Inheritance of Pattern: Analysis from Phenotype to Gene

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SYNOPSIS. The form and pattern of multicellular organisms are developmental phenotypes. They are long term processes rather than static structures. They involve myriad events at multiple locations. The efficient encoding of such phenotypes is analyzed here in two stages. First, the complex developmental behavior is broken down so it can be accounted for by cell or tissue rules. The most effective rules have the instantaneous character found in time-based differential equations. When integrated over time and space, the rules produce the behavior. Second, the cytological and nuclear basis of the rules is sought. One thus studies a complex phenotype in terms of its successive antecedent causes, refining understanding as one gets closer to the genome.

The approach is applied here to phyllotactic (leaf placement) patterns. Leaves may be alternating in a plane, whorled, or in a helical arrangement. In all three cases a new leaf forms as an arc-like bulge at a site apical to a small number of neighboring leaves. The leaf-forming sites are irregularities in the pattern of cellulose reinforcement in the surface of the apical dome. Two organ-level rules combine to produce new leaf sites. First, each established leaf develops a single reinforcement field, with gently curved reinforcement lines, on the region of the dome just above the leaf. Second, where parts of two or three such fields abut on the dome they combine to make the irregularity for the next leaf. Hence a given reinforcement pattern on the dome produces a leaf; the action of the leaves in turn reestablishes the reinforcement pattern. The cellular basis of generating a reinforcement field appears to be a cytoskeletal response to excessive stretch, brought on by rapid growth of adjacent leaf bases. The large scale patterns are thus traceable to cytoskeletal phenomena and from there to genes involving microtubular behavior.

INTRODUCTION

This essay consists of two parts. First, the general problem of analyzing the inheritance of pattern, in all organisms, is addressed. It will be concluded that analysis from the phenotype, via antecedent biophysical causes, toward the gene can be particularly effective. Second, this approach is applied to the study of the patterns found in plant shoots and flowers. It will be concluded that leaves are formed through cyclic biophysical activities in the surface plane at the tip of the shoot. These phenomena can be traced to cytoskeletal behavior and hence to the genome.

SPECIFIC DIFFICULTIES TO THE STUDY OF FORM AND PATTERN

The difficult aspects of studying the inheritance of form and pattern can be readily recognized. The outstanding one is that form and pattern are not simple tangible things. Rather, they are end-products of a long progression starting at, or before, fertilization. The organism inherits the whole progression, not simply the end product. There are myriad pertinent events, occurring in a reproducible fashion. Furthermore, the events occur at specific locations over many microns, or millimeters, of space. Form and pattern will be called developmental phenotypes. They are continuous integrations, over much time and space. This distinguishes them from other kinds of phenotypes.

The distinction among phenotypes is seen in a simple two by two matrix. One axis determines whether the phenotype is small (described in terms of macromolecules) or big (described at the cell or organ level). The other axis deals with whether the phenotype is positive, concerning the presence of something, or is negative, concerning the lack of something. Developmental phenotypes are both big and posi-
tive. It is noteworthy that they are by far the least understood. The nature of understanding phenotypes in general will be briefly reviewed; then the best approach for developmental phenotypes will be addressed.

*Understanding means a “one to one” correspondence*

Understanding a phenotype usually means finding a one-to-one correspondence between features of the genome, i.e., base pair sequence, and features of the phenotype. This is relatively easy for the three non-developmental classes of inherited features. The presence or absence of an enzyme was the phenotype that enabled Beadle and Tatum to give rise to the field of biochemical genetics. They provided the famous duality, “One gene, one enzyme.” The steps in between, now elaborated with removal of introns and with post translational modification, are not mysterious. The issue of one-to-one correspondence has gotten ever more exact. The interaction of proteins with other molecules is now analyzed in terms of point for point correspondence between details of surfaces of paired molecules, with precision at the atomic level (Smith et al., 1986). This is the realm of reductionism where detailed molecular correspondence is safely anticipated. Small phenotypes, positive or negative, present no fundamental difficulties.

The tactic “isolate and simplify” has been extraordinarily successful in clarifying the gene to phenotype relation at the molecular level.

The large, but negative, phenotypes are also relatively well understood, but within an obvious limit. The gene for winglessness in *Drosophila*, for example, could be studied in detail. The amino acid sequence of its wild-type product could be deduced. Its functional, or essential, parts could be ascertained. One could thus learn those specific features of the genome which, when absent, will cause failure of the wing to appear. The limit is that this does not tell us, in any satisfying way, what actually makes the wing arise in the wild type. The challenge of explaining a large and positive phenotype requires that two major things be accounted for: the sequence of the many contributing events, and their distribution in space.

*Methods for one to one specification of position*

Questions about position are readily handled within the macromolecular domain by specificity of molecular binding. Scaling up this concept to the point where cell-to-cell binding generates pattern is an important idea for development (Edelman, 1986, 1987). However, both animals and plants generate organs by the local folding of a coherent epithelium or epidermis, without major change of cell contacts, so there must be more to positional issues than specific binding by freely moving cells.

