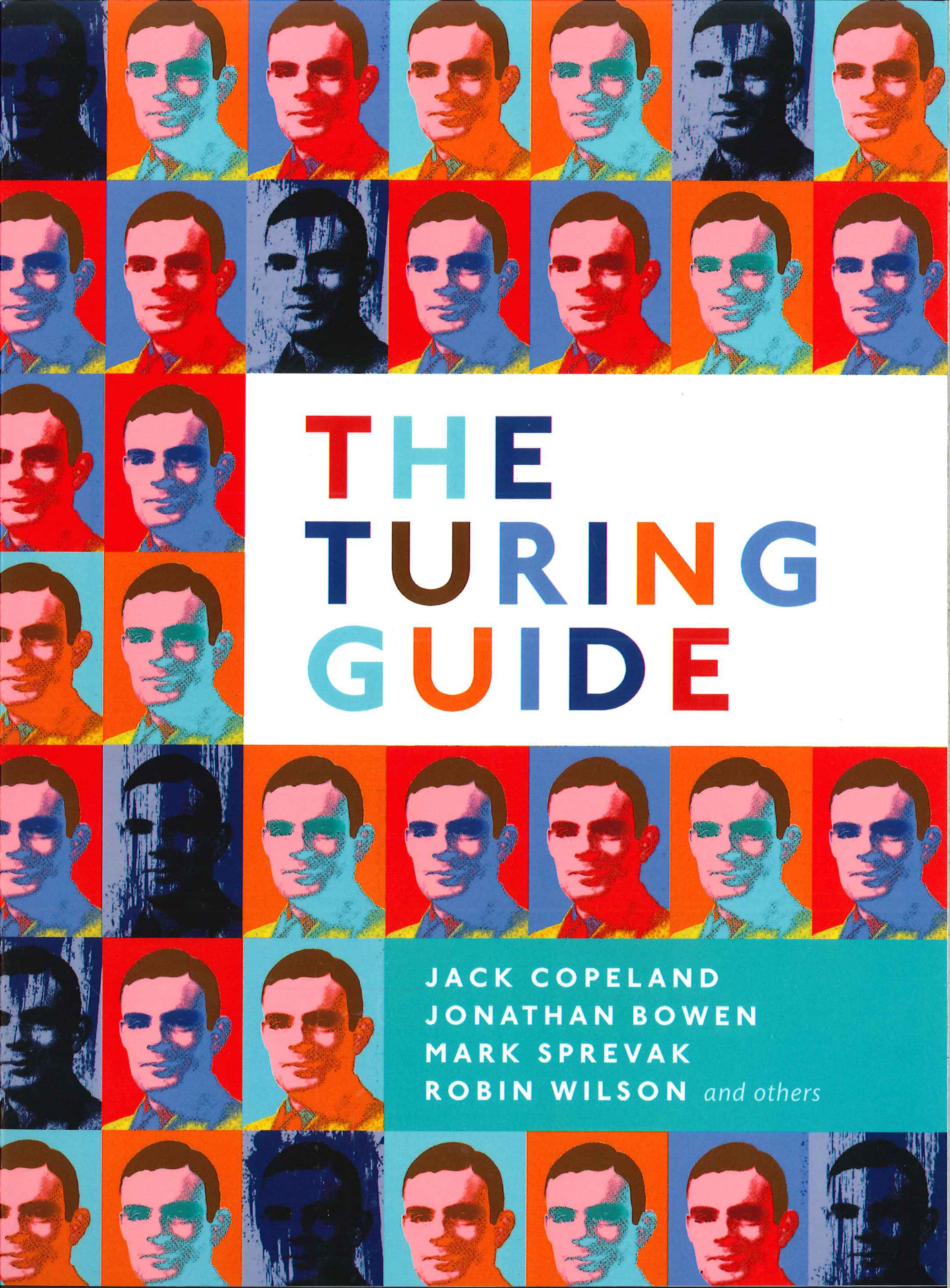

THE TURING GUIDE

JACK COPELAND
JONATHAN BOWEN
MARK SPREVAK
ROBIN WILSON *and others*

THE TURING GUIDE

B. JACK COPELAND
JONATHAN P. BOWEN
MARK SPREVAK
ROBIN WILSON
and others

OXFORD
UNIVERSITY PRESS

CHAPTER 34

Turing's theory of morphogenesis

THOMAS E. WOOLLEY, RUTH E. BAKER, AND PHILIP K. MAINI

In 1952, Turing proposed a mathematical framework for understanding certain very interesting chemical reaction systems.[1] He described a rather counter-intuitive chemical mechanism, and showed that it could generate patterns in chemical concentrations. He coined the term 'morphogens' for the chemicals composing his mechanism, and he hypothesized that morphogens instruct cells to adopt different fates. Which future is 'selected' by the cell depends on the concentrations of morphogens to which the cell is exposed. Thus a new field of research was born, leading to novel mathematical developments and to new biological experiments. However, researchers continue to hunt for a biological example of Turing's mechanism in which the morphogens can be identified. Here we briefly review sixty years of research inspired by Turing's seminal paper.

Introduction

This chapter is a non-mathematical introduction to the mathematical techniques that allow us to understand the mechanisms behind the formation of biological patterns, such as the development of stripes on the skin of a zebra. In particular, we can extract general rules from the mathematical models, and these aid us in identifying places where such patterns could be found. We highlight the successes of Turing's theory, discuss its applicability to particular real-life examples, and explain the potential solutions that it offers to problems thrown up by recent advances in biology.

The core idea of Turing's theory is to take two stable (or stabilizing) processes and combine them. What will you get? Intuitive reasoning suggests that the outcome is a stable system. Turing showed that this is sometimes the *wrong answer*.

Turing's equations

The theory is that patterns arise as the consequence of an observable population, such as skin cells, responding to diffusing and reacting populations of chemicals, such as proteins. These chemicals are known as 'morphogens' and the process of generating biological complexity is known as 'morphogenesis'. Multiple different types of morphogen can be present, and they react with each other in order to create products that cells can use and/or respond to. What cells do is determined ultimately by their genes, but gene expression—which genes are 'turned on'—is determined by a multitude of signals. In Turing's theory the most important signal is the morphogen.

