Transductions to Generate Plant Form and Pattern: An Essay on Cause and Effect

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ABSTRACT

Many complex processes can be broken into transduction steps where one state is converted to another by a well-defined activity. One difficulty for analysis is that transductions occur in chains or networks. Another, of primary concern here, is that a single transduction can be complex. Some such transductions can efficiently explain phenomena often thought to be summations or orchestrations of many simple transductions. Pattern formation is in this category. For a wide range of transductions one can define cause and effect in a differential equation. In its integral one can define the before and after states. The main experimental tactic to characterize unknown transductions is co-variation. The before state (input) is altered, change in the after state (output) is assayed. Thus an unknown transduction, with cause and effect embodied in the differential, is investigated through long-term changes in its integral. This is fully practical when all of the integral is known or readily surmised, as in simple discrete biochemical transductions. As causal differential expressions become complex, their integrals become more versatile in generating output because this changes not only with variation in the expression itself but also with boundary conditions and limits. These very features, however, make such a function increasingly intractable to discovery by co-variation. Only a small part of the integral is embodied in the before and after states; the remainder is not readily surmised. Accordingly, in contrast to reliance on the role of controls to deduce unknown simple transductions, the complex ones are generally established through formalization of the differential nature of the process itself.

INTRODUCTION

The development of plant form, as well as its predictable modulation by gravity or light, must reflect a reliable causal chain between a plant’s genome and its structure. In contemporary terms this chain, or network, consists of transduction steps. This essay will examine the concept of cause and effect for transductions which apply to morphogenesis and pattern.

A single transduction step is broadly defined as any reliable conversion of a system, from one state to another, which is caused by a well-defined activity. Molecular examples would be the amination of an acid to convert it to an amino acid, or the phosphorylation of an enzyme (Fig. 1). Chains of transductions involving sequential covalent chemical changes are well understood. Their solution formed the basis of the “one geneNone enzyme” doctrine which initiated molecular genetics (Beadle and Tatum, 1941). One may ask, are there limitations which impede the extension of this concept to broader issues, and, if so, how can the limitations be dealt with?

The Challenge

As one shifts from biochemistry to physiology, the relative comprehension of causal sequences is much reduced (Fig. 2). For example, an obscure transduction chain connects the influence of gravity to the bending of a root. Shifting further, to the organismic or developmental level, a still more obscure chain connects the presence of excess copies of the knotted gene in tobacco with the induction of ectopic adventitious shoots near the mid-ribs of the leaves (Sinha and Hake, 1993). A mutation in Arabidopsis leads to a supernumerary embryo on the suspensor (Somerville, unpublished). Similarly, a mutation of Drosophila brings on the production of supernumerary eyes on the legs (Habler et al., 1995). An obvious challenge is to characterize the critical transductions in such sequences. Note that these chains must contain at least one step where gene products, initially soluble (mRNA), are causally connected to solid tissue configuration. That is, among many other things, the transduction chain must account for a phase change.

The Tactic

Transductions are commonly studied by the tactic of co-variation. One alters an input to a process and assays variation in the output. The input change presumably alters the “cause” which brings on a change in the output, the “effect”. In some cases the pertinent variables can be measured and/or changed continuously and non-invasively (e.g., growth rate, light absorption, gas evolution, turgor pressure). Photosynthesis is particularly amenable to being studied “on line”. In most cases, however, particularly in mutation studies involving development, this is not possible. The alteration step occurs well before the response and the assay often consumes the sample. We will deal with this situation.

With Beadle and Tatum, the input variation was mutation at the genome level, the assay was of compounds in a pathway of synthesis of a vitamin (peridoxine). When one deals with a single transduction step; the co-variation is often qualitative (on or off), or at least roughly proportional (directly or inversely). When one deals with a complex transduction chain, as in the latter examples above, the co-variation obviously spans many steps between a very remote “cause” and the assayable “effect”. In such cases the tactic is to employ the
Simple Transductions

**Fig. 1. Simple Transduction Steps.** (a) In a conversion, compound A (substrate) is converted to compound C (product) through the presence of effector, b. (b). Evidence for such a transduction. The compound A changes its mass and gains label, in the presence of b, becoming C. (c) In summation, the primary effector b is made active/inactive by the presence/absence of a ligand on a receptor site on b.

combined intervention and assay, i.e., co-variation, at various internal sites in the chain or network to resolve it. The effectiveness is dependent on the sites of intervention and assay being close together, ideally bracketing a single step (Fig. 2). In the best case, one has many mutations in the genome and many sequential markers for the screen.

A Unifying Framework

Co-variation has been extraordinarily widely applied. In the more complex systems, many insights have been gained, and vast numbers of control elements have been thoroughly demonstrated in molecular detail. But in many cases, e.g., phyllotaxis, gastrulation, etc., "how it all fits together" is remarkably obscure. This shortcoming is especially glaring when the output is geometrically complex at the tissue and organ level. There the "fitting together" problem reflects two difficulties: Not only are a multitude of pertinent relatively simple transductions present but, potentially equally seriously, certain single ones may be extremely complex.

