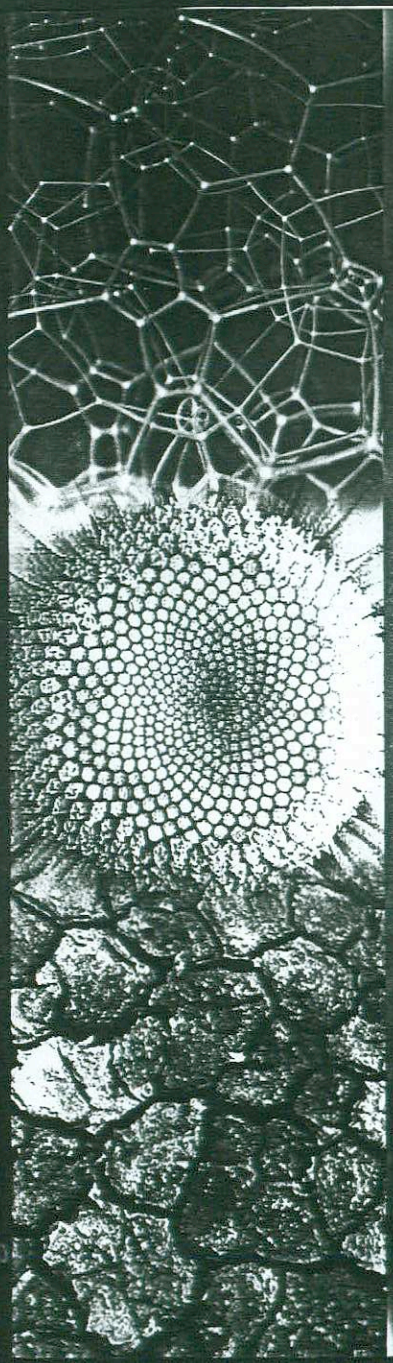
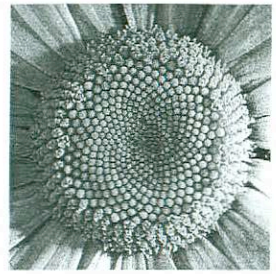


THE SELF-MADE TAPESTRY

Pattern formation in nature

Philip Ball

BODIES



... and after another long time, what with standing half in the shade and half out of it, and what with the slippery-slidy shadows of the trees falling on them, the Giraffe grew blotchy, and the Zebra grew stripy, and the Eland and the Koodoo grew darker, with little wavy grey lines on their backs like bark on a tree trunk; and so, though you could hear them and smell them, you could very seldom see them, and then only when you knew precisely where to look.

Rudyard Kipling
The Just So Stories

When Rudyard Kipling explained how the animals of Africa acquired their markings, he was tapping into a universal mythology. The Native Americans, for instance, have their own tales of how the skunk became endowed with its two-tone tail. When people see patterns in nature as striking as these, they want some means of explaining them.

Darwin's theory of evolution gave us an answer for the modern age, and it was not so different in essence from Kipling's: the patterns enhance the animal's chances of survival. In the tree-dappled light of a tropical forest, a spotted leopard can merge with the surroundings, giving it a better chance of sneaking up on its prey. A striped zebra is better hidden amongst the vertical striations of the long grass and bushes on the veldt (Fig. 4.1), and an insect patterned to resemble a flower is at less risk from predators. In addition, patterns help animals to recognize other members of their species, an obvious requirement if the species is to propagate.

But as I explained in the introduction, this kind of explanation, while correct, is incomplete and ultimately rather unsatisfying. I shouldn't be surprised if some of you prefer the 'just so' explanation, which at least has something to say about how the stripes and spots *got there*. (The leopard's spots, you may recall, are the fingerprints of a solicitous Ethiopian.) A Darwinian explanation says nothing about this—it merely suggests that, once there, these patterns will stay because the animal is better off with them. Can it be that evolution

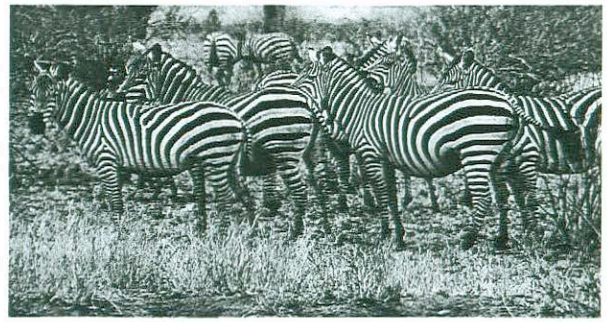


Fig. 4.1 Zebras in Kenya. (Photo: Michael and Sandra Ball.)

found its tortuous way to the striped zebra via a slow, gradual and random accumulation of mutations towards stripe-giving genes? What, then, were the intermediate stages? Were there proto-zebras that exhibited some other kinds of pattern? Darwinism in itself provides little guidance for answering such question—it does not allow us to deduce which patterns are possible and which are not, but simply provides a mechanism that explains why some persist in certain species but not others (differences in habitat, for example). Natural selection does not tell us what is on the palette; it is a tool for retrospective rationalization, and rarely if ever for prediction. Does nature really have an infinite choice of skin patterns, or must it select from just a few? And how do each of those arise?

These are some of the questions that I will look at in this chapter. We shall see that the puzzle of surface markings on organisms is ultimately bound up with the much broader matter of how bodies themselves are shaped, and why they take on the forms that they do. In a sense, similar mechanisms may be at play in both cases.

Yet while surface markings, like those on pelts and shells, are immediately recognizable as patterns, we might imagine that the *shapes* of bodies are functional rather than representational. Specific features would seem to be dictated by specific functions, they are tools rather than flags or camouflage. Surely we have eyes and hands not because these are elements of a body's 'pattern' as such but because we need them to see and to manipulate our environment? To put this another way: a particular stripe of a zebra's skin marking is merely a consistent part of the pattern, whereas (setting aside the bilateral symmetry of the body) a particular limb, like our left arm, doesn't obviously 'follow' from the form of the rest of our bodies—it seems to be a specialized element, not a generic one. We will see, however, that it can make a kind of sense to regard our body plans as biological patterns, albeit very complex and refined ones that derive from the sequential and hierarchical subpatterning of simpler patterns. That this is so perhaps becomes more apparent by considering the forms of plants, which tend to have a higher degree of symmetry than mammals. A plant too has limbs, but these are much more obviously arranged in a regular, somewhat symmetrical manner. And it isn't so hard to identify animals to which the same applies—a starfish, for instance. There is a fertile tension inherent in the question of whether the form of living organisms should be regarded either as a haphazard assembly of components that together make up an evolutionarily viable being, or instead as a highly complex, spontaneously patterned form.

Frozen waves

If I am to be chronological, we must begin this exploration of biological pattern formation with just about the hardest question one could ask of it: how a body plan emerges in a fertilized egg. But the British mathematician Alan Turing, who considered this problem in the early 1950s, was one of the brightest scientific minds this century has seen, and didn't have much fear of hard questions.

Turing's work takes a decidedly tangential angle to much of the mainstream research on biological development, although in recent years the two points of view have shown signs of converging. We know that different tissues and organs in a multicellular body are characterized by differences in the genes that are 'active' in their constituent cells—basically, the cells of (say) the liver make use of some different genes (to manufacture different proteins) from the cells of bone marrow, even though the genetic content of both is the same. The cell types in these organs are said to be *differentiated*, and to show different regimes of gene *expression* (gene-to-protein conversion). Geneticists interested in development commonly seek to identify these differences in gene expression, and to investigate the protein products to see what role the proteins play in the function of the tissues.

This is a reductionist approach that helps us to understand the consequences of cell differentiation. It also shows us where to look for explanations of the origin of that differentiation: in the switching on or off of certain genes. But it is an approach that runs out of steam when we pare the problem of development back to its starting point. For in the beginning, an embryo is just a ball of identical cells, each with the same genetic constitution. How do the initial differences in gene expression arise from this uniform ball?

What we are faced with here is a question of symmetry breaking: somehow the (roughly) spherical symmetry of the multicellular embryo gets broken such that different parts of it follow different developmental pathways to become a head, a heart, a toe. What breaks the symmetry of the embryo? This is the fundamental question of morphogenesis, the study of the development of biological form.

At the beginning of the 1950s, symmetry breaking was not a new idea in physics, but no one had given much thought to how it might be relevant in chemistry or biology. At that time, Alan Turing was working on mathematical problems associated with computer theory at the University of Manchester. His work in this field was to become seminal, and underpins much of the present-day research into artificial intelligence. During the Second World War Turing was set to work as a code-breaker, and some of the techniques that he developed for unravelling German naval messages are still classified today. For his contributions in this area Turing was held in high esteem by the British intelligence organization, but his knowledge was also regarded as a national secret.

Turing's ultimate dream was to make a thinking machine, an artificial brain. His interest in brain structure and development led him to ponder on broader questions of biological development, and ultimately to the issue of morphogenesis. In 1952 he published a paper describing a hypothetical chemical reaction that could generate spontaneous symmetry breaking, leading to stable spatial patterns, in an initially uniform mixture of chemical compounds. This, he suggested, might provide a model for how patterning takes place in an initially spherical fertilized egg. Entitled 'The chemical basis of morphogenesis', this paper is undoubtedly one of the most influential in the whole of theoretical biology.

One of the remarkable things about Turing's idea was that he proposed that diffusion of the chemical species (called morphogens) through the medium in which they were dispersed could be the driving force for symmetry breaking. This goes against intuition: normally diffusion is seen as a mechanism for producing uniformity, for smoothing out inhomogeneities in a system. It was almost as if he was suggesting that diffusion could cause thoroughly dispersed ink in water to condense into concentrated ink droplets, rather than the reverse.

But in Turing's chemical system, diffusion is acting in competition with another process, namely an autocatalytic chemical reaction. It is, in other words, an example of a reaction–diffusion system. We saw in the last chapter how these systems can generate nonstationary patterns, such as the travelling target and spiral waves of the Belousov–Zhabotinsky (BZ) reaction, when the system possesses the property of excitability. Turing showed that under certain conditions, *stationary* patterns can also arise in excitable reaction–diffusion systems. These generally take the form of spots or stripes of differing chemical concentrations.

Turing considered a process in which some chemical compound, say A, undergoes an autocatalytic reaction to generate more of itself: the rate at which A is generated depends on the amount of A already present. But within his scheme, A also activates in some way the formation of a compound B that inhibits the formation of more A. The key element for obtaining spatial patterns is that A and B diffuse through the reaction medium at different rates, so the effective ranges of their respective influences are different. This means that the A's and B's can dominate in distinct regions.

When Turing formulated this scheme, he had to rely on mathematics that could be performed with a pencil and paper, and so he chose to represent the chemical processes using the simplest mathematical equations possible. This forced upon him some compromises to get around the rather artificial results that these simple equations could sometimes generate. In particular, Turing had to make his equations *linear*, which implies that effects are proportional to their causes. Linear equations are easier to solve than non-linear ones, but in Turing's case it meant that the solutions—the distributions of his hypothetical chemical species—were unstable against perturbations. Although he was able to show that the scheme was capable of generating spatial patterns, Turing clearly felt hindered by the intractability of a more sophisticated analysis. He suggested that, while the difficulties were probably too great to allow for any all-embracing theory of pattern formation in these schemes, perhaps a 'digital computer' would enable one to investigate a few particular cases more accurately.

But it was only after his landmark paper was published that Turing seems to have begun to perceive the real key to his patterning mechanism, which is that it represents a competition between *activation* by compound A and *inhibition* by compound B. Moreover, the inhibitor B must diffuse more rapidly than A for patterning to occur. Thus, while activation and autocatalytic production of A is a localized process, inhibition of A by B is *long-ranged*, because once formed in the vicinity of A, B can rapidly diffuse away to inhibit the formation of A elsewhere. At the same time, this rapid diffusion of B ensures that it does not inhibit the local formation of A—it is removed from the vicinity too quickly (Fig. 4.2). The whole scheme represents a subset of reaction–diffusion processes called activator–inhibitor systems.

What happens, then, is that once random fluctuations in the initial concentration of A trigger spots of enhanced A production through autocatalysis, the inhibitor B is produced and rapidly diffuses away to

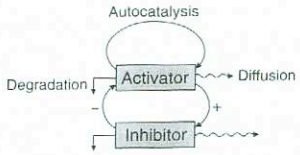
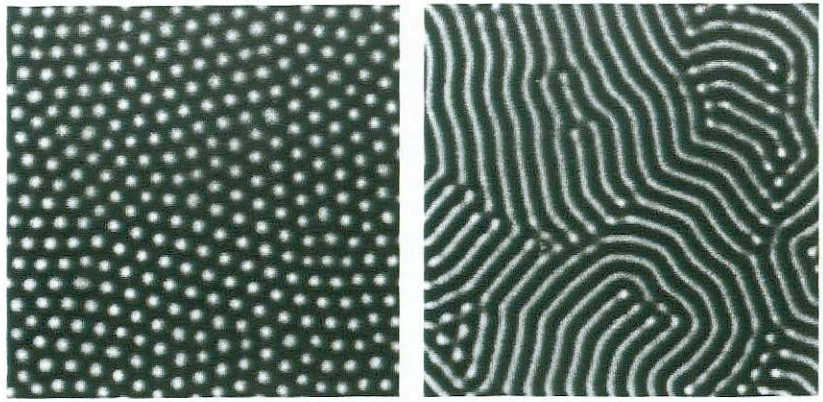


Fig. 4.2 How an activator–inhibitor scheme works. The activator generates more of itself by autocatalysis, and also activates the inhibitor. The inhibitor disrupts the autocatalytic formation of the activator. Meanwhile, the two substances diffuse through the system at different rates, with the inhibitor migrating faster.

suppress formation of the activator in the immediate surroundings. So an array of isolated spots of A is created, surrounded by regions rich in B in which formation of A is suppressed (Fig. 4.3*a*). If there are processes that remove the end products of the reaction at a steady rate and supply fresh sources of the reactants needed to make A and B, this pattern of spots can remain stable indefinitely. Alternatively, the regions of A production can merge into stripes that trace out a maze-like network (Fig. 4.3*b*).

