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HIERARCHICAL MODELS FOR SPATIAL PATTERN FORMATION IN BIOLOGY

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ABSTRACT

We review some recent work investigating a hierarchy of patterning processes in which a reaction-diffusion model forms the top level. In one such hierarchy, it is assumed that the boundary is differentiated, and it is shown that this can greatly enhance the robustness of the patterns subsequently formed by the reaction-diffusion model. In the second, a spatial heterogeneity in background environment is first set-up by a simple gradient model. The resulting patterns produced by the reaction-diffusion system may be isolated to specific parts of the domain. The application of such hierarchical models to skeletal patterning in the tetrapod limb is considered.

Keywords: Pre-patterns, boundary conditions, limb development, robustness, reaction-diffusion.

1. Introduction

The central goal in developmental biology is to understand how various processes in the embryo conspire to produce the spatial patterning of cellular differentiation which leads to the vast range of pattern and structure observed in the adult. The formation of structure is termed morphogenesis and the role of modelling in morphogenesis is to suggest possible scenarios for how various physical and chemical processes interact to produce pattern.

Broadly speaking, there are two main types of models in embryology: chemical prepattern models, which propose that a spatially heterogeneous pattern in some chemical is set-up, and that cells then differentiate in response to this prepattern according to some interpretation mechanism; and mechanochemical models, which propose that the physicochemical interactions between cells and their environment set-up a spatial pattern in cell density. In mechanochemical models it is hypothesized that high density cell aggregates then differentiate to form structures. The book by Murray [18] presents a comprehensive review of both types of model and contains the relevant references.

Many of the above models concentrate on a specific patterning mechanism. However, morphogenesis is composed of a complex hierarchy of processes in which patterning mechanisms at one level regulate those at higher levels. This was recognised

by Turing himself who remarked, in his classical 1952 paper, that "most of an organism, most of the time, is developing from one pattern into another, rather than from homogeneity into a pattern.' [25]. In this paper we review some recent work on two such hierarchical models. In the first, an initial mechanism sets up different types of boundary conditions for a reaction-diffusion model. In the second, the properties of the chemicals in a reaction-diffusion system are controlled by the spatial distribution of a chemical set-up by an underlying model.

Turing [25] showed that a system of reacting and diffusing chemicals could be driven unstable and evolve to a spatially heterogeneous pattern in the chemical concentrations. Turing called these chemicals morphogens. Assuming that cells differentiate if the morphogen concentration lies above a particular threshold value, this prepattern would then result in spatially-patterned cell differentiation. Turing's mechanism provides one means of generating positional information [26].

Reaction diffusion models have been proposed as possible pattern generators in a variety of biological contexts. However, it is well known that Turing models can exhibit multiple stable steady state solutions and that pattern selection can be sensitive to initial conditions and small variations in parameter values [3]. Thus, Turing models are unreliable pattern generators and therefore inadequate for cases in which pattern formation occurs via a robust sequence of transitions, such as in skeletal patterning in the tetrapod limb. Furthermore, the patterns produced by Turing systems are symmetric across the domain and therefore the structures they specify are essentially the same. However, in some applications, the structures observed biologically are intrinsically different.

Turing's model for pattern formation has been extensively analysed mathematically and numerically for the case in which all chemicals are assumed to satisfy the same type of boundary condition pointwise on the boundary. Typically, these are either zero flux or fixed at the uniform steady state concentration. We call these boundary conditions scalar boundary conditions. In Sec. 2, we review some results for the case of non-scalar boundary conditions, wherein each species satisfies different boundary conditions at any point on the boundary. We compare the properties of the solutions of the scalar case with those of the non-scalar case and show that boundary conditions can greatly influence the form and robustness of solutions.

Most applications of Turing systems assume that the background environment is spatially homogeneous, that is, all model parameters are assumed constant across the domain. However, there is now substantial experimental evidence to suggest that in some systems, such as the tetrapod limb, environmental inhomogeneity may play an important role in regulating pattern. In Sec. 3 we consider how an underlying spatial prepattern in a control chemical which modifies morphogen diffusivity can influence the patterning properties of the Turing model. In Sec. 4 we consider an application to skeletal development in the tetrapod limb.

