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Some Mathematical Models for Biological Pattern Formation

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7.1 Introduction

The study of growth and form is central to the understanding of many areas in the physical, chemical and biological sciences. D'Arcy Thompson was one of the key figures in the early studies of this area. He recognized that although classification in biology was extremely important, it was also crucial to develop theories to explain observed traits. In the latter half of this century, advances in this area have been made by understanding a few systems in depth, which serve as paradigms for the broader picture in which Thompson was interested. Of course, while pattern is not form, both are closely related.

Perhaps the most famous example of spatio-temporal pattern formation, and one of the earliest studied, is the Belousov–Zhabotinsky reaction, in which bromate ions oxidize malonic acidic in a reaction catalysed by iron which, depending on its state, can assume two different colours – reddish orange or blue (Johnson and Scott, 1996). By observing the colour changes, one can see that this reaction exhibits a wide range of spatio-temporal patterning, such as propagating fronts, spiral waves, target patterns and toroidal scrolls. Such oscillatory and wave-like patterns also arise in physiology, for example in the propagation of electrical activity in the heart, and in biology, for example, waves of cAMP that initiate cell aggregation in the slime mould. Spatial patterns have recently been shown to arise in the CIMA reaction (Maini *et al.*, 1997).

The formation of spatial pattern in developmental biology is a central issue and

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is still not resolved. Although genes play a crucial role in embryology, a study of genetics alone will not be sufficient to help us understand how the physicochemical properties of embryonic material interact to produce the complex spatio-temporal signalling cues which ultimately determine cell fate. An ideal tool for studying such complex non-linear interactions is mathematical modelling, and there is now a vast literature on such models (Murray, 1993). In Section 7.2 some models for biological pattern formation will be classified and their applications discussed. In Section 7.3 the coupling of such models will be illustrated by two examples: the formation of feather germs in dorsal chick skin, and aggregation in cellular slime mould. In Section 7.4 the role of domain growth will be analysed in the context of the spatio-temporal patterning of tooth primordia in alligators and in pigmentation patterning in fish. Finally, Section 7.5 contains a discussion of future goals and areas of research.

7.2 Simple Models

A number of models have been proposed to account for spatio-temporal patterning phenomena and regeneration in several areas of developmental biology. In this section, I broadly classify these models. I term them *simple models* because in later sections I will consider the coupling of such models. But from a mathematical viewpoint, they can be far from simple!

7.2.1 Chemical prepattern models

Chemical prepattern models assume that a spatial pattern in some chemical (termed a morphogen) is set up and cells respond to this pattern by differentiating accordingly. Wolpert (1969) proposed a simple model in which a source–sink mechanism, coupled with diffusion and degradation, led to a spatial gradient in a single morphogen. He proposed that this provided *positional information* for cells, which differentiated according to a series of threshold values.

A more complicated spatial pattern can be set up by the interaction of two chemicals. Turing (1952) showed that a system of two chemicals reacting and diffusing could evolve to a spatial pattern in chemical concentration. Such a pattern consisted, in its simplest one-dimensional form, of a series of peaks and troughs. These models may be couched in terms of partial differential equations, or discretized and analysed as cellular automata (Bard, 1981).

These models differ in the sense of cell interpretation of the chemical prepattern. In Wolpert's model, the chemical prepattern is set up by a simple process which can only produce a simple gradient. To use this gradient to generate a complicated pattern, it is hypothesized that a complex series of thresholds exist and cells have the machinery to interpret multiple thresholds. In Turing's model, complex spatial patterns arise due to a complex chemical interaction, but the interpretation of the prepattern is via a single threshold (Nagorcka, 1989).

Turing's model takes this form:

$$\frac{\partial u}{\partial t} = D_u \nabla^2 u + f(u, v) \tag{7.1}$$

$$\frac{\partial v}{\partial t} = D_v \nabla^2 u + g(u, v) \tag{7.2}$$

where $u(\underline{x}, t), v(\underline{x}, t)$ denote chemical concentrations at position $\underline{x}$ and time t, D_u, D_v are diffusion coefficients and f, g are reaction kinetics. These equations are solved subject to certain initial conditions, typically random perturbations about a uniform steady state, and boundary conditions, which are typically periodic, zero flux, or fixed. The forms of the kinetic terms may be derived in a number of ways.

Phenomenological Models

The kinetic terms are chosen such that one of the chemicals is an *activator*, the other an *inhibitor*. An example is the Gierer–Meinhardt model (1972):

$$f(u, v) = \alpha - \beta u + \gamma u^2 / v, \tag{7.3}$$

$$g(u, v) = \delta u^2 - \mu v, \tag{7.4}$$

where $\alpha, \beta, \gamma, \delta$ and μ are constants.