The mathematical equations describing morphogenesis not only model the chemical reactions themselves, but also the essentially random motion of the morphogens as they diffuse. The so-called 'diffusion equation' is incredibly important for understanding all undirected random motion; for example, heat conduction in solids, drainage of water through soil, and gases spreading through the air.[2] The basic equation states that material is conserved throughout the region of diffusion, and that a diffusing chemical always tends to spread out equally but does so in a random way with no preferred direction. The equation also tells us that the rate of change of chemical concentration at a specific position (on a zebra's skin, for example) is given by the 'local balance' of the flow of material into and out of the location in question, with no matter being either created or destroyed.

The big idea

The flash of inspiration that led Turing to suggest the pattern-forming mechanism now bearing his name is a complete mystery. Indeed, because the underlying assumptions of his work are so counter-intuitive, it is a testament to his genius that he found such an important result in a place where no one would think to look.

Turing focused his research on *stable reaction systems*: systems that tend to a constant concentration of morphogens everywhere in the system. On their own, these stable chemical systems cannot produce long-term patterning. We are all familiar with the fact that allowing inert substances to diffuse together does not give rise to patterning; for example, if we put a drop of red ink into water and neither heat nor stir the liquid, diffusion causes the ink to spread out uniformly through the water over time. In other words, after a sufficiently long time no part of the water is darker red than the rest—there is no pattern.

At this point, common sense tells us that if we have a system of stable reactions and simply allow the chemicals to diffuse around, we would not expect any interesting behaviour: we would eventually see a constant concentration of morphogens everywhere, and no pattern. This is because we have a stabilizing mechanism (diffusion) acting on a set of already stable reactions. However, Turing postulated that, when coupled with certain reactions, diffusion could in fact lead to a patterned state: this is called *diffusion-driven instability*. Starting from an unpatterned state, the system comes to exhibit persistent patterns.

To illustrate this idea, let us use a fictitious example involving 'sweating grasshoppers'.[3] Suppose that numerous grasshoppers inhabit a field of dry grass. Suddenly, a fire starts burning somewhere and spreads out into the dry grass. The grasshoppers try to avoid the fire as much as possible, fleeing randomly around the field. As the grasshoppers move, they generate moisture in the form of sweat. This sweat prevents the fire from penetrating into areas of high grasshopper density.

