

Transductions for the Expression of Structural Pattern: Analysis in Sunflower

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The transductions to initiate and propagate pattern were investigated in the sunflower head. The spiral pattern emerges as new florets form in regular centripetal sequence on the flat disc. The spiral lines of florets arise as rows of simple bumps. Each bump splits to become a small bract and a circular disc flower. This topographical progression was described by a scanning electron microscopic technique applied to living tissue. The suitability of various theories to explain the progression was examined. Because no periodic cell specialization was seen by scanning electron microscopy prior to pattern emergence, a mechanism that produces pattern in uniform tissue by spontaneous physical buckling (folding) was examined further. Key configurational changes of development were reproduced in models using the buckling assumptions. In further testing, a young head was physically constrained to cause it to grow as an oval. Pattern was modified as predicted. Unexpectedly, organ character changed as well. In localized regions, the folding was abnormal; the typical dyad floret, bract and flower, was replaced by a single large bract. This anomaly is known in mutants of sunflower; hence, the physical treatment induced a phenocopy. We concluded that (1) buckling is a strong candidate to be the process producing organ pattern, and (2) the accompanying topographical changes can be prerequisite to organ differentiation.

INTRODUCTION

The genetically determined characteristics of shoots may be placed in three categories that differ in the manner of describing, and potentially explaining, the phenotype. In the first category, here termed biochemical (including macromolecular), the feature is characterized by the amount of a particular substance such as a pigment, hormone, or transcription factor. In mathematical terms, the trait is a simple continuous variable. A second category, called geometrical, deals with form and pattern. In contrast to the first category, the phenotypic character here is often partly described by whole numbers, e.g., of organs, a discrete or discontinuous variable. An example is the influence on phyllotaxis by the mutation *pallida* in snapdragon (Harrison, 1964). The mutation increases the frequency of seedlings with three leaves at a node (angular separation 120°) compared with the normal pair of leaves (angular separation 180°). The leaves are presumably biochemically identical. Because this phyllotactic pattern is first evident at the stem apex as a pattern of primordia (bumps), it is a topographical feature in three dimensions and can be characterized, quantitatively, only by using several variables. The third group, called combined, involves both categories one and two, usually in close coordination. In such cases, the geometrical changes correlate with biochemical ones. Homeotic mutants of flowers are good examples of the third category (Coen and

Meyerowitz, 1991). A given whorl in a mutant flower develops a whole number of organs that differ in geometry (but not necessarily in number) from the wild type, as well as in biochemical composition. For example, in *deficiens* of snapdragon and in *green pistillate* of tomato, stalked stamens are replaced by sheetlike carpel walls (Sommer et al., 1990; Rasmussen, 1993).

For the first, or biochemical, category, there is no doubt about the nature of the transduction chain connecting genome to phenotype. Here, molecular binding and catalysis are the pertinent processes. The "before" and "after" states of each transduction are molecular (e.g., DNA sequence is transduced to amino acid sequence). In such chains, the controlling factors and the responding system are similar; the influence of controls is explainable by molecular inhibition or promotion. For the geometrical characters present in the second and third categories, however, the transduction chain is not established. Because these features are ultimately controlled by genes, the early steps in the causal chain must involve molecules in solution. The molecules diffuse, lacking any permanent pattern. At some point, however, changes in the nature and activity of such diffusing molecules must lead to predictable changes in the geometry of solid tissue. For this transition, the character of the controlling elements and of the responding system are markedly different. This paper addresses two issues: (1) the nature of the key transduction step from diffusing chemical influence to physical form and pattern (topography) and

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(2) the basis of the typical coordination between biochemical and geometrical features seen in the combined category. This coordination may involve the reverse of the transduction in issue (1), i.e., an influence of structural configuration upon subsequent molecular synthesis.

The approach was threefold. First, we described the course of development of geometrical detail on the surface of the head of sunflower using a replica technique for scanning electron microscopy that images living cells. The details were compared qualitatively with the predictions from three theories of pattern formation. One theory, based on physical buckling (bending) as a spontaneous response to uneven stress in a flat plane, was selected for further testing. Second, we examined whether the explicit assumptions of buckling theory, well developed in engineering, could reproduce selected episodes of configuration change seen on the sunflower head. This is a quantitative test of the plausibility of buckling theory for morphogenesis. Third, experimental physical constraints were applied to the developing head to modify the buckling process. The effects on pattern and organ identity are described. Our simplest interpretation of the data is that physical buckling is a key transduction step. Also, consequences of buckling are likely to influence gene expression and thus be involved in the coordination seen in the third, or combined, category of shoot characters. Background on pattern formation and on morphogenesis in sunflower precedes the Results section.

Background: Theory of Pattern

Pattern formation is a long-standing problem in the theory of development (Sinnott, 1960; Held, 1992). In each of the three main theories, the key step, the origin of periodicity of tissue structure, has a markedly different basis (e.g., biological, chemical, or physical). To facilitate comparison, we present the steps by which each theory converts a model band of undifferentiated cells into a row of three differentiated primordia. In each case, the initial state has simple nonperiodic chemical features derived from antecedent molecular activity. In each case, the row of primordia arises synchronously.

Positional Information Theory

This theory (Held, 1992) assumes that a gradient of diffusing molecules is evaluated by cells so that cell position is defined by a specific concentration, as shown in Figure 1A. Cells at certain concentrations become unique and, through cellular activity such as periclinal division and growth, bring on overt morphogenesis. There is no periodicity in the diffusing compound that serves as reference, but there is development of a periodic prepattern of special cells anticipating the ultimate topography. The final step, usually not defined in detail, is a congruent transfer of pattern from unique cell groups in a plane to unique three-dimensional morphology. The initiation of pattern is based on cell interpretation of smooth gradients, and hence is biological. A version of this theory is effectively applied

to explain bands of specific gene expression in early *Drosophila* development (St. Johnston and Nüsslein-Volhard, 1992) and has been applied to plants (Holder, 1979).

