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Pattern Regulation in Epimorphic Fields

Cells may make use of a polar coordinate system for assessing their positions in developing organs.

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

Classical embryological analysis has led to the concept of a developmental unit, which Weiss (*1*) and others have called the field. It can be defined operationally as the domain within which changes in the presumptive fates of cells (regulation) can occur in response to surgical manipulation. In several organisms it has been shown that up to a certain stage (for example, up to the gastrula stage in the amphibian) the whole embryo can regulate in response to the removal of parts and it therefore constitutes a single field (the primary field). But later surgical interventions have more localized effects, restricted to developmentally autonomous parts of the embryo which we will call secondary fields (*2*). Examples of secondary fields are the developing limb buds and eye, ear, and heart primordia in amphibian embryos, and the appendages and imaginal disks of developing insects.

Following the removal of parts of a field, regulation of the presumptive pattern of differentiation may result in the regeneration of missing elements or in the duplication of elements already present in the fragment. It can occur by epimorphosis, in which pattern elements are added during growth with little change in the remaining part of the pattern, or by morphallaxis, in which regulation involves remodeling of the remaining part of the field to form a miniature but complete pattern (*3*). Most primary embryonic fields seem to regulate by morphallaxis, whereas secondary fields in general show epimorphic regulation.

Fields can also be given a rigorous theoretical definition in terms of Wolpert's positional information theory (*4*). Wolpert proposed that in studying the formation and regulation of spatial patterns of differentiation, we make a distinction between the events by which cells are assigned positional values (positional information) according to their physical locations in the coordinate system of a developing field, and the subsequent responses of the cells (interpretation of positional information) resulting in specific cytodifferentiation. Stern (*5*) had previously proposed a similar distinction between an underlying "prepattern" and the cellular competence to respond. Distinguishing positional information from the cells' response to it is justified on the grounds that genetic mutations can affect the two events separately, and that different patterns of cytodifferentiation can apparently result from the same underlying map of positional values because of differences in the interpretation event (*4*, *6*–*8*). In terms of positional information theory, the field can be defined as a set of cells which have their positions specified with respect to the same coordinate system (*4*).

In this article, we propose a model which accounts formally and in a simple and unified way for the kinds of developmental regulation seen in the secondary fields of both vertebrates and invertebrates. We will discuss in detail the regulative behavior of the limbs of amphibians and of hemimetabolous insects, and of the imaginal disks of *Drosophila*, systems which have been extensively investigated. We expect the model to be applicable to other secondary fields, but its applicability to situations where regulation does not occur [for example, the limbs of higher vertebrates during the later stages of outgrowth and in the mature animal (*9*)] or is limited [the early limb bud of chicks (*10*)] is at present difficult to test. We do not present a detailed molecular model for pattern formation; rather, we consider how the regulative behavior of tissues can be explained in terms of rules for the behavior of individual cells. The problem can subsequently be reduced to consideration of molecular mechanisms to explain cellular behavior.

The model we present here is restricted to two dimensions. This is justified in the case of imaginal disks and insect appendages since we are only considering the cuticular patterns secreted by epithelial sheets, and it is also not unrealistic for amphibian appendages, as we shall show later. In fact, it might be generally true that patterns are established in two dimensions rather than

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three; that is, in sheets or layers of cells rather than in solid masses of tissue. The three-dimensional morphology of embryos and of secondary field derivatives develops by means of folding, shaping, and growth of cell layers (as in gastrulation, neurulation, and imaginal disk evagination), and their three-dimensional integration is accomplished by specific inductive relationships between cell layers, as in epithelio-mesenchymal interactions (*11*), rather than by three-dimensional pattern formation in solid tissue.

Description of the Model

The unique definition of cell positions in a two-dimensional array requires spatial variation in at least two different parameters. There are various two-dimensional coordinate systems by which position could be specified in terms of distance or angle from points or lines, but we propose that positional information in epimorphic fields is specified in terms of polar coordinates (Fig. 1). One component of positional information is a value corresponding to position on a circle, and the second component is a value for position on a radius. Of course, the number of values given in both sequences in Fig. 1 is arbitrary, as is the assumption that the positional values in each sequence are equally spaced. In amphibian and cockroach legs the outer circle represents the proximal boundary of the limb field, while the field center is at the distal tip of the limb. In the imaginal disks of *Drosophila* the outer circle represents the disk boundary, and in the case of disks which produce appendages, the center is the presumptive distal tip of the appendage (*12*).

The two rules we propose for the behavior of cells in epimorphic fields are as follows.

1) Shortest intercalation rule. When normally nonadjacent positional values in either the circular or the radial sequence are confronted in a graft combination or as a result of wound healing, growth occurs at the junction until cells with all the intermediate positional values have been intercalated; then growth ceases. The circular sequence is continuous and the position 12/0 does not imply a boundary having unique properties; it arises inevitably when labeling a circle (such as a clockface) with numbers. This continuity of the circular sequence means that there are two possible sets of intermediate values between any two nonadjacent positional values. For example, juxtaposition of cells with values 3 and 6 gives two possible sequences of intermediate values: 3 (4, 5) 6 and 3 (2, 1, 12/0, 11, 10, 9, 8, 7) 6. A critical stipulation of the model is that when cells with nonadjacent positional values in the circular sequence are brought into contact, intercalation is always by the shorter of the two possible routes.

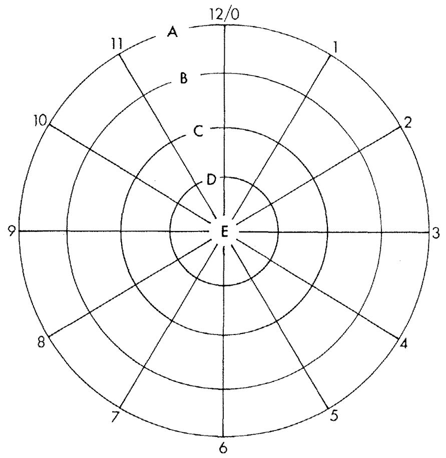


Fig. 1. Polar coordinates in a positional information field. Each cell is assumed to have information with respect to its position on a radius (*A* through *E*) and its position around the circle (0 through 12). Positions 12 and 0 are identical, so that the sequence is continuous. [From (*64*)]

2) Complete circle rule for distal transformation. The entire circular sequence at a particular radial level may undergo distal transformation to produce cells with all of the more central (distal) positional values (*13*). We propose that this distal transformation occurs only when cells with a complete circular sequence of positional values are either exposed at an amputation site or generated by intercalation.

We now consider the experimental evidence from hemimetabolous insect legs, from the imaginal disks of *Drosophila*, and from regenerating amphibian limbs, and show that these different systems exhibit an intriguing uniformity of behavior when considered in terms of the proposed model.

Evidence from the Legs of Hemimetabolous Insects

Shortest intercalation. The shortest intercalation rule is most clearly illustrated by deletion and grafting experiments on the larval cockroach leg. Removal of a narrow longitudinal strip of integument (cuticle plus epidermis) from any location around the circumference of the femur in a larva results in the cut edges healing together, thereby confronting cells which are normally nonadjacent. This results in localized growth and intercalation; in subsequent larval stages the leg regains its normal size and the deleted structures are regenerated (Fig. 2) (*14*).

Grafting a rectangular piece of integument to an abnormal position around the circumference, without changing its proximal-distal position, again confronts normally nonadjacent cells at the edges and ends of the graft (Fig. 3, a and b) (*15, 16*). In a variety of different combinations, this stimulates growth leading to intercalation of the structures which normally lie between host and graft circumferential positions by the shorter of the two possible routes [Fig. 3, a(ii) and b(ii)]. When the confrontation is between opposite circumferential positions there is no shorter route and such limbs may intercalate either of the half-circumferences separating host and graft positions (*17*). Grafting between femur and tibia gives similar results (*16, 17*), suggesting that cells at corresponding circumferential positions in different leg segments have the same positional values in the circular sequence. These experiments further indicate that the intercalated structures are derived from both host and graft (*17*).

Proximal-distal intercalation also occurs in the cockroach leg; association of normally nonadjacent levels within a leg segment results in localized growth and intercalary regeneration of the intermediate structures. Combining a distal graft level and a proximal host level results in a normally orientated regenerate (Fig. 4a), whereas combining a proximal graft and a distal host level produces a regenerate with reversed proximal-distal polarity (Fig. 4b) (*18–20*). In these and all of the other experiments we discuss here, the polarity of pattern elements is consistent with the direction of the sequence of positional values; we know of no case where one can be altered independently of the other. Grafting between pro- and metathoracic legs (*20*), between differently pigmented species (*21*), and between different cuticle color mutants (*22*) has shown that intercalary regenerates are derived from both host and graft.

These results are all accounted for by the shortest intercalation rule applied to the circular or radial component of positional information. However, a complication arises in that the radial sequence appears to be repeated in each segment. Hence combination of midtibia and midfemur, for example, provokes no intercalary regeneration (Fig. 4c), whereas combination of proximal tibia and distal femur results in an intercalary regenerate approximately half a segment long, in this case derived mostly from the femur (Fig. 4d) (*19*, *20*).

