Nonlinear Eigenvalue Problems in the Stability Analysis of Morphogen Gradients

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This paper is concerned with several eigenvalue problems in the linear stability analysis of steady state morphogen gradients for several models of Drosophila wing imaginal discs including one not previously considered. These problems share several common difficulties including the following: (a) The steady state solution which appears in the coefficients of the relevant differential equations of the stability analysis is only known qualitatively and numerically. (b) Though the governing differential equations are linear, the eigenvalue parameter appears nonlinearly after reduction to a problem for one unknown. (c) The eigenvalues are determined not only as solutions of a homogeneous boundary value problem with homogeneous Dirichlet boundary conditions, but also by an alternative auxiliary condition to one of the Dirichlet conditions allowed by a boundary condition of the original problem. Regarding the stability of the steady state morphogen gradients, we prove that the eigenvalues must all be positive and hence the steady state morphogen gradients are asymptotically stable. The other principal finding is a novel result pertaining to the smallest (positive) eigenvalue that determines the slowest decay rate of transients and the time needed to reach steady state. Here we prove that the smallest eigenvalue does not come from the nonlinear Dirichlet eigenvalue problem but from the complementary auxiliary condition requiring only to find the smallest zero of a rational function. Keeping in mind that even the steady state solution

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needed for the stability analysis is only known numerically, not having to solve the nonlinear Dirichlet eigenvalue problem is both an attractive theoretical outcome and a significant computational simplification.

1. Introduction

Morphogens (also called ligands in some cases) are molecular substances that bind to cell surface receptors and other molecules. The gradients of different morphogen-receptor concentrations are known to be responsible for the patterning of biological tissues during the developmental phase of the biological host. For a number of morphogen families including Dpp in the wing imaginal disc of Drosophila fruit flies, it is well established that the concentration gradients are formed by morphogens transported from a localized production site and bound to cell surface receptors downstream (see [3], [4], [8], [16], and other references cited in [10]). Recently, the mechanism of morphogen transport has been re-examined by both theoreticians and experimentists, resulting in considerable uncertainty regarding the role of diffusion in transporting morphogens and in other transport mechanisms being suggested as replacements. The observations against diffusive transport are summarized and addressed in [10] on the basis of the results from a quantitative analysis of two mathematical models describing the known morphogen activities in the wing imaginal disc of Drosophila fruit flies. Each of these takes the form of a system of partial differential equations and auxiliary conditions defining an initial-boundary value problem (IBVP). The first group of results from this quantitative study reported in [10] shows that *diffusive models* of morphogen transport can account for much of the data obtained on biological systems including those that have been used to argue against diffusive transport. When observations and data are correctly interpreted, they not only fail to rule out diffusive transport, but actually favor it.

The mathematical underpinning of the case for diffusive transport of morphogens made in [10] is provided in a two-part report. In the first part [12], we focused on the morphogen activities in the extracellular space of the Drosophila wing imaginal disc corresponding to the model designated as System B in [10]. Specific results presented there include a necessary and sufficient condition for the existence of nonnegative, monotone decreasing steady state concentration gradients, the characterization of the shape of these gradients, and their stability with respect to small perturbations. In the second part of the report [11], we address the mathematical problems associated with a formulation that explicitly accounts for the effects of endocytosis and receptor synthesis and degradation in the intracellular compartments, designated as System C in [10].

One remarkable outcome of our analysis of System C shows that the governing boundary value problem (BVP) for the steady state behavior of this more realistic model (in the case of a uniform receptor synthesis rate) may be reduced to the corresponding BVP for System B with the amplitude parameter β and the shape parameter ψ for that basic system replaced by the corresponding amplitude and shape parameters β_ω and ψ_ω (see Sections 2 and 4 of this paper). The equivalence of the two steady state BVP implies that we do not have to be concerned with internalization when investigating additional biological processes impacting morphogen gradients in Drosophila mentioned in [10], except for determining an effective degradation rate for the extracellular formulation of the problem. There is also a similar formal equivalence between the eigenvalue problem for the linear stability analysis of the steady state behavior of System B and that of the more complex System C. This equivalence allows us to establish asymptotic stability for the more complex system by the same method used for System B in [12].

