REACTION-DIFFUSION SYSTEMS AND EXTERNAL MORPHOGEN GRADIENTS: THE TWO-DIMENSIONAL CASE, WITH AN APPLICATION TO SKELETAL PATTERN FORMATION

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Abstract. We investigate a reaction-diffusion system consisting of an activator and an inhibitor in a two-dimensional domain. There is a morphogen gradient in the domain. The production of the activator depends on the concentration of the morphogen. Mathematically, this leads to reaction-diffusion equations with explicitly space-dependent terms. It is well known that in the absence of an external morphogen, the system can produce either spots or stripes via the Turing bifurcation. We derive first order expansions for the possible patterns in the presence of an external morphogen and show how both stripes and spots are affected. This work generalizes previous one-dimensional results to two dimensions. Specifically, we consider the quasi-one-dimensional case of a thin rectangular domain and the case of a square domain. We apply the results to a model of skeletal pattern formation in vertebrate limbs. In the framework of reaction-diffusion models, our results suggest a simple explanation for some recent experimental findings in the mouse limb which are much harder to explain in positional-information-type models.

1. Introduction

The problem of pattern formation in developmental biology is concerned with how complex spatial structures in tissues and organs arise during embryonic development through the interaction of cells with their environment and with each other.

It has been shown experimentally that the action of gradients of signalling molecules is important for development [1]. Examples of such gradients include the protein bicoid in Drosophila, or Sonic Hedgehog in vertebrate limbs.

One of the simplest theories of how these gradients determine patterning is the concept of positional information [1]: Cells “sense” the local chemical concentration and determine their location within the developing tissue based on this. They then execute a genetic program depending on their location.

Another paradigm for pattern formation is the Turing mechanism in reaction-diffusion systems [2, 3]. Under certain conditions, the interplay of diffusion and reaction of two or more chemicals can lead to the emergence of patterns in the concentration of the chemicals. Reaction-diffusion models have been proposed for several different phenomena such as the patterning of animal skins, the spacing of hair follicles and the process of skeletal pattern formation in embryonic vertebrate limbs [4, 3, 5]. In many of these situations, chemical gradients are known to exist and to play an important role.

In [6], we set out to investigate the interaction of reaction-diffusion models with a modulating chemical gradient. In a generic model, there are two chemicals, an inhibitor and an activator molecule, which are secreted by cells, diffuse, decay, and react with each other. There is also a chemical gradient present whose action is to increase the rate at which the activator molecule is expressed by cells. Assuming a linear gradient, the resulting equations are

\[
\frac{\partial U}{\partial t} = F(U) + D\nabla^2 U + x \begin{bmatrix} \varepsilon_a U \\ 0 \end{bmatrix},
\]  

(1.1)
where \( U(x,t) = (u(x,t), v(x,t)) \) is a vector of chemical concentrations, \( F(U) \) describe the reaction kinetics, \( D = \text{diag}(D_u, D_v) \) is the matrix of diffusion coefficients, and \( \varepsilon_c a \) is some constant. For an analytical treatment, we assumed that the parameter \( \varepsilon_c \) is “small”.

The above equations were studied in one spatial dimension in [6]. The results can be summarized as follows: In the parameter region where a Turing pattern is possible, adding a “small” chemical signal essentially does not change the wave number of the pattern. It changes the baseline of the pattern to a linear function which increases towards the source of the external signal, as one would expect. The amplitude of the pattern either increases or decreases towards the source, or, somewhat unexpectedly, it may have a minimum somewhere in the interval. See also the concentration patterns in Figure 3 below, where we apply a two-dimensional analogous result to a simple caricature model of skeletal pattern formation in vertebrate limbs.

We also considered in [6] the parameter region where the system (1.1) cannot produce Turing patterns in the absence of an external morphogen (that is when \( \varepsilon_c = 0 \)). Maybe surprisingly, for a large subregion of this parameter region, the system can produce Turing-like patterns if the external morphogen is present, that is for nonzero \( \varepsilon_c \). For instance, taking the activator/inhibitor diffusivity ratio \( d = D_v / D_u \) as the bifurcation parameter, it is well known that for \( d > 1 \), there are no Turing patterns in generic reaction-diffusion systems. Indeed, this is the case when the activator diffuses faster than the inhibitor. However, in the example involving an external morphogen considered in [6], the system can produce “Turing-like” oscillatory patterns for values of \( d \) up to approximately 5.48. A caveat is that the amplitude is very small for larger values of \( d \).

