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Phil. Trans. R. Soc. Lond. A 1994 **347**, 661-676
doi: 10.1098/rsta.1994.0073

Threshold bifurcation in tissue interaction models for spatial pattern generation†

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Experimental evidence indicates that tissue interaction plays an essential role during skin pattern formation. Here we focus on the mathematical aspects of two specific tissue interaction models. Introducing interaction mechanisms into the traditional pattern formation models is not only biologically consistent, but also leads to results agreeing more closer to those observed in embryogenesis. We specifically examine the bifurcations from spatially simple solutions to spatially complex patterns. In both models this increased complexity in solution is obtained by increasing the effect of the interaction mechanism through a certain threshold. The role of tissue interaction in sequential patterning is also considered.

1. Introduction

The generation and onset of spatial patterns in the early stages of embryonic development has been widely studied, with some success, by using a variety of mathematical modelling approaches (see, for example, Murray (1989) for a good review of the subject). Typically, reaction-diffusion equations, chemotaxis equations, and mechanochemical force balance equations are used for describing the various mechanisms.

It is well known (Gilbert 1988) that communication between different groups of cells and tissue types plays a crucial role during morphogenesis. However, to date, most of the models proposed are concerned only with very specific tissues and little attention has been given to modelling tissue interaction. We shall show here that the introduction of tissue interaction into these classical models has far reaching consequences for explaining morphogenetic structure formation.

We specifically focus on two aspects of tissue interaction by considering two different models for vertebrate skin pattern formation. In the model of Cruywagen & Murray (1992) uniform skin tissue can only be excited into spatial structure in the presence of tissue interaction. Furthermore, the interaction effect must be larger than a certain threshold before the bifurcation to structure occurs.

The second model we consider is that of Shaw & Murray (1990). Here the interaction mechanism is not crucial for simple pattern formation. It can, however, excite the skin tissue into producing far more complex patterns than normally exhibited by such models. Again there is a threshold behaviour – bifurcation

† This paper was produced from the authors' disk by using the TEX typesetting system.

Phil. Trans. R. Soc. Lond. A (1994) **347**, 661–676
Printed in Great Britain

from simple to complex patterns occurs only when tissue interaction becomes dominant enough.

We examine the type of patterns which these tissue interaction models can generate. By specifically looking at linear bifurcations from multiple eigenvalues, we demonstrate that a very simple tissue interaction model can produce highly complex spatial patterns.

Skin patterns are frequently laid down sequentially, as a wave of skin determination sweeps across the tissue. We show that tissue interaction is not only important for specifying the complexity of spatial structure, it is often necessary for understanding how complex patterns are laid down in such a sequential manner.

2. Tissue interaction

Vertebrate skin is composed of two layers, the epidermis and the dermis. The epidermis consists of a sheet of tightly packed epithelial cells which exhibits viscoelastic properties. The dermis on the other hand is made up of mesenchymal cells which can move in the extracellular matrix – essentially an array of fibres and collagen. These layers are separated by a thin sheet of tissue called the basal lamina.

A variety of model mechanisms have been proposed for explaining pattern formation in either of these two layers. However, not only does pattern formation appear to occur almost simultaneously in both layers, biological evidence indicates that there is, in fact, active communication between the layers and that, furthermore, pattern formation can only occur in the presence of both dermis and epidermis (see Murray *et al.* 1993 for references).

We explain here a theoretical scenario of how the dermal–epidermal communication could operate. This is based on the experimental results of Chuong & Edelman (1985) and Gallin *et al.* (1986) on chick feather germ formation. It is assumed that, during epidermal pattern formation, a certain factor is produced by the epithelial layer. This factor, which could, for example, be a chemical, is represented by e in figure 1. The signal, e, is relayed through the basal lamina to the dermis where it alters the dermal pattern formation mechanism. Similarly, it is assumed that in the dermal layer a factor, represented by s in figure 1, is produced. This signal is likewise relayed via the basal lamina to the epidermis to influence the pattern formation process there.

In view of the work of Chuong & Edelman (1985), the actual factors involved could be chemicals, produced by the cells in the respective layers, which are relayed across the basal lamina via diffusion. Alternatively the factors could be mechanical signals, with active stresses being transferred through the physical attachments that connect the two layers. Folkman & Mosconi (1978) showed that mechanical forces could have a major effect on cell behaviour. Electrical signals have also been suggested as a possible means of communication. It is possible that a combination of the above three mechanisms is operating.

Depending on the mechanism in each of the respective layers, three different types of interaction are theoretically possible. For example, dermal and epidermal mechanisms that are not capable of generating patterns independently could be coupled. Alternatively, a pattern generating mechanism in one layer could interact

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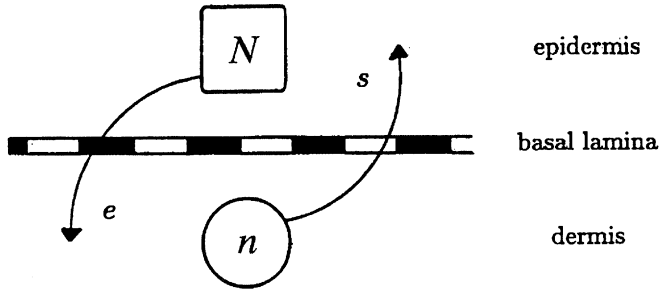

Figure 1. A schematic diagram of a tissue interaction mechanism. The dermal cells n, produce a factor s, which is relayed to the epidermis and influences the pattern formation mechanisms there. Similarly a factor e, produced by the epidermal cells N, is relayed to the dermis where it acts on the dermal pattern formation mechanisms.

with one which does not have that capability. Finally, dermal and epidermal mechanisms, both with the ability for generating spatial patterns independently, could be coupled.