2. The Role of Boundary Conditions

The role of boundary conditions on the form and stability of the solutions of a one-dimensional reaction-diffusion equation was analysed in [8]. The two species Turing model in one space dimension takes the form

$$\begin{aligned} u_t &= \nu u_{\xi\xi} + f(u,v,p) \\ & \qquad\qquad \text{in } (0,1) \\ v_t &= \nu\delta v_{\xi\xi} + g(u,v,p), \end{aligned} \tag{1}$$

with boundary conditions

$$\begin{aligned} \theta_1 \tfrac{\partial u}{\partial n} &= \rho(1-\theta_1)(\theta_3 u^s - u) \\ & \qquad\qquad \text{for } \xi = 0,1 \\ \delta\theta_2 \tfrac{\partial v}{\partial n} &= \delta\rho(1-\theta_2)(\theta_3 v^s - v), \end{aligned} \tag{2}$$

where $u(\xi,t)$ and $v(\xi,t)$ are nondimensionalised chemical concentrations at position ξ and time t, with $\xi \in [0,1]$; $\nu = D_1/\omega L^2$, $\delta = D_2/D_1$, where D_1 and D_2 are the dimensional diffusion coefficients of u and v respectively, ω^{-1} is a typical reaction time scale, and L is a measure of the domain length. The functions f and g are rational polynomials which model the reaction kinetics and p denotes the vector of kinetic parameters. In this system, all the parameters are assumed constant throughout the domain. 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. However, if $(\theta_1,\theta_2,\theta_3) = (1,0,1)$, then u satisfies homogeneous Neumann boundary conditions, and v satisfies homogeneous Dirichlet conditions. This is an example of a non-scalar boundary condition.

Many properties of the solutions of the Turing model for scalar boundary conditions are well known [19]. For comparison, one can analyse the non-scalar case in a number of ways. The steady state problem may be analysed using the numerical package AUTO [7]. This scheme calculates the steady states and their stability as a specified parameter varies. By varying the parameters θ_i, it is possible to homotop between various types of boundary conditions. The time evolution problem can be investigated using a combination of numerical integration, linear analysis, and nonlinear bifurcation analysis. Imposing non-scalar boundary conditions greatly increases the mathematical complexity of the problem. For example, standard linear analysis cannot be used as the choice of appropriate spatial eigenfunctions is no longer obvious. However, a modification of the standard linear analysis can be used for certain special cases [8].

Different choices of f and g in (1) model different reaction schemes for the Turing system and the effect of different types of boundary conditions can be investigated

by focussing on a particular reaction-diffusion system. Using a combination of the analyses outlined above, results for different boundary conditions can be compared as a particular parameter is varied. Dillon *et al.* [8] considered the parameter L as the bifurcation parameter, and observed a number of differences which can be summarised as follows (for the simplified glycolysis model [2], for which $f(u,v,p) = \beta - \kappa u - uv^2, g(u,v,p) = \kappa u + uv^2 - v$, where β and κ are fixed parameters):

- For the case of scalar boundary conditions, a minimum domain length is required for a spatially non-uniform steady state to exist. For certain non-scalar boundary conditions, however, a stable, non-constant, steady state can exist at arbitrarily small L.
- For the scalar case, the bifurcation diagram increases in complexity with increasing L. For the non-scalar case, the range of admissible solutions decreases and hence the complexity of the bifurcation diagram is greatly reduced.
- For the scalar case there are regions in parameter space wherein more than one stable steady state solution exists. For the non-scalar case the problem of multiplicity of solutions 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.

Ngwa [20] has shown that these results also hold for the Schnakenberg model [22]. Moreover, he solved the time evolution problem on a growing domain. For the scalar case, as L grows, it moves into the parameter regime where more than one stable steady state exists. Which state is selected will depend on the solution profile of the morphogens as L moves into this region. Consequently, one cannot predict *a priori* which pattern will be exhibited. However, with certain non-scalar boundary conditions, the problem exhibits a unique stable steady state (which changes with L) for a large range of L and therefore this problem does not arise.

3. Composite Model

To investigate how the second hierarchical scheme may influence the patterning properties of a reaction-diffusion model, a composite model with scalar boundary conditions in which the spatial variation in diffusion coefficients of the morphogens is controlled by a regulatory chemical c was considered by Maini *et al.* [16]. They assumed, as before, that the diffusion coefficient of u was constant, but that the diffusion coefficient of v was modulated by c. This could, for example, reflect a change in gap junction permeability for v due to the presence of c [21].