Hypothetical Models

Derived from a hypothetically proposed series of chemical reactions. For example, Schnakenberg (1979) proposed a series of trimolecular autocatalytic reactions involving two chemicals:

$$X \underset{k_2}{\overset{k_1}{\rightleftharpoons}} A, \quad B \xrightarrow{k_3} Y, \quad 2X + Y \xrightarrow{k_4} 3X.$$

Using the law of mass action and denoting the concentrations of X, Y, A and B by u, v, a and b, respectively, we have

$$f(u, v) = k_2 a - k_1 u + k_4 u^2 v, \tag{7.5}$$

$$g(u, v) = k_3 b - k_4 u^2 v \tag{7.6}$$

where $k_1, \ldots, k_4$ are (positive) rate constants. Assuming that there is an abundance of A and B, a and b can be considered to be approximately constant.

Empirical Models

The kinetics are fitted to experimental data. For example, the Thomas (1975) immobilized-enzyme substrate-inhibition mechanism involves the reaction of uric acid (concentration u) with oxygen (concentration v). Both reactants diffuse from a reservoir maintained at constant concentrations u_0 and v_0, respectively, onto a

membrane containing the immobilized enzyme uricase. They react in the presence of the enzyme with empirical rate $V_m uv/(K_m + u + u^2/K_s)$, so that

$$f(u,v) = \alpha(u_0 - u) - \frac{V_m uv}{K_m + u + u^2/K_s}, \tag{7.7}$$

$$g(u,v) = \beta(v_0 - v) - \frac{V_m uv}{K_m + u + u^2/K_s} \tag{7.8}$$

where α, β, V_m, K_m and K_s are positive constants.

Actual Chemical Reactions

Although it was in 1952 that Turing predicted the spatial patterning potential of chemical reactions, this phenomenon has only recently been realized in actual chemical reactions. Therefore, it is now possible in certain cases to write down detailed reaction schemes.

The first Turing patterns were observed in the chlorite–iodide–malonic acid starch reaction (CIMA reaction) (Castets *et al.*, 1990; De Kepper *et al.*, 1991). The model proposed by Lengyel and Epstein (1991) stresses three processes: the reaction between malonic acid (MA) and iodine to create iodide, and the reactions between chlorite and iodide and between chloride and iodide. These reactions take the form

$$\mathrm{MA} + \mathrm{I_2} \rightarrow \mathrm{IMA} + \mathrm{I^-} + \mathrm{H^+}$$

$$\mathrm{ClO_2} + \mathrm{I^-} \rightarrow \mathrm{ClO_2^-} + \frac{1}{2}\mathrm{I_2}$$

$$\mathrm{ClO_2^-} + 4\mathrm{I^-} + 4\mathrm{H^+} \rightarrow \mathrm{Cl^-} + 2\mathrm{I_2} + 2\mathrm{H_2O}.$$

The rates of these reactions can be determined experimentally. By making the experimentally realistic assumption that the concentrations of malonic acid, chlorine dioxide and iodine are constant, Lengyel and Epstein derived the following model:

$$\frac{\partial u}{\partial t} = k_1 - u - \frac{4uv}{1+u^2} + \nabla^2 u$$

$$\frac{\partial v}{\partial t} = k_2\left[k_3\left(u - \frac{uv}{1+u^2}\right) + c\nabla^2 v\right]$$

where u, v are the concentrations of iodide and chlorite, respectively, and k_1, k_2, k_3 and c are positive constants.

General results on the patterning properties of reaction-diffusion equations can be found in the books by Britton (1986), Edelstein-Keshet (1988), Fife (1979), Grindrod (1996), Murray (1993) and Segel (1984).

7.2.2 Cell movement models

Cell movement models assume that a spatial pattern arises in cell density, and cells then differentiate in a density-dependent manner. There are a number of ways in which

cell aggregation can occur. For example, cells can move in response to gradients in certain chemicals (chemotaxis) and it has been shown by a number of authors that such cell–chemical interactions can lead to spatial pattern formation (Keller and Segel, 1971; Maini *et al.*, 1991). These models involve reaction and diffusion, but spatial patterning arises due to the advective term introduced by chemotaxis. The typical model takes this form:

$$\frac{\partial n}{\partial t} = D_n \nabla^2 n - \nabla.(\chi(u) n \nabla u) + f(n, u) \tag{7.9}$$

$$\frac{\partial u}{\partial t} = D_u \nabla^2 u + g(n, u), \tag{7.10}$$

where $n(\underline{x}, t), u(\underline{x}, t)$ denote cell density and chemoattractant concentration, respectively, at position $\underline{x}$ and time t, D_n, D_u are diffusion coefficients, f, g are terms incorporating production and degradation and $\chi(u)$ is the chemotactic sensitivity.

Cells can also respond to mechanical cues, such as movement in response to advective forces exerted by cells on a common substratum, or due to movement up gradients in adhesive activity (haptotaxis). The first model incorporating these ideas was proposed by Oster *et al.* (1983) and since then such models have been extensively studied (Murray, 1993). The mathematical equations of these models are quite different. They consist of conservation equations for cells and extracellular matrix, which take the general form of the equations above, but the main difference is the force-balance equation, which describes the behaviour of a viscoelastic material.

