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Distal Regeneration and Symmetry

Susan V. Bryant, Vernon French, Peter J. Bryant

Summary. A revision of the "polar coordinate model" shows how pattern formation in diverse regenerating systems can be understood in terms of strictly local cell interactions.

The way in which the activities of the cells of an embryo are coordinated in space and time is one of the great remaining enigmas of biology, and it is a problem which has not, so far, benefited significantly from recent advances in the methodology of molecular biology. Instead, our efforts at understanding the formation of spatial pattern in developing animals are still comparable to the pre-Mendelian stage of genetics; we are still searching for the formal "rules" by which we can predict the behavior of embryos under various experimental treatments. Only when we understand these principles at a formal level, do we expect to be able to frame appropriate questions for a molecular analysis.

Although we seek to understand the events occurring in normal embryonic development, it is the bizarre mistakes made by the developing organism, either spontaneously or in response to experimental manipulations, that are most instructive, just as mutant genes were instructive in gaining an understanding of the functioning of the normal genome. The details of many naturally occurring abnormalities were described as long ago as 1894 by Bateson (*1*), who was the first to attempt to define a set of rules to describe the symmetry relationships of extra appendages. Since then, the abnormalities resulting from experimental manipulation have been thoroughly analyzed in several experimental systems. We showed several years ago (*2*) that most of the pattern regulation (developmental responses to surgical intervention) seen in the appendages of amphibians and cockroaches as well as the imaginal discs of *Drosophila* could be understood in terms of a single set of rules, the polar coordinate model. Some parts of this model made use of local interactions between cells, but one of the rules (the "complete circle rule") implied that more long-range interactions could occur between cells. In this article, we propose an alternative formulation taking into account recent experimental findings, and the revised model involves only interactions that occur on a strictly local level between cells and their nearest neighbors.

The Polar Coordinate Model

We begin from Wolpert's (*3*) idea that spatial patterns result from cells acquiring information about their physical positions in the developing cell population. We consider this positional information to be specified along polar coordinates in two dimensions (that is, in a cell layer), so that each cell would have a positional value with an angular and a radial component. In the limb or imaginal disc, the radial sequence of positional values corresponds to the (presumptive) proximal-distal axis of the appendage, with the distal tip at the center of the field (Fig. 1), while the circumferential sequence of positional values corresponds to the circumferential position on a cross section at each proximal-distal level.

The most important feature of this model is the proposition that tissues have the general property of intercalation. When cells from two normally nonadjacent radial or circumferential positions are confronted with one another as a result of either grafting or wound healing, the discontinuity in positional values stimulates local cell division, and during this growth, those positional values are generated which normally lie between the confronted positions. If the confronted cells differ in circumferential position, then intercalation generates the set of positional values that separates the two points by the shorter, rather than the longer, of the two possible circumferential routes (the "shortest intercalation rule"). This proposition is supported by direct evidence from studies of the cockroach leg (*4, 5*) and from indirect evidence from studies of imaginal discs and amphibian limbs (*2*). An important consequence of intercalation is that it results in patterns that show continuity, in which all pattern elements are adjacent to their normal nearest neighbors or to extra copies of themselves, even though globally the pattern may be bizarre (*6*). It is important to note that the systems we discuss are those which show epimor-

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phic pattern regulation (7), where continuity is achieved by the addition of new cells with appropriate positional values by localized growth from the starting fragment. In morphallactic systems (7) such as hydra and various early embryos, continuity can be achieved by modification of cell fates, which does not require growth (8).

In the second rule of the polar coordinate model, the "complete circle rule," we proposed that whenever a complete circumference of positional values is exposed or generated (by amputation, grafting, wound healing, or intercalation) at a given proximal-distal (that is, radial) level, then growth occurs; and during this growth, all of the more distal presumptive parts are generated (distal transformation) (9). A corollary to this is that without a complete circumference, distal transformation should not occur. However, several recent experiments have established the ability of incomplete, symmetrical "double-half" circumferences to support some degree of distal transformation. They have led, as we discuss below, to a better understanding of distal transformation. In general, we conclude from the evidence presented here that distal transformation is not an all-or-none response but a graded one that depends on (i) the number of circumferential positional values present at the base of the outgrowth and (ii) their mode of interaction.

Model for Distal Outgrowth: The Distalization Rule

When either the distal or proximal parts are removed from an amphibian or cockroach leg, or an imaginal wing disc of *Drosophila*, subsequent growth generates the parts of the pattern that are normally distal to the cut edge. When this growth occurs from a proximal piece, the result is regeneration of the missing distal parts, whereas a distal piece will duplicate the existing distal parts. In order to emphasize that this kind of pattern formation always generates more distal elements, it has been termed distal transformation (9). However, since the addition of distal parts appears to depend on the addition of new cells rather than alteration of old ones, we call the phenomenon distal outgrowth.

We propose that the basic cell interaction leading to distal outgrowth is between cells from different circumferential positions that come together toward the wound center as the amputation site heals (Fig. 2a). These confrontations between normally nonadjacent cells lead to circumferential intercalation according to the shortest intercalation rule, and if a complete circle of positional values was present at the amputation site, a new complete circle will be generated by this mechanism. An important point is that, although a specific kind of wound healing is used as an example in Fig. 2a, the same outcome would be predicted by practically any other set of circumferential confrontations that might occur from a wound that consists of a complete circle of positional values. Any kind of wound closure must involve cell displacements that lead to the generation of more complete circles.

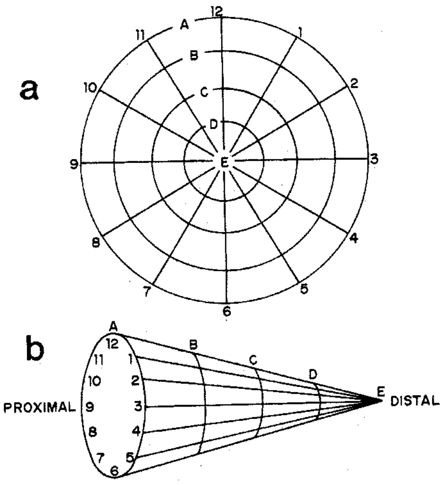


Fig. 1. Polar coordinates of positional information in an epimorphic field. Each cell is assumed to have information with respect to its position on a radius (A–E) and on a circumference (0–12). Positions 0 and 12 are identical, making the circumferential sequence of positional values continuous. In (a) the field is depicted as a flat field, as it might be arranged in an imaginal disc, with the proximal part of the field at the edge and the distal part in the center. In (b) the field is shown as it might be arranged on the surface of an appendage, with the proximal part of the field at the base of the cone and the distal point at the tip.