The best known proposal for specifying location on a large scale uses Positional Information, a concept originated by Wolpert (1971). This approach is “holistic” rather than reductionist. Molecular detail is foregone in return for an operational grip on the whole large scale situation. The idea is that position can be specified by a coordinate system, X and Y, as in analytical geometry. Rather than have each value on each axis be determined by a different gene product, which would presumably soon exhaust the coding capacity of the genome, the idea is that a continuous spatial gradient of single gene product, a morphogen, gives consecutive values along each axis. With two perpendicular morphogen gradients, a cell reads its position accurately in two dimensions. The model proposes that a transduction ensues whereby the cell does the appropriate thing for its position.

Because different things happen sequentially at the same location, a weakness of this approach is that the time sequence at a given location must also be specified. The ingenious economy evident in the handling of the position problem is not evident for
the treatment of timing. Hence Positional Information, at least in this simple form, handles only one half of the problem. One must also deal efficiently with time sequences.

**One to one specification of sequential activity**

The way that sequential activity is inherited is no great problem, in principle, within the molecular domain. The complicated sequence of events that determines whether a virus will be latent or lytic is clarified in a recent book on Lambda (Ptashne, 1986). Sequence in time is readily explained at the molecular level by the progression of a polymerase in one direction from a promoter site. The subsequent transcription and translation of new polymerases or regulatory molecules then initiates transcription at new sites. Time is required to build up the proper concentration of a regulator to turn off, or on, certain initiation sites. The encoding of a specific time sequence of molecular activity is not difficult to envision.

It is tempting, therefore, to extrapolate from the valuable dictum of "one gene, one enzyme" to assume "one gene activation, one developmental event." The difficulty is that, in large scale development, there are far too many developmental events. There are more specific neuronal connections ($10^{12}$) made during the development of the human nervous system than there are base pairs ($10^{10}$) in the human genome (Johnson, 1987). There must be an economy in specifying the sequence of cellular events. There is no question that gene activity in the broad sense is ultimately behind all developmental phenomena. The questionable assumption is that sequential gene activation is a broad enough concept to account for the developmental sequences. There is long standing evidence that additional phenomenology is involved.

The unicellular marine alga *Acetabularia* can "decide" to produce a reproductive, rather than a vegetative, cap structure even in the absence of its nucleus (Puiseux-Dao, 1970). This shows that not all important developmental decisions are made at the level of concurrent gene activation. It is clearly possible to have sequential development while gene activation states are constant.

Gene states normally do change during development and it is useful to have information on stage-specific transcription or translation. The limitation here, however, is that a protein's appearance is important either to the concurrent development or to any later stage. By the same token, a given developmental process, such as the sequence of reactions of a sea urchin egg upon fertilization, is a function of many proteins, previously produced at various times. The sequence of their production need have no simple correspondence to the sequence of the developmental process in question. Thus the finding of molecular correlates does not necessarily provide a full explanation for sequential development.

In brief, there has been difficulty in accounting for spatial detail and progression of time sequence, as independent developmental problems. Various one-to-one concepts, found in positional information, sequential gene activation, etc., have not been entirely satisfactory. The major problem is that most one-to-one concepts simply relate one change to another and hence lack a built-in compounding property. This last is necessary if one is to encode, with high efficiency, a multitude of events. We turn now to another one-to-one concept, one which has this compounding property. Furthermore, it can deal with specification of time sequence and position concurrently.

**A one to one relationship which has generative properties**

This section advocates, for developmental studies, the use of another kind of one-to-one relationship, that between a time-based differential equation and its
Figs. 1, 2. Specification of sequences by time-based differential relationships. Fig. 1. The exponential time course of increase in cell number $N$ is the integral of the well known differential equation below. The constant $k$ is the relative growth rate. Fig. 2. A progression in cell proportions, toward highly elongate form, is shown in A. Length is L and width is W. The slope on the double log plot in B is the coefficient linking fractional increment in length to fractional increment in width, as in equation C. This means that, for example, an 8% increase in length will always be accompanied by a 2% increase in width, as in D. (From Green and Poethig, 1982, with permission)

One case of the efficient encoding of a sequence by a differential equation is well known. The exponential growth curve for a colony of cells is generated by integrating the equation $dN/dt = kN$, where $N$ is the number of cells and $k$ is a constant. A reproducible time course for population growth is tersely encoded (see Fig. 1).

This equation can be converted to a verbal form. It can be expressed as a rule which operates for a population over small (differential) time steps. The rule is that during each step the population size is increased by an increment proportional to the present population size. There is no difficulty in visualizing the biological reality and pertinence of the rule. On the one hand its integration leads to the reproducible population growth. On the other, the rule comes from the fact that the cells are asynchronous and have a common cell cycle duration. This duration, which determines the value of $k$ and hence the exact form of the curve, reflects the speed with which the cells complete the cycle under the conditions at hand.

