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Morphogenesis and Evolution

Abstract

The believe that the genome in the fertilized egg constitutes the complete set of instructions for morphological development is not consistent with the experimental evidence or theoretical modelling. This is demonstrated by an analysis of morphogenesis in the giant unicellular alga *Acetabularia acetabulum*, which involves an explicit mathematical model, studied by computer simulation. The key to an understanding of morphological development is the dynamics of calcium-cytoskeletal interaction. Genes are involved in specifying the parameters of this system, but robust morphogenesis arises from the dynamic coupling between this and the growth process, which results in a hierarchical cascade of symmetry-breaking bifurcations whereby the complex adult form emerges from the egg. Morphogenesis in viable species probably arises from the coupling between different dynamic modes in a hierarchically organised system.

Introduction

Viewed from the molecular and genetic levels, development is an extremely complex process. Thousands of genes and their products are involved in the orderly sequence of events that transform and fertilized egg of a particular species into the coherent intricacy of the adult form. What do we need to know to understand such a process? Is it really necessary to identify all the relevant genes involved, to map their changing patterns of activity in the developing embryo, and to decode the combinational language of gene interactions? Is the key to morphogenesis to be found in a genetic program?

The predominance of the genetic paradigm in contemporary biology encourages the belief that the patterns observed in the morphology and the behaviour of organisms is a result of effective genetic algorithms that can discover, in an immense search space, improbable genetic programs that are then stabilized by the adaptive success of the equally improbable organisms that they generate. In contrast to this view, I shall suggest that the space of possible biological forms, though certainly very large, may be much smaller

than that suggested by the size of genetic program space; and that the role of natural selection in determining biological form may be much less than is often assumed. There is nothing in the least original about this position. Eighty five years ago, in the introduction to his celebrated volumes 'On Growth and form', D'Arcy Thompson (1917) had this to say: 'So long and so far as "fortuitous variation" and the "survival of the fittest" remain engrained as fundamental and satisfactory hypotheses in the philosphy of biology, so long will these satisfactory and specious causes tend to stay severe and diligent enquiry ..... to the great arrest and prejudice of future discovery.' So I am simply taking up D'Arcy's theme and developing it in a contemporary context, using insights that have come from complex dynamic analysis and computer simulation that were not available to him. However, the message is essentially the same.

The importance of this view within a medical context relates to the claims made on behalf of the Human Genome Project by apologists such as Delisi (1988), who says the following: 'This collection of chromosomes in the fertilized egg constitutes the complete set of instructions for development, determining the timing and details of the formation of the heart, the central nervous system, the immune system and every other organ and tissue required for life.'

As will become evident in my analysis of morphogenesis by the use of an explicit mathematical and computer programme to describe the generation of a biological form, this statement is simply wrong. It makes a category error about what is required to understand the formation of biological structures during development.

Morphogenesis

It is a good strategy to study a system that is basically simple, but is just complex enough to present a non-trivial example of the problem under investigation. The organism whose life cycle is shown in figure 1 is one that has served developmental biologists well since it was first cultured in the laboratory by Hammerling (1931). It is the giant unicellular alga, *Acetabularia acetabulum*, whose habitat is the shallow waters around the Mediterranean.

The life cycle can be taken to start with the conjugation of two flagellated, haploid isogametes (no males or females in this species), which form a roughly spherical, diploid zygote about 50 μm in diameter. The spherical symmetry is broken by the formation of a stalk that grows upwards and a branching root-like structure, the rhizoid, that anchors the alga to rocks on the sea floor. The single nucleus remains in a branch of the rhizoid while the stalk grows and, after several weeks, starts to produce delicate branching elements (bracts) in rings called verticils or whorls. These can be seen in more detail in figure 2, which shows an alga at 10-12 weeks that has just

