5. Spatial and Functional Organization

Coupled Models for Spatial Organization in Development

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Several simple models have been proposed to account for spatial organization in early development. Recently, a number of models have been proposed in which such patterning mechanisms are coupled. Here, we present a review of some of the latest theoretical results on such systems. In particular, we consider two cases: in one, the coupling of two mechanisms greatly enhances the robustness of the patterns produced; in the other, a hierarchical model exhibits spatially asymmetric patterns that are consistent with certain experimental observations.

1 Introduction

Spatial organization in early development is one of the central issues in embryology. Many models have been proposed to account for spatial pattern formation in such diverse areas as skeletal patterning in the vertebrate limb, the marking on animal coats, the formation of regular patterns in tissue organ primordia, segmentation patterns in *Drosophila*, aggregation and differentiation in *Dictyostelium Discoideum*, to name but a few. Two important models are chemical pre-pattern models, which propose that a spatial pre-pattern is set up in some chemical concentration to which cells respond by differentiating if the concentration breaches a threshold value [24]; and mechanochemical models, which propose that the mechanical and chemical interaction of cells with their environment leads to a spatial pattern in cell density and cells within high density aggregates then differentiate. The pattern formation potential of these models has been widely studied. The book by Murray [17] is an excellent and detailed review of this work.

The patterns produced by these models, however, are unable to capture essential experimental observations in some cases and this has prompted a number of authors to consider the pattern formation potential of more complicated models in which mechanisms are coupled together. This views spatial organization as a hierarchy of events. For example, many of the above models, such as reaction-diffusion models for chemical pre-patterns, exhibit patterns that increase in complexity with increasing domain size. Thus they are unable to account for patterns that exhibit a large degree of scale-invariance. For example, the slug stage of the slime mould *Dictyostelium Discoideum* is composed of pre-stalk and pre-spore cells. These cell populations are in a ratio that remains fixed over several orders of magnitude in slug length. Othmer and Pate [21] considered a modified reaction-diffusion system in which the diffusion coefficient of one of the chemicals was determined by a control chemical which, itself, satisfied a reaction-diffusion equation. They showed that this modified model could exhibit patterns that were scale invariant.

In skin morphogenesis, certain organs occur in patterns that are composed to two very different wavelengths. This appears to be inconsistent with standard models in which a preferred wavelength is selected. Nagorcka et al. [18] considered a tissue interaction model for the formation of skin organs. They showed that coupling a reaction-diffusion model with a mechanochemical model could lead to complex pattern formation in which the pattern was composed of two spatial patterns, each having very different wavelengths. More recently, Cruywagen et al. [5] considered a tissue interaction model in which each mechanism alone was incapable of producing pattern, but when coupled, the full model produced a diverse range of patterns.

One of the major criticisms of the application of reaction-diffusion (or Turing models [23]) to pattern formation in embryology is that the patterns they generate are too sensitive to variation in parameter values or initial conditions for them to realistically account for robust patterning mechanisms [1]. Moreover, Turing patterns are symmetrical in the sense that each peak in concentration has the same height and the wavelength is constant across the domain, at least near to the bifurcation point, whereas many patterns in embryology are asymmetrical. In Section 2 we present two modifications of the Turing model in one spatial dimension. In Model 1, the role of boundary conditions is investigated. It is shown that appropriately selected boundary conditions can greatly enhance the robustness of the resulting patterns. Model 2 is a hierarchical model in which the diffusion coefficients of a reaction-diffusion system are determined by a control chemical. It is shown that this model exhibits spatially asymmetric solutions. An application of these results is presented in Section 3 and both models are discussed in Section 4.

2 Generalised Turing Models

The generalised nondimensionalised Turing model for two species in one space dimension takes the form

$$u_t = \gamma f(u, v, p) + (D_u u_x)_x$$
$$\text{in } (0,1) \qquad (1)$$
$$v_t = \gamma g(u, v, p) + (D_v v_x)_x$$

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with boundary conditions

$$\theta_1 \frac{\partial u}{\partial n} = \rho(1-\theta_1)(\theta_3 u^s - u) \quad \text{for } x = 0,1 \tag{2}$$

$$\delta\theta_2 \frac{\partial v}{\partial n} = \delta\rho(1-\theta_2)(\theta_3 v^s - v) \ ,$$

where $u(x,t)$ and $v(x,t)$ are nondimensionalised chemical concentrations at position x and time t, with $x \in [0,1]$; γ is a nondimensional parameter proportional to the dimensional length L of the domain, and the diffusion coefficients of u and v are D_u and D_v, respectively. The functions f and g are rational polynomials which model the reaction kinetics and p denotes the vector of kinetic parameters. The parameters $\theta_i \in [0,1]$, $i = 1,2,3$ are homotopy parameters, and u^s and v^s denote the uniform steady state values of morphogen concentrations, that is, $f(u^s, v^s, p) = g(u^s, v^s, p) = 0$.