Distal transformation. After amputation in a larval stage, during subsequent stages many insect legs regenerate the structures normally distal to the amputation site, even from an originally proximal-facing cut surface created by reversing part of the leg in the long axis (Fig. 4e) (*23*, *24*). Distal regeneration can also occur from both components of a graft-host junction if they do not heal together after grafting (Fig. 4f) (*20*, *22*). We interpret these results as distal transformation of the radial sequence (*25*). Bohn (*23*) combined two external or internal longitudinal tibia halves in bilateral symmetry and found either no distal regeneration or very limited regeneration from the symmetrical amputation surfaces. This result is consistent with the idea that a complete circular sequence of positional values must be present at the stump in order for regeneration (distal transformation) to occur.

Supernumerary regeneration. The model can provide an explanation for the occurrence, number, position, orientation, and asymmetry of supernumerary regenerates arising from graft-host junctions in insect legs (*26*). When a longitudinal strip of integument is grafted into an abnormal circumferential position, supernumerary legs are produced from the ends of the graft in some combinations (Fig. 3b) but not in others (Fig. 3a), as predicted by the model. In combinations where graft, host, and the values intercalated at the longitudinal edges of the graft comprise two symmetrical copies of part of the circular sequence, intercalation occurs at the ends of the graft to restore the continuity of longitudinal rows of pattern elements [Fig. 3a(iii)] (*15*). Where graft, host, and the tissue intercalated at the longitudinal edges comprise a complete circumference, distal transformation occurs at the ends of the graft, forming supernumerary regenerates [Fig. 3b(iii)] (*17*).

After a left leg is grafted to a right stump (or vice versa) two supernumerary regenerates are formed from the graft-host junction (*23*, *26–28*). If the anterior-posterior axis of the graft is reversed relative to that of the host, supernumeraries arise from anterior and posterior and their orientation is like that of the host [Fig. 5a(i)], whereas the supernumeraries arise internally and externally after graft internal-external axis reversal [Fig. 5b(i)]. The model predicts that intercalation will occur at the graft-host junction between the confronted values of the circular sequence, always intercalating the missing values by the shorter route. The shorter route is different on the two sides of each point of maximum incongruity, hence at these points a complete circumference is generated [Fig. 5, a(ii) and b(ii)]. Distal transformation will occur from these complete circular sequences to form supernumerary regenerates, their orientation and handedness being determined by the direction in which intercalation occurs in the adjacent regions of the graft-host junction. Figure 5, a(ii) and b(ii), shows the graft-host junction after anterior-posterior and internal-external axis reversal, indicating the correctly predicted positions and orientations of the supernumeraries.

After reversal of both transverse axes by 180° rotation in the cockroach leg, Bullière (*28*) and Bohn (*23*, *27*) found

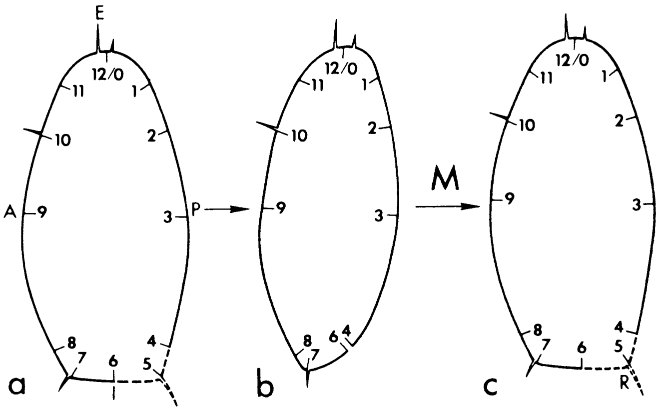


Fig. 2. Intercalary regeneration in the circular sequence after removal of a longitudinal strip of the cockroach leg. Schematic cross section of the femur, distal view. Letters *A*, *I*, *P*, and *E* denote anterior, internal, posterior, and external faces; numbers 0 to 12 physical positions around the circumference, corresponding (arbitrarily) to positional values 0 to 12 in the circular sequence (Fig. 1). (a) Removal of epidermis between positions 4 and 6 (dashed line) leads to (b) the cut edges healing together and (c) intercalary regeneration (dashed line) of the missing values. The regenerated structures (*R*) are visible after molting (*M*).

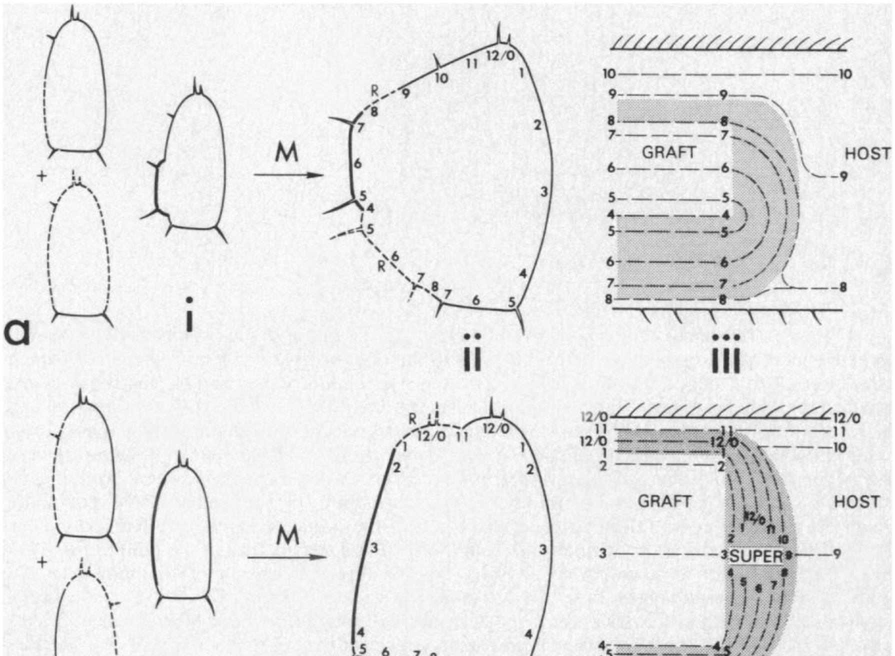


Fig. 3. Intercalary regeneration in the circular sequence after transplanting longitudinal strips of the cockroach leg. (a) Graft of internal face of the left femur, or (b) graft of posterior face of the right femur, into the anterior face of the left femur. In each case (i) and (ii) are schematic cross sections through the graft, while (iii) is an anterior view of the distal end of the graft. (i) Graft combination: dashed lines represent discarded portions, and bold line represents the graft. (ii) Result after two molts (*M*). Positional values (dashed lines, *R*) have been intercalated between graft (bold line) and host by the shorter route. (iii) At the distal end of the graft there is intercalation (shaded area) to restore normal sequences of positional values. Dashed lines connect points of equal positional values in the circular sequence. In (a) such intercalation does not lead to the generation of a complete circumference, but in (b) it does, and distal transformation occurs to form a supernumerary limb (*super*) at the distal end of the graft.