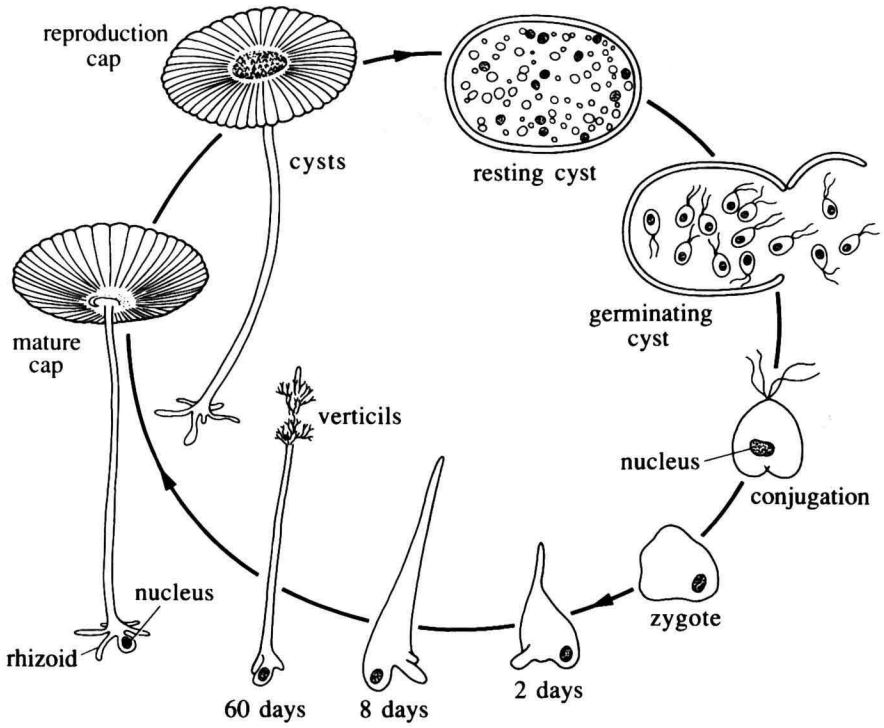

Figure 1: The life cycle of Acetabularia acetabulum.

produced a little structure at the centre of the last whorl – a cap primordium. This grows into the mature cap, about 0.5-1.0 cm in diameter, while the stalk at this stage is between 3 and 5 cm. long. To the uninitiated eyes, the cap looks multicellular, but the whole organism is a single giant cell that has undergone a fairly complex pattern of morphogenesis.

After several months the alga goes into reproductive mode, the single nucleus dividing many times to produce thousands of haploid nuclei that stream into the cap and differentiate into flagellated gametes within cysts that are released as the cap wall dissolves away under the action of enzymes. Little hatches in the cysts then open to release the gametes, and the life-cycle starts anew. How are we to understand this life-cycle, which is one of the simpler kind? In studying this in laboratory, it is more convenient to work with regenerating algae. If the cap is cut off, it is regenerated following exactly the same morphogenetic sequence as during normal development: tip growth, whorl formation, cap formation. The process is highly reproducible, allowing for systematic experimental investigation.