Turing himself never used the terms 'activator' and 'inhibitor' however; instead, he regarded compound B as a rapidly diffusing 'poison'. It was not until 1972 that Hans Meinhardt, then at the Max Planck Institute for Virus Research in Tübingen, Germany, and his colleague Alfred Gierer had the insight that short-ranged activation and long-ranged inhibition are the principal elements of Turing's patterns. With the benefit of computers to do the number-crunching, Meinhardt and Gierer were able to formulate Turing's mechanism using more complicated—and more physically motivated—non-linear equations, and to show how they could be plausibly related to the kinds of processes known to take place during real biological patterning and development.

Because of their stationary nature, it is tempting to regard Turing patterns as a kind of end product of the reaction, as if they were spatial differences in composition that have become 'frozen in' to the reaction mixture just as bubbles become frozen into ice. This is not so, however. The patterns are non-equilibrium patterns, and are *dynamic* in the sense that they are sustained by constant motion and reaction of the chemical compounds in the mixture. Similarly, the oscillation inside an organ pipe excites a fixed pattern of varying air density even though the molecules in the air continue to move around. The point is that Turing's patterns are maintained—the symmetry is broken—only so long as the system is driven *away* form equilibrium. They arise



a *b*

Fig. 4.3 The activator–inhibitor scheme can generate disordered patterns of spots and stripes. The composition of the system is different in the light and dark regions. (Images: J. Boissonade, University of Bordeaux.)

spontaneously from a homogeneous medium in a symmetry-breaking instability as the driving force away from equilibrium is increased.

By curious coincidence, Turing formulated his ideas on pattern formation in chemical systems at just the same time that Boris Belousov in the Soviet Union was discovering the peculiar oscillatory behaviour that some chemical reactions can exhibit. It was in 1951 that Belousov first observed the colour-changing properties of a chemical cocktail that was later to be refined into the BZ mixture. As we saw in the previous chapter, Belousov received scant reward for this discovery during his lifetime; but for his own contribution to chemical pattern formation, Turing was to see even less recompense. In the same year that he published his paper on pattern formation, he was arrested for sexual offences when an investigation into a burglary at his home led to the disclosure that he was homosexual. This supposed crime was enough for Turing to be compelled to take 'corrective' hormone treatment, and to be regarded as a security risk, placing restrictions on his freedom to travel. In 1954 the disgrace and constraints deriving from these charges led the 42-year-old Turing to commit suicide by ingesting cyanide.

Making striped paint

For almost twenty years after Turing published his paper, nothing happened. No new field of chemical pattern formation was born. Biologists studying morphogenesis took no heed. Yet today Turing's ideas are hailed as seminal. Why the delay?

For one thing, Alan Turing was years ahead of his time. The buzzwords that encapsulate the behaviour in his theoretical reaction–diffusion system—complexity, pattern formation, symmetry breaking, non-equilibrium systems, non-linearity—were either unheard of or regarded as rather specialized and obscure branches of science in the 1950s. It is only when the climate is right, when a sea change has taken place, that a genuinely new scientific idea can find wide acceptance. In the 1950s, almost all of chemistry was concerned with equilibrium processes, and most of mathematical science looked at non-linear problems with horror.

But there was also the matter of whether Turing's theoretical ideas had any relevance to the real world. He had to make a number of approximations to make his equations manageable, leaving open the question of whether real chemical systems would indeed show this kind of behaviour. Creating Turing patterns in a real reaction proved to be a tremendous challenge, and for a time it began to look as if they might be merely mathematical phantoms. As for the relevance to morphogenesis—that is still an open question, although as we shall see, there are compelling reasons to believe that some of the exquisite surface markings of animals are Turing patterns or something very much like them.

When in the late 1960s and early 1970s chemists began to learn about the BZ reaction, the connection between this and Turing's reaction–diffusion scheme began to emerge. The connection was made more concrete in 1971 when Zhabotinsky (in Puschino, USSR) and Arthur Winfree (then at the University of Chicago) independently observed spiral waves in the BZ reaction. Winfree subsequently showed that the spiral is the result of an activator–inhibitor pattern-formation process in the poorly mixed BZ mixture. But although the BZ patterns are comparable to Turing structures insofar as they are both the result of non-linearities, autocatalysis and feedbacks in the chemical reactions that produce them, the mechanism that gives rise to BZ chemical waves is *not* the same as the instability that leads to Turing structures. For one thing, the BZ patterns are *travelling* waves, whereas Turing's patterns are stationary. In other words, the combination of localized activation and long-ranged inhibition is not by itself sufficient to guarantee stationary Turing patterns. They will be produced only if the response of the inhibitor to changes in the activator concentration is rapid (which means, in effect, that the processes that remove the inhibitor must be fast relative to those that remove the activator). If, on the other hand, the inhibitor sticks around for a long time, the system has a tendency to undergo BZ-like oscillations—and such oscillations can occur even if there is no difference between the rates of diffusion of the various chemical species, whereas such a difference is essential to Turing's mechanism. Travelling waves are generated, meanwhile, if the inhibitor diffuses less rapidly than the activator. And these waves need to be initiated by some local disturbance to the medium, rather than arising from a spontaneous, 'global' symmetry-breaking instability. From the perspective of morphogenesis these differences are critical, because Turing was looking to explain how stable, fixed structures can arise spontaneously in embryos.

In 1968 Ilya Prigogine and René Lefever from the University of Brussels, stimulated by the Prague conference at which the BZ reaction was given its first public airing to Western scientists, proposed a hypothetical scheme for an oscillatory reaction. This model, later

Box 4.1: The Brusselator

There are four steps in the Brusselator, which involve interconversions of molecules A, B, C, D, X and Y. A and B are the reactants, C and D the products, and X and Y are intermediates in this transformation:

$$A \rightarrow X \quad (4.1)$$

$$B + X \rightarrow C + Y \quad (4.2)$$

$$2X + Y \rightarrow 3X \quad (4.3)$$

$$X \rightarrow D. \quad (4.4)$$

Notice that equation 4.3 is autocatalytic, since two molecules of X give rise to three. Less obviously, it is also susceptible to inhibition, since the compound Y is needed for the reaction to occur but is consumed in the process. In other words, the fact that the reaction generates more X does not necessarily result in exponential growth because this X cannot itself undergo equation 4.3 if there is no Y left in the vicinity. Thus the rate at which Y diffuses to 'feed' equation 4.3 is crucial to the whole process, and this has the same significance as the diffusion of the inhibitor species in Turing's scheme.

called the Brusselator (see Box 4.1), possesses strong similarities to the Oregonator developed in 1974 by chemists at Oregon State University to account for the BZ reaction (page 55). Because it includes autocatalytic feedback, the Brusselator shows oscillations and bifurcations like those of the BZ reaction. But when it proceeds in an incompletely mixed system in which the various chemical species diffuse through the medium at markedly different rates, instabilities can occur that give rise to spatial patterns—variations in composition from place to place—which take the form of stationary Turing-type stripes and spots.

The hypothetical Brusselator scheme made Turing's ideas a little more concrete, and showed that oscillatory reactions similar to the BZ reaction (though not the BZ reaction itself!) might be able to produce Turing patterns under the right conditions. But it was not until 1990 that this was demonstrated experimentally. Patrick De Kepper and co-workers at the University of Bordeaux carried out an oscillatory chemical reaction involving chlorite and iodide ions and malonic acid in a thin layer of gel that was continuously fed from opposite directions with fresh reagents. This reaction, called the CIMA reaction, was developed by De Kepper and colleagues in the early 1980s as an alternative to the BZ reaction. Its oscillatory and pattern-forming behaviour can be made apparent by adding a colour-change indicator, starch—this changes from yellow to blue when it captures and binds the tri-iodide ions (I_3^-) involved in the reaction. Although in many ways similar to the BZ reaction, the CIMA reaction is closer to Turing's scheme because it has an explicit activator and inhibitor—the iodide and chlorite ions, respectively.

To turn an activator–inhibitor system into one capable of forming Turing structures, the two species must be made to diffuse through the reaction medium at very different rates. This is hard to arrange, and explains why it took so long to find a suitable experimental system: in water, just about all small molecules and ions diffuse at more or less the same rate. But the Bordeaux researchers were able to introduce very different rates of diffusion in the CIMA reaction by conducting it in a polymer gel. The molecules of the starch indicator are themselves large polymers, and so they get entangled in the network of the gel with their captive tri-iodide ions. The chlorite ions (the inhibitor species) pass through the gel network unheeded; but the iodide ions (the activator species) are slowed down considerably, because they can keep getting stuck to the immobile starch/tri-iodide groups.

The researchers saw a colour change from blue to yellow along a strip where the various reagents meet and react. Under the right conditions this band broke up into rows of dots (Fig. 4.4). That these dots represented

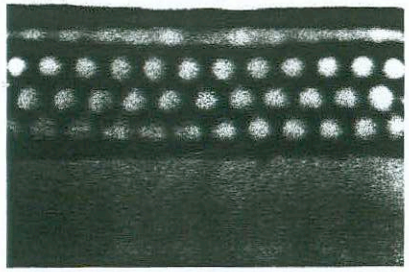


Fig. 4.4 Turing patterns in the CIMA reaction. The dot pattern is restricted to a strip where diffusing reagents meet. (Photo: J. Boissonade, University of Bordeaux.)

a genuine Turing pattern was confirmed by Irving Epstein of Brandeis University and coworkers in 1991, who performed theoretical calculations, based on the known mechanism of the CIMA reaction and the measured differences in diffusion rates of the activator and inhibitor. They showed that they could reproduce the patterns seen experimentally by invoking the Turing mechanism.

The next challenge was to grow Turing patterns over large areas. This was achieved in 1991 by Qi Ouyang and Harry Swinney from the University of Texas at Austin—their two-dimensional lattices of Turing structures contained thousands of the yellow dots (Fig. 4.5). The researchers showed that the pattern disappeared if the gel was warmed above 18°C, and that it reappeared when the gel was cooled. This abrupt and spontaneous patterning in response to a gradual change in conditions is what is expected of a Turing structure. Ouyang and Swinney were also able to demonstrate another of the enticing predictions of the Turing instability: the possibility of forming new stable patterns by changing the reaction conditions. By increasing the iodide concentration or lowering the malonic acid concentration, they broke the symmetry in a new way, forming stripes instead of spots (Plate 4). Their chemical leopard was transformed into a chemical tiger.

You will no doubt have noticed that the patterns in Fig. 4.4 and Plate 4 are ordered, whereas the spots and stripes in the theoretical calculations of a Turing system depicted earlier (Fig. 4.3) are less regular. Both periodic and disordered patterns are possible, depending on the precise parameters of the chemical reactants (such as their diffusion rates) and the nature of the way in which the patterns grow. I shall return to this point later. Notice, however, that in both cases a more or less uniform separation is maintained, on average, between the features (spots or stripes) of the pattern. This separation is set largely by the rate at which the inhibitor diffuses away from the centres of activation.

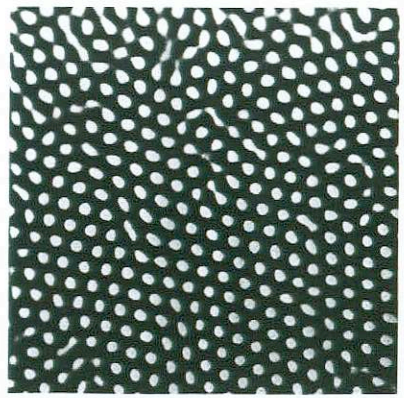


Fig. 4.5 Extended Turing patterns in the CIMA reaction. (Photo: Harry Swinney, University of Texas at Austin.)

Coming alive

Swinney and colleagues, working with John Pearson from the Los Alamos National Laboratory, have found a curious kind of chemical pattern that might be considered a hybrid of the travelling waves of the BZ reaction and the stationary spots of Turing structures. In another type of oscillatory reaction–diffusion system called the ferrocyanide–iodate–sulphite reaction they have observed spots that grow and divide like replicating cells. Pearson first saw these life-like spots in numerical simulations of a reaction–diffusion system on the computer, in which they blossomed when the diffusion rates of the various chemical components were ascribed certain values. Swinney's group then discovered conditions under which the replicating spots would manifest themselves experimentally (Plate 9). The spots do not appear spontaneously; their formation has to be triggered by perturbing the mixture locally, for example, by shining ultraviolet light onto a part of it. The spots grow from a roughly circular shape, elongating into a dumbbell shape as they get bigger until finally they split into two circular spots, which then repeat the sequence (Fig. 4.6). But just as in life, these systems cannot support too abundant a birth rate. If the spots get too overcrowded, they annihilate each other—they 'die'.

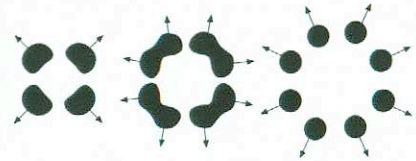


Fig. 4.6 Growth and division of replicating chemical spots. (After: Pearson *et al.* 1993.)

Skin deep

There is now no doubt that Turing's ideas about chemical pattern formation were visionary ones. But do they have anything at all to do with morphogenesis, which was what motivated him to propose his reaction– diffusion model in the first place? At present, this question remains open. Although some biologists are convinced that spontaneous patterning processes akin to, if not identical to, the Turing instability lie at the heart of the most fundamental aspects of embryo development, many regard the whole idea as an as-yet untested hypothesis that at best amounts to a curious sideshow. But one area of developmental biology in which Turing's ideas have made an undeniable impression is in the formation of the surface patterns of the living world: the leopard's spots (Plate 10), the zebra's stripes, the giraffe's blotches. Turing has become biology's answer to Kipling.

The beauty of all this is that the diverse range of pelt patterns and markings can be explained with the *same* basic mechanism. To my mind, this alone is a very good reason to believe that Turing was on the right track. William of Ockham would have reminded us that if we can account not only for the leopard's spots but also for the zebra's stripes and the giraffe's dapples with the same theoretical model, that is surely more satisfactory than having to construct a different model for each. The idea arguably makes sound evolutionary sense too: it economizes on the amount of (genetic) information needed to produce the pattern. The location and size of each of a zebra's stripes does not have to be specified by a personalized, paint-by-numbers genetic plan; all that the genes have to record is the blueprint for making the activator and inhibitor substances at the right stage in development.

The pelt patterns of mammals are mosaics of just a few colours—they are defined by hair colours that are either white, black, brown, or yellow/orange. The origin of these individual colours is well understood. The colour of the hairs that grow from a particular region of the skin is determined by pigment-producing cells called melanocytes that sit in the innermost layer of the skin's epidermis. The pigment, called melanin, is a light-absorbing protein that passes from the melanocyte into the hair. It comes in two forms: eumelanin, which turns hair black or brown, and phaeomelanin, which turns it yellow to orange.