In the present paper, we generalize the analysis from one to two spatial dimensions, which is of course more relevant to applications in physical or biological modeling. We also apply our results to a simple model of chondrogenesis in vertebrate limbs. We propose that our previous results can explain in a reaction-diffusion framework certain experimental findings concerning the action of the signalling molecule Sonic hedgehog (SHH). In mouse embryos, the availability of SHH in non-SHH-producing cells was genetically modified. This lead to a shallower SHH gradient. The observed result was the loss of a middle finger [7]. These results are hard to explain in a simple positional information model, but we show how these results are consistent with a reaction-diffusion model in conjunction with the action of SHH as an “external” morphogen.

2. Model equations and Turing bifurcation

We analyze reaction-diffusion equations with a spatially dependent term of the form

\[
\frac{\partial U}{\partial t} = x \begin{bmatrix} \varepsilon_c a \\ 0 \end{bmatrix} + AU + D \nabla^2 U + Q(U,U) + C(U,U,U) + h.o.t.
\]  

(2.1)

where \( U(t,x) = (u(t,x) - u_0, v(t,x) - v_0) \) is a vector of concentrations of two chemicals at position \( x = (x,y) \), offset by some constant concentrations \( u_0, v_0 \), respectively. (The concentrations \( u_0, v_0 \) form a spatially homogeneous steady state if \( \varepsilon_c = 0 \), see below.) \( A \) is a matrix, \( Q \) and \( C \) are quadratic and cubic terms, respectively. The matrix \( D \) is the diffusion matrix:

\[
D = \begin{bmatrix} d & 0 \\ 0 & 1 \end{bmatrix}
\]

Here the inhibitor diffusion coefficient is scaled to 1 and \( d \) denotes the ratio of the activator and the inhibitor diffusion coefficients. The first term on the right hand side describes a spatially dependent kinetics term, where \( a \) is a constant and \( \varepsilon_c \) is a small parameter.

We consider the above equations on a rectangle \( R = [0, L_x] \times [0, L_y] \) and no-flux conditions

\[
\frac{\partial U}{\partial x} \bigg|_{x=0,L_x} = 0, \quad \frac{\partial U}{\partial y} \bigg|_{y=0,L_y} = 0.
\]  

(2.2)

We consider certain additional geometric conditions on the rectangle \( R \) describing cases relevant for models of biological development below in section 3.
We are primarily interested in the steady state $U(x)$ given by
\begin{equation}
0 = x \left[ \begin{array}{c}
epsilon \alpha \\ 0 \end{array} \right] + AU + D \nabla^2 U + Q(U, U) + C(U, U, U) + \text{h.o.t.}
\end{equation}
Note that for $\epsilon = 0$, the system has a spatially homogeneous steady state solution $U_0 = (0, 0)$, corresponding to the spatial homogeneous steady state $u(x, t) = u_0$, $v(x, t) = v_0$.

Now suppose we keep $\epsilon = 0$ for now and fix all parameters except $d$, the ratio of diffusion coefficients. For some critical value $d = d_0$, the steady state solution $U_0 = (0, 0)$ becomes unstable and a Turing bifurcation occurs for $d < d_0$. We introduce the small parameter $\epsilon_d$ by writing
\[ d = d_0 - \epsilon_d^2 d_2 + \cdots. \]
Correspondingly, we write in matrix notation
\[ D = D_0 - \epsilon_d^2 D_2 + \cdots, \]
where $D_0 = \text{diag}(d_0, 1)$ and $D_2 = \text{diag}(d_2, 0)$. We can then write the steadys state solution as an asymptotic expansion in the two small parameters $\epsilon_c$ and $\epsilon_d$:
\begin{equation}
U(x) = \epsilon_d U_{01}(x) + \epsilon_c U_{10}(x) + \epsilon_d^2 U_{02}(x) + \epsilon_c^2 U_{20}(x) + \epsilon_d \epsilon_c U_{11}(x) + \cdots.
\end{equation}
The following two assumptions are made for the linearization matrix $A$, guaranteeing a Turing bifurcation [3, 6]:
\begin{enumerate}
\item Without spatial variation, $A$ corresponds to a stable dynamical system, i.e. both eigenvalues of $A$, $\gamma_1$ and $\gamma_2$, satisfy $\text{Re}(\gamma_1) < 0$ and $\text{Re}(\gamma_2) < 0$. So
\[ \text{trace}A = \gamma_1 + \gamma_2 < 0, \quad \det A = \gamma_1 \gamma_2 > 0. \]
\item For $d > d_0$, $A$ is stable with respect to any spatially varying perturbation with wave number $k$, i.e.
\[ \det(A - k^2 D) > 0 \quad \text{for all } k, \text{ for } d > d_0. \]
For $d < d_0$, $A$ is unstable with respect to some spatially varying perturbation with wave number $k$, i.e. for some $k > 0$, $A - k^2 D$ is unstable.
\end{enumerate}
The critical ratio of diffusivities $d_0$ is given by the conditions
\[ \det(A - k_0^2 D_0) = 0 \text{ for some critical wave number } k_0, \]
\[ \det(A - k^2 D_0) > 0 \text{ for } k^2 \neq k_0^2. \]
Thus there is a Turing bifurcation at $d = d_0$; a Turing pattern with wave number $k_0$ appears. Note that $D_0^{-1} A$ has repeated eigenvalue $k_0^2$ with only one corresponding eigenvector; thus $D_0^{-1} A$ is not diagonalizable. Let $V_1$ be the eigenvector corresponding to $k_0^2$ and $V_2$ be a vector that satisfies $(D_0^{-1} A - k_0^2 I) V_2 = V_1$. Since $V_1$ and $V_2$ are linearly independent, we have $P^{-1}(D_0^{-1} A) P = \Lambda$, where $P = [V_1 \ V_2]$ and
\[ \Lambda = \begin{bmatrix} k_0^2 & 1 \\ 0 & k_0^2 \end{bmatrix}. \]
Note that the above discussion of the Turing bifurcation is valid in the absence of an external morphogen gradient, that is, for $\epsilon_c = 0$ in the system (2.1). In section 4, we investigate what happens at the critical diffusion ratio $d_0$ in the presence of an external morphogen gradient, that is, when $\epsilon_c$ is nonzero. We will see that the situation depends on the geometry of the domain.

