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Spatial and Spatiotemporal Pattern Formation in Generalised Turing Systems

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Abstract—Reaction-diffusion, or Turing, models have been proposed to account for a number of pattern formation phenomena in early development. However, there are a number of crucial morphogenetic phenomena that contradict the standard Turing model. Here we review three generalisations of the Turing model and show how they can be applied to two such phenomena. We discuss how these generalisations can provide insight to the processes underlying patterning in these cases.

Keywords—Boundary conditions, Robustness, Limb development, Spatially varying parameters, Tooth morphogenesis.

1. INTRODUCTION

One of the main areas of research in developmental biology seeks to understand the key processes and mechanisms that underlie morphogenesis, the formation of structure and form within the embryo. Broadly speaking, there are two main classes of models. In one, it is hypothesized that a chemical prepattern is set up, either via a source-sink model [1], or due to diffusion-driven instability (DDI) in a reaction-diffusion system (Turing systems [2]). It is then proposed that cells interpret this prepattern by differentiating only where the chemical (or morphogen) concentrations lie above or within specified threshold values. Hence, the observed spatial pattern of cell differentiation is thought to overlie the chemical prepattern. In the second, it is proposed that due to the mechanochemical interactions between cells and their external environment, a spatial pattern in cell density arises. It is then proposed that cells in high density aggregates differentiate [3,4]. The book by Murray [5] provides an excellent and detailed review of the above models.

In this paper, we review some recent work on generalised Turing systems in one dimension and consider their application to two areas of morphogenesis, namely, skeletal patterning in the chick limb, and tooth morphogenesis in the alligator. These two examples exhibit many developmental phenomena that occur in general so that an understanding of the underlying mechanisms giving rise to pattern in these specific examples, may have widespread application. Sections 3 and 4 present two generalisations of the standard Turing model and their application to limb development is discussed in Section 5. In Section 6, we present a further generalisation and detail its application to tooth morphogenesis in the alligator. Section 7 discusses the biological implications of these generalised Turing models.

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2. THE TURING MODEL

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

$$\begin{aligned} u_t &= \gamma f(u,v,P) + (D_u u_x)_x, \\ v_t &= \gamma g(u,v,P) + (D_v v_x)_x, \end{aligned} \qquad \text{in } (0,1), \tag{1}$$

with boundary conditions

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

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 model the reaction kinetics and typically take the form of rational polynomials. The vector P denotes the 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, $f(u^s,v^s,P) = g(u^s,v^s,P) = 0$.

In the standard Turing model, the parameters D_u, D_v and P are taken as constant and the boundary conditions are either zero flux, that is, $(\theta_1,\theta_2,\theta_3) = (1,1,\cdot)$, or fixed at the steady state, that is, $(\theta_1,\theta_2,\theta_3) = (0,0,1)$. We refer to these two types of boundary conditions as scalar boundary conditions [6].

The standard Turing model has been extensively analysed and applied to several developmental phenomena. One of the major criticisms of the application of this model 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 [7]. 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 primary bifurcation points, whereas many patterns in embryology are asymmetrical. Furthermore, this well-ordered generation of pattern is inconsistent with certain patterns which form in a complex spatiotemporal sequence.

We will consider three generalisations of the above model which address the above issues. In the first, we consider the role of different types of boundary conditions. In the second, we examine the effects on the patterns due to a spatially-varying diffusion coefficient, and in the third, the effects of spatiotemporal variation in one of the kinetic parameters.

3. GENERALISATION 1

Dillon *et al.* [6] analysed the Turing model with constant parameter values for several different types of boundary conditions, corresponding to different values of $(\theta_1,\theta_2,\theta_3)$. This increases enormously the complexity of the analysis of the model. For example, for the nonscalar boundary conditions $(\theta_1,\theta_2,\theta_3) = (1,0,1)$, the eigenfunctions of the linearised equations about the uniform steady state are no longer simple sines and cosines. Furthermore, for many nonscalar boundary conditions there is no uniform steady state. For example, if u^s is nonzero, then for $(\theta_1,\theta_2,\theta_3) = (0,1,1)$, the system does not have a uniform steady state.

In [6], the Turing model for nonscalar boundary conditions was extensively analysed numerically by solving the time evolution equations and by using the numerical bifurcation package AUTO [8]. The particular Turing model studied was the simplified glycolysis model, where $f(u,v,P) = \beta - \kappa u - uv^2$, $g(u,v,P) = \kappa u + uv^2 - v$, and β and κ are fixed parameters. The

analysis revealed a number of key differences between the solutions for scalar boundary conditions and those for nonscalar boundary conditions. Two examples of these differences follow here.