Whether or not melanocytes produce melanin seems to be determined by the presence or absence of certain chemicals in or just below the epidermis. It is not yet known, however, what these chemicals are. During the late 1970s James Murray, a mathematician then working at the University of Oxford, proposed that the distribution of these chemical 'triggers' takes on a characteristic pattern owing to a Turing-like interaction of activator and inhibitor species during the first few weeks of embryogenesis. Thus at a very early stage the embryo acquires a 'pre-pattern' of chemical morphogens, which is later read out by the melanocytes when they respond to the presence or absence of these morphogens by making or failing to make pigments. It is rather like the trick in which a pattern of invisible ink, like lemon juice, is made visible by the heat of a candle flame.

To verify this model, one would at least have to identify the morphogens and their distribution in a pre-pattern in the growing embryo. This has not yet been achieved. But Murray took a different approach: he asked whether, if the basic mechanism were correct, it could produce the kinds of pattern features seen in nature.

The precise spatial pattern produced by a reaction–diffusion system that undergoes a Turing instability depends on a number of factors, such as the relative diffusion rates of the activator and inhibitor species. We saw above that different patterns can be produced by changing the reaction conditions (such as the temperature). Another strong influence on pattern selection is the size and the shape of the region in which the chemical process is occurring. This may seem a little odd—the outcome of most chemical reactions does not depend on whether the reaction is conducted in a narrow test tube or a round-bottomed flask. But the point about Turing patterns is that they are expressions of a wave-like modulation of the concentration of reacting species throughout the system, and like sound waves in an organ pipe, the kinds of wave (the *modes*) that can be supported are dependent on the dimensions of the container in which they are set up.

There is, in fact, a minimum size for which a reaction–diffusion system can generate a spatial pattern at all. The characteristic size of a feature of the pattern—the diameter of a spot, for instance—is determined by the diffusion rate (the 'range', if you like) of the activator. So if the system as a whole is about the same size as this, no pattern is evident—the concentrations are uniform throughout. As the system grows, increasingly complex patterns can be formed as the number of modes that can be supported increases (Fig. 4.7). (Although this analogy with acoustic standing waves is visually appealing, it should not be taken

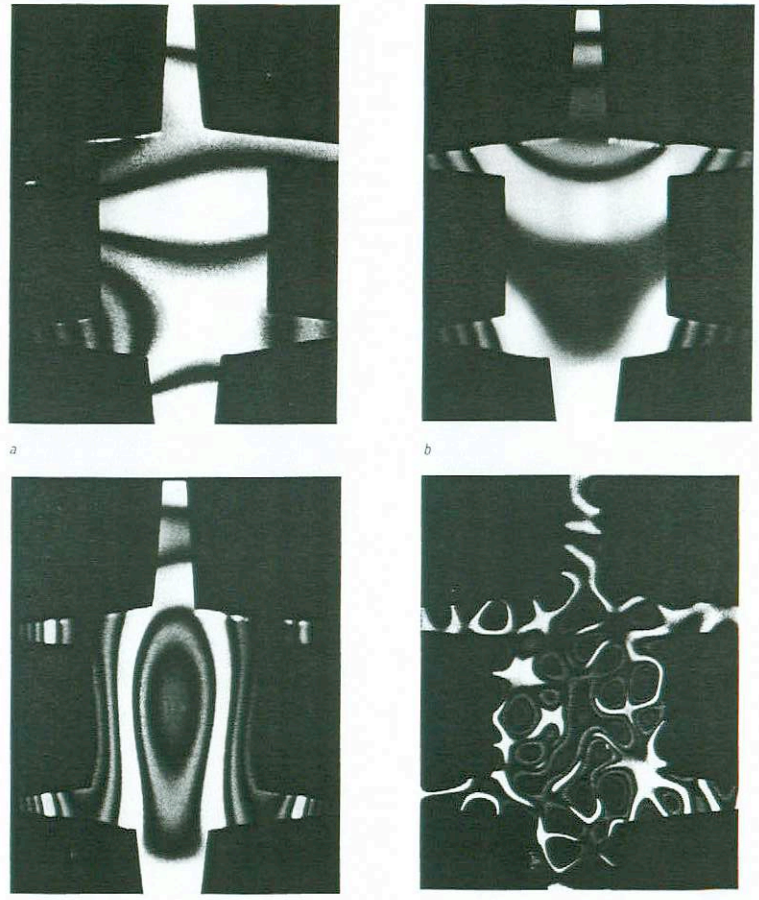


Fig. 4.7 The patterns formed by activator–inhibitor schemes depend on the size of the system: larger systems can support more 'modes', and so exhibit more complex patterns. This is analogous to the complexity of the vibrations excited by sound waves in surfaces of different sizes. Shown here are the acoustic vibrations excited in plates shaped to represent the body surfaces of mammals. The excitation increases in frequency from (*a*) to (*d*), which is equivalent to increasing the size of the plate. (Photos: James Murray, University of Washington, Seattle.)

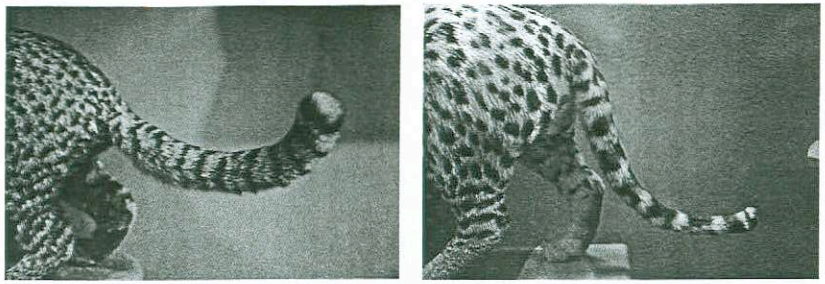


Fig. 4.8 The patterns on animal tails may be either spots or bands, but bands always appear as the tail tapers towards the end—as seen here for a Geoffroy's cat (left) and an ocelot (right).

too literally. Standing waves such as those shown in Fig. 4.7 do *not* arise in the same way as Turing patterns—there is no local activation and long-ranged inhibition involved.)

Murray investigated whether this dependence on size and shape might plausibly account for the differences in pattern seen amongst animal tails. The tail is a good feature to study, since it can be modelled mathematically to a good approximation as a tapering cylinder, a fairly simple shape. Tail patterns come in just two basic varieties: bands running around the circumference, or spots. But just about all patterned tails end in a series of bands (Fig. 4.8).

When Murray performed calculations to see what patterns a reaction–diffusion system would generate on tapered cylinders, he found that both bands and spots could be produced. If the model tail is small, only bands are formed—these are essentially a one-dimensional pattern, since the variation in colour (that is, in pigment-stimulating activator chemicals) occurs only in one direction, along the tail's axis. If the tail is larger, however, more complex modes can be supported, and the patterns become two-dimensional (spots), varying around the circumference of the cylinder as well as along the axis (Fig. 4.9). So a transition from bands to spots may take place along the tail as it widens from the tip, just as is seen in the cheetah and leopard.

Murray found that inter-species differences between tail patterns can also be rationalized in terms of the known embryonic forms of the animals. The tail of the genet, for instance, is always banded along its entire length, whereas that of the leopard is mainly spotted, with bands just at its tip. To judge from the similar shape of the adult tails, there is no obvious reason why this

Fig. 4.9 The patterns produced on tapering cylindrical 'model tails' by an activator–inhibitor scheme depend on their size and shape. Small cylinders support only bands (stripes) (*a*), whereas spots appear on larger cylinders (*b*) as they widen. On a more slowly tapering tail (*c*), the transition from bands to spots is more clear. (After: Murray 1990.)

should be so; but in the respective embryos, the tail of the genet is thin and almost uniform in diameter and so supports only bands, whereas the embryonic leopard tail is fairly short and sharply tapered, and so will allow spots.

If indeed the markings of the adult animal are laid down by a chemical pre-pattern in the very young embryo, the timing of this pre-patterning stage can be crucial, since the size and shape of the embryo changes fast. This fact may be reflected in the differing stripe markings of the zebras *Equus burchelli* and *Equus grevyi*:

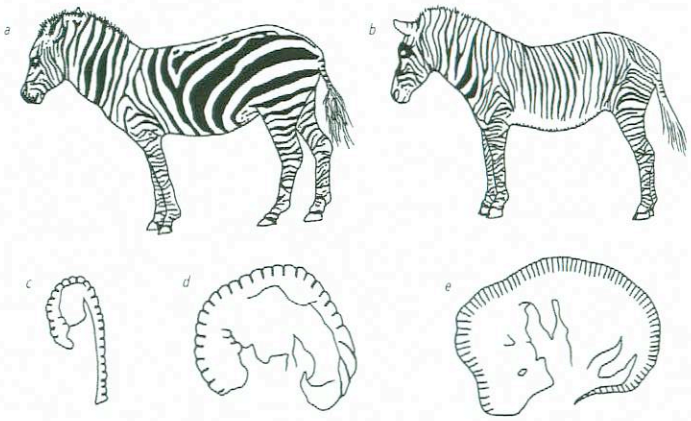


Fig. 4.10 The adult zebra *Equus grevyi* (*b*) has more and narrower stripes than the adult *Equus burchelli* (*a*). This is thought to be because the striped 'pre-pattern' is laid down on the embryo of the latter at an earlier stage: after 21 days for *Equus burchelli* (*c*), but after 5 weeks for *Equus grevyi* (*e*). The smaller embryo supports fewer stripes, and so by the time it is of comparable size (*d*), its stripes are wider. (Drawings by the author, after Murray 1989.)

the stripes of the former are broader and less numerous than those of the latter (Fig. 4.10). There is evidence to suggest that *Equus burchelli* acquires its pattern several weeks earlier in the gestation period than the latter. The same chemical mechanism, producing stripes of the same width, would then give the smaller *Equus burchelli* embryo (Fig. 4.10*c*) fewer stripes than the larger *Equus grevyi* (Fig. 4.10*e*). So when both have grown to a comparable size, the former has broader stripes than the latter.

I should add a cautionary note here: stripes are in fact not all that easy to make in Turing-type models, since they have a tendency to break up into spots. Murray assumed that stripes could survive in his model, but in

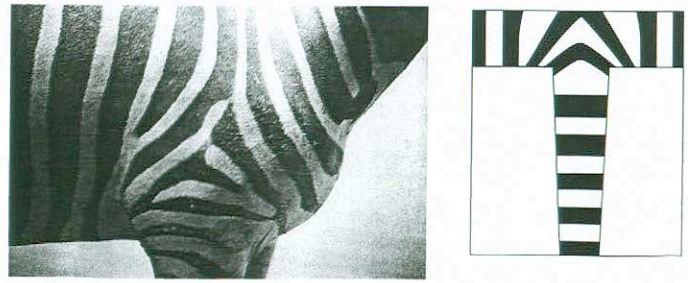


Fig. 4.11 The scapular stripes of a zebra, where the leg meets the body, form a kind of chevron pattern (*a*), which is reproduced in an activator–inhibitor model with this idealized geometry (*b*).

practice extra ingredients are commonly needed to ensure this. For example, stripes may be stabilized if there is an upper limit to the rate of autocatalytic production of the activator, so that this reaction can become 'saturated'.

Murray ventured to look at the patterns that would be generated by reaction–diffusion systems in more complicated geometries, such as the junction of the leg and body of a zebra. Here the same kind of modification of the stripe pattern is seen in all zebras—a kind of chevron pattern in which the bands of the leg blend with the stripes of the body. These markings are called scapular stripes (Fig. 4.11*a*). Murray considered a simplified two-dimensional approximation to the shape of the leg-body junction, and found that a system that generated stripes in the body and bands in the leg would also produce the chevron pattern at their junction (Fig. 4.11*b*).

So within Murray's model, if the chemical parameters in the reaction–diffusion system are much the same for all species (an assumption that is not unreasonable but not firmly supported either), then the size and shape of the embryo at the time of pre-patterning exert a dominant influence on the eventual pattern. One implication of this is that small animals with short gestation periods should have less complex pelt patterns than larger animals, because their smaller embryos support fewer modes. On the whole this seems to be borne out, perhaps most dramatically by the honey badger and the Valais goat, which exemplify the simplest kind of non-uniform colouration of all: an abrupt division into a white and a black half.

But it turns out that the apparent complexity of a pattern diminishes at the other end of the size range too, when the animal becomes very large. This is because, as more and more modes become possible on the patterned embryo, the features start to merge as the dividing lines between them become squeezed out. Thus, for instance, giraffes have very closely spaced spots with narrow light boundaries (Fig. 4.12); and elephants and hippopotami have no markings at all. More, in terms of skin markings, is less.

The giraffe patterns that Murray's model generates are blobs with rounded edges (Fig. 4.13*a*)—simply bloated versions of the leopard spots. But this is arguably not an accurate depiction of the spots on real giraffes, which—as you can see from Fig. 4.12—are more like irregular polygons separated by roughly straight lines of unpigmented hair. Hans Meinhardt, now at the Max Planck Institute for Developmental Biology in Tübingen, Germany, and colleague André Koch have developed a more sophisticated reaction–diffusion model that eliminates this deficiency. Their model incorporates an activator–inhibitor system in which the diffusion constants of the activator and inhibitor do not differ too greatly. Then, as I mentioned

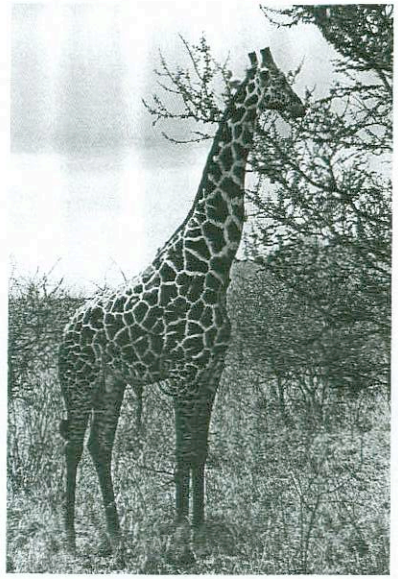


Fig. 4.12 On large animals like the giraffe, the pelt pattern consists of very large features that almost merge, with narrow boundaries between them. (Photo: Michael and Sandra Ball.)