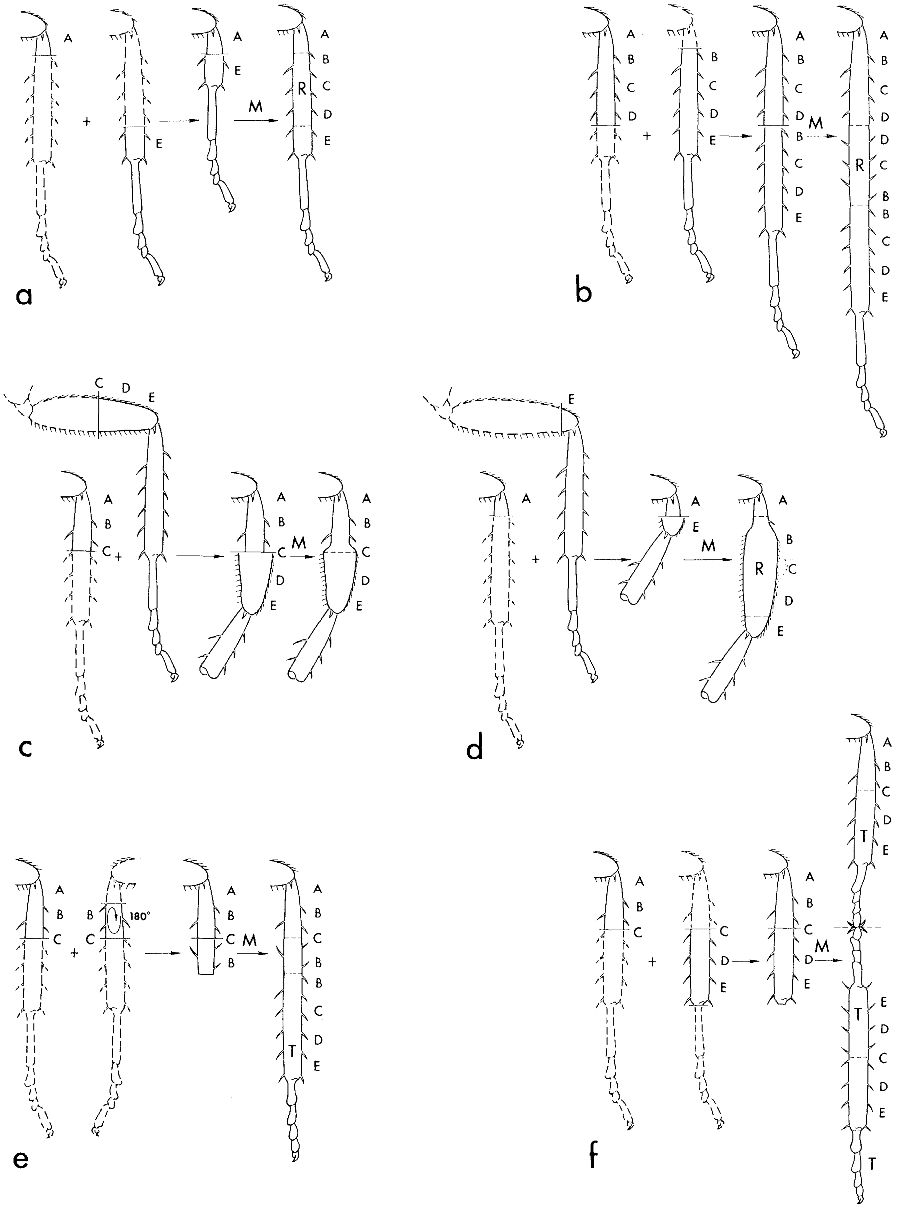


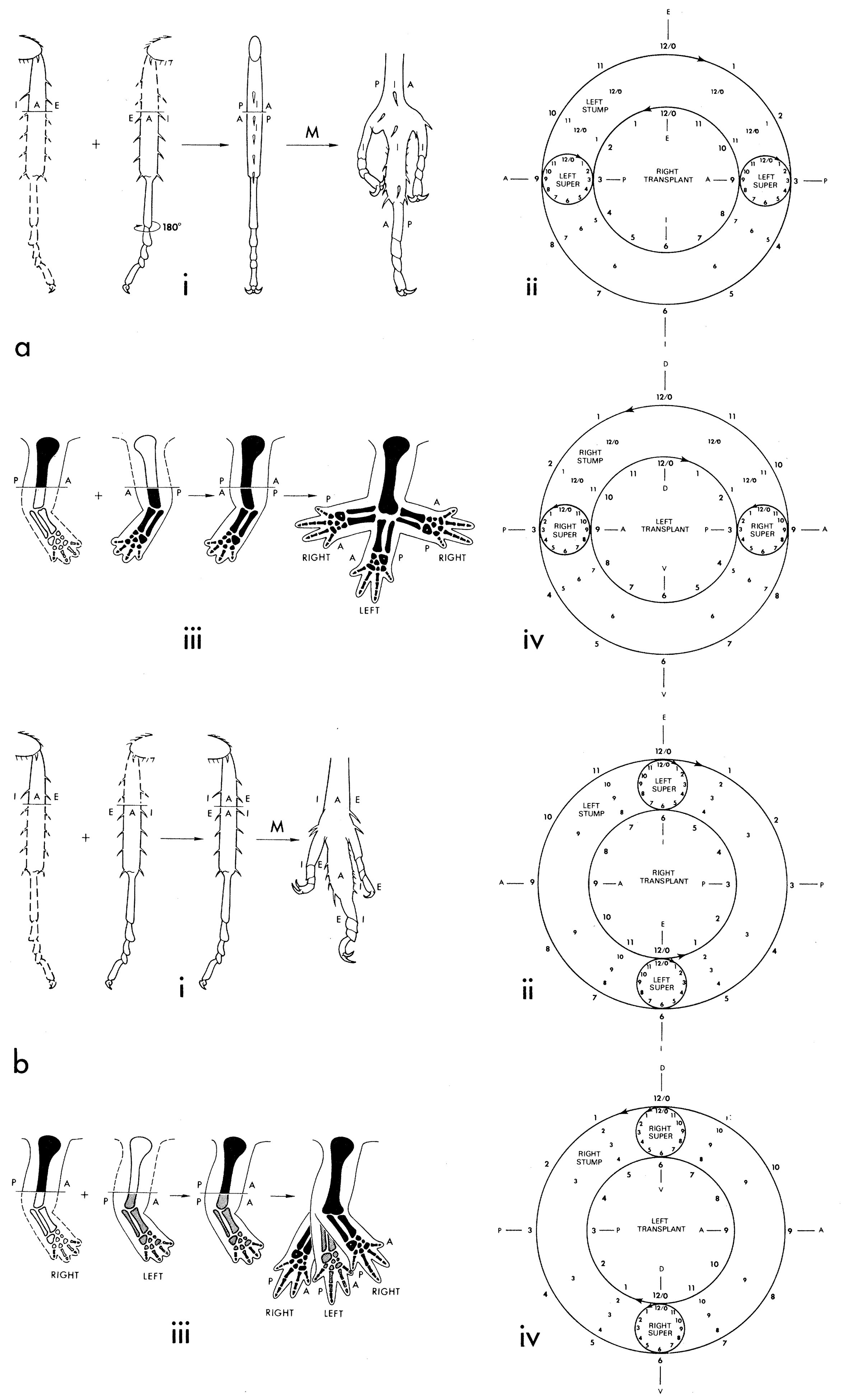
Fig. 4 (above). Intercalary regeneration and distal transformation in the proximal-distal (radial) sequence after grafting together different levels of the tibia or tibia and femur of the cockroach leg. Letters *A* to *E* denote physical levels of the leg segment corresponding to positional values *A* to *E* in the radial sequence (Fig. 1). In each case, the diagram shows the graft combination and the result after two molts (*M*). (a) to (d) Intercalation (*R*) occurs between normally nonadjacent positional values in the proximal-distal sequence when the grafts are between tibia and tibia (a and b) or between tibia and femur (d), but no intercalation occurs between homologous positional values in the tibia and femur (c). (e) Transformation (*T*) from an originally proximally facing cut surface occurs following reversal of part of the radial sequence. (f) Transformation (*T*) occurs from the distal cut surface and from the graft and the host when the grafted parts fail to heal and interact at a junction. Fig. 5 (opposite). Application of the model to the production of supernumerary limbs in cockroaches and amphibians following contralateral transplantations with either (a) anterior-posterior or (b) external-internal (dorsal-ventral) axes opposed. (i) Diagram of the experiment and the major result in cockroaches (*23*, *26–28*). Letters *I*, *A*, *E*, and *P* denote internal, anterior, external, and posterior surfaces; *M* denotes molt. Supernumerary regenerates are formed at the points of axial incongruity. (iii) Diagram of the experiment and the major result in the newt *Notophthalmus viridescens* (*60*, *64*). In this case regeneration blastemas, rather than parts of mature limbs, are transplanted. The dorsal surface is solid black, the ventral surface stippled; *A* and *P* denote anterior and posterior surfaces. Supernumerary regenerates have formed at the points of axial incongruity. (ii) and (iv) Schematic cross section of graft-host junction, distal view; outer circle, host circumference; inner circle, graft circumference. (The diameters of the graft and stump are shown to be different for clarity only.) The circular sequence is marked around the circumference by numbers 0 to 12. Numbers between the circles are values generated by intercalation (by the shorter route) between the different confronted positional values of host and graft. The shorter route is different on the two sides of each point of maximum incongruity, so a complete circular series is generated at that position. Subsequent distal transformation leads to the production of a supernumerary limb. The arrangement of the positional values around the supernumerary limbs is determined by the direction of intercalation in the adjacent regions of the graft junction and is a consequence of the shortest intercalation rule. The direction of the intercalation adjacent to the supernumerary limbs gives their handedness and orientation. As can be seen, both supernumerary regenerates are of stump handedness, oriented in the same way as the limb stump, and they are in mirror image symmetry with the transplant. Abbreviations: *super*, supernumerary limb; *V*, *I*, *A*, *D*, *E*, and *P*, ventral, internal, anterior, dorsal, external, and posterior.

either no supernumerary or two supernumerary regenerates (one left- and one right-handed), forming in various positions and with various orientations. Supernumeraries were not found after 90° rotation (*23*, *28*). We have shown (*26*) that the shortest intercalation rule can account satisfactorily for these results (see Fig. 6).

In addition to these results on cockroaches, similar supernumerary regenerates are produced after contralateral leg transplantation or 180° rotation in stick insects (*29*), hemipterans (*30*), lepidopterans (*31*), and spiders (*32*), and after similar operations on the anal cerci of crickets (*33*) and earwigs (*34*).

Organization of the base of the cockroach leg. Bohn (*35*) found that a leg would still regenerate after complete removal of the entire leg including its most proximal segment, the coxa. By extirpating different amounts of tissue anterior and posterior to the base of the coxa, he found that a leg can be produced by a confrontation between the sclerites anterior to the coxa (the trochantin and praecoxa) and a membranous zone posterior to the coxa (Bohn's leg-inducing membrane or LIM; see Fig. 7a). The sclerites of one segment are separated from the LIM of the next anterior segment by another membranous zone, which Bohn called the sclerite-inducing membrane or SIM since a confrontation between it and the sclerites resulted in duplication of the sclerites. The base of the leg was thus envisaged as comprising several qualitatively distinct transverse zones.