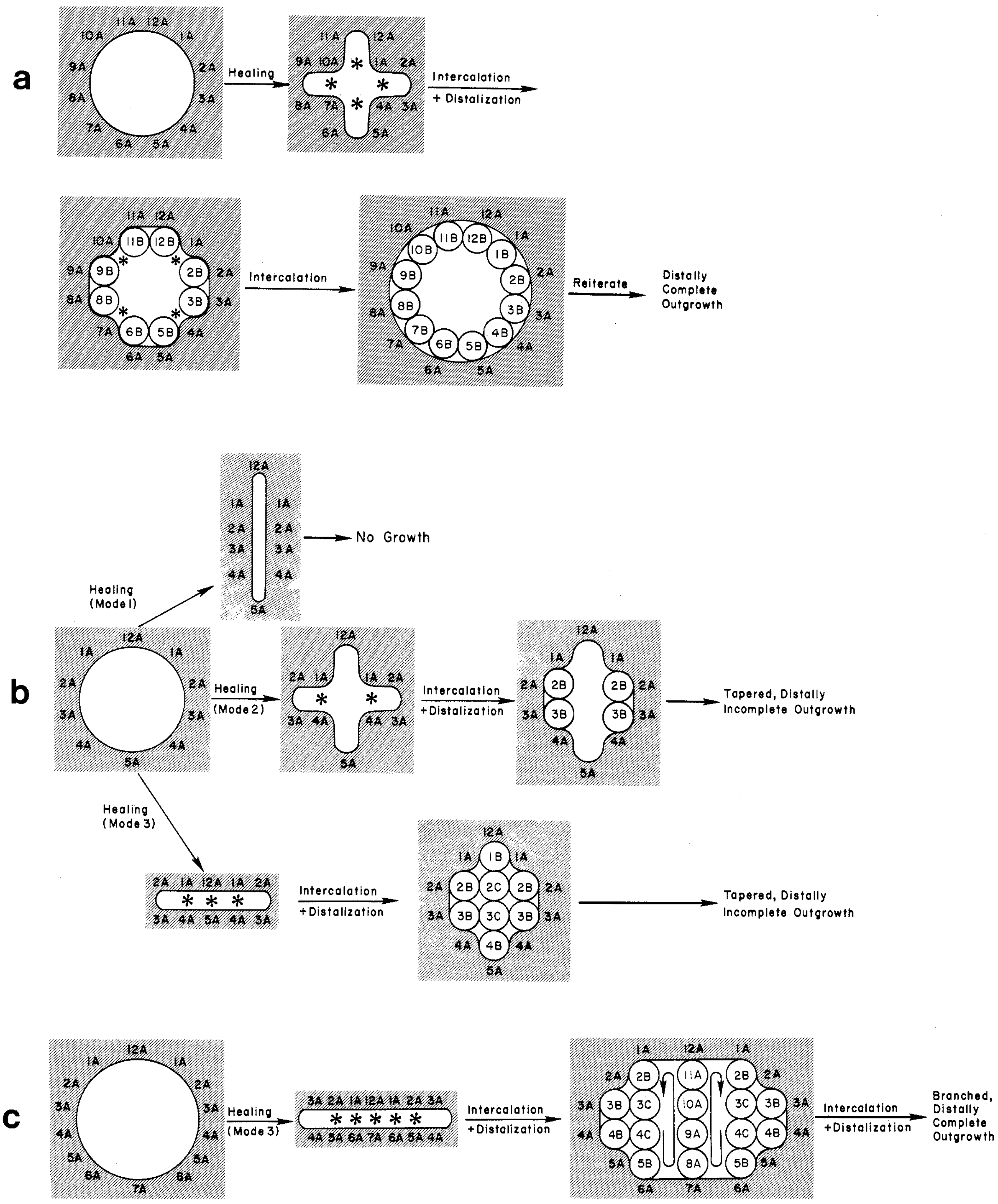
In order to achieve distal outgrowth the new cells generated during circumferential intercalation at the growing tip of the appendage must adopt positional values that are more distal than those of the preexisting cells at the wound edge. We propose that this comes about as a result of a strictly local interaction as follows: during intercalation, a newly generated cell will normally adopt a positional value which is intermediate between those of the confronted cells. However, *if this represents a positional value that is identical to that of a preexisting adjacent cell* (as in the case in Fig. 2a), *then the new cell is instead forced to adopt a positional value that is more distal than that of the preexisting cell.* Thus the new circle in Fig. 2a is at the B rather than the A level. We will call this the distalization rule. For simplicity, we will assume that the new cells adopt the positional value which is only one step more distal, as shown in Fig. 2a, but this is not crucial since proximal-distal intercalation will fill in any gaps that would be formed by any less regular process. Repeated rounds of circumferential intercalation with distalization, with some provision for stopping at the distal tip, will give an outgrowth which is both circumferentially and distally complete.

For surgically created symmetrical fields such as "double-half" limbs in amphibians or cockroaches, the above model predicts that distalization may occur from the symmetrical partial circumferences. However, the extent of distalization will depend on the orderliness and direction of wound healing at the amputation site, and on the number of different positional values present at the cut edge.

Figure 2b shows how the extent of distalization from symmetrical partial circumferences would depend on the mode of wound healing at the amputa-

Fig. 2. Model of distal outgrowth from asymmetrical and symmetrical wound surfaces. (a) An asymmetrical wound surface. The tissue remaining after removal of B, C, D, and E levels of the pattern is shaded and the wound edge is outlined by the circle. This diagram could represent an imaginal disc with the center removed or the stump of an amphibian or cockroach leg after amputation of the terminal parts. It is proposed that during the process of wound healing, different parts of the circumference come into contact, and the second diagram shows one way in which this might occur. Circumferential intercalation (*) produces cells with positional values identical to those of preexisting adjacent cells, and hence the new ones are forced to the next most distal level B (distalization rule). Subsequent intercalation completes the B level, and reiteration of the whole process generates the remaining distal levels. In outgrowth from complete circumferences, the process is essentially independent of variations in the directions of wound healing. (b) A symmetrical wound surface. When the starting configuration is symmetrical as in a double-half limb, the outcome depends on the kind of wound healing that occurs. Mode 1 healing gives no positional value confrontations to stimulate intercalation and thus no distal outgrowth occurs. In contrast, modes 2 and 3 give limited distalization yielding symmetrical and distally incomplete outgrowths. Mode 3 gives a more complete outgrowth than mode 2. (c) A symmetrical wound surface. When the starting configuration consists of two symmetrical copies of more than half of the circumference, the shortest intercalation rule predicts that certain kinds of wound healing [mode 3 of (b)] will lead to the production of two symmetrical complete circles. This will give rise to a diverging, branched, distally complete outgrowth.

tion site. With mode 1 healing, where all healing is across the line of symmetry, there are no positional value confrontations to stimulate intercalation, and no growth or distalization is expected. The opposite extreme is mode 3, where the maximum degree of positional value confrontation occurs. In Fig. 2b, the first round of circumferential intercalation after mode 3 healing gives only four different positional values on each side at the B level compared to six on each side at the A level. At the C level there are only two positional values, and no further distalization is possible because all positional value confrontations have been resolved. The positional values that are lost at each stage are always those on the midline, and the result is a tapered or convergent symmetrical outgrowth which is distally incomplete. We have