It has also been proposed that cells move to minimize energy (Cocho *et al.*, 1987a, b; Steinberg, 1970; Sulsky *et al.*, 1984). Such models can be set up mathematically and solved to show cell sorting and patterning behaviour consistent with a number of experimentally observed phenomena.

7.2.3 Applications

Turing assumed that the chemicals in his reaction-diffusion system were growth hormones, so the spatial pattern in chemical concentrations would result in spatially non-uniform growth and hence pattern. He applied his theory to account for whorled leaves and to growth-induced shape changes in the early embryo which he proposed could account for gastrulation. Since his seminal paper, reaction-diffusion models have been proposed to account for a vast number of patterning processes in Nature, too great to be completely reviewed here, so here are a few examples to give a flavour of the applications.

Geirer and Meinhardt (1972) used their model to account for pattern formation in *Hydra* and their model was also consistent with several of its regenerative properties. Reaction-diffusion models have been used to account for compartmentalization in insect development and to provide an explanation for the occurrence of various mutants (Meinhardt, 1982). However, for *Drosophila* it now appears that patterning is due to a cascade of protein interactions rather than to a reaction-diffusion mechanism.

Reaction-diffusion models have been applied to shell patterns (Meinhardt, 1995) and to butterfly wing pigmentation patterns (Nijhout, 1990). Reaction-diffusion and cell movement models have been applied to animal coat markings (Bard, 1981; Cocho

et al., 1987a, b; Murray, 1981; Murray and Myerscough, 1991; Murray *et al.*, 1990) and to skeletal patterning in the limb (Maini and Solursh, 1991).

Although these models are based on very different biological assumptions, many of them share common properties. For example, the patterning in reaction-diffusion and in many cell movement models arises from the interaction of *short-range activation* and *long-range inhibition*. On the one hand, this has the disadvantage of making it very difficult to use models to distinguish between mechanisms; on the other hand, it does mean that one can make general conclusions and predictions that are *mechanism-independent*. This leads to the idea of *developmental constraints*: only certain patterns are possible, regardless of the mechanism (Oster and Murray, 1989).

7.3 Coupled Models

Many pattern formation phenomena arise as the result of the coupling of mechanisms. In some cases the mechanisms on their own do not produce pattern, it is the interaction that leads to pattern; in other cases each subsystem can exhibit patterns, and the coupling leads to complex patterning. In this section I consider an example from each class of model.

7.3.1 Feather germ formation

The formation of skin organs, e.g. hair, teeth, feathers and sweat glands, has been widely studied. The skin is composed of two basic layers, the epidermis and the dermis, which are separated by a thin layer of tissue called the basal lamina. In many cases these layers interact during organ formation. An example of this is the formation of feather germs, which determine the future sites of feathers. A feather germ consists of an epidermal thickening, termed a placode, overlying a condensation of cells in the dermis, termed a papilla. On the chick back, these form along the dorsal midline and then propagate out on either side, eventually forming a hexagonal lattice of feather germs.

A number of models have been proposed to account for this tissue interaction. For example, Nagorcka *et al.* (1987) considered a model of two coupled reaction-diffusion systems, while Shaw and Murray (1990) used a coupled mechanochemical system. Both models were shown to exhibit spatial patterns. These tissue interaction models have the common property of being able to produce patterns independently in the dermis and the epidermis. However, experimental studies show that the dermis cannot produce coherent patterns without the presence of the epidermis and vice versa. In other words, tissue interaction is crucial for the patterning process.

In an attempt to address this issue, Cruywagen and Murray (1992) proposed a mechanochemical model in which epidermal cells produce a chemical that diffuses into the dermis, where it acts as a chemoattractant for dermal cells, while the dermis secretes a chemical that diffuses into the epidermis, where it serves to enhance the tractional forces exerted by epidermal cells. The full model consists of seven partial differential equations, but using some approximations and quasi-steady-state assumptions on the chemical equations, it may be reduced to the following system of two equations (I refer the reader to the original paper for full details of the experimental motivation and mathematical derivation of the model equations):

$$\nabla^2\theta - \beta\nabla^4\theta + \nabla^2\left\{\frac{\tau n^2}{1+cn^2}\right\} = \rho\theta \tag{7.11}$$

$$\frac{\partial n}{\partial t} = D\nabla^2 n - \nabla\cdot(n\nabla\alpha(1-\theta))\,. \tag{7.12}$$

The first equation is derived by considering the epidermis as a viscoelastic sheet and then assuming that the viscous terms are set to zero. This leads to an equation for the dilation θ, where β, τ, c and ρ are constants. A further approximation yields the relation $N = 1 - \theta$, for the epidermal cell density N. Dermal cell density is denoted by n and the third term in the first equation incorporates the enhancement of cell traction in the epidermis due to the chemical produced by the dermal cells. The second equation models dermal cell diffusion and chemotaxis, where the effect of the epidermally secreted factor on dermal chemotaxis is incorporated into the second term on the right-hand side. This model has been extensively studied (Cruywagen *et al.*, 1992; 1994a, b) and shown to exhibit many of the properties of the full model. Crucially for this model, neither submodel on its own can exhibit spatial patterning. It is the coupling of the submodels that leads to pattern formation. It has been shown that this model can exhibit propagating patterns consistent with those observed experimentally (Figure 7.1).