3. Geometric Conditions on the Domain

We now describe certain conditions on the rectangular domain $R = [0, L_x] \times [0, L_y]$. So the length of $R$ is $L_x$ and the width is $L_y$. A class of functions satisfying the boundary conditions (2.2) consists of functions of the form $\cos(m \pi x / L_x) \cos(n \pi y / L_y)$ where $m, n \in \mathbb{Z}_0^+$ are nonnegative integers. The dispersion relation for $m, n$ for Turing patterns is the equation
\begin{equation}
\left( \frac{m \pi}{L_x} \right)^2 + \left( \frac{n \pi}{L_y} \right)^2 = k_0^2, \quad m, n \in \mathbb{Z}_0^+.
\end{equation}
(A reminder of the derivation of this relation is given in section 4.) Here \( k_0 \) is the critical wave number, see above. The existence of solutions \((m, n)\) and the number of solutions to the dispersion relation depend on the geometry of the domain \( R \). We now consider two classes of rectangles \( R \) which are of interest for applications in modeling.

3.1. Small width: Quasi-one-dimensional case. In the first case, we consider those pairs of length \( L_x \) and width \( L_y \) for which the only solution of (3.1) is

\[
  n = 0, \quad m = m^* \quad \text{for some } m^* \in \mathbb{Z}_0^+.
\]

This is the case for instance if \( L_y \) is small, in particular if \( L_y < \pi/k_0 \). So we are dealing with domains that can be described as “long”, narrow rectangles. The external morphogen gradient runs along the long side of the rectangle. Shapes which are well approximated by long thin rectangles are encountered in several cases in developmental biology, for instance as the neural tube in developing vertebrates. Another example is the thin zone of undifferentiated mesenchymal cells beneath the apical ectodermal ridge of the embryonic chick limb bud. There is an SHH gradient along this zone. (See also section 7.)

3.2. Square. The second case is a square: \( L_x = L_y = L \). We also assume that the resulting equation

\[
  m^2 + n^2 = \left( \frac{k_0 L}{\pi} \right)^2
\]

has only two solutions, namely

\[
  m = m^*, n = 0 \quad \text{or} \quad m = 0, n = m^*
\]

for some \( m^* \in \mathbb{Z}_0^+ \).

For general reaction-diffusion equations in the absence of an external morphogen gradient, this case was analyzed by Ermentrout in [8], see also [9]. It is shown that there are two possible resulting Turing patterns, either stripes or spots. Details on the results and how the external morphogen gradient affects them are given in the following section below.

4. Results: Interaction of Turing pattern and external gradient

To determine the linear approximation of \( U \) in the form of (2.4), we compute analytically the first order terms in \( \varepsilon_d \) and \( \varepsilon_c \), \( U_{01} \) and \( U_{10} \), respectively.