3. Pattern formation through tissue interaction

Here we consider the tissue interaction model proposed by Cruywagen & Murray (1992). The model is based on the experimental results of Chuong & Edelman (1985) and Gallin *et al.* (1986) who examined the role of tissue interaction during chick feather germ morphogenesis.

A mechanochemical system is used for modelling the epidermis as a viscoelastic sheet (see, for example, Murray 1989 for a review). The system consists of a force balance equation describing the passive viscoelastic stresses present in the tissue and a traction term responsible for the active tissue stresses. This equation is coupled with an equation to model epithelial cell conservation. The field variable $\boldsymbol{u}$ is used to represent the epithelial material displacement, N represents the epithelial cell density and n the dermal cell density.

The mechanochemical force balance equation is

$$\nabla\cdot\left\{\overbrace{\mu_1\frac{\partial\epsilon}{\partial t}+\mu_2\frac{\partial\theta}{\partial t}\boldsymbol{I}}^{\text{viscous stress}}+\overbrace{\epsilon-\beta_1\nabla^2\epsilon+\nu'(\theta-\beta_2\nabla^2\theta)\boldsymbol{I}}^{\text{elastic stress}}+\overbrace{\frac{\tau s^2(n)}{1+cs^2(n)}}^{\text{active traction}}\right\}=\overbrace{\rho\boldsymbol{u}}^{\text{body forces}},\tag{3.1}$$

where μ_1 and μ_2 are the shear and bulk viscosities respectively, $\nu'=\nu/(1-2\nu)$ with ν Poisson's ratio, $\boldsymbol{I}$ is the unit tensor, $\epsilon=\frac{1}{2}(\nabla\boldsymbol{u}+\nabla\boldsymbol{u}^{\mathrm{T}})$ the linear strain, and $\theta=\nabla\cdot\boldsymbol{u}$ the dilation (see, for example, Landau & Lifshitz 1970). The parameter ρ reflects the strength of the attachments of the epidermis to the surrounding tissue, while β_1 and β_2 measure the long range elasticity.

The active traction term depends on a chemical signal, s, received from the dermis. It is assumed that the chemical is produced by the dermal cells. For simplicity a linear dependence on the dermal cell density is assumed, that is $s(n)=k_sn$, where k_s is the positive production rate. The specific functional form chosen for the traction term models the switchlike behaviour of the active

contraction (see Murray & Oster 1984). The sharpness of the switch is controlled by the parameter c, while the strength is controlled by τ.

Since epithelial cells are not motile, the only contribution to cell flux is convection and so the epithelial cell conservation equation is taken as

$$\frac{\partial N}{\partial t} = -\overbrace{\nabla \cdot N\frac{\partial \boldsymbol{u}}{\partial t}}^{\text{convection}}. \tag{3.2}$$

For the dermal layer, where the cells are more loosely arranged and are motile, a cell chemotaxis model is used. Active chemotaxis is balanced by cell diffusion, so the dermal cell conservation equation can be expressed as

$$\frac{\partial n}{\partial t} = \overbrace{\nabla \cdot D\nabla n}^{\text{diffusion}} - \overbrace{\nabla \cdot \{n\alpha\nabla e(N)\}}^{\text{chemotaxis}}, \tag{3.3}$$

where D is the positive cell diffusion coefficient, α is the positive chemotaxis constant and e is the chemo-attractant diffusing from the epidermal layer to the dermal layer. The chemo-attraction is thus induced by the signal received from the epidermal layer. We assume that this signal is produced by the epidermal cells and that it is linearly proportional to epidermal cell density, so $e(N) = k_e N$, where k_e is a positive constant measuring the production rate.

We can reduced the force balance equation (3.1) to a scalar equation in the dilation, θ, by taking the divergence of both sides. Secondly, since the strains in the tissues during the formation of skin organ primordia are small, we can use a small strain approximation to simplify the epithelial cell density equation. Hence we can linearize the epithelial cell conservation equation (3.2) about the steady state $N = 1$ which, on integration, gives $N = 1 - \theta$. By substituting this relationship into (3.1) and (3.3) a small strain version of the model is obtained, namely,

$$\mu\frac{\partial}{\partial t}\nabla^2\theta + \nabla^2\theta - \beta\nabla^4\theta + \tau\nabla^2\left(\frac{n^2}{1+cn^2}\right) = \rho\theta, \tag{3.4a}$$

$$\frac{\partial n}{\partial t} = D\nabla^2 n - \nabla \cdot \{\alpha n\nabla(1-\theta)\}, \tag{3.4b}$$

where k_s^2 has been incorporated into the parameters τ and c, k_e has been included in the parameter α, $\mu = \mu_1 + \mu_2$, $\beta = \beta_1 + \beta_2$, and μ, β, τ and ρ have been divided by $(1+\nu')$. However, since viscosity effects are believed to be small, we set μ equal to zero.

The specific tissue geometry considered is idealized as a rectangular domain $\boldsymbol{B}$ of dimensions L_x and L_y. On the boundaries we impose the zero-flux conditions

$$(\eta \cdot \nabla)n = 0, \quad (\eta \cdot \nabla)\theta = 0, \quad (\eta \cdot \nabla^3)\theta = 0, \quad \text{for } (x, y) \text{ on } \partial\boldsymbol{B}, \tag{3.5}$$

where η is the unit normal vector on the domain boundary $\partial\boldsymbol{B}$. These conditions ensure that the dermal and epidermal cell densities are conserved. Note that dermal and epidermal cell division is not included in the model, since these do not appear to be of significance in the type of skin organ formation which we are considering.