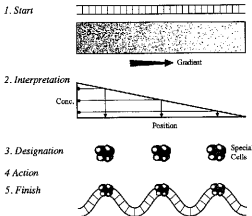
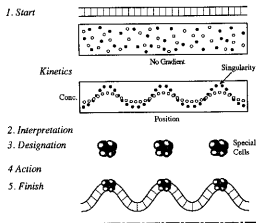
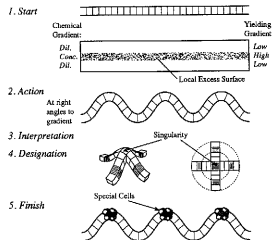
Reaction-Diffusion Theory

The reaction-diffusion theory (Harrison, 1993) assumes no initial gradient. It is postulated that evenly distributed activator and inhibitor molecules interact spontaneously to produce a stable undulating concentration profile. This is a chemical prepattern (e.g., three peaks in a row). This pattern is used to designate separate groups of special cells whose activity produces the physical pattern (Figure 1B). This conversion has the attractive feature that similar concentrations (peaks) give similar structures. There is a congruent transfer of pattern from unique cells to unique morphology. The nature of this last step is often left undefined (Meinhardt, 1982). Pattern initiation is chemical.

Buckling Theory

Buckling theory (Green, 1992) assumes that a simple chemical gradient exists across the initial strip (Figure 1C). This chemical gradient is first converted congruently to a physical yielding gradient, just as growth hormone gradients are considered to produce physical wall loosening gradients (Ray, 1993). The simple nonperiodic expansion gradient in the initial flat plane is converted by spontaneous physical buckling (folding or undulating) into a periodic three-dimensional configuration of the surface. The conversion is well known in engineering and is explained by the plate bending equations of von Kármán (Szilard, 1974). The 90° relation between gradient and pattern is appropriate because in plant apices the histological gradients (e.g., central and peripheral zones) are radial, whereas the primordia arise along the circumference. In the buckling proposal for the transduction, the special cells associated with the tip of each bump are considered to arise later. This is by mechanisms sensitive to the folding process, e.g., an activation by stretch receptors (Hsieh et al., 1992) or to the new topography itself (Green, 1989). Note that pattern initiation and morphogenesis are combined in a single step. In contrast to the other theories, no periodic prepattern of special cells in the flat plane is necessary for the origin of the undulating morphology.

All three theories can be modified to account for the propagation of pattern because adjacent preexisting structure can influence the initiation process. To explain pattern extension in phyllotaxis, it has been traditional to assume that formed organs produce a diffusing inhibitor. New organs are rapidly established by special cells when and where new sites of minimum inhibition appear (Mitchison, 1977; Schwabe, 1984). There are many successful simulations using this chemical inhibition concept. This mechanism is compatible with the first two theories for the origin of pattern, which are relatively well established. There are several rationales for investigating whether the newer third theory is also compatible with a "mutual

A Positional Information**B Reaction Diffusion****C Buckling**

repulsion" mechanism. First, with the possible exception of cells failing to express *knotted* in corn (Smith and Hake, 1992), special cells have not been recognized to anticipate pattern in shoots. Hence, a theory not requiring them is of interest. Second, because shoot organs typically arise from several scores of cells (Smith and Hake, 1992), a theory that has the initiation process involving an area, rather than an idealized point (e.g., Douady and Couder, 1992), is more appropriate. Third, the known suppressing effect of a bump in a physical sheet on the bending nearby may account for the spacing phenomena normally attributed to chemical inhibition.

Background: Sunflower

The disc region of the capitulum or head of sunflower is an example of the best-known plant pattern: the famous Fibonacci array of intersecting spiral rows of organs (Jean, 1988). The sunflower pattern is unusual because the units, florets, are not single organs such as leaves. Rather, each floret is a highly reduced branch system consisting of a peripheral (abaxial) small bract and an axillary circular flower. The disc is thus a highly compressed inflorescence developing in a plane. The issue of expression of pattern applies at three scales. At the largest scale are the spiral lines of elements. There are two sets of such spiral parastichies, as shown in Figure 2A. The inward extension of such lines of florets is the phenomenon of phyllotaxis. Next is the development of the element itself, the floret, which splits to become bipartite (Figure 2B). Finally comes the emergence of the floret's two parts, the bilaterally symmetrical bract and the radially symmetrical flower (Figure 2C). At this smallest scale, pattern is a manifestation of organ

Three proposals are shown for how an initial chemical influence leads to the conversion of a simple band of cells into a row of three differentiated primordia (stage 5). Action refers to the stage producing physical periodicity (undulation).

(A) Positional information. A gradient of a "morphogen" molecule (1) is interpreted quantitatively (2) by all cells. Those with certain values become special (3) and bring on the upward folding of tissue (4). The periodicity first originates in the cellular interpretation step.

(B) Reaction diffusion. A uniform mixture of activator and inhibitor (open and closed circles) spontaneously produces three peaks of high concentration (1). The peaks define three groups of cells (2 and 3), which bring on upward folding (4). Periodicity first arises through chemical kinetics.

(C) Buckling. The initial chemical gradient is across the band (1) and leads to an imbalance of tendency to expand in the flat plane. Dilute regions are low yielding; concentrated regions are high yielding. This unbalanced physical state brings on the spontaneous undulation of the band, giving periodicity (2). Assuming the cells are transversely reinforced (fine lines in 4), coherent reinforcement (dotted lines) is possible around the base but not at the tip of dome where alignments conflict: cells there become different (4).

Conc., concentrated; Dil., dilute.

Figure 1. Potential Transductions To Connect Chemical Influence to Organ Pattern.