4.1. Determination of \( U_{01} \). In either case 3.1 (small width) or 3.2 (square), collecting the \( \varepsilon_d \) terms, we get

\[
  D_0^{-1} A U_{01} + \nabla^2 U_{01} = 0. \quad (4.1)
\]

Making the ansatz

\[
  U_{01} = \sum_{m,n} \cos \frac{m\pi}{L_x} \cos \frac{n\pi}{L_y} u_{m,n} \quad (4.2)
\]

and plugging this back into (4.1) leads to the dispersion relation

\[
  \left( \frac{m\pi}{L_x} \right)^2 + \left( \frac{n\pi}{L_y} \right)^2 = k_0^2. \quad (4.3)
\]

By assumption on the geometries, we now have the following results:

Result 4.1. (Small width) Under the assumption of subsection 3.1, we have

\[
  U_{01} = \hat{c}_1 \cos(k_0 x) V_1 \quad (4.4)
\]

where \( \hat{c}_1 = \pm \sqrt{-\frac{\alpha_1}{\alpha_3}} \), with \( \alpha_1 = \langle k_0^2 D_2 V_1, V_1 \rangle \) and

\[
  \alpha_3 = \langle Q(V_1, A^{-1}Q(V_1, V_1)), V_1 \rangle + \frac{1}{2} \langle Q(V_1, (A - 4k_0^2 D_0)^{-1}Q(V_1, V_1)), V_1 \rangle - \frac{3}{4} \langle C(V_1, V_1, V_1), V_1 \rangle.
\]
Here $V_1$ is an eigenvector of $D_0^{-1}A$ corresponding to the eigenvalue $k_0^2$, and $V^\perp$ is a vector that is perpendicular to $D_0V_1$ as defined at the end of section 2. The bracket $\langle \cdot, \cdot \rangle$ denotes the inner product of two vectors.

This result follows immediately from the one-dimensional case in [6]. The square case is treated in [8]:

**Result 4.2. (Square)** Under the assumption of subsection 3.2, the general solution can be written as

$$U_{01} = \left( \hat{c}_1 \cos \frac{m\pi}{L} x + \hat{c}_2 \cos \frac{m\pi}{L} y \right) V_1.$$  \hspace{1cm} (4.5)

Here $\hat{c}_1$ and $\hat{c}_2$ are two coefficients. As shown in [8], only the following three possibilities can occur:

- case 1: $\hat{c}_2 = 0$ ("vertical stripes")
- case 2: $\hat{c}_1 = 0$ ("horizontal stripes")
- case 3: $\hat{c}_1 = \hat{c}_2$ ("spots")

There are explicit expressions for the nonzero coefficients given in [8].

### 4.2. Determination of $U_{10}$

**Result 4.3.** In either of the two cases of subsection 3.1 and 3.2, the following first order expansion in $\varepsilon_c$ holds:

$$U_{10} = \left( c_1 \cos k_0 x + c_2 \sin k_0 x + \frac{c_4}{2k_0} x \cos k_0 x \right) V_1 + (c_4 \sin k_0 x)V_2 - x(\beta_1 V_1 + \beta_2 V_2),$$  \hspace{1cm} (4.6)

where $c_2 = \frac{\beta_1}{k_0} - \frac{\beta_2}{2k_0}$ and $c_4 = \frac{\beta_2}{k_0}$. The coefficient $c_1$ is given by the solution of a linear algebraic equation given explicitly in section 5.2 of [6], which we omit here for brevity.

5. Derivation of Result 4.3

In the following we derive Result 4.3 using its one-dimensional counterparts derived in [6]. Assuming the uniqueness of solution, we discuss Results 4.1 and 4.2 one by one.

**Result 4.1:** This is identical to the one-dimensional analysis shown in [6].

**Result 4.2:** Collecting the $\varepsilon_c$ terms, we get

$$AU_{10} + D_0 \nabla^2 U_{10} = -x \begin{bmatrix} a \\ 0 \end{bmatrix}.$$  \hspace{1cm} (5.1)

Its general solution, satisfying the boundary conditions (2.2) reads

$$U_{10}(x, y) = c_0 \cos \left( \frac{m\pi}{L} y \right) V_1 + U_{10}^x = U_{10}^x + U_{10}^y,$$  \hspace{1cm} (5.2)

where

$$U_{10}^x = \left( c_1 \cos k_0 x + c_2 \sin k_0 x + \frac{c_4}{2k_0} x \cos k_0 x \right) V_1 + (c_4 \sin k_0 x)V_2 - x(\beta_1 V_1 + \beta_2 V_2)$$  \hspace{1cm} (5.3)

with $c_2 = \frac{\beta_1}{k_0} - \frac{\beta_2}{2k_0^2}$ and $c_4 = \frac{\beta_2}{k_0^2}$ and $A^{-1} \begin{bmatrix} a \\ 0 \end{bmatrix} = \beta_1 V_1 + \beta_2 V_2$.