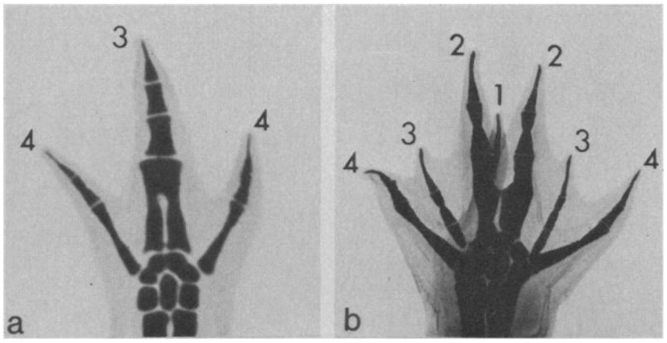
arbitrarily adopted a model with 12 circumferential and 5 radial positional values, and models with different ratios of the two would predict different degrees of distalization. If, for example, there were only three radial positional values, then the mode 3 example in Fig. 2b would be distally complete. An intermediate type of wound healing is mode 2, in which some healing occurs across the midline and some along the midline. Mode 2 healing again produces convergent symmetrical outgrowths, but in this case, they are less distally complete than with mode 3.

Another variable that is expected to affect the kind of outgrowth from a symmetrical circumference is the number of positional values on either side of the line of symmetry. In cases where each side of the symmetrical circumference contains less than half of a complete circumference, as in the examples in Fig. 2b, intercalation can never generate the missing parts of the circumference, and the outgrowths are likely to be convergent and distally incomplete. In contrast, in cases where there is more than half of a complete set of positional values on each side of the line of symmetry, intercalation can replace all of the missing parts of the circumference on both sides. This will lead to a divergent symmetrical outgrowth or, in extreme cases, to two complete distal outgrowths that are mirror images of each other, as shown in Fig. 2c. Of course, mode 1 or mode 2 healing in the example shown (Fig. 2c) would not lead to a divergent outgrowth.

The distalization rule replaces the "complete circle rule" (*2*), and the differences in degree of distal outgrowth under various circumstances now follow simply from the geometrical properties of complete circles compared with double partial ones. How this model can be used to account for distal outgrowth in symmetrical and asymmetrical circumferences in different epimorphic fields is discussed below.

Amphibian Limbs

As we have already argued (*10*), positional information in the urodele limb seems to be primarily a property of the connective tissue, in particular the dermis. Hence, in the following discussion, when we consider interactions between cells with different positional values, we refer to cells of the connective tissue. These cells dedifferentiate after limb am-



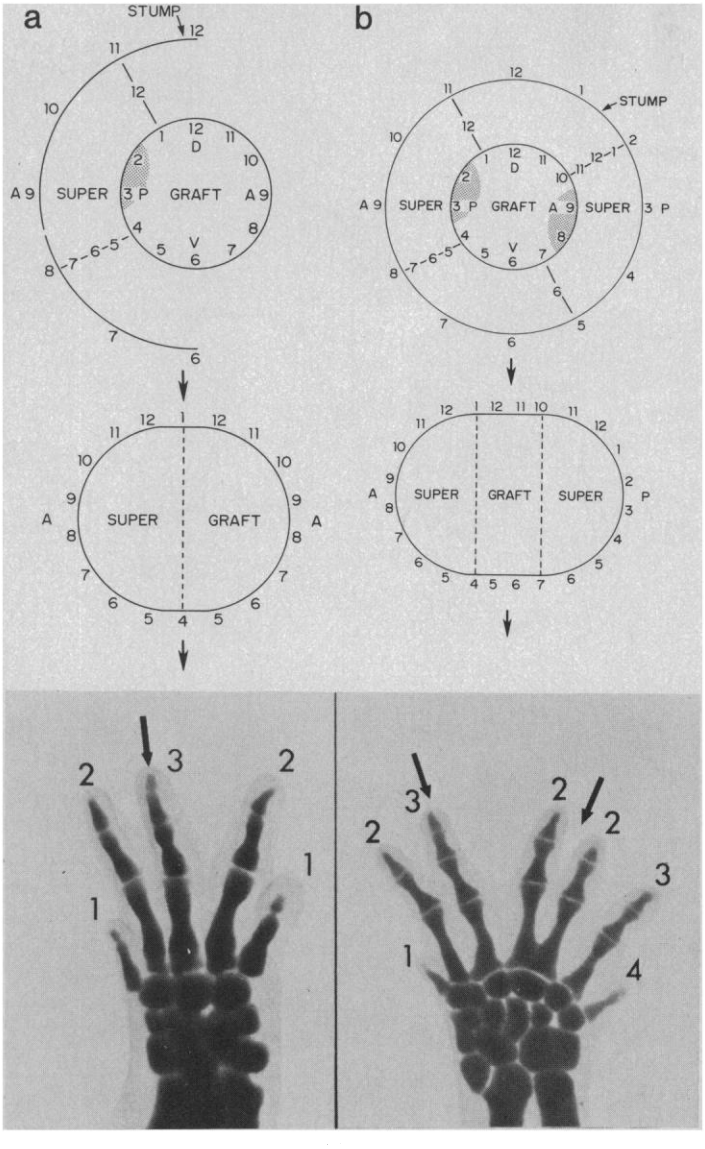


Fig. 3 (above). Symmetrical axolotl regenerates. In (a), the regenerate is convergent, and in (b), it is divergent. Both limbs formed from double-posterior symmetrical stumps. In (a), the limb was amputated in the upper arm 5 days after surgery and this symmetrical, convergent limb regenerated [from (*15*)]. In (b) a regenerate which formed on a symmetrical double-posterior limb was reamputated within the regenerated portion, and the photograph shows the resulting divergent regenerate [from (*14*)]. Thus the positional values at the site of amputation were similar in each case, but the pattern of cellular interactions occurring on the wound surface led to the different outcomes. In (a), mode 2 healing (Fig. 2b) is assumed to have occurred, whereas in (b) the result is explained by mode 3 healing and the intercalation of missing positional values, as illustrated in Fig. 2c. [Courtesy of *Developmental Biology*] Fig. 4 (right). (a) The formation of a symmetrical, double-anterior regenerate from a fused graft and supernumerary limb in the newt. Limbs such as these are sometimes formed when very immature regenerates are transplanted to the contralateral limb stump (*20*). The top diagram illustrates the stump and graft with intercalated positional values between them. Cell death in the shaded region of the graft would eliminate posterior positional values that would not necessarily be replaced. The middle diagram shows the positional values that would be present at the base of the combined graft and supernumerary limb. Distal outgrowth from this symmetrical base would proceed to give the symmetrical regenerate shown at the bottom. The line of symmetry separating graft and supernumerary parts of the outgrowth is indicated by an arrow on the hand skeleton. (b) The formation of an "expanded" right hand (*20*) in the newt. Such limbs sometimes form after contralateral transplantation of an immature regenerate. The top diagram shows the stump and graft with intercalated positional values between them. Shaded areas represent proposed zones of cell death in the graft which would not necessarily be replaced by intercalation. The middle diagram shows the positional values assumed to be present at the base of the outgrowth. Distal outgrowth from such a base could lead to a limb such as that shown at the bottom, where the central portion of the grafted hand is flanked on each side by supernumerary outgrowths arranged in mirror-image symmetry to the remaining portion of the graft. Arrows in the bottom part of the figure indicate lines of symmetry. [Courtesy of *Developmental Biology*]