When $(\theta_1, \theta_2, \theta_3) = (1,1,\cdot)$ the boundary conditions (2) reduce to homogeneous Neumann conditions (zero flux), and when $(\theta_1, \theta_2, \theta_3) = (0,0,1)$ we have Dirichlet conditions fixed at the uniform steady state. These two types of boundary conditions are referred to as scalar boundary conditions [7]. If D_u and D_v are constant, then the above system, with scalar boundary conditions, is the standard Turing model that has been widely studied. We now consider two variations of the standard model.

Model 1

The standard Turing model is clearly a very special of the above general model. For example, if $(\theta_1, \theta_2, \theta_3) = (1,0,1)$, then u satisfies homogeneous Neumann boundary conditions, and v satisfies Dirichlet conditions fixed at the uniform steady state for v. This is an example of a non-scalar boundary condition. The effects of such non-standard boundary conditions was investigated in [7] for (1) with constant diffusion coefficients. It is important to note that the mathematical analysis becomes much more complicated as soon as one moves away from the standard case. For example, for this particular set of non-scalar boundary conditions, the eigenfunctions of the linearised system are no longer simple sines or cosines. Moreover, for the case $(\theta_1, \theta_2, \theta_3) = (1,0,0)$, for example, if v^s does not equal 0 then the system does not have a uniform steady state. There are, however, some special cases in which a non-standard linear analysis can be carried out (see [7]). The time evolution model can be analysed by solving the system numerically. The steady state problem can be investigated using numerical bifurcation packages such as AUTO [6] which calculate the steady states and their stability as a bifurcation parameter is varied. For the case of scalar boundary conditions, it is well known that a minimum domain length is required for a spatially non-uniform steady state to exist and the bifurcation diagram and steady states increase in complexity with increasing domain length. Furthermore, multiple stable steady states are possible. An investigation of the corresponding properties for non-scalar boundary conditions was carried out in [7] for the kinetics $f(u,v,p) = \beta - \kappa u - uv^2$, $g(u,v,p) = \kappa u + uv^2 - v$, where β and κ are fixed parameters, corresponding to a simplified glycolysis model. It was found that for certain non-scalar boundary conditions, a stable, non-constant, steady state can exist at arbitrarily small L. For most non-scalar boundary conditions, the range of admissible solutions decreases and hence the complexity of the bifurcation diagram is greatly reduced. Solutions are less sensitive to changes in domain size and are more robust to changes in other parameters and in initial conditions.

These results hold for several types of reaction kinetics. For example, the Schnakenberg model [22], where $f(u,v,p) = a - u - u^2 v$, $g(u,v,p) = b - u^2 v$, a and b are constant parameters, was analysed in [20] and shown to exhibit similar properties. Furthermore, the time evolution problem for these kinetics was simulated for the case where γ was taken as a function of t to represent a growing domain. For scalar boundary conditions, as domain length increases, the steady state problem exhibits a multiplicity of stable states. For the time evolution problem, one therefore has a mode selection problem in which the pattern exhibited for a certain domain size will depend critically on the initial pattern, which, in turn, will depend on the rate of domain growth. However, with certain non-scalar boundary conditions, the problem of multiplicity of stable solutions can be totally eliminated for a large range of domain length. Therefore, as the domain grows, it can only evolve to the single pattern appropriate to that domain length.

Model 2

We now consider the scalar boundary condition case in which the diffusion coefficients are spatially varying. Several authors have analysed Turing models with spatially-varying parameters [8, 10–12, 14] but there are few analytic treatments for the case of spatially-varying diffusion coefficients [13]. Here, we consider a hierarchical model in which the diffusion coefficients of u and v depend on a control chemical c, which itself satisfies the reaction diffusion equation

$$c_t = \nu^2 c_{xx} - \Theta^2 c \tag{3}$$

subject to the boundary conditions

$$c_x(0,t) = 0 \ , \quad c(1,t) = c_0 \ , \tag{4}$$

where ν^2 and Θ^2 are, respectively, the nondimensionalised diffusion coefficient and the rate of linear degradation of c. The motivation for this equation comes from the application to skeletal patterning along the anterior-posterior axis of the limb bud in which there are known to be gradients of several chemicals which influence morphogenesis. Assuming further that the diffusion coefficients depend linearly on c, this is the simplest possible composite model.