One principal result of this paper is the formulation and analysis of a morphogen gradient system of intermediate complexity, designated as System R. It allows for receptor synthesis and degradation but not endocytosis. With the results in [11], such an extracellular model is now seen to be adequate for the study of additional morphogen processes without the complication of internalization. Not surprisingly, the analysis of the steady state behavior of System R is again formally equivalent to those of Systems B and C. We note here that the eigenvalue problems associated with the linear stability analysis of these three systems have the following common features: (a) The steady state solution, which occurs in the coefficients of the relevant differential equations of the stability analysis is only known qualitatively and numerically. (b) Though the governing differential equations are linear, the eigenvalue parameter appears nonlinearly in the differential equations as well as in the boundary conditions after reduction to a problem for a single unknown. (c) The eigenvalues, already proved to be all positive, are determined not only as solutions of a homogeneous BVP in differential equations with homogeneous boundary conditions of the Dirichlet type, but also by an alternative auxiliary condition (to one of the Dirichlet conditions) arising from the dynamics of morphogen production at the source end of the solution domain. Nonlinear eigenvalue problems of the kind described in Ref. [2] arise frequently in applications (see [6] and [9]). But the three features mentioned above have made the stability analysis less straightforward.

The most interesting feature in our problems, however, is a novel consequence of the alternative auxiliary condition pertaining to the slowest decay rate of transients. We prove that the smallest eigenvalue does not come from the nonlinear Dirichlet eigenvalue problem but from the alternative auxiliary condition requiring only the determination of the smallest zero of a rational function. The development and proof of this theoretically important and

computationally useful result will be given first for System B in Subsections 2.3 and 2.4. Some of the intermediate results for this system are known exactly and explicitly, enabling us to focus on dealing with the main mathematical difficulty. The more involved analysis of the analogous problem in Section 3 for System R is more typical for other systems with receptor synthesis and degradation. The experience gained from the analysis of the simpler problem of System B makes it possible to deal mainly with issues not already addressed in the simpler system.

Only results for a one-dimensional version of these models will be analyzed herein. The same models in two and three dimensions previously formulated in [12] can be analyzed similarly as demonstrated in [12] and [11] for the various results pertaining to the steady state behavior of the morphogen gradients.

2. Diffusion, reversible binding, and degradation

In [10], we focus on a Drosophila wing disc so that comparison can be made with both the recent measurements of [3] and [16] and the conclusions based on a "diffusion and receptor-binding" model considered by [8]. More specifically, we simplified the development of the wing imaginal disc of a Drosophila fly to a one-dimensional reaction-diffusion problem in which morphogen is introduced at the rate v at one end, the border between the *anterior* and *posterior* compartment of the disc, and absorbed at the other end, the edge of one of these compartments. Let $a(x, t)$ be the concentration of the diffusing morphogen specie (Dpp) normalized by the fixed uniform concentration R_0 of cell receptors at a dimensionless distance x, a fraction of the distance $X_{\max}$ from the point source of morphogen production (at $x = 0$) to the edge of the posterior chamber, and dimensionless time t, in units of $X_{\max}^2/D_L$ where D_L is the diffusion coefficient. Let $b(x, t)$ be the corresponding *normalized* concentration of morphogens bound to cell receptors. The simplest model of morphogen gradient formation developed and analyzed in [10] and [12] (designated as System B in [10]) consists of the following dimensionless system of nonlinear differential equations of the reaction-diffusion type governing the rates of change of free and bound morphogens:

$$\frac{\partial a}{\partial t} = \frac{\partial^2 a}{\partial x^2} - h_0 a(1 - b) + f_0 b \quad (0 < x < 1, t > 0), \tag{1}$$

$$\frac{\partial b}{\partial t} = h_0 a(1 - b) - (f_0 + g_0)b \quad (0 \leq x \leq 1, t > 0). \tag{2}$$

