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Bones, feathers, teeth and coat markings: a unified model

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Pattern formation

The formation of structure within the developing embryo is perhaps the most important and remarkable phenomenon in science. From the almost uniform mass of dividing cells in the very early stage of development, emerges the vast range of pattern and structure observed in animals. The skin, for example, forms many specialised structures such as hair, scales, feathers and glands, as well as antlers and horns. Butterfly wings exhibit spectacular colours and patterns and many animals develop dramatic coat markings.

Although genes play a key role, genetics says nothing about the actual *mechanisms* which produce pattern and structure as an organism develops from egg to embryo to adult form – knowing the dictionary does not mean that we know Shakespeare. The development of structure and form is called morphogenesis and consists of a complex interaction of mechanisms. In spite of a vast amount of research the mechanisms involved are still not fully known and are the source of intense interest and controversy amongst experimentalists and theoreticians.

Turing's breakthrough

In 1952, the British mathematician, Alan Turing[1], wrote a ground

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breaking paper in which he showed that spatial patterns could arise spontaneously. Remarkably, he showed that *diffusion could drive a chemical system unstable, leading to pattern where no prior pattern existed*. This was a totally counter-intuitive result because our experience of diffusion is that it is a *stabilising* process. For example, drop some blue ink into a glass of water. At first, one observes a pattern – a blob of blue ink. After a while, the pattern disappears and the mixture is now a uniform light blue colour. Diffusion has destroyed the pattern. Now consider two chemicals reacting with each other. Let one be self-activating. Left alone, it would continue to catalyse the production of itself. Suppose that it is in the presence of another chemical which inhibits its growth. Then a stable equilibrium can be reached in which both chemicals are at a constant concentration level. Now suppose that we allow both chemicals to diffuse but, crucially, we assume that the activator diffuses more slowly than the inhibitor. The system destabilises as now there is not enough inhibitor to control the growth of the activator. The result is a pool of high activator and low inhibitor concentration. This phenomenon is now known as diffusion-driven instability. Turing showed, mathematically, that the system could in fact exhibit more complex patterns. One of his applications was to the growth of tentacles in hydra. Assuming that one of the chemicals in this reaction-diffusion system was a growth hormone, then regions of high concentration would lead to growth of tissue. In this way, he viewed the reaction-diffusion system as setting down a chemical pre-pattern to which cells responded, leading to pattern at the morphological level. As a result, these chemicals are known as morphogens.

Turing in action

For many years, Turing models were the source of a great deal of controversy. While theoreticians analysed the properties of the patterns exhibited by the model, experimentalists pursued Turing structures in nature, without success. Finally, in the late 1980s, the breakthrough was made and Turing patterns were discovered in chemistry[2,3] (see Figure 1).

An alternative approach to pattern formation

In the Turing, or reaction-diffusion (RD) approach to embryological pattern formation, it is hypothesised that cell density is uniform and the spatial pattern is in the chemical concentration. An alternative approach – the cell movement (CM) approach – hypothesises that

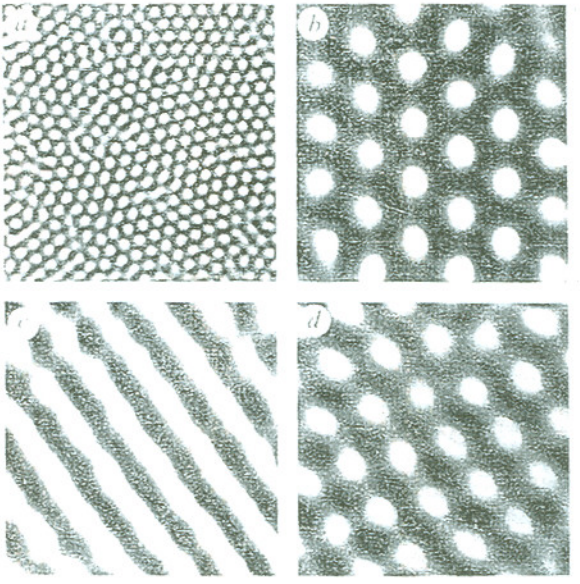

Fig. 1. Different types of stationary chemical (Turing) patterns in the chlorite-iodide-malonic acid reaction (from Nature, **352**, *610–612). Bar represents 1 mm.*