Despite a wide range in their complexity, it will be argued that all transductions can fit into a common formal framework. Because change is involved, a transduction must deal with time. Thus, explicitly or implicitly, it must involve something changing with time, i.e., dv/dt. With a finite time bracket between intervention and assay, the differential equation is studied through its integral. This perspective, involving both a differential equation and its integral, allows three everyday conceptual dualities to be specified. These are: "before and after" states, "immediate cause and immediate effect", "control element (effector) and responding system". The central assumption of co-variation is that by studying before and after one can characterize cause and effect as well as effector and responding system. There are intrinsic limitations to this approach.

A Preview of the Progression in Complexity

There will be four examples illustrating the connection of the three common pairs of terms to calculus. At the same time, the practicality of co-variation analysis will be addressed. The examples increase in complexity as follows: a) The familiar biochemical transductions (switches) will be shown to be extreme, but well justified, reductions to algebra from a more universal calculus-based relation. b) When the functional differential
Analysis by Co-variation: Within a Chain

![Diagram of transduction chain]

**Fig. 2.** Analysis of a Transduction Chain between Genome and Phenotype. The general analytical tactic of co-variation is to intervene at an early step and to assay for a parallel change at a later stage. Ideally, the before and after stages bracket a single transduction (e.g., biochemistry). In physiological studies more steps are included in the analytical bracket. In studies where the intervention is by mutation and the assay is in morphology, the number of steps in the co-variation bracket is presumed to be very large.

A statement for the transduction involves more than just proportionality, the co-variation tactic encounters ambiguity and the switch character is gone. c) For analysis of a process as simple as growth in cell number, i.e., increase by compound interest, all features of calculus apply. This provides two new avenues for the experimental intervention. In addition to the parameters within the differential equation itself, there is control of the outcome through change in the limits of integration and in the constants of integration. This diversity of input, for potentially the same output, adds ambiguity to any co-variation study attempting to characterize an unknown transduction of this type. d) For de novo pattern formation, even in the simplest possible cases, a very complex integration is involved (Harrison, 1993). The same three classes of control parameters as above are involved, but now these parameters are functions, rather than simple scalar entities.

As the differential equations become more complex, they can become extraordinarily effective at explaining, upon integration, wide variations in form and pattern. Unfortunately, this attractive feature automatically makes the equation difficult to be discovered by co-variation. This reciprocity, between explanatory power and ready discoverability, will be developed through four examples.

**A PROGRESSION OF FOUR TRANSDUCTION TYPES**

1. **Familiar Transductions: Proportionality and Summation**

   1. **Conversion (Proportionality)**

      These transductions apply best to discrete steps as occur in metabolism, gene action, etc. They will be exemplified in their simplest possible form. A typical transduction step has already been briefly described (Fig. 1). The sense of it is that A is converted to C by means of effector b. For example, protein A is phosphorylated (from labeled ATP) by enzyme b to become modified protein C. This would be assayed by a band on a gel becoming unique by the incorporation of radioactive phosphate, also by changing mass slightly. If the amount of A is considered constant, the amount of C is...
Transduction as Integration
Class I

(a) Conversion

\[ \begin{align*}
\text{Conversion} \\
A & \xrightarrow{b} C \\
\text{Before} & \xrightarrow{\text{After}} 
\end{align*} \]

(b) Proportional Covariation

\[ C \propto b \]

(c) The Switch

\[ \begin{align*}
\text{before} \quad \text{negligible} \\
\text{unitize} \quad + \\
\text{After} & \xrightarrow{\text{present or on}} \\
\text{absent or off} & \xrightarrow{\text{Before}} \\
\text{b} & \xrightarrow{\text{C}} \\
\end{align*} \]

(d) The Differential:

\[ \frac{dC}{dt} = b \cdot \frac{A}{\text{Effector} \cdot \text{Responding System}} \]

(1) Effect

\[ \frac{dC}{dt} = b \cdot \frac{A}{\text{Effector} \cdot \text{Responding System}} \]

(2) Cause

(3) The Integral:

\[ C = b \cdot A \cdot t + C_0 \]

Simplification:

\[ C = b \cdot A \rightarrow C \propto b \]

Fig. 3. Class I. Simple Transductions. The conversion in diagram (a), the co-variation of product C with effector b in (b), and the switching action of b in (c) are derived from the simple differential equation in (d). Change in C is proportional to b, when A is constant. When the integration is made (d), the two equations allow precise definition of "cause and effect", "effector and responding system", and "before and after". In practice, co-variation, comparing before and after, is used to deduce the other two dualities. In these simple transductions the integral can be reduced to a simple product to give proportional co-variation. The simplification is to unitize time and to neglect the constant of integration. This makes the integral and differential equations equivalent. If b is active/inactive, the transduction becomes a switch (c).

Genotype

A Network of Simple Transductions

Phenotype

Fig. 4. Resolving a Transduction Network. Co-variation study must be localized to identify branches. Convergence (summation) occurs when two or more transductions influence either the substrate level (start of each arrow) or the effector (ball in the middle of an arrow). Divergence occurs when a conversion transduction make more than one product. Feedback and back reactions are potential complications.
proportional to the amount of \( b \), so the equation is:

\[
C = b \cdot A \quad (1)
\]

That is, the more \( b \), the more \( C \). Or, the presence of \( b \) "switches on" \( C \). Some transductions involve different effectors for the forward and reverse directions and hence are not simple. We will deal only with the case where strong directionality is present and the kinetics are zero order. The role of \( b \) is as a catalyst; it lowers the free energy of activity through unstable mutual binding of molecular surfaces. The transduction is "spontaneous" because, over-all, there is a net loss of useful energy.