Bohn's results are in excellent agreement with the predictions made by our model, if it is assumed that the leg field extends into the leg base as in Fig. 7a. The most proximal positional values of the leg field are assumed to occupy the anterior edge of the basal sclerites anteriorly, and the region of the LIM posteriorly. The leg field would be separated from those of other segments by the region of the SIM. Within this most proximal zone of the leg field the rules for cellular interaction are, as before, intercalation in the circular and radial sequences, and distal transformation from complete circular sequences. Consider a few of Bohn's experiments (*35*).

a
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1) Extirpation of the coxa plus part of the posterior (Fig. 7b) or anterior (Fig. 7c) region of the field will result in wound closure, the formation of a complete circular sequence by intercalation, and hence distal transformation (leg regeneration), as is observed.

2) Extirpation of the coxa plus the complete posterior region of the field (Fig. 7d) will not result in leg regeneration because more than half of the positional values in the circular sequence have been eliminated. We assume that wound healing occurs by contraction of the cut edge, as with imaginal disks (discussed below). Confrontation of different positional values in the circular sequence leads to intercalation which results in mirror-image duplication of the anterior values; a duplication of the sclerites is observed. Similarly, extirpation of coxa plus the anterior field would result only in duplication of the posterior values (Fig. 7e); no leg is regenerated but there are no markers in this area, so no duplication is visible.

3) Extirpation of the zone separating the meso- and metathoracic leg fields (Fig. 7f) results in healing of the posterior region of one leg field to the anterior region of the next leg field; intercalation will lead to the formation of a complete circumference of reversed anterior-posterior polarity, and distal transformation will produce a reversed supernumerary leg, as is observed.

One difficulty with gross extirpation experiments is the possibility of variation in the mode of healing, and this may account for variability in the results of some of Bohn's experiments. However, Bohn (*36*) also performed a large number of grafting operations on the leg base, and almost all of the results are compatible with this polar coordinate model, which provides a unified interpretation of the organization of both the basal region of the leg and the remainder of the appendage.

Evidence from Imaginal Disks

Although extensive studies have been made of the growth of imaginal disk tissues of *Drosophila* cultured in adult flies, it is only recently that systematic studies have been made of the regulative properties of specific disk fragments. We now consider these regulative properties in relation to the proposed model.

When a disk fragment is transplanted to a larval host, it undergoes metamorphosis with the host (having no opportunity for growth) and discloses its presumptive fate in the structures which are differentiated. In this way, fate maps of the leg and wing disks have been shown to be more or less concentric with the presumptive proximal structures at the periphery and the distal tip of the appendage close to the center (*37, 38*).

If a disk fragment is transplanted into an adult host it grows but does not differentiate until retransplanted into a host larva. Using this technique it has been shown many times that during growth different disk fragments regenerate the missing structures or produce a mirror-image duplicate of the presumptive pattern already present. Furthermore, for a large number of different cuts it has been shown that bisection of a disk gives one regenerating fragment and one duplicating fragment (*38–42*). In the most extensively analyzed case, that of the wing disk (*38*) it was possible to localize a point in the center of the disk which defines the direction of regeneration and duplication. When the disk is cut into three pieces, fragments which have a cut edge facing away from the center undergo regeneration, while those having a cut edge which faces toward the center undergo duplication. However, the presence of the center in the fragment is not a prerequisite for regeneration, since fragments bounded by two cut edges on the same side of the center show regeneration from one side and duplication from the other. Neither does the presence of the center guarantee regeneration, since four-sided central fragments undergo duplication (*38*).

The finding of regeneration and duplication in complementary fragments of imaginal disks led to the idea of a gradient of positional values within the disk (*38, 40*). It was suggested that from any one level on the gradient, growth allows the generation of lower positional values but not of higher values. Addition of the same set of positional values to the two halves of the gradient comprises regeneration in one case and duplication in the other. This gradient model accounted well for the behavior of fragments of the wing disk if it was assumed that there were multiple, different gradients radiating out from the center (*38*). However, several results are inconsistent with this formulation. First, it was shown that cen-

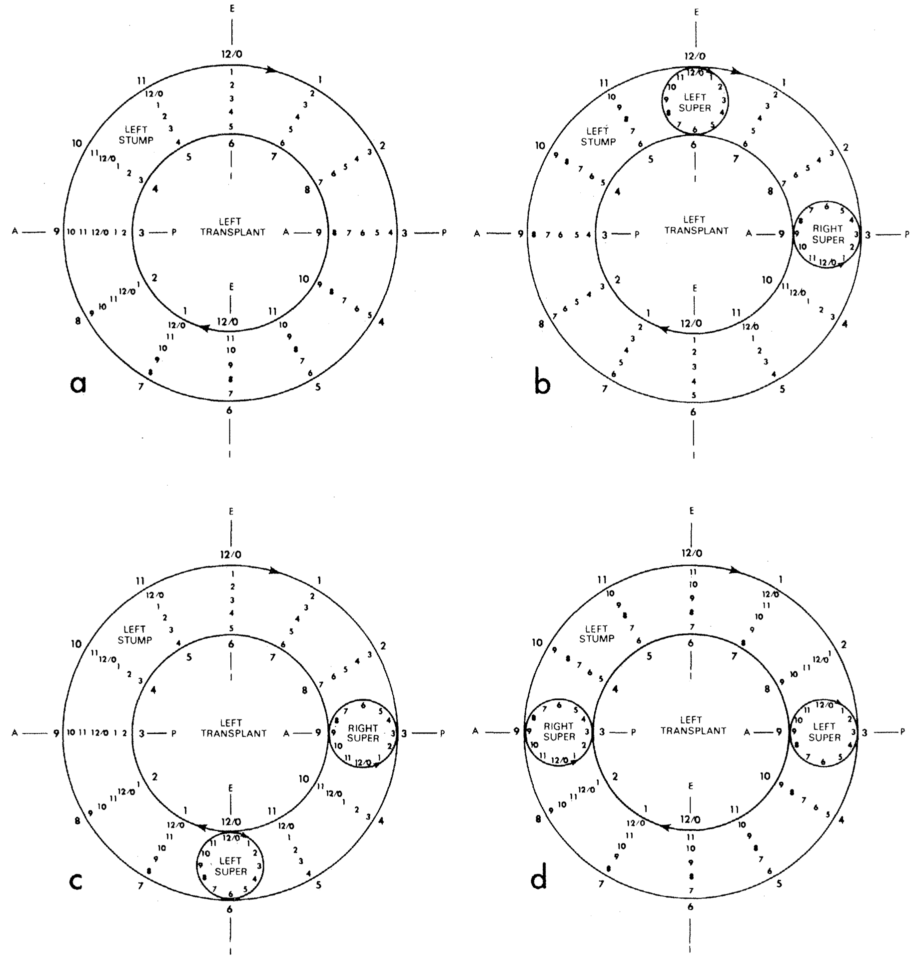


Fig. 6. Transformation from complete circular sequences generated by intercalation between graft and host after reversal of graft anterior-posterior and internal-external axes (180° rotation) in the cockroach. Conventions are as in Fig. 5. Depending on the direction of intercalation, there may be formation of no complete circular sequences (a), or sequences at any two positions, such as adjacent (b and c) or opposite (d).