7.3.2 Cell aggregation in slime mould

The life cycle of the cellular slime mould *Dictyostelium discoideum* serves as an excellent paradigm for morphogenesis and has for many years attracted the interest of developmental biologists and theoreticians. Under favourable conditions, the amoebae feed on bacteria in the soil and divide. Starvation triggers a developmental programme which is initiated by cell–cell signalling via the extracellular messenger cyclic 3′5′-adenosine monophosphate (cAMP). The chemotactic response to this signal leads, through the phenomenon of cell streaming, to the formation of a multicellular organism composed typically of 10^4 to 10^5 cells. This organism passes through an intermediate motile (slug) phase during which cells differentiate into prespore and prestalk types, before developing a fruiting body, aiding the dispersal of spores from which new amoebae develop under favourable conditions. The comparative simplicity of morphogenesis in *Dictyostelium* has made it an attractive model system for the study of self-organization, and many of the molecular and cellular mechanisms which are involved in cell aggregation, collective movement and differentiation have now been identified.

A large body of experimental and theoretical work exists on the molecular mechanisms of the cAMP signalling dynamics, and models incorporate the detailed biochemical dynamics of the binding of extracellular cAMP to its surface receptors (Devreotes, 1989; Goldbeter, 1996; Tang and Othmer, 1994).

The incorporation of diffusion of extracellular cAMP into these models yields a description of the signalling dynamics in a stationary cell layer, which is a valid approximation for the situation at the beginning of cell aggregation (Tyson *et al.*, 1989; Monk and Othmer, 1990; Tang and Othmer, 1995). This leads to a coupled system of reaction-diffusion equations in which the variables are extracellular cAMP