The chemical c, which is the control chemical, is assumed to be secreted at one end of the domain, diffuse and be degraded throughout the domain, and satisfy a zero flux condition on the other boundary. The appropriate nondimensionalised equation for c is

$$c_t = d^2 c_{\xi\xi} - \Theta^2 c \tag{3}$$

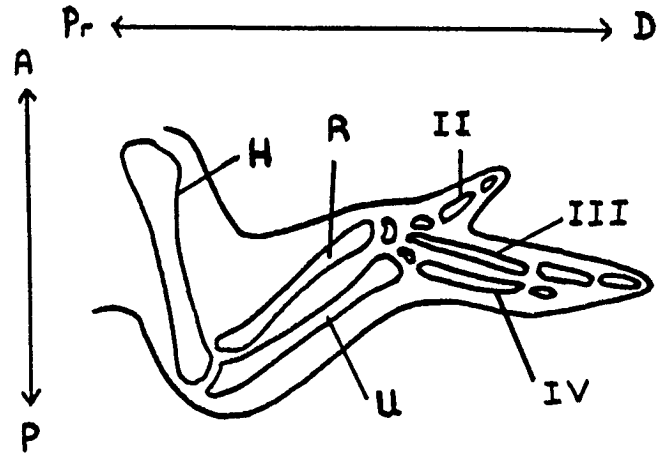


Fig. 1. Diagrammatic representation of the skeletal pattern within the chick limb. H — humerus, R — radius, U — Ulna, II, III, IV — digits, A — anterior, P — posterior, Pr — proximal, D — distal.

subject to the boundary conditions

$$c_\xi(0,t) = 0, \qquad c(1,t) = c_0\,, \tag{4}$$

where d^2 and Θ^2 are, respectively, the nondimensionalised diffusion coefficient and the rate of linear degradation of c.

Assuming that this reaction-diffusion equation reaches a stable equilibrium on a fast time scale during which insignificant changes in morphogen concentration take place, then the equilibrium distribution of c is $c_0 \cosh(\Omega\xi)/\cosh\Omega$, where $\Omega = \Theta/d$. A further assumption, that the diffusivity of v is directly proportional to c, implies that the composite model has the form

$$\begin{aligned} u_t &= \nu u_{\xi\xi} + f(u,v,p) \\ & \qquad\qquad \text{in } (0,1) \\ v_t &= \nu(\delta(\xi)v_\xi)_\xi + g(u,v,p), \end{aligned} \tag{5}$$

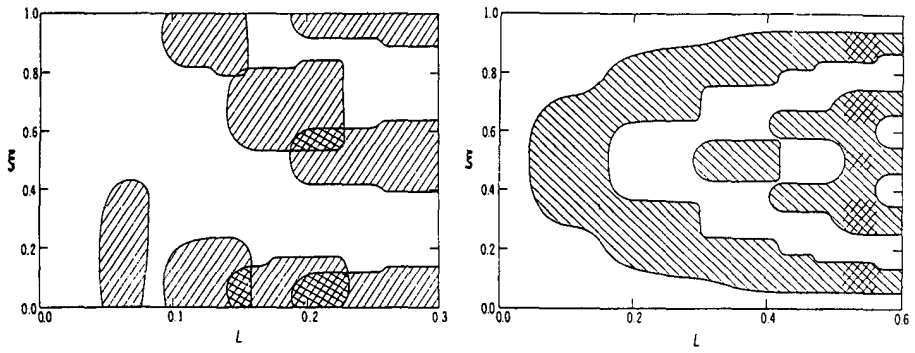
where $\delta(\xi) = \alpha c_0 \cosh(\Omega\xi)/\cosh\Omega$, and α is a constant.

The introduction of spatially varying parameters greatly increases the complexity of the reaction-diffusion system, even for the case of scalar boundary conditions. There have been a number of numerical studies of the effects of spatially varying reaction terms in reaction-diffusion systems [9, 13, 14] and of spatially varying diffusion [12]. A mathematical analysis in which one of the reaction terms is spatially varying was carried out in [1] and [11]. Recently, we carried out a linear stability analysis for (5) with a step function approximation to the diffusion coefficient and determined, analytically, regions in parameter space wherein the system can be driven unstable and evolve to spatially heterogeneous patterns [16]. It was shown that (5) exhibited qualitatively similar behaviour to that of the simpler system.

In particular, the solutions of (5) are not symmetric, but have a spatially-varying amplitude of oscillation; the explicit form of this dependence can be obtained from linear analysis for the case of the step function approximation to the diffusion coefficient [4]. A consequence of this spatial variation in amplitude is that patterns can be isolated to certain regions of the domain. A typical spatial pattern is illustrated in Fig. 3(a).