The verbalization of this rule allows recognition of the key features of useful, or generative, developmental rules. First, the rule couples an action (incrementing the population size) to a condition (the present size of the population). Both the condition and the response are in the same language ($N$). The rule looks neither forward nor backward; it is an isolated instantaneous coupling, pertaining only to "now." Also, the results of all previous activity, i.e., previous increments, are carried forward as cell numbers accumulate. Thus an instantaneous action is based on current conditions, and all previous activity is carried forward. Thus one can interpret developmental progression as equivalent to mathematical integration, over time and space.

There are now two major "one to one's"
to be worked out: between the development and the rules and between the rules and the genome. It is evident that the rules need to be understood early in the analysis. To do so, it is easier to reduce the developmental progression to rules than to somehow start with the rules. The advantage is that reducing the phenomenology to rules is equivalent to differentiating an integral, i.e., differential calculus. Starting with the rules, and proceeding toward the developmental sequence is doing integral calculus, a process with the complication of constants of integration and boundary conditions. Integral calculus is taught to us later, for a reason. Two more examples should make the reader at home with looking at development as an integration, and with studying it through differentiation.

The development of the giant internode cell of the pondweed *Nitella* involves the growth of a small tuna-fish-can shaped cell into a giant telephone-pole shaped cell (Green and Poethig, 1982). The final cell is several times broader than the original cell. The developmental phenotype here is thus a progression of ever more elongate cells, as shown in Figure 2A. The reduction of this sequence “to rule” is very easy. A double log plot of cell height vs. girth shows that the intermediate cell proportions fall on a straight line with a slope of about 4, as in Figure 2B. This means that growth in height and in girth can be treated as two compound interest rates, kept in a fixed ratio. The rate for height is four times that for girth as in Figure 2C, D. The simple relation works only if the rates are those for continuously compound interest.

The verbalization of this instantaneous activity is straightforward. Starting with a square, for simplicity, the rules say that the percentage increment in height will be four times the percentage increment in girth. Thus in one small step the height goes up, say, 8%, girth, 2%. (Actually the increments are infinitesimal, but in the same ratio.) Over the first time step this converts the square to a rectangle. In the next time step the rules are applied to the rectangle, not the original square, so the axial ratio of the cell quickly increases giving an ever more elongate outline.

The progression for cell shape has been reduced to a rule which dictates a constant bias in fractional extension, favoring height, for each interval. The remaining explanation of this phenotype must link this directionally biased growth to the genome. The nature of the connections comprising the link is illustrated in Figure 3.

The bias in stretch rate, height vs. girth, is explained by strong transverse reinforcement of the cell by cellulose (Fig. 3C). The reinforcement restricts the natural increase in girth. This connection is made through the physics of directionally reinforced cylinders. The transverse reinforcement is in turn a function of the transverse alignment of cytoplasmic microtubules whose orientation appears to govern that of the cellulose (see Gunning and Hardham, 1982). The maintenance of a transverse configuration of microtubules in the cortical cytoplasm of a longitudinally growing cell is a challenge yet to be explained. Strain alignment should pull the microtubules longitudinally. Possibly the microtubules maintain their alignment by adhering to the cell membrane and by minimizing their own girth through mutual sliding (Green and Poethig, 1982). At any event, there must be rules that keep the microtubules transverse. The composition of the microtubules, and their special behavior, is a function of proteins. At this point the causal chain reaches the domain where molecular phenomenology is relatively well understood.

From the diagram in Figure 3, there are 7 stages in understanding the final phenotype: shape progression of a single cell. The over-all chain is based on six one-to-one relations between successive stages. It is seen that the mode of conversion of one
A long range one-to-one correspondence is thus fully understood only when all the intermediate conversions in the chain are known. When read from gene to phenotype, the chain shown in Figure 3 is causal. When read from phenotype to genome, the chain is seen in the advocated direction for analysis, particularly for the stages remote from the gene. A final example illustrates the utility of “reduction to rule” for a developmental sequence at the tissue level. The example comes from Thompson (1942) and deals with the sequence of cleavage in a flattened egg. As shown in Figure 4, many partitions subdivide the original circular egg into a complex, yet reproducible pattern. The direct specification of the timing, location, and angle of each cleavage would of course require large amounts of information in the genome. Thompson’s point is that the sequence, in both time and space, falls out from a short set of rules, the rules being constant throughout. The set of rules, or generative algorithm, is in the special instantaneous format where an action is coupled to a condition.

The rules are that a) the new partition halves the area of the parent cell, b) new
partitions meet old walls at 90°, three at a point, and c) the new wall is the shortest one that meets the other two criteria. The rules apply to all cells, all the time. The time step is one cell cycle.

The first two cycles, A and B, are easy to anticipate. At the third, C, however, the solution is not obvious. The first inclination, to continue with a radial wall, violates rule one; the second inclination, to put in a circumferential wall, violates rule three. The oblique arc shown fits all requirements. Thereafter, the process continues to yield a configuration which ultimately has hints of a cortex/medulla arrangement. There will always be one three-sided cell in each quadrant. The constant rules breed increasing complexity because the boundary conditions, the cell outlines, keep changing. Thus one generates change from constancy, giving fundamental economy in encoding a progression.