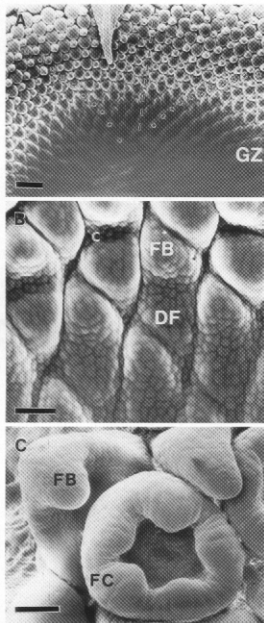


Figure 2. Casts of Replicas of the Disc Region of Living Sunflower Capitula.

(A) Low power. The developmental progression is from tissue without surface differentiation, below, to well-formed dyads, above. The generative zone (GZ) is an arclike region in between. For the florets at the periphery, the small spherical object is the tip of the bract; the large one is the disc flower. A single large involucre bract is above. Two spiral parastichies (asterisks) and a head radius (dashed line) are shown. Bar = 200 μ m.

(B) Developing florets. Each starts as a parallelogram delimited by pairs of parallel parastichy lines (lower florets). At the same height on the page, the creases are equally distinct. A transverse crease (C) develops in the upper, older florets. The upper (abaxial) part of each floret

identity. Sequential stages in this development of geometrical detail are encountered as one examines tissue ever farther from the center of the head, as shown bottom to top in Figure 3A.

The pioneering work of J.H. Palmer and his associates (e.g., Palmer and Steer, 1985) shows that in young sunflower heads floret initiation occurs on an expanse of tissue that is devoid of obvious pattern. The inward passage of a circular "generative zone" converts this apparently featureless tissue into the characteristic array of complex organs. Their work has shown, remarkably, that new generative zones can be initiated by vertical cuts in the undifferentiated region. Bracts appear at both sides of the cut, apparently as a wound response. Comparable large bracts can appear throughout the central area in certain mutations (Schuster, 1985), so the cuts induce apparent phenocopies. Florets are then made in either a centripetal or centrifugal direction on the head (Palmer and Marc, 1982; Hernández and Palmer, 1988). The florets are normal but their pattern is irregular. The disc center is therefore demonstrably labile with regard to the pattern of elements to be formed and to the geometrical features within the organs in the pattern (flowers versus bracts). Thus, the sunflower is attractive for the study of how pattern is expressed.

RESULTS

Progression in Surface Topography

Development of surface detail within the generative zone is followed by examining structures outward, radially, from the center of the developing head, as shown in Figure 3A. The first evidence of a primordium is its appearance as a subtle tissue undulation, approximately in line with two rows (parastichies) of older undulations. The initial bump pattern is roughly sinusoidal (crest, trough, crest) in a circumferential direction, as well as in the radial direction. High-resolution scanning electron microscopy images of the central region were examined for any pattern of variation in cell outline, defined by anticlinal walls, that would anticipate the pattern of the bumps. None was observed. Another test for an anticipatory prepattern is to look for special activity at a primordium site, activity that might not be evident in an image taken at a single time. In a time series of surface images, we examined retrospectively the site of origin of a single bump, looking for unusual surface histology (Figures 3B to 3D). None was detected.

Soon after initiation, the bump appeared somewhat like a comet, with a high point toward the periphery of the capitulum

becomes a disc flower bract (FB), and the lower part becomes a disc flower (DF). Bar = 40 μ m.

(C) A dyad floret at high power. A leaflike floral bract (FB) is peripheral to a disc flower with a floral corolla (FC). Bar = 50 μ m.

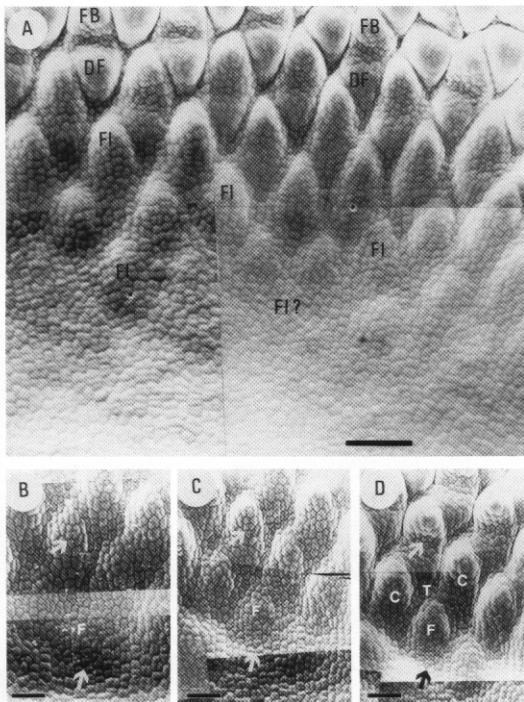


Figure 3. Scanning Electron Microscopy Casts of Living Generative Zones.

(A) The transition from cells undifferentiated in surface features, below, to dyad florets, above, is a continuous elaboration of topography. There is no periodic cell configuration anticipating bump formation. The center of the head lies off the image; it would be below the figure. FI, floret; FB, floral bract; DF, disc flower. Bar = 100 μ m.

(B) to (D) Sequential daily images of a given generative area. Arrows point to individual cells. Part of the region between them develops into a floret (F). There is no visible surface cell activity associated with the floret initiation. The field bulges with sinusoidal periodicity, C (crest), T (trough), C (crest), horizontally across the field as well as vertically. Bars = 50 μ m.

and a vanishing "tail" toward the head center (Figure 3A). The high point is the oldest part of the bump, because the generative zone moves inward. At any given distance from the center of the head, the sharpness of the creases delimiting the bump was found to be comparable. With increasing distance of the bump from the center, this sharpness accentuated until each bump was delimited by a parallelogram of well-defined creases (Figure 2B). These parallelograms were in a configuration that extended the large-scale pattern. When a spiral array was examined, the florets were skew parallelograms (Figure 2B). When simple alternate packing was seen, the florets were rhomboidal (Figure 3A, center).