It will now be shown that this common working concept of a transduction is a simplification of an integration (Fig. 3). The convenient switch concept, and the idea of proportional variation, bypass the role of time. In reality, however, time must be involved. The process is thus viewed as the conversion of A to C at a given rate determined by \( b \). Thus,

\[
dC/dt = b \cdot A \quad (2)
\]

Two dualities of the three concept-pairs can be identified. The immediate "effect" is \( dC/dt \) (making \( C \)); the "cause" is \( b \cdot A \) (\( b \) acting on \( A \)); the "control element" or effector is \( b \); the "responding system" is \( A \). Going through the simple integration allows one also to identify "before and after" and to make simplifications. If the equation is rearranged to \( dC = b \cdot A \cdot bt \) and integrated, the result is algebraic:

\[
C = b \cdot A \cdot t + C_0 \quad (3)
\]

In this integral, \( C_0 \) is "before"; and \( C \) is "after". Using the differential (2) and the integral (3) together, the three dualities of nomenclature are defined.

To obtain proportionality, two simplifications are made. \( C_0 \) is considered negligible and \( t \) is considered unity. Thus the integral expression reduces to \( C = b \cdot A \) where more \( b \) simply gives more \( C \). One has one equation with one unknown, \( b \). In this context, co-variation study, where a reduction in \( b \) gives a parallel reduction in \( C \), could readily produce the pertinent function. The mathematics of change, i.e., cause and effect in the differential, has been made identical with the mathematics of before and after, found in the integral. For the "switch" interpretation, both \( b \) and \( C \) are considered to be on a present or absent basis. The equivalence of the differential and integral expressions, of great utility here, is a very special case.

2. Transduction by Summation

The transductions above are readily influenced by another type of simple interaction. For example, the control element \( b \) may itself be subject to control. Thus \( b \) may be an enzyme, or a membrane. Here the responding system is a receptor site and the immediate effector is a ligand (Fig. 1(c)). This illustrates specification of change in terms of the stable mutual binding of molecular surfaces. For example, many causal agents may combine to turn on or off a transcription; here the transductions converge, or sum. In the conversion transductions, the mathematics reduced to multiplication; in summation it reduces to addition/substraction. The simplicity results from the discrete nature of the change. If the transduction is functionally isolated, before and after analysis is as effective as obtaining information "on line". Complexity in this category, as idealized here, lies not primarily within the transduction, but rather among transductions.

3. Compounding Several Transductions

Aggregates of transductions can take the form of a chain (Fig. 2). Details of a chain are found by co-variation studies closely spaced along the chain. This was the breakthrough of Beadle and Tatum in 1941. In molecular genetics, the ideal is to find a gene product influencing the immediate "before" condition and a marker to assay the immediate "after". A more general problem is that of solving a network (Fig. 4). When, on the one hand, the product of a single transduction, e.g., a transcription factor, influences many different promoters, the transduction chain diverges. When, on the other, a single transduction requires input from several antecedent transductions, several chains converge. A striking example of convergence/divergence (as well as summation) is found in the genetics of organ identity in flowers (Coen and Meyerowitz, 1991). For example, the specification of a petal requires the co-occurrence (summation) of two types of gene products. The same type of gene product can be involved, as one of a pair of factors, in more than one organ type (divergence).

With sufficiently closely spaced co-variation studies, the details of any network (or cycle) could presumably be deduced. Note that one must resolve whether an antecedent transduction influences input (A), assumed to be normally in excess, or acts on the effector (b). To optimize resolution, the investigator tries to carry out co-variation in isolated sub-systems (e.g., isolated Golgi) and adds all possible ancillary information (e.g., in situ localization of expression, testing for cell autonomy of a process, etc.). Many contemporary studies employ this strategy. For example, the lysogeny-lysis decision in phage has been described as a large number of antecedent transductions (some complex) which form a network (McAdams and Shapiro, 1991). The most complex transductions series which is well understood is the cascade of events in vision (Stryer, 1991). Highly complex network behavior in biological systems is a study in itself. It has been analyzed in terms of Boolean algebra (Kauffman, 1993).