(1) For scalar boundary conditions, it is well known that a minimum domain length is required for spatially nonuniform steady states to exist. For certain nonscalar boundary conditions, however, spatially nonuniform steady states can exist for arbitrarily small domain length. Moreover, using variational techniques, it can be shown that some of these states are stable (see [6] for full details).

(2) For scalar boundary conditions, the complexity of the form of the spatially nonuniform steady states increases with domain length and, for sufficiently large length, multiple stable steady states are possible. For most nonscalar boundary conditions, the range of admissible solutions decreases, and hence the complexity of the bifurcation diagram is reduced. As a result, solutions are less sensitive to changes in domain size (and in other parameters) and initial conditions, that is, the robustness of the admissible solutions is greatly enhanced.

These results have been shown to hold for other types of reaction kinetics [9].

4. GENERALISATION 2

We now consider the Turing model for the case of scalar boundary conditions in which we impose spatial variation on the diffusion coefficients. Although Turing models with spatially-varying parameters have been analysed by a number of authors (for example, [10–14]), the case of spatially-varying diffusion coefficients has received less attention [15]. Here, we assume that one or both of the diffusion coefficients D_u and D_v are functions of 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

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

where ν^2 and Θ^2 are, respectively, the nondimensionalised diffusion coefficient and the rate of linear degradation of c. This form is motivated from considering the application to skeletal patterning along the anterior-posterior axis of the chick limb bud in which there are gradients of several key chemicals which influence morphogenesis. The simplest possible generalisation of the standard model in this case is to assume that either one or both of D_u and D_v depend linearly on c. A further simplification can be made if we assume that the c equation is on a faster timescale compared to the equations for u and v. One can then substitute the equilibrium distribution $c_0 \cosh(\Omega x)/\cosh\Omega$ for c, where $\Omega = \Theta/\nu$.

The mathematical investigation of this system is again nonstandard. A linear analysis can be carried out for the case in which the equilibrium distribution for c is approximated by a piecewise linear function. In this way, it is possible to delimit regions in parameter space wherein different types of patterns can arise [16]. In this case, the imposed asymmetry in diffusion coefficients leads to asymmetrical patterns. The asymmetry can take two forms (for full details see [16,17]).

(1) The pattern peaks have almost constant amplitude but their wavelength varies across the domain.

(2) The amplitude of the peaks varies markedly across the domain while the wavelength stays apparently constant throughout the domain. This results in a pattern that is isolated to one part of the domain.

Detailed numerical simulations show that these results carry over for the full system (with the diffusion coefficients varying continuously with space).

5. APPLICATION TO SKELETAL PATTERN FORMATION IN THE CHICK LIMB

We now consider the significance of the above generalisations of the Turing model by applying the model to the formation of skeletal pattern in the chick limb. This is a widely studied problem both theoretically and experimentally (see [18] for review). The actual process of pattern formation in the chick limb occurs at a very early stage, when the limb has the shape of an almost cylindrical bud, that is, before the complex geometry of the limb develops. Pattern formation essentially occurs along two axis: the front to back axis, or anterior-posterior (AP) axis, and the axis pointing outward from the limb, namely the proximal-distal (PD) axis. The skeletal pattern along the PD axis follows the transitional sequence 1-2-3, corresponding to the humerus, the radius and ulna, and the digits, of which there are three in the chick wing bud. The extra elements appear to be accommodated by a widening of the AP axis. Although it may be possible to generate this sequence using the standard Turing model, the parameters of the model would have to change in a very precise manner [19]. This is because of the sensitive nature of the standard Turing model to perturbations in initial conditions and/or parameter values. However, as discussed in Section 3, imposing certain nonscalar boundary conditions can greatly enhance robustness. In fact, it can be shown that the required sequence of pattern can be generated easily and robustly as domain length L changes [6]. 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 coefficients.

The above model does not capture the asymmetry of the skeletal elements along the AP axis. This can be achieved, however, by considering the generalisation presented in Section 4. In that case, the control chemical c essentially partitions the domain into pattern-forming and nonpattern-forming subdomains. The intuitive understanding of this result is quite clear. Noting 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 subdomains are "large," and therefore pattern-forming, while others are "smaller" than the minimum domain length necessary for pattern formation. Combining both generalisations, one can show that the corresponding generalised Turing model can exhibit solutions which are consistent with the patterns observed in normal development [20].

We now consider a recent experiment which contradicts the standard Turing model but is consistent with Generalisation 2 above. In this experiment [21], double anterior chick limbs were formed by replacing the posterior section of a host limb bud with the anterior part of a donor limb bud, in such a way that the resultant limb bud was the same size as a normal limb bud. The double anterior limb formed two humeri despite being the same size as a normal limb bud which produces only one humerus. This is inconsistent with the traditional Turing model which predicts that the complexity of the pattern formed depends crucially on the length of the domain. However, it is wholly consistent with Generalisation 2 as the result can be interpreted as combining two pattern-forming subdomains [22].