In (1)–(2), the parameters h_0, f_0, and g_0 are the on-rate constant $k_{on} R_0$, off-rate constant k_{off}, and degradation rate constant k_{deg} normalized by $X_{\max}^2/D_L$. The

nonlinear reaction-diffusion system (1) and (2) is augmented by the boundary conditions

$$x = 0 : \frac{\partial a}{\partial t} = v_0 - h_0 a(1 - b) + f_0 b, \quad x = 1 : a = 0 \tag{3}$$

for $t > 0$ and the homogeneous initial conditions

$$t = 0 : a = b = 0 \quad (0 \le x \le 1). \tag{4}$$

(Note that a flux term may be included in the boundary condition at $x = 0$ but numerical results obtained show that such an addition contributes only a small perturbation for a sufficiently large shape parameter ψ.) In these relations, the dimensionless per unit receptor concentration production rate v_0 is the actual production rate per unit receptor concentration v/R_0 normalized by D_L/X_{max}^2:

$$v_0 = \frac{v/R_0}{D_L/X_{max}^2}.$$

Our primary concern with System B is whether it can sustain stable steady state morphogen concentration gradients and how the gradients depend on the biological rate parameters. We summarize the known results for the steady-state problem in the next subsection.

2.1. Time-independent steady state solution

We denote a time-independent steady-state solution of (1)–(3) by $\bar{a}(x)$ and $\bar{b}(x)$. For this steady state solution, we have $\partial\bar{a}/\partial t = \partial\bar{b}/\partial t = 0$ so that these equations and boundary conditions become

$$\frac{d^2\bar{a}}{dx^2} - h_0\bar{a}(1 - \bar{b}) + f_0\bar{b} = 0 \quad (0 < x < 1), \tag{5}$$

$$h_0\bar{a}(1 - \bar{b}) - (f_0 + g_0)\bar{b} = 0 \quad (0 \le x \le 1), \tag{6}$$

$$v_0 - h_0\bar{a}(0)[1 - \bar{b}(0)] + f_0\bar{b}(0) = 0, \quad \bar{a}(1) = 0. \tag{7}$$

We can use (6) to eliminate $\bar{b}$ from all other relevant equations to obtain a BVP for $\bar{a}$ alone:

$$\frac{d^2\bar{a}}{dx^2} = \frac{g_0\bar{a}}{\bar{a} + \alpha_0}, \quad \bar{a}(0) = \frac{v_0\alpha_0}{g_0 - v_0}, \quad \bar{a}(1) = 0 \tag{8}$$

with

$$\bar{b}(x) = \frac{\bar{a}(x)}{\bar{a}(x) + \alpha_0}, \quad \alpha_0 = \frac{f_0 + g_0}{h_0} = \frac{K_{off} + K_{deg}}{K_{on}R_0}. \tag{9}$$

Upon setting

$$\beta = \frac{v_0}{g_0}, \quad \bar{\beta} = \frac{\beta}{1 - \beta}, \quad \psi \equiv \mu^2 = \frac{g_0}{\alpha_0}, \quad \bar{a} = \bar{\beta}\alpha_0 A, \qquad (10)$$

the BVP (8) for $\bar{a}(x)$ may be written as

$$A'' = \frac{\psi A}{1 + \bar{\beta} A}, \quad A(0) = 1, \quad A(1) = 0, \qquad (11)$$

where $(\cdot)' = d(\cdot)/dx$. It is evident from the development above that the normalized steady state morphogen concentrations $A = \bar{a}/(\bar{\beta}\alpha_0)$ and $\bar{b}$ depend only on the two parameters β and $\psi \equiv \mu^2$. Moreover, the value of the normalized ligand-receptor concentration at the source point $x = 0$ is simply β:

$$\bar{b}(0) = \frac{v_0}{g_0} \equiv \beta. \qquad (12)$$

In terms of the original biological parameters, we have

$$\beta \equiv \frac{v_0}{g_0} = \frac{v}{k_{\text{deg}} R_0}, \quad \psi \equiv \frac{g_0}{\alpha_0} = \frac{k_{\text{deg}}}{k_{\text{deg}} + k_{\text{off}}} \frac{x_{\text{max}}^2}{D_L} k_{\text{on}} R_0. \qquad (13)$$