the pattern actually occurs in cell density. In other words, due to mechanical and chemical cues, cell density forms a spatial pattern and cells in high-density aggregates then differentiate. We illustrate the difference between these models with an application to skeletal patterning in the limb (see below for more details). The RD approach would explain the development of the humerus as follows: due to diffusion-driven instability, a pre-pattern in a morphogen forms that is high in the central core of the developing limb bud. Cells in the central core experience this high concentration, which triggers a genetic switch causing them to secrete cartilage. The CM approach would say that a high density of cells forms in the central region of the limb. The mechanical conditions within high-density aggregates then triggers a genetic switch in these cells, causing them to produce cartilage[4].

Developmental constraints

Although based on very different biology, the above models are similar in two crucial ways. Firstly, they view pattern to form as a result of *self-organisation* – the system spontaneously generates pattern. Secondly, their mathematical analysis reveals that the patterns they

produce are very similar. In other words, the patterns produced are mechanism-independent. If patterns in developmental biology are really the result of self-organisation, a key question is, what controls the pattern? These ideas are illustrated in the applications below.

Application 1: Animal coat markings

In 1981, James Murray[5–7], a mathematical biologist at Oxford at the time, hypothesised that a RD system sets up a morphogen pre-pattern in which one of the morphogens causes the cells to secrete pigment. He showed (see Figure 2) how the resulting patterns depended on the size of the domain and on the geometry. As the domain

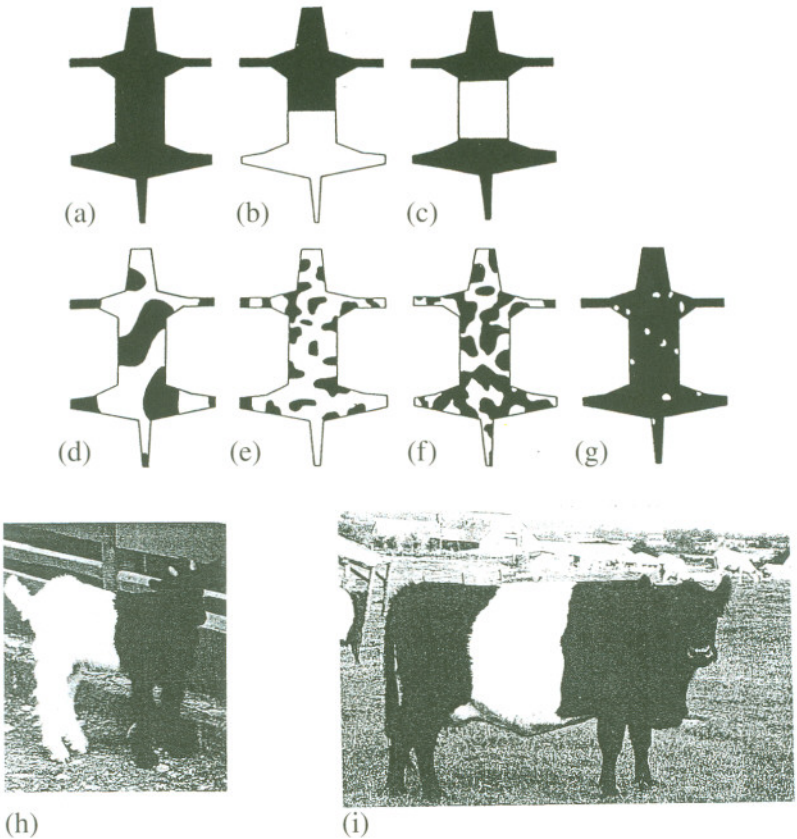

Fig. 2. *Effect of body surface scale on the spatial patterns formed by an RD mechanism. Scale increases from (a) to (g). (Pictures rescaled). (h) Young Valais goat, (i) Belted Galloway. (Figures (a) to (h) from ref. 7).*

increases in size, more complex patterns are possible. If the domain geometry narrows, then less complex patterns are the only possibility. In particular, *whereas it is possible to have a spotted animal with a striped tail, it is not possible to have a striped animal with a spotted tail* (see Figure 3). This is an example, of a developmental constraint.