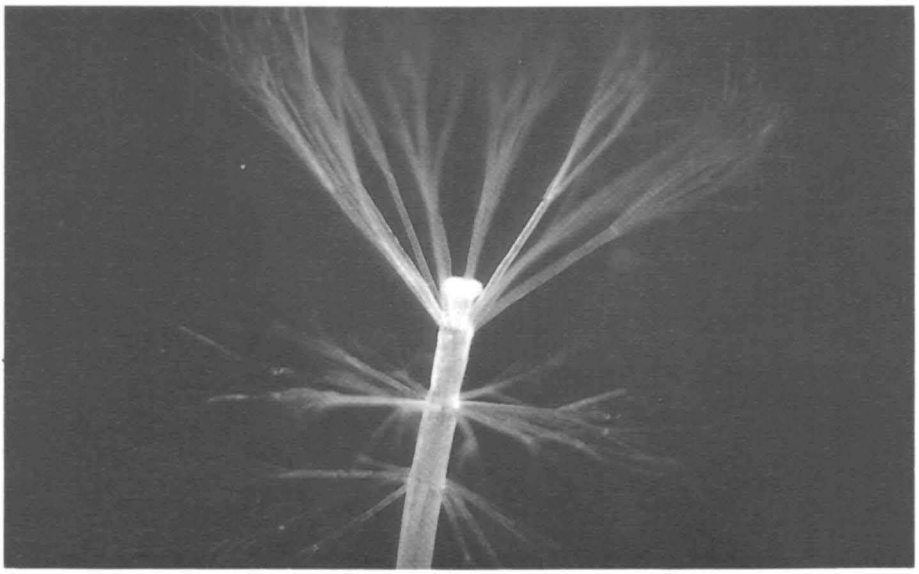

Figure 2: Formation of a cap primordium, after three whorls.

Modelling Morphogenesis

Our research depended upon the work of many others (cf Brachet and Bonnotto, 1970) but our particular interest in morphogenesis led us to study those factors and processes that were particularly involved in growth and form. It emerged that calcium is particularly important (Goodwin and Pateromichelakis, 1979; Goodwin et al, 1983; Harrison and Hillier, 1985) and this led us to a study of the dynamic properties of the cytoskeleton, the network of filaments in the cytoplasm whose mechanical state is modulated by calcium. Calcium exerts its influence by a variety of means: it activates enzymes such as gelsolin that cuts microfilaments; it is involved in initiating contraction of actomyosin filaments; and it influences the polymerization of microtubules and microfilaments. These properties of the cytoskeleton and the affects of calcium derive largely from work on organisms other than *Acetabularia* whose cytoskeletal network may be simpler, lacking microtubules and actomyosin even though the alga can make both tubulin and a myosin epitope which are involved in nuclear division and cyst morphogenesis (Menzel et al, 1992). The effects of calcium on the cytoskeleton in plants is described by Williamson (1984), Shelanski (1989), and by Menzel and Elsner-Menzel (1989). The way in which calcium affects the mechanical state of the cytoplasm (measured by its elastic modulus) is shown qualitatively in figure 3.

The cytoplasm forms a thin shell just inside the cell wall whose shape changes during regeneration in the manner shown schematically in figure 4.

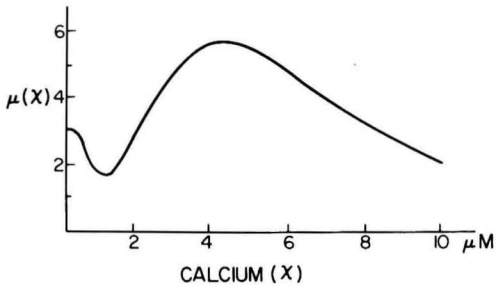

Figure 3: Variation of elastic modulus with free Ca^{2+} concentration (micromolar). Qualitative relations only.

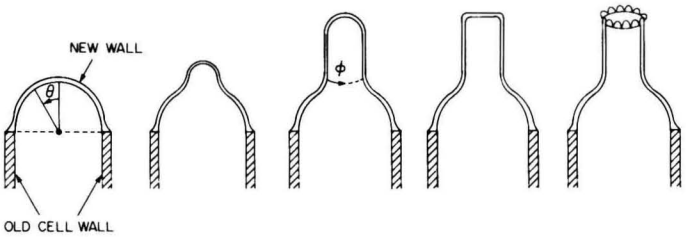

Figure 4: Representation of the morphogenetic changes taking place during regeneration of Acetabularia; hemispherical dome; tip formation; growth; tip flattening; whorl formation.