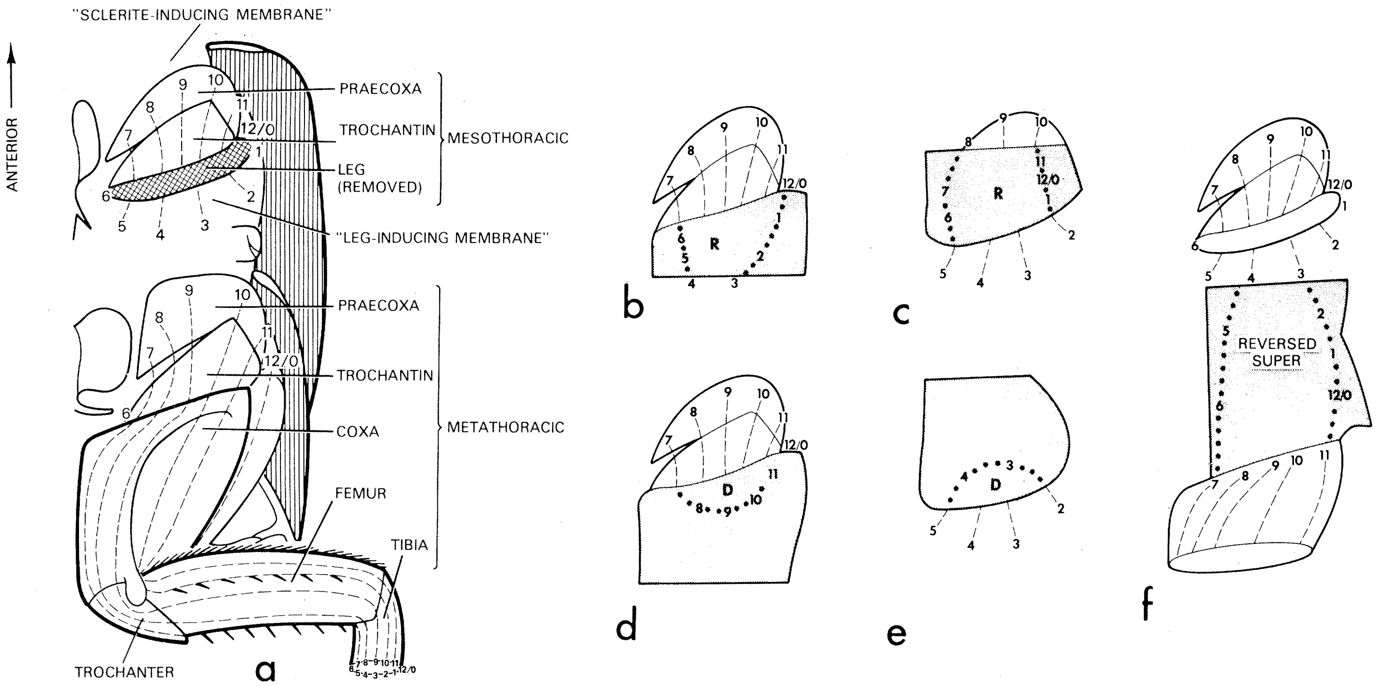


Fig. 7. (a) Organization of the base of the cockroach leg, after Bohn (*35*), with the addition of a hypothetical circular sequence of positional values at the proximal boundaries of the mesothoracic and metathoracic leg fields. Dashed lines connect points of equal positional value in the circular sequence [based on the results of French and Bullière (*16*) and French (*17*) on the tibia and femur, and of French (*14*) on the coxa]. The mesothoracic leg is removed at the base of the coxa (crosshatched). (b to f) An interpretation of some of the results of Bohn (*35*); see text for details. Abbreviations: *R*, leg regeneration; *D*, duplication of regions of the leg base. *Reversed super* means regeneration of a supernumerary leg with opposite handedness.

tral fragments of the wing disk, containing the center and bounded by four cut edges, undergo duplication instead of the expected regeneration, and that the complementary peripheral fragment can regenerate the center (*43*). Second, the four different 90° sectors show duplication, whereas each complementary 270° sector regenerates. This result is not explicable in terms of the predicted behavior of each free edge, since a given edge would have to undergo regeneration under some circumstances and duplication under others. Third, it has now been demonstrated that the regulative behavior of a fragment can be modified by mixing it with fragments from other regions of the disk (*44*). Thus, the presumptive notum region of the wing disk, when cultured alone, shows duplication, but when mixed with presumptive wing it can regenerate wing structures, a phenomenon analogous to intercalary regeneration in cockroach and amphibian limbs. Intercalary regeneration was also demonstrated between anterior and posterior wing disk fragments (*44*).

The present model can explain these results if the regulative behavior of a fragment is not merely a function of the independent behavior of free cut edges, but is the result of interaction between the cut edges after they fuse together during wound healing. Wound healing occurs very rapidly in imaginal disk fragments, probably before much growth has occurred (*45*). In the case of 90° and 270° sectors, the two radial cut surfaces apparently heal together during the first 2 days of culture (Fig. 8).

We assume that regulation occurs in the circumferential direction as a result of the apposition of different circumferential positions by wound healing. Then the shortest intercalation rule accounts simply for the regeneration of 270° sectors and duplication of 90° sectors (Fig. 9a). It also accounts for the behavior of wing disk segments which are bounded by a single cut edge, if we assume that wound closure occurs by contraction along the cut edge, as in Fig. 9b. Again, the evidence indicates that wing disk segments do show the appropriate type of wound healing (*45*).

It has been shown (*41*) that in the leg disk, in contrast with the wing disk, the upper medial quarter of the disk can regenerate the remainder, while the complementary three-quarter sector duplicates. This is consistent with the model if there is a nonuniform spacing of positional values in the leg disk, such that more than half of the values are crowded into the upper medial quarter of the disk (Fig. 9c). There is, of course, no reason to assume uniform spacing, and there is evidence of nonuniform growth of the leg disk during metamorphosis (*37*). As discussed below, nonuniform spacing of positional values is also suggested by the results of certain types of grafting experiments on regenerating amphibian limbs. The model predicts that the periphery of the disk should undergo distal transformation to regenerate the center, which is true of the leg disk (*41*) and wing disk (*43*) and that central fragments should duplicate (Fig. 9d), which was shown to be the case in the wing disk (*38*).

The experimental evidence indicates that the complete circle rule is followed during regulatory growth in both leg and wing disks. Schubiger (*41*) cultured half leg disks from which the presumptive distal regions ("end knob") had been removed. He showed that the lateral half undergoes duplication, and usually fails to regenerate distally, whereas the medial half, which is able to regenerate the missing lateral half, also regenerates the distal structures at high frequency. Similarly, when "cap" and "base" pieces lacking the end knob are cultured, the cap piece, which regenerates the base, shows distal regeneration, whereas the duplicating basal piece usually does not. In the case of the wing disk, it is known for a variety of pieces from the edge of the disk that regeneration toward the center does not occur during duplication of the fragment (*38*), although such regeneration does occur from an annular fragment (*43*).

The genital disks produce bilaterally symmetrical adult derivatives, and in such cases the model predicts that fragments generated by cuts parallel to the

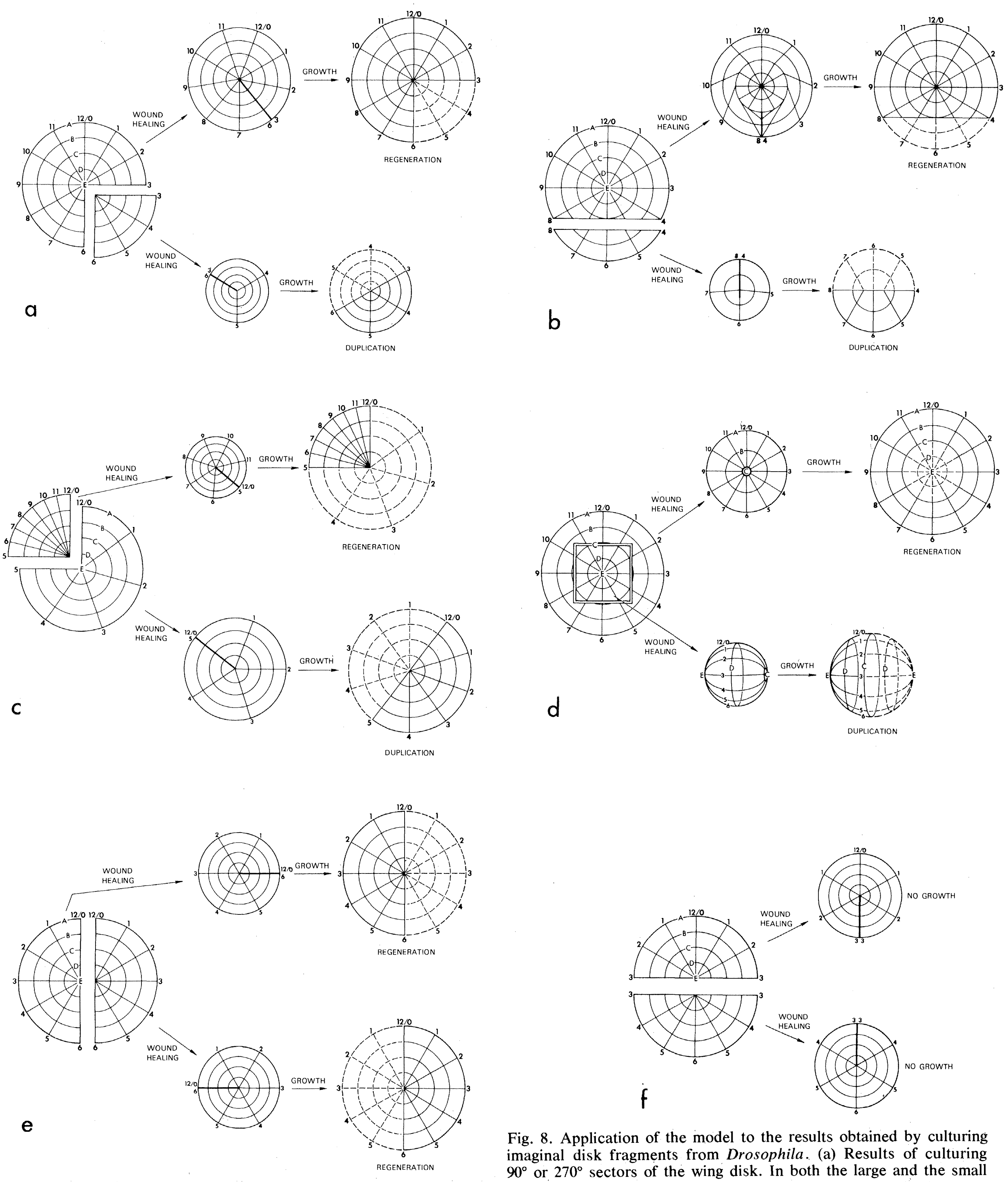


Fig. 8. Application of the model to the results obtained by culturing imaginal disk fragments from *Drosophila*. (a) Results of culturing 90° or 270° sectors of the wing disk. In both the large and the small sector, the cut edges heal together, in each case bringing position 3 next to position 6. Shortest intercalation in both cases leads to the production of intercalary values 4 and 5. In the 90° sector this constitutes a duplication of existing positional values, whereas in the 270° sector it constitutes regeneration of the missing positional values. (b) Results obtained following bisection of the wing disk. Both of the fragments heal by contraction along their cut edges, in each case bringing positions 8 and 4 together. Shortest intercalation leads to the production of positions 5, 6, and 7. In the smaller fragment, the intercalary structures constitute a duplication of existing positional values, and in the larger fragment they constitute a regenerate. (c) Results of culturing different fragments in the leg disk. It is assumed in this case that positional values in the circular sequence are nonuniformly spaced. By the same reasoning as in (a), the 90° sector and the 270° sector will regenerate and duplicate respectively. (d) Results obtained by culturing central and peripheral fragments of the wing disk. A complete circular sequence of positional values is present at each contracted cut edge; transformation results in duplication of the central fragment and regeneration of the peripheral fragment. (e and f) Results obtained by culturing fragments of the bilaterally symmetrical genital disk. (e) Cuts parallel to the axis of symmetry result in regeneration or duplication, and when the cut is medial, as shown here, regeneration is indistinguishable from duplication. (f) Cuts perpendicular to the axis of symmetry do not lead to the confrontation of different positional values, so neither regeneration nor duplication occurs.