(*a*) *Linear stability analysis*

Cruywagen & Murray (1992) considered only one-dimensional spatial domains. Since the epithelial and dermal cell condensations which form during early skin morphogenesis are small, it is appropriate to examine equations (3.4) in the vicinity of the bifurcation point to spatial patterns. In this section we therefore determine what type of two-dimensional patterns a linear stability analysis would predict.

The two-dimensional problem bifurcates to spatial pattern at either a simple eigenvalue or a multiple eigenvalue. Of particular interest is the solutions that arise via bifurcation from a multiple eigenvalue, since, as far as we know, this has not been widely studied in pattern formation mechanisms. Instead of only single mode patterns evolving, various modes can interact to produce mixed mode patterns.

Equations (3.4) admit the spatially uniform steady states

$$n = 0, \quad \theta = 0 \quad \text{and} \quad n = 1, \quad \theta = 0,$$

of which only the second is biologically relevant. Linearizing about this steady state gives

$$\rho\theta = \nabla^2\theta - \beta\nabla^4\theta + P\nabla^2 n, \quad \frac{\partial n}{\partial t} = D\nabla^2 n + \alpha\nabla^2\theta, \tag{3.6}$$

where θ and n now denote small perturbations from the non-trivial steady state, and

$$P = 2\tau/(1+c)^2.$$

We look for solutions to the linear system of the form

$$\boldsymbol{w} = \begin{pmatrix} \theta \\ n \end{pmatrix} \propto \exp(\mathrm{i}\boldsymbol{k}\cdot\boldsymbol{x} + \lambda(k^2)t),$$

where $\boldsymbol{k}$ is the wave vector, $k^2 = \boldsymbol{k}\cdot\boldsymbol{k}$ and $\lambda(k^2)$ is the temporal growth rate of the disturbance. Substituting $\boldsymbol{w}$ into the linearized system (3.6) leads to the dispersion relation

$$\lambda(k^2) = -c(k^2)/b(k^2), \tag{3.7}$$

where

$$b(k^2) = \beta k^4 + k^2 + \rho, \quad c(k^2) = \beta D k^6 - (P\alpha - D)k^4 + \rho D k^2.$$

The uniform steady state is linearly unstable if and only if $\mathrm{Re}\lambda(k^2) > 0$ for a positive eigenvalue k^2. So, from (3.7) it follows that the steady state is unstable if and only if $c(k^2) < 0$ for some $k^2 > 0$.

It is easy to show that if $P\alpha - D > 0$ then the uniform steady state loses stability at the point where the critical equality

$$(P\alpha - D)^2/4\beta D^2\rho = 1,$$

holds. Instability occurs when the left-hand side becomes larger than 1. The corresponding critical eigenvalue at this point is

$$k_c^2 = \sqrt{(\rho_c/\beta)}. \tag{3.8}$$

It is important to remember that tissue interaction is reflected through the

parameters α and P. If either is zero no unstable eigenvalues are possible and hence no pattern formation can result. Two-way interaction is thus essential for pattern formation.

Increasing the effect of the interaction mechanism by increasing either α or P destabilizes the system and can excite it into spatial pattern formation. However, from (3.8) we see that the wavenumber of the evolving pattern is independent of the interaction parameters.

Since the zero-flux boundary conditions (3.5) constrain k^2 to discrete values we can examine the specific patterns predicted by the linearized equations (3.6). Solutions to the linear system (3.6), satisfying the boundary conditions (3.5), are

$$\boldsymbol{w}(\boldsymbol{x},t) = \begin{pmatrix}\theta\\ n\end{pmatrix} = \sum_{\tilde\phi,\tilde\psi} p(\tilde\phi,\tilde\psi)\begin{pmatrix}1\\ M(k^2)\end{pmatrix}\exp(\lambda(k^2)t)\cos\phi x\cos\psi y,$$

where

$$M(k^2) = \frac{-\alpha k^2}{\lambda(k^2)+Dk^2}.$$

The wave vectors $\boldsymbol{k}$ are now discrete and take the form $\boldsymbol{k} = (\phi,\psi)^{\mathrm{T}}$ where

$$\phi = \tilde\phi\pi/L_x, \quad \psi = \tilde\psi\pi/L_y, \quad \tilde\phi,\tilde\psi = 0,1,2,\ldots, \tag{3.9}$$

and $k^2 = \phi^2+\psi^2$. The values of $p(\tilde\phi,\tilde\psi)$ is determined by a Fourier transform of the initial conditions.

The spatially heterogeneous solution that emerges for large time is the sum of the modes

$$p(\tilde\phi,\tilde\psi)\begin{pmatrix}1\\ M(k^2)\end{pmatrix}\cos\phi x\cos\psi y,$$

corresponding to the *mode pairs* $(\tilde\phi,\tilde\psi)$ for which

$$\lambda\left(\left(\pi\tilde\phi/L_x\right)^2+\left(\pi\tilde\psi/L_y\right)^2\right) > 0.$$

Specifically we consider examples in which only one of the discrete eigenvalues, say k_c^2, is unstable. Depending on the domain size, k_c^2 could be either a simple or a multiple eigenvalue. If k_c^2 is a multiple eigenvalue one or more of the mode pairs $(\tilde\phi,\tilde\psi)$ from the above sequence (3.9) satisfy the expression

$$k_c^2 = \left(\tilde\phi\pi/L_x\right)^2+\left(\tilde\psi\pi/L_y\right)^2.$$

For the square domain, $L_x = L_y$, examples of mode pairs corresponding to a simple eigenvalue are

$$\{(1,1)\},\{(2,2)\},\{(3,3)\}.$$

Double eigenvalues correspond to the sets of mode pairs $\{(0,1),(1,0)\}$, $\{(0,2),(2,0)\}$, $\{(1,2),(2,1)\}$, while a triple eigenvalue would, for example, correspond to $\{(5,5),(1,7),(7,1)\}$. The eigenvalue k_c^2, corresponding to the set of mode pairs $\{(0,5),(5,0),(3,4),(4,3)\}$, is a quadruple eigenvalue and so on.

