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Organizer, organize thyself

1.1 PATTERN, PATRON, PROCESS

This book is about the great mystery of how living organisms develop the shapes and proper relative positions both of the whole organism and of all the parts that make it up. These are large-scale structures, ranging from parts of a cell visible by optical microscopy up to gross anatomy. My primary interest is not in the structures themselves, most of which remain after life has departed and are indeed commonly described from studies of dead material. Nor is my attention given mainly to structural details on the molecular scale within the provinces of the biochemist and molecular geneticist, details that could be regarded as the ultimate limits of anatomy, cutting-up that has reached the atomic scale. I am concerned mainly with how the tiny, partly fragmentary and partly one-dimensionally ordered genetic beginnings are transformed into the three-dimensional organism by the processes of development. This book is about processes, as they occur during life and make up a large part of what distinguishes living from inanimate matter.

The proper study and description of processes may involve diverse branches of the physical and chemical sciences (Harrison 1993, Chap. 8), but must quite often be primarily based on dynamics. That is the kind of explanation of developmental events to which I have devoted most of my effort, and is the main topic of this book. The postulation of ways in which diverse and complex shapes can readily be generated by dynamics is as old as the double helix model of DNA (Turing 1952). But the study of developmental dynamics still attracts only small numbers of researchers. Therefore this book is more about unanswered questions than about established explanations. My only certainty, for this as part of a generality for most fields of science, is that processes must in the end receive as much attention as structures if we are to understand the workings of the universe and all its parts.

Visitors to the increasingly fuzzy three-way intersection of science, pseudo-science and science fiction often wonder what life is like if it exists somewhere else in the universe, in forms that have originated quite independently of those on Earth. Is the DNA–RNA-protein molecular basis of all known life a unique set of building materials that will inevitably be found wherever there is anything that can be recognized as alive? My guess is that the essence of life does not reside in such molecular detail, indeed not in anything structural, but in processes and their dynamics, most particularly those that make up the development of the organism, its maintenance by assimilation of foodstuffs and excretion of waste products, and its reproduction. When Isaac Newton looked at the skies, he envisaged and established laws of motion that are the same for little things on Earth and huge objects in space. He and his scientific successors had no philosophical scruples against applying these laws to objects of unknown composition, and establishing that the laws worked very well to explain an enormous diversity of motions. They remain the primary basis of modern science, and explain the motion of the moon equally well whether its mass is made up of green cheese, Dante's nuns who failed to keep their vows, or a glob of interstellar rocky garbage. The cosmologist expects Newtonian concepts of motion and gravity and their Einsteinian modifications to apply to everything, including dark matter of still unknown composition. (Some readers of a philosophical bent may perhaps have the word Newtonian attached to a concept called 'clockwork universe', regarded as inadequate and out-of-date. I am not here concerned with that. The application of Newton's laws to whatever tiny, little or big bit of matter one happens to be considering is as timely today as when he formulated the laws.)

Does biological development yet await its Newton despite half a century of non-linear dynamics, just as evolutionary ideas had to await Charles Darwin despite the thinking of such 'transformists' as Lamarck, St-Hillaire and Erasmus Darwin up to half a century earlier? I hope not. The history of the evolutionary concept involved a most unusual sequence. The first step beyond data in the scientific method is supposed to be the formulation of a type of law defined as generalization from data. That the evolutionary succession is a valid generalization was recognized by some, but not generally accepted until Charles Darwin provided a mechanism for how it can happen. That is the second kind of law, theoretical explanation, and is supposed to arise out of already accepted generalizations.

In biological development, there is now quite a different situation. There is a plenitude of both data and theory; but they seem to be looking at each other from opposite sides of an apparently unbridgeable gulf. This is not at all what was expected by D'Arcy Thompson (1917, 1942, 1961). His title, *On Growth and Form*, clearly implied a preconception that form was a manifestation of the processes of growth, and that this would soon (after 1917) become a major direction for the advance of biology. Part of his basis for this was that he thought the possible limits of microscopy to have been almost reached. (The electron is to blame for many aspects of our lives today.) In the event, twentieth-century biology became in great part the quest for nanoanatomy, if I may be excused for using such a word, in the forms both of electron microscopy and of the molecular structure of the genome. The spectacular advances in this field have quite overwhelmed what could have been (and I hope some day will become) other large fields of biology much closer to the spirit of D'Arcy Thompson.