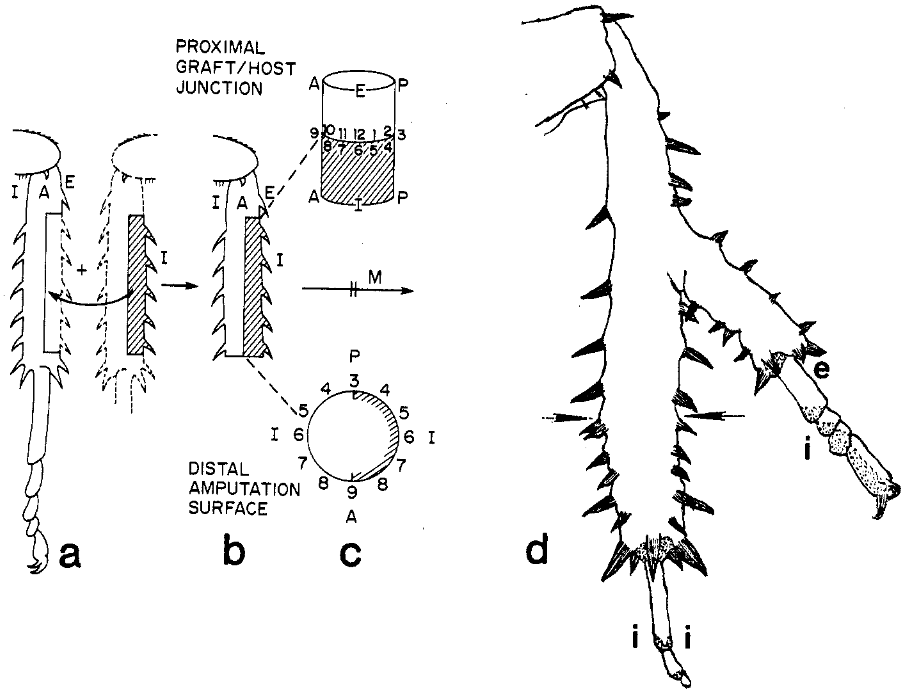


Fig. 5. Regeneration from a double-internal amputation surface in the cockroach tibia. The letters *A*, *P*, *I*, and *E* denote anterior, posterior, internal, and external faces of the tibia, and circumferential positions are labeled 1 to 12. Grafting the internal half of a left tibia (hatched) in place of the external half of a right tibia (a) and then amputation through the graft (b), creates a complete circumference (c) at the proximal limit of the graft and a symmetrical double-internal circumference at the amputation site. (d) The result after two molts (*M*) with the proximal confrontation regenerating a normal complete tarsus with internal adhesive pads (*i*) and the external articulatory condyle (*e*), while the amputation site (marked by arrows) regenerates a symmetrical distally incomplete tarsus with two sets of internal pads (*i*).

putation, and in company with dedifferentiating cells of other tissue types, accumulate in the center of the wound area under the apical ectodermal cap (*11*). In moving from the periphery toward the center of the wound, they come into contact with connective tissue cells from which they were previously separated. Distal outgrowth from a normal asymmetrical amphibian limb stump will then occur as in Fig. 2a.

Experiments have been performed in both newts and axolotls to test the ability of surgically constructed symmetrical upper arms to undergo distal outgrowth (*12, 13*). When limbs with two symmetrically arranged anterior, posterior, dorsal, or ventral halves were constructed and allowed to heal for 30 days or more prior to amputation, distal outgrowth either did not occur or was very incomplete both distally and circumferentially, giving a highly convergent regenerate. Convergent regenerates may result from growth after mode 2 healing, whereas those cases where no distal outgrowth occurred may result from mode 1 healing (Fig. 2b). When symmetrical double-posterior (but not double-anterior) axolotl limbs were amputated immediately after construction rather than after a period for wound healing between the two halves, the majority formed symmetrical distally complete regenerates (*14*). This may be because the lack of tissue continuity across the line of symmetry prevented interaction and hence loss of midline positional values. Additional evidence that each half can behave autonomously is provided by the formation of half regenerates in some cases (*14*). Symmetrical double-posterior limbs provided with an intermediate time (5 to 10 days) for healing produced an intermediate type of convergent limb, usually with three digits (Fig. 3a) (*15*).

Regenerates from surgically constructed symmetrical lower legs (*16*) or lower

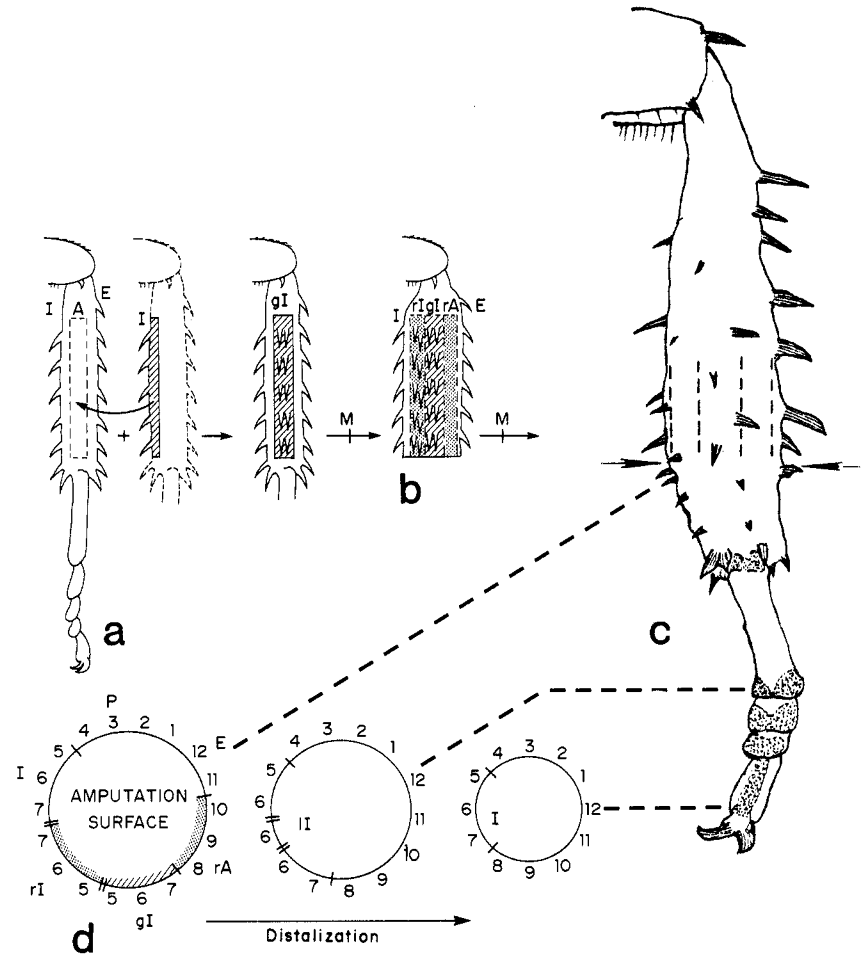


Fig. 6. Regeneration from a cockroach tibia amputation surface including two extra copies of the internal face. The internal face (hatched) is grafted to the anterior face of the donor tibia (a) which is left for one molt (*M*) when another internal face (*rI*) (shaded) and an anterior face (*rA*) (shaded) have been intercalated along the edges of the graft (*gI*) removing discontinuities in circumferential position. The tibia is then amputated through the graft (b), and (c) shows the regenerated distal structures; (d) the circumference at the level of amputation with three copies of the internal face separated by two lines of symmetry (double lines) where positional values may be lost during distalization, to give an abnormally large internal face (*II*) and eventually a normal internal face (*I*).