4. Biological Application

We now consider the application of Turing models to skeletal patterning in the vertebrate limb. Figure 1 illustrates the pattern of skeletal elements within the chick limb. The elements are laid down along the proximal-distal (PD) axis in a 1-2-3 transition sequence and they are asymmetrical along the anterior-posterior (AP) axis. In Fig. 2 we compare the transition sequence for the scalar Turing model with that of the non-scalar model as the parameter L increases. The latter can reliably and robustly generate the transition sequence observed along the PD axis. Thus, one can capture the PD behaviour of the skeletal pattern by imposing suitable non-scalar boundary conditions on the Turing model. Note that when the skeletal pattern is laid down, the limb bud is cylindrical with an elliptical cross-section and does not have the complicated geometry of the adult wing.



(a) (b)

Fig. 2. The subintervals of $(0,1)$ in which the v-component of the solution to (1) for glycolysis kinetics exceeds a fixed threshold concentration, as the parameter L increases, with β and κ fixed at 1.0 and 0.001, respectively. (a) The scalar case where u and v satisfy zero flux boundary conditions; (b) The non-scalar case where u satisfies zero flux boundary conditions and v is fixed to zero on the boundary. Clearly for (b) a simple threshold mechanism can reliably produce the sequence 1-2-3- ... of pattern elements as the parameter L increases, whereas this is impossible for the standard case with scalar boundary conditions. If the corresponding time evolution equations are solved on a growing domain one obtains the same solutions to those observed in (a) and (b) [20]. Note that in (b) there is no discontinuity between successive elements. During early limb development successive proximal-distal elements initially appear continuous but separate from each other at a later stage.

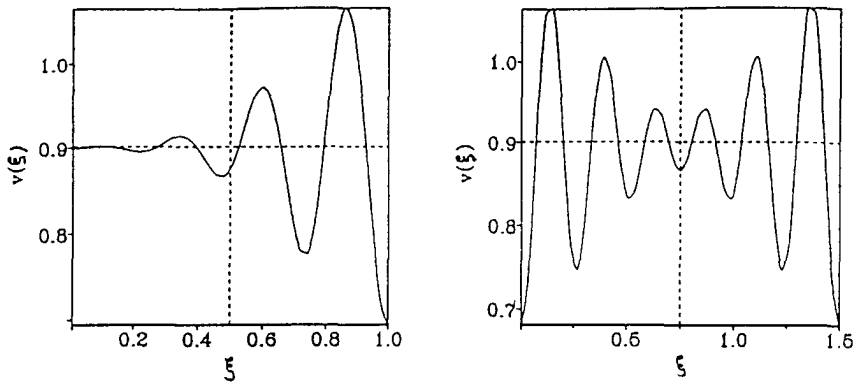


Fig. 3. The v component of the solution of the composite model for the case of Schnackenberg kinetics [22], where $f = a - u - u^2 v$, $g = b - u^2 v$, a and b are constant parameters fixed at 0.1 and 0.9, respectively, for different boundary conditions on the control chemical c and different domain lengths, L. (a) c satisfies the boundary conditions (4), and $L = 1$. (b) c satisfies the boundary conditions (6), and $L = 1.5$. For a fixed threshold concentration (- - - -), the prepattern in (a) specifies three distinct elements, while (b) predicts a complete duplication of six elements, two of each kind specified in (a).

To capture the asymmetry along the AP axis, we consider the composite model. In this model, the underlying spatial pattern in control chemical influences the position and amplitude of peaks in morphogen concentration. Therefore, the positional information supplied by these morphogen profiles could lead, via cell differentiation, to the specification of asymmetrically patterned elements, whose position within the domain is controlled by the spatial distribution of c. For example, Fig. 3(a) shows that for a suitably chosen threshold concentration, the composite model can specify three skeletal elements, corresponding to the digits, which are intrinsically distinct because of the varying concentrations of morphogen to which they are each exposed. This is in contrast to the standard reaction-diffusion system with spatially uniform parameters, which produces identical and equally spaced elements. The asymmetry in the solution profiles of the composite model is a consequence of the spatial gradient in the control chemical c. Such gradients do exist in the limb [24], and appear to emanate from the zone of polarising activity, or ZPA, which is a specialised group of cells occurring at the posterior margin of the developing limb bud. Grafting a donor ZPA to different positions along the AP axis of a host chick limb leads to the formation of additional digits and stimulates growth along this axis [23]. If, in our composite model, we assume that the ZPA is the source of the control chemical c, then grafting a donor ZPA onto the anterior margin of the limb corresponds to creating a new source of the chemical c. As this graft stimulates growth, c now satisfies (3) with boundary conditions