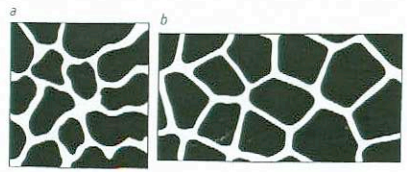


Fig. 4.13 A simple activator–inhibitor model for the giraffe's patterns produces large blotchy features that are only a crude approximation to the real pattern (*a*). A more sophisticated model in which travelling waves of activator and inhibitor throw a biochemical 'switch' to trigger pigment production generates more realistic polygonal shapes (*b*). (Images: (*a*) after Murray 1990; (*b*) after Koch and Meinhardt 1994.)

on page 81, the system does not generate stationary Turing patterns but travelling waves, rather like those of the BZ reaction. These waves become translated into a fixed spatial pattern by the interaction of the reaction–diffusion system with a biochemical switch: when the concentration of activator exceeds a certain threshold at any point in space, a chemical is generated there that stimulates melanocytes into producing melanin. Once this switch is thrown, it stays that way—melanin is produced even if the production of the activator subsequently ceases.

The production of activator is assumed to be initiated at several random points throughout the system. Chemical waves of activator then spread outward from these initial points, triggering melanin production as they go. But where the wavefronts meet, they annihilate each other, just as we see in the BZ reaction (Fig. 3.3). These annihilation fronts define linear boundaries between each domain of activator production, and so the system breaks up into melanin-producing polygonal domains separated by unpigmented boundaries (Fig. 4.13*b*)—a much closer approximation to the pattern seen on real giraffe pelts.

Meinhardt and Koch found that with a little fine tuning of model parameters they could also obtain a better approximation to the leopard's pattern too—these are commonly not mere blobs of pigmented hairs but rings or crescents (Plate 10); their model could generate structures like this (Fig. 4.14). Models of this sort, which involve two interacting chemical systems instead of the single reaction–diffusion system considered by Murray, are clearly able to produce much more complex patterns.

Hard stuff

Anyone who is happy to accept with complacency the view that animal markings are simply determined by Darwinian selective pressures has a surprise in store when they come to consider mollusc shells. The patterns to be seen on these calcified dwellings are of

Fig. 4.14 The leopard's spots are in fact mainly crescent-shaped features. An activator–inhibitor scheme that involves two interacting chemical patterning mechanisms can reproduce these shapes. (After: Koch and Meinhardt 1994.)

exquisite diversity and beauty, and yet frequently they serve no apparent purpose whatsoever. Many molluscs live buried in mud, where their elaborate exterior decoration will be totally obscured. Others cover their shell markings with an opaque coat, as if embarrassed by their virtuosity. And individual members of a single species can be found exhibiting such personalized interpretations of a common theme that you would think they would hardly recognize each other (Fig. 4.15).

Ultimately these patterns are still surely under some degree of genetic control, but they must represent one of the most striking examples of biological pattern for which there are often next to no selective pressures.* While this means that their function remains a mystery, it also means that nature is given free reign: she is, in Hans Meinhardt's words, 'allowed to play'.

It is tempting to regard shell patterns as analogous to the spots and stripes of mammal pelts, and some are indeed apparently laid down similarly in a global, two-

* There is nothing anti-Darwinian in this, however, since Darwin's theory does not insist that all features be adaptive.

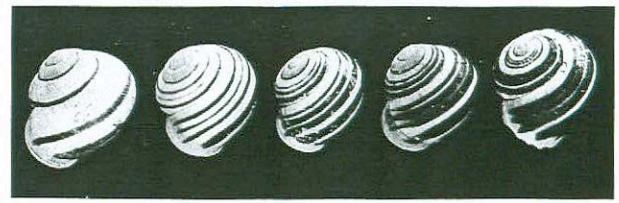


Fig. 4.15 Shell patterns in molluscs can exhibit wide variations even amongst members of the same species. The shells of the garden snails shown here bear stripes of many different widths. (Photo: Hans Meinhardt, Max Planck Institute for Developmental Biology, Tübingen.)

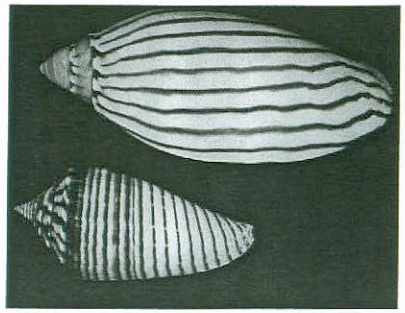


Fig. 4.16 Stripes that run parallel to and perpendicular to the axis of the shell reflect profoundly different patterning mechanisms: in the former case (*top*), the stripes reflect a patterning process that is uniform in space but periodic in time; while the latter case (*bottom*) represents the converse. (Photo: Hans Meinhardt.)

Fig. 4.17 Oblique stripes are the result of travelling waves at the growth edge, periodic in both space and time. (Photo: Hans Meinhardt.)

dimensional surface-patterning process. But most are intriguingly different, in that they represent a historical *record* of a process that takes place continually as the shell grows. For the shell gets bigger by continual accretion of calcified material onto the outer edge, and so the pattern that we see across the surface of the shell is a trace of the pigment distribution along a one-dimensional line at the shell's edge. Thus stripes that run along or around the growth axis (Fig. 4.16), while superficially similar, are in fact frozen time-histories of qualitatively different patterning processes: one in which a spatially periodic pattern along the growing edge remains in place as the shell grows, the other in which bursts of pigmentation occur uniformly along the entire growth edge followed by periods of growth without pigmentation. Stripes that run at an oblique angle to the growth direction, meanwhile, are manifestations of a *travelling* wave of pigmentation that progresses along the edge as the shell grows (Fig. 4.17).

Thus we can see that shell patterns can be the product both of stationary patterns, analogous to Turing patterns, and of travelling waves, analogous to those in the BZ reaction—arising in an essentially one-dimensional system.

Hans Meinhardt has shown that both types of pattern can be reproduced by a model in which an activator–inhibitor process controls the deposition of pigment in the calcifying cells at the shell's growing edge. The stripe patterns in the lower shell of Fig. 4.16, for instance, are a manifestation of a simple, periodic stationary pattern in one dimension (Fig. 4.18*a*), an analogue of the two-

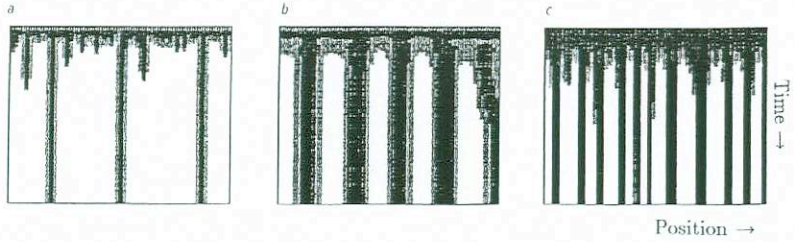


Fig. 4.18 Stripes perpendicular to the growth edge of the shell are the result of one-dimensional spatial patterning at the edge. The pattern gets 'pulled' into stripes as the shell edge advances (*a*). If the activator diffuses more rapidly, the stripes broaden (*b*). When the concentration of the activator rises until it 'saturates' (becomes limited by factors other than long-ranged inhibition), the spacing of the stripes becomes irregular (*c*). (Images: Hans Meinhardt.)

dimensional spot pattern of Fig. 4.2. The width of the stripes and the gaps between them can be acutely sensitive to the model parameters, particularly the relative diffusion rates of activator and inhibitor (Fig. 4.18*b*, *c*). So differences between members of the same species, like those seen in Fig. 4.15, might be the result of differing growth conditions, such as temperature, which alter the diffusion rates. Alternatively, Meinhardt has shown that such intra-species irregularities can arise if the pattern at the shell's growing edge becomes frozen in at an early stage of growth, for example if the communication between cells via diffusing chemical substances ceases.

As the pattern on a shell is a time-trace of the pattern on a growing edge, the full two-dimensional pattern depends on how the edge evolves. For example, the bands in Fig. 4.16 and the spoke-shaped patterns in Fig. 4.19 may be the result of just the same kind of periodic spatial pattern on the growing edge, except that in one case the edge curls around in a spiral and in the other it expands into a cone. When, however, the perimeter length of the edge increases as in Fig. 4.19, the change in dimension may introduce new features into the pattern, just as we saw earlier for the change in scale of patterned mammals. That is to say, as the expansion of the edge separates two adjacent pigmented regions, a new domain may be supportable between them (recall that the average distance between pattern features in an activator–inhibitor system tends to remain the same as the system grows). That would account for the later appearance of new stripes in the conical shell shown on the right in Fig. 4.19.

When Meinhardt's activator–inhibitor systems give rise to travelling waves, the resulting trace on the shell is a series of oblique stripes, as an activation wave for pigmentation moves across the growing edge. We saw how such waves can be initiated in the two-dimensional BZ reaction from spots that act as pacemakers, sending out circular wavefronts. In one dimension these pacemaker regions emanate wavefronts in opposite directions along a line. So the resulting time-traces are inverted V shapes whose apexes point away from the growth edge. When two wavefronts meet on an edge, they annihilate one another just like the target patterns of the BZ reaction, and we then see two oblique stripes converge in a V with its apex *towards* the growth edge (Fig. 4.20*a*). Both features can be seen on real shells (Fig. 4.20*b*). This shows that even highly complex shell patterns can be produced by well-understood properties of reaction–diffusion systems—the complexity comes from the fact that we are seeing the time-history of the process traced out across the surface of the shell.

Occasionally one finds shells that seemed to have had a change of heart—that is to say, they display a beautiful pattern that suddenly changes to something else entirely (Fig. 4.21). An activator–inhibitor model can account for the patterns before and after the change, but to

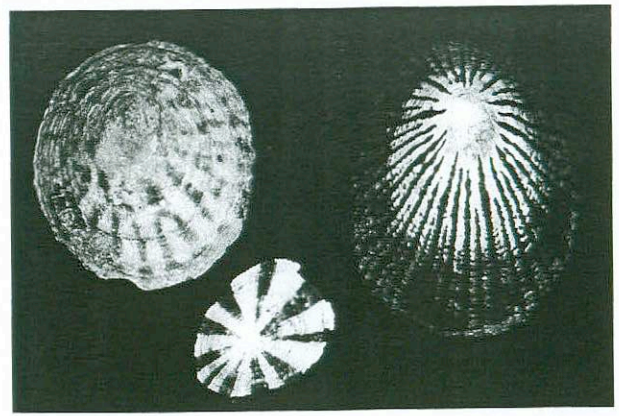
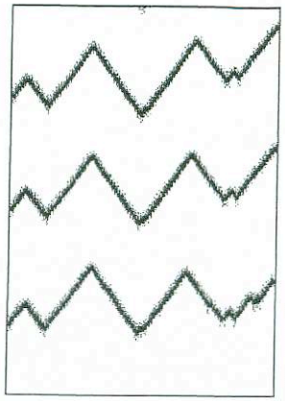
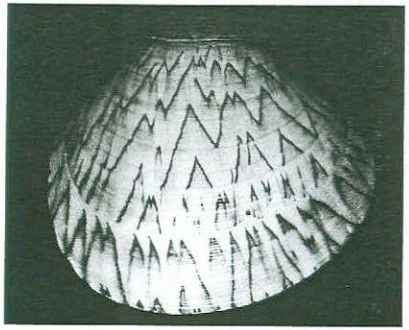


Fig. 4.19 When the shell's growth edge traces out a cone instead of a spiral, a one-dimensional periodic pattern at the edge becomes a radial 'spoke' pattern. As the edge grows in length, new pattern features may appear in the spaces between existing spokes (*right*). (Photo: Hans Meinhardt.)



a



b

Fig. 4.20 Annihilation between travelling waves in an activator–inhibitor model leads to V-shaped patterns (*a*), as seen on the shell of *Lioconcha lorenziana* (*b*). (Photo: Hans Meinhardt.)

account for the change itself we need to invoke some external agency. It seems likely that shells like this have experienced some severe environmental disturbance—perhaps the region became dry or food became scarce—and as a result the biochemical reactions at the shell's growing edge were knocked off balance by the tribulations of the soft creature within (remember that it is this creature, not the shell itself, that is ultimately supplying

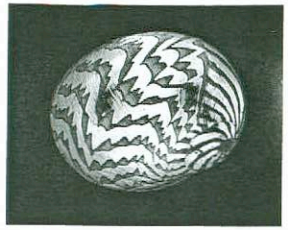


Fig. 4.21 Sudden changes in environmental conditions can restart the patterning process on shells, creating abrupt discontinuities in the pattern. (Photo: John Campbell, University of California at Los Angeles.)

the materials and energy for shell construction!). This sort of perturbation can 'restart the clock' in shell-building, and the pattern that is set up in the new environment may bear little relation to the old one. Like all good artists, molluscs need to be left alone in comfort to do the job well.

But is it real?

Biologists are hard to please. However striking might be the similarity between the patterns produced by these reaction–diffusion models and the real thing, they may say that it could be just coincidence. How can we be sure that the Turing mechanism is really at work in these creatures?

Ultimately the proof will require identification of the morphogens responsible, and that still has not been done. But in 1995, Japanese biologists Shigeru Kondo and Rihito Asai from Kyoto University staked a claim for a Turing mechanism in animal markings that was hard to deny. They looked at the stripe markings of the marine angelfish, a beautiful creature whose scaly skin bears bright yellow horizontal bands on a blue background. It is common knowledge that a reaction– diffusion system can produce parallel stripes; but what is different about the angelfish is that its stripes do not seem to be fixed into the skin at an early stage of development—they continue to evolve as the fish grows. More precisely, the pattern *stays more or less the same* as the fish gets bigger—smaller fish simply have fewer stripes. For example, when the young angelfish of the species *Pomacanthus semicirculatus* are less than 2 cm long, they each have three stripes. As they grow, the stripes get wider, but when the body reaches 4 cm there is an abrupt change: a new stripe emerges in the middle of the original ones, and the spacing between stripes then reverts to that seen in the younger (2-cm) fish (Fig. 4.22). This process repeats again when the body grows to about 8 or 9 cm. In contrast, the pattern features on, say, a giraffe just get bigger, like a design on an inflating balloon.