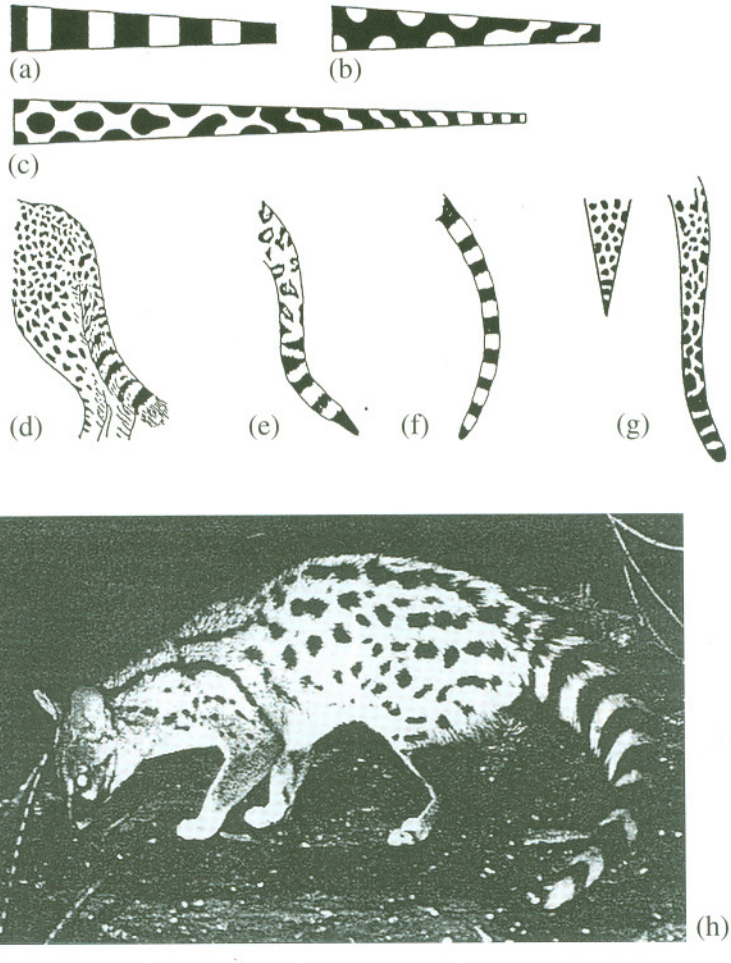

Fig. 3. (a)–(c) *The effect of domain geometry on the patterns exhibited by a RD system. As the domain narrows, there is a transition from spots to stripes. (d) Typical tail markings from an adult cheetah. (e) Typical adult jaguar. (f) Pre-natal tail markings in a male genet. (g) Typical markings on the tail of an adult leopard. (h) Genet, showing transition from a spotted body to a striped tail. (From ref. 7).*

The RD model equations bear similar solutions to the equations that govern the vibration of a membrane. Therefore, if one could visualise the displacement of a vibrating plate using colour, one should see animal coat markings on an appropriately shaped plate. This is indeed the case (see Figure 4).

One of the intense controversies in pattern formation concerns the model mechanism that produces pattern – is it reaction-diffusion or cell movement? In most cases, present experimental technology cannot answer this question. However, in coat markings, there is strong evidence that the patterning occurs due to the movement of pigment cells. Hence, a cell movement model is more realistic, biologically.