$$c(0,t) = c(L,t) = c_0, \tag{6}$$

where L is the new domain length. The distribution of the control chemical, and hence the diffusion coefficient of v, is now determined by

$$\delta(\xi) = \alpha c_0 \cosh(\Omega(\xi - L/2))/\cosh(\Omega L/2). \tag{7}$$

Figure 3(b) shows that the system can generate a mirror symmetric pattern which has six concentration peaks of varying amplitude for the case in which the ZPA graft increases the domain size by 50%. Together with the same threshold mechanism as before, the model predicts the formation of a duplicate set of additional digits, which is consistent with experimental results (see [15] for a review of experimental results on the limb). On smaller domains (corresponding to transplanting donor ZPA to a more posterior site) the model predicts the formation of fewer digits, as is also observed experimentally. Note that the standard Turing model also predicts that additional digits will form if the domain size is increased, but it cannot specify which digits are duplicated. In contrast, the composite model exhibits a prepattern in morphogen concentration which can distinguish between the different types of digit formed.

5. Discussion

We have reviewed recent work that considers two modifications of the traditional one-dimensional reaction-diffusion model. It has been shown that imposing appropriate non-scalar boundary conditions can lead, in a robust and controlled manner, to a sequence of transitions that closely resembles those observed in skeletal patterning along the proximal-distal axis in the developing limb. Although domain length L was taken as the transition parameter it should be noted that this parameter occurs in the dimensionless grouping $\nu = D_1/\omega L^2$. Therefore the sequence of transitions illustrated in Fig. 2 could be generated by changes in any of the parameters that occur in this dimensionless quantity, for example, due to changes in diffusion coefficient. This could arise due to a change in gap junction permeability [6].

Imposing a background gradient in the diffusion coefficient of one of the morphogens results in asymmetrical patterns consistent with those observed along the anterior-posterior axis of the limb. It is now known that retinoic acid occurs in a graded distribution along the AP axis. Furthermore, there is recent experimental evidence that retinoic acid modulates gap junction permeability and hence diffusion of chemicals through cells [5, 17].

Recently, it was shown that recombinant double-anterior limb buds produce two humeri [27]. As the recombinant limb bud was the same size as a normal limb bud, the standard Turing or mechanochemical model would predict that the pattern should be normal, that is, one humerus should form. Hence this result suggests that the humeral element must have been determined prior to the stage at which the experiment was performed. As there was no sign of any pattern in cell density at this stage, the authors concluded that skeletal patterning in the limb could not possibly be due to a mechanochemical mechanism. However, the

composite model described in Sec. 3 is consistent with this observation [16]. As the mechanochemical and Turing models share a number of important mathematical properties, we conjecture that this result also holds true for the mechanochemical model. Therefore, an alternative explanation of the result in [27] is that skeletal patterning in the limb is due to a hierarchical process. At a low level of this hierarchy is a gradient mechanism which can partition the domain into pattern-forming and non-pattern-forming subdomains. This simple pattern is then elaborated upon by a more complicated mechanism higher up in the hierarchy which, in turn, leads to a more complex pattern. That mechanism could well be of mechanochemical type.

One of the important consequences of the model described in Sec. 2 is that by essentially combining two patterning mechanisms, namely one which differentiates the boundary so that it is an impermeable membrane to one morphogen and a sink to the other, with a reaction-diffusion model greatly enhances the robustness of the latter. This is a specific example of the more general hypothesis recently proposed by Goodwin *et al.* [10] which suggests that morphogenesis is intrinsically robust due to the dynamic coupling between different patterning mechanisms.

The scenario for skeletal pattern formation in the limb presented in this paper does not capture the temporal sequence of development along the AP axis, but this may be due to cells responding to the morphogen prepattern in a time-specific fashion. Moreover, it proposes that the AP asymmetry in pattern is due to a spatially varying diffusion coefficient. Such patterns could also be set-up by spatially varying reaction kinetics parameters or, in two-dimensions, by an asymmetrical domain. However, noting that the diffusion coefficient and the length scale are intrinsically linked, we have, in effect, considered the second case. Moreover, experimental observations do show that diffusion is asymmetrical across the AP axis.

We have considered the pattern forming processes along the PD and AP axis as separate, uncoupled processes. This is a common assumption which provides useful insights and yields predictions which are consistent with many experimental observations. However, there are a number of experiments which suggest that a fully realistic model for spatial patterning must be two- or three-dimensional, incorporating the ideas presented here but also introducing interactions between specific specialised regions in the limb. This is presently under investigation.

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