Each parallelogram developed a transverse crease (top of Figures 2B and 3A). Its long axis was parallel to the circumference of the head, in contrast to the creases that delimited the parallelogram, which were oblique. The subdividing crease was thus parallel to the generative front and across the length of the floret. The peripheral (older) bump became a bilaterally symmetrical small disc bract, arching toward the head center. The inner bump was round, becoming a circular flower with five petals (Figure 2C). There is thus a continuous cascade of folding that transforms an apparently featureless field of surface cells into bumps that extend a large scale pattern of complex elements.

Simulation of Certain Steps in the Progression

We investigated the possibility that the observed sequence in sunflower reflects phenomenology known in engineering for inanimate sheets. Buckling, often a sudden yielding, is the bulging response of a flat sheet to an imbalance of surface expansion in its plane. An inanimate plate, when confined at its margin and subjected to increasing pressure from below, will develop a succession of bulges in a well-defined pattern (Szilard, 1974; Green, 1992). In plants, it is possible that the tunica layer (Esau, 1953) behaves as an expanding sheet, the corpus exerts pressure, and the patterned primordia are the resulting bulges.

Although buckling is an exceedingly common phenomenon, e.g., giving rise to the saddle shape in a potato chip and to wrinkles in wet skin, quantitative prediction of shape change by buckling requires very advanced mathematics. To make the computation practical, there are restrictions on the format used (e.g., boundaries need to be straight; deformations must be small). Hence, the simulations will deal only with excerpts of the developmental progression.

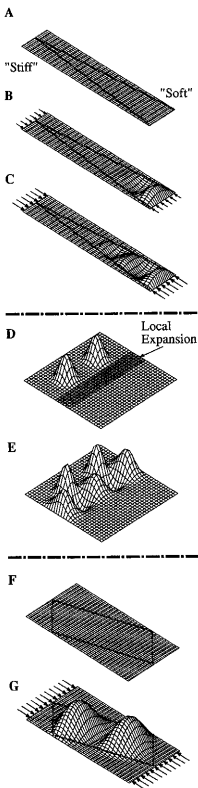
The gradual emergence of pattern from "nothing," as shown in Figure 3A, is characteristic of shoot meristems (e.g., Green, 1992). The corresponding origin of a graded row of bumps in a plate, as an undulation with the amplitude increasing from zero, can be simulated. In the simulation, the tissue is treated as a continuum with properties only of thickness and stiffness. A *de facto* increasing gradient in extensibility, corresponding to time since passage of the generative front (e.g., bottom to top in Figure 3A), was incorporated by making the tissue patch

be a long triangle. Its base would be toward the periphery of the head. Plates of such outline bend more readily in their broader regions. The growth process itself (producing excess surface) was incorporated by applying radial compression, as shown in Figure 4A. After modest compression, an undulation was seen at the broad end; it "vanished" as the narrow end was approached (Figure 4B). As additional compression was applied, the surface retained the original bumps and extended the undulation inward (Figure 4C). This resembled the inward radial advance of the generative front as seen in successive images in Figures 3B to 3D.

The propagation of a two-dimensional array with alternating elements, as seen in nature and as shown in Figure 3A, was simulated under different boundary conditions for technical reasons. Here, two bumps were artificially added at the start, as shown in Figure 4D. The expansion activity of the generative zone was simulated by adding the equivalent of heat to a localized region interior to the pair of bumps. This gave a band of uniform local expansion. The resulting undulation produced three new bumps exactly out of phase with the original two. This simulation showed in an inanimate system that configuration *per se* (the initial bumps) can influence adjacent areas to fold so as to extend an alternating pattern. In the model, the "inhibitory" action of the initial bumps, relative to positioning the new bumps (Figure 4E), was brought on by the plate's tendency to minimize the rate of change in curvature over space. The descending side of a bump continued as the descending side of a depression. The minimal strain energy response could thus propagate alternating pattern. Note that whole numbers of old bumps induced whole numbers of new bumps. Thus, physical undulation can provide discrete numbers of structures.

In these simulations, the surface was free on both sides. In the plant, the tunica layer has corpus on one side and nothing on the other. This condition can be simulated by the addition of pressure and an "elastic foundation" to one side of the sheet. This asymmetry enhances the bumps and reduces the depressions to give a more realistic topography. Primordia can arise in widely separated fashion. However, when only an isolated excerpt of the pattern can be simulated, the pressure influences the slope and phase of the undulation at the lateral boundary, an artifact. Hence, until the completion of the simulation of an entire circular dome where an artificial lateral boundary doesn't exist, the undulations are presented in their simplest, undamped form.

Finally, the subdivision of a model floret was simulated. An initially skew parallelogram was subjected to compression (to generate excess surface) along the head radius (Figure 4F). The resulting buckling into two similar bumps (Figure 4G) mimicked crease formation *in vivo*. The bumps arose simultaneously. In the plant, however, the abaxial bump forms first and has a different fate from the adaxial bump. Selected conversions of morphology were thus reproduced under the simple assumption that the response is one of a confined sheet reacting to conditions where, locally, it has excess surface. No assumptions involving diffusion or cell behavior were involved.



In Vivo Responses to Altered Head Geometry

As shown in Figures 5A and 5B, young round heads, which were confined between fixed parallel bars, grew into ovals. The generative zone separating the smooth inner head region also became oval. The new rows of primordia, instead of being spiral, became relatively straight (Figures 5C and 5D). The rows were roughly parallel to the constraint.

In certain regions, particularly those near the long sides of the oval, the details of surface folding were unusual. Instead of primordia being delimited by parallel creases of equivalent clarity and depth (Figure 2B), the two sets of creases differed. One set was strong and delimited rows of primordia; the other set of creases, "breaking up" the row, was extremely weak in comparison (Figure 5D). The strong creases were roughly parallel to the constraint, as expected assuming surface expansion continued between the fixed boundaries, developing new folds. This was seen in all five heads given the experimental constraint. It was not seen in five controls. In regions with such folding, many primordia formed as rows of single large bracts, the blades nesting roughly normal to the row, with no indication of flower formation, as shown in Figures 6A and 6B. The bracts were roughly twice the normal size. In regions at the ends of the oval, normal bipartite florets were seen (Figures 6C and 6D). The very central region of constrained heads had parallel rows of organs (Figure 6E) or had disorganized florets (Figure 6F). Individual heads imaged when small, as shown in Figure 7A, were examined later. They showed mature bracts in the unusual location and in an unusual stacking pattern (Figures 7B to 7D).