The solution (5.2) satisfies the boundary conditions (2.2) for any value of $c_0$ and $c_1$, so $c_0$ and $c_1$ have to be determined by the quadratic and cubic terms in the $\varepsilon_c$ expansion of $U$. Collecting the $\varepsilon_c^2$ terms, we get

$$AU_{20} + D_0 \nabla^2 U_{20} + Q(U_{10}, U_{10}) = 0$$  \hspace{1cm} (5.4)

The nonhomogeneous terms in equation (5.4) take the following form

$$Q(U_{10}, U_{10}) = Q(U_{10}^x, U_{10}^x) + 2Q(U_{10}^x, U_{10}^y) + Q(U_{10}^y, U_{10}^y).$$

Therefore equation (5.4) has a particular solution

$$U_{20}^p = U_{20}^{xx} + U_{20}^{xy} + U_{20}^{yy}.$$
where $U_{30}^{x}(x), U_{30}^{xy}(x, y)$ and $U_{30}^{yy}(y)$ are the particular solutions to

\[
AU_{20}^{x} + D_0 \partial^2_x U_{20}^{x} + Q(U_{10}^{x}, U_{10}^{x}) = 0, \\
AU_{20}^{xy} + D_0 \partial^2_y U_{20}^{xy} + Q(U_{10}^{xy}, U_{10}^{xy}) = 0
\]

and

\[
AU_{20}^{yy} + D_0 \partial^2_y U_{20}^{yy} + Q(U_{10}^{yy}, U_{10}^{yy}) = 0 \tag{5.5}
\]

respectively.

Furthermore, collecting the $\varepsilon c^3$ terms, we get

\[
AU_{30} + D_0 \nabla^2 U_{30} + 2Q(U_{10}, U_{20}) + C(U_{10}, U_{10}, U_{10}) = 0 \tag{5.6}
\]

which has a particular solution

\[
U_{30}^p = U_{30}^{xx} + U_{30}^{xy} + U_{30}^{yy}
\]

where $U_{20}^{x}(x), U_{20}^{xy}(x, y)$ and $U_{20}^{yy}(y)$ are the particular solutions to

\[
AU_{30}^{xx} + D_0 \partial^2_x U_{30}^{xx} + 2Q(U_{10}^{x}, U_{20}^{x}) + C(U_{10}^{x}, U_{10}^{x}, U_{10}^{x}) = 0, \\
AU_{30}^{xy} + D_0 \partial^2_y U_{30}^{xy} + 2Q(U_{10}^{xy}, U_{20}^{xy}) + 2Q(U_{10}^{xy}, U_{20}^{xy}, U_{20}^{xy}) = 0
\]

and

\[
AU_{30}^{yy} + D_0 \partial^2_y U_{30}^{yy} + 2Q(U_{10}^{yy}, U_{20}^{xx}) + C(U_{10}^{yy}, U_{10}^{yy}, U_{10}^{yy}) = 0 \tag{5.7}
\]

respectively.

The following one-dimensional result was derived in [6]: Let $V^\perp$ be a vector perpendicular to $D_0 V_1$ and let $U_{01} = \tilde{c}_1 \cos(k_0 x)$ be the solution to

\[
AU_{01} + D_0(U_{01})_{xx} = 0 \tag{5.8}
\]

with the boundary conditions

\[
(U_{01})_x \bigg|_{x=0,L} = 0.
\]

Solving

\[
AU_{03} + D_0(U_{03})_{xx} + D_2(U_{01})_{xx} + 2Q(U_{01}, U_{02}) + C(U_{01}, U_{01}, U_{01}) = 0 \tag{5.9}
\]

with

\[
AU_{02} + D_0(U_{02})_{xx} + Q(U_{01}, U_{01}) = 0, \tag{5.10}
\]

for $U_{03}$ to satisfy the boundary conditions, $\tilde{c}_1$ must solve the algebraic equation

\[
\alpha_1 \tilde{c}_1 + \alpha_3 \tilde{c}_1^3 = 0
\]

with $\alpha_1 = \langle k_0^2 D_2 V_1, V^\perp \rangle$ and

\[
\alpha_3 = \langle Q(V_1, A^{-1} Q(V_1, V_1)), V^\perp \rangle + \frac{1}{2} \langle Q(V_1, (A - 4k_0^2 D_0)^{-1} Q(V_1, V_1)), V^\perp \rangle - \frac{3}{4} \langle C(V_1, V_1, V_1), V^\perp \rangle.
\]

The nonzero solutions are $\tilde{c}_1 = \pm \sqrt{-\frac{\alpha_1}{\alpha_3}}$.