Inside the shell of cytoplasm is a liquid-filled cavity, the vacuole, within which salts and small molecules accumulate, producing an osmotic pressure on the cell wall that keeps the cell in a state of turgor, and gives it a flexible rigidity. Our model consisted of three components only: wall, cytoplasm, and vacuole. The most important of these is undoubtedly the cytoplasm, and it was necessary to derive equations that describe its mechanical properties, the way in which calcium is regulated in the cell, and how calcium influences the state of the cytoplasm. The derivation, carried out by Goodwin and Trainor (1985), followed quite closely an earlier model of cytoskeletal-calcium dynamics described by Oster and Odell (1983). This is based upon the theory of viscoelastic media in which strain (degree of stretching or compression of the cytoplasm) is the primary variable, the theory being adapted to the properties of the cytoplasm, particularly its dependence on calcium. Equations for calcium concentration, which is regulated at resting levels of fractions of a micromole in all eukaryotic cells, describe the combined effects of calcium-binding proteins and membrane pumps, as presented in Hart et al (1989) and Briere and Goodwin (1990). These equations are mutually coupled so that not only do changes in free

calcium concentration affect the strain in the cytoplasm, but cytoplasmic strain influences the concentration of free calcium. As a result, the model has the same properties as Turing systems: spatial patterns of strain and calcium concentration can arise spontaneously as a result of bifurcation and symmetry-breaking in the dynamics of the system. In consequence, within a region of cytoplasm such as the regenerating tip it is possible for changes of state and shape to occur starting from initially uniform conditions. Our equations are not the same as Turing's, since no chemical reactions are involved, but they belong to the same class of partial differential equations describing excitable media.

The basic reason for the capacity of the model to bifurcate and generate spatial patterns comes from the interaction between calcium and the cytoskeleton. Wherever free calcium happens to increase in concentration from resting levels, the cytoplasm softens because of the effect of calcium on cytoplasmic elastic modulus (figure 3). The cytoplasm is closely apposed to the cell wall, which is under pressure from the vacuole, so the cytoplasm is always under tension (being stretched). Wherever it softens, it will stretch more (positive strain), which results in release of more calcium from the bound or sequestered condition, as assumed in the model. So there is a positive feedback loop that results in a local run-away increase in cytoplasmic strain and free calcium concentration, initiating a spatial non-uniformity of state. This increase is then opposed by the reversal of the elastic modulus-calcium relationship (figure 3) when free calcium increases beyond about 5μM, and by diffusion of calcium, so the pattern gets stabilized. Conversely, wherever free calcium fluctuates below resting levels there is reduced cytoplasmic strain accompanied by more binding and sequestration of free calcium. These are the dynamic properties that underlie spontaneous pattern formation in the model, as in other models of this type (cf Murray, 1989),

In order for the shape of the cell to change, it is necessary for the pattern of cell well synthesis to change in response to patterns in the cytoplasm. A growth algorithm was defined in accordance with known properties of wall growth in plants based upon strain (Cleland, 1971; Green et al, 1971). Coupling of wall to cytoplasmic state was assumed to result from wall softening (decrease in wall elastic modulus) as a function of underlying cytoplasmic strain via a mechanism such as a strain-activated membrane pumps. The exact nature of this coupling is not yet experimentally established.

Biologically speaking, our model is extremely simple: an elastic wall capable of growth, an excitable cytoplasm whose state influences that of the wall, and a vacuole that exerts constant pressure on the wall. Mathematically, the model is complex and non-linear. To observe its behaviour in three dimensions it was necessary to carry out a finite element simulation. This was done in collaboration with Christian Briere (Briere and Goodwin, 1988). The regeneration domain was described by a set of elements, each of

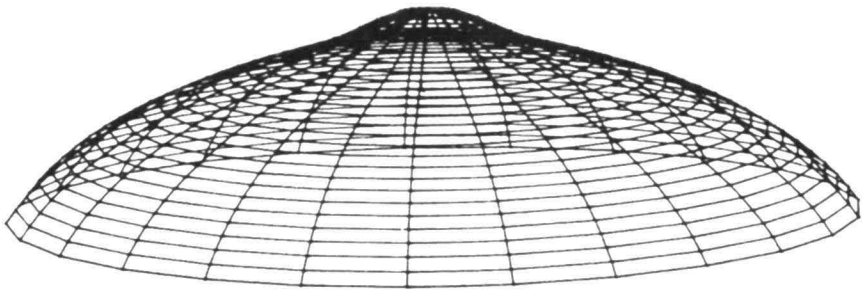

Figure 5: Computer simulation of regeneration in which the cytoplasm and the cell wall are described as shells made up of finite elements which obey equations describing their dynamics as mechanochemical or elastic media, respectively, showing tip initiation.