On three of the five manipulated heads, individual trumpet flowers that were the size of ray florets but had the symmetry

The three-dimensional images are derived on the assumption that an inanimate surface folds to accommodate excess surface within it in a way that minimizes the strain energy of the sheet. There are no assumptions involving cell activity or diffusion.

(A) A long triangular strip, inside the dark lines, will be subject to an in-plane load along its axis. The triangularity gives a gradient of response (high at right). This simulates the presumed gradient of extensibility in the generative zone, which in this example would be moving from right to left.

(B) At low load (arrows), the broader part buckles into an undulation that falls to zero to the left.

(C) At a higher load, the undulation extends farther, still with decreasing amplitude. This production of the morphological gradient resembles the development of natural undulations in Figures 3B to 3D.

(D) The initial surface has two bumps artificially built in as shown. Nonperiodic expansion is artificially generated in the indicated zone.

(E) The resulting minimal energy surface has three new bumps alternating with the original two. The latter set the phase of the new undulation. This parallels the spacing process in Figure 3.

(F) The subdivision of the floret is simulated. Compression (analogous to growth) is applied along a radius to an initially flat skew parallelogram (dark lines).

(G) The three-dimensional image of the resulting minimal energy response is shown. Note that the new crease is at right angles to the radius. This is the case in nature (Figure 2B).

Figure 4. Selected Topographical Transitions on the Capitulum Surface.

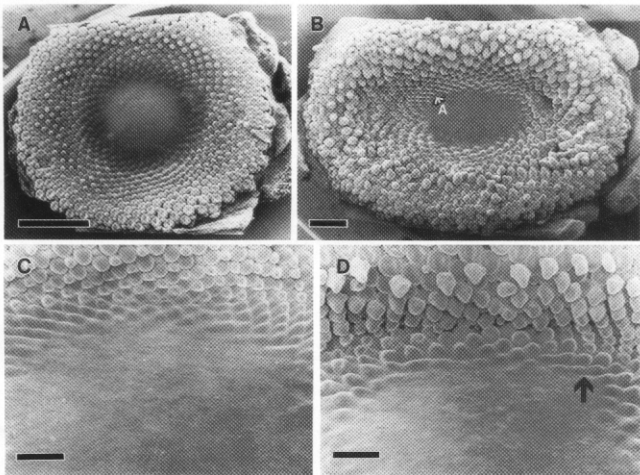


Figure 5. Constraint Experiments on Capitulum Development.

The flower heads and primordia are casts from living plants. Some casts are trimmed by straight cuts (the plant is not cut).

(A) Initial round configuration with central undifferentiated region.

(B) Large oval shape after 9 days of capitulum growth. "A" denotes the area seen in detail in Figures 6A and 6B.

(C) Detailed view to the right of "A" in (B) showing that, at left and right, the parastichy lines are relatively straight and in the horizontal direction, roughly paralleling the constraints.

(D) The same region 48 hr later, showing that many new bulges do not form parallelograms (arrow).

Bars in (A) and (B) = 1 mm; bars in (C) and (D) = 250 μ m.

of disc flowers appeared (Figure 7D). Trumpet flowers have been reported as genetic anomalies for the ray area, but apparently not for the disc region of the head (Fick, 1976).

DISCUSSION

Interpretation of Normal Development and the Physical Simulations

Our study of the normal development of topography on the capitulum surface showed that the large-scale structural

pattern is initiated as a gradual undulation of surface to form bumps. This pattern was not anticipated by a corresponding pattern of special cells with features detectable by scanning electron microscopy. It is possible that an anticipatory prepattern of activity not involving surface features exists prior to the undulation. The anticipation of a primordium by a periclinal division below the site is sometimes assumed (Esau, 1953), but this is not widespread. Bump formation can frequently precede such divisions (Tiwari and Green, 1991). The statement that divisions in deeper layers initiate floret formation in sunflower (Hernández and Palmer, 1988) was based on an image of a clearly developed floret and, hence, we interpret the term "initiate" to mean "accompany."

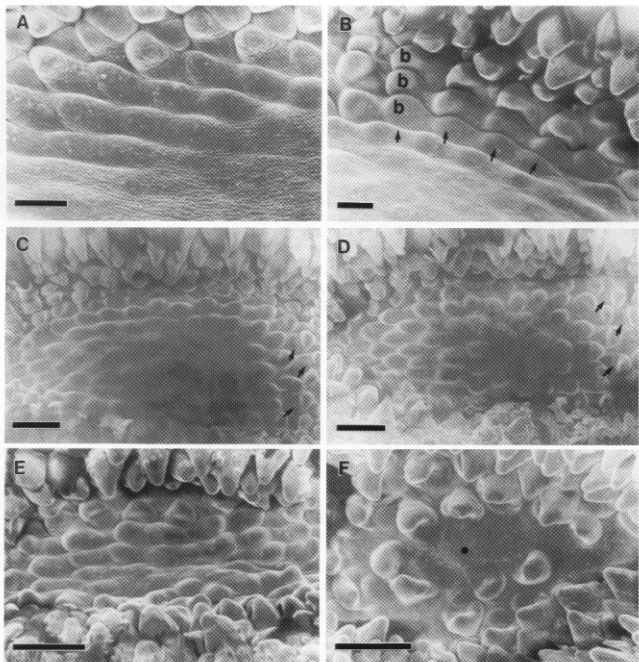


Figure 6. Anomalous Pattern and Organ Formation in Constrained Capitula.