Therefore, for $U_{30}^{yy}$ in equation (5.7) to satisfy the boundary conditions, we must have

\[
c_0 = 0
\]

since equations (5.5) and (5.7) are the special case of equations (5.10) and (5.9) with $D_2$ being the zero matrix.

It remains to determine $c_1$ in (5.3). Note that as $U_{10}^{y} = 0$, we may choose $U_{20}^{xy} = U_{20}^{yy} = 0$, and thus also $U_{30}^{xy} = U_{30}^{yy} = 0$. Therefore, the equations (5.4) and (5.6) reduce to ordinary differential equations in $x$ only (as opposed to partial differential equations in $x$ and $y$). Thus the one-dimensional analysis from [6] carries over to this case, giving the value of $c_0$ as stated in Result 4.3.
6. Examples and Numerics

We performed numerical tests to verify the validity of the analytic first order expansions $U_{10}$ and $U_{01}$ as given in (4.4), (4.5) and (4.6), respectively. Our approach was to compare the resulting first order approximation (2.4) to the numerically computed steady state solution as given by (2.3). We chose the Schnakenberg equations [3] as the reaction kinetics, so that we have the following set of equations:

\begin{align*}
\frac{\partial u}{\partial t} &= a(1 + \varepsilon_{c}x) + u^{2}v - u + d\nabla^{2}u \quad (6.1) \\
\frac{\partial v}{\partial t} &= b - u^{2}v + \nabla^{2}v. \quad (6.2)
\end{align*}

Here $a, b$ are two parameters and as before we write $d = d_{0} - \varepsilon_{d}^{2}d_{2} + \ldots$, where $d_{0}$ is the critical value of the diffusivity $d$. So a Turing bifurcation occurs at $\varepsilon_{d} = 0$.

We found that solving the full parabolic equations with a simple explicit forward Euler scheme was not accurate enough for the numerical computation of the steady state. Instead, we computed the solutions for the discretized steady state equation by means of Newton’s method, using the first order analytic expansion as the initial state. This worked better. For $\varepsilon_{c} = 0$ and approximately $10^{-2} < \varepsilon_{d} < 10^{-4}$, the results are very satisfactory. Similarly, for $\varepsilon_{d} = 0$ and approximately $10^{-3} < \varepsilon_{c} < 10^{-4}$, the results are acceptable. See Figure 1. However, if both $\varepsilon_{d}$ and $\varepsilon_{c}$ are nonzero, the fit is not nearly as good. Presumably, the discrepancy between the numerical solution and the first order approximation is due to the second order terms. By making $\varepsilon_{d}$ and $\varepsilon_{c}$ smaller, this effect would be reduced, but the numerical method does not work well for approximately $\varepsilon_{d} < 10^{-4}$ and $\varepsilon_{c} < 10^{-4}$, giving an amplitude that is much too low. This may be because Newton’s algorithm approaches the constant equilibrium solutions to the steady state equations if the amplitude of the nonconstant steady state is too low.

Overall, the results are convincing that the analytic first order terms are in fact correct. However, in general, speed and robustness suffer compared to the situation in one (spatial) dimension [9]. So while for the limited purposes of this section these methods are sufficient, it appears that for more complicated kinetics terms, more accurate and sophisticated numerical methods would be needed. Examples from the large literature are higher-order finite difference methods [10], or finite element methods such as the ones that have been successfully used for reaction-diffusion equations on moving domains [11, 12].

7. An application to a model skeletal pattern formation

We now show how our results may be useful in applications to models of skeletal pattern formation.

Vertebrate limbs develop from small buds of undifferentiated mesenchymal cells. As the bud grows out from the body wall, these unspecialized cells differentiate into various tissues, including cartilage, which acts as the template for the bony skeleton. The skeletal anatomy of the limb consists of several bones arranged in a specific pattern. The nature of the mechanisms of control for this pattern continues to be widely studied [13]. Indeed, pattern formation in the limb is often regarded as a paradigm for organogenesis [1, 14].

Several models have been proposed for the basic mechanisms. (For a survey, see [15].) Some of the most prominent ones are based on various positional information mechanisms [1] and reaction-diffusion mechanisms [14, 16].