Note that $\beta = v_0/g_0$ characterizes the relative strength of the morphogen production rate and the degradation rate, and $\psi = h_0 g_0/(g_0 + f_0)$ characterizes the relative strength of the effective morphogen-receptor binding rate and morphogen diffusion rate. The following existence, uniqueness, and monotonicity theorem for steady state morphogen concentrations was proved in [12] by the monotonicity method [1, 14, 15]:

THEOREM 1. *A unique pair of (time-independent) strictly decreasing steady state solutions $\bar{a}$ and $\bar{b}$ for (5)–(7) exists in [0,1] if and only if $g_0 > v_0$ (so that $\beta < 1$).*

The differential equation for $A(x; \beta, \psi)$ is second order and autonomous; hence an exact solution is possible. However, this exact solution is in the form of a quadrature with an unknown parameter in the integrand to be determined by a boundary condition. It is neither informative nor computationally useful. On the other hand, with β much less than unity, we may seek a parametric series expansion of the steady state solution in powers of $\beta(\simeq\bar{\beta})$. The following leading term perturbation solutions for $\bar{a}$ and $\bar{b}$ were obtained in [12]:

$$\bar{a}(x; \beta, \psi) \sim \frac{v_0}{\psi} \left\{ \frac{\sinh(\mu(1 - x))}{\sinh \mu} + O(\beta) \right\},$$

$$\bar{b}(x; \beta, \psi) \sim \beta \left\{ \frac{\sinh(\mu(1 - x))}{\sinh \mu} + O(\beta) \right\}. \qquad (14)$$

Thus *the effect of increasing ψ is to steepen the gradient of the concentrations* with *the gradient becoming a boundary layer* if $\mu \gg 1$. On the other hand, when $\bar{\beta} \gg 1$, we have as a leading term perturbation solution in $1/\bar{\beta}$

$$\bar{a}(x; \beta, \psi) \sim \frac{v_0}{\psi(1 - \beta)} \left\{ (1 - x) + O(1/\bar{\beta}) \right\} \tag{15}$$

while $\bar{b}(x; \beta, \psi) \sim 1$ except for a boundary layer correction adjacent to the sink at $x = 1$.

2.2. Stability of the steady state solution

With the existence of a unique time-independent steady state ligand-receptor concentration gradient with all the right properties established, we want to know if it is stable. For a linear stability analysis, we consider perturbations from the steady state solution in the form

$$a(x, \tau) = \bar{a}(x) + e^{-\lambda \tau} \hat{a}(x), \quad b(x, \tau) = \bar{b}(x) + e^{-\lambda \tau} \hat{b}(x) \tag{16}$$

where the time-independent portion of the perturbations, $\hat{a}$ and $\hat{b}$, are negligibly small compared to the corresponding steady state solution. After linearization, the two differential equations for a and b require that $\hat{a}$ and $\hat{b}$ satisfy

$$-\lambda \hat{a} = \hat{a}'' - h_0(1 - \bar{b})\hat{a} + (f_0 + h_0\bar{a})\hat{b}, \tag{17}$$

$$-\lambda \hat{b} = h_0(1 - \bar{b})\hat{a} - (f_0 + g_0 + h_0\bar{a})\hat{b}. \tag{18}$$

We solve (18) for $\hat{b}$ in terms of $\hat{a}$, making use of $\bar{b} = \bar{a}/(\bar{a} + \alpha_0)$ and $\alpha_0 = (f_0 + g_0)/h_0$ to get:

$$\hat{b} = -\frac{h_0[1 - \bar{b}(x)]}{\lambda - [h_0\bar{a}(x) + g_0 + f_0]}\hat{a}$$

$$= -\frac{h_0(g_0 + f_0)}{[h_0\bar{a} + f_0 + g_0][\lambda - (h_0\bar{a} + g_0 + f_0)]}\hat{a}. \tag{19}$$