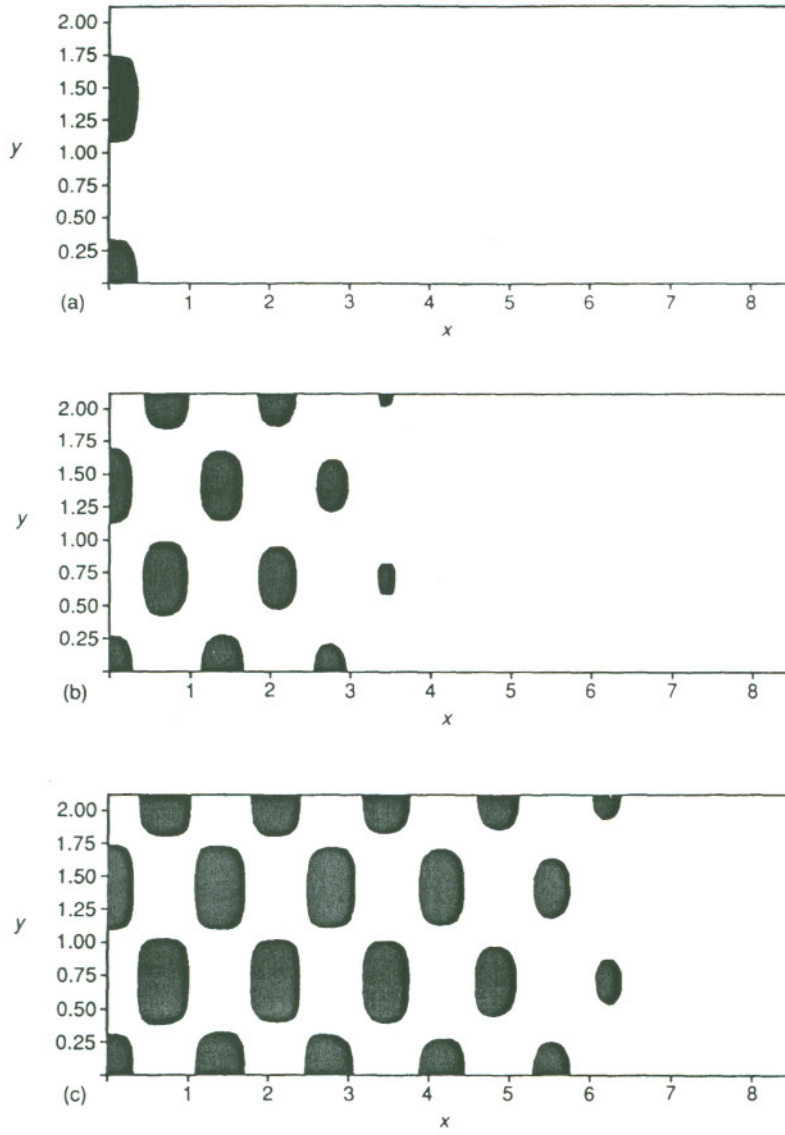

Figure 7.1 Sequential pattern formation in the caricature model (7.11)-(7.12) for tissue interaction. (a) Initially a single row consisting of spots of high cell density is specified at one end of a rectangular domain. (b, c) As the system evolves, the pattern propagates with more rows added sequentially. In this way the mechanism converts a simple one-dimensional pattern into a complicated two-dimensional form. Regions of high cell density are shaded. (Reproduced with permission from Cruywagen *et al.*, 1992)

concentration and density of cAMP receptors. This model is of excitable type and exhibits spiral waves and target patterns of cAMP concentration.

To model cell streaming, one must couple cell movement and chemotaxis to this excitable system. So far, this has been modelled in two different ways. The first approach consists of modelling discrete cells equipped with cAMP-dependent movement rules coupled to a finite-difference approximation for the continuous cAMP dynamics (Dallon and Othmer, 1997; Kessler and Levine, 1993; Van Oss *et al.*, 1996). A second approach is to approximate the cell distribution by a continuous density, resulting in a system of coupled partial differential equations for the cell density and the cAMP dynamics (Vasiev *et al.*, 1994; Höfer *et al.*, 1995a, b). The former approach has the advantage that one can follow distinct cells and compare their detailed motion with that of amoebae in experiments. The latter modelling approach allows one to use the extensive analytical machinery of partial differential equation theory to fully understand how each process and parameter affects model behaviour. However, both models rely on the same mechanism – a chemotactic cell response to an excitable system involving extracellular cAMP and cAMP receptors.

Consider the continuum model of Höfer *et al.* (1995). The nondimensionalized model takes the following form:

$$\frac{\partial n}{\partial t} = \nabla \cdot (\mu \nabla n - \chi(v) n \nabla u) \tag{7.13}$$

$$\frac{\partial u}{\partial t} = \lambda[\phi(n) f_1(u, v) - (\phi(n) + \delta) f_2(u)] + \nabla^2 u \tag{7.14}$$

$$\frac{\partial v}{\partial t} = -g_1(u) v + g_2(u)(1 - v), \tag{7.15}$$

where n, u and v denote cell density, extracellular cAMP concentration and fraction of active cAMP receptors, respectively. We motivate each equation in turn. The first equation is the conservation equation for cell density. It is assumed that cell movement has a random component, with cell diffusion coefficient μ, and a chemotactic component, with sensitivity denoted by $\chi(v)$. To account for adaptation, the latter is taken to be of the form $\chi(v) = \chi_0 v^m/(A^m + v^m)$, $m > 1$, where χ_0 and A are positive constants. Hence an appreciable chemotactic response requires a minimal fraction of active receptors, yet the response saturates for a large fraction of active receptors. Note that many models of chemotaxis assume χ to be a constant. Under that assumption, cells would respond to a pulse of chemoattractant by moving towards the wave in the wavefront, then moving with the wave in the waveback, resulting in a net movement away from the source of attractant, rather than towards it. This is the so-called chemotactic wave paradox (Soll *et al.*, 1993). The form of $\chi(v)$ chosen above resolves this paradox (Höfer *et al.*, 1994).

The second and third equations are a simplified model of the cAMP–cell receptor dynamics modified by cell density effects (Martiel and Goldbeter, 1987). The first term on the right-hand side of the second equation accounts for cAMP production by assuming that the rate of cAMP synthesis per cell is $f_1(u, v) = (bv + v^2)(a + u^2)/(1 + u^2)$. This models autocatalytic cAMP production with saturation, mediated by cAMP binding to active receptors. The rate of cAMP degradation per cell is taken to be $f_2(u) = du$. The cell density dependence is reflected in the factor

$\phi(n) = n/(1 - \rho n/(K + n))$, and δ accounts for cAMP degradation in the absence of cells (Höfer *et al.*, 1995a). The parameters a, b, d, ρ, K, δ and λ are all positive constants.

The first term on the right-hand side of the third equation accounts for receptor densitization and, assuming the law of mass action, $g_1(u) = k_1 u$. The second term on the right-hand side models resensitization of the desensitized $(1 - v)$ fraction of receptors, at the constant rate $g_2(u) = k_2$. The parameters k_1 and k_2 are positive constants.

Good estimates of most of the parameters are available from the experimental literature (Höfer *et al.*, 1995b), and substituting these values into the model, we find that the model captures the key features of the aggregation process. A typical aggregation sequence is shown in Figure 7.2.