A large variety of linear patterns can develop from these sets of linearly unstable modes. Since the dermal cell density solution n, differs only by the constant factor $M(k_c^2)$ from the epithelial dilation θ, its solution is qualitatively similar.

For simplicity we shall therefore only examine the dilation solutions θ, of the linear problem (3.6).

In general, single mode pair solutions give rise to rhombic spatial patterns, since the linear time independent solution which emerges is of the form

$$\theta(\boldsymbol{x}) = \cos\phi x \cos\psi y, \tag{3.10}$$

where $\boldsymbol{k}_c = (\phi, \psi)^{\mathrm{T}}$ is the discrete wave vector satisfying the zero-flux boundary conditions (3.5). (The solution (3.10) has been scaled so that $\theta(0,0) = 1$.) Expression (3.10) can be rewritten as

$$\theta(\boldsymbol{x}) = \tfrac{1}{2}(\cos(\phi x + \psi y) + \cos(\phi x - \psi y)),$$

which, in terms of polar coordinates (r, ϑ), is

$$\theta(r,\vartheta) = \tfrac{1}{2}(\cos\{r(\phi\cos\vartheta + \psi\sin\vartheta)\} + \cos\{r(\phi\cos\vartheta - \psi\sin\vartheta)\}). \tag{3.11}$$

Furthermore, by considering (ϕ, ψ) as variables and transforming them to polar coordinates $(\kappa, \frac{1}{2}\varphi)$, expression (3.11) simplifies to

$$\theta(r,\vartheta) = \tfrac{1}{2}(\cos\{\kappa r\cos(\vartheta - \tfrac{1}{2}\varphi)\} + \cos\{\kappa r\cos(\vartheta + \tfrac{1}{2}\varphi)\}),$$

where

$$\kappa = \sqrt{(\phi^2 + \psi^2)} = \sqrt{k_c^2}, \qquad \varphi = 2\arccos(\phi/\sqrt{k_c^2}).$$

We thus see that φ represents the rhombic angle of the solution and that the solution is invariant under a rhombic rotation, that is

$$\theta(r,\vartheta) = \theta(r,\vartheta+\pi) = \mathcal{R}\theta(r,\vartheta) = \theta(r,\vartheta),$$

where $\mathcal{R}$ is the rhombic operator.

If $\varphi = \frac{1}{2}\pi$ or $\varphi = \frac{3}{2}\pi$, then $\phi = \psi$ and a square- or chess board-type pattern evolves, which is a special case of the rhombic pattern.

The simplest non-homogeneous pattern possible on the two-dimensional rectangular domain is the roll, which occurs when either $\phi = 0$ or $\psi = 0$ in the rhombic solution (3.10). The roll is invariant under a rotation of π.

All the linear patterned solutions, arising from a simple eigenvalue, tessellate the plane, since they satisfy

$$\Gamma(\boldsymbol{x} + j\omega_1 + l\omega_2) = \Gamma(\boldsymbol{x}),$$

where $\Gamma = (n, \theta)^{\mathrm{T}}$ is the solution of the system, j, l are integers and ω_1, ω_2 are appropriately chosen independent vectors.

Patterns corresponding to multiple eigenvalues are not usually tessellations of the plane. A much richer class of mixed mode patterns does, however, exist. For example, when we have a double eigenvalue k_c^2, with the two corresponding wave vectors, say $(\phi_1, \psi_1)^{\mathrm{T}}$ and $(\phi_2, \psi_2)^{\mathrm{T}}$, satisfying the zero-flux boundary conditions (3.5), two rhombic patterns interact, thus

$$\theta(\boldsymbol{x}) = \alpha\cos\phi_1 x\cos\psi_1 y + \beta\cos\phi_2 x\cos\psi_2 y,$$

where α and β are real numbers so that $\alpha + \beta = 1$. (This scales the time independent solution so that $\theta(0,0) = 1$.) Specifically, if we consider a square domain of dimensions $(3,3)$ and isolate the unstable eigenvalue $k_c^2 = 40\pi^2/9$, then the corresponding unstable mode pairs are $(2,6)$ and $(6,2)$. The time independent solution to the linear problem, where $\alpha = \beta = \frac{1}{2}$, is as illustrated in figure 2.

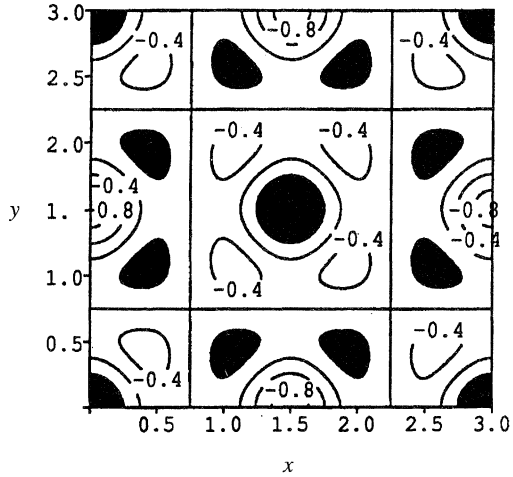

Figure 2. The linear double mode complex patterned solution corresponding to the mode pairs (2,6) and (6,2) plotted on the square domain (3,3); see expression (3.10). Here $\alpha = \beta = \frac{1}{2}$. Regions where the solution is larger than 0.8 are shaded.