Upon substituting (19) into (17), we obtain

$$\hat{a}'' + [\lambda - q(x, \lambda)]\, \hat{a} = 0, \tag{20}$$

where

$$q(x, \lambda) = \frac{h_0(f_0 + g_0)}{h_0\bar{a} + f_0 + g_0} \frac{\lambda - g_0}{\lambda - g_0 - f_0 - h_0\bar{a}}. \tag{21}$$

A corresponding development of the boundary conditions (3) leads to the following two homogeneous boundary conditions for $\hat{a}(x)$:

$$K(\lambda)\hat{a}(0) = 0, \quad \hat{a}(1) = 0 \tag{22}$$

where

$$K(\lambda) = \lambda - \frac{h_0(1-\beta)(\lambda - g_0)}{\lambda - [h_0\bar{a}(0) + g_0 + f_0]}$$

$$= \lambda - \frac{h_0(1-\beta)(\lambda - g_0)}{[\lambda - (1+\bar{\beta})(g_0 + f_0)]}. \tag{23}$$

The equation $K(\lambda) = 0$ has two solutions given by

$$\lambda = \frac{\sigma \pm \sqrt{\sigma^2 - 4h_0 g_0(1-\beta)^3}}{2(1-\beta)} \tag{24}$$

$$= \frac{\sigma \pm \sqrt{\tilde{\sigma}^2 + 4h_0(1-\beta)^2(\beta g_0 + f_0)}}{2(1-\beta)} \equiv \begin{Bmatrix} \lambda_1 \\ \lambda_0 \end{Bmatrix} \tag{25}$$

with

$$\sigma = f_0 + g_0 + h_0(1-\beta)^2, \quad \tilde{\sigma} = f_0 + g_0 - h_0(1-\beta)^2. \tag{26}$$

Note that for either solution, we can always obtain a nontrivial solution of the ODE (20) and the second boundary condition in (22) with the first boundary condition trivially satisfied because we have $K(\lambda_k) = 0$, $k = 0$ or 1.

We see from (25) that the zeros are real and from (24) that they are positive. We have also shown in [12] that the eigenvalues of the homogeneous boundary value problem defined by the differential equation (20) and the homogeneous Dirichlet boundary conditions

$$\hat{a}(0) = 0, \quad \hat{a}(1) = 0 \tag{27}$$

must also be positive. As the eigenvalues of (20) and (22) consist of those of the eigenvalue problem (20) and (27) and the zeros of $K(\lambda) = 0$, we have the following theorem:

THEOREM 2. *The eigenvalue problem* (20) *and* (22) *has only positive eigenvalues. Hence the unique steady state morphogen gradients, $\bar{a}$ and $\bar{b}$, are asymptotically stable.*

In addition to the zeros of $K(\lambda) = 0$ being positive, we can say more about their locations. We see from (23) that $K(\lambda)$ has only one singularity (a simple pole) at

$$\lambda = (1+\bar{\beta})(g_0 + f_0) \equiv \lambda_c \tag{28}$$

with $K(0) < 0$ and $K(\lambda) \to \infty$ as $\lambda \to \lambda_c$. Hence, $K(\lambda)$ has at least one real (positive) zero in the interval $0 < \lambda < \lambda_c$. In fact, the following stronger statement can be made:

LEMMA 1. $K(\lambda)$ *is a monotone increasing function of* λ *in* $0 \leq \lambda < \lambda_c$ *and hence has only one positive real zero in that interval given by* (*see* (24)):

$$\lambda_0 = \frac{\sigma - \sqrt{\sigma^2 - 4h_0 g_0 (1 - \beta)^3}}{2(1 - \beta)}. \tag{29}$$