The challenge of solving intricate networks of transductions is recognized. Another challenge, however, arises as the individual transduction becomes complex. It still connects input to output rigorously, but the tactic of characterizing an unknown transduction by co-variation studies which span it, rapidly becomes more difficult.
Class II: Non-proportional Algebra

Plant Cell Growth

The Unifying Differential Equation:

\[ \frac{dL}{dt} = S \cdot \frac{H \cdot Ex}{H + Ex} \]

Potential Covariation Ambiguities

(b) Poor Covariation

Rate: \( H = Ex \)

Ideal

Ex or H

(c) Good Covariation

Rate: \( H \cdot Ex \) or \( Ex \cdot H \)

Ideal

Ex or H

Alternative Limiting Factors

H₂O

Water Entry (H) Limits

Soft Wall

OR

Solute

Wall Yielding (Ex) Limits

Stiff Wall

Fig. 5. Class II. A Non-Proportional Transduction. The conversion of a short plant cell to a longer one can be described by the differential equation in (a). Increase in cell length, \( L \), is related to solute effect (\( S \)), permeability to water, \( H \), and wall extensibility, \( Ex \). There is always good co-variation of rate with \( S \). If \( H \) and \( Ex \) are roughly equal in magnitude, co-variation is poor over a wide range of \( H \) and \( Ex \) (b). If either \( H \) or \( Ex \) is large relative to the other, there is near ideal co-variation of rate with the smaller parameter but not with the larger (c). The two extremes, where \( H \) or \( Ex \) is small, are illustrated in (d) and (e) respectively. This equation was not deduced from co-variation but rather from an effort to reconcile the opposing role of turgor in wall extension (turgor helps) and water entry (turgor hurts).

II. Non-Proportional Transduction.

The rate of plant cell growth, of central interest in physiology (tropisms) and morphogenesis, is subject to myriad control factors (hormone levels, \( O_2 \), salinity of the medium, etc.). For example, it is increased by auxin, decreased by osmotic inhibition, etc. Cell growth is thus a candidate for applying co-variation to deduce a functional relationship in a physiological context.

The various agents must act on some kind of formal transduction that gives added length to a cell. An inspired differential equation, by Lockhart (1965), serves to connect such diverse controls to the response (change in growth rate). His equation, simplified here, originated as an effort to reconcile two contradictory proportionality concepts, each dealing with turgor pressure. On the one hand, turgor pressure should promote growth because a strong cell wall must be extended by it. On the other, the necessary concurrent entry of water into the cell is inhibited by high turgor because it raises the water
potential (Ray et al., 1972). By itself, neither proportional co-variation statement can be challenged. But both cannot co-exist in the cell as simple proportionality. Turgor cannot help and hurt the growth process at the same time. Lockhart solved this dilemma by combining both concepts into a single equation in which turgor was eliminated. It was represented by the factors that controlled it. As in Fig. 5a. a simple form of the equation is: rate = (solute conc.) * (wall extensibility*H₂O conductivity)/ [wall extensibility + H₂O conductivity] (4) or
dL/dt = S*(Ex-H)/(Ex+H) (5).

L is cell length. Ex is extensibility, and H is hydraulic conductivity. For the present argument about complexity within a single transduction (increase in length), this equation is ideal. We will use it only in the differential form for "on line" analysis. Assume that each parameter can be altered independently; also that each can be measured continuously (non invasively).

The equation predicts proportional co-variation between rate and S, solute concentration. However, co-variation of rate with either Ex or H is complex. If Ex and H are comparable in magnitude, only modest co-variation is detected over a wide range (Fig. 5(b)). One might therefore conclude that the only significant parameter is S. If, however, either Ex or H is small relative to the other, there will be good co-variation of rate with the smaller parameter (Fig. 5(c)). For example, let Ex = 1 and H = 10, giving a rate of 10/11 = 1.0. If the larger H is doubled, one gets 20/21 also = 1.0, little effect. If, however, the smaller Ex is doubled, one gets 20/12, almost a doubling in rate. Thus strong co-variation need not be found with either important parameter, and if found, could point to one or the other formally equivalent factor as a "unique" control. Without exhaustive co-variation, and knowledge of algebra, even "on line" co-variation might miss the true symmetry in the potential roles of Ex and H. The difficulty is that even with S being constant, the differential equation represents a broad family of curves connecting rate to Ex, one curve for each value of H. If one is dealing with a largely steep curve, Ex appears important, if a flat curve, Ex appears unimportant. Only exhaustive co-variation, showing the steep and flat parts of each curve, would reveal the nature of the function.

The physiological validity of the relationship is obvious at the extremes. A cell with a weak wall but with an impermeable membrane would not grow (Fig. 5d). The same is true for a cell with a very strong wall and a highly permeable membrane (Fig. 5e). Testing, in light of the Lockhart equation, shows that usually Ex is small relative to H, and co-varies with rate.

This example illustrates that a known differential expression explains any co-variation (top down). But, deducing the equation from data, even if taken on-line and non-invasively, can be difficult. In this case the equation came not from co-variation but from realizing that two opposing proportionalities could be reconciled in the proper differential expression. In the next category of transduction data is taken before and after a finite time interval. Deduction of an unknown function by experiment must be based on information about its integral.

III. Transduction via Simple Integration

A simple transduction involving a non-trivial integration is found in accounting for an increment in cell number in the case of a cell colony growing in unlimited medium (Fig. 6a,b). The outstanding difference from case I is that the mathematics no longer deals with "before and after" simply in the sense of a switch. The difference from case II is that now we deal with the integral to take data for the transduction. We confront the common situation where the essential cause and effect is embodied in the differential while we are constrained to the data on the before and after conditions only. The aim here is to make clear the limitations on deducing an unknown differential relation from co-variation studies involving only its integral.