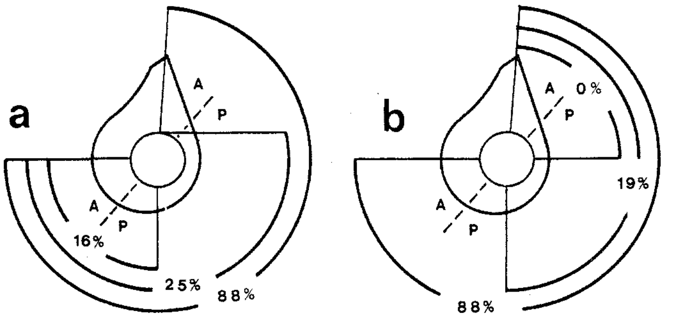


Fig. 7. The frequency of distal outgrowth (percentage of claws) in various duplicating proximal sectors of the *Drosophila* first leg disc, cultured in adult hosts before metamorphosis. [From (*27*); courtesy of *Developmental Biology*]

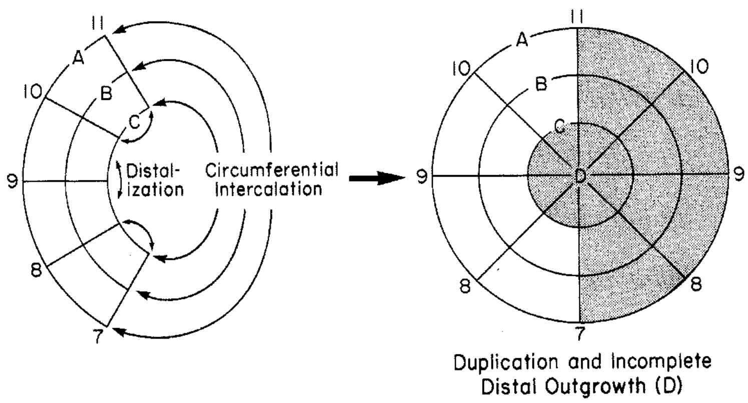
arms (*17*) were in almost all cases distally complete although they were deficient in the circumference. In these cases, since amputation was performed close to the distal tip of the pattern (that is, lower arm or leg), fewer rounds of intercalation would be required to form all of the missing distal levels than would be the case with a more proximal amputation. Hence, even though midline positional values may be lost during distal outgrowth, as in the upper arm experiment, the most distal positional values could be reached.

The model predicts that the number of positional values in the symmetrical limb will influence the extent of distal outgrowth, and this effect may explain a number of other experimental results. For example, investigators (*13–15*) have reported that double-posterior axolotl upper arms regenerate more structures than double-anterior upper arms, and Stocum (*16*) found the same disparities between double-anterior and double-posterior thighs. This result would be expected if the posterior physical half of the axolotl upper limb contains more positional values than the anterior half (*18*).

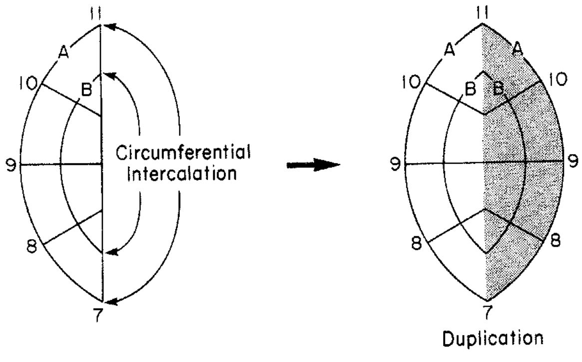
Missing positional values can sometimes be regenerated from symmetrical limb stumps. Holder, Tank, and Bryant (*14*) amputated double-posterior axolotl upper arms immediately after construction, and found that symmetrical double-posterior regenerates were usually formed. When these regenerates were subsequently reamputated in their lower arms, symmetrical regenerates again formed, but in almost all cases the pattern diverged and was more complex than in the original regenerate (Fig. 3b). In a few cases, even the most anterior digit (digit 1) was formed on the midline of the symmetrical regenerate. Similar reamputation of double-anterior regenerates was not performed because they form too little new structure to make this feasible. The results on double-posterior limbs can be explained if the posterior half of the axolotl upper arm contains more than half of the circumferential positional values. During the first regeneration, predominantly mode 2 healing (Fig. 2b) would give a convergent regenerate. However, some of the first regenerates formed as many as six digits, suggesting mode 3 healing. During the second regeneration, however, a predominantly mode 3 healing would give a divergent regenerate as in Fig. 2c. This may happen more readily in the flattened blastema formed after the reamputation at the lower arm level than in the initial upper arm blastema.

The regenerative ability of a different type of symmetrical limb has been examined by Slack and Savage (*19*), who constructed, by surgical manipulation, double-posterior limbs in the axolotl embryo. These embryonically derived symmetrical limbs regenerated distally complete symmetrical limbs when they were amputated or reamputated. If the embryonically derived double-posterior limbs contain a double set of more than half of the positional values, midline positional values may, in fact, be lost during distal outgrowth; but given the large starting number of circumferential positional values, the process could continue until the distal limb pattern had been regenerated. Some midline loss does appear to take place during regeneration since most of the regenerates (77 percent) had fewer skeletal elements than the originals. In other cases the regenerates formed divergent patterns with more digits than in the original limb (*19*). Whether a regenerate from a symmetrical stump converges or diverges would depend on the number of different positional values present at the amputation plane as well as on the pattern of interactions between the early blastema cells.