If the grasshoppers move too slowly then the whole field will burn, and there will be no resulting pattern. But if the grasshoppers escape death by moving faster than the fire can spread, then some patches of grass will burn, while other areas, crammed with grasshoppers, become saturated by moisture and do not burn. As a result, the field develops a pattern composed of sections of burnt and unburnt grass. Essentially, this pattern arises due to Turing's mechanism (Fig. 34.1).

Turing was (as usual) ahead of his time in his thinking, and his ideas lay dormant for quite a while. More recently, however, the concept of diffusion-driven instability has led to numerous avenues of further investigation, both theoretical and experimental.

In the particular case of two interacting species (like the fire and the sweating grasshoppers), two mathematical biologists, Alfred Gierer and Hans Meinhardt, clarified Turing's mechanism by identifying one species as an 'activator' (the fire in our case, since it produces more fire and activates the grasshoppers to sweat) and one species as an 'inhibitor' (the sweat, since it prevents fire from occurring).[4] They also showed that, for patterning to occur, the inhibitor must diffuse more rapidly than the activator (the grasshoppers must flee more quickly than the fire advances).

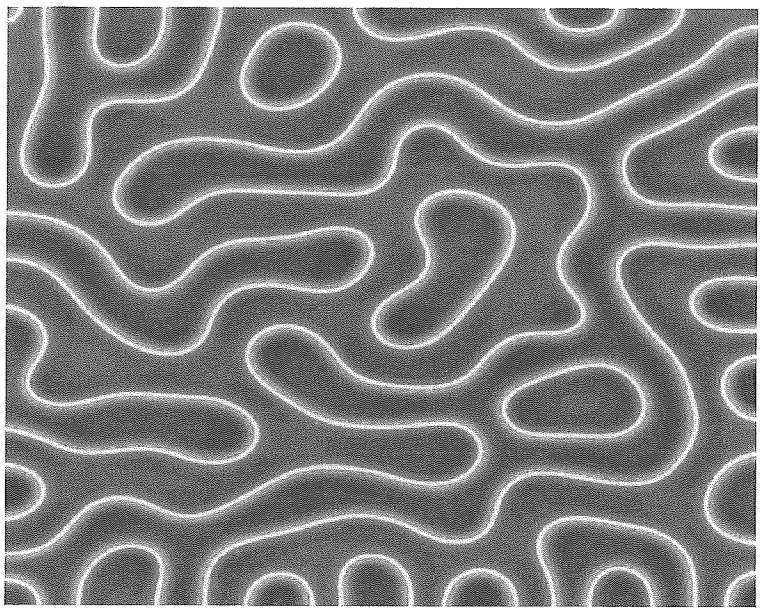

Figure 34.1 An example of a two-dimensional striped Turing pattern.

Thomas E. Woolley.

Do Turing patterns exist in nature?

Nearly forty years after their existence was first theorized, researchers constructed chemical Turing patterns.[5,6] This was quickly followed by the development of a corresponding mathematical model.[7] It is impossible to overstate the importance of these achievements. These researchers showed for the first time that Turing patterns were not merely theoretical. Moreover, their work spurred many other chemists to find reaction systems that give rise to patterns in chemical concentration.

Even though the theory and chemistry of such chemical reaction systems is now well documented, the existence of Turing patterns in *biology* is still controversial. Many biochemical gene products may in fact be Turing morphogens. For example, there is strong evidence to suggest that, during limb formation, certain cell growth factors (proteins that stimulate cell division) act as Turing activators, and although in some cases their complementary inhibitors have been identified, definitive proof that the resulting patterns are Turing patterns still eludes us. To give another example, the regular patterns of arrangement of hair follicles in various mammals also suggest the presence of Turing's mechanism.[8]

Two recent studies of the development of mice have produced strong evidence that Turing patterns might accurately describe a number of biological systems. Jeremy Green and his fellow researchers were the first experimental group to claim to have shown that two proteins, 'fibroblast growth factor' (FGF) and 'sonic hedgehog' (Shh), could act as Turing-style morphogens.[9]

Neither of these proteins is unique to the mouse system that Green and his group studied. Shh was in fact first identified in fruit flies, and acquired its name through experiments showing that fruit fly embryos developed small pointy protrusions, like the quills of a hedgehog, when Shh was inhibited. More recently, it has been discovered that Shh is essential in the development of not only the brain and spinal cord but also the teeth.[10] FGF proteins are known to play important roles in wound healing and the development of blood vessels and neurons.