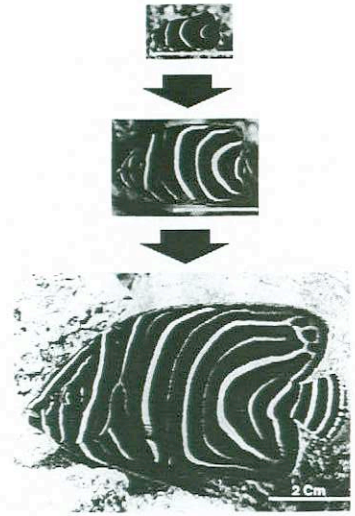


Fig. 4.22 As the angelfish grows, its stripes maintain the same width—so the body acquires more of them. This contrasts with the patterns on mammals such as the zebra or cheetah, where the patterns are laid down once for all and then expand like markings on a balloon. (Photo: Shigeru Kondo, Kyoto University.)

This must mean that the angelfish's stripes are being actively sustained during the growth process—the reaction–diffusion process is *still going on*. One would expect that, if the fish were able to grow large enough (to the size of a football, say), the effect of scale evident in Jim Murray's work would kick in and the pattern would change *qualitatively*. But the fish stop growing much short of this point.

Kondo and Asai were able to reproduce this behaviour in a theoretical model of an activator–inhibitor process taking place in a growing array of cells. This is more compelling evidence for the Turing mechanism than simply showing that a process of the same sort can reproduce a stationary pattern on an animal pelt—the mechanism is able to reproduce the growth-induced expansion of the pattern too.

But the researchers went further still. They looked also at the angelfish *Pomacanthus imperator*, which has rather different body markings. The young fish have concentric stripes that increase in number as the fish grows, in much the same way as the stripes of *P. semicirculatus*. But when the fish become adult, the stripes reorganize themselves so that they run parallel to the head-to-tail axis of the fish. These stripes then multiply steadily in number as the fish continues to grow, so that their number is always proportional to body size, and the spacing between them is uniform. New stripes grow from branching points which are present in some of the stripes—the stripe 'unzips' along these branching points, splitting into two (Fig. 4.23*a*). The calculations of Kondo and Asai, using the same reaction–diffusion model as for *P. semicirculatus*, generated this behaviour exactly (Fig. 4.23*b*). Their model also mimicked the more complex behaviour of branching points located at the dorsal or ventral regions (near the top and bottom

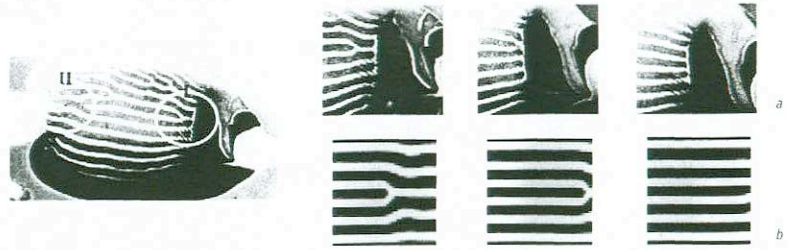


Fig. 4.23 The 'unzipping' of new stripes in *Pomacanthus imperator* (*a*; region I on the left) can be mimicked in a Turing type model (*b*). (Photos: Shigeru Kondo.)

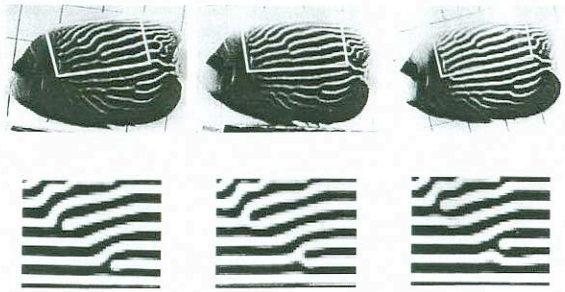


Fig. 4.24 Complex pattern reorganization in the dorsal and ventral regions of *Pomacanthus imperator* (*a*), is also captured by the model (*b*). (Photos: Shigeru Kondo.)

of the body) (Fig. 4.24). What is more, there was a rough correspondence between the relative times taken for these different transformations in the real fish and in the calculations (where 'time' means number of steps in the computer simulation).

It is hard to imagine that, given this ability of the reaction–diffusion model to generate the very complex rearrangements of the fish stripes, the model is anything but a true description of the natural process. Kondo and Asai pointed out that since the reaction–diffusion process is apparently still going on in the adult fish (whereas it is assumed to take place only during the embryonic pre-patterning stage in patterned mammals), it might be a lot easier to identify the chemical species—the activator and inhibitor molecules—responsible in this case. That would provide incontrovertible proof that Alan Turing truly guessed how nature makes her patterns.

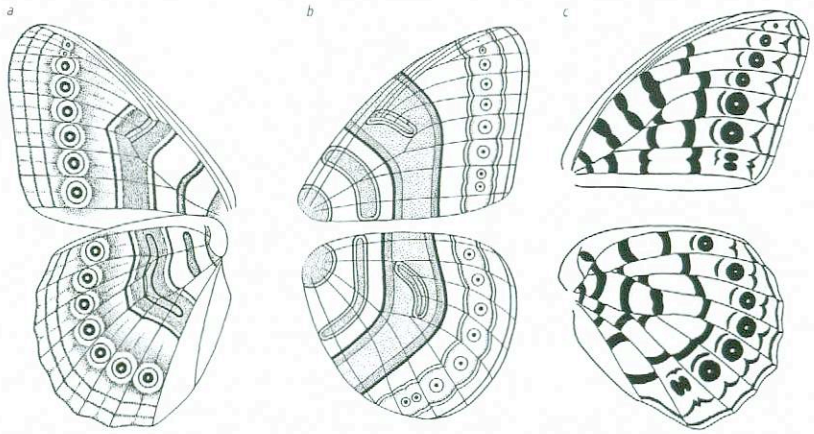


Fig. 4.25 The nymphalid ground plans of (*a*) Schwanwitsch and (*b*) Süffert represent the Platonic ideal of all butterfly and moth wing patterns. They both contain features from which almost all observed patterns can be derived. An updated version of the ground plan (*c*) takes more explicit account of the effect of wing veins. (Images: H. Frederik Nijhout, Duke University.)

On the wing

The animal-marking patterns considered so far are two-tone affairs: they involve the production of a single pigment by differentiated cells. But the natural world is replete with far more fanciful displays that are enough to make a theorist despair. Consider, for instance, the butterfly (Plate 11), whose wings are a kaleidoscope of colour. Not only is the range of hues fantastically rich, but the patterns seem to have a precision that goes beyond the zebra's stripes: they are highly symmetrical between the two wings, as though each spot and stripe has been carefully placed with a paint brush. Can we hope to understand how these designs have been painted?

That question was squarely faced in the 1920s by B.N. Schwanwitsch and F. Süffert, who synthesized a tremendous variety of wing patterns in butterflies and moths into a unified scheme known as the nymphalid ground plan. This depicts the most common basic elements observed in wing patterns in a single universal blueprint, from which a huge number of real patterns can be derived by selecting, omitting or distorting the individual elements. Although Schwanwitsch and Süffert developed their schemes independently, they show a remarkable degree of consistency (Fig. 4.25). The basic pattern elements are series of spots, arcs and bands that cross the wings from the top (anterior) to the bottom (posterior) edges. These top-to-bottom features are called symmetry systems, because they can be regarded as bands or sequences of discrete elements that are approximate mirror images around a symmetry axis that runs through their centre (Fig. 4.26). Even the most complicated of wing patterns can generally be broken down into some combination of these three or four symmetry systems lying side by side—although sometimes they are so elaborated by finer details that the relation to the ground plan is by no means obvious.

No butterfly is known that exhibits all of these elements, however; rather, the nymphalid ground plan represents the maximum possible degree of wing patterning that nature seems able to offer. The full range of wing patterns can be obtained by juggling with the size, shape and colour of selected elements of the plan.

The building blocks that make up these patterns are tiny scales on the wing surface that overlap like roofing tiles. Each scale has a single colour, so that looked at close up, every pattern has the 'pixellated' character of a television image (Fig. 4.27). Some of the colours are produced by chemical pigments—the melanins that feature in animal pelt markings, and other pigment molecules that give rise to whites, reds, yellows and occasionally blues (the latter are derived from plant pigments). But some scales acquire their colours by means of physics, not chemistry. They have a microscopic ribbed texture which scatters light so as to favour some wavelengths over others, depending on the match between the wavelength of the light and the spacing of the ribs. Most green and blue scales generate their colours this way, and it can result in the iridescent or silky appearance of some wing surfaces.

The wing pattern is laid down during pupation, when the surface cells of the developing wing become programmed to produce wing scales of a certain colour (whether it be by the production of pigments or of a particular surface texture). The challenge is to understand how this programming is carried out so as to express the characteristic distributions of spots and bands that each species selects from the nymphalid ground plan.

One important consideration is that the overall pattern appears to be strongly modified by the system of veins that laces the wing. Süffert's initial scheme did not

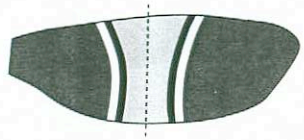


Fig. 4.26 The central symmetry system, a series of bands that runs from the top to the bottom of the wing. The mirror-symmetry axis is denoted by a dashed line.

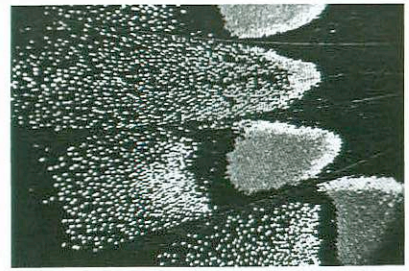


Fig. 4.27 The wing patterns of butterflies and moths are made up from overlapping pigmented scales, each of a single colour. (Photo: H. Frederik Nijhout, Duke University.)

take this into account, but Schwanwitsch appreciated the importance of the veins. In some species, in fact, the wing pattern simply outlines the vein pattern with a coloured border. In general the stripes that cross the wing from top to bottom (particularly the broad band down the centre, called the central symmetry system: Fig. 4.26) are offset where they cross a vein. Schwanwitsch called these offsets dislocations, by analogy with the dislocations of sedimentary strata where they are cut by a geological fault. H. Frederik Nijhout of Duke University has proposed an updated version of the nymphalid ground plan which features these dislocations at veins much more prominently (Fig. 4.25*c*).

This classification of pattern elements helps immeasurably when we come to attack the question of how the patterns arise, because it means that we can focus on the handful of basic symmetry systems, and only afterwards need we worry about how these have become elaborated into the distorted forms that they might take in particular species. Take the central symmetry system, for example. In 1933 A. Kühn and A. von Engelhardt performed experiments to try to understand how this pattern element on the wings of the moth *Ephestia kuhniella* (Fig. 4.28*a*) came into being. The organization of this pattern—the fact that the bands run unbroken (albeit dislocated by the vein structure) from the anterior to the posterior wing edge—implies that the signal triggering it must be non-local: it must pass from cell to cell. So what happens if cell-to-cell communication is disrupted? To find out, Kühn and von Engelhardt cauterized small holes in the wings of the moths during the first day after pupation to present an obstacle to between-cell signalling. They found that the coloured bands became deformed around the holes (Fig. 4.28*b*). After studying the effect of many such cauteries on different parts of the wing, they proposed that the bands of the central symmetry system represent the front of a propagating patterning signal—a 'determination stream'—which issues from two points, one on the anterior and one on the posterior edge (Fig. 4.28*c*).

This was a remarkably prescient idea, anticipating the idea of a diffusing chemical morphogen that triggers pattern formation. But Kühn and von Engelhardt didn't get it all right. For a start, a closer look at their cautery studies suggests that there are *three* sources of morphogen, not two, all of which lie on the mirror-symmetry axis of the central symmetry system. But more importantly, whereas they saw the bands as wavefronts, recent experiments suggest instead that the patterning is triggered when a smoothly varying concentration of the diffusing morphogen (not a sharp wavefront) exceeds a certain threshold and throws some kind of biochemical switch that induces a particular colouration.

Jim Murray has devised a reaction–diffusion system to model these experiments in which a morphogen, which switches on a particular gene in the wing cells, is released from two sources on the anterior and posterior wing edges. He found that the boundary of the gene-activated region of the wing mimicked the shapes of the deformed stripes quite well (Fig. 4.28*d*). Frederik Nijhout proposes that the cauterized holes don't just

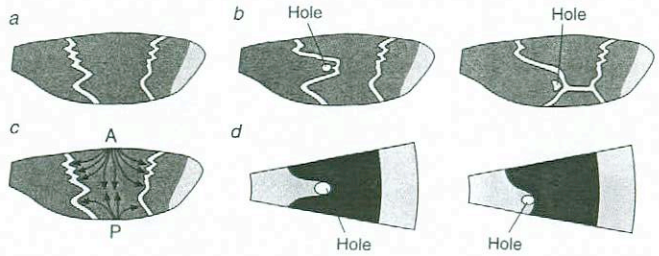


Fig. 4.28 (*a*) The moth *Ephestia kuhniella* has a central symmetry system defined by two light bands. (*b*) Kühn and von Engelhardt investigated the formation mechanism of these bands by cauterizing holes in pupal wings and observing the effect on the pattern. (*c*) They hypothesized that the disruptions of the pattern can be explained by invoking 'determination streams' of some chemical morphogen issuing from centres located on the anterior (A) and posterior (P) edges of the wing. (*d*) There is some correspondence between the pattern boundaries in these experiments and those generated in an idealized model in which a reaction–diffusion system switches on genes that fix the pattern. (After: Murray 1990.)

present obstacles to morphogen diffusion—they actually soak it up (that is, they are a morphogen *sink*). A model based on this assumption can explain all of the experimental results.

The idea that patterning is orchestrated by morphogen sources and sinks underpins all work on butterfly wing patterns today. Moreover, it appears that these sources and sinks are restricted to just a few locations: at the wing veins, along the edges of the wing, and at points or lines along the midpoint of the 'wing cells', the compartments defined by the vein network. Moreover, whereas Kühn and von Engelhardt assumed that their 'determination streams' issued across the whole wing, it is now clear that each wing cell has its own autonomous set of morphogen sources and sinks. So explaining the wing pattern as a whole can be reduced to the rather simpler problem of explaining the pattern in each wing cell, which is copied more or less faithfully from wing cell to wing cell.