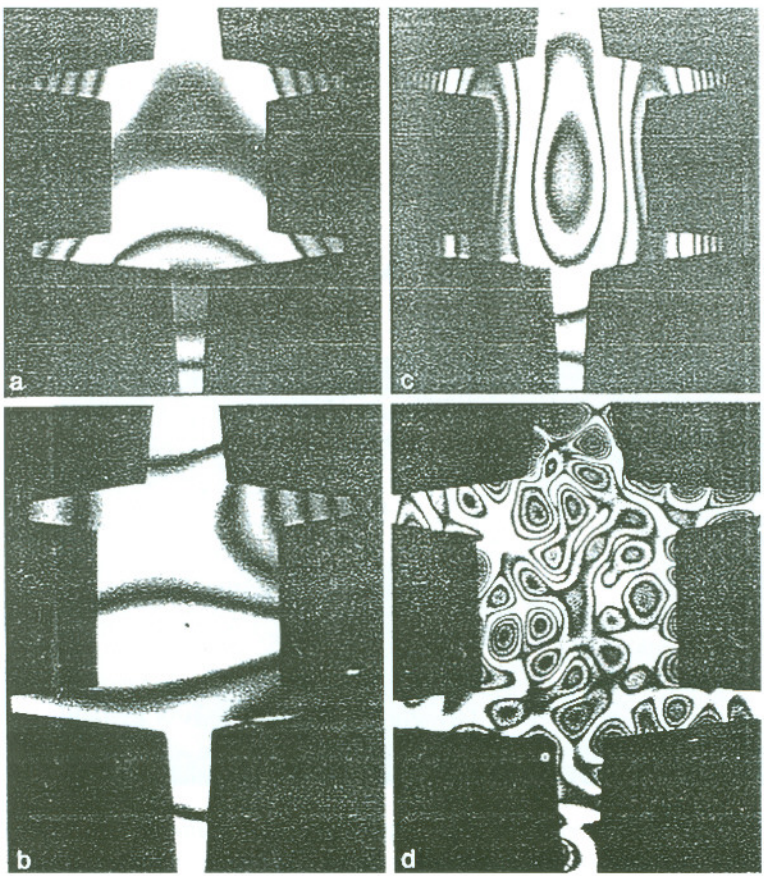

Fig. 4. *Sequence of holographic interferograms on a plate excited by sound waves of increasing frequency from (a) to (d). (From ref. 7). (a) and (c) resemble zebra stripes, (d) resembles leopard spots.*

However, due to the mathematical similarity between RD and CM models, the above patterns can be produced also by a CM model.

Detailed analysis of these models show that they can exhibit many of the coat patterns observed on animals. However, there are certain patterns that they cannot exhibit (see Figure 5). Recently, it has been shown that a composite model incorporating a reaction-diffusion mechanism with cell movement can reproduce these patterns (K. Painter, P.K. Maini and H.G. Othmer, in preparation)

Application 2: Skeletal patterns in the vertebrate limb

Both RD and CM models produce patterns that can be highly sensitive to slight changes in geometry and model parameters. This makes these models unrealistic as models for robust patterning phenomena, such as the development of skeletal patterns in the limb. The standard models assume that the domain boundary is a passive impermeable membrane. Recent studies have shown, however, that if the boundary is considered to play an active role in patterning by, for example, being a source or a sink of morphogen, then the patterns become highly stable and one can robustly generate the sequence of patterns observed in the limb[8] (see Figure 6). A model prediction, therefore, is that disrupting the boundary will lead to a disruption of the pattern. Experimental evidence supports this prediction.

In 1990 experimentalists at the University College and Middlesex School of Medicine[9] carried out an experiment to disprove that skeletal patterning occurred as the result of an RD system. In the early stages of limb development, before any pattern was visible, they created a symmetrical limb bud by combining two half limb buds. The resultant limb bud was the same size as a normal limb bud, yet developed two humerus instead of one. As the domain size was normal, an RD system would predict that one should get a single humerus. This experiment clearly contradicts the standard RD model. However, a modification of the model that assumes diffusion across the limb is not constant exhibits exactly the behaviour observed in this experiment[10]. Experimental evidence strongly suggests that such a diffusion gradient does indeed exist in the limb[11,12].

Application 3: Feather germ formation

Much experimental work has been devoted to understanding the sequence of patterning events that determine the position of feathers.