line of symmetry will either regenerate or duplicate depending on the position of the cut (Fig. 9e), but if the cut is perpendicular to the line of symmetry there will be no regeneration or duplication (Fig. 9f). The first prediction is amply borne out by the work of Hadorn *et al.* (46), and we have tested the regulative abilities of four fragments of the male genital disk produced by horizontal cuts and have observed neither regeneration nor duplication (47).

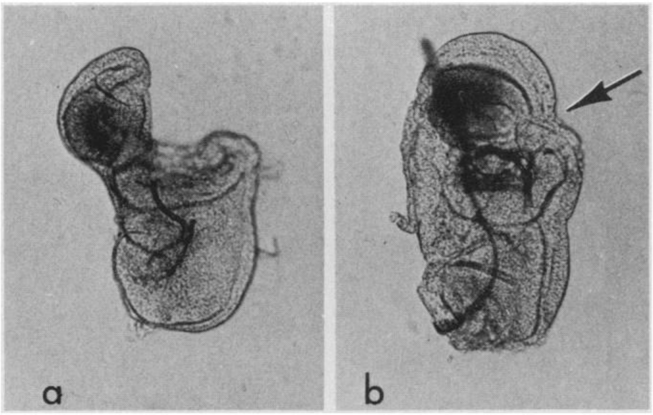
Evidence from Amphibian Appendages

Location of positional information. Unlike *Drosophila* imaginal disks and cockroach legs, amphibian limbs cannot be regarded as two-dimensional epithelial sheets. The limb is a three-dimensional structure with a central core of bone, surrounded by a cylinder of muscle and covered by dermis and epidermis. Nevertheless, our two-dimensional model for pattern regulation is adequate to account for many of the regulative phenomena in amphibian appendages. Experiments on developing limbs have shown that the information for the limb pattern initially resides in a circular disk of mesodermal cells (48–50). When presumptive limb bud mesoderm is grafted to other regions of the embryo without its overlying epidermis, a limb develops (in cooperation with nonlimb epidermis) in which the anterior-posterior organization is the same as that of the original graft. Presumptive limb epidermis without limb mesoderm cannot support the development of a limb. Further support for the premise that positional information may only be specified in two dimensions comes from recent experiments by Carlson (51). When the position of muscle or dermis within a mature axolotl limb is changed and the limb is amputated through the reoriented region, regenerates with multiple distal limb elements are produced. Such abnormal regenerates would be expected if muscle and dermis were to carry positional information and behave according to the rules for epimorphic regulation described here. However, Carlson also found that bone and epidermis do not produce similar effects on limb morphogenesis when their positions with respect to their surroundings are altered. Hence, it seems as though positional information for both development and regulation in the axolotl limb may be specified in two dimensions rather than three; that is, in a flat disk of mesoderm in the presumptive limb region or in a hollow cylinder of mesoderm in the mature limb.

Distal transformation. It is well known that the mature limbs and tails of urodeles can regenerate distal structures after amputation. In the tail, the accuracy of regeneration has been demonstrated by showing that the number of vertebrae regenerated is proportional to the number removed by amputation (52). Distal regeneration can also occur from originally proximal-facing surfaces of amphibian limbs (53) and tails (54). In these experiments, limb or tail segments are grafted so as to reverse their normal proximal-distal orientation, allowing distal levels to survive and undergo pattern regulation, whereupon duplicate distal parts are regenerated. Distal regeneration also occurs from the proximal-facing stump of larval anuran tails in culture (55).

Distal transformation is also shown by the developing limb field and early limb bud of the embryo. When presumptive limb bud material is excised, a limb bud can frequently reform from the remaining surrounding tissue (48). But if only the peripheral portions of the limb field, the presumptive girdle rudiments, are excised, they are not regenerated by more central regions of the field (56).

Evidence that distal transformation will occur only from a complete set of positional values in the circular sequence comes in part from experiments performed by Carlson (57) and Lheureux (58) on x-irradiated limbs, which do not usually regenerate when amputated. If an irradiated limb stump is provided with a cuff of nonirradiated skin (epidermis and dermis) in which the complete circular sequence of positional values is present, distal regeneration will occur. However, if such a cuff of skin, although physically complete, contains only a small part of the circumference, then distal



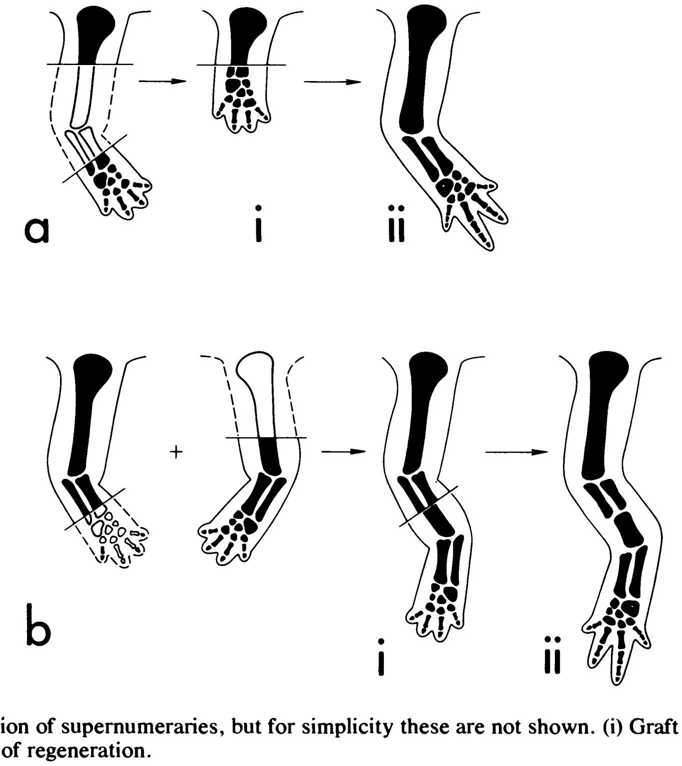


Fig. 9 (left). Wound healing in a 270° sector of the *Drosophila* wing disk (45), (a) after 1 day of culture in an adult abdomen and (b) after 2 days of culture. The two cut edges have fused together. Fig. 10 (right). Intercalation in the proximal-distal (radial) sequence in *Notophthalmus viridescens*. (a) After grafting a blastema (ranging in age from early bud to early digits) from a level in the distal half of the lower arm to a stump in the proximal half of the upper arm. (i) Graft combination. (ii) Schematic representation of resulting limb at the end of regeneration, showing intercalation. (b) After grafting a blastema (varying in age from early bud to early digits) from a level in the proximal half of the upper arm to a stump in the distal half of the lower arm. No intercalary regeneration is observed. This operation is performed between left and right limbs and frequently leads to the production of supernumeraries, but for simplicity these are not shown. (i) Graft combination. (ii) Schematic representation of resulting limb at the end of regeneration.

transformation fails to occur. Since the quantity of tissue available to participate in regeneration is approximately the same in each of these cases, it appears that distal transformation is dependent on the presence of cells comprising a complete circular sequence of positional values at the level being tested. Experiments have shown that compound limbs (double anterior, double posterior, double dorsal, or double ventral) are unable to regenerate distally after amputation (*59*), as predicted by the model.

Shortest intercalation. Intercalation in the proximal-distal sequence has been demonstrated in regenerating amphibian limbs (*60, 61*). When a forearm blastema is transplanted to the upper arm there is considerable dedifferentiation of the stump and transplant, and intercalation occurs so that the resulting limb contains all the proximal-distal levels (Fig. 10a). Similar results have been obtained using the tail (*52*). Stocum (*61*) has presented evidence showing that some of the cells in the intercalary growth in limbs come from the stump. Grafting an upper arm blastema to a lower arm stump does not evoke intercalary regeneration (Fig. 10b) (*60, 61*).