Proof: It suffices to calculate $dK/d\lambda$ to obtain

$$\frac{dK}{d\lambda} = 1 + \frac{h_0(f_0 + \beta g_0)}{[\lambda - (1 + \bar{\beta})(f_0 + g_0)]^2} = 1 + \frac{h_0(1 - \beta)^2(f_0 + \beta g_0)}{[(f_0 + g_0) - (1 - \beta)\lambda]^2},$$

which is positive for all λ in $0 \leq \lambda < \lambda_c$. As $K(\lambda)$ changes sign in that interval, it has one crossing, which is at the smaller root λ_0 of the two zeros of $K(\lambda) = 0$ given in (29). ∎

2.3. The smallest eigenvalue of the Dirichlet eigenvalue problem

Although knowing the eigenvalues being positive is sufficient to ensure the (linear) stability of the steady state morphogen concentration gradients, we want to know the order of magnitude of the smallest eigenvalue. It would give us some idea of how quickly the system returns to the steady state after a small perturbation. As parametric studies require that we repeatedly compute the time evolution of the concentration of both free and bound morphogens from their initial conditions, the value of the smallest eigenvalue will also give us some idea of the decay rate of the transient behavior and the time to steady state.

To find the smallest eigenvalue of (20) and (22), we need to compare the smallest zero λ_0 of $K(\lambda) = 0$ with the smallest eigenvalue λ_s of the Dirichlet eigenvalue problem (20) and (27). We already have the former; but instead of trying to determine the latter, our goal in fact is to show that $\lambda_s > \lambda_0$ without actually obtaining λ_s explicitly. In this subsection, we will obtain some preliminary results toward this final goal.

Let $\lambda(>0)$ be an eigenvalue of (20) and (27) and $\hat{a}(x)$ the corresponding eigenfunction. Upon multiplying ODE (20) for this eigen-pair by $\hat{a}(x)$ and integrating by parts, we obtain the following Rayleigh quotient-like relation for λ after observing the boundary conditions (27):

$$\lambda \int_0^1 (\hat{a})^2 \, dx = \int_0^1 (\hat{a}')^2 \, dx - \int_0^1 \left[\frac{h_0(\lambda - g_0)}{\delta_B(x; \lambda)} \right] (\hat{a})^2 \, dx, \tag{30}$$

where

$$\delta_B(x; \lambda) = \{1 + \bar{\beta} A(x)\} \{(f_0 + g_0)[1 + \bar{\beta} A(x)] - \lambda\}. \tag{31}$$

The following key result follows from (30) and the first mean value theorem for integrals (see p. 107, [13]):

LEMMA 2. *There exists some $\xi = \xi(\lambda)$ in (0,1) for which*

$$\lambda \int_0^1 (\hat{a})^2 \, dx = \int_0^1 (\hat{a}')^2 \, dx - \left[\frac{h_0(\lambda - g_0)}{\delta_B(\xi; \lambda)} \right] \int_0^1 (\hat{a})^2 \, dx. \tag{32}$$

Proof: The conclusion is a straightforward application of the first mean value theorem for integrals on page 107 of [13] by taking in that theorem

$$\alpha(x) = \int_0^x (\hat{a})^2 \, dx, \quad f(x) = \delta_B(x; \lambda). \tag{33}$$

∎

We now rewrite (32) as

$$K_1(\lambda, A_\lambda) \int_0^1 (\hat{a})^2 \, dx = \int_0^1 (\hat{a}')^2 \, dx, \tag{34}$$

where $A_\lambda = A(\xi(\lambda))$ and

$$K_1(\lambda, \eta) = \lambda + \frac{h_0(\lambda - g_0)}{\{1 + \bar{\beta}\eta\}\{(f_0 + g_0)(1 + \bar{\beta}\eta) - \lambda\}}. \tag{35}$$

The relation (34) is seen to be the Rayleigh quotient for $K_1(\lambda, A_\lambda)$. With the auxiliary conditions (27), it is well known (see [17]) that

$$\frac{\int_0^1 (\hat{a}')^2 \, dx}{\int_0^1 (\hat{a})^2 \, dx} \geq \pi^2. \tag{36}$$

Therefore, we have:

LEMMA 3.

$$K_1(\lambda, A_\lambda) \geq \pi^2 \tag{37}$$

with $K_1 = \pi^2$ only when $\hat{a}$ is a multiple of $\sin(\pi x)$.