The developmental biology I discuss in this book can be categorized in a sub-discipline often called 'pattern formation'. But I do not distinguish between pattern and form, nor in any clearly defined way between pattern formation, morphogenesis, epigenesis and indeed most of biological development. All are concerned with essentially the same thing: the unfolding in space of the organization of the organism, something that is not in the first instance spatial, nor intrinsically structural. The essence of living organization is that a multitude of processes start running in sequences and in relationships to each other that permit their mutual support and minimize mutual destructive interactions with each other. But as part of the strategy of both necessary separations and proper couplings of these processes, living organisms develop significant shapes that announce pictorially the existence of this organization of continuing events. Spatial compartmenting is one of the most powerful methods used by organisms to convert a complexity of processes into a set of mutually interacting but semi-independent simplicities. Each of these simplicities is a pattern; and their mutual organization on many levels is a multitude of patterns.

Here, I am using the word 'pattern' to refer primarily to an abstraction in our minds, a pattern of interaction of processes. We may convert this into a picture that Nature has not drawn in the organism – for instance, a diagram of biochemical cycles. But Nature has gone beyond this, using the biochemistry as an artist to paint a self-portrait, the shape of the organism and its parts, which we can often appreciate statically as a pattern in the pictorial sense.

Is this, the obvious appearance of the biological world that everyone knows, perhaps more to be thought of as the abstraction, where the hidden but essential and down-to-earth reality is actually the set of processes generating the shapes? Of course, living shapes are most easily seen in very practical terms as enabling the organisms to run, swim, eat, use sunlight, extract nutrients from the soil and so forth. But all of these functions are ultimately ways that the organism goes about maintaining the organization of its processes of life. Spatial, pictorial pattern is a showing forth of the existence of these processes. The German word corresponding to pattern, 'muster', is derived from the Latin 'monstrare', to show.

But the English word is derived from the Latin 'pater', father, and the meaning of 'pattern' up to the sixteenth century was rather similar to 'patron'. This raises the question: when organization is evident, where may we find the Organizer? Formulations of this question go back at least to Aristotle, who wrote in his *Generation of Animals*:

> It would appear unreasonable to suppose that anything external fashions all the individual parts, the viscera or any others, because unless it is in contact it cannot set up any movement, and unless it sets up a movement no effect can be produced by it. Therefore there is already present in the foetus itself that which is either an integral part of it or separate from it. To suppose it is some other thing, and separate from it, is not reasonable. If it were, the question arises: when the animal's generation is complete, does this something disappear, or does it remain within the animal? We cannot detect any such thing, which is in the plant or animal and yet is no part of the organism. (Translation slightly modified by Harrison and Holloway from that of Peck 1942)

This quotation is from a few pages of Aristotle's work that are commonly referred to as indicating a belief in epigenesis (*ab initio* generation of its shape by each new organism) in contrast to preformation (pre-existence of the shape in miniature, e.g. the idea of a 'homunculus' within the human sperm). Aristotle did not use the word epigenesis, which was a nineteenth-century invention of von Baer. But the question in the quotation is very clear, and is the earliest statement I am acquainted with on the problem of self-organization. How can the organizer and the organized be identical? Twenty-three centuries after Aristotle, this is still being asked. For instance, in a review of wing and leg morphogenesis in the fruit fly *Drosophila*, Serrano and O'Farrell (1997) restate essentially the same question in the words: 'One of the most mysterious features of morphogenesis is that structures form themselves as they grow ... How are the actions of morphogens coordinated with the process of growth?'