There is no need to keep the rules in a simple fixed relationship. For example, in the present case, cells below a certain threshold size might always “change” rule #3 to call for the longest wall that would meet the other conditions. Such a shift in behavior could well require new gene products. The logical encoding of such behavior can nonetheless be reduced to constant rules. In the present example, the third rule would simply be rephrased to allow two conditions. It would read: if bigger than a certain size, make the shortest wall; if smaller than a certain size, make the longest wall. In this way the coupling of instantaneous relationships becomes flexible, as a flow scheme. Interactions between cells can also be incorporated. Whether new gene products are required for a given shift in behavior is a function of the response term. The shift from straight to curved walls at division three presumably would not require a shift in gene expression; a shift from shortest to longest wall presumably would. The format allows development to be broken down into its essential components without presupposing any details of mechanism.

DEVELOPMENT AS AN INTEGRATION:

CONCLUSIONS

First, the genes pertinent to a given developmental process are all those responsible for the rules to be valid. This is bound to be a large number. Further, the sequence by which the pertinent genes are activated need bear no parallel to the sequence by which the development occurs. Nonetheless the tie in between the genome and development can be pursued effectively, by relating specific genetic changes to variations in the generative scheme.

A second conclusion is that the requirement to account for specificity in both space and time can be satisfied concurrently. The spatial features are specified by the sequential following of rules of a geometrical nature.

A third is that the generative rules, despite being strictly operational, constitute a sufficient explanation of what is going on. The rules generate the progression provided they can be followed. They will account for development just as Mendel’s rules provided prediction for progeny ratios. Like Mendel’s laws, the rules invite subsequent refinement for mechanism. Sufficiency, in the form of somewhat abstract rules, is gained while one is still lacking molecular specificity. The reciprocal trade-off is found in some molecular correlations in development. The molecule itself is known in detail. What it does, is often not. The molecule is “implicated” in the process; this connection does not, in itself, provide a sufficient explanation.

A fourth conclusion, related to the above, is that analytical strategies which use data directly coupling a specific agent (inhibitor, stimulator, signal, etc.) to a change in a final developmental phenotype usually have to contend with relative ignorance of context. By analogy, one understands a specific key, and a keyhole, but does not
Fig. 5. The three major phyllotactic patterns. Leaves are numbered in order of their initiation. A. Distichous is a zig-zag pattern in a plane. In top view, the pattern has two ranks; each is called an orthostichy. B. Whorled. Two or more organs are initiated together at a node, successive whorls "nest" with new organs bisecting the angle between pairs of older organs. C. Spiral. The top view of a meristem is shown twice. In (1), three clockwise spirals, parastichies, pass through all the leaves. In (2), five counter clockwise spirals pass through all the leaves. The phyllotaxis is called 3:5 in this case. The divergence angle between consecutive leaves is the Fibonacci angle, about 137.5°.

thereby automatically understand the workings of the lock. Such analyses often presume that a summing of many such couplings will explain the development. When the causal chain has an integration step, however, simple summing is inappropriate and interpretation can be obscure. An alternate strategy, advocated here, is to analyze from the phenotype through antecedent causes, thereby analyzing the activity of the "lock" in terms of its functional components, which often have differential character.

This approach will be illustrated in Part II, an analysis of the large scale patterns in plant development. The geometry of shoot behavior will be reduced to rules pertaining to tissue behavior. The tissue behavior can be traced to cytoskeletal phenomena, and finally to the genome.

THE INHERITANCE OF PATTERN IN PLANT SHOOTS

A widespread developmental phenotype is the regular arrangement of shoot structures on the axis of plants. The spiral, or
helical, deployment of reproductive structures seen in sunflower heads and pine cones has fascinated observers since antiquity. These spectacular spiral examples should not overshadow the fact that virtually all shoot structures, vegetative or reproductive, are produced in a regular pattern of one sort or another (Schwabe, 1984). As a first step in seeking the mechanism of their production, it is necessary to characterize the patterns. Three major ones, described in Esau, 1977, are shown in Figure 5.

There are three major phyllotactic patterns

The simplest arrangement of organs is termed distichous (two ranked), or alternating, left and right, in a plane (Fig. 5A). This zig-zag pattern is typical of many monocots including grains such as corn. Distichy is found in the iris, the famous “Traveler’s palm,” etc. Ivy and pea are dicotyledonous examples.

The second category is called whorled (Fig. 5B). Here, more than one leaf, or organ, is produced at the same time and at the same height on the apical dome, ideally. Whorls of two opposite leaves, with the pairs successively rotated by 90°, form a decussate arrangement. This pattern is found in maple trees, snapdragons, and mint plants. Whorls of three, rotated successively by 60° (“tricussate”), are found in the oleander shrub. Many simple flowers have their floral organs in successive whors: sepals, petals, stamens, carpels. There are whors of three in the floral parts of the tulip and iris; whorls of five are found in many simple flowers of the succulent family, Crassulaceae.