which obeyed the equations of the model. These collectively defined the geometry of the domain in accordance with intrinsic properties such as elastic modulus, and pressure such as that exerted by the vacuole (cf figure 4). The boundaries of the regeneration domain were fixed, as they are by the old cell wall in a regenerating alga, and the initial conditions were specified with all the variables starting from spatially uniform patterns. Parameters were chosen so that the cytoplasm was in the bifurcation range where spatial patterns could arise spontaneously, and with values that resulted in wavelengths of variables that were smaller than the domain over which morphogenesis was to occur (the regeneration domain of figure 4). This was simply to ensure that interesting patterns could arise. The model was then allowed to do its own thing, to follow whatever morphogenetic trajectories were available to it.

Morphogenetic Dynamics

The first pattern that developed in the model was the spontaneous formation of gradients in free calcium concentration and in cytoplasmic strain with maxima at the tip of the regeneration domain. As a result of this, the wall softened at the top and underwent an elastic deformation due to vacuolar pressure (figure 5). This was followed by growth of the wall and an extension of the tip. After a period of growth another change occurred. Instead of gradients in cytaplasmic variables with maxima at the tip, an annulus of elevated free calcium and strain occurred a short distance back from the tip.

This is shown in figure 6. This result explained something we had frequently observed in regenerating algae but had never understood. Just before a whorl is initiated, the normally conical tip flattens, as shown in figure 4. The model suggested that this was due to the transition from calcium and strain gradients with maxima at the tip, to an annulus, maxi-

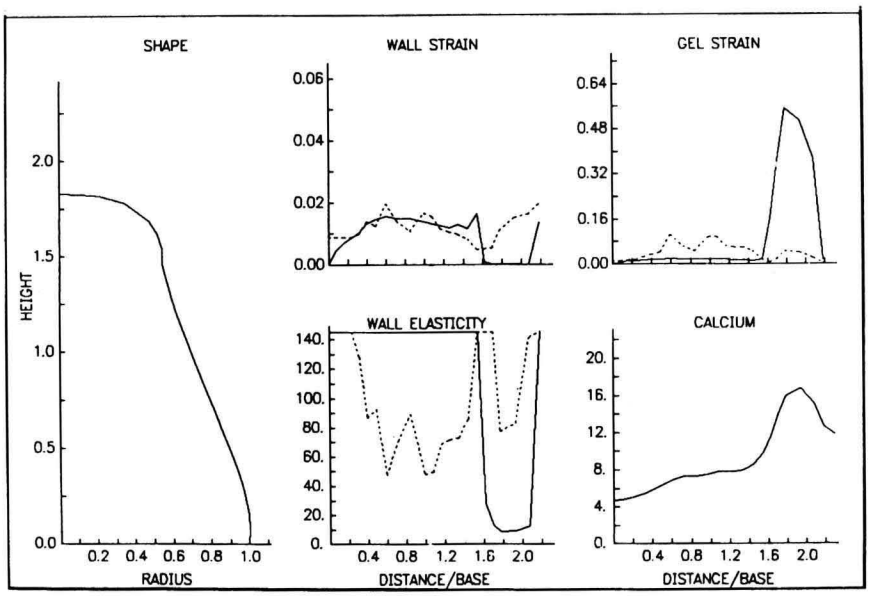

Figure 6: Tip flattening and calcium annulus formation.