Finally, a different class of results can also be understood in terms of this new model. When advanced regenerates of newts were transplanted from the left to the right limb to oppose anterior and posterior positions of graft and host (*20*), supernumerary limbs arose in the graft-host junction, as would be expected according to the polar coordinate model (*2*, *21*). These supernumerary limbs were of opposite handedness to the grafted limb and were usually clearly separated from it. However, when young blastemas were transplanted, no supernumerary limbs developed, but, instead, the regenerate was variously modified to form a



Duplication and Incomplete Distal Outgrowth (D)



Duplication

Fig. 8 (left). Circumferential intercalation between the two ends of a proximal arc of an imaginal disc produces duplication if less than half of the circumferential positional values are present. At the same time, distalization occurs from the inside wound surface, to give a symmetrical distal outgrowth which is distally incomplete in this case. New tissue is shaded. Fig. 9 (right). When a straight-sided fragment of an imaginal disc contains less than half of the circumferential positional values, it undergoes duplication during growth. No distalization is expected. New tissue is shaded.

double-anterior, double-posterior or expanded limb of stump handedness (*20*). These "modified" limbs probably represent fused supernumerary and grafted limbs after death of part of the graft at either its anterior or posterior edge. Cell death was, in fact, observed after transplantation of these very young blastemas (*20*). A double-anterior regenerate can be considered as a graft in which cells died on the posterior edge, fused to an anterior supernumerary outgrowth (Fig. 4a). The double-anterior fused structure is symmetrical and would be capable of distal outgrowth, as was discussed earlier. Similarly, the double-posterior regenerates can be viewed as a result of loss of some anterior positional values in the graft, followed by distal outgrowth from the fused (graft-supernumerary) symmetrical blastema. Finally, the "expanded" limbs, with basically stump handedness, can be seen (Fig. 4b) as a combination of a graft which lost both anterior and posterior tissue, plus supernumerary limbs on both sides. Two lines of symmetry are present in such an outgrowth.

Cockroach Legs

Cockroach leg stumps can be constructed with a variety of abnormal numbers and arrangements of circumferential positional values (*5*, *22*), and the structures that regenerate from them strongly support the present model of distal outgrowth.

The cockroach leg can be described in terms of four faces: anterior, posterior, internal, and external. A length of the internal half of a right tibia was transplanted in place of the external half of a left tibia and allowed to heal (*22*) (Fig. 5). When the leg was amputated through the graft, the regenerate that formed from the double-internal stump was also double-internal and was distally incomplete. In contrast, a normal asymmetrical tibial apex and complete tarsus grew out from the proximal graft-host junction. Double-external tibial circumferences constructed in a similar way very rarely formed any terminal regenerates, although complete distal structures were produced from the proximal graft-host junction as before. Experiments similar to the above performed by Bohn (*23*) produced similar results.

A symmetrical double-internal or double-external wound surface can also be produced in the cockroach leg by extirpating a V-shaped sector extending halfway into the side of the tibia. This experiment (*23*) produced results strikingly similar to those just outlined for double-half limbs. After removal of an internal sector, the wound surfaces sometimes produced a symmetrical, double-internal, distally incomplete outgrowth, whereas a double-external wound surface was less capable of regeneration, only rarely producing distal tibial elements and never producing tarsus.

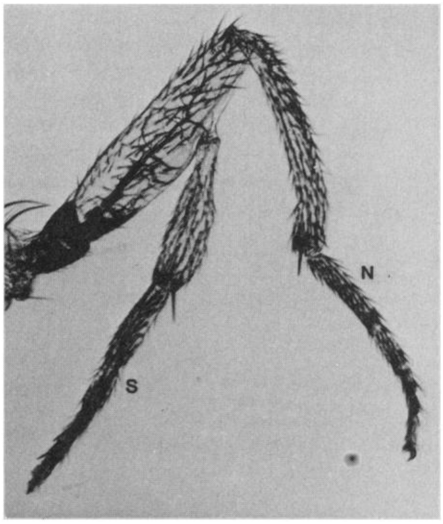


Fig. 10. A triplication of parts of the second leg in *Drosophila* after a surgical cut made in situ in the imaginal disc of the larva (*32*). The branch on the right (*N*) is of normal symmetry, whereas that on the left (*S*) consists of symmetrical copies of the ventral part of the leg, and is assumed to represent an outgrowth from the symmetrical wound surface. [Courtesy of *Developmental Biology*]

These two sets of results show that symmetrical double-half limb stumps in the cockroach, exactly as in the amphibian, can undergo limited outgrowth to produce symmetrical and distally incomplete regenerates. The occurrence and extent of distal outgrowth may, again, depend on the directions of wound healing during regeneration. Thus, wound healing between disparate positions would sustain distalization, whereas healing between identically specified cells in the two symmetrical halves would prevent further growth (Fig. 2b). The superiority of double-internal over double-external wound surfaces in producing distal regenerates could be a result of differences in the predominant directions of wound healing in the two cases, or it may suggest that the internal half of the tibia, as defined by the experimental cuts, carries more positional values than does the external half. Grafting procedures that evoke supernumerary regeneration suggest a fairly uniform spacing of circumferential positional values in the cockroach tibia (*2*), and therefore indicate that the first of these two explanations is the more likely to be correct.

Regeneration was also studied from stump circumferences that included extra copies of some of the positional values (*22*). A strip of tibia was grafted into a different circumferential site on the host tibia (Fig. 6a) and, after healing and intercalary regeneration along the longitudinal edges of the graft (Fig. 6b), the tibia was amputated through the graft. Figure 6c shows the formation of a circumference including two extra copies of the internal face, and the structures regenerated after amputation. In all cases, a distally complete regenerate was formed, and the extra internal faces present in the stump were progressively lost from the more distal levels (*22*). Hence the regenerate's tibial apex was abnormally large, and enlarged or multiple internal adhesive pads were found on the first and second tarsal segments; but the third and fourth tarsal segments and claws were normal in all cases.

If distal outgrowth depends on successive rounds of intercalation between cells with different circumferential values which are confronted at the site of

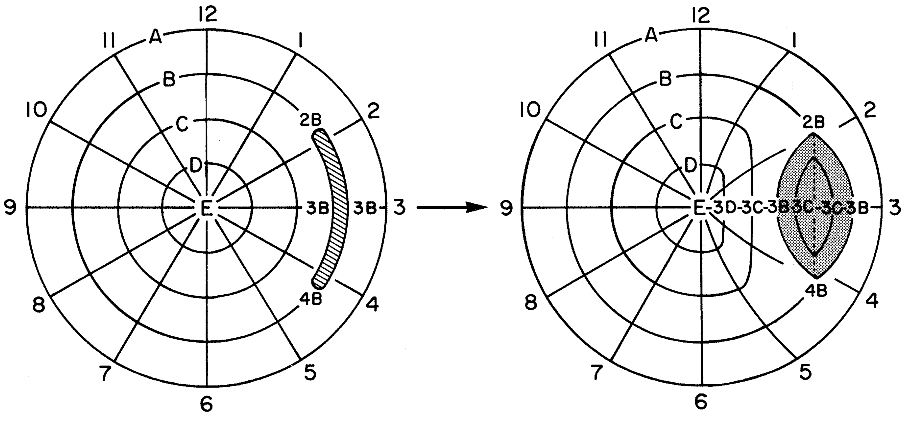


Fig. 11. Diagram showing how a cut in an imaginal disc (left) produces a symmetrical wound surface (hatched) which could lead to (right) a symmetrical, distally incomplete outgrowth (shaded) by the distalization process.

healing, then the double-half cockroach stumps would be expected to lose positional values on the midline and give convergent distally incomplete regenerates as in Fig. 2b. Similarly, the extra positional values would be expected to taper out during regeneration (Fig. 6d), leaving the normal complete circumference distally.