The pertinent differential equation here, analogous to those in I and II is:

\[ \frac{dN}{dt} = k \cdot N \]  (6)

N is the number of cells and k is the relative growth and compound interest rate. Most clearly, k is the immediate controlling factor (itself of course dependent on many other things) and N is the responding system (Fig. 6c). The immediate effect is dN/dt; the immediate cause is k*N (k acting on N).

To define "before and after", for a given increment of growth, one has to use the integral. Rearranging (6) and adding integral signs, we get:

\[ \int_0^T (dN/N) = \int_0^T (k \cdot dt) \]  (7)

The integral (Fig. 6d) is:

\[ \ln N = k \cdot (t2-t1) + \ln N_o \]  (8)

where N₀ is the initial value. Taking anti-natural logarithms, the equation becomes:

\[ N = e^{k \cdot (t2-t1)} \cdot N_o \]  (9)

The integral N is the "after" condition; N₀ is the "before" condition. The increment is: N - N₀ = e^{k \cdot (t2-t1)} \cdot N₀

The potential controlling parameters governing the increment, the outcome of a particular transduction, are now obviously three, in contrast to the single one in class I. As noted, k is an immediate controlling factor. For any given change, two other controls equally well govern the outcome. One is N₀, the constant of integration, the other obvious one is the duration of the time period, t₁ to t₂. In the equation for the increment there are four parameters. In the most ambiguous situation one would know only two, N₀ and N. In this particular case the time interval is readily knowable and thus the value of k can be easily calculated. Hence this known equation, with four independent parameters, is such that the differential can be found from before and after information (N₀, N) from the integral. This is provided that there is
Class III: Transduction as Simple Integration

Autocatalytic Growth

![Graphs and Equations](image)

(c) The Differential Eq:
Applies during transduction

\[
\frac{dN}{dt} = k \cdot N
\]

(d) The Integral:

\[
\ln N = k \cdot (t_2 - t_1) + \ln N_0
\]

(e) A Symbol
Covariation: Three Categories of Input or Control

Fig. 6. Class III. Transduction as Simple Integration. The conversion here is a change in cell number, \( N \), over a time interval \( t_2 - t_1 \). This is graphed linearly (a) or semi-logarithmically (b). The cell population grows autocatalytically by the equation in (c). Cause and effect (1), effector and responding system (2) are identified in the differential equation. The integral (d) identifies before and after (3). Analysis by co-variation is impeded by the fact that a given change in \( N \) can reflect a change in three parameters: the function, the limits of integration and the constant of integration. Thus in (a) an increase in \( N \) from \( x \) to \( z \), compared to one from \( x \) to \( y \) could reflect a change in growth rate constant \( k \) the duration of the time interval, or the initial value of \( N \). This versatility of the integration process makes difficult the deduction of the details of the differential equation on the basis of knowledge only of "before" and "after". The practicality of co-variation is thus reduced compared to that in simple transductions.

supplemental information on the time interval.

The deduction of an unknown comparable differential equation with four variables, on the basis of limited information on its integral, is another matter. Experimentally, one would alter the system and measure change in output. The basic ambiguity is that one would not know if the alteration affected the values in the function itself, the limits of integration, or the constants of integration (singly or in combination). As shown in Fig. 6b, in comparing \( x \rightarrow y \) with \( x \rightarrow z \), the additional increase in \( N \) could equally well be dependent on change in any or all three of the kinds of input. Thus the tactic of co-variation, on its own, is not attractive for the characterization of unknown transduction schemes of this type. The differential equation here (or its equivalent) was presumably arrived at more directly, from a realization that cells
make new cells in proportion to cell number. The essence of the process itself was formalized. This may be the best way to arrive at differential expressions when information about the integral is far from complete.

The effect of the constant of integration is significant. In the semi-log plot, only a displacement of the function by changing $N_e$ is evident. Note, however, that when the graph is linear (Fig. 6a), changing $N_e$ alters the form of the function as well as the intercept. This feature of calculus, that changing a single constant (a "mere" constant of integration) can change the shape of an integral function everywhere, is not generally appreciated. It is typical for complex differential equations. This makes difficult interpretation of the change in shape of an integral function, in response to experiment. Such a change does not necessarily reflect a change in the differential function. The versatility as one goes from differential to integral means ambiguity as one goes from integral to (unknown) differential.

IV. Morphogenetic Transduction-Complex calculus

Some geometrical transitions which are easy to state verbally (e.g., change of phyllloaxic pattern, gastrulation) are recognized as being highly complex. By our definition, they are transductions. There are two approaches to trying to clarify the mechanism. In one, accounting for the complexity can be attempted "brute force" i.e., by assuming myriad transductions of type I. These would somehow be orchestrated over both time and space to produce the observed change. This point of departure requires considerable ingenuity (and complex mathematics) for the orchestration. This strategy is often thought to be the only entry to such problems, with co-variation study being the only obvious tactic. An alternative view is that in such processes there may be only one key transduction, but it is very complex and hence unlikely to be found by co-variation. We will now deal with one such case, drawing in an appropriate complex integration from an analogous system.