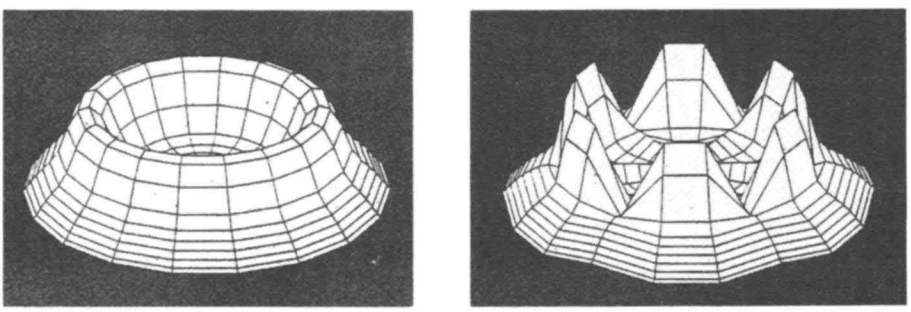

Figure 7: Bifurcation of the calcium annulus to a whorl prepattern

mum wall softening and therefore maximum wall curvature then occuring on this annulus resulting in the flattened tip.

The next stage should be whorl formation. Could the calcium annulus break symmetry and generate a whorl prepattern? The result is shown in figure 7: under perturbation, the annulus bifurcates into a series of peaks which have the symmetry of a whorl. These changes of calcium pattern, from gradient to annulus to a ring of peaks accompanying the morphogenetic sequence from tip to whorl formation, have been observed experimentally by Harrison et al (1988) using the calcium probe chlorotetra-

cycline, which measures membrane-bound calcium. It is to be expected that free calcium will show similar changes, but this remains to be demonstrated.

The model gave further results. As growth continued and the tip continued to extend, the calcium annulus became unstable and collapsed back to a gradient, with a conical tip forming. After a further period of growth an annulus formed again, with the possibility of bifurcating to a whorl prepattern. This sequence is reminiscent of the intermittent production of whorls that occur during algal growth. We were unable to carry out a full simulation of whorl formation because the finite element model is not sufficiently robust to allow stable growth on the fine scale of individual bracts of a whorl. This requires a substantial extension and refinement of the modelling program that has not yet been achieved. Also, we have not yet obtained a structure that looks like a cap. What we do get is an enlarged terminal structure that grows laterally but is not nearly as flat as a cap. It appears that more anisotropy is required in the model so that strain levels increase around the rim of the cap more than those elsewhere, a type of hoop strain that has been proposed by Green (1987, 1989) for leaf morphogenesis in higher plants.

Morphogenetic Attractors and Evolution

Despite these limitations of the model, the results obtained are of considerable interest. The sequence of tip formation, growth, tip flattening, whorl initiation, intermittent repeat of this process as growth continues, and terminal cap-like structure is a result simply of the cycle of cytoplasmic dynamics causing a change of shape which then acts back on dynamics. This defines a moving-boundary process, which is the category to which morphogenesis belongs. Our results suggest that what we are seeing is the expression of a dynamic attractor in morphogenetic space, a robust pattern-generator that arises naturally and inevitably in a system organized as are the interacting shells of wall and cytoplasm, the latter having excitable dynamics.

What is the role of genes in this process? They specify parameter values within a range that allows a morphogenetic trajectory to unfold to a viable form. We do not yet know how large this domain is in parameter space. But the ease with which we found suitable parameter values suggest that it is extensive – a large basin of attraction seems to exist. We do know that we can change most of the parameters without significantly affecting behaviour, so the morphogenetic sequence is quite robust. Our conjecture is that it is generic in this space – a structurally stable trajectory. If this is the case, then there should be many species of algae in the taxon containing *Acetabularia* that all share the same basic morphology. And this is indeed the case. The *Dasycladales* are an ancient group of giant unicellular green algae most of which are known from fossil remains of calcified cell walls that extend back at least to the Cambrian Era, 570 million years ago. These, together with the

20 or so living species, are described in a recent monograph by Berger and Kaever (1992). All species have a rhizoid and an axis with bracts, most of them produced in whorls. In the majority of species the bracts are the gametophores (where the cysts and gametes form) and no caps are produced, so *Acetabularia* belongs in this respect to a minority class. However, it continues to produce whorls despite their sterility and apparent lack of function, being transient structures that are shed. We know also that the algae can grow perfectly well without whorls, whose production can be prevented by simply reducing the calcium concentration in the sea-water (Goodwin et al, 1983), and these whorl-less stalks can then make caps and produce cysts simply by restoring calcium to normal levels. So why does *Acetabularia* produce whorls of sterile bracts?