The third category is the most famous and has obvious spiral features. We will deal only with the common Fibonacci spiral forms. Examples include oaks, many palms, willows, mustards, and in fact most plants. The spirals are termed “Fibonacci” in honor of a mathematician who is associated with a series of numbers used in characterizing the various spiral patterns. The series goes: 1, 1, 2, 3, 5, 8, 13, 21, etc. It is generated by adding two consecutive members to give the next member. In many spiral patterns, such as that of the florets in a sunflower head, the eye is caught by two sets of spirals of opposing sense (right vs. left handed). If all the spiral lines of one sense are counted, and compared with the number in the other set, it is typical to find the two numbers to be consecutive members of the Fibonacci series (Fig. 5C). Simple helical structures such as a pine cone will be low in the series, e.g., 5:8. Sunflower heads may be high, such as 34:55.

When treated as a fraction, successive pairs of numbers approach the “Golden Ratio” which cuts a circle into two arcs of approximately 222.5° and 137.5° (about 62%, 38%). This ratio is special, or “Golden,” because the whole circumference is to the big arc as the big arc is to the little arc. The developmental significance is that the small arc, about 137.5°, is the typical angle between successive organs in most spiral forms. This is called the divergence angle. Divergence is 180° in distichous patterns; it is 90° for pairs in a decussate pattern.

Phyllotactic patterns are variations on a single generative theme

All proposals for the basic mechanism of phyllotaxis have the form of logical loop. Special sites on the apical dome make leaves; recently formed leaves somehow determine the location of new leaf sites. The leaves’ influence must act “inward” toward the center of the dome, against the general “outward” flow of all the cells on the dome. Any explanation of the production of the three patterns must involve such cyclic reciprocating activity.

There are reasons to believe that many important features of the phenomenology are shared in the three cases. Most obviously, the typical product of the activity, a leaf, has the same bilaterally symmetrical features regardless of the phyl-
Phyllotaxis. Further, in some cases the same plant can often shift from one pattern to another. For example, *Eucalyptus globulus* shifts from decussate to spiral after the tree reaches a certain state of maturation; similarly, ivy shifts from distichous to spiral (Rogler and Hackett, 1975). Upon flowering, many plants shift phyllotaxis to make a flower which is whorled. For example, the mustard family typically has spiral phyllotaxis in the vegetative form. The flowers, however, have 4 petals in a whorl. The iris shoot is distichous; the flowers have striking three-fold whorled symmetry.

Additional evidence for shared causation among phyllotactic systems comes from the work of Snow and Snow (1935). A shallow diagonal cut across the apical dome of *Epilobium*, a decussate plant, produces two half shoots each with spiral phyllotaxis. It is clear that the same genome can code for a variety of patterns. In light of these experiments, which brought on spirality surgically, it seems likely that the same state of gene activity can produce more than one pattern. Probably the shallow cut in *Epilobium* disturbed physical boundary conditions, rather than changed states of developmental gene activation, to shift the pattern. One thus expects that the three major patterns are variations on the same generative scheme.

Phyllotactic patterns are two dimensional. It is assumed that the basic mechanism of their development is also. The search for a plausible mechanism will proceed in two steps. First, the behavior will be reduced to rules described at the level of surface growth and histology. The rules will then be reduced to plausible biophysical and cellular mechanisms.

**Rules for Organ Behavior in Phyllotaxis**

A highly characteristic feature of a phyllotactic pattern is the divergence angle between successive leaves. As already noted, it is commonly assumed that a new leaf site is determined by the position and activity of recently formed leaves. Three empirical rules, describing developmental activity at the shoot meristem surface, can generate the dictichous and whorled patterns; related rules can account for spiral phyllotaxis.

In all cases, the leaf arises as a crescent-shaped ridge whose concave side faces the tip of the smooth apical dome. The primordium thus has a left tip, a central region, and a right tip. Rule one, for dictichous phyllotaxis, is that early in its development, each leaf is involved in the production of a small group of parallel cell files running radially on the region of the dome just above the center of the leaf base. These will be called "central distal files" or CDF (Fig. 6). Rule two is that the ends of the ridge grow circumferentially, as pincers, on the dome, until encountering CDF from an older leaf. The resulting arc-shaped ridge is the base of the new leaf. Rule three is that a new leaf arises as a ridge when the left and right ends of a growing leaf base encounter central distal files. The tips of leaf base number \(n\) encounter the central files of leaf \((n - 1)\), the next older, to initiate leaf \((n + 1)\), as shown in Figure 6. The right tip of the old leaf base influences formation of the left tip of the new leaf, and the left, the right. The leaf-base growth occurs as a pincer's movement. This makes each growing leaf base serve as an angular bisector of the available circumference, defined as the circular arc length between central distal files, in this case 360°. The biophysics of converting a primordium to a leaf is addressed in Green (1986) and will not be covered here.