One of the simpler morphogenetic changes in plant development is the transition from a globular to a heart-shaped embryo. A humble, yet profound, analogous geometrical change is the formation of a potato chip (Fig. 7a). Here the "before" condition is a flat slice of potato. The "after" is a saddle-shape. In that case, just as in the dicot embryo, there arise two wavelengths of variation in elevation around the ring: alternating crests and troughs for a total of two each. Another inanimate example of the spontaneous production of spatial periodicity is seen when a long knitted band is made with a central stripe of more densely spaced stitches. Released from stretch, the band develops regular undulations extending from end to end (Green, 1994). This transition resembles the production of rows of close-packed lateral roots induced by auxin (Laskowski, 1996), also rows of close-packed new leaves in regenerating Graptopetalum (Green, 1996). If the row is closed to make a circle, this transduction mimics the simultaneous emergence of five sepals in a ring in the flower of Anagallis (Hernandez et al., 1991).

Theory

We now investigate the possibility that the inanimate physical analogs provide the key to the morphogenetic transitions in the plant. That is, we assume the mechanism is not one where a summation of cell responses causes the morphogenesis of a tissue (Fig. 7b), but rather that the mechanism lies at the level of the tissue itself, viewed as a continuum (Fig. 7c). Engineers have established the basis of the buckling phenomenon in continuous sheets. For example, a saddle-shape can arise physically when a disk develops an imbalance in surface area. In the case of the potato chip, during cooking the rim stiffens while the center shrinks. The center is under tension, the rim under compression. The spontaneous physical response to this imbalance is to minimize the stress (and deformation) gradients by bending in 3-D. This results in a structure where the rate of change of curvature, with position, is minimized (Fig. 7c). Note that this stabilizing activity is not the "active effort" of many sub-districts of the system, but rather is a spontaneous "passive reaction" of a whole tissue, under stress. The inevitable energy input for the spontaneous process has been made previously.

Assuming that the process of buckling applies in the plant (i.e., in the tunica of the apical meristem), two suppositions must be made: first, gradients in biochemical activity would be converted to gradients in physical properties of the tissue; second, stress would be generated by differential growth. These factors would combine to provide the non-periodic "before" condition. Spontaneous physics would then produce the periodic "after" configuration (see Green et al, 1996a,b). There would be a vast preamble of biological activity involving class I transductions to set the stage; i.e., to provide the material and stress components for the differential equation. Note that such a transduction accomplishes the conversion of chemical (soluble) input, the remote cause, to the specific configuration of a solid, the effect.

The equation for buckling is imported from physics and engineering. Co-variation done long ago (stressing a plate and observing the bending), combined with advanced mathematical abstraction, presumably led to formulation of the pertinent differential equations (Szilard, 1974). To cope with the mathematical intricacy, we will use only one equation. Also, the same simplification used in case I is applied here. Time is removed from the picture by considering the transduction to be a single step, the attainment of structural equilibrium. The end of one such step is consolidated and becomes the start of the next step.

As with case III, a single differential equation applies during the entire step. For computation, the basic
Fig. 7. Class IV. Transduction via Complex Integration. The conversion changing a non-periodic surface to an undulating one occurs in the formation of a potato chip (a), as it makes a saddle. Such a change could be viewed as the orchestrated summation of multiple simple transductions (b) or as a single transduction of a single continuous unit (c). We treat it as the latter. As in class I, time is eliminated by considering the transduction to be a step. For the similar case of a bar under compression (c), the sense of the transduction is that applied stress (cause) will tend to increase deflection of any curved region until the increasing structural consequences of deflection (effect), i.e., reluctance to bending and/or being displaced, equalize the stress. When the effect cancels the cause, the step is over. (d) (1) At the differential level, cause and effect is addressed in terms of a point (i.e., whether it goes up or down). The immediate effector is applied stress. (2) The immediate responding system involves the shape and physical properties of the bar (or plate). (3) For the long-term process, the before state is the initial nearly flat topography. The after state, the integral, is the final topography. The three categories of input for co-variation study can be recognized. They are terms in the function, limits of integration, and constant of integration. The latter two categories are now functions, not simple parameters as in class III. Deduction of this type of differential function by co-variation appears exceedingly unlikely.
situation for the surface of a plant embryo (or a chip) is simplified. The before condition is assumed to be a flat disk subject to in-plane stress. The after condition is a saddle-shape whose development reflects increasing opposition to the in-plane stress. This increasing resistance (morphogenesis) brings the structure into mechanical equilibrium, ending the step. A comparable situation applies to a bar attached to a bed of springs. It undulates after being compressed at its ends (Fig. 7c). Mathematical analysis, greatly simplified, is easier for the bar. It will be given now (Fig. 7d).

The master linear equation (Szilard, 1974), applying along an axis x, is:

\[ D \nabla^2 \omega + Q \nabla^2 (\omega + \omega_0) + k \omega \cdot p = 0 \]  

where

- \( D \) is flexural rigidity.
- \( Q \) is in plane stress.
- \( k \) is the spring constant of a cushion, and
- \( p \) is a pressure normal to the system. \( \nabla \) is the Laplacian operator (a second derivative). Immediate cause and effect are dealt with at the differential level. Here one restricts attention to the fate of a single point. does it go up or down? The immediate cause pushing it, say, up is that in-plane stress is deflected by small curvature to give an upward force. The immediate effect is an increasing resistance to upward movement in the bending structure. When the effect,
namely downward tendency, equals the cause, an upward tendency, the step is over.