It is also important to examine more closely another claim of the authors. They state that because there was no sign of any pattern in cell density at the time the experiments were performed, it follows that skeletal patterning in the vertebrate limb can not be due to a mechanochemical mechanism. However, as the mechanochemical model shares many similarities with the Turing model, we conjecture that a generalisation of the mechanochemical model similar to that proposed in Section 4 for the Turing model 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 [23]. That model may be a chemical prepattern or a mechanochemical model.

6. APPLICATION TO TOOTH MORPHOGENESIS

In the above biological example, the pattern forms in a simple temporal sequence in which, due to the increase in domain size with time, more complex spatial patterns are laid down. We now consider an example of complex spatiotemporal pattern formation, namely that of 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 [24–26] so that there is ample experimental data on which to base a realistic mathematical model.

We focus on the patterning of teeth in the lower jaw of the alligator (see [27] for full details). The alligator jaw has left-right symmetry so that one need only consider one side of the jaw. Moreover, to a very good approximation, one can think of the jaw as being essentially a 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, so we shall focus only on the first seven tooth primordia. They are formed 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.

It appears that when a tooth primordium forms, it inhibits, for a certain length of time, tooth primordium formation nearby. We now show how a generalised form of the Turing model can exhibit this type of behaviour. We consider the Schnakenberg model [28], where $f(u,v,P) = a - u - u^2v$, $g(u,v,P) = b - u^2v$, but we assume that the source of the morphogen u is now determined by the concentration of a control chemical c. Specifically, we assume that $a = hc$, where h is a positive constant. We assume further that the control chemical c diffuses and is degraded, linearly. Hence, the equation for c takes the form

$$c_t = D_c c_{xx} - \delta c, \tag{5}$$

and we impose the boundary conditions

$$c_x(0,t) = 0, \qquad c(1,t) = c_0; \tag{6}$$

that is, there is a source of c at the posterior end ($x = 1$) of the jaw, with zero flux at the anterior end. We assume that the morphogens u and v have zero flux boundary conditions at both ends. We incorporate domain growth into the equations by assuming that jaw length grows at a constant rate r. On rescaling the domain to $[0,1]$, this results in the diffusion terms being multiplied by the factor e^{-2rt} and contributes a dilution term $-ry$ to the kinetics of each reaction-diffusion equation, setting $y = u, v, c$, respectively.

Now for c large, the Schnakenberg system does not exhibit diffusion-driven instability (DDI). Therefore, the domain length has to grow sufficiently, and c decay to sufficiently small values, so that DDI can occur. We can choose parameters (see [27] for full details) such that DDI occurs in the anterior part of the domain, and a single peak in u forms. We assume that this then marks tooth primordium 1. We assume further that this tooth becomes another source of c. If we continue to run the model we find that c falls below the level required for DDI in a region posterior to tooth 1, so that tooth 2 forms there. In a similar fashion, tooth 3 forms posterior to tooth 2. However, at this point, the domain has grown sufficiently so that c next breaches the threshold anterior to tooth 1, and this is where tooth 4 forms. Continuing this simulation, it can be shown that, for a wide range of parameter values, the model can generate the correct spatiotemporal sequence of the first seven teeth. The upper jaw also exhibits a spatiotemporal sequence of tooth formation, but the sequence is different to that in the lower jaw. However, the

model can also generate this sequence, by a simple change in parameter values. Moreover, the model can make a number of experimentally testable predictions on the results of removing or implanting tooth primordia.

7. DISCUSSION

In this paper, we have considered three generalisations of the standard Turing model. We have shown how generalising the type of boundary conditions imposed on the standard Turing model can greatly increase the robustness of certain solutions. This may be considered to be a particular example of the more general hypothesis of Goodwin *et al.* [29], which suggests that morphogenesis is intrinsically robust due to the dynamic coupling between different patterning mechanisms.

The second generalisation assumes that the diffusion coefficients of the morphogens vary spatially. This is not an unrealistic assumption. In fact, it has been shown, via dye spreading experiments, that such spatially varying diffusion does occur along the AP axis of the vertebrate limb [30,31].

The third generalisation consists of spatiotemporal variation of the kinetics. This was proposed to describe the complex spatiotemporal patterning that occurs during tooth primordia formation in the developing alligator jaw. This model gives rise to specific biologically testable predictions which should help us to gain an increased understanding of the mechanisms underlying this developmental phenomenon.

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