The same set of rules will also perpetuate the whorled pattern of primordia. There is only a trivial difference. In dictichous plants, the pertinent right and left tips encountering the distal files come from the same leaf; in whorled plants they come from two different leaves (Fig. 7). This difference need have no substantive effect on the
Phyllotaxis: Orthogonal Patterns

Fig. 6-9. Generative schemes for the orthogonal patterns. Leaves are numbered in order of their origin. These are top views, showing crescent-shaped leaf bases. Each leaf base has a central portion (C), and left (L) and right (R) tips. Pincers-like growth is shown by small arrows. Prominent cell files lying inward to the central region of a leaf are central distal files (CDF). Figs. 6, 7. At the organ level, three rules suffice to perpetuate the patterns. a. New leaves grow as pincers until meeting CDF. b. Leaves produce their own CDF. c. A new leaf starts interior to where the pincers encounter CDF and grows in the opposite sense. In these cases the leaf-base growth bisects the available circumference (i.e., that between CDF). Leaf initiation is at a site on the bisector of the angle made by the growth arrows of the arriving leaf-base tips. Figs. 8, 9. Reinforcement patterns (lines) on apical domes in relation to cyclic initiation of leaves. Each leaf has associated with it a reinforcement field with lines of cellulosic reinforcement roughly tangential to the inner face of the leaf base. Fig. 8. A distichous apex. Below center, a small portion of the field from leaf 1, CDF, plus nearby portions of the left and right parts of the field of leaf 2, combine to give a three-membered U-shaped reinforcement pattern at the site for leaf 3. This is a leaf-site field. Presumably the buckling that forms the new leaf is fostered by the relatively high curvature at this site. Buckling occurs along the reinforcement lines (lines of least resistance) and smooths the three-membered reinforcement irregularity into a smooth arc, the new leaf. This can explain how the angle between R and L tips of leaf 2 is bisected by the new leaf 3 as in Figure 6. Subsequent pincers-like growth of leaf 3 is based on the buckling continuing along lines of reinforcement. Fig. 9. Arguments identical to those for the distichous dome suffice to perpetuate a whorled (decussate) pattern. R and L fields now come from different leaves.
mechanism. In both cases the scheme results in the repeated bisection of the available circumference by the new leaf, to give the divergence. While this available circumference is 360° in distichous phyllopectaxis, it is 180° or less in whorled phyllopectaxis. In both distichous and whorled forms, the leaves lie on orthostichies. These are straight radial lines that later become vertical. Along these lines there is continuity of central distal files. The files pass up one side and down the other side of a leaf (along the mid-rib region), and then pass through a gap between the leaves before passing over a subsequent leaf.

The corresponding generative algorithm for plants with Fibonacci spiral patterns is less obvious. Unlike the whorled forms, the two leaves which are lateral neighbors to a future leaf site are not at the same height on the dome (same distance from its center). Unlike whorled and distichous, no obvious bisection of an angle is carried out. Rather, a new leaf forms with its center at or near the Golden Section of the angle between the two neighboring leaves. The size of this angle is a function of the rank of the meristem in the Fibonacci series. If low, e.g., 3:5, the angle is about 84.5°; in higher forms, e.g., 8:13, the angle will be smaller. In all cases the Golden Section of the angle is taken, dividing it into arcs which are about 62% and 38% of the original. The new leaf forms on this off-center dividing line, closer to the older neighboring leaf.

A suitable generative algorithm, obviously provisional, is that two adjacent leaves “cause” a new leaf to arise at the Golden Section of the angle between them. Thus in Figure 10, leaves 2 and 4 “section” the angle between them to initiate leaf 7 at about 32° from leaf 2. This new leaf’s base will expand laterally as a pincer’s movement, as in the scheme for distichous and whorled. The radial distance of the new leaf from the center of the dome is predicted by its plastochron age difference from the two neighbors. In 3:5 phylloectaxis the plastochron ratio (fractional radial displacement per cycle) is about 1.2. Hence, the new leaf will arise at (1/1.2)^3 of the radial distance to the younger leaf, (1/1.2)^5 of the radial distance to the older.

If the production of the three phyllopectic patterns is a “variation on a theme,” then one must show how similar phenomenology can give a Bisection of an angle when the lateral leaf structures are at the same height on the dome and a Golden Section when two lateral leaf structures are at different heights. To address the question of how older leaf primordia could “section” an angle in any fashion, one must have more detailed knowledge of the biophysics of the leaf primordium and the apical dome.

Primordium formation relates to patterns of reinforcement direction

The specific “sectioning” of an angle by a small number of older leaves is manifest as a local buckling of the dome surface at the particular angle. This initiates a leaf. The analysis here will characterize the buckling and then explore how the older leaves could influence its angular location.

Over-all, the dome surface is reinforced by cellulose microfibrils (Green, 1985). The lines of reinforcement, passing over many cells, are roughly concentric on the dome, i.e., the radius of curvature of the reinforcement lines generally increases with distance from the dome center. A leaf primordium arises when a crescent region of this surface buckles, forming a crease on the inner side of the fold. At the site of buckling the reinforcement lines appear to be arcs, facing the dome center, which are somewhat more curved than expected at that distance from the dome center. Such regions are called leaf-site fields (Fig. 8).