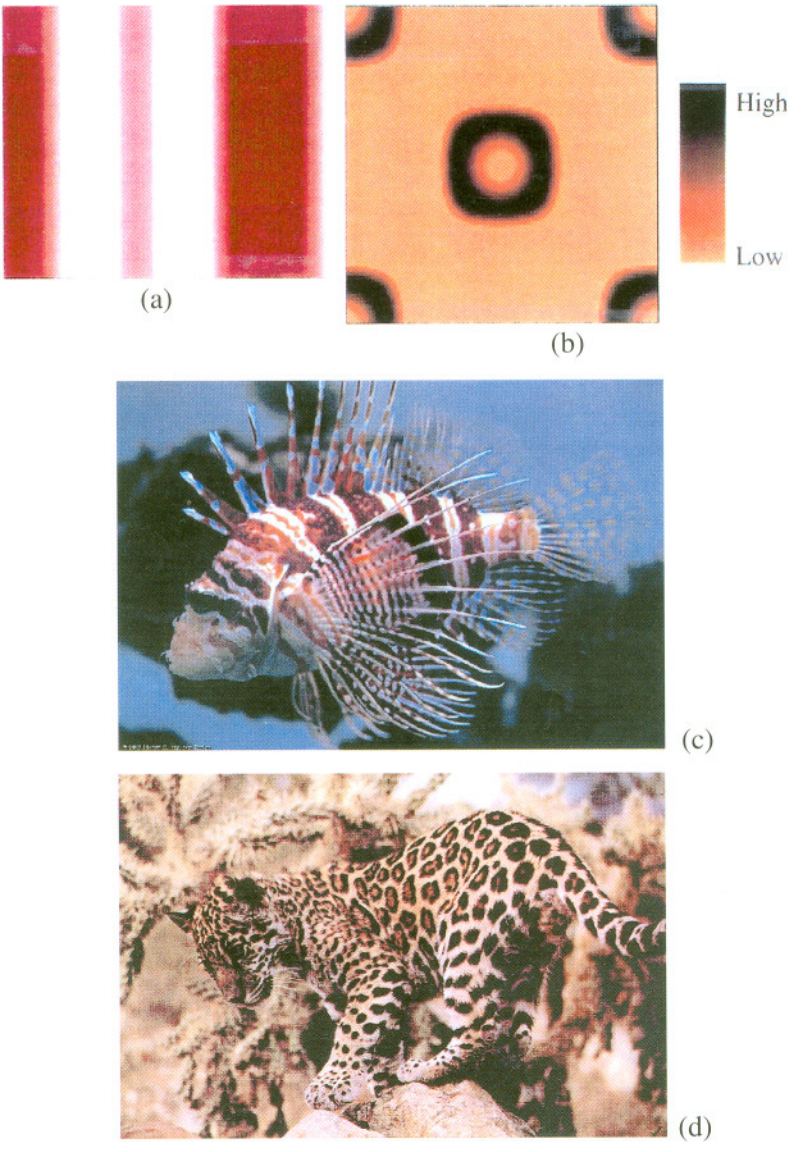

Fig. 5. (a) *A composite RD-CM model can exhibit broad and narrow stripes, similar to those observed on the Lion fish taken from p-vd-meulen@geocities.com ww.geocities.com/yosemite/7147/Copyright © Pieter S. van der Meulen. (c). This type of pattern cannot be exhibited by either model alone. (Red denotes high cell density). (b) The composite model can also capture finely detailed patterns that elude the individual models. Compare with patterns on the jaguar taken from http://sunsite.sut.ac.jp/multimed/pics/feline/WC-Young.jaguar. jpg (d). (K. Painter, P.K. Maini and H.G. Othmer, in preparation).*

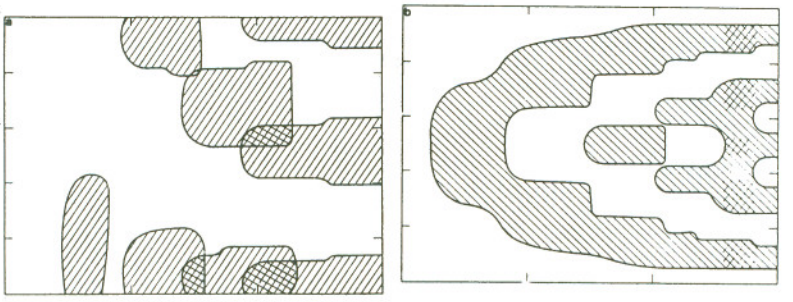

Fig. 6 (a) *Sequence of patterns formed by the standard RD model with passive boundary conditions. (b) Other boundary conditions can lead to a 1–2–3–4–5 sequence of patterning similar to that observed during skeletal development in the limb. (From ref. 8). Note that the joints are continuous here. This is how the pattern is first observed experimentally. The appearance of distinct joint regions occurs at a later stage.*