The ingredients of a model for wing patterns can therefore be specified by a kind of hierarchical dismemberment of the full pattern. First, the nymphalid ground plan provides a kind of template onto which all actual patterns can be mapped, so that the underlying nature of pattern elements can be discerned. Then this pattern is regarded as an assembly of autonomous wing cells, each of which is itself a collection of pattern elements such as stripes and eyespots (ocelli) which are induced by 'organizing centres', sources and sinks of morphogens. The morphogens are assumed to diffuse through the wing cell, throwing biochemical switches where they surpass some critical threshold. And these organizing centres can lie only at the wing cell midpoints or at their edges (at veins or wing tips).

A general model for patterning that takes these principles as its starting point has been developed by Nijhout. It attempts to solve two mysteries: how do various combinations of sources and sinks create the vast array of pattern elements that we see, and how do these sources and sinks arise in the first place from a uniform sheet of cells?

The first question is the easier one, because Nijhout found that simply by selecting various combinations of sources and sinks located at the specified places he could obtain an endless variety of pattern features. He developed a 'toolbox' of sources and sinks that determine the concentration contours of a diffusing morphogen throughout the wing cell (Fig. 4.29*a*). As any of

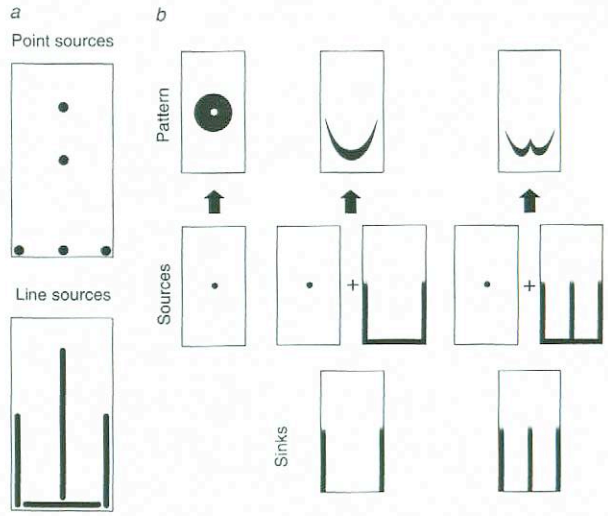


Fig. 4.29 A set of sources and sinks of morphogen (*a*) in an idealized wing cell (here shown as a rectangular unit with veins at the edges and the wing edge along the bottom) can be combined to generate many of the pattern features observed in nature (*b*). (After: Nijhout 1991.)

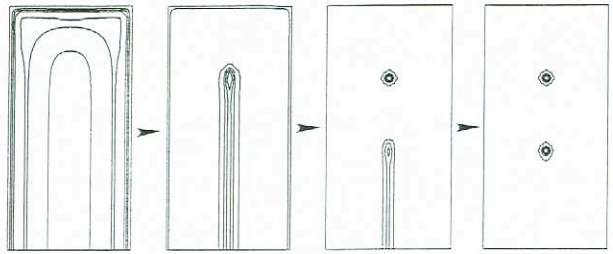


Fig. 4.30 The elements of the toolbox in Fig. 4.29*a* can be produced from an activator–inhibitor model in which an activator is released from the wing veins. The pattern of activator production (shown as contours) changes over time to a central line that retracts to leave isolated spots. (Images: H. Fredrick Nijhout.)

these contours can in principle represent the threshold above which the patterning switch is thrown, a single combination of 'tools' can generate a wide range of pattern features (Fig. 4.29*b*). Amongst these are most of those that appear in nature—and some that do not! What are we to deduce from the latter—that the model is flawed, or that butterflies don't make use of the full 'morphospace' of patterns available to them? The second possibility is quite feasible, because there may be certain types of pattern that simply don't help the evolutionary success of the creature.

So how are the sources and sinks put in place? This is a question that involves spontaneous symmetry breaking in the wing cell, and to answer it Nijhout invokes the activator–inhibitor scheme. To begin with, the only 'special' places in the wing cell are the edges, at the veins and at the wing tips. But of the tools in Fig. 4.29*a*, only one (the line source along the wing edge) tracks one of these special locations fully. Nijhout has shown that all of the other tools can be produced by an activator–inhibitor scheme in which an activator diffuses from the vein edges into an initially uniform mixture of activator and inhibitor. At first, this leads to *inhibition* of activator production adjacent to the veins (Fig. 4.30). Then a region of enhanced activator production appears down the wing cell midpoint. This retracts towards the wing cell edge, leaving one or more point sources of activator as it goes. The number and location of sources depends on the model parameters—the rates of diffusion and reaction. This model suggests that the location and shape of morphogen sources is therefore determined by the time during development when the pattern of the activating substance gets 'fixed' into a source region.

To really verify this model, we'd need to identify and to track the development and behaviour of putative morphogens. Ultimately this is a question of genetics—

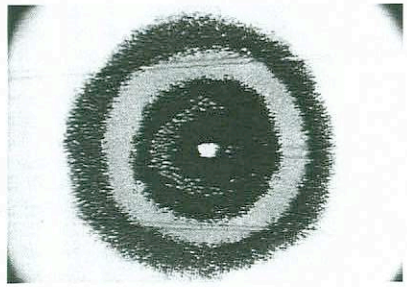


Fig. 4.31 The eyespot pattern is found on many butterfly and moth wings. It probably serves to alarm potential predators. (Photo: H. Frederik Nijhout.)

both the production of the morphogen and its influence on wing scale colour are under genetic control. Many genes have been identified that control certain pattern features in particular species, for example, by changing colours, adding or removing elements or changing their size. But how the genes exert this effect via diffusing morphogens is in general still poorly understood. One of the best studied pattern features is the eyespot or ocellus, a roughly circular target pattern (Fig. 4.31). These markings appear to serve as a defence mechanism, startling would-be predators with their resemblance to the eyes of some larger and possibly dangerous creature. The centre of the eyespot is an organizing centre that releases a morphogen, which diffuses outwards and programmes surrounding cells. Experiments by Sean Carroll of the Howard Hughes Medical Institute in Wisconsin and colleagues have elucidated the genetic basis of the patterning process. They found in 1996 that

a gene called *Distal-less* determines the location of the eyespots. The gene is turned on (in other words, the Distal-less protein encoded by the *Distal-less* gene* begins to appear) in the late stages of larval growth, while the butterfly is still in its cocoon. That the *Distal-less* gene is involved in this process is something of a surprise, since in arthropods like beetles it is known to have a completely different role, determining where the legs grow.

Expression of the Distal-less protein occurs initially in a broad region around the tip of the wing, and the protein spreads by diffusion. Gradually, the production of the Distal-less protein becomes focused into spots, which define the centres of the future eyespots. This focusing is similar to that seen in Nijhout's model for the formation of morphogen sources (Fig. 4.30). Once the focal points have been defined, they serve as organizing centres for the formation of the concentric rings—and it seems that the Distal-less protein now does the organizing. It becomes expressed in an expanding circular field centred on the focal point, and this signal somehow controls the developmental pathways of surrounding cells, fixing within them a tendency to produce scales of a different colour to the background (Fig. 4.32). This process of differentiation of scale-producing cells around the eyespot focus is still imperfectly understood. But it seems clear that the diffusing morphogenetic signal (whether this be the Distal-less protein itself or some

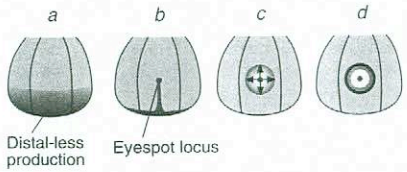


Fig. 4.32 The formation and positioning of eyespot patterns is initiated by a gene product called Distal-less. This protein is at first produced over a broad region around the edge of the developing wing (*a*). It then becomes focused into narrow bands down the midpoint of one or more wing subdivisions (defined by the pattern of veins), ending in a spot which will form the centre of the eyespot (*b*). From this central locus issues a signal comprised of one or more other morphogens, which diffuse outwards (*c*) and eventually induce differentiation of the wing's scale cells into differently pigmented rings (*d*).

* The names of genes are conventionally spelt in italics, while the protein products derived from them have the same name but in normal typeface.

other gene product activated by it) controls the pattern but not the colour of the marking, since eyespot foci transplanted to different parts of the wing produce eyespots of different colours.

To me, one of the most astonishing things about the whole wing-patterning scheme is the way that evolution employs it as a paint-box to create highly specialized pictures. Some butterfly species have evolved patterns that mimic those of other species, because the latter are unpalatable to the former's predators. This kind of so-called Batesian mimicry is good for the mimic but bad for the species it imitates, once predators begin to wise up to the possibility of deception. So the two patterns become involved in a kind of evolutionary race as the mimic attempts to keep pace with its model's tendency to evolve a new set of colours. And the dead-leaf butterfly displays a particularly inventive use of the nymphalid ground plan, which it has gradually distorted and dislocated until the wing pattern and colouration acquire the appearance of a dead leaf—an example of a universal pattern corrupted into camouflage.

Written on the body

What, at last, of the patterns of body plans, which stimulated Turing in the first place? Can the complicated blueprint for our human shape really be imprinted on an embryo by chemicals that are blindly diffusing and reacting, activating and inhibiting?

This topic shows how a little knowledge can simply make life harder. In the eighteenth century no one was troubled by the question of how babies grow from embryos, because it was assumed that, naturally enough, all creatures start life as miniature but fully formed versions of their adult selves, and just grow bigger. People, it was thought, grow from microscopic homunculi in the womb, which possess arms, legs, eyes and fingers perfect in every detail. The problem with this idea, which was rather swept under the carpet, is that it entails an infinite regression: unless you are prepared to accept the formation of pattern from a shapeless egg at *some* stage, you have to assume that the female homunculi contain even smaller homunculi in their tiny ovaries, and so on for all future generations.

During the eighteenth century this idea was gradually dispensed with, but only in favour of an alternative that was really no more attractive. It assumed that egg cells need not be fully formed homunculi but were instead imbued with an invisible pattern that would find gradual expression as a mature organism. This was not

much of an advance because it still begs the question of where that patterning might come from.

To go from a spherical fertilized egg to a newborn baby, you have to break a lot of symmetry. Turing's mechanism provides a way to do that, but there is no reason to suppose that it is unique. Today's understanding of morphogenesis suggests that here, at least, nature may use tricks that are at the same time less complex and elegant but more complicated than Turing's reaction–diffusion instability. It seems that eggs are patterned and compartmentalized not by a single, global mechanism but by a sequence of rather cruder processes that achieve their goal only by virtue of their multiplicity.

The reference grid of a fertilized egg, which tells cells whether they lie in the region that will become the head, a leg, a vertebra or whatever, *is* apparently painted by diffusing chemicals. But there is no global emergence of a Turing-style pattern to differentiate one region form another; rather, the chemicals merely trace out monotonous gradients: high near their source and decreasing with increasing distance. A gradient of this sort differentiates space, providing a directional arrow that points down the slope of the gradient. Each of the chemical morphogens has a limited potential by itself to structure the egg, but several of them, launched from different sources, are enough to get the growth process underway by providing a criss-crossing of diffusional gradients that establish top from bottom, right from left. In other words, they suffice to break the symmetry of the egg and to sketch out the fundamentals of the body plan.

The idea of gradient fields as organizers of initial morphogenesis can be traced back to the beginning of this century: in 1901 Theodor Boveri advanced the idea that changes in concentration of some chemical species from one end of the egg to the other might control development. Experiments involving the transplantation of cells in early embryos led the eminent biologist Julian Huxley to propose in 1934 that small groups of cells, called organizing centres or organizers, set up 'developmental fields' in the fertilized egg that are responsible for the early stages of patterning over much larger regions. Transplanting these organizers to different parts of the fertilized egg was found to lead to new patterns of subsequent development, suggesting that the organizers exercise an influence on the cells around it while growth is occurring—the egg need not be prepatterned before fertilization.

In 1969 the British biologist Lewis Wolpert moulded these ideas into a form that underpins most research on morphogenesis today. Wolpert asserted that the diffusional gradients of morphogens emanating from organizing centres provide *positional information*, letting cells know where they are situated in the body plan. Above a concentration threshold the morphogens switch on genes that set in train a series of biochemical interactions, leading to ever more patterning of the local environment and differentiation of cells into different tissue types.

One problem with the idea of a simple diffusional gradient as the patterning mechanism, however, is that once the single-celled egg has begun to divide into a multicelled body, the diffusing morphogens face the barrier of cell membranes. How can a gradient progress smoothly from cell to cell?

In the most extensively studied of developmental systems, the fruit fly *Drosophila melanogaster*, this problem does not arise. The fruit fly egg is unusual in that it does not become compartmentalized into many cells separated by membranes until a relatively late stage in the growth process, by which time much of the essential body plan is laid down. Like all developing eggs, the fruit fly egg makes copies of its central nucleus, where the genetic storehouse of DNA resides; but whereas in most organisms these replicated nuclei then become segregated into separate cells, the fruit fly egg just accumulates them around its periphery. Only when there are about 6000 nuclei in the egg do they start to acquire their own membranes.

For this reason, morphogens in the fruit fly embryo are free to diffuse throughout the egg in the first few hours after it is laid. After a short time, the egg develops

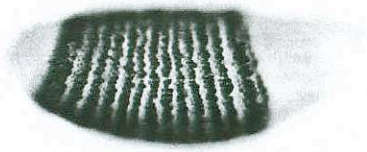


Fig. 4.33 The embryos of the fruit fly develop stripes soon after fertilization which eventually define the different body compartments. (Photo: Peter Lawrence, Laboratory for Molecular Biology, Cambridge; from Lawrence 1992.)

stripes (Fig. 4.33). These evolve into finer stripes, and as the egg begins to become divided into separate cells, these stripes mark out regions that will subsequently become different body segments: the head, the thorax, the abdomen and so forth.

As this striped pattern suggests, the first breaking of symmetry takes place along the long axis of the ellipsoidal egg. This is called the anterior–posterior axis, the anterior being the head region and the posterior the tail. The initial segmentation process seems to be controlled by three genetically encoded signals: one defines the head and thorax area, another the abdomen, and a third controls the development of structures at the tips of the head and tail. When the respective genes are activated, they generate a morphogen that then diffuses from the signalling site throughout the rest of the egg.