The basic idea of positional information models is that cells are informed of their position within the limb by an external spatially dependent signal. The cells respond by executing an individual position-dependent program leading to the formation of spatial structures. One way to provide such positional information is by morphogen gradients. By sensing the local concentration of a morphogen, cells can “infer” their spatial position. (For example, in two spatial dimensions, the local concentration of two morphogens with non-parallel gradients would determine the position uniquely.)

Several gradients appear within the developing limb at various times during development, most prominently a gradient of the protein Sonic Hedgehog (SHH). SHH is expressed in both the mouse and the chick in a region at the posterior (i.e., toward the tail-end of the embryo) junction of the limb bud with the body wall, called the Zone of Polarizing Activity (ZPA). It forms an anteriorposterior (i.e., high at the prospective little finger, low at the prospective thumb) gradient. A portion of the protein has
Figure 1. Three examples for case 1: “small width” (see subsection 3.1). Shown in the upper row are three-dimensional plots of the activator concentration on a rectangle of length $L_x \approx 29.83$ and width $L_y = L_x/10$. The bottom row shows cross-sections for $y = \text{const}$ for the same cases. On the bottom, the first order approximation is shown as a solid line, whereas the results of numerical solution of the full nonlinear equations are shown by the symbols “◦”. Left: $\varepsilon_d = 10^{-3}, \varepsilon_c = 0$; center: $\varepsilon_d = 5 \times 10^{-3}, \varepsilon_c = 10^{-3}$, right: $\varepsilon_d = 0, \varepsilon_c = 10^{-4}$. The plots illustrate the transition between patterns whose dominant cause is the diffusive instability of the Turing mechanism (left) to patterns whose dominant cause is the external gradient (right). Note that this transition acts on the amplitude of the pattern, whereas the frequency is little affected. In this example, as the steepness $\varepsilon_c$ of the external gradient increases, the amplitude of the pattern changes from a constant (on the left) to a function whose minimum is in the interior of the domain. The numerical results yield that the first order approximations are a good fit for the left and right cases, but the fit is less satisfactory if both $\varepsilon_d$ and $\varepsilon_c$ are of comparable size. This is probably due to the second and higher order terms. For the graphs, Schnakenberg kinetics as given in (6.2) were used with $a = 0.1, b = 0.9, d = d_0 - \varepsilon_d^2$ with $d_0 \approx 0.1167$, length $L_x = 16\pi/k_0 \approx 29.83$, where $k_0 \approx 29.83$ is the critical wave number for the Turing bifurcation. A Newton scheme for the steady state was used for the numerical solution with $n = 200$ sample points.

been shown to freely diffuse across the limb bud [17], and introduction of a source of SHH in the anterior region of the limb bud causes mirror image duplication of the digits [18]. For this and other reasons, SHH has been seen as a strong candidate for a morphogen in a proposed positional-information mechanism.

In reaction-diffusion models, the characteristic pattern is laid out by a Turing-type mechanism. The transitions between different patterns (from “humerus” to “radius/ulna” to “digits”) are proposed to be governed by changes in the geometry of the domain of activity, or by some other mechanism. The identity of the activator and inhibitor molecules is elusive, although there are several candidates [19, 20]. Advocates of this type of mechanism acknowledge that morphogen gradients such as the SHH gradient, play a role in the pattern formation process, but it is argued that their role is that of a secondary, “fine tuning” mechanism that is only important after the basic arrangements of the cartilage condensations have been laid out by the reaction-diffusion mechanism [21].

Certain experimental findings on the role of SHH, described below, are in conflict with a simple positional information-type mechanism for specifying digit identities. For this reason, more complicated mechanisms which involve both spatial and temporal SHH gradients have been proposed [7, 22, 23]. We show here how these findings can instead be explained quite naturally in the reaction-diffusion framework using our results from previous sections.
Figure 2. Three examples for case 2: square domain (see section 3.2), here in the case where the Turing pattern consists of “spots” (left). As in Figure 1 three-dimensional plots of the first order approximations of the activator concentration on a square domain of length and width $L_x = L_y = L \approx 29.83$ are shown. Again, the plots illustrate the transition between patterns whose dominant cause is the diffusive instability of the Turing mechanism (left) to patterns whose dominant cause is the external gradient (right). In this case, this means the transition from a Turing “spots” pattern to a stripe pattern with spatially varying amplitude. The explicit data was as follows: Left: $\varepsilon_d = 0.01, \varepsilon_c = 0$; center: $\varepsilon_d = 0.002, \varepsilon_c = 0.01$; right: $\varepsilon_d = 0, \varepsilon_c = 0.01$. As in Figure 1, Schnakenberg kinetics were used with $a = 0.1, b = 0.9, d = d_0 - \varepsilon_d^2$ with $d_0 \approx 0.1167$ were used.