Drosophila Imaginal Discs

The appendages of the *Drosophila* adult are derived from essentially embryonic epithelial sacs in the larva known as imaginal discs. Fate maps of the leg and wing imaginal discs of *Drosophila* show that the distal tip of the adult structure is derived from somewhere near the center of the disc, while successively more proximal adult parts arise from successively more peripheral locations in the disc (*24, 25*). When the central (presumptive distal) part of either of these discs is removed and the remaining peripheral fragment is grown in an adult fly before transfer to larvae for metamorphosis, the missing distal parts are regenerated (*25, 26*). If the presumptive distal part itself is cultured, at least in the case of the wing disc it also generates a new set of distal elements in mirror symmetry to the original set (*25*).

The ability of partial circumferences of presumptive proximal tissue to undergo distal outgrowth has been tested in both leg (*27, 28*) and wing (*29*) discs. In these experiments the central area of the disc is removed and various arcs of the proximal annulus are cultured before metamorphosis. Some such fragments (in the theoretical interpretation, those containing more than half of the circumferential positional values) regenerate the entire circumference and, as expected, show distal outgrowth. Others (those thought to contain less than half of the circumferential positional values) produce a duplicate partial circumference in which the two patterns are in mirror-image symmetry (*26–29*). This circumferential regeneration and duplication can be understood as a result of intercalation between different parts of the presumptive pattern, which come together during wound healing and behave according to the shortest intercalation rule (*2*).

Some presumptive proximal fragments, which show duplication and therefore are assumed to contain less than half of the circumferential positional values, nevertheless undergo various degrees of distal outgrowth (*27, 29*). These results, then, provide a test of the circumferential requirements for distal outgrowth which is analogous to the use of symmetrical double-half limb stumps in cockroaches and amphibians. The results from the leg disc (Fig. 7) show clearly that the probability of distal outgrowth increases with the proportion of the circumference present in the starting fragment (*27*). In the wing disc also, the highest frequencies of distal regeneration were seen in those fragments which contained a large part of the circumference (*29*). These results are easily interpreted by the proposed distalization model (Fig. 8). After wound healing, circumferential intercalation generates a duplicate of the starting fragment, whereas healing between cells along the inner margin of the starting fragment leads to distalization. A mirror-image duplicate of the distally regenerated structures will be produced either by intercalation between original and duplicate patterns, or by distalization from the duplicate. In either case, the result is a symmetrical regenerate in which distal completeness (or probability of completeness) will depend on the fraction of circumference present originally and on the mode of wound healing of the fragment.

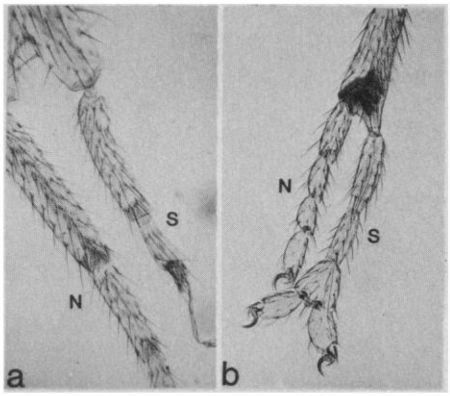


Fig. 12. Symmetrical outgrowths (*S*) of parts of the *Drosophila* leg produced by a 48-hour temperature pulse of 29°C applied to late third instar *l(1)ts-726* larvae (*42*). (a) Convergent, distally incomplete third leg outgrowth. (b) Divergent, distally complete first leg outgrowth. *N*, leg of normal asymmetry.

Other conditions may have to be met in order for distal outgrowth to occur from these fragments. Thus, Schubiger and Schubiger (*27*) suggest that both anterior and posterior compartments of the disc (*30*) must be present for distal outgrowth to occur, and in the wing disc the ventral part of the anterior-posterior compartment boundary seems to be required for distalization (*29*).

Schubiger and Schubiger (*27*) reported occasional examples of duplicating patterns that appear to diverge to two complete distal circumferences (tarsi) from proximal bases which, since they show circumferential duplication, must contain less than half of the positional values. These examples can be explained if we assume that the starting fragment contained less than a half-circumference at its proximal limit (and therefore duplicated circumferentially) but more than a half-circumference at its distal limit (and therefore diverged distally).

Simple circumferential intercalation suffices to produce a complete duplicate of a straight-edged fragment (Fig. 9). No distalization is expected in this case, since the new cells being generated are not usually adjacent to preexisting cells with identical positional values. Thus, the generally conservative type of duplication observed in these fragments (*25*) is accounted for (*31*).

Some further information on pattern regulation in the leg disc comes from surgical operations performed in situ on developing larvae (*32*). Complete bisection of the leg disc gave one regenerating and one duplicating fragment, as in the transplantation studies (*26*). However, these operations sometimes failed to completely sever the disc, and pattern triplications were obtained in several cases (Fig. 10). Such a triplication consisted of a normal, asymmetrical and complete leg in normal orientation together with a symmetrical outgrowth. These results are not easily explained by the original polar coordinate model (*2*), but the modification proposed here allows them to be interpreted. They can, in fact, be considered as exactly analogous to the results of sector incisions on cockroach legs described earlier. An incision in the leg disc, which is closed at both ends, would have a symmetrical wound surface that could produce a symmetrical regenerate (Fig. 11) as in the cockroach experiments. Whether the regenerates diverged or converged would depend on how much of the presumptive circumference was present at the wound site and on the speed and directions of wound healing before and during growth. Triplication of wing-disc derivatives with symmetry relationships similar to those in the leg have been produced by surgical operations or irradiation in *Drosophila* (*32, 33*) and by surgical operations in *Ephestia* (*34*).