Both the ancient and the modern formulation of the question contain, however, what I believe to be the germ of the answer. Aristotle's references to 'setting up a movement', and Serrano and O'Farrell's use of the words 'form', 'grow', 'actions' and 'process' direct our attention towards dynamics as the field of work that should lead to the desired explanations. My purposes in writing this book are two-fold: to indicate some of the diversity of developmental phenomena; and to describe those advances in dynamic theories of pattern formation, mainly from 1952 onwards, which I believe to be the first few steps along the road towards discovering unifying principles underlying the experimental diversity.

This book may be read for general interest. But I hope also to encourage more scientists of all backgrounds to take active part in the quest for these unifying principles. To those with physical and chemical backgrounds who are accustomed to studies on clean samples containing no more than a handful of different substances participating in one or two reactions (or none at all), I suggest that they should not be afraid of systems containing hundreds of thousands of chemicals. If such a system is alive and in good health, it is not dirty but indeed almost the ultimate in cleanliness. The reacting systems are so organized that they not only avoid interfering with each other but actively assist each other and defend each other against contamination. To biologists who are accustomed to meticulous description of detail in diverse organisms, I suggest that they should not be so completely preoccupied with getting the details right as to neglect the presence of new unities to be found. Much of this search involves highly selective use of items from the available genetic and biochemical knowledge pool, rather than any attempt to put all of it together into a single computer program.

1.2 BREAKING OUT

For anyone setting out upon this quest, I present the painting on the cover as an appropriate heraldic blazon. The original hangs in a hall of the University of British Columbia library. It is *Emergent Image* by the late Jack Shadbolt, and was described by the artist as representing a butterfly breaking out of the cocoon. The picture therefore has the primary characteristic that must be shown in any good description of development: it moves. Many pictures of biological pattern, whether made photographically or by an artist, are derived from the study of dead material fixed for microscopy, and so convey to the viewer the cessation of movement that is death or fixation. But this picture is of pattern formation. To me, it shows much more than the emergence of a preformed structure from an external restraint. It strongly evokes the emergence of an adult organism from the whole sequence of development within the earlier stages of the organism.

Clearly, the motion in the picture is from left to right and involves increasing size. But the starting motif on the left, a set of wiggly lines, is not a molecular microstructure such as the DNA in an egg. Rather, it remarkably suggests a type of elementary spatial pattern that has attracted attention in biology, chemistry and theoretical biophysics, all at dates later than Shadbolt's painting. That is, a set of parallel stripes, one of the simple motifs for patterning in 2-D. Shadbolt shows wavy stripes. That waviness indeed turns up in computations from stripe-forming dynamic models (Lyons and Harrison 1992a; cover picture of Harrison 1993), in non-living chemical pattern-forming systems such as the CIMA (chlorite–iodide–malonate) reaction (Ouyang and Swinney 1991), and in at least one living pattern that shows clear indications of dynamic generation (Kondo and Asai 1995; this book, Fig. 9.8).

The motif is repeated in the striking pattern of white and dark stripes in an elongated oval. This strongly suggests the first action in an insect egg that gives rise to the body segmentation characteristic of the Insecta (Chapter 8). Nature does its painting of the earliest embryological events with particular RNAs and proteins. But it is a recurrent theme of this book that Nature's palette is huge, and that the same kinds of painting processes can be done with very diverse materials. Striped patterns can appear from the earliest signs of segmentation in an embryo, to postnatal organization of the visual system in a mammalian brain (Fig. 9.7), to the coat pattern of a zebra. Examples are multitudinous; but are the modes of formation of any one category of patterns equally numerous, or are there just a handful of them, or is there just one, as Newton's laws of motion apply to everything? My inclination is to the view that the answer will finally be 'a handful', but that to seek them it is going to be most useful to have the unifying idea in mind.

And then Shadbolt's picture explodes to the right. This clearly indicates breaking out of the cocoon, but I would add 'amongst other things'. There is an ambiguity in the middle space. The fragments from the left seem not necessarily to be falling into discard, but just as probably to be moving to the right, to build the butterfly. Strength of movement is well shown, but very little of detail in organizing the organism. This book is about how to start trying to fill in that great gap in our knowledge.