An obvious answer is that these structures result from the generic dynamics of morphogenesis in this group of organisms, all of which share the same basic organization. They are produced not because they are useful but because they are natural. This is the way we explain forms in nature – the shape of a diamond crystal, vortices in liquids, elliptical trajectories of planetary movement about the sun. These are the structurally stable forms generated by particular dynamic processes. In biology the notions of function or adaptation or natural selection are often used to explain organismic form, but these ideas address only the question why a structure persists. They fail to explain why the structure exists – i.e., what kind of dynamic process makes the form possible as one of its stable solutions. This gets at the generative roots of the problem.

Is there a genetic program that directs the morphogenetic sequence in *Acetabularia?* That genes are involved in the process there is no doubt – they set the values of the parameters. How many genes are involved we do not know, but certainly every one of the 26 parameters must involve the contributions of many genes. For example, the effective diffusion constant of free calcium in the cytoplasm, one of the sensitive parameters that affects the dynamics, is dependent upon the concentration and affinity constants of any protein that binds calcium such as calmodulin, calcitonin, and any analogue of troponin involved in the cytoskeleton. Concentration regulation of proteins involves other genes, so there could be as many as 10 genes involved in specifying this one parameter. The restoring force of the cytoplasm or the elastic modulus of the cell wall depends an dozens of gene products that contribute to the mechanical properties of these cellular components. We could probably account for several hundred genes in our model, all acting polygenically on the parameters. Not one of these do we need to know in detail, since we are looking for generic properties – classes of process, types of form that can be generated. Of course, the more detailed information we have, the more accurate the model. But the clue to robust morphogenesis may be in the coupling between different components of the overall process. An excitable cytoplasm generates an initial gradient that is

stabilised by plastic deformations of the tip, whose growth then makes possible the appearance of the next mode, an annulus. The geometry then changes again with tip flattening, creating the conditions for a third bifurcation to the pre-whorl pattern, which is stabilised by further changes of shape as the bract primordia grow into a whorl. Thus a hierarchical cascade of sequential symmetry-breaking occurs and complex form arises from a dynamically stable trajectory. The principles here can be generalised to a conjecture that morphogenesis in viable species is an intrinsically robust process that arises from the coupling between different dynamic modes in a hierarchically organised system (Goodwin, 1993; Goodwin et al, 1993). A number of different candidates for this hierarchical, coupled system are emerging (e.g., Hunding et al, 1990; Lacalli, 1990; Mittenthal et al, 1992). Out of this and other work on these complex systems may come the resolution of the tension between dynamic models and genetic programs that combines both in a new category of morphogenetic field that can be used as the basis for a classification of the generic forms available to life. This would open the door to a rational taxonomy of living forms (Ho. 1990) and provide a context for understanding how evolution has explored morphospace (Gould, 1991).

The Evolution of Generic Forms

From the perspective outlined above there emerges a fairly obvious conjecture about the morphological products of evolution. All conserved aspects of biological structure may be the generic forms generated by morphogenetic fields (Newman and Comper, 1990; Goodwin, 1990;Goodwin et al, 1993). These include the basic body plans of the different phyla, such regulaties as the patterns of leaf production in higher plants (phyllotaxis), and homologous structures such as tetrapod limbs. Take the question of phyllotaxis. Leaves are generated by the growing tip of a plant by a multicellular structure, the meristem, in which growth and form are linked as they are in *Acetabularia*. There are only three basic patterns of this process, as shown in Figure 8. Leaves can be produced one at a time on opposite sides of the growing tip (distichous phyllotaxis) as in the grasses (monocotyledons); they can be produced in groups of 2,3, or more, with alternating positions at successive nodes (whorled phyllotaxis); or the leaves are generated singly at a fixed angle (average 137.5°), resulting in a spiral (spiral phyllotaxis). Why should these patterns be so constrained? The possibility is that these are the only stable solutions of this morphogenetic process, a conjecture for which Green (1987,1989) has convincing experimental evidence and Douady and Couder (1992) have provided an elegant model.