Green and his group were in fact researching the growth of ridges in the mouths of mice, specifically ridges in the palate. Their work is all the more suggestive because they were able to derive a mathematical model involving Turing's mechanism that reproduced the mouth ridge pattern of normal mice. They also showed that their model could predict the way that the ridge patterns changed when the activity of the morphogens was increased or decreased in experiments.

Shortly after this work on mouth ridges, it was also shown that Turing systems could explain the development of toe spacing in mice. In particular, the effects on toe development of so-called Hox genes were explored.[11] The prevailing theory had been that higher doses of Hox proteins would cause extra toes to form, and so eliminating Hox gene activity would reduce the number of toes. However, as Hox genes were eliminated, it was found that *more* toes formed, fourteen in the most extreme case. The overall paw size remained unchanged, though, meaning that as the number of toes increased they became thinner.

It seems, then, that the Hox gene system can control the *spacing* of the Turing pattern. Although the mathematical theory of this process reproduces the experiments very well, there remains a significant problem—namely, that Hox genes do not diffuse and so are not actually morphogens in Turing's original sense. This means that if this research is to fit with the Turing hypothesis, the genes must somehow be signalling to their environment by means of a mechanism that acts *like* a diffusive agent.

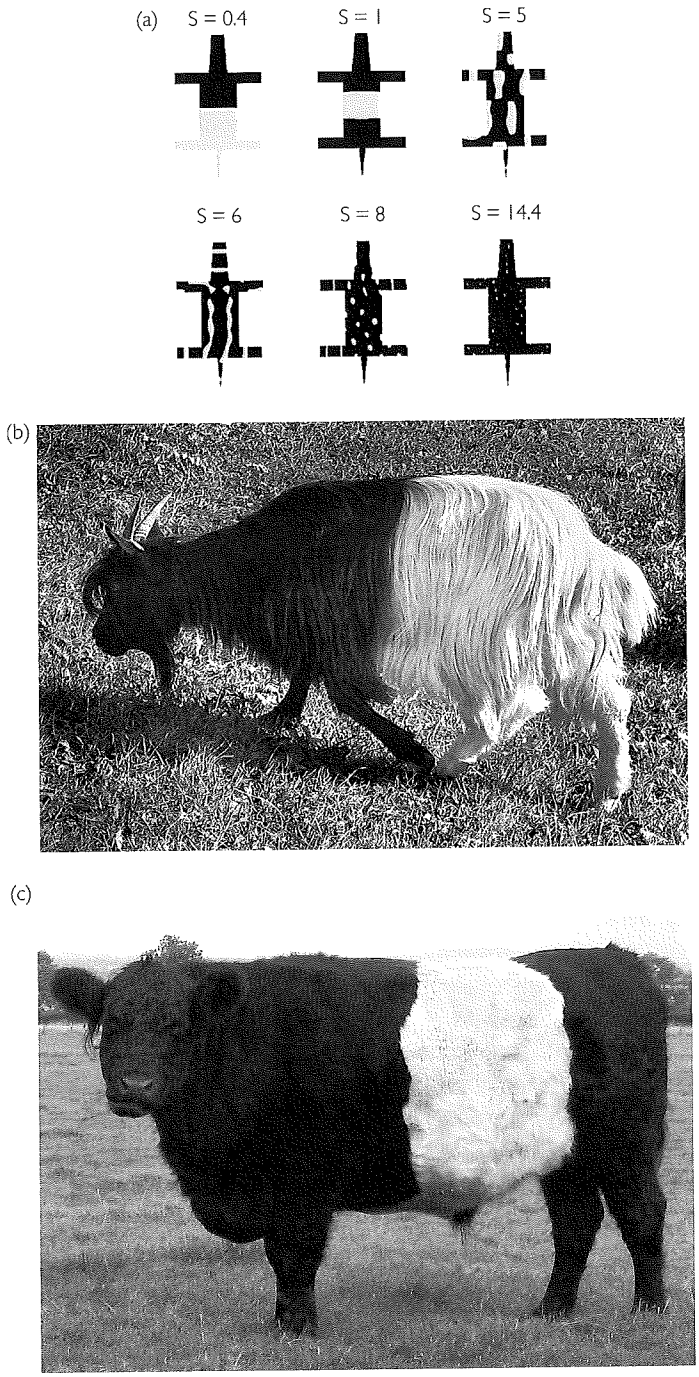

Figure 34.2 (a) As the size of the host surface changes, the pattern changes too: each animal skin has been scaled to be the same size, with a scale factor S denoting the magnification. (b) A Valais goat. (c) A Galloway cow.