Mixed mode patterns can vary considerably depending on the modes interacting. There is, however, a simple tessellation of the plane that can be generated by two interacting modes. When the two wave vectors are

$$\begin{pmatrix}\phi_1\\ \psi_1\end{pmatrix} = \begin{pmatrix}\kappa\\ \sqrt{3}\,\kappa\end{pmatrix}, \qquad \begin{pmatrix}\phi_2\\ \psi_2\end{pmatrix} = \begin{pmatrix}2\kappa\\ 0\end{pmatrix}, \tag{3.12}$$

or

$$\begin{pmatrix}\phi_1\\ \psi_1\end{pmatrix} = \begin{pmatrix}\sqrt{3}\,\kappa\\ \kappa\end{pmatrix}, \qquad \begin{pmatrix}\phi_2\\ \psi_2\end{pmatrix} = \begin{pmatrix}0\\ 2\kappa\end{pmatrix}, \tag{3.13}$$

and $\alpha = \frac{2}{3}$, $\beta = \frac{1}{3}$ a hexagonal pattern results. With wave vectors as in (3.12) the solution can be written as

$$\theta(\boldsymbol{x}) = \tfrac{1}{3}[\cos\kappa(\sqrt{3}y + x) + \cos\kappa(\sqrt{3}y - x) + \cos 2\kappa x],$$

which, in terms of polar coordinates (r, ϑ), is

$$\theta(r,\vartheta) = \tfrac{1}{3}[\cos\{2\kappa r\sin(\vartheta + \tfrac{1}{6}\pi)\} + \cos\{2\kappa r\sin(\vartheta - \tfrac{1}{6}\pi)\} + \cos\{2\kappa r\sin(\vartheta - \tfrac{1}{2}\pi)\}].$$

The polar coordinate form shows the invariance under a hexagonal rotation, that is invariance to rotation by $\frac{1}{3}\pi$, thus

$$\theta(r,\vartheta) = \theta(r,\vartheta + \tfrac{1}{3}\pi) = \mathcal{H}\theta(r,\vartheta) = \theta(r,\vartheta),$$

where $\mathcal{H}$ is the hexagonal rotation operator.

As an illustrative example we assume the unstable eigenvalue $k_c^2 = 4\pi^2$ on the domain $(2\sqrt{3}, 2)$. The corresponding mode pairs are $(6, 2)$ and $(0, 4)$ so that the two wave vectors have the required form (3.13), where $\kappa = \pi$. The resulting pattern is shown in figure 3. Note that hexagonal solutions not only satisfy zero-flux conditions on the boundaries of a rectangular domain, but do so also on all the hexagonal symmetry boundaries.

Naturally, when we have a triple or higher multiple eigenvalue, a much richer and more complex range of linear patterns is possible.

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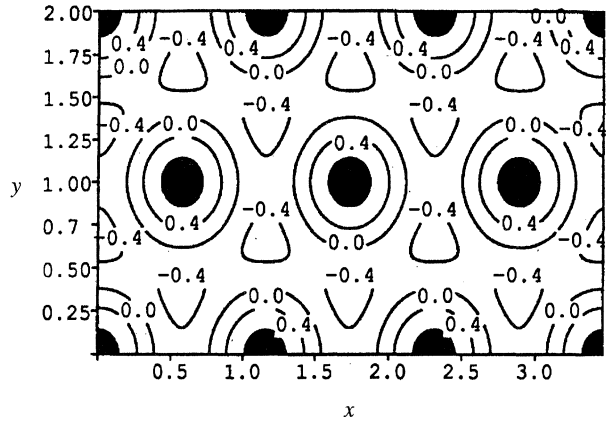

Figure 3. A contour graph of the linear hexagonal pattern corresponding to the mode pairs (6,2) and (0,4) plotted on the rectangular domain $(2\sqrt{3}, 2)$; see expression (3.13). Regions where the solution is larger than 0.8 are shaded.

We have only considered linear solutions in this section. From our numerical results, using the full nonlinear system, it seems, however, that the solutions to the linear system (3.6) very closely resemble those of the corresponding nonlinear equations (3.4) when we are in the neighbourhood of the bifurcation point. That the linear solutions give a very good indication of the nonlinear results can, for example, be seen in Cruywagen & Murray (1993) where a rhombic pattern produced by the model is presented.

4. Complex pattern formation and tissue interaction

We briefly consider here the tissue interaction model of Shaw & Murray (1990). They coupled two well-known pattern generating mechanisms used for modelling skin pattern formation. Unlike the model of Cruywagen & Murray (1992), patterned solutions are possible in the absence of tissue interaction. However, adding the effect of tissue interaction dramatically changes the nature of these patterned solutions as we shall see below.

The epithelial pattern formation is modelled by the Schnakenberg reaction-diffusion system (see, for example, Murray 1989). It is assumed that hypothetical chemicals provide *positional information* (Wolpert 1981) for epithelial morphogenesis. If we represent the concentration of the two chemicals by V and W, the system can be written as

$$\frac{\partial V}{\partial t} = A - V + V^2 W + D_V \nabla^2 V - \delta\theta, \tag{4.1 a}$$

$$\frac{\partial W}{\partial t} = B - V^2 W + D_W \nabla^2 W. \tag{4.1 b}$$

where D_V and D_W are the positive diffusion rates and, A and B are positive constants related to the production and kinetics of the chemicals. Note that an additional term, $-\delta\theta$, has been added to the first equation to account for the dermal to epidermal tissue interaction. The variable θ denotes the dermal cell

dilation and the parameter δ reflects the strength of the coupling between the layers.