The immediate cause of buckling is thought to be that the peripheral regions of the growing dome surface exert pressure on the more central regions; stresses there are relieved when local buckling
occurs. The crease of the buckling occurs along the reinforcement lines, the path of least resistance. The new fold has the appropriate curved bilateral symmetry of a new leaf and faces the center of the dome. Subsequent pincer-like growth of the leaf base also follows the reinforcement lines.

With this view of the actual bulging process, the remaining issue is to find how action of the older neighboring leaves could set up the proper reinforcement irregularity at the appropriate location for a future primordium. Such a bilaterally symmetrical site must have a highly curved reinforcement pattern with its concave side facing the center of the dome.

New leaf-site fields arise as composites of parts of older fields

It has been observed that established leaves have multicellular fields of aligned reinforcement on the dome surface between the leaf and the center of the dome (Green, 1985). These fields arise, apparently, as a cytoskeletal response to the very rapid lateral growth of the primordium when it first formed. The leaf stretches the nearby dome, and the dome is thought to respond by modifying its structure to make a field. A leaf field is modeled by drawing circular arcs of constant radius distal to each leaf, using the distance from the leaf base to the dome center as the radius. Each field has a left, central, and right portion (Fig. 8). Once initiated, the curvature of reinforcement in an established leaf field slowly decreases as growth displaces the leaf from the center.

The proposed mechanism for leaf initiation is that if each older leaf has a reinforcement field interior to it, new leaf-site reinforcement patterns can arise by the combining of parts of older fields. While each older reinforcement field is itself of gentle curvature, parts of two or more established fields can combine, in an initially disjointed fashion, to give a leaf site field of sharper curvature. The disjointed character would disappear as buckling occurs and an arc-like ridge is formed.

The pattern for field combination to make leaf-sites is straightforward for distichous and whorled patterns. In the case of distichous phyllotaxy, a curved three-membered U configuration is formed at the leaf site (heavy bracket in Fig. 8). The base of the U comes from the central field of the leaf which is two cycles older than the leaf being formed. The left and right sides of the U come from the right and left fields, respectively, of a leaf one cycle older. Alignment in the left and right fields can be extrapolated to make a V. The new surface buckles along this V thereby “bisecting” the angle between the lateral reinforcement fields and, through subsequent pincers growth, also the available circumference. For leaves in whorls, the local situation is the same except that the two lateral sides of the U come from different leaves, not one leaf (Fig. 9).

A closely related process can operate in the spiral forms. Here, however, a Golden Section of an angle is taken and only two leaves are involved. Instead of a three-membered U or bracket configuration forming the leaf-site irregularity, a two-membered V pattern is formed directly. Both patterns can smooth into arcs of reinforcement which face the center of symmetry.

The interaction of reinforcement fields in spiral forms is modeled in planar diagrams. The alignment of the reinforcement fields in nature is known only approximately (Green, 1985). The most attractive explicit assumption is, as before, that the curvature at the base of each of the two older leaves is characteristic of each field. Many lines with this radius of curvature are drawn in front of two neighboring leaves, as in Figure 11. This is done by putting the compass point at the center of symmetry of the dome to draw the first arc at the leaf’s base. Subsequent arcs are drawn by moving the compass point farther from
In spiral forms, successive leaves are at the Fibonacci angle which makes the Golden Section (38%–62%) of a circle. A new leaf, e.g., 7, appears at the Golden Section of the “available circumference” (84.5°) between centers two leaves adjacent in space but not consecutive in age. The leaf then grows as a pincers, giving its base the typical bilateral symmetry.

**Fig. 10.** In spiral forms, successive leaves are at the Fibonacci angle which makes the Golden Section (38%–62%) of a circle. A new leaf, e.g., 7, appears at the Golden Section of the “available circumference” (84.5°) between centers two leaves adjacent in space but not consecutive in age. The leaf then grows as a pincers, giving its base the typical bilateral symmetry.

**Fig. 11.** Interpretation of the reinforcement pattern on a dome with spiral phyllotaxis (*Ribes*: Green, 1985). Reinforcement lines are constructed, for each leaf, with constant curvature equal to that found at the leaf base. Successive lines are drawn as the compass point is moved along a diameter passing through the leaf and the dome center. The two fields abut along the diameter on which leaf 7 will form. Both fields are skew relative to the dome center. Nonetheless they combine to form a broad V-shaped reinforcement irregularity similar to the U-shaped one in other forms (heavy V). The V faces the dome center, approximately. Its alignment is also seen in the sharper V made by normals to the arcs. The angle of the bisector of this localized reinforcement irregularity approximates the angle which takes the Golden Section of the larger arc between leaves 2 and 4. The correspondence is exact at a point, X, peripheral to the leaf site. It is possible that the reinforcement pattern at the point X is the critical biophysical input to initiate a leaf. Repeated leaf initiation at the local Golden Section (e.g., between leaves 2 and 4) should perpetuate a spiral pattern. The cyclic generation of crescent-like reinforcement irregularities is proposed as the common basis for phyllotactic patterns in general.