(a) Thomas E. Woolley. (b) Taken by B. S. Thurner Hof and posted to http://commons.wikimedia.org/wiki/File:Walliser_Schwarzhalsziege_051071.jpg. Creative Commons licence. (c) Posted to Flickr by amandabhslater at http://www.flickr.com/photos/15181848@N02/2874880039/. Creative Commons licence.

Cows, angelfish, and tapirs

Why is Turing's diffusion-driven instability such an attractive mechanism for describing the development of pattern and form? First, it is simple: the production of the patterns relies on the natural tendency of molecules to diffuse and react. Second, the mechanism has inbuilt features that control the spacing of the patterns. These inbuilt features give Turing patterns a number of characteristic properties. For example:

- in order for patterns to form, the host surface needs to be larger than a specific critical size
- provided that the host is large enough to support patterns, no external 'input' is needed to specify the pattern—the process is self-regulating.

This means that Turing patterns can be highly regular over large distances without any external input.

Both these features are illustrated in Fig. 34.2(a), where we see the effect of increasing the size of an animal's skin. At first the skin is too small to support patterning, but as the skin gets bigger a qualitative change in behaviour occurs, leading to a skin with different concentrations of morphogen at the front and back. As the skin continues to increase in size further bifurcations occur, causing the pattern to become more complex, eventually leading to a maze-like pattern and then, finally, isolated spots. These transitions can be compared with the different patterns observed on the Valais goat (Fig. 34.2b) and the Galloway cow (Fig. 34.2c): the small goat only has one transition, whereas the larger cow has two.

Biologists Shigeru Kondo and Rigito Asai extended these results linking size and pattern by studying animals that grew in size while their skin patterns developed.[12] In experiments involving the marine angelfish *Pomocanthus* they observed that, as the size of the angelfish doubles, new stripes develop along the skin in between the old ones, so producing nearly constant spacing between the stripes. This constant spacing in the patterns on the fish's skin suggests that a Turing-like mechanism is responsible for the development of the pigmentation making up the pattern. Applying this picture to human growth might seem to imply, alarmingly, that we should develop more heads or limbs as we grow from childhood. But human cells can select a fate only during a brief time interval, usually at the embryonic stage, after which existing structures simply grow in size, rather than new structures forming.

Yet the natural world is not always as simple as these results might suggest. To give one example, the coat pigmentation of the Brazilian tapir refuses to be understood as a Turing pattern (Fig. 34.3). This is because the pattern becomes *more* complex on the thinner limb regions, contradicting Turing's theory. Furthermore, only baby Brazilian tapirs are patterned. As the animals mature, their coat markings disappear, leaving a uniform grey colour. For such patterns to be consistent with Turing's theory, we would need to postulate that the 'inputs' to the pattern-making processes on the limbs differ from those on the body and that, moreover, the inputs change over time, causing an evolution from a patterned condition to no pattern. Alternatively, the changes in the tapir's skin pattern may indicate that Turing's mechanism is not universal: these changes may occur in a regime that simply cannot be characterized by Turing's theory.

Figure 34.3 A baby tapir.

Posted to Flickr by ekilby at https://www.flickr.com/photos/ekilby/9375324236/. Creative Commons licence.

More problems—and solutions

One criticism of Turing's mechanism is that it often requires a possibly implausible 'fine-tuning' of biological parameters.[13] Moreover, the diffusion rates of the activator and inhibitor chemicals must vary greatly—something that is unlikely in practice. However, these problems become less severe if there are more than two reacting species. The two-species Turing system is generally only a caricature of the underlying biology. Systems in which three or more morphogens interact are more realistic.

Another criticism is that Turing's mechanism produces patterns that lack robustness. This means that minor perturbations in the starting state of the process, the geometry of the host surface, or the boundary conditions of the process, may greatly influence the final pattern.[14] In certain cases, such as animal skin patterns, the resulting individuality could be a positive outcome. However, when dealing with the toes of mice, for example, the mechanism should ensure that the normal pattern of toe spacing is produced reliably.