It is found that the initial uniform state is unstable from the outset, suggesting that the coupled dynamics of cAMP wave propagation and cell movement exhibit a patterning instability perpendicular to the direction of wave propagation. An analytically tractable caricature of the above model has been proposed by Höfer *et al.* (1995b) from which it is possible to derive explicit conditions on the parameters for the uniform steady state to go unstable. The dispersion relation predicts the wavelength of the fastest-growing unstable mode, and agrees closely with the results of numerical simulations and experimental observations.

A more detailed numerical study of the model reveals that this minimal model, based on biochemical and mechanical mechanisms established at the cellular level, can account for a number of other important properties. For example, our numerical simulations show that low initial cell densities lead to the formation of a central hole. The formation of a central hole is observed experimentally, if the system is treated with caffeine (Steinbock and Müller, 1995). Caffeine treatment lowers the excitability of the system, and in the model this corresponds to lowering λ. This is equivalent, in some sense, to lowering the cell density n. Another key experimental prediction of the model is discussed in Section 7.5.

7.4 The Role of Domain Growth

In many developmental systems, domain growth does not play a role in pattern *formation*, it simply results in already established patterns growing in size. However, in some cases, domain growth is crucial for the pattern *selection* process. Here are two such examples.

7.4.1 Alligator tooth primordia

Experimental evidence suggests that jaw growth plays a crucial role in the spatial positioning of tooth primordia in *Alligator mississippiensis*. These primodia are either resorbed or differentiate to form functioning teeth. It also appears that existing primordia inhibit neighbouring primordium formation. Although there is substantial literature on dentition development in vertebrates, the mechanism of primordium morphogenesis is still not understood. In this case the role of mathematical modelling is to determine whether the processes thought to be of most significance (here domain growth and inhibition) are sufficient to produce the observed patterns.

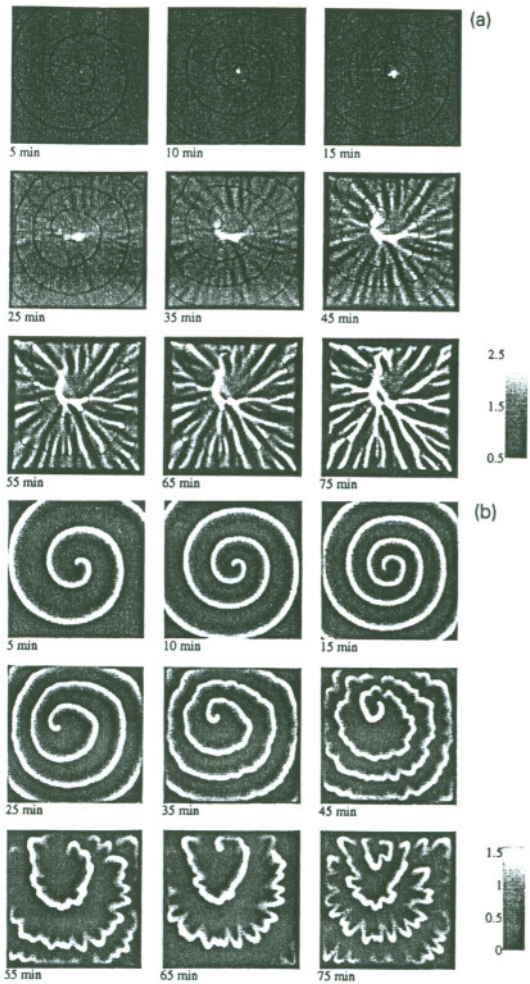

Figure 7.2 Spatio-Temporal evolution of (a) cell density, and (b) cAMP concentration in a numerical simulation for equations (7.13) to (7.15). (Reproduced with permission from Höfer *et al.*, 1995b)

The formation of primordia in the lower jaw is symmetric about the midline, so one can consider only half the lower jaw. The first seven primordia are initiated in the spatio-temporal sequence $7-3-6-2-5-1-4$ where the 4 denotes the anterior-most primordium (the 4th primordium to form) and 7 denotes the posterior-most primordium (the 7th to form). Kulesa *et al.* (1996) investigated the spatial patterning properties of the following model (I refer the reader to the original paper for full details on the experimental background, motivation and derivation of the model):

$$\frac{\partial u}{\partial t} = \gamma(hc - u + u^2 v) - ru + \exp(-2rt)\frac{\partial^2 u}{\partial x^2} \tag{7.16}$$

$$\frac{\partial v}{\partial t} = \gamma(b - u^2 v) - rv + d\ \exp(-2rt)\frac{\partial^2 v}{\partial x^2} \tag{7.17}$$

$$\frac{\partial c}{\partial t} = -\delta c - ru + p\ \exp(-2rt)\frac{\partial^2 c}{\partial x^2}. \tag{7.18}$$

This is the non-dimensionalized version of the model on the spatial domain $[0,1]$, where u and v satisfy zero flux boundary conditions, $c(0,t) = c_0(t)$, with c satisfying zero flux at $x = 1$.