It is intriguing that, at the new leaf site, the V faces almost directly toward the center of symmetry. The error is about 3 degrees in 3:5 phyllotaxis. In a peripheral region below the leaf site, the V pattern faces the center of symmetry exactly (X in Fig. 11). It is suggested, as before, that the “sectioning” of the angle between the two leaves takes place by a physical buckling at a V-like junction of the two lateral fields, the V facing the center of the dome. Because the two fields are each skew on the dome, this bisection relative to the fields makes an approximate Golden Section of the angle between the two leaves (see Fig. 11).

Why is the explanation approximate? First, it might be necessary for the perti-
Inheritance of Pattern

We have evidence that the symmetry of buckling forces to exist in a region behind the future crease, rather than at the leaf site itself. If so, the explanation may be precise. Second, the model assumes strict circular arcs for the reinforcement fields; the real lines of reinforcement may be parabolic, elliptical, etc., in nature. This distinction could lead to a solution exactly at the leaf site. Finally, the model is constructed on a plane, whereas most domes are curved surfaces. It is hoped that ultimately this type of biophysical phenomenology will be able to explain the origin of a leaf site in terms of both its angle and its radial distance from the center of symmetry.

Discussion

The development in spiral and non-spiral forms appears to be based in a common generative scheme. A reinforcement irregularity is formed through the recombination of reinforcement fields from recently formed organs. The field becomes a precursor to the new organ. The reinforcement irregularity has several important features: it is bilaterally symmetrical, faces the center of symmetry of the dome, and has differences on its upper and lower sides because only the concave (upper) side will buckle to form a crease. These features, seen on the dome, anticipate the major aspects of the organ to come. These features have their biophysical antecedents in the action of growing organs on the dome. In this way the logical loop that “dome structure makes leaves,” and “leaves make dome structure,” is completed.

The diversity of phyllotactic patterning can be initially reduced to an organ-level algorithm where activity of two or three leaves determines a new leaf site. In distichous and whorled forms the angle between two leaf base tips is bisected; in spiral forms the angle between two leaves is sectioned in the golden proportion, the new leaf appearing nearer the older member of the original two. It is postulated that in all cases the immediate morphogenetic function of a growing new primordium is the same: to produce a reinforcement field distal to itself, on the dome. In all patterns the combination of parts of several such fields produces a new, arc-reinforced, leaf-site field. The differences between patterns relate to initial conditions or secondary features of leaf base growth. These determine the geometry in which the cyclic combination occurs.

The genetic basis of the production of a reinforcement field apparently relates to cytoskeletal activity. Microtubules are thought to govern the orientation of the cellulose reinforcement (Gunning and Hardham, 1982; Lloyd, 1982). An attractive hypothesis for the generation of a reinforcement field on the dome is that the excessively rapid transverse stretch of dome tissue distal to the new primordium orients cell division, and also cellulose reinforcement, to run parallel to the lines of stretch.

The gene products which give the cytoskeletons this orientation capacity would therefore be responsible for the ability to carry out phyllotaxis in general. The gene products responsible for any specific type of phyllotaxis, i.e., those that relate to boundary conditions and growth behavior of leaf bases, are more obscure. It is likely that their identification will require further refinement of the generative activity of apical domes. The hierarchical analysis is summarized in Table 1.

It is a major point of this article that developmental progressions, including cyclic ones such as phyllotaxis, can be interpreted as the consequence of the following of rules which have differential character. That is, the rules couple a response to a condition. Once identified, the rules can be investigated to find their cytological and nuclear basis. A second major point is that such rules, and their ultimate basis in the genome, may often be more readily ascer-
Table 1. Summary of analysis: the major patterns.

<table>
<thead>
<tr>
<th>Distichous</th>
<th>Whorled</th>
<th>Spiral</th>
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<tbody>
<tr>
<td>Decussate</td>
<td>Decussate</td>
<td>Decussate</td>
</tr>
<tr>
<td>Tricussate</td>
<td>Tricussate</td>
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</table>

1. New leaf arises with a divergence equal to the Bisector of a whole circumference.

2. Leaves bisect the "available circumference" (i.e., the arc between older leaves).

- Organ rules
  - Basis of bisection:
    - a. Leaves make central distal files
    - b. Leaf bases grow as pincers and stop at central files
    - c. New leaves arise where pincers stop

- Tissue rules
  - 3. Leaf arises as localized crescent-shaped buckling, along special pre-established cellulose reinforcement lines (lines of least resistance). The reinforcement irregularity is a V or U with concave side facing the center of shoot symmetry.
  - 4. The various phyllotaxies regenerate these irregularities through the juxtaposition of parts of older single reinforcement fields. The mode of cyclic combination determined the particular phyllotaxy.
  - 5. Single reinforcement fields arise on the dome, each field distal to a recently formed leaf.

- Cell rules
  - 6. Reinforcement fields arise in cells on the dome apparently in response to rapid circumferential stretch by nearby leaf base growth.
  - 7. This traces to a presumed cytoskeletal response.
  - 9. RNA.
  - 10. DNA. Other genes pertain to secondary factors bearing on level #4 to generate particular patterns.

- References
INHERITANCE OF PATTERN