(A) and (B) Sequential daily images. Note that one set of creases, roughly horizontal, is pronounced. This pronounced set runs parallel to the constraint and the generative front. The other set is seen only as subtle undulations on a ridge. The structure buckles into primordia, which are unusual in that they become single large bracts (b) rather than dyad florets. The bracts are in register (arrows) rather than alternating with disc flowers. Bars = 100 μ m.

(C) and (D) Development over 2 days of the central region of the receptacle. Note that many normal dyad florets are formed at right (arrows). In this region the generative front would pass roughly at right angles to the prominent folds as in untreated heads. Bars = 250 μ m.

(E) Central region with parallel folds. Bar = 250 μ m.

(F) Central region with many bract primordia. Note that all lamina are at right angles to the capitulum center (black dot). This presumably reflects a causal relation between orientation of the line of the generative front and that of the blade. Bar = 250 μ m.

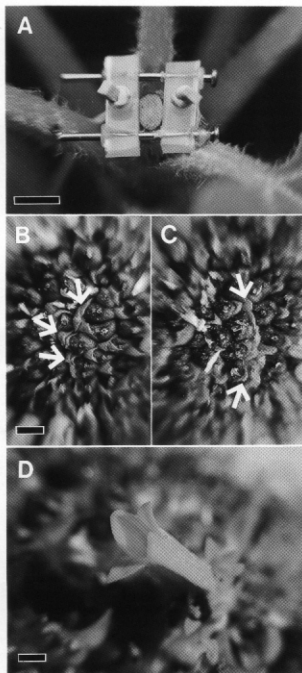


Figure 7. Experimental Setup and Resulting Abnormalities.

(A) The device used to constrain young capitula, seen after 9 days of treatment, showing the head's oval shape.

(B) Anomalies in a mature capitulum with flower corollas removed. Three bracts subtend two ovaries (upper arrow). Bracts nest together (lower arrows). This is the same head depicted earlier in Figures 6A and 6B.

(C) Comparable to (B), but in another region of the capitulum. Between the arrows is an anomalous row of bracts.

Anticipation of a primordium's emergence by localized chemical activity is indicated by the report that cells about to form a leaf in maize fail to express the *knotted-1* gene (Smith and Hake, 1992). Such a prepattern could be generated by a reaction-diffusion mechanism. However, anticipatory periodic activity was not evident in scanning electron microscopy studies in sunflower and at present is not widely evident by other criteria in other systems. For example, homeotic genes for floral organ identity are usually described as acting in a ring rather than as spots. The buckling theory, which does not require a periodic prepattern of any sort, is therefore a plausible format for interpretation.

In sunflower, we observed an increasingly intricate folding of the surface. The continuous progression was compatible with a repeated buckling process. The product of one round of folding, creases, could serve as the initial boundary conditions (local constraint at the periphery) for the next round of bulging (Green, 1992). We suggest that in nature this qualitative cycle is capable of repeating on a large scale, in the generative front, to extend the double spiral pattern. It could repeat at ever smaller scales to produce topographical features within the elements of the pattern.

Simulations

The simulations using buckling theory were interpreted to show that selected episodes in the natural pattern-forming progression can be reproduced, as far as epidermal configuration is concerned, on the basis of explicit physical properties of sheets. The simulation required input only with regard to yielding properties, thickness, and boundary conditions for an inanimate plate. Thus, although the plant epidermis is living and cellular, this may be incidental to the immediate folding mechanism. The establishment of the prerequisites would of course be biological. Our proposal is that simple expression gradients are converted to physical yielding gradients, congruently, and that the response to the yielding gradient is the process that initiates periodicity. Thus, variation in expression of pattern would become manifest through variation in the antecedents to the buckling process. In contrast to most theories for phyllotaxis (Schwabe, 1984), the key event would not be a local activation at a point on a uniform field, but rather the emergence of a tissue undulation over an area. This process could work equally well at the intracellular level, e.g., producing the rays in the cap of the alga *Acetabularia* (Harrison, 1993) and the undulations often seen in anticlinal epidermal walls of leaves. The qualitative description and the quantitative simulations combine to suggest that a mechanism involving buckling and creases is plausible for pattern formation.

(D) An unusual "disc trumpet" apparently not previously seen on other discs. It has the dimensions of a ray floret with the radial symmetry of a normal disc flower.

Bars in (A) and (B) = 5 mm; bar in (D) = 3 mm.

A Generative Front in the *Drosophila* Eye?

The development of the facet pattern in the eye of the fruit fly presents a striking parallel to that seen for the florets in the sunflower head. In the eye, a moving furrow is equivalent to the generative front in that its passage converts a field of unorganized cells into a highly regular alternating pattern of recognizable units. The array becomes the hexagonally packed ommatidia (Wolff and Ready, 1991). Whereas transduction for cell differentiation in the eye is well understood once the overall pattern is clear (number 8 cells induce their neighbors), how the pattern starts is obscure. A prepattern grid of appropriately spaced number 8 cells has apparently not been reported. The pattern arises gradually "from nothing," as in the sunflower. The first sign is an alternating pattern of rosettes of cells on the surface; some cells assume a pie-section shape around a point to make the rosette. Assuming rosette formation is sensitive to topography, it is possible that the alternating pattern is propagated through a buckling process, as shown in Figures 4D and 4E.

Interpretation of the Constraint Experiment

The treatment of simply constraining expansion in one direction is expected to accentuate folds normal to that direction (parallel to the constraint boundaries). This apparently occurred on the sunflower head. Straight rather than spiral rows developed. Because diffusion processes are not directly affected by curvature (or stress), one would expect a reaction-diffusion mechanism to perpetuate the double spiral pattern despite the constraint.