Assuming that this reaction-diffusion equation reaches a stable equilibrium on a fast time scale during which insignificant changes in u and v concentrations take place, the equilibrium distribution of c is $c_0 \cosh(\Omega x)/\cosh \Omega$, where $\Omega = \Theta/\nu$.

As an example, we chose the Schnakenberg model for the kinetics for u and v. A mathematical investigation of this system is again non-standard. If one approximates the diffusion coefficient to be piecewise constant a linear analysis can be carried out which determines the parameter space in which various types of patterns can form [2]. The patterns are now asymmetrical. For the case where both diffusion coefficients are piecewise constant the patterns produced have almost constant amplitude but their wavelength varies across the domain [3]. For the case in which only one of the diffusion coefficients depends on c, the patterns produced can be isolated to parts of the domain, with an amplitude that can vary markedly across the domain [2]. Detailed numerical simulations show that these results carry over for the full system (with the diffusion coefficients varying continuously with time).

3 Application

We now consider the biological application of the above modified Turing models to skeletal patterning in the vertebrate limb. This is a widely studied problem both theoretically and experimentally (see [15] for review). The skeletal elements within the limb are laid down at a very early stage within the limb bud, before the complicated geometry of the bud has developed. Pattern formation essentially occurs along two axes: anterior-posterior (AP) and proximal-distal (PD). The limb grows outwards under the influence of a specialised ridge structure at the tip known as the apical ectodermal ridge (AER). Removal of this ridge terminates growth and leads to limbs in which the skeletal structure has been truncated. The AP pattern is controlled by a specialised zone at the posterior margin known as the zone of polarising activity (ZPA). The skeletal pattern along the PD axis follows the transitional sequence 1-2-3... the extra elements being accommodated by a widening of the AP axis. It may be possible to generate such a transitional sequence using the standard Turing model, but only if the parameters of the model are changed in a very precise manner [19]. However, using Model 1 we can show that the sequence can be generated easily and robustly as domain length L changes [7]. It is important to note that as the parameter L occurs in a nondimensionalised parameter which also includes diffusion coefficients, such a sequence could also arise due to appropriate changes in diffusion coefficient.

The above model does not capture the asymmetry of the skeletal elements along the AP axis. This behaviour can be obtained from Model 2, in which the domain can be essentially partitioned by the concentration of the control chemical, c, into pattern-forming and non-pattern-forming subdomains. The intuitive explanation of this result is quite clear. Bearing in mind that the diffusion coefficient and domain length are closely linked, changing the diffusion coefficient corresponds to varying the length. Therefore a spatially-varying diffusion coefficient essentially rescales the domain in such a way that certain sub-domains are larger, and therefore pattern-forming, than others which are non-pattern-forming.

Recently it was shown [25] that experimentally formed double anterior limbs exhibit two humeri despite being the same size as a normal limb bud which produces only one humerus. Note that this is inconsistent with the traditional Turing model which predicts that the pattern formed depends on the length of the domain. However, it is wholly consistent with Model 2 as the result can be interpreted as combining two pattern-forming subdomains [16]. It should also be noted that, because at the stage this experiment was performed there was no sign of any pattern in cell density, the authors concluded that skeletal patterning could not be due to a mechanochemical mechanism. However, as the mechanochemical model shares many similarities with the Turing model, we conjecture that a modification of the mechanochemical model along the lines of Model 2 would result in patterns consistent with this experiment. Hence a different interpretation of this result is that skeletal patterning in the limb is the consequence of a patterning hierarchy of mechanisms in which a gradient type model first partitions the domain into a set of subdomains on which a more complex model acts. That model may be a chemical pre-pattern or a mechanochemical model.

4 Discussion

In this paper we have considered two modifications of the classical Turing model. We have shown that these modifications can generate robust patterns and asymmetrical patterns in one dimension. Model 1 may be thought of as a specific example of the more general hypothesis recently proposed by Goodwin et al. [9] which suggests that morphogenesis is intrinsically robust due to the dynamic coupling between different patterning mechanisms. Model 2 assumes that the diffusion coefficient of chemicals varies spatially. Such a phenomenon has been shown experimentally along the AP axis of the vertebrate limb [4]. We are presently extending the analysis of these models to higher dimensions. Preliminary analysis of Model 2 on a two-dimensional domain shows that a variety of new patterns emerges, one of which is a sequence of parallel stripes and spots. These results will be presented in a forthcoming publication.

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