In response to this criticism, we can point out that researchers have in fact demonstrated realistic ways of generating robust patterns. For example, it has been shown that robust patterns can be generated when realistic forms of growth are included in the model.[15] When the surface is growing, the tightly controlled initial patterns evolve through intermediate stages until their final form is created. Turing foresaw this. As he said in his 1952 paper:

> Most of an organism, most of the time, is developing from one pattern into another, rather than from homogeneity into a pattern.

Although Turing knew that there were limitations to his model, this fortunately did not stop him from publishing his ideas. He thought that as the paradigm was extended, and generalized to match reality more closely, many of the problems would be surmounted.

One particular problem that is still not well understood is that of time delays in the chemical reactions involved. When considering a mathematical formulation of biology, researchers usually assume implicitly that the chemical reactions occur instantaneously. But this is not true. When dealing with gene products, the delays involved in the production of reactants can be significant—of the order of minutes to hours. Yet if we include time delays in the mathematical equations, what we see is a catastrophic collapse of the ability of the Turing mechanism to generate patterns.[16] Although the addition of randomness has been shown to regenerate the patterns in certain specific cases,[17] this conundrum has still not been completely solved. Indeed, these studies may indicate that the detailed biological processes used in generating patterns are ones in which delays are minimal.

Conclusions

Experimental biology continuously pushes the boundaries of knowledge forward. Our desire for a better quality of life and to live longer has led to a prioritization of the biosciences. However, experimentation, and the linear verbal reasoning implicit in that approach, can lead us only so far: to extend our biological insight we need a fundamental understanding of the complex non-linear feedback interactions inherent in living systems. This is no longer a new idea, but after Turing introduced it, it lay dormant for some time, eclipsed by the long-lasting excitement surrounding the gene theory revolution that was started by the Cambridge researchers Francis Crick and James Watson in 1953, the year after Turing published his ideas on morphogenesis.

Here we have shown that some relatively simple ideas give us a mathematical framework for understanding the formation of complex patterns. These ideas afford a way of comprehending the creation of the natural beauty of pigmentation patterns in animal skins, and give us insight into many developmental features, such as toe development in mice, which in turn can then be translated to human development.

Turing's theory of morphogenesis has been highly successful in illuminating mechanisms that may underlie a wide range of patterning phenomena. But we should not forget that the theory is a *simplification* of the underlying biology. Turing's picture of diffusing chemicals driving a system to form patterns via chemical interactions may not be exact. In fact, recent work suggests that Turing's morphogens may actually be cells themselves: certain types of cells involved in pigmentation patterning in fish have been shown to interact with each other in the same way that Turing hypothesized his morphogens to interact.[18]

In this chapter we have only scratched the surface of a huge topic. Turing's model has been extended to three spatial dimensions, and sources of biological randomness have been included.[19] This has led to new theories and generalizations. It would certainly be a mistake to think that, because of its long history, Turing's idea has run its course. There are plenty of questions to motivate and intrigue a new generation of minds. As Turing said:

> We can only see a short distance ahead, but we can see plenty there that needs to be done.

In the light of recent biological evidence, Turing's original ideas may not stand up in detail, yet the levels of abstraction and detail in his model were absolutely appropriate at the time he

formulated it. The modern range of experimental and theoretical extensions of his 1952 model show that he was on the right track. These extensions strongly support his key claim that a complete, and physically realistic, model of a biological system is not necessary in order to explain specific key phenomena relating to growth.

Turing's theory provides a nice illustration of a general point that the mathematical biologists George Box and Norman Draper put so succinctly:[20]

> All models are wrong, but some are useful.

Turing put the point this way, when discussing the application of his mathematical ideas to biology:[21]

> [My model] will be a simplification and an idealization, and consequently a falsification. It is to be hoped that the features retained for discussion are those of greatest importance in the present state of knowledge.

Turing's theories lay dormant for a long time, because mathematics and biology were not ready for such counter-intuitive ideas. If it had not been for his premature death, just two years after he published his ideas on morphogenesis, how much further might he have developed his theory? How much closer might we now be to solving one of nature's greatest mysteries? How much more useful might our (still wrong) models be?