The shortest intercalation rule for the circular sequence has not been directly tested in amphibian limbs. However, experiments by Harrison (*48*) and Swett (*62*) in which half of the embryonic limb field was removed, or in which the two halves were separated by a tissue barrier, indicate that the reciprocal halves of the field behave differently. For example, isolated ventral or anterior half-limb fields often fail to form limbs, whereas isolated dorsal or posterior half-limb fields usually form normal or duplicated limbs. This suggests that intercalation can occur in the circular sequence, and that the ability of a half-limb field to form a complete limb depends on whether a complete sequence of positional values can be generated. Similar experiments performed on regenerating limb blastemas (*63*) did not reveal any differences in the behavior of reciprocal halves of blastemas. This is probably because half-blastemas developing in situ can always reconstruct a complete circular sequence of positional values by virtue of their contact with the adjacent normal limb stump.

Supernumerary regeneration. The shortest intercalation and complete circle rules together can account for the occurrence, location, handedness, and orientation of supernumerary limbs which are formed after certain grafting procedures. If a limb blastema is grafted to the contralateral limb stump such that the anterior-posterior axes are misaligned, a maximum of two supernumerary limbs form from anterior and posterior regions of the graft junction [Fig. 5a(iii)] (*60*). Similarly, when a blastema is grafted to the contralateral limb stump such that the dorsal-ventral axes of the graft and stump are misaligned, again a maximum of two supernumerary limbs are formed, but this time in dorsal and ventral locations [Fig. 5a(iii)]. When a limb blastema is rotated 180° and replaced on the same limb stump, both anterior-posterior and dorsal-ventral axes of graft and stump are misaligned. Once more, a maximum of two supernumerary limbs are formed and they are located in the anterior-ventral and posterior-dorsal positions with respect to the axes of the limb stump at the graft junction (Fig. 11) (*64*).

The results of the contralateral grafts are exactly analogous to those obtained with cockroaches and can be explained in the same way [Fig. 5, a(iv) and b(iv)]. The handedness and axial orientation of the supernumerary limbs are the same as those of the stump. Both handedness and orientation are defined by the direction in which intercalation occurs in the adjacent regions of the graft junction.

In contrast to the variable results in cockroaches, the two supernumerary limbs formed after 180° rotation of blastemas in amphibians are found in constant circumferential positions. A simple way to account for this is to assume that positional values in the circular sequence have slightly nonuniform spacing, as shown in Fig. 11. There are then only two locations around the graft junction where the two possible routes of intercalation involve the same number of positional values. Intercalation occurs in opposite directions at each side of these locations, generating complete circular sequences from which distal transformation will occur to form separate super-

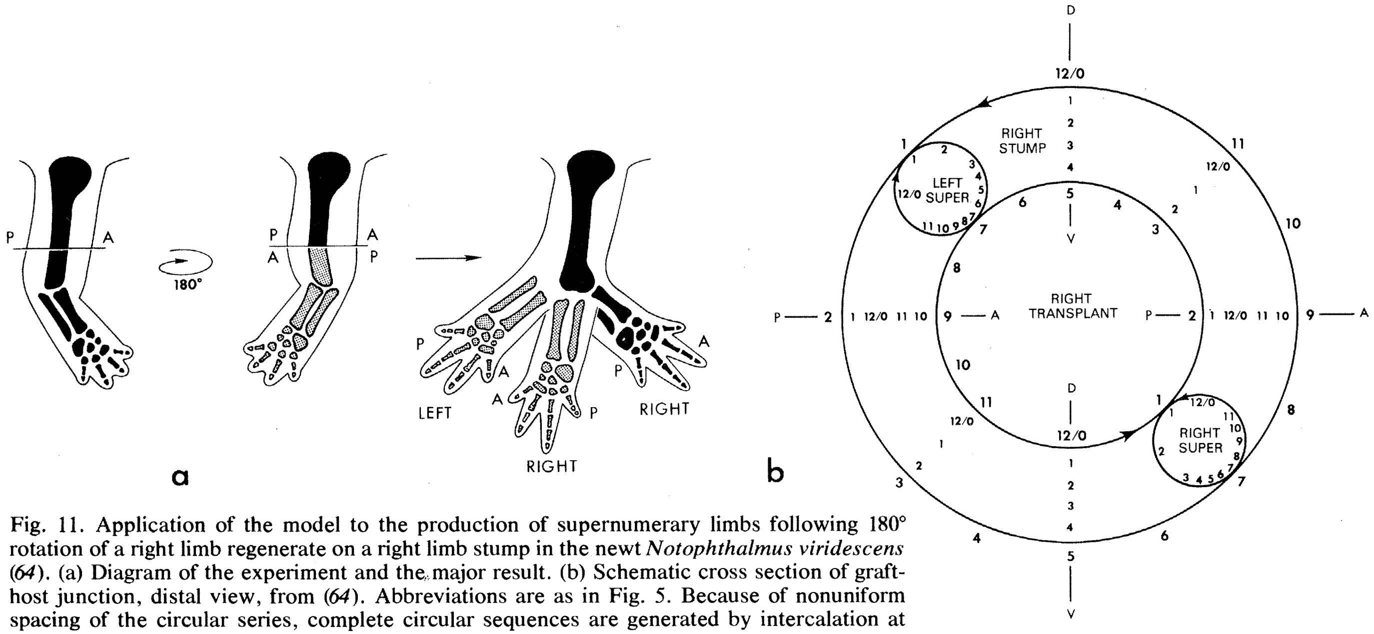


Fig. 11. Application of the model to the production of supernumerary limbs following 180° rotation of a right limb regenerate on a right limb stump in the newt *Notophthalmus viridescens* (*64*). (a) Diagram of the experiment and the major result. (b) Schematic cross section of graft-host junction, distal view, from (*64*). Abbreviations are as in Fig. 5. Because of nonuniform spacing of the circular series, complete circular sequences are generated by intercalation at anterior-ventral and posterior-dorsal locations only. The arrangement of positional values around the supernumerary limbs, which is a consequence of the shortest intercalation rule, gives the handedness and orientation of these structures. As can be seen, the posterior-dorsal supernumerary is a left hand, a mirror image of the transplant. The anterior-ventral supernumerary limb is a right limb which is normally oriented with respect to the limb stump, and it is not a mirror image of the transplant. Dark shading, dorsal; light shading, ventral.

numerary limbs. One supernumerary limb is of the same handedness and orientation as the stump, whereas the other is of opposite handedness. It is important to note that the assumption of slight nonuniformity in positional value spacing has no effect on the predictions made for the contralateral grafts, other than to change very slightly the predicted positions of supernumerary regenerates.

The model also allows one to understand the production of supernumerary limbs following transplantation of developing amphibian limb buds. Harrison (*49, 65*), Swett (*62, 66–68*), and others [see (*64*)] transplanted limb buds of various ages in various axial orientations either to a limb region of the embryo (orthotopic transplants) or to a flank region (heterotopic transplants). In many instances, supernumerary limbs were produced. If the principles discussed above for the production of supernumerary limbs are applied to the developing limb bud, it becomes apparent that certain grafting operations would be expected to lead to the formation of supernumerary limbs, whereas others would not. Bryant and Iten (*64*) have categorized many of these early experiments into these two groups, and have found that in experiments in which the model would predict the occurrence of supernumerary limbs, 59 percent of the animals ($N > 1700$) formed one or more supernumerary limbs. In experiments in which the model would not predict the formation of supernumerary limbs, only 5 percent of the animals ($N > 800$) formed supernumerary limbs. Thus, the occurrence of supernumerary limbs in these classical experiments agrees very well with the results expected according to the model presented here.

Initial Establishment of Epimorphic Fields

The secondary fields which we have discussed develop at particular locations in the primary morphallactic field which comprises the whole early embryo: the insect blastoderm (*69*) and the amphibian blastula and early gastrula (*2*). The available evidence suggests that secondary fields acquire a polar epimorphic character very early and that they are initiated by the sequential establishment of certain key positional values. Intercalation between these initial positional values then controls the growth of the field during development.

In the case of imaginal disks, several workers have studied the normal development of pattern by removing disks from young larvae and causing them to undergo metamorphosis prematurely by implanting them into old larvae. It is then assumed that the structures which are differentiated give an indication of which positional values had been established at the time of explantation. When a leg disk from a second instar larva is caused to metamorphose, only the most proximal and distal structures are differentiated, but disks from older larvae are able to produce the intermediate structures (*70*). This could, of course, occur by the same mechanism of positional value intercalation that we are proposing for regulative development. Similar studies have been done with the eye-antenna disk, but the results are somewhat contradictory (*71*). Studies on duplication and deletion resulting from damage to imaginal disks at the blastoderm stage or during the first larval instar (*72*) suggest that the basic polar organization of imaginal disks is established very early, when they each consist of only 10 to 20 cells.

Our model provides a close link between pattern formation and growth control in imaginal disks. We propose that growth is stimulated by the apposition of positional values which are ordinarily nonadjacent. Thus, growth during normal development will occur until the pattern is complete, and during culture of a disk fragment growth will occur until either regeneration or duplication is complete. All small fragments of disks will duplicate and then cease to grow, whereas dissociation and reaggregation will stimulate growth until intercalation of positional values is complete. All of these predictions are in accord with the experimental findings (*73*).