The head/thorax morphogen is a protein called bicoid, which is produced when the gene that encodes this protein is switched on. Production of the bicoid protein takes place at the extreme anterior end of the egg, and the protein diffuses through the cell to establish a smoothly declining concentration gradient (Fig. 4.34*a*). To transform this smooth gradient into a sharp compartmental boundary (which will subsequently define the extent of the head and thorax regions), nature exploits the kind of threshold switch that I have described earlier. Below a certain threshold concentration, bicoid has no effect on the egg, but above this threshold the protein binds to DNA and triggers the translation of another gene into its protein product, called hunchback. (More accurately, the bicoid protein promotes the formation of the intermediary hunchback RNA molecule from the *hunchback* gene on the chromosome—it is the RNA that is ultimately translated into a corresponding protein.) In this way, a smooth gradient in one molecule (bicoid) is converted into an abruptly stepped variation in another (the hunchback RNA) (Fig. 4.34*b*, *c*).

You may have noticed that this patterning mechanism seems to have cheated on the question posed at the outset: how does an initially uniform cell break its symmetry? OK, so the cell in this case is not quite so uniform—it already has a long axis and a short axis. But why should bicoid suddenly be produced at one end and not the other, or indeed in any one region of the cell and not others? The answer seems to be that the egg is acted on from outside in an asymmetric manner. Although the egg itself is initially a single cell, it begins its development as a part of a multicellular body. The single 'germ cell' that will grow into the egg becomes

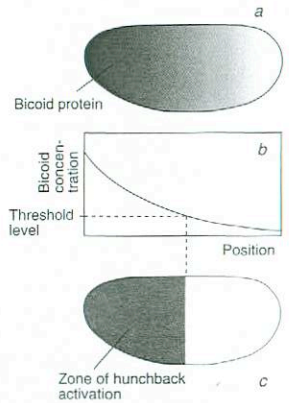


Fig. 4.34 The initial patterning of the fruit fly embryo is controlled by a protein called bicoid, which diffuses along the cell from the anterior end to set up a concentration gradient (*a*). Where the concentration surpasses a certain threshold, the bicoid protein triggers the formation of the so-called hunchback protein (*b*, *c*). Thus the smooth gradient in bicoid gives rise to an abrupt boundary of hunchback expression.

attached to follicle cells before fertilization, and within this assembly the follicle cells and other specialized entities called nurse cells provide nutrients for the egg cell's growth. The nurse cells deposit RNA encoding the bicoid protein at the anterior tip of the egg while they are still attached to one another, and the bicoid RNA starts to generate bicoid protein as soon as the cell is fertilized. So you see, I'm afraid that there is no wondrous spontaneous symmetry-breaking here as there is in Turing's mechanism—instead, a broken symmetry is passed from generation to generation.

The patterning of the posterior region of the fruit fly egg is controlled by a morphogen called the nanos protein. (*Nanos* is Greek for dwarf, and what with hunchbacks too, you can imagine that there are unfortunate deformities associated with the malfunctioning of these genes.) At some stage after longitudinal segmentation has taken place by the action of these morphogens, the egg has to break another symmetry, between top (where the wings will go) and bottom (where the legs and belly are). This is called the dorsoventral axis, and its direction is defined by a protein called dorsal. The mechanism by which dorsal does its job is rather more complicated than bicoid or nanos, however. The

top–bottom gradient is not one in concentration of the dorsal protein—which is actually more or less uniform throughout the egg—but in the protein's location. Towards the bottom, it segregates more strongly into nuclei than into the cell's watery cytoplasm, while the reverse is true towards the top. There appears to be an underlying signal of still uncertain nature that determines whether or not the dorsal protein can find its way into the many nuclei in the egg; this signal is activated from the bottom (ventral) edge of the embryo. Again, the initial impulse for this symmetry-breaking signal seems to come from outside the cell—from a concentration gradient in some protein diffusing through the extracellular medium, which transmits its presence to the egg's interior by interactions at the cell membrane. The way the dorsal morphogen does its job is more complicated too. It is a double switch: above a certain threshold it inhibits the formation of RNA from a pair of developmental genes, whereas above a still higher threshold it promotes RNA formation from a second pair of genes. These gene products are themselves then involved in switching on other developmental processes. Moreover, other molecules called cofactors appear to be able to modify a gene's response to a morphogen, and the cofactors can establish their own concentration gradients. Already we are starting to see why molecular biology seldom lends itself to simple conceptual models: before too long, just about any biological process reveals itself as a sequence of many highly specific steps, in which proteins interact through convoluted pathways to regulate each other's formation.

Do these same initial processes of morphogenetic patterning by chemical gradients apply to other organisms, including us? It seems highly probable that they do, although as I say, most other organisms face the obstacle of cell-to-cell communication in early embryonic development. While there are probably chemical signalling molecules that act as morphogens by switching genes on or off according to their local concentration, they are presumably transmitted from their source region in a stepwise manner—one cell parcelling them out to another—rather than by smooth diffusion. Lewis Wolpert has proposed that morphogens make their way from clusters of cells called zones of polarizing activity (ZPAs) to convey positional information to surrounding cells. It was thought for some time that the small molecule retinoic acid might be a morphogen for limb development in vertebrates, as it appeared to be released from a ZPA at the posterior edge of the developing wing bud of chicks to define the front and back ends (anteroposterior axis) of the wing. But whether retinoic acid indeed has this role is still an open question.

Leg pulling

Not everyone, however, believes that development has to bow entirely to this kind of rigid genetic control. Jim Murray, working with George Oster from the University of California at Berkeley, has postulated a model for structuring and patterning of the body plan at much later stages of an organism's development that involves spontaneous instabilities much like those that give rise to chemical Turing patterns. Murray proposes that structures such as the characteristic hierarchical branching of limb bones or the regular positioning of feathers and scales are a consequence of the interplay of chemical signalling between cells and the mechanical forces that arise in response to these. There are two types of tissue cell: epithelial cells, which aggregate into sheets that constitute the fabric of skin and tissue, and mesenchymal cells, which can pull themselves around using finger-like protrusions called filopodia. Mesenchymal cells will move in response to a variety of stimuli, including gradients in chemical concentrations, in electric fields and in adhesive interactions with a substrate.

Murray and Oster's 'mechanochemical' model of morphogenesis proposes that these signalling mechanisms, particularly those involving chemical gradients set up by diffusion, cause mesenchymal cells to clump together. The traction forces caused by this aggregation, as the cells pull on the surrounding medium, can then establish instabilities that lead to further patterning. For instance, Murray and Oster propose that during limb development a spontaneous instability creates an aggregation of cells along the central axis of an initially uniform cylindrical limb (Fig. 4.35*a*), which will thicken into cartilage and eventually be mineralized into bone. This process is akin to the formation of a single Turing stripe. But the slightest ellipticity in the cross-section of the central cylindrical aggregate makes it unstable: the traction forces act to accentuate this ellipticity, making the limb flatten out (Fig. 4.35*b*). At a certain point, the flattening induces a symmetry-breaking bifurcation of the central condensation, causing it to branch (Fig. 4.35*c*). A subsequent cascade of bifurcations creates the segmentation of the aggregate into the characteristic bone patterns seen in limbs (Fig. 4.35*d*, *e*). Moreover, as a central aggregate gets longer and thinner, mechanical instabilities arise in the longitudinal

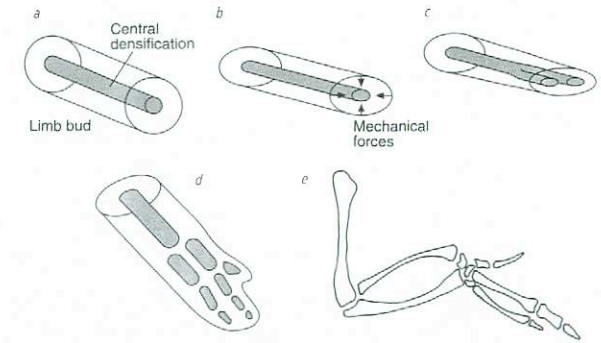


Fig. 4.35 A spontaneous instability in a developing limb bud, due to mechanical forces exerted by cells on their neighbours, creates an increase in cell density along the central axis of the cylindrical bud (*a*). Any deviation from a perfectly circular cross-section (ellipticity) is accentuated by the mechanical forces, causing the limb bud to flatten (*b*). When this flattening exceeds some threshold, a bifurcation takes place to produce two axes of densification (*c*). Subsequent bifurcations and segmentations (*d*) produce the structures that become cartilage and then bone, as seen in the limb of a 10-day-old chick (*e*).

direction (along the axis) which create segmentation of the digits.

Within this picture, the characteristic pattern of limb bones seen in many diverse large animals (Fig. 4.36)—

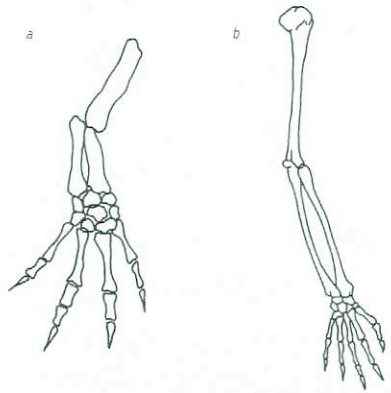


Fig. 4.36 The same sequence of bifurcations and segmentations as in Fig. 4.35*e* is seen in the bone structure of the limbs of many animals, including the salamander (*a*) and humans (*b*).

the division of a single radius into a bifurcated ulna and then into a series of segmented digits—is posited as an inevitable outcome of the physical forces that are acting, not a structure determined arbitrarily by genetics. The model of Murray and Oster—which, incidentally, has been advanced on a far more rigorous mathematical basis than the qualitative description given here—can also account for the polygonal patterning of feathers in birds and scales in fish and reptiles. Feathers are initiated from 'primordia', areas of thickening of the embryonic bird's epidermis caused by an aggregation of underlying dermal cells in the skin. The primordia are arrayed in roughly hexagonal patterns (familiar from the skin of the Christmas turkey), and in Murray and Oster's model these patterns are the mechanochemical equivalent of hexagonal Turing patterns (Fig. 4.3*a*), arising through spontaneous symmetry breaking.

If Murray and Oster are even partly right, these processes suggest that there are certain 'fundamental' structures of organisms that are not at all determined by the arbitrary experimentation and weeding out that evolution is thought to involve. Instead, these structures have an inevitability about them, being driven by the basic physics and chemistry of growth. If life were started from scratch a thousand times over, it would every time alight on these fundamental structures eventually. Within the parlance of modern physics, they are

attractors—stable forms or patterns to which a system is drawn regardless of where it starts from. Within this picture are echoes of the ideas of the eighteenth-century zoologist Etienne Geoffroy de St Hilaire, who believed that there might be certain ideal, Platonic forms in living organisms, from which all other forms are derived by modifications of greater or lesser extent.

This is an extremely contentious idea, since at face value it challenges one of the central tenets of Darwin's theory: that evolution advances by selection from a pool of random mutants. The concept of morphogenetic attractors introduces an element of determinism to this randomness. But even if the protagonists of this concept turn out to be validated, that would not by any means bring Darwin tumbling from his pedestal. There is absolutely no question that natural selection operates in the real world and that it has produced the tremendous variety of organisms with which we share the planet. The idea that this process of mutation and selection might be modulated by other factors is not by any means new in itself, and is hard to doubt. Geological forces have undoubtedly shaped the evolution of the living world: continental drift has isolated subpopulations of species and caused them to diverge, for example, and ice ages and at least one huge meteorite impact have profoundly altered survival prospects in the prehistoric world. No one argues, meanwhile, that nature's palette is not constrained by the rules of physics and chemistry. If the formation of patterns by symmetry-breaking proves to pose limitations on evolutionary choices, that will add just one more nuance to Darwin's towering achievement.

Patterns in bloom

Probably the best candidate system for the identification of Platonic forms in development is the arrangement of leaves on a plant stem. It isn't hard to imagine all sorts of ways in which leaves could be placed up the stem; but if you go out into the garden or park you will soon discover that there are just three basic patterns. Something seems to be placing rather severe constraints on the options.

Most commonly (in 80% of plant species), leaves execute a spiral up the stem, with each leaf displaced above the one below by a more or less constant angle

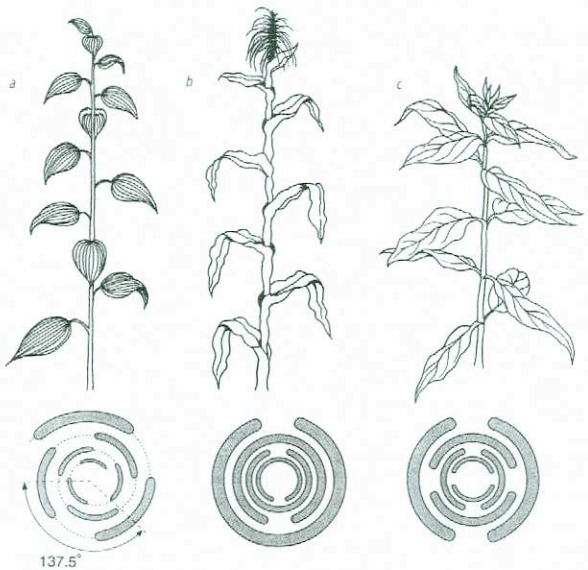


Fig. 4.37 Three distinct patterns can be identified in the arrangement of leaves around plant stems (phyllotaxis): (*a*) spiral, (*b*) distichous and (*c*) whorled. Below each drawing I have shown a schematic representation of the leaf pattern seen from above, with successive leaves depicted as smaller the farther they are down the stem.

(Fig. 4.37*a*). The potato plant, for instance, has this arrangement. The angle of offset is close to 137.5° in many different species, an observation that begs for an explanation. There is, we shall see, something a little spooky about this angle. A second arrangement, called distichous, places successive leaves on opposite sides of the stem, usually with the leaf wrapped almost fully around the stem (Fig. 4.37*b*). We could regard this as a form of spiral in which the offset angle is 180°. The third pattern, called whorled, has little clusters (whorls) of leaves—two or more—at regular intervals up the stem, with each whorl offset so that it sits over the gaps of the whorl below. A common whorled pattern juxtaposes two leaves 180° apart offset at an angle of 90° from the two below (Fig. 4.37*c*). Mint has this arrangement, and so does the stinging nettle. The formation of these leaf patterns is called phyllotaxis ('leaf ordering'), and it turns out to have some remarkable mathematical properties.