A feather germ, or primordium, the site of a feather, consists of two structures – a placode, which is an aggregation of cells in the outer layer of skin (epidermis), overlying a papilla, which is a condensation of cells in the lower layer of skin (dermis). On the dorsal surface of chickens, for example, a row of feather germs is first laid down along the dorsal midline. The pattern then propagates outward, with primordia forming in subsequent rows at interdigitating points, leading to a rhombic pattern. In this case, there is a definite tissue–tissue interaction between the epidermis and dermis. It has been shown that a mathematical model accounting for cell movement in both layers, with interaction, can exhibit this type of pattern[13,14] (see Figure 7). A key result to come out of this model is that when the first row of primordia form, the mechanical stress field exerted by cells automatically causes cells in the next row to aggregate at interdigitating points. This provides a mechanism, therefore, of setting up a complex two-dimensional spatial pattern by needing only to specify a simple one-dimensional pattern.

Application 4: Tooth development in the alligator

An example of complex spatiotemporal pattern formation is tooth morphogenesis in the vertebrate jaw of the alligator, *Alligator mississippiensis*. This is a process of complex self-organisation, where both domain growth and pattern inhibition play crucial roles, and it has been the source of detailed experimental investigation[15–17], so

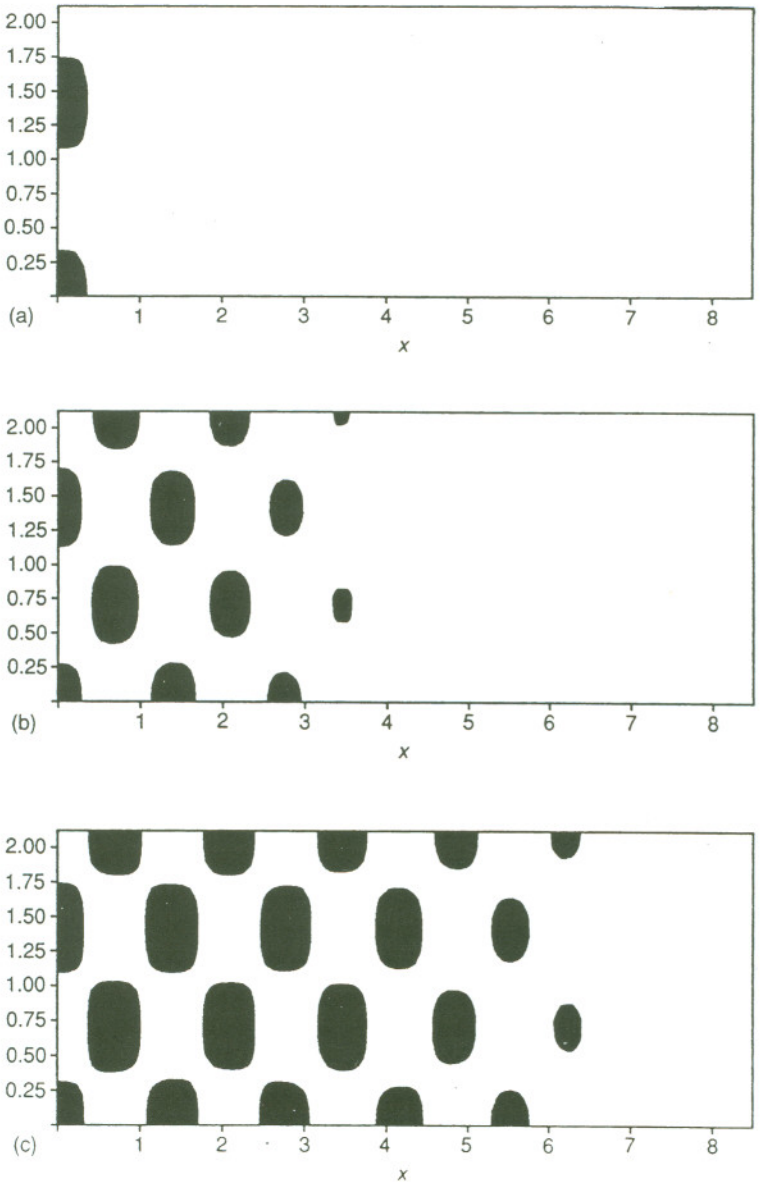

Fig. 7 *Sequential pattern formation exhibited by a tissue-tissue interaction model for feather primordium formation. Beginning from a simple one-dimensional pattern, (a), a complex two-dimensional pattern, (b) and (c), evolves with the characteristic rhombic structure observed on the dorsal surface of the chick. (From ref. 13).*

that there is ample experimental data on which to base a realistic mathematical model.