Several workers (*35, 36*) have discovered that pattern duplications can be produced at high frequencies by applying pulses of high (restrictive) temperature to developing larvae of strains carrying mutations that cause cell death at the restrictive temperature. Two mutations of the *su(f)* locus have been studied in detail: *l(1)ts-726* (*35, 37–39*) and *l(1)mad^{ts}* (*40*). Direct evidence for tem-

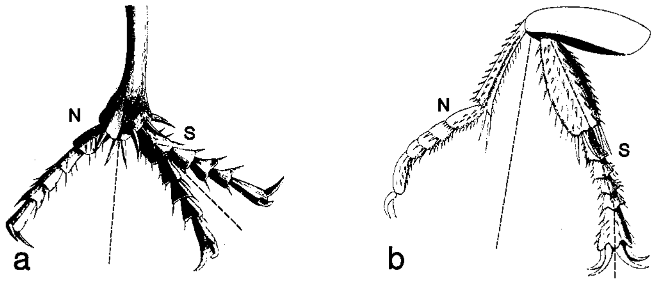


Fig. 13. Naturally occurring branched beetle legs, from Bateson (*1*). In both cases the symmetry relation between the original and extra parts conform to "Bateson's Rules" but in (a) the supernumerary limbs (*S*) are complete and almost separate, while in (b) they are complete but fused together. [Courtesy of Macmillan Press, Ltd.]

perature-induced cell death in localized patches has been obtained for *l(1)ts-726* (*35, 40*), whereas Jurgens and Gateff (*41*) argue against such a mechanism for $l(1)mad^{ts}$. However, the pattern abnormalities caused by these two alleles are so similar that it seems likely that they cause similar primary defects. The most common result of an early restrictive-temperature pulse is leg duplication, where the abnormal leg comprises a bidorsally (*41*) or bilaterally (*39*) symmetrical partial leg with the ventral or medial structures, respectively, missing. Histological work (*35, 40*), as well as genetic mosaic analysis (*38*), indicates that the part of the pattern with normal handedness represents the surviving tissue after degeneration in the original leg disc, whereas the duplicate pattern is produced by growth that occurs after the degeneration.

Another abnormality produced mainly by later restrictive-temperature pulses applied to these mutants is triplication, resembling that described above as resulting from surgery in situ. Such an abnormality can be considered as a normal leg from which a symmetrical outgrowth has arisen (Fig. 12) (*42, 43*). The outgrowths are often distally incomplete and converging (*39, 41, 42, 43*), but sometimes distally complete and diverging (*42, 43*). Whereas the simple duplications found in these mutants are probably a result of degeneration extending to the edge of the imaginal disc, it seems likely that the triplications arise from an internal zone of degeneration analogous to a partial cut in the surgical operations (Fig. 11). We would then assume that the outgrowth represents the outcome of variable and symmetrical distalization from the symmetrical margin of the degenerated zone (*44*).

Although the *su(f)* alleles affect primarily the leg and eye-antenna disc, similar effects are known for the wing disc. The mutations sd^{UCl} and *vg* cause duplication of the notum, apparently in response to degeneration of the wing blade (*45*), whereas the lethal pair *costal* produces a symmetrical, distally incomplete outgrowth of the anterior wing margin, possibly as a result of degeneration in the imaginal disc (*46*).

Bateson's Rules

In 1894, Bateson (*1*) assembled a compendium of information on supernumerary limbs and other pattern abnormalities in animals collected from nature. He found that triple extremities were one of the most common of the abnormalities and noted that the three distal patterns always followed certain rules of symmetry. The appendage next to the one in the normal position was always a mirror image of it, and the one farther from the normal was always a mirror image of the nearer. Bateson constructed a mechanical model to illustrate this principle, which also demonstrated that the symmetry relationships applied to longitudinal planes of symmetry at various directions in the appendage. It is easy to see that these are exactly the symmetry relationships of the outgrowths and normal parts of surgically damaged cockroach legs and of the three elements of the triplications produced by surgical damage or temperature-sensitive cell-lethal mutations in *Drosophila* imaginal discs. In most cases, the extra structures comprised separate and complete sets of distal structures (Fig. 13a) but sometimes they were fused into a complete double structure (Fig. 13b).

Conclusions

Experiments on regenerating newt limbs, cockroach legs, and *Drosophila* imaginal discs continue to produce strikingly similar results, suggesting a common mechanism for pattern formation and regulation in these epimorphic fields. As we showed previously (*2*), various experiments lead to the conclusion that cells in these developing systems form structures in accordance with their positional information, which is specified in two dimensions, along polar coordinates rather than along orthogonal (anterior-posterior, dorsal-ventral, and medial-lateral) axes. Insect cuticular patterns are essentially two-dimensional, being formed by the single layer of surface epidermal cells; but it is still not clear precisely how the internal limb patterns of muscles and (in the amphibian limb) cartilages are formed. We assume that these patterns are based on the two-dimensional positional information in the superficial cell layers, but clearly there must be some additional cues concerning "depth" within the limb.

Wherever a complete circle of positional information is exposed at an amputation site or generated by intercalation, a complete distal regenerate can form. What we have seen is that some distal outgrowth can occur from proximal sites that do not constitute complete circles of positional information but rather are symmetrical partial circles. Stocum (*16*) has previously suggested that a relationship may exist between the fraction of the circumference which is present proximally and the probability or degree of distal outgrowth which occurs. We suggest that the observed relationships are those expected of a mechanism where (i) new cells at the growing distal tip are specified circumferentially by intercalation between cells at the most distal preexisting level, and (ii) each new cell is forced to adopt a more distal positional value if its assigned value would otherwise be identical to that of a preexisting adjacent cell. This mechanism involves only nearest-neighbor cell interactions but accounts for distal outgrowth from complete circles as well as the variable, symmetrical, and often incomplete outgrowths formed from symmetrical double-partial circles. In many cases we have proposed that the precise form of pattern regulation and the extent of distal outgrowth depends on how the wound surfaces come together during healing. Where possible, the healing process should be studied directly (*47*).

Pattern regulation in amphibian and insect appendages is epimorphic (*7*), occurring within new tissue produced at an amputation site or a graft/host junction. Evidence for local growth during pattern regulation is available for both amphibians and insects (*48*). We have assumed the simple, extreme form of epimorphosis where cellular positional values are stable except in the daughter cells (possibly only one daughter cell) from a division provoked by discontinuity in positional information of abutting cells. Others have suggested that regeneration in amphibian (*49*) and insect (*50*) limbs involves an initial morphallactic (*7*) phase during which changes in positional value may spread back sequentially from the cut edge or junction, followed by an epimorphic phase characterized by cell proliferation within this band of altered tissue. Clearly, this could occur by a slight modification of the present model, and need involve only nearest-neighbor interactions (*49*).

Appendages develop from secondary embryonic fields and we have suggested (*2*) that the same cellular interactions may govern the initial formation of the pattern as well as its later behavior in regeneration. Secondary fields form from regions of the primary field within which pattern regulation may be purely morphallactic (*2*), as indicated by work on the early embryos of both amphibians (*51*) and insects (*52*).

There are still very few clues indeed about the molecular basis for positional information. However, most of the global features of pattern regulation in appendages can now be explained in terms of strictly local interactions between cells and their nearest neighbors. This suggests that in the search for a mechanism, we should seriously consider the possibility that positional information, at least in epimorphic secondary fields, may be a property of the cell surface. Pattern regulation may therefore depend upon interactions between adjacent cell surfaces (*49*), rather than the long-range diffusion or diffusion-reaction systems which have been postulated to account for the behavior of morphallactic systems (*53, 54*).

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55. Supported by grants HD 06082 and AG 01979 from the National Institutes of Health and by the Science Research Council. We thank many of our colleagues for helpful discussions during the development of this manuscript, especially N. Holder, L. Iten, J. Girton, and S. Strub. We thank the colleagues mentioned in the text for permission to quote their unpublished results. We also thank W. Fox, B. Hsei, M. Killacky, K. Fouts, and L. Wolcott for technical assistance.