For the dermis a mechanochemical model akin to the one described in the previous section is used. It consists of three equations: a force balance equation for modelling the forces within the extracellular matrix and two conservation equations, one for dermal cell density and the other for the extracellular matrix density.

As before the variable n is the dermal cell density, ρ is the extracellular matrix density and $\boldsymbol{u}$ the displacement of the matrix, caused by forces deriving from the cell traction. The cell conservation equation is

$$\frac{\partial n}{\partial t} + \overbrace{\nabla \cdot \left(n \frac{\partial \boldsymbol{u}}{\partial t} \right)}^{\text{convection}} = 0, \tag{4.2}$$

the matrix conservation equation is

$$\frac{\partial \rho}{\partial t} + \overbrace{\nabla \cdot \left(\rho \frac{\partial \boldsymbol{u}}{\partial t} \right)}^{\text{convection}} = 0, \tag{4.3}$$

while the matrix force balance equation is

$$\nabla \cdot \left\{ \overbrace{\mu_1 \frac{\partial \epsilon}{\partial t} + \mu_2 \frac{\partial \theta}{\partial t} \boldsymbol{I}}^{\text{viscous stresses}} + \overbrace{\epsilon + \nu' \theta \boldsymbol{I}}^{\text{elastic stresses}} + \overbrace{\frac{\tau n}{1 + \lambda n^2} (\rho + \beta \nabla^2 \rho)(1 + \gamma(V - V_0)) \boldsymbol{I}}^{\text{active traction}} \right\} = \overbrace{s \boldsymbol{u} \rho}^{\text{body forces}}, \tag{4.4}$$

where μ_1, μ_2, ν', I, ϵ and θ are as in equation (3.1). Again τ measures the strength of the active traction. Here the parameter λ accounts for cell–cell contact inhibition in high cell populations. Traction also depends on the matrix density ρ, with an added long range contribution $\beta \nabla^2 \rho$, where β (> 0) measures the strength of the long-range traction. The dermal to epidermal interaction is introduced through the $\gamma(V - V_0)$ term, where V_0 is the uniform steady state of the chemical V and γ is a measure of the strength of the interaction. The parameter s reflects the attachment of the extracellular matrix to the surrounding tissue.

As in the model of Cruywagen & Murray (1992) the above set of equations can again be reduced by using the biologically justified small strain approximation. From equations (4.2), (4.3) we see that both cell density, n, and matrix density, ρ, can be approximated by $1 - \theta$. Substituting these relationships into the force balance equation (4.4), and after linearizing the right-hand side, again using the small strain assumption, the divergence of the resulting equation gives a reduced model in terms of the dilation θ,

$$s\theta = \mu \frac{\partial}{\partial t} \nabla^2 \theta + \nabla^2 \theta + \tau \nabla^2 \left(\frac{(1 - \theta)}{1 + \lambda (1 - \theta)^2} \left(1 - \theta - \beta \nabla^2 \theta \right) \left(1 + \gamma (V - V_0) \right) \right), \tag{4.5}$$

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where for algebraic convenience, $\mu = \mu_1 + \mu_2$ and μ, τ and s have been divided by $(1 + \nu')$.

As explained in § 2, tissue interaction is introduced via dermal and epidermal factors operating across the basal lamina to influence the behaviour of the adjacent layer. In this model the epidermal factor is responsible for modifying the traction which the dermal cells exert on the extracellular matrix. This modification is assumed to be in the form of a multiplier, $(1 + \gamma(V - V_0))$, of the active traction term in the force balance equation (3.1). The dermal factor in turn is assumed to alter the production rate of the morphogen V, which is introduced by adding $-\delta\theta$ to the equation (4.1 *a*) for morphogen V.

(*a*) *Linear stability analysis*

A linear stability analysis can again be used to predict the final nonlinear solutions and, furthermore, can highlight the role of tissue interaction in the model. The homogeneous steady state of the reduced system (4.1) and (4.5) is given by

$$V_0 = A + B, \quad W_0 = B/(A + B)^2, \quad \theta_0 = 0.$$

We first consider the model in the case when the epidermis and dermis are uncoupled, thus γ and δ are both zero. Two independent dispersion relations, one for each layer, result.

The dispersion relation for the linearized epithelial model is

$$\sigma^2(k^2) + I(k^2)\sigma(k^2) + H(k^2) = 0, \tag{4.6}$$

where

$$I(k^2) = (D_V + D_W)k^2 + \left((A + B)^2 + \frac{(A - B)}{(A + B)}\right),$$

$$H(k^2) = D_V D_W k^4 + \left(D_W \frac{(A - B)}{(A + B)} + D_V(A + B)^2\right) k^2 + (A + B)^2.$$

This gives a typical parabolic dispersion relation and if, say, $A < B$, then bifurcation to spatial structure can occur through diffusion driven instability by increasing the value of D_W through a critical threshold.

The dispersion relation derived for the dermal mechanochemical equation (4.5) is

$$\mu k^2 \sigma(k^2) + G(k^2) = 0, \tag{4.7}$$

where

$$G(k^2) = \frac{\beta\tau k^4}{1 + \lambda} + \left(1 - \frac{2\tau}{(1 + \lambda)^2}\right) k^2 + s.$$

Again a parabolic-type dispersion relation results, and bifurcation to spatial pattern occurs, for example, by increasing the traction parameter, τ, through a critical threshold.