The immediate effect is the in-plane stress, the immediate responding system is the entire structure; its topography and its physical properties. The integral is:

$$\omega(x) = \frac{p}{k} + C_1 e^{i\lambda x} + C_2 e^{i2\lambda x} + C_3 e^{i3\lambda x} + C_4 e^{i4\lambda x} + f(x) \omega(x)$$

The C's are constants of integration involving limits and boundary conditions. The before and after states are defined for a particular case. Here \(\omega_0(x)\) is a flat bar (with irregularities); it is the "before". "After", \(\omega(x)\), is undulating topography along the bar. Above, we considered immediate cause and effect (at a point). If we now consider the conversion of a straight bar to an undulation, or a flat disk to a saddle, as the over-all effect of a given transduction, then the over-all cause is the integration as applied to the initial system. Cause and effect in this broad sense requires all three input categories. This is the form in which the transduction is accessible to experimental co-variation. Cause and effect in the narrow, or immediate, sense involves only the differential function.

**Application to Pattern Generation**

The extensive ability of a single transduction to specify pattern de novo is shown in Fig. 8a. Here an annulus, flat except for random "noisy" bumps on its surface, is converted by uniform in-plane stress to a ring of 25 alternating humps and depressions ("organs"). The same process propagates pattern. In Fig. 8b a comparable naive annulus is interior to an undulating one as produced in Fig. 8a. There is common slope at the interface but no other shared feature. Upon stress, the inner annulus makes a ring of "organs" exactly out of phase (alternating) with those of the first annulus. This mimics the production of alternating whorls in simple whorled flowers, e.g., the iris. The common slope is the critical boundary condition causing the alternation. The transductions for the two annuli are otherwise identical.

The non-intuitive role of the limits of integration is shown in Fig. 9. In the vertical column A-C, the sole variation is in the width of the annulus. Note that increasing width (c) leads to Y-shaped "organs" rather than single undulations (A). More dramatically, in the column D-F, increasing width changes a pattern of mainly circumferentially long ridges and valleys (d) to checkerboard pattern (e) and a return to long ridges/valleys (f). The non intuitive role of boundary conditions is also striking in Fig. 9. The sole difference between a figure in the left column and the corresponding figure at right is that the outer margin is clamped flat at left and in free to rotate, i.e., is hinged, at right.

The pair of buckling equations (Green and Rennich, 1996) can thus be regarded as a master formal differential relationship. Its diversity in generation of pattern upon integration (without change of any parameter in it) is brought out through variation in dimensions and the physical state of the boundaries. Clearly such an integration competes with the notion that geometrical complexity needs to be generated by the operation of patterned controls on a geometrically passive responding system. Here the geometrical detail arises solely through the transduction of uniform in-plane stress, equivalent to growth, into a topographical response. The complexity (including the periodicity) resides in the responding system (Fig. 7d).

It should be clear from the above illustrations that co-variation, in ignorance of the function, is exceedingly unlikely to lead to the discovery of such functions. This is aggravated by the fact that the boundary conditions and limits are no longer simple values, as in case III, but are function. Actually in Fig. 8c., the boundary condition is a function (position) of a derivative (slope).

The four examples (I to IV) have illustrated increasing capacity for specification using differential equations. The difficulty in arriving at an unknown function by co-variation increases in parallel. This latter reflect the intrinsic problem of deducing a differential equation on the basis of only partial information on its integral.

Complex transductions are likely to be in series and/or parallel with other transductions, simple or complex. This compounds the difficulty in trying to deduce the nature of complex developmental phenomena, e.g., phyllotaxis, on the basis of co-variation studies which involve intervention at the level of the genome and assay in the anatomy of the shoot apex. A diagram (Fig. 10) presents a model network involving various types of transductions. Within this array, co-variation studies of increasing potential ambiguity (challenge) are designated by brackets with increasing numbers.

**DISCUSSION**

This essay has presented the view that, because transductions involve change over time, they can all be analyzed in relation to a differential equation and its integral. The essence of the process of is embodied in the differential equation. Experimental analysis with characterization done before and after an interval involves primarily the integral. Within this perspective, there are three major conclusions.

First, there is a range in complexity and versatility of the pertinent equation. Discontinuous processes such as steps in biochemical synthesis are well described by simple equations, often equivalent to switches. As the complexity of the pertinent differential equation increases, so does its versatility and potential explanatory power. Thus a single transduction, e.g., buckling, can account for detailed morphogenetic behavior over a large area (pattern formation). This mechanism contrasts with the common assumption that such activity is to be explained by a great many independent transductions which are orchestrated, e.g., by "positional information". Thus there may be one.
Fig. 9. The Role of Limits and Boundary Conditions in a Complex Transduction. (a)-(c). The outer margin of the annulus (width = 0.2) is clamped, the inner is hinged. Increasing the width of the annulus leads to a Y-shaped “fusion” of undulations, reducing the number at the inner margin. Fused primordia are common in plants. (d)-(f). Both margins are hinged (simply supported). (d). Width is 0.2. The simple hump pattern seen in (a) is replaced by undulations involving three nodes (two 1/2 wavelengths), thereby largely splitting the annulus circumferentially. The humps are replaced by long (legend continued on next page)
circumferential ridges (trenches), small in number. (e). The annulus width of 0.25 does not match either 2 or 3 half wavelengths. The annulus is split circumferentially, as above, but there is now regular undulation around the circumference. This is in alternating whorls. (f). Width is 0.3. 4 nodes (3 x 1/2 wavelengths) are compatible with annulus width. The annulus is trisectioned, circumferentially, largely by long ridges (trenches) very small in number.

