundamental distinctions between regulation and regeneration. The same conclusion has often been drawn from the results of grafting and amputation experiments on functional amphibian limbs, larval limb buds and embryonic limb fields (see, for example, Bryant & Iten 1976). Basic cellular properties of intercalation and distalization may be involved in forming the leg pattern in the embryo, elaborating it through normal growth and responding to a disturbance at any developmental stage (French *et al.* 1976).

Cellular positional values may be much more stable in large differentiated limbs than in tiny embryonic limb rudiments (Slack & Savage 1978), so there may be differences in the extent to which growth is directly involved in the formation of new pattern elements. Morgan (1901) first used the term *morphallaxis* where 'a part is transformed directly ... without proliferation at the cut surfaces' and *epimorphosis* where 'a proliferation of material precedes the development of the new part', but he cautioned that 'the two processes are not sharply separated, and may even appear combined in the same form'. Pattern regulation in insect legs is broadly epimorphic, occurring within new tissue produced at an amputation site or a graft junction. There is direct evidence for local growth in imaginal discs (Abbott *et al.* 1981; Dale & Bownes 1980) and both direct (Bullière 1972) and indirect (e.g. figure 3) evidence from cockroach legs. However, careful histological studies are still needed to establish *how far* the stimulation of division extends from the cut or junction. We have assumed that simple, extreme form of epimorphosis where positional value only changes in the daughter cells (or one daughter cell) from a division provoked by a discontinuity between the values of abutting cells. There is no evidence that growth is so strictly localized and, indeed, Bullière (1972) claims that a tiny regenerate forms by reorganization of the distal part of the cockroach leg stump *before* any growth occurs. It is possible that a positional discontinuity may cause a change in values that spreads back from the cut edge or junction, and is then followed by cell division within this band of altered tissue (Maden 1977). Clearly this would require further slight modifications of the rules of the polar coordinate model, but it need only involve interactions between adjacent cells.

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REFERENCES (French)

Abbott, L., Karpen, G. & Schubiger, G. 1981 Compartmental restrictions and blastema formation during pattern regulation in *Drosophila* imaginal leg discs. *Devl Biol.* (In the press.)

Bateson, W. 1894 *Materials for the study of variation.* London: Macmillan.

Bohn, H. 1965 Analyse der Regenerationsfähigkeit der Insekten-extremität durch Amputations- und Transplatationsversuche an Larven der afrikanschen Schabe *Leucophaea maderae* Fabr. (Blattaria). II. Mitt. Achsendetermination. *Wilhelm Roux Arch. EntwMech. Org.* **156**, 449–503.

Bohn, H. 1970 Interkalare Regeneration und segmentale Gradienten bei den Extremitäten von *Leucophaea*-Larven (Blattaria). I. Femur und Tibia. *Wilhelm Roux Arch. EntwMech. Org.* **165**, 303–340.

Bohn, H. 1971 Interkalare Regeneration und segmentale Gradienten bei den Extremitäten von *Leucophaea*-Larven (Blattaria). III. Die Herkunft des interkalaren Regenerates. *Wilhelm Roux Arch. EntwMech. Org.* **167**, 209–221.

Bownes, M. 1975 Adult deficiencies and duplications of head and thoracic structures resulting from microcautery of blastoderm stage *Drosophila* embryos. *J. Embryol. exp. Morph.* **34**, 33–54.

Bryant, P. 1971 Regeneration and duplication following operations *in situ* on the imaginal discs of *Drosophila melanogaster*. *Devl. Biol.* **26**, 637–651.

Bryant, P. 1975 Pattern formation in the imaginal wing disc of *Drosophila melanogaster*: fate map, regeneration and duplication. *J. exp. Zool.* **193**, 49–78.

Bryant, S., French, V. & Bryant, P. 1981 Distal regeneration and symmetry. *Science, N.Y.* **212**, 993–1002.

Bryant, S. & Iten, L. 1976 Supernumerary limbs in amphibians: experimental production in *Notophthalmus viridescens* and a new interpretation of their formation. *Devl Biol.* **50**, 212–234.

Bullière, D. 1971 Utilisation de la régénération intercalaire pour l'étude de la détermination cellulaire au cours de la morphogenèse chez *Blabera craniifer* (Insecte Dictyoptère). *Devl Biol.* **25**, 672–709.

Bullière, D. 1972 Étude de la régénération d'appendice chez un insecte: stades de la formation des régénérates et rapports avec le cycle de mue. *Annls embryol. morph.* **5**, 61-74.

Dale, L. & Bownes, M. 1980 Is regeneration in *Drosophila* the result of epimorphic regulation? *Wilhelm Roux Arch. EntwMech. Org.* **189**, 91–96.

French, V. 1978 Intercalary regeneration around the circumference of the cockroach leg. *J. Embryol. exp. Morph.* **47**, 53–84.

French, V. 1980 Positional information around the segments of the cockroach leg. *J. Embryol. exp. Morph.* **59**, 281–313.

French, V. 1981 Leg regeneration in insects; cell interactions and lineage. *Am. Zool.* (In the press.)