Recently, a mathematical model has been developed to account for the patterning of teeth in the lower jaw of the alligator[18]. As the alligator jaw has left–right symmetry, one need only consider one side of the jaw. Moreover, to a very good approximation, one can think of the jaw as being essentially an one-dimensional domain going from posterior (back of the jaw) to anterior (front of the jaw). Teeth arise as the result of tooth primordia which are clumps of cells in the jaw mesenchyme which mark where future teeth will form. The sequence in which these primordia form is very complex. For example, the first seven tooth primordia form along the posterior–anterior axis in the sequence 7–3–6–2–5–1–4. That is, the first tooth (tooth 1) forms near the anterior end of the jaw. The second tooth primordium to form is posterior to the first tooth, and primordium 3 forms posterior still. By this stage, the jaw has elongated sufficiently for tooth 4 to form anterior to tooth 1. Teeth 5, 6 and 7 then form in a posterior sequence. This sequence of patterning can not be captured by the above simple models.

It appears that when a tooth primordium forms, it inhibits, for a certain length of time, tooth primordium formation nearby. By incorporating a third chemical, assumed produced by tooth primordia, into the RD framework, and allowing the domain to grow, this behaviour is captured by the model (Figure 8). Moreover, the model can make experimentally testable predictions on the effects of removing early primordia.

Summary

A first necessary criterion to be met by any model for pattern formation is that it must be able to reproduce the patterns it purports to model. The above examples show how simple ideas from self-organisation can produce spatial patterns of varying complexity that are consistent with those observed experimentally. Second, the model must be consistent with the results of experimental manipulation. Third, the model must make experimentally testable predictions. In this way, a mathematical model can help to elucidate the underlying biochemical and biophysical mechanisms of pattern formation. I have tried to illustrate these ideas with the above examples. These examples also show the breadth of patterning phenomena that can be captured by these models. In animal coat markings, the patterns are laid down simultaneously. In limb development, the skeletal elements are laid down sequentially. The application to feather germ