In this model, u and v are components satisfying a modified Schnakenberg (1979) kinetics with the source of u depending on the chemical c, which itself diffuses and is degraded linearly. The other linear degradation terms represent dilution due to the assumption that the domain is growing exponentially with constant growth rate r. Domain growth also modifies the diffusion coefficients by the factor $\exp(-2rt)$ when the domain is non-dimensionalized to a fixed domain.

In this model, the source term can be taken as the bifurcation variable. As this decreases below a critical value, diffusion-driven instability occurs and a spatially varying pattern in u and v is set up. Initially, parameter values are chosen so that the source profile is such that everywhere in space it is above the critical bifurcation value. As the domain grows, the c profile becomes sufficiently low for diffusion-driven instability to occur in the u, v system and u evolves to a one-peaked pattern. It is then assumed that this peak marks a tooth primordium and initiates the production of c, which acts as an inhibitor to further primordium initiation. This ensures that the second primordium forms sufficiently far away from primordium 1. Kulesa *et al.* showed that this mechanism could lead to the correct spatio-temporal initiation of the first seven tooth primordia. Hence they conclude that the interaction of inhibition and domain growth is a plausible mechanism for generating this complex sequence. Such a mathematical model can be used to make predictions about the outcome of experimental manipulations.

7.4.2 Pigmentation patterning in fishes

Unlike mammalian skin patterns, which simply enlarge with body growth, patterns on certain fishes change *qualitatively* with domain growth. A striking example of this is the stripe pattern on the marine angelfish *Pomacanthus*. As the fish grows, its stripes grow wider apart until they are about twice as far apart as in the juvenile case. At this stage, new stripes appear between the existing stripes in such a way as to restore the original interstripe spacing. The new stripes are thinner than existing stripes but

they gradually broaden.

Kondo and Asai (1995) considered a simple reaction-diffusion model on a one-dimensional growing domain and showed that it exhibited similar mode-doubling behaviour to that observed in an one-dimensional anterior–posterior cross-section of *Pomacanthus* but there was one crucial drawback – their model predicted that all stripes had the same width.

Recently, Painter *et al.* (1999) have considered a generalized Turing model for this phenomenon on a two-dimensional domain which represents the surface of the fish. In their model, the pigmentation pattern is assumed to arise as a result of cell movement (this is consistent with results on zebrafish). They consider a typical reaction-diffusion system coupled to a cell movement equation of the form

$$\frac{\partial n}{\partial t} + \nabla.n\underline{v} = D_n\nabla^2 n - \nabla.[\chi_u(u)n\nabla u + \chi_v(v)n\nabla v] + n(r_x + r_y) \tag{7.19}$$

where $\underline{v} = (r_x x\mathbf{i} + r_y y\mathbf{j})$ is the uniform velocity field generated by growth, r_x and r_y are growth rates in the x and y directions, respectively, u and v are the concentrations of the chemicals in the reaction-diffusion system, n is pigment cell density and $\chi_u(u)$ and $\chi_v(v)$ are the chemotactic sensitivities.

They show that this system can produce the mode-doubling phenomenon observed in *Pomacanthus*, with the inserted stripes being narrower than the existing stripes. The reason behind this behaviour is that as the domain grows, the pattern in the underlying reaction-diffusion system undergoes mode doubling with stripes all of the same size. However, under the biologically realistic assumption that cell movement is on a longer timescale than chemical diffusion, the cell rearrangement induced by this new pattern occurs very slowly, resulting in the new inserted stripes being thinner than the existing stripes, broadening only gradually. More generally, this model system is using chemotaxis as a novel positional information interpreter.

7.5 Discussion and Future Directions

Development of spatial pattern and form is unquestionably one of the central areas in biology, as was recognized by D'Arcy Thompson. It has led to genuine interdisciplinary research which has advanced many areas of science and mathematics. For example, the study of parabolic systems of partial differential equations was largely motivated by Turing's paper. The rich behaviour of these systems has resulted in the development of a large body of mathematical theory. Cell movement models pose different mathematical questions, such as blow-up and collapse (Othmer and Stevens, 1997), while mechanical models are a system of mixed hyperbolic/parabolic/elliptic equations for which very little theory exists.

While the study of pattern formation has been the source of a great number of mathematical problems, it is reasonable to ask what role mathematics has to play in pattern formation. It is clear that pattern formation in many areas arises as the result of a complex interaction of mechanisms. The language of mathematics is ideally suited to understanding non-linear interactions and it is only through mathematical and computational approaches that one can predict the outcome of such complex interactions. This allows one to make predictions that are experimentally testable. Of course, no mathematical model can take into account *every* process:

approximations and simplifications have to be made. In this respect, mathematics is like any other scientific tool – it has its limitations, but it can be used in conjunction with experimental tools to help elucidate the underlying mechanisms involved in pattern formation.