The artist can paint pictures that tell a story of movement. But much of the information to which a biologist must devote extensive attention is from dead and fixed material and quite lacks that artistry. In my previous book (Harrison 1993, Chap. 1) I mentioned the story of the conductor Franz Lachner, who complained of the score of Wagner's *The Flying Dutchman* about 'the wind that blows out at you wherever you open the score'.

I pointed out that only an expert musician can feel this wind when looking silently at the printed score, and suggested that biologists need to acquire a similar expertise in seeing motion in electron micrographs. Subsequently, I have become involved in a project on the possible mechanism of some rather spectacular pattern formation that happens during chromatin condensation in sperm of some species. This is described in Section 7.2.2 of this book. It has given me direct experience in trying to ferret out time-sequence information from electron micrographs, in which each one was necessarily made from a different killed specimen. The experience has only reinforced my previously expressed view.

But it has become commonplace to have continually moving pictures in any ordinary room. The natural habitat of these organisms is the computer screen, and they are classified as screen-savers. I can't stand them. When I am not using my computer, it sits there with a screen as uniformly dark grey as volcanic ash before life has moved back on to it. This is because I find screen-savers immensely distracting. I want to watch them and work out what is going on. At one time, I had a screen-saver of tropical fish (of diverse hitherto unknown species, I think) wandering across the screen, sometimes reversing direction, disappearing at all four boundaries and sometimes reappearing at the same point or on the opposite side. This show was rapidly destroying my scientific endeavours; I had quite a lot of statistics compiled on the movements of these fish, and was approaching questions of whether each one had a continuous existence or whether they were created and annihilated, and so forth, when a hard drive crash finally rescued me. The point of this story is that I had no interest in the details of the program that was running this show. All I wanted to know was what things were in the algorithm that the program was written to implement; and I had hope of working this out from observations.

My attitude to developmental phenomena is much the same. If an organism has anything close to an equivalent of a computer program, it is the genome, a mass of detailed statements in which it is quite usual for a single trivial error to have catastrophic consequences. The programs that people in my research group (especially Stephan Wehner and David Holloway) have written for computations of simultaneous three-dimensional chemical patterning and growth for branching processes in plants comprise about 20 000 lines in the C programming language. A single error in one line can have consequences as bad as those of a mutation in a genome.

What is the way to go about studying how the developmental event happens: to go for the genome by studying mutations, which is like trying to write the computer program from the effects of diverse errors made without the observer knowing just what they are? (Great advances in genetics have been made this way, before there was any way of making known chemical changes in the genome.) Or, to study larger-scale features of the event and its unfolding in time, and to try therefrom to write the algorithm, a much briefer thing than the program? My answer to this question is that both must be done. It is best to start building a bridge from both ends.

1.3 KRESPEL

The early nineteenth-century stories of E. T. A. Hoffmann are best known from the use made of three of them in Offenbach's opera. One of these concerns a Councillor Krespel and his daughter Antonia. In the original story (translation, Kent and Knight 1969), much more is told of the eccentricities of the father. To construct a new house, he had the masons build, on a square foundation, four walls with no apertures. He had them halt when they had reached a particular height, and then specified, one by one, the places for material to be knocked out for windows and doors, and the exact size of each of these. There was never any architect's plan. 'Everything had to be done on the spot, according to the orders of the moment. In a short time a completely finished house was standing, presenting a most unusual appearance from the outside – no two windows being alike, and so on – but whose internal arrangements aroused a very special feeling of ease.'

This episode can surely be read as a good metaphor for one of the ways that living things go about constructing themselves, in contrast to the conventional architectural method of following a plan by leaving all the necessary gaps in the structure as it is built from the ground up. An organism is completely bounded from the start of development, and the processes continually take note of these boundaries, as Krespel kept on looking at the walls to see what he would do next. Because of this kind of interaction with what is being built, Krespel, though he is looking at the house from outside, is metaphorically close to that Organizer that is part of what is being organized and yet cannot be part of it, the concept that so puzzled Aristotle.