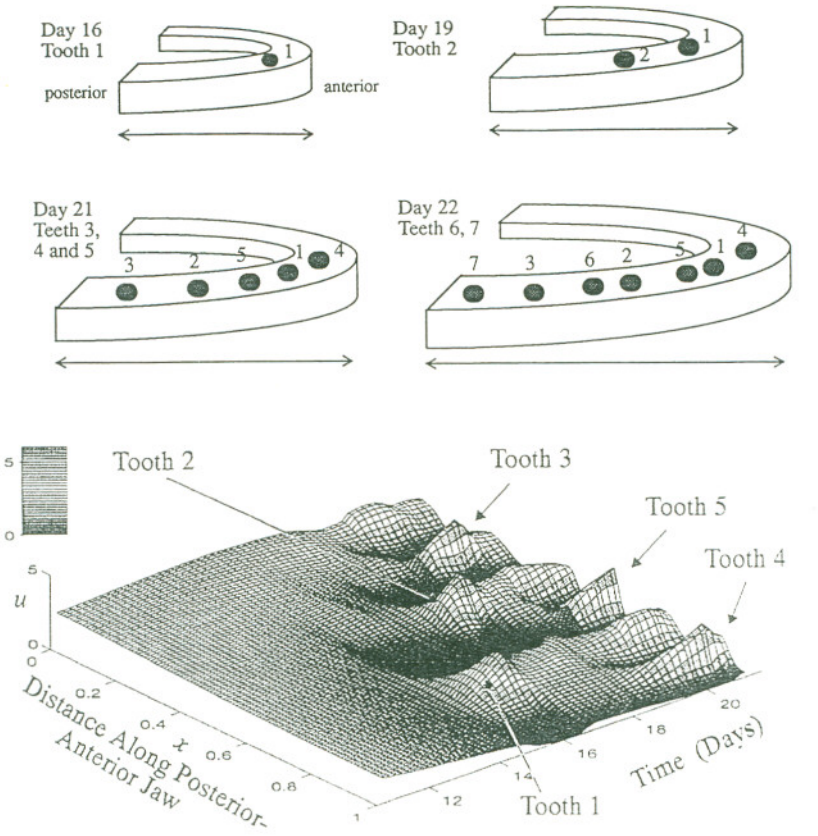

Fig. 8 (a) Spatial sequence of first seven teeth (lower jaw) in Alligator mississippiensis. (From ref. 15). (b) Space-time plot showing how this sequence may be generated using the model of Kulesa et al. (ref. 18) (only first five teeth shown, for clarity).

formation illustrates complex sequential regular pattern formation, while the application to tooth primordium formation illustrates sequential irregular pattern formation.

Many other patterning phenomena have been studied (see, for example, ref. 7). Pattern formation is one of the central issues in developmental biology and intense interdisciplinary research involving experimentalists and theoreticians is beginning to help us understand how this phenomenon occurs. A detailed understanding of normal development is a necessary first step to the understanding of abnormal development and, hopefully, will help medical science combat developmental defects.

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References

1. Turing, A.M. (1952), *Phil. Trans. R. Soc. Lond.*, **B237**, 37–72.
2. Castets, V. Dulos, E. Boissonade, J. & De Kepper, P. (1990) *Phys. Rev. Lett.* **64**(24), 2953–2956.
3. Ouyang, Q. & Swinney, H.L. (1991) *Nature*, **352**, 610–612.
4. Maini, P.K. & Solursh, M. (1991) *Int. Rev. Cytol.*, **129**, 91–133.
5. Murray, J.D. (1981) *Phil. Trans. R. Soc. Lond.*, **B295**, 473–496.
6. Murray, J.D. (1981) *J. theor. Biol.*, **88**, 161–199.
7. Murray, J.D. (1989) *Mathematical Biology*. Springer-Verlag, Berlin.
8. Dillon, R. Maini, P.K. & Othmer, H.G. (1994) *J. Math. Biol.*, **32**, 345–393.
9. Wolpert, L. and Hornbruch, A. (1990) *Development*, **109**, 961–966.
10. Maini, P.K. Benson, D.L. & Sherratt, J.A. (1992) *IMA J. Math. Appl. Med. Biol.*, **9**, 197–213.
11. Brummer, F. Zempel, G. Buhle, P. Stein, J.-C. & Hulser, D.F. (1991) *Exp. Cell. Res.*, **196**, 158–163.
12. Coelho, C.N.D. & Kosher, R.A. (1991) *Dev. Biol.*, **148**, 529–535.
13. Cruywagen, G.C. & Murray, J.D. (1992) *J. Nonlinear Sci.*, **2**, 217–240.
14. Cruywagen, G.C. Maini, P.K. & Murray, J.D. (1992) *IMA J. Math. Appl. Med. Biol.*, **9**, 227–248.
15. Westergaard, B. & Ferguson, M.W.J. (1986) *J. Zool. Lond.*, **210**, 575–597.
16. Westergaard, B. & Ferguson, M.W.J. (1987) *J. Zool. Lond.*, **212**, 191–222.
17. Westergaard, B. & Ferguson, M.W.J. (1990) *Am. J. Anat.*, **187**, 393–421.
18. Kulesa, P.M. Gruywagen, G.C. Lubkin, S.R. Sneyd, J. Maini, P.K. Ferguson, M.W.J. & Murray, J.D. (1996) *J. theor. Biol.*, **180**, 287–296.