Presently, biology offers mathematics a range of challenges. At one extreme are the areas such as slime mould, in which many of the processes are known and data on parameters available. Here the role of modelling is to make precise experimental predictions on the effects of manipulating small details in the system. At the other extreme, in which most areas lie, are systems in which very little is known. In such cases the role of modelling is to see what processes are sufficient to produce the patterning; for example, in the case of alligator teeth it has been shown that domain growth, coupled with inhibition, is sufficient to produce the required sequence of pattern. This does not mean that this *is* the mechanism, it only says that it is a candidate mechanism. It also makes experimentally testable predictions.

For a model to be of use, it must provoke experimentation. An example of this is in the skeletal development of the chick limb. Both reaction-diffusion and cell movement models predict that the complexity of pattern is intimately linked to the size of the domain. To test this theory, Wolpert and Hornbruch (1990) constructed double anterior chick limbs which had the same size as a normal chick limb. They found that the double anterior limbs gave rise to two humeral bones, even though the limb was the same size as a normal limb, which only produces a single humerus. This contradicts the model predictions. Maini *et al.* (1992), however, showed that if there was a spatially varying diffusion coefficient, then a reaction-diffusion system could predict such a pattern. At the same time as this work was being carried out, Brümmer *et al.* (1991) showed that there was indeed a gradient in diffusivity across the limb bud.

Another use of mathematical models is to provide alternative explanations for observed phenomena. For example, in slime mould, it is observed in situ that as streaming proceeds, the wave speed and wavelength of the spiral patterns decrease (Gross *et al.*, 1976). Previously this has been explained by assuming that biochemical changes must be occurring in the cell–cAMP system. However, an alternative, and much simpler explanation comes out of the modelling. As cell streams grow, they alter the propagation conditions for the cAMP waves. This is initially equivalent to increasing λ in equation (7.14), leading to an increase in excitability of the medium. This in turn leads to an increase in the rotation frequency of the spiral core. As a result (Tyson and Keener, 1988) the wave speed and wavelength of the spiral patterns decrease. Hence the model shows that complex biochemical changes are not necessary to account for the observations.

One of the key areas of future study is domain growth. An understanding of how this can be incorporated into the modelling and the effects that it has on the patterns exhibited by models is an exciting new area to be investigated. Preliminary studies show that the mode-doubling phenomenon mentioned in Section 7.4 is very robust. If one wishes to generate a certain number of stripes on a large domain using reaction-diffusion theory then, due to the multiplicity of stable solutions on large domains, the pattern produced may be very sensitive to initial conditions. However, if we start off with a small domain and let it grow, then the correct stripe pattern can be generated robustly. Alternatively, boundary conditions can be used to select certain patterns

(Dillon *et al.*, 1994).

Another important area for study is the effect of domain geometry on the patterns predicted. Kauffman *et al.* (1978) solved a reaction-diffusion system on an ellipse and showed that the nodal lines of chemical concentration closely matched the compartmental division lines observed in *Drosophila* wing imaginal disc. However, Bunow *et al.* (1981) showed that if the equations were solved on a more realistic geometry, the nodal lines differed significantly from those on the ellipse. It is surprising that so little work has been done on the role of domain shape. However, with increased computer power, this is becoming easier to do. The effect of domain shape on patterns in fish is presently being analysed (Varea *et al.*, 1997, Madzvamuse, 1999). The advances in computer technology mean that such studies are now more feasible.

Until now most models have been couched in terms of coupled systems of partial differential equations, in which quantities such as cell density are modelled as continuum variables. This allows one to use the methods of applied mathematics to investigate solution behaviour. However, the continuum approximation is not a good one in the limit of low density, when cells really behave as discrete entities. Furthermore, advances in biotechnology now enable single cell trajectories to be followed in some systems (e.g. slime mould). Therefore, in such areas, models considering cells as discrete entities are more appropriate. This leads to coupled systems of discrete ordinary partial differential equations and a whole new area of mathematics.

In most cases thus far, modelling has been phenomenological. Recently Othmer and Stevens (1997) have investigated in detail how one can derive models at the macroscopic cell population level by considering processes for movement at a microscopic cellular level. In this way they were able to show how different movement rules at a local level could result, for example, in the different phenomenological forms of chemotactic sensitivity commonly used. Such a modelling approach leads to a greater degree of insight about the processes which are present.

Although I have focused here on differential equations, integral equations can also be used to model pattern formation. In this framework, a convolution kernel is used to model the patterning interaction. Recently Sekimura *et al.* (1999) used a novel model of this form to account for the formation of stripe patterns in scale cells in the butterfly wing. In particular, they showed how a spatial gradient in a parameter can robustly select rows of the correct orientation over other rows or spots.

In conclusion, growth and form provide a seemingly endless source of interesting and novel mathematical problems, while mathematics can be used as a tool to explore different mechanisms and processes underlying these phenomena. For the area to advance, we must have truly interdisciplinary research efforts involving biological experimentation, mathematical modelling and computational investigations. The recent advances in biotechnology, coupled with the rapid increases in computational power, now make such progress a real possibility.

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