In further contrast to the normal head, in which all folds at the same distance from the head center have equivalent clarity, the constrained heads had strong folds roughly parallel to the constraint and weak folds at right angles to it (Figures 6A and 6B). This imbalance appeared to have maximal consequences at local areas in which the generative front ran roughly parallel to the strong creases. Here, the subdivision of the floret failed. The floral cascade, both geometrical and molecular, did not take place. Alternative scenarios for the differential folding, e.g., that the constraint generally activates stretch receptors that alter transduction chains to bring on the changed topography, are considered unlikely. The reason is that the response was localized and directional. It correlated well with oriented features of the treatment and the generative zone. That stretch reception could be involved as a consequence of the abnormal folding (organ identity) is quite possible. It is proposed that folding phenomena are likely to be significant early steps in establishing organ identity as well as in determining large-scale pattern.

In this study, the origin of the unexpected large bracts could have a biophysical explanation. In the normal head, the generative front, presumably an expansion stimulating region, first interacted with the narrow end of a parallelogram-shaped

ground. This end made a small bract; its axil was the normal site for flower initiation. In localized regions in the physically modified case, the generative front encountered the broad side of the forming parallelogram, possibly stimulating growth along this entire side and hence transforming the whole bump into a bract. Any folding appropriate to splitting the primordium and initiating the flower might be precluded. This failure to split would not occur generally in the head; hence, most florets would be normal.

How physical processes could influence later gene expression is an open question, but the evidence here for such a link suggests that coordinated development can be considered as a cascade involving alternating influences. On the one hand, chemical patterns are transduced into morphology; on the other, morphology influences chemical activity, including gene expression. A scheme of this sort for sunflower, incorporating the buckling cycle between two steps in a biochemical cycle, is given in Figure 8. A key feature in the scheme is that a new bump in the generative zone has two actions. By its configuration, the bump physically influences the position of later bumps. By its maturation and production of expansion-promoting substances, the bump ensures that new bumps will form nearby. In the proposed scheme, the physical aspect determines where primordia form; the chemical aspect governs whether they will form. The two main reciprocal activities will now be discussed with reference to other systems.

Gene Expression Influencing Structure via Buckling

That gene expression can influence structure is attested to by every morphological mutant. In some cases, a specific role for buckling phenomena can be readily envisioned. As noted, in the *pallida* mutation of snapdragon the normal pairs of leaves (cotyledons) are replaced by nodes of three leaves. This occurs in up to 21% of the cases (Harrison, 1963). In buckling theory, the number of bumps (wavelengths) in a strip or annulus is roughly determined by the ratio of length to breadth of the band. Variation in whole numbers of organs in a whorl could, thus, be brought on by a change in the proportions of the active region. Many flowers with unusually large numbers of parts are big. Tomato flowers with the mutation *fasciated* have roughly four times the wild-type number of carpels; the gynoecium region has four times the area of the wild type (Szymkowiak and Sussex, 1992). In *perianthia* of Arabidopsis, larger peripheral floral zones could simply give more sepals and petals (Chasan, 1993). Alternatively, increasing the expanding tendency could reduce the wavelength for buckling and give added undulations within a constant dimension (Hicks and Sussex, 1970).

Interesting cases potentially involving buckling are found among homeotic floral mutants. In *deficiens* (*globifera* allele) of snapdragon, the third whorl of the flower, instead of forming five isolated stamens, expands into an undulating band that becomes carpeloid (Figure 26 in Sommer et al., 1990).

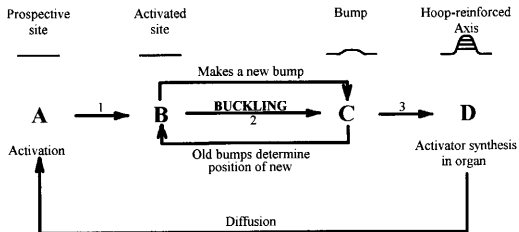


Figure 8. Summary of the Proposal for Key Steps in the Expression of Pattern.

There are four stages (A to D), connected by three conversion steps. A chemical influence (A) is converted to a local imbalance in tendency of surface to expand (B) by step 1. This chemical influence and the expansion tendency are not periodic. In step 2, a spontaneous physical process, buckling, produces a periodic bump pattern (C), which relieves the previous physical imbalance. This buckling process is influenced by preexisting bumps so that new bumps alternate with the old, propagating a pattern. In step 3, the bump (C) matures to its mature configuration (D), which has transverse reinforcement by cellulose (horizontal lines). This final stage produces the chemical influence that reinitiates the cycle. The activity of the generative zone in sunflower is proposed to reflect the repetition of such a scheme, which is cyclic for both chemical and physical components.

In a mutant of *petunia* (shown as "pin" in Figure 5 of Green, 1992), a more extreme case of failure of whorls to produce individual organs is seen. Some whorls persist as circular ridges; others develop as collars. A similar collar structure is seen in the *pin-formed* mutant of *Arabidopsis* and in embryos where auxin transport has been inhibited (Liu et al., 1993). In all cases, the failure of an annulus to "break up" into discrete organs could result from the failure of normal buckling to occur in the circumferential direction.

In the above instances, genetic variation would act through its influence on the initial conditions for buckling. In other mutants, there could be an interplay with organ identity factors already present. For example, in the *blind* mutation of *petunia*, stamen tips appear on petals. Assuming applicability of the concentric annulus model for floral organ identity (Coen and Meyerowitz, 1991), it is possible that the organ boundaries set by buckling in this mutant do not coincide with the boundaries of the annuli of gene expression. Folds could encompass more than one organ identity zone and thus give the combined phenotype. Variations in buckling phenomena, superimposed on other forms of spatial specification, could thus be involved in various morphological mutants.

Structure Influencing Gene Expression (Biosynthesis)

To deal effectively with development in sunflower, it is necessary to postulate an influence of new structure on gene expression, or at least biosynthesis. Two reasons for the postulate are based on normal development. The simplest

suggestion to explain the repeated production of florets by a moving generative front involves a diffusing "tissue softening" influence coming from recently made primordia. This generates a zone of increased yielding. Also, to explain further cycles of development within a primordium, some cyclic chemical control of yielding properties on smaller scales seems unavoidable. In the constraint experiment, a role for structure acting on expression is needed to explain the apparent phenocopies where the expected gene expression for a flower is eliminated.