Since we can isolate a specific critical eigenvalue, say k_c^2, for either of these dispersion relations, the class of patterns that can arise is exactly the same as those discussed in the linear analysis of the previous section.

Here, however, the dermal and epidermal models are capable of producing patterns independently and completely unrelated patterns could develop in the respective layers. This is, however, biologically unrealistic. By introducing tissue

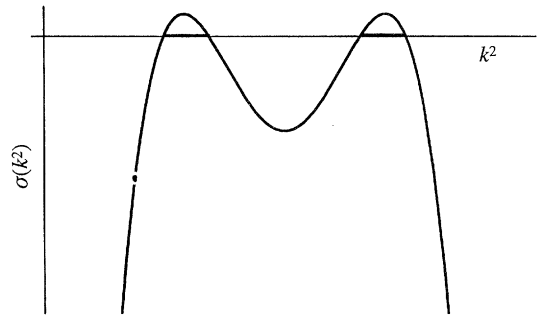

Figure 4. A typical dispersion relation for the tissue interaction model (4.1) and (4.5) with two ranges of unstable wave numbers, indicated by the heavy lines. In this case the dispersion relation is completely symmetric and the unstable modes would have the same linear growth rate. This is, however, not true in general.

interaction, the two pattern generators are coupled and this excites the two sets of equations into producing highly complex, but intimately related, patterns as we shall see below.

When either γ or δ are non-zero we have a single dispersion relation for the composite system of equations (4.1) and (4.5), namely,

$$[\sigma^2(k^2) + I(k^2)\sigma(k^2) + H(k^2)]\,[\mu k^2\sigma(k^2) + G(k^2)] - \frac{\gamma\delta\tau}{1+\gamma}\,(\sigma(k^2) + J(k^2))\,k^2 = 0, \qquad (4.8)$$

where

$$J(k^2) = D_W k^2 + (A+B)^2.$$

Note that this equation is the product of the dispersion relation (4.6), of the epithelial model and the dispersion relation (4.7), of the dermal model, with an additional term that reflects the two-way interaction.

As in § 3, the value of the interaction parameters, γ and δ, are crucial to the resulting solutions. Shaw & Murray (1990) demonstrated that if either or both are positive, two different ranges of unstable eigenvalues can actually be isolated as is shown in figure 4. The stability of these eigenvalues depends on the product of the two interaction parameters. By increasing $\gamma\delta$ the ranges of unstable eigenvalues are increased. By decreasing this product both ranges of unstable modes decrease and do indeed vanish if for $\gamma\delta = 0$ ($\gamma \neq 0$ or $\delta \neq 0$) there are no unstable modes.

When $\delta = 0$ ($\gamma = 0$) the above dispersion relation only holds for the dermal equation (4.5) (epidermal equation (4.1)), while the original uncoupled dispersion relation (4.6) ((4.7)) is valid for the epidermal (dermal) model. In effect one of the pattern generators is decoupled, but still influences the patterns of the other generator through the interaction term which acts as a *forcing* term.

By choosing the parameters appropriately one could isolate two marginally unstable eigenvalues. The solution to the linear system would therefore be a superposition of the patterns corresponding to these two unstable eigenvalues.

As we have already shown in the previous section, a single unstable multiple eigenvalue gives rise to extremely complex patterns, since the eigenvalue decomposes into multiple wave vectors that satisfy the boundary conditions. By coupling

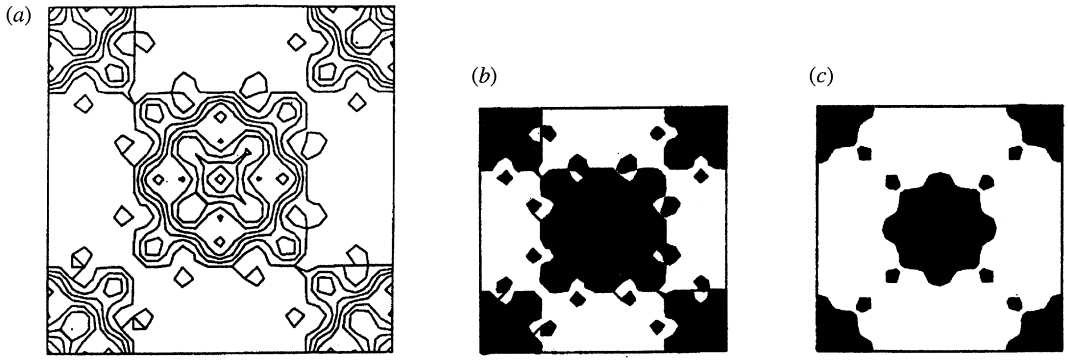

Figure 5. A typical steady-state pattern in the epithelial chemical concentration V, that results from solving the model (4.1) and (4.5) numerically in the vicinity of the bifurcation point. (*a*) Contours spaced at intervals of 0.002. (*b*) Regions where V is larger than 1.000 are shaded. (*c*) Regions where V is larger than 1.004 are shaded (from Shaw 1989).

two pattern generating mechanisms, each with its own unstable, simple or multiple, eigenvalue, the complexity of the linear solutions is drastically increased. The number of interacting modes is now the sum of the multiplicity of the two independent eigenvalues. That this is indeed the case is evident from numerical simulations of the nonlinear system. A typical result is shown in figure 5.

Interestingly, the interaction serves a dual purpose in this model, since not only does it excite the tissue into producing highly complex patterns, it also couples the two independent pattern generating mechanism to give one resulting pattern for both layers. It therefore has a global stabilizing effect by reducing the number of apparently possible patterns. This has important implications for embryogenic patterning (see Murray 1993 for a detailed discussion).