When I first observed these arrangements for myself, I assumed that they were clever adaptations selected because they give the leaves maximum exposure to sunlight. You can be sure that arrangements that failed to do this would be selected *against*, but a closer investigation of phyllotactic patterns reveals that there must be more here than Darwinian selection from a random pool of possibilities. They have a mathematical structure in which we can surely see the fingerprint of some *physical* mechanism at work.

The arrangement of leaves along a stem provides us with a somewhat distorted version of the true growth pattern, which becomes extended along the stem axis. Plants grow from the tip of the stem, where one finds a

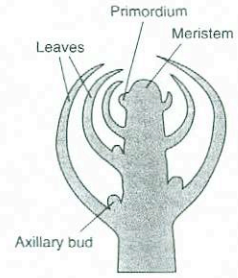


Fig. 4.38 The pattern of phyllotaxis is determined at the tip of the growing stem (the meristem), where the leaf buds (primordia) are initiated. (After: Koch and Meinhardt 1994.)

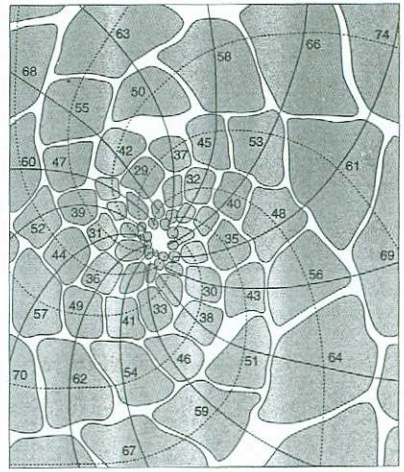
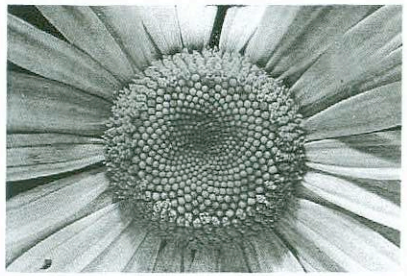
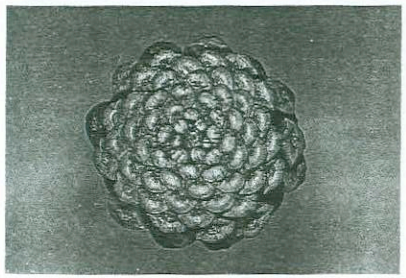


Fig. 4.39 The pattern of spiral phyllotaxis in the monkey puzzle tree. Here I show the projection of the pattern onto a two-dimensional plane, looking down the axis of the branch. Leaves are numbered consecutively from the youngest, and the two systems of spirals (solid and dashed lines) indicate leaves that are in contact with one another. (After: Goodwin 1994.)

bud of multicellular tissue called the meristem. Here cells are multiplying rapidly, and just behind the advancing tip (the apex), side buds called primordia begin to protrude one by one. These will subsequently develop into leaves (Fig. 4.38). There is a roughly constant time interval, called the plastochrone, between the formation of successive primordia, with a typical duration of one day. The leaf pattern is determined by *where* around the boundary of the apex the primordia appear. As the stem grows upwards, the positions of successive primordia trace out a spiral when seen from above. One can see this more clearly by projecting the leaf positions onto a plane perpendicular to the stem (Fig. 4.39). Here some of the leaves are numbered according to the sequence in which they developed, and lines are drawn through leaves that are in contact with one another. These trace out *two* systems of spirals, which twist in opposite directions. The double-spiral pattern is more immediately evident when the primordia develop not into leaves but into florets in a flower head (Fig. 4.40), since in that case they remain all in the same plane.



a



b

Fig. 4.40 The double spiral pattern of phyllotaxis is particularly clear in the arrangement of florets in a flower head (*a*) and of leaflets in a pine cone (*b*). (Photos: Scott Camazine, Pennsylvania State University.)

Golden wonder

The regularity of these spiral patterns has long been seen as the expression of mechanical laws that govern phyllotaxis. W. Hofmeister proposed in 1868 that each new primordium appears periodically on the apex boundary at an interval equal to the plastochrone, and in a position corresponding to the largest gap left by the preceding primordia. In other words, the primordia are simply trying to pack efficiently, just like atoms in a crystal. In 1904, A.H. Church took this idea further in a book called *On the Relation of Phyllotaxis to Mechanical Laws*, from which Fig. 4.39 is derived. And in 1979 H. Vogel performed computer calculations which showed that the preferred angle of 137.5° allows for the optimal packing of primordia placed sequentially along a spiral. Yet there is a richness to the spiral patterns for which these simple packing considerations cannot fully account.

Travelling out along any one of the lines in Fig. 4.39, you will find that the leaf numbers differ from one another by eight along the dashed lines and by 13 along the solid lines. This construction permits a classification of the phyllotaxis pattern—it is denoted (8, 13). Examples from other monkey puzzle branches show other phyllotactic relationships—(5, 8), for instance, and (3, 5). To a mathematician, these pairs of numbers have a familiar ring. They are all adjacent pairs in a well-known mathematical sequence called the Fibonacci sequence, first defined in 1202 by the Italian mathematician Leonardo of Pisa, nicknamed *Filius Bonacci* or Fibonacci. Each term in the sequence is constructed by adding together the previous two, starting with 0 and 1. Thus, 0 + 1 = 1, and the first three terms are 0, 1, 1. The next is 1 + 1 = 2, then 1 + 2 = 3, then 2 + 3 = 5 and so on. The series runs 0, 1, 1, 2, 3, 5, 8, 13, 21, 34 ... .

Straight away we can see the adjacent pairs (3, 5), (5, 8) and (8, 13). But it turns out that the phyllotaxis classifications of leaves, petals or floret patterns in *any* plant species correspond to pairs in this series. A corollary of this is that the number of petals on most flowers corresponds to a Fibonacci number: buttercups have five, marigolds have 13, asters 21.

More mathematical spookiness follows. The ratio of successive terms in the Fibonacci series gets closer and closer to a constant value the further along the series one progresses: 8/13 = 0.615, for example, and 13/21 = 0.619. This ratio approaches a value of 0.618034 to the first six decimal places. This number was well known to the ancient Greeks, who knew it as the Golden Section. It can also be expressed as $(\sqrt{5}-1)/2$, where $\sqrt{5}$ is the square root of 5. To the Greeks, this was a harmonious, almost mystical constant of nature. If you want to draw a rectangle that can be subdivided into a square and a smaller rectangle with the same proportions as the original one (but reduced in scale), the ratio of the two sides must be equal to the Golden Section (Fig. 4.41*a*). These proportions were considered by the Greeks to be pleasing to the eye, and they based the dimensions of many temples, vases and other artefacts on this ratio. There is a long-standing idea that for a perfectly proportioned human body the ratio of the height of the navel to the total height (and also some other bodily proportions) is equal to the Golden Section. It is also related to the logarithmic spiral (Chapter 1), which is traced out by the extremities of a series of rectangles growing in the successive proportions of the Fibonacci sequence (Fig. 4.41*b*). The Golden Section is commonly held to be one of nature's 'special' numbers, like π or e—but one particularly intimate to the geometry of life.

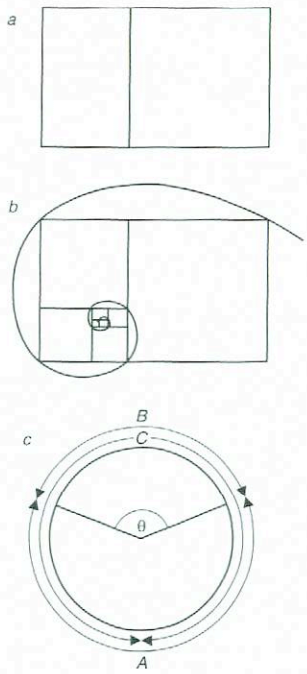


Fig. 4.41 There is a rectangle of unique proportions that can be divided up into a square and a smaller rectangle that has the same proportions as the larger one (*a*). The ratio of equivalent sides of the larger and smaller rectangles is equal to the Golden Section. If we continue to divide the smaller rectangles in the same way, their equivalent corners trace out a logarithmic spiral (*b*). The Golden Angle is the angle at the apex of a segment of a circle of circumference C that sweeps out an arc of length B such that $B/A = A/C$ (*c*). This angle (θ) is about 137.5°.

Now, the Golden Section has a 'Golden Angle' associated with it. This is most easily visualized by dividing a circle into a segment whose perimeter stands in the same ratio to the rest of the circle as the latter's perimeter does to the circumference of the whole circle (Fig. 4.41*c*). The Golden Angle is that at the apex of the segment. And it is equal to 137.5°—the angle at which successive leaves are commonly offset along a plant stem in spiral phyllotaxis! This correspondence between the most common phyllotactic divergence angle and the Golden Angle was first identified, to their surprise, by the mathematicians L. and A. Bravais in 1837.

If this all seems like number-juggling akin to the numerology of end-of-the-world prophets, rest assured that it is mostly an expression of the same basic fact. Once we have established that leaves spiral up a stem with offsets of the Golden Angle, then all the rest—the relationship to the Fibonacci series and to the Golden Section—follows. Ian Stewart explains why in his book *Nature's Numbers*.

Phyllotaxis, therefore, contains a hidden mathematical pattern for which we are unlikely to find an explanation by rooting around in the genetics of plant developmental biology. It seems likely that there is some more universal basis to these observations.

That this is so was impressively demonstrated by the French physicists Stéphane Douady and Yves Couder in 1992. They performed an experiment in which they dropped tiny droplets of a magnetic fluid onto a disk covered with a film of oil, on which the droplets floated. The apparatus sat in a vertical magnetic field, which polarized the magnetic particles and caused them to repel one another. The researchers also applied a horizontal magnetic field, which was stronger at the periphery of the disk than at its centre—this pulled the

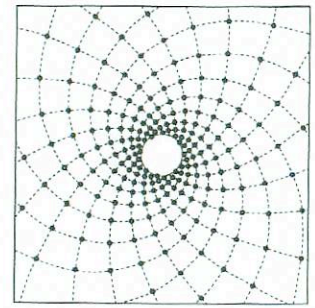


Fig. 4.42 Magnetic droplets moving from the centre to the edge of a round dish while repelling one another trace out spirals of the same kind as those observed in phyllotaxis. Yet here there are clearly only physical forces at play. (After: Douady and Couder 1992.)

droplets outwards towards the edge. Thus, as the droplets fell one by one, they were pushed out to the edges of the disk while repelling one another. When the droplets were added at a fast enough rate, they travelled outwards to form a spiral pattern just like those seen in phyllotaxis (Fig. 4.42), with successive droplets diverging at an angle of about 137.5°. Interestingly, when the rate of droplet addition was low enough, successive droplets diverged at 180° instead (since in this case each droplet was simply repelled by the previous one, the others being too far away)—the pattern then corresponds to distichous phyllotaxis (Fig. 4.37*b*). Under some conditions other divergence angles were seen, which correspond to other, more rare divergence angles seen between leaves that exhibit spiral phyllotaxis.

All very well—except that growing plants are not magnetic droplets! But what Douady and Couder were setting out to test was the idea that phyllotaxis at the Golden Angle is preferred because it allows the optimal packing together of primordia arranged around a spiral on the meristem. They suggested that their experiment, in which the droplets repel one another along spiral trajectories, reproduces these same packing effects. Their findings imply that a plant need not somehow 'know' from the outset that 137.5° spiral phyllotaxy is the best choice—on the contrary, the *dynamics* of the growth process automatically select this angle. If you like, each plant 'finds out' this solution as it grows.

This brings us back to attempts to capture the dynamics of patterned biological growth using reaction–diffusion models. Can such models reproduce the spiral phyllotaxis patterns?

There are at least two separate positioning mechanisms at work in this process. One must tell the primordia how far apart they should be along the stem's axis. This mechanism in effect specifies the interval between inception of primordia—the plastochrone. The other mechanism specifies where around the stem's circumference the primordia should develop—say, at a 137.5° angle from the primordium below for the case of a typical spiral phyllotaxis pattern. This is called the azimuthal position.

Experiments on plant growth dating back to the 1940s have shown that the axial position of a primordium is controlled by a chemical mechanism—specifically by plant hormones that are produced at the apex and transported towards the roots. Hans Meinhardt and André Koch have used this observation as the basis of a reaction–diffusion model in which the hormones act as inhibitors that repress primordia formation in a given region of the stem until the tip has grown far enough beyond this region for the hormone concentration to fall below a certain threshold value. Once this long-ranged inhibition becomes sufficiently weak, some local activator molecules switch on cell proliferation to induce the budding of a primordium.

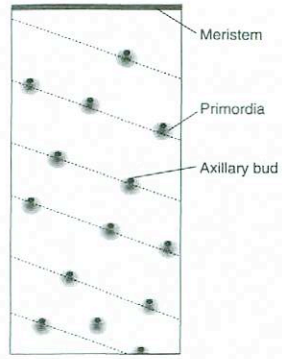


Fig. 4.43 Spiral phyllotaxis can be generated in a reaction–diffusion model of patterning on a cylindrical plant stem, here shown rolled out into a flat sheet. The spiral sequence of primordia is indicated by dashed lines. New primordia develop below the meristem at the top of the cylinder. (After: Koch and Meinhardt 1994.)

In this model, a second activator–inhibitor mechanism controls the azimuthal position of the primordia. As this position is influenced by long-range inhibition, primordia cannot pack too closely together, just as spots in a Turing pattern cannot come too close. In a sense, this is an expression of the packing effects first suggested by Hofmeister. Meinhardt and Koch carried out calculations to find the primordia patterns that their model would produce on an idealized plant stem modelled as a narrow, hollow cylinder. They found that the primordia (and thus ultimately the leaves) became positioned along a spiral winding up the stem in a (2, 3) phyllotaxis pattern—one of the Fibonacci pairs observed in nature (Fig. 4.43). By making some simple and reasonable assumptions about how cells differentiated around the primordia, Meinhardt and Koch were even able to account for the formation of the little 'secondary' buds called axillary buds seen just above the developing leaf where it joins the stem in real plants. (I'm told to remove these from my tomato plants to ensure a good yield of tomatoes, and have often wondered why they were there in the first place.)

There is no direct evidence for this pattern-forming mechanism in phyllotaxis, although the role of plant hormones suggests that it is not unreasonable. But it shows that even quite complicated body shapes in living organisms can be plausibly explained by the chemical processes of self-organization and spontaneous pattern formation that Alan Turing dreamed up over four decades ago.