Much interest has recently centered around the progressive establishment of clonal restrictions (compartmentalization) during the development of the wing disk (*74*). A major clonal boundary separates presumptive posterior from presumptive anterior tissue at the beginning of disk development, and later, clonal boundaries separate ventral from dorsal, and so on, to generate a number of clonally separate subcompartments within the disk. During normal development even a rapidly growing clone will not cross an already formed compartment boundary. There are some indications of a similar anterior-posterior clonal separation of cells in the cockroach leg (*14, 26*). However, during regeneration and duplication of disk fragments, cells can generate structures in other compartments (*38, 44*). There is evidence that the anterior-posterior boundary is often observed during intercalation, but in some cases cells from one compartment proliferate into the other compartment (*44*). The relevance of compartmentalization to the processes of intercalation and transformation which we describe here remains to be established.

The normal development of pattern in amphibian limbs has been studied by transplanting limb buds at different stages of their development to abnormal locations in the embryo. At the earliest of the stages studied (*50*) presumptive limb bud mesoderm was removed from embryos at the slit-blastopore stage and transplanted to the presumptive flank region. Regardless of the orientation of the grafted mesoderm in its new environment, the anterior-posterior axis of the limb which develops conforms to the original polarity of the graft. It was not until later in development that a limb bud would retain its original dorsal-ventral polarity, following grafting in reverse orientation (*66, 75–77*). Later experiments by Swett (*67*) showed that only dorsal tissue is of importance in establishing this axis. Experiments on other developing organs (*78*) have demonstrated a similar sequence in the establishment of the orthogonal axes of these secondary fields. This fact need not, however, lead inevitably to the conclusion that positional information in such fields is specified by means of orthogonal coordinate systems. Rather, it is possible that anterior and posterior positional values in the circular sequence are established first, followed later by dorsal and then ventral values. All intermediate values could be established by intercalation, leading to growth in diameter of the limb bud, followed by distal outgrowth (transformation), according to the complete circle rule.

MacDonald (*79*) has independently developed a polar model for pattern specification in the amphibian retina. He argues that sequential orthogonal polarization could reflect the formation of several reference points for the establishment of a polar coordinate system, rather than that positional value within the field must be specified along orthogonal axes.

Possible Classes of Molecular Mechanism

We do not present here a detailed molecular mechanism for the epimorphic field, but merely comment briefly on the suitability of several general classes of mechanism.

Many previous attempts to understand pattern formation have been based on gradient models: it is assumed that there is a spatial gradient of some cellular parameter in the tissue, so that the local value of the parameter provides positional information (*4, 19, 80*). A linear concentration gradient of a diffusible substance could be established by maintaining a source of material at one boundary and a sink at the other. More complex gradient models have been proposed, but they need not concern us here. Almost all involve the assumption that cells at the boundary have special metabolic or permeability properties.

While gradient models can and have been used to explain the regulative behavior of limbs in the proximo-distal direction (*23, 81*) they are inadequate to account for the regulative behavior we have described in the circular direction. A gradient must have low and high points but, as we have seen, the circular sequence of positional values behaves as though there were no discontinuities or regions with unique "boundary" properties. Two gradients with opposite polarity could be joined around a circle with no discontinuities, but to uniquely specify every position would require at least two different double gradient systems orientated at an angle to each other. Each point could then be specified by the values of two parameters. However, such mechanisms do not readily account for the shortest intercalation rule (*82*).

The phase-shift model proposed by Goodwin and Cohen (*83*), with appropriate modification, provides an attractive model for regulation in the circular sequence. All cells at a given radial level could undergo metabolic oscillation with the same amplitude and frequency, but cells at different positions in the circular sequence could differ in a regular manner in phase. A total phase shift of 360° around the circumference would then give a circle of positional values with no discontinuities. Although it is not obvious how this kind of positional information could be interpreted by cells, the behavior of coupled oscillators (*84*) does allow such a model to account for the shortest intercalation rule. The radial component of positional information could, of course, be encoded as the amplitude of the oscillation (*85*).

Summary

We have described a formal model for pattern regulation in epimorphic fields in which positional information is specified in terms of polar coordinates in two dimensions. We propose that cells within epimorphic fields behave according to two simple rules, the shortest intercalation rule and the complete circle rule, for both of which there is direct experimental evidence. It is possible to understand a large number of different behaviors of epimorphic fields as a straightforward consequence of these two rules, and the model therefore provides a context in which to view many of the results of experimental embryology. Although we have confined our discussion to cockroach legs, the imaginal disks of *Drosophila*, and regenerating and developing amphibian limbs, the fact that the model can explain regulative behavior in such evolutionarily diverse animals suggests that it may have general applicability to epimorphic fields. The predictions which the model makes should make it possible to assess its applicability to other developing systems, and to investigate the cellular mechanisms involved.

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NEWS AND COMMENT

Squall in Chesapeake: Marine Institute Rammed by Junk Charge

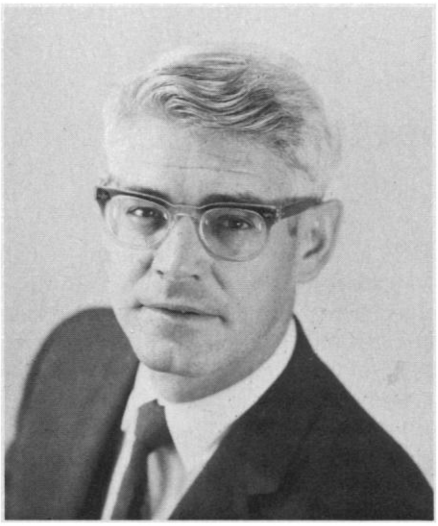
In an astounding series of legal moves, Virginia officials are prosecuting one of the nation's prominent marine scientists, William J. Hargis, Jr., director of the Virginia Institute of Marine Sciences (VIMS) and chairman of the National Advisory Committee on Oceans and Atmosphere (NACOA). Friends of Hargis say that the charges against him are so flimsy that they must have been brought in retaliation for his warnings about the hazards of the pesticide Kepone in local waters, which have angered state authorities. Hargis will stand trial in Gloucester County Circuit Court in Gloucester, Virginia, on 1 and 7 September.

On 6 July, a county grand jury indicted Hargis on two counts of embezzlement. One charge alleges that in October Hargis "fraudulently" disposed of a diesel engine which belonged to the state by allowing a carpenter, Jim Taylor, employed by VIMS, to take it home.

The second charge alleges that Hargis committed embezzlement when he asked VIMS to rectify its leave records from 1971 onward, so that 130 hours of annual leave and 37 hours of sick leave would be restored to him.

Supporters of Hargis argue that no crime took place on either matter. They say that Taylor, seeking some parts from the engine for his own use, had agreed with Hargis aide John B. Pleasants to donate free labor to VIMS in return. He took the whole engine home, they say, to dismantle it and get the parts he wanted. Besides, they say, supply laws of the state of Virginia allow Hargis, as the state's agent at VIMS, to dispose of scrap property as he sees fit. VIMS acquired the engine as part of a generator-diesel unit, which it bought for $177 in 1971; the engine had stood outdoors, rusting, since that time. In March, when police began asking questions about the engine, Taylor returned it, together with all its parts, to VIMS (see photo) and signed an affadavit to that effect.

On the matter of annual leave, supporters of Hargis say that, when he was appointed to NACOA in 1971, he assumed that NACOA business was an outside activity and took annual leave when attending meetings. But in January, the VIMS Board of Administration ruled that Hargis' NACOA business was part of his duties as director of VIMS. Gilbert L. Maton, chairman of the board, wrote in a memo that Hargis "has been authorized to adjust his official working schedule so as to participate in the required activities of NACOA." Thus, say his defenders, when Hargis asked for adjustment of his leave records, he was following orders and thus he had no intent of defrauding anybody.



William J. Hargis, Jr.

Other scientists who have heard of the Hargis case have been angered by the situation. One, Roscoe Meadows, of Deepsea Ventures, Inc., has helped to organize a defense fund to pay Hargis' legal fees which have been estimated at between $20,000 to $40,000—an amount well beyond his total annual income of approximately $30,000.* Another is William J. Nierenberg, a former Chairman of NACOA and an equally prominent oceans adviser. To Nierenberg, the charges sound as though they might have been politically motivated. "If you want to be political you can really sock a civil servant, even just by making him incur legal fees. Bill [Hargis] is really in a jam," he said.

Some VIMS staffers say that the whole matter may have arisen out of the personal antipathy of J. K. Adams, a state police investigator, toward Hargis, which developed while Adams was investigating another VIMS matter late last year. Adams was assigned to look into charges that another VIMS scientist, John L. Dupuy, was engaged in a conflict of interest situation. Although Adams appeared to have finished the Dupuy investigation in December, VIMS staffers say that he "hung around" for several more months, often interviewing Hargis at length and asking other staffers apparently unrelated questions whose common theme always seemed to be Hargis. "He was always barging in on people and reading them their rights," says one staffer. "He wouldn't let you have witnesses present or tape-record the interview." It appears to have been

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*The William J. Hargis Defense Fund, P.O. Box 391, Gloucester Point, Virginia 23062.