5. Tissue interaction and sequential pattern formation

The above models can explain patterning phenomena that occur simultaneously, everywhere, in the prospective tissue. Often, however, embryonic patterns and structure develop sequentially. This is, for example, the case in skin pigmentation in alligators (see Murray *et al.* 1990). The stripelike patterns seen on alligators are laid down sequentially from the head to the tail.

Chick feather germs also develop sequentially (Sengel 1976). An initial row of feather germs is laid down along the dorsal midline. New rows are then added laterally at equal time intervals. For a more detailed discussion of these and other examples refer to Cruywagen *et al.* (1993).

Modelling these phenomena is, however, not an easy task. Nagorcka (1986) made a first attempt and demonstrated how tissue interaction could be the key to understanding these processes. He introduced a switch mechanism that travels across the skin to make it sequentially competent for pattern formation. Parameter values are selected so that bifurcation to spatial pattern could only occur inside the switch boundary. Nagorcka (1986) argued that the bifurcation parameter should be involved in the tissue interaction mechanism so that by switching interaction on, the model becomes capable of generating patterns. Since patterns

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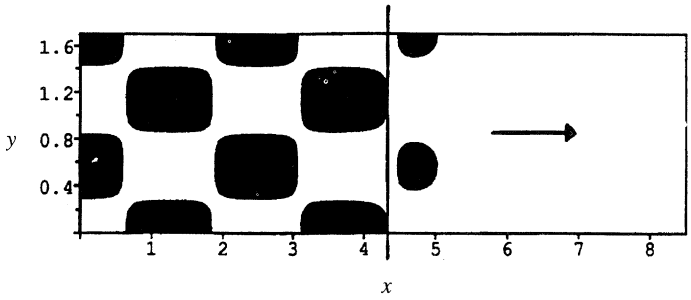

Figure 6. Sequential pattern formation in the reduced caricature tissue interaction model (3.4) with a moving switch mechanism. Initial conditions are random perturbations about the non-trivial homogeneous steady state specified over the whole domain. Regions of high cell density ($n > 1.0$) are shaded.

are only possible in the region where the tissue interaction mechanism is switched on, they are laid down sequentially along with the travelling switch mechanism.

Cruywagen *et al.* (1993) extended the idea of a switch mechanism further by using the model of Cruywagen & Murray (1992) discussed in § 3. They considered rectangular domains and as switch mechanism had a vertical line moving from one end of the domain to the other. As the switch mechanism travels out (see figure 6) a small narrow strip of tissue initially becomes capable of pattern formation. The first patterns are laid down in this strip and are thus in effect one-dimensional. They demonstrated that the initial pattern that is laid down on the quasi-one-dimensional domain actually dictates the resulting pattern on the two-dimensional domain.

Although the model of Cruywagen & Murray (1992) is phenomenologically very close to the biology it can only produce very simple sinusoidal patterns in one dimension. Since it has a parabolic-type dispersion relation, only one range of eigenvalues can be isolated of which only the dominant one grows an persist. Only simple two-dimensional patterns would result from such a one-dimensional pattern.

The model of Shaw & Murray (1990) can in fact account for complex patterns in one dimension. With the dispersion relation (4.8), as shown in figure 4, one can isolate two independent eigenvalues. Since two dominant wave numbers, one each from the two ranges of unstable eigenvalues, grow, mixed mode patterns develop in one dimension. As the switch travels out, these patterns persist and mixed mode patterns are laid down on the two-dimensional domain.

Thus for the sequential formation of complex patterns a more involved model such as Shaw & Murray's (1990) tissue interaction model is necessary.

6. Discussion

Ever since the crucial importance of tissue interaction in embryonic development has been demonstrated experimentally, it has become necessary to adapt the more traditional pattern formation models to try to understand the mechanistic implications of these new results. We have examined two models in which the tissue interaction mechanisms are used to couple the usual mechanochemical, chemotaxis and reaction diffusion systems for skin morphogenesis. With these

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models we were able to offer an explanation for some of the experimentally observed results which previous models failed to address.

The model of Cruywagen & Murray (1992), discussed in § 3, explains why skin pattern formation only occurs in the presence of both dermal and epidermal tissue. They proposed that a tissue interaction mechanism is needed and has to be dominant enough to excite uniform tissue into active pattern formation.

By using the model of Shaw & Murray (1990) we showed in § 4 that by coupling two pattern generators, each with its own characteristic pattern, the system is excited into forming far more complex structures than the usual sinusoidal patterns. With this model we could thus explain the formation of complex patterns that are seen, for example, on reptile skin.

Although many embryonic patterns are laid down sequentially, good models for explaining this process are still lacking. It seems, however, natural in modelling such sequential processes, to use tissue interaction as the switch mechanism, which turns pattern formation on. Furthermore, as explained in § 5, dermal–epidermal communication is also necessary for modelling the sequential formation of very complex patterns.

Note that only a few tissue interaction studies have been carried out so far and further investigation is necessary. For example, what would happen if two mechanochemical models or two reaction diffusion models interact in a spatial context? It is however, evident, that interaction mechanisms can excite tissue into interesting behaviours, which could lead to the explanation of hitherto unsolved problems in morphogenesis.

This work was in part supported by a grant from the U.S. National Institute of Health (RR 01243–12), Simulations Research Facility in Circulatory Mass Transport and Exchange. G.C.C. also thanks the Philip Endowment of the University of Washington and the South African Foundation for Research Development for their financial support.

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