The fact that more than 80% of higher plant species have spiral phyllotaxis may then arise primarily from the sizes of the attractors for these

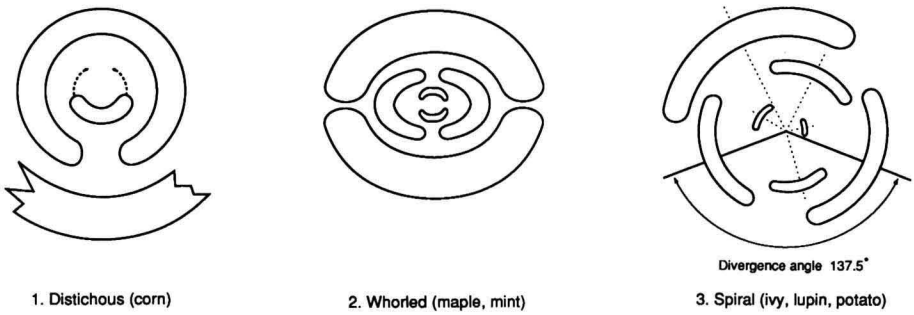

Figure 8: The three basic patterns of leaf phyllotaxis.

different solutions. The idea here is simply that all three patterns produce perfectly satisfactory leaf arrangements from the point of view of catching sunlight, respiration, transpiration, and so on, so that the functional aspects ('fitness') of all forms are roughly equivalent. The frequencies of these different patterns in nature may then simply reflect their differential probability as measured by the sizes of the attractors. Furthermore, the different patterns are rationally united as different solutions of the same morphogenetic process: they are transformations within a particular generative dynamic. So taxonomy (relationships of similarity and difference) and differential abundance can both be explained within a single dynamic perspective. Instead of being based upon history (genealogy) and function, classification is then based upon generative dynamics, which includes environmental influences and the study of dynamic stability, as previously discussed. By putting development back into evolution at a fundamental level, we lose nothing of value in the study of gene action (parameters, hierarchical dynamics) or natural selection (stability), but we gain the whole dimension of rational taxonomy (form and transformation) and *sufficient* explanations of biological phenomena, rather than just the description of necessary conditions (genes, survival). So we can begin to talk about the evolution of generic forms, and to consider the possibility of an evolutionary theory that involves the dynamics of development as the generative origins of morphological species, their systematic relationships, and their intrinsic stability.

Morphogenesis and Medicine

Finally, we can ask what all this has to do with medicine. There is an important sense in which biology serves medicine, and a reductionist biology serves a reductionist medicine, the dominant medical model. This is a very powerful approach to problems of human health and disease, but it has severe limitations. The ones I have focussed on relate to morphogenetic

processes of reproduction and regeneration. Clearly a knowledge of genes and their products is insufficient to understand how such processes occur and why particular forms are generated. I have focussed attention on plant rather than animal form, but the principles are the same and there are models similar to the ones I have described that apply to such processes as tetrapod limb morphogenesis (Oster at al, 1985) and evolution (Shubin and Alberch, 1988). The conclusion is that understanding the parts does not give one an understanding of how the whole organism is generated, which requires additional principles of spatial and temporal organisation that are embodied in the properties of morphogenetic fields. Regeneration and healing are expressions of these principles of generating and restoring the whole organism, and are therefore relevant to medical understanding and practise. A biology of wholes is related to, but clearly goes beyond, a biology of parts.

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