There are two plausible classes of mechanism for this influence by structure. The transduction could involve either the structure *per se* or the activity that makes the structure. In the first category are physiological consequences of morphology (e.g., better gas exchange at crests rather than troughs of an undulating surface). There is also the possibility that a static shape can be "interpreted" by cytoskeletal interactions to recognize unique sites in topography. As shown in Figure 1C, columns of parallel-hoop reinforced cells can all have their transverse reinforcement alignments (microtubule arrays) parallel at the base of a dome, as shown by dotted circles. This is not possible at the top where alignments conflict. Cells able to have coalignment of their cytoskeleton with neighbors would have one fate. Those unable to coalign, i.e., those at the top, would have another (Green, 1989).

The other category, in which physical activity influences expression, currently receives much attention. A prime candidate in the present context would be an action of local expansion and shear on stretch receptors (Hsieh et al., 1992). Shear is predicted at leaf sites (Selker et al., 1992). Shear could influence ion fluxes and start transduction chains, which could readily

influence gene expression. Touch can induce the expression of calmodulin-related genes (Braam and Davis, 1990). There are many examples of mechanical influences on development in animals. Most tissue culture cells will not divide without mechanical attachment to a solid substrate; bone ossification is promoted by mechanical shear acting on osteocytes (Carter et al., 1988), and subtle shear forces can stimulate the metabolism of cells (Frangos et al., 1988). Mechanotransduction has been shown to occur at the cell surface (Wang et al., 1993). There is thus no scarcity of potential links from physical phenomena to gene expression. With plausible links in both directions (chemistry to physics, physics to chemistry), one can view morphogenesis as involving cyclic interactions between expression and morphogenetic activity. We conclude that the buckling phenomenon, occurring after biological activity has established the appropriate physical conditions, is a candidate to be a key process in the transductions that initiate and propagate pattern. Further, the undulation process, or the resulting structure, is likely to have an influence on subsequent synthesis. Such reciprocal exchanges, occurring in a cascade, could explain how geometrical and biochemical features normally develop in tight coordination.

METHODS

Plant Cultivation

Sunflower plants (*Helianthus annuus* cv Cargill 208A) (Cargill Hybrid Seeds, Fargo, ND) were grown in 2-L plastic pots containing a soil-peat mixture (1:1) in chambers at Stanford University (Stanford, CA). The growth conditions were an 18-hr photoperiod at $580 \mu\text{mol m}^{-2} \text{sec}^{-1}$ light intensity at the canopy height, with 28°C day-night temperature. Plants were watered daily and fertilized to maintain an optimal level of nutrients.

Starting at floral stage 5 of Palmer and Marc (1982), i.e., 33 to 35 days after seedling emergence, the last formed apical leaves and involucre bracts were removed and the incipient floral meristem (receptacle) exposed. The generative zone was separated by 8 to 10 normal florets from the area of removed bracts. Hernández and Palmer (1988) have shown that bract removal does not affect the subsequent development of the inflorescence to maturity.

Sequential Scanning Electron Micrographs of the Meristem

Using the technique of Green and Linstead (1990), sequential replicas of a single receptacle surface were obtained during flower formation. This method does not affect development of a growing apex over periods of more than 10 days (Hernández et al., 1991). The exposed sunflower meristem surface was covered daily with a viscous impression mixture (Kerr's Mirror 3; Kerr Manufacturing Co., Romulus, MI) to make a primary replica. After 15 min of polymerization, the now firm replica was gently removed with tweezers. To protect the apex from desiccation, the receptacle meristem was immediately covered with a cap made from wet tissue paper and aluminum foil. The mold was inverted and affixed to a slide, and a cast was made. The molds were completely filled with resin (Spurr, 1969) and placed in an oven at 60°C for 48 hr. After hardening, the casts were trimmed, mounted on scanning

electron microscope stubs, and sputter coated. They were examined in a scanning electron microscope (model 505; Philips, Eindhoven, The Netherlands) at 10 kV and photographed. This replica method was applied daily to 20 flowering plants for a period of 15 days. Structure on such plants was indistinguishable from that seen in plants exposed to the method for the first time.

Application of Lateral Constraint

In five plants, lateral constraint was applied to capitula by a specially devised clamp made with parallel plastic bars supported by metal pins, as shown in Figure 7A. This treatment was applied after removing the last formed apical leaves and involucre bracts in capitula at floral state 6 (36 to 38 days after emergence). Over a period of 9 days, this procedure induced the growth of an initially circular capitulum (Figure 5A) into an oval-shaped one (Figures 5B and 7A). Molds and casts were made daily throughout, and the casts were examined by scanning electron microscopy as described above. Control capitula remained circular as they grew.

Computer Simulation

Minimal strain energy configurations satisfy the two differential equations of von Kármán (Szilard, 1974). The simulations involve the specification of an initial configuration and load as the "before" situation. The transduction to the "after" condition is the production of the surface that is the minimal energy configuration that accommodates the load. The deformation is elastic and is assumed to correspond to a small step of growth.

The von Kármán equations involve fourth derivatives and are nonlinear. Their solution is expedited by use of the fast Fourier transform. The initial topography, yielding properties, and boundary conditions are translated into Fourier space. That is, surface topography is encoded in the form of an interference pattern between evenly spaced grid lines in perpendicular directions. Once in the Fourier domain, the response of the surface to load is more readily calculated. Successive approximation methods have to be used. Once convergence is obtained, the new Fourier coefficients are used to construct the topography of the resulting surface (C.-H. Wu, unpublished data). The methodology is evolving and current procedures are available upon request. They are the Ph.D. dissertation project of Mr. Cheng-Hsiu Wu, Department of Mechanical Engineering, Stanford University, Stanford, CA. The work is under the direction of Prof. Charles Steele.

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