By writing, above, 'one of the ways', I imply that there is a restriction on the generality of the metaphor. This restriction is essentially to developing organisms that do not yet have parts irrevocably committed to specific fates, but are still completely capable of 'regulation' (in the biologist's sense of that word). In the animal kingdom, amniote embryos have this capacity when they have, e.g. as many as 20 000–60 000 cells in the chick embryo (Streit *et al*. 2000). In the plant kingdom, development continues throughout life. The building of a flower, comprising many events of pattern formation, morphogenesis and differentiation of cells and organs, starts only at the culmination of the life cycle, when a meristematic region becomes specified as floral, and Krespel arrives to take charge.

But a major inadequacy of Hoffmann's metaphor is that the boundaries of the house do not expand. All of Krespel's eccentricity could not enable him to start with the walls of a bungalow and end up with a cathedral. Likewise, the greater part of the work on dynamics of biological pattern formation to date has been done for regions of fixed size, shape and boundary conditions. This restriction is stated explicitly, for instance, in Meinhardt (1982) and Nicolis and Prigogine (1977). And it is evident from the contents of those books that an enormous amount of work highly relevant to how biological development happens can be done without stepping outside that restriction. Indeed, many pattern-forming events take place in embryos that cannot feed until those events have happened, and cannot grow until they can feed.

There are other instances in which one cannot avoid the fact that the four walls of Krespel's house should be continuously growing as he chooses places for windows and doors. These include nearly all the events of plant development, and much of later animal development, such as the formation of limbs, multilobed glands, lungs, etc. For these, the question of coordination of pattern formation and growth must be addressed. In what follows, I try to emulate Krespel's level of eccentricity in two ways. First, I deal with this more complex kind of development before treating patterning in regions of fixed size and shape. Here, the method in my madness is that I want to raise questions as much as to provide answers, and phenomena of simultaneous patterning and growth are best to draw out a good map of question-marks.

Second, I concentrate first on plant development, recommending it as a highroad into most of the territory on that map. Though the biology of our own kingdom is inevitably a main preoccupation of the human species, many generalities of biology have been discovered first in plants. Therein, the cell was first seen by Robert Hooke, long before it was recognized as a component of all life. The nucleus also was seen first in plants; and where would our knowledge of genetics have reached today without the old plant-breeder, Gregor Mendel?

But back to Krespel. When he asked for things to be done in what seemed to be a strange order, he was nevertheless meticulous for each new task in specifying precise dimensions, stopping wall-building when it had reached the height of a two-storey house, and giving exact sizes for each door and window. This is a common feature of biological development. The components of living action, chiefly chemical reactions, transport processes and physical forces, are capable of measuring distances without the intervention of human intelligences and their elaboration of measuring methods. When I get to my favourite topic of chemical-kinetic mechanisms, I show that this property is capable of quite straightforward explanation. (This was treated with somewhat more mathematics in my earlier book, Harrison 1993). Again, the main point is: does any postulated mechanism possess this property, when the biological events being considered clearly have it?

In relation, however, to the general strategy of explaining how development controls itself, the significant feature of Krespel as a metaphor for the self-organizer is that he made his judgements of size and position in relation to a larger structure already grown. That aspect of development is a major theme of this book. A pattern is an entity occupying all or part of the space of an existing entity. Where, then, do we find the metaphor for the much more popular approach of tracing developmental events from the spatially tiny store of information in the genome? That, surely, is within Krespel himself. His eccentricity of method and piecemeal decision-making deny the existence of a preformed architectural plan. Likewise, the genome holds the potential for production of substances without a preformed plan of where they are to go. But a seemingly planned large-scale structure takes form. The dynamics of the planning are within the organism, and their reality no less than that of the structures to which they lead.