In [7], the authors report on an experiment in mouse embryonic limbs in which the processing of SHH was genetically modified so that the SHH diffusion coefficient was decreased and the resulting SHH gradient was shallower. The result was the loss of one of the middle digits, digit 2, in the developed embryo, whereas the other digits appeared relatively little affected.

As pointed out by the authors, this result contradicts a simple spatial positional information model. Indeed, for such a model, one would expect that a shallower gradient should affect digits that require the highest levels of SHH, not one of the middle fingers. The investigators thus proposed a modified temporal-positional information mechanism, in which the duration of exposure to SHH influences digit specification [7].

We show here how a combined reaction/diffusion and external morphogen model may explain these findings quite naturally. In a simplified “caricature” model, a reaction-diffusion system sets up a pre-pattern, which is then translated by cells into a pattern of pre-cartilage cells. If the local activator concentration is above a certain threshold, cells condensate, while in places where the local concentration is below the threshold, no condensation forms. Initially proposed in [24], later models based on this idea have been increasingly more sophisticated cell biologically and mathematically (see for example [5, 16, 25, 26, 27]). However, the simple threshold model remains useful for an intuitive understanding and will be used in the example below.

As described in the previous section, the interplay of a reaction-diffusion system with the “external” morphogen gradient leads to a slightly perturbed sine pattern. This is somewhat reminiscent of interference phenomena in waves. The amplitude of patterns is now spatially dependent and may have a minimum somewhere within the domain. We illustrate in Figure 3 how this phenomenon may lead to a loss of an interior finger in the very simple caricature model sketched above. (In this example, the “lost” finger is digit 4.) As seen, decreasing the morphogen signal corresponds to decreasing the parameter $\varepsilon_c$. Since the minimum of the amplitude of the Turing wave pattern is at the second “finger”, decreasing $\varepsilon_c$ in this example means that the second wave peak from the right is the first to fall below the threshold. The result is a loss of the corresponding finger.

The reaction kinetics and parameters in the model equation were chosen for illustrative purposes and have no direct correspondence to experimentally determined quantities. However, the lesson of this example is that the effect of an external morphogen gradient on a reaction-diffusion patterning mechanism is subtle: while the wave number of the Turing pattern is essentially unaffected, the amplitude is spatially varying, leading typically to a minimum somewhere in the interior of the domain. Thus, when changing
parameters, the apparent “loss” of structures inside the domain, as opposed to loss of structure on the boundary of the domain, seems to be a natural effect in such systems.

8. Conclusions and future prospects

The one-dimensional results from [6] concerning the effects of an external morphogen in an activator-inhibitor reaction-diffusion system were generalized to two spatial dimensions. This covers the “quasi-one-dimensional” case where the width of the domain is small compared to the length and the square domain case where the system can produce both stripes or spots. One of the important properties is that as the external gradient becomes steeper, the amplitude changes from a spatially constant function to a function which typically has a minimum in the interior of the domain. See Figures 1 and 2. We then applied these results to reaction-diffusion models of skeletal pattern formation in embryonic mouse limb. We sketched a new mechanism to explain in this framework the experimental finding that decreasing the steepness of the SHH gradient results in the loss of a middle finger in the developed embryo.

It would be interesting to explore these ideas in more depth using existing sophisticated models involving physical properties of cells, more detailed reaction kinetics and more realistic geometries [16, 27, 26]. Another problem to consider is the combined effect of two or more external morphogen gradients, or the case in which the external chemical is produced at more than one location, leading to a morphogen concentration with two or more peaks.

References

Figure 3. An illustration of the mechanism by which the loss of a middle finger can occur in a caricature model of limb development. As the external morphogen gradient becomes less steep, corresponding to a decrease of $\varepsilon_c$, the local activator concentration at the second peak falls below the threshold concentration. The left-hand figures show the steady state activator concentrations in an anterior-posterior cross section. The right-hand side shows the corresponding patterns induced by those regions where the activator concentration is above the threshold. Thus the dark regions represent pre-cartilage condensations, the precursors to the digits. While there are all 5 digits in the first four rows, in the fifth row, there are only 4 digits; a middle digit is “lost” (i.e., fails to form). On the right-hand pictures, the horizontal direction is the anterior-posterior direction, and the vertical direction is the proximo-distal direction. (The left hand side shows first order expansions with parameters $a = 0.2, b = 0.3$ and fixed $\varepsilon_d = 0.01$. The anterior-posterior length of the domain is $L = 21.35$. The threshold value was chosen as $T = 0.516$.)

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