French, V., Bryant, P. & Bryant, S. 1976 Pattern regulation in epimorphic fields. *Science, N.Y.* **193**, 969–981.

Girton, J. 1981 Pattern triplications produced by a cell-lethal mutation in *Drosophila. Devl Biol.* **84**, 164–172.

Girton, J. & Russell, M. 1980 A clonal analysis of pattern duplication in a temperature-sensitive cell-lethal mutant of *Drosophila melanogaster. Devl Biol.* **77**, 1–21.

Haynie, J. & Bryant, P. 1976 Intercalary regeneration in the imaginal wing disc of *Drosophila melanogaster. Nature, Lond.* **259**, 659–662.

Karlsson, V. 1980 Distal regeneration in proximal fragments of the wing disc of *Drosophila. J. Embryol. exp. Morph.* **59**, 315–323.

Kauffman, S. 1981 Epimorphic pattern regulation. (Submitted.)

Lewis, J. 1981 Simpler rules for epimorphic regeneration: the polar co-ordinate model without polar co-ordinates. *J. theor. Biol.* **88**, 371–392.

Maden, M. 1977 The regeneration of positional information in the amphibian limb. *J. theor. Biol.* **69**, 735–753.

Mittenthal, J. 1981 The rule of normal neighbours: a hypothesis for morphogenetic pattern regulation. *Devl Biol.* (In the press.)

Morgan, T. 1901 *Regeneration.* New York: Macmillan.

Penzlin, H. 1963 Uber die Regeneration bei Schaben (Blattaria). I. Das Regenerationsvermogen und die Genese des Regenerats. *Wilhelm Roux Arch. EntwMech. Org.* **154**, 434–465.

Postlethwaite, J. 1978 Development of cuticular patterns in the legs of a cell-lethal mutant of *Drosophila melanogaster. Wilhelm Roux Arch. EntwMech. Org.* **185**, 37–57.

Przibram, H. 1931 *Connecting laws in animal Morphology.* London: University of London Press.

Reinhardt, C., Hodgkin, N. & Bryant, P. 1977 Wound healing in the imaginal discs of *Drosophila.* I. Scanning electron microscopy of normal and healing wing discs. *Devl Biol.* **60**, 238–257.

Russell, M. 1974 Pattern formation in the imaginal discs of a temperature sensitive cell-lethal mutant of *Drosophila melanogaster. Devl Biol.* **40**, 24–39.

Russell, M., Girton, J. & Morgan, K. 1977 Pattern formation in a *ts*-cell-lethal mutant of *Drosophila*: the range of phenotypes induced by larval heat treatments. *Wilhelm Roux Arch. EntwMech. Org.* **183**, 41–59.

Sander, K. 1971 Pattern formation in longitudinal halves of leaf hopper eggs (Homoptera) and some remarks on the definition of 'Embryonic regulation'. *Wilhelm Roux Arch. EntwMech. Org.* **167**, 336–352.

Schubiger, G. 1971 Regeneration, duplication and transdetermination in fragments of the leg disc of *Drosophila melanogaster. Devl Biol.* **26**, 277–295.

Schubiger, G. & Schubiger, M. 1978 Distal transformation in *Drosophila* leg imaginal disc fragments. *Devl Biol.* **67**, 286–295.

Shelton, P., Truby, P. & Shelton, R. 1981 Naturally-occurring abnormalities (Bruchdreifachbildungen) in the chelae of three species of Crustacea (Decapoda) and a possible explanation. *J. Embryol. exp. Morph.* **63**, 285–304.

Slack, J. 1980 A serial threshold theory of regeneration. *J. theor. Biol.* **82**, 105–140.

Slack, J. & Savage, S. 1978 Regeneration of mirror symmetrical limbs in the axolotl. *Cell* **14**, 1–8.

Steiner, E. 1976 Establishment of compartments in the developing leg imaginal discs of *Drosophila melanogaster. Wilhelm Roux Arch. EntwMech. Org.* **180**, 9–30.

Stocum, D. 1980 Intercalary regeneration of symmetrical thighs of the axolotl, *Ambystoma mexicanum. Devl Biol.* **79**, 276–295.

Strub, S. 1977 Developmental potential of the cells of the male foreleg disc of *Drosophila.* I. Pattern regulation in intact fragments. *Wilhelm Roux Arch. EntwMech. Org.* **181**, 309–320.

Strub, S. 1979 Leg regeneration in insects: an experimental analysis in *Drosophila* and a new interpretation. *Devl Biol.* **69**, 31–45.

Weiss, P. 1939 *Principles of development.* New York: Holt, Reinhart & Winston.

Williams, G. & Caveny, S. 1980 A gradient of morphogenetic information involved in muscle patterning. *J. Embryol. exp. Morph.* **58**, 35–61.

Winfree, A. 1980 *The geometry of biological time.* New York: Holt, Reinhart & Winston.

Wolpert, L. 1969 Positional information and the spatial pattern of cellular differentiation. *J. theor. Biol.* **25**, 1–47.

Wright, D. 1979 Segmental pattern of *Oncopeltus.* D.Phil. thesis, University of Cambridge.

Wright, D. 1981 (In preparation.)