Morphogenesis in Sunflower

Transduction Network

Covariation Brackets

Fig. 10. Hypothetical Transduction Network. Simple transductions (Class I) are small solid arrows; complex transductions (Class IV) are long open arrows. Genome (start) is below. There are two main phenotypes in this example, the sunflower head. First, the early one, is the spiral pattern of humps on the head. Second, the late one, is the ring of five petals produced on each hump in the disc. The increasing apparent impracticality of co-variation study to characterize a progression is shown by the increasing numbers in circles. The difficulties are of two sorts: the interactions of many transductions in series and in parallel (many small arrows), also the complexity within a single transduction (open arrow). Note that the latter requires input of three kinds. Such a transduction may also produce output of several kinds (e.g., buckling may open ion channels, change biochemistry), initiating new transductions. For the analysis of Class IV transductions, the advantage of intervention at the genome level may be outweighed by the distance, in terms of steps, between input variation and the assayed output.
master transduction at the level of the tissue. The individual cells, at least in such a buckling phenomenon, need not to "know their position" in order to respond. Further, the buckling process provides an explicit way of connecting gene activity, present transiently in diffusing compounds, into the expression of solid tissue configuration. Thus increasing capacity for specification is associated with increasing complexity (higher order) of the equation.

A second conclusion is that, as the differential equations show broader capabilities, they become more difficult to discover by co-variation. There are two reasons. First, in nature, a complex one is likely to follow many simple biochemical and physiological transductions, in a causal net. The output of such activity becomes the material input for the major single transduction. To attempt the co-variation analysis of a single complex transduction, all this antecedent information is necessary. Second, even if all the input information is obtainable, there remains the intrinsic difficulty that it may enter the unknown function in three different ways, often not obvious a priori. These are: terms in the differential expression itself, the limits, and the constant of integration. Control of the outcome, the entity assayed, can be equally based on all three. When the equation is higher order (e.g., fourth derivatives), the constants of integration include derivatives, usually functions of them. The potential ambiguity between change in input and change in output, the basis of analysis by co-variation, is enormous.

While the tactic of co-variation has been remarkably effective in many transduction chains where single steps can be adequately circumscribed, it encounters great impediments where the isolation is difficult and the unknown equation is complex. Experiments invariably provide information in the form of insight, clues, cues, etc., but it is often not well defined. Thus it may not effectively sum to give a clear characterization. In light of this, the three great advantages of varying the input to an unknown process by mutation (strong control of a process, molecular specificity of the intervention, and high reproducibility) may be outweighed by the fact that the intervention occurs far from the process of interest. The tactic of discovery by co-variation of controlling parameters thus appears to have impediments not only reflecting the network nature of transductions, but also others reflecting the mathematics of complex cause and effect within a single transductions.

The third conclusion is that, in light of the above difficulties, unknown complex transductions are more effectively approached, or discovered, by an emphasis on what the process is like as against on what controls it. The complex pattern-forming equations for chemistry, reaction diffusion, were formulated by Turing in the 1950's (Harrison, 1993). In his remarkable mind the key feature of de novo pattern formation, e.g., in hydra and in whorls of plant organs, was that a ring of uniform amplifier. Thus when structural/chemical undulations of the right wavelength occurred at random, these would be selectively amplified, and then fitted to fill the available space. The equations he developed were such that a high derivative process, diffusion, inhibited the production of short wavelength undulations, a scalar factor (of chemical inhibition) suppressed long wavelengths. The key feature of the process was formalized. The mathematics is nonlinear partial differential equations (2nd order).

To connect the present buckling process (Class IV transduction) to plant morphogenesis, a parallel was noted between the saddle configuration of the apex of an opposite-leafed plant, or a heart-shaped embryo, and that of a potato chip. The equations for the patterned buckling of a plate (chip) had been worked out. They are more complex than reaction-diffusion equations because they are fourth order. They are simpler, however, in the sense that time is not included. Thus the pertinent equations were found by analogy with an inanimate system. The constants of integration are derivatives (slope, curvature) which, surprisingly, makes them tractable. One can bend a tissue non-invasively. Hence this complex relationship is testable through variation in boundary conditions.

In summary, development is likely to contain single transductions whose basis involves a wide range of complexity of cause and effect relationships. All transductions can be viewed as integrations of a differential equation. A tactic for the efficient characterization of the simpler ones, involving intervention and subsequent assay, is employed extensively. It encounters multiple but well defined ambiguities when applied to the complex transductions. For analyzing the complex phenomena, the common emphasis on the control of a process (studied by changes in the integral) could well be replaced, or at least supplemented, by emphasis on the nature of the process itself (embodied in the differential expression).

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