In particular, we have $K_1(\lambda_s, A_{\lambda_s}) \geq \pi^2$ because the eigenfunction $\hat{a}_{\lambda_s}$ associated with the smallest eigenvalue λ_s of the Dirichlet eigenvalue problem (20) and (27) is generally not proportional to $\sin(\pi x)$. It is possible to establish a relation between λ_s and the smallest zero $\tilde{\lambda}_s$ of

$$K_1(\lambda, A_{\lambda_s}) = \pi^2. \tag{38}$$

This relation is a consequence of the following lemma:

LEMMA 4. *For every $\eta \in [0, 1]$, $K_1(\lambda, \eta)$ is a monotone increasing functions of λ and therefore $K_1(\lambda, \eta) = \pi^2$ has only one zero in $[0, \lambda_\pi(\eta))$, where*

$$\lambda_\pi(\eta) = (f_0 + g_0)(1 + \bar{\beta}\eta) \leq \lambda_c \qquad (39)$$

is the only singularity (a simple pole) of $K_1(\lambda, \eta)$ and λ_c is the only singularity (a simple pole) of $K(\lambda)$ given in (28).

Proof: With η as a parameter, it is a straightforward calculation to find

$$\frac{dK_1}{d\lambda} = 1 + \frac{h_0[f_0(1 + \bar{\beta}\eta) + g_0\bar{\beta}\eta]}{[1 + \bar{\beta}\eta][\lambda - (1 + \bar{\beta}\eta)(f_0 + g_0)]^2}. \qquad (40)$$

As η is nonnegative, the right-hand side of the relation above is positive for λ in the interval $[0, \lambda_\pi(\eta))$. With $K_1(0, \eta) < 0$ and $K_1(\lambda, \eta) \to +\infty$ as $\lambda \to \lambda_\pi(\eta)$ for $\eta \geq 0$, $K_1(\lambda, \eta)$ has exactly one (positive) zero in the interval $0 \leq \lambda < \lambda_\pi(\eta)$ for $\eta \geq 0$. At the same time, we have for $\eta \leq 1$

$$\lambda_\pi(\eta) \leq (f_0 + g_0)(1 + \bar{\beta}) = \lambda_c;$$

the lemma follows. ∎

Lemma (4) assures us that $\tilde{\lambda}_s$ is the only zero of (38) in $[0, \lambda_\pi(A_{\lambda_s}))$. It is therefore also the smaller of the two zeros of (38) given that both zeros must be positive and $K_1(\lambda, A_{\lambda_s})$ in $[0, \lambda_\pi(A_{\lambda_s}))$ is a monotone increasing function of λ for $0 \leq \lambda < \lambda_\pi(A_{\lambda_s})$. We then have the following useful inequality between $\tilde{\lambda}_s$ and the smallest eigenvalue λ_s of the Dirichlet eigenvalue problem (20) and (27):

LEMMA 5. $\tilde{\lambda}_s \leq \lambda_s$.

Proof: There are two cases: (a) For $\lambda_s \geq \lambda_\pi(A_{\lambda_s})$, the result follows from $\tilde{\lambda}_s \leq \lambda_\pi(A_{\lambda_s})$. (b) For $0 < \lambda_s < \lambda_\pi(A_{\lambda_s})$, it follows from (37) and the definition of $\tilde{\lambda}_s$ that

$$K_1(\lambda_s, A_{\lambda_s}) \geq \pi^2 = K_1(\tilde{\lambda}_s, A_{\lambda_s}). \qquad (41)$$

Since $0 < \tilde{\lambda}_s, \lambda_s \leq \lambda_\pi(A_{\lambda_s})$, the desired result is an immediate consequence of (41) and Lemma (4) with $\eta = A_{\lambda_s}$. ∎

2.4. The decay rate of transients

We are ready to show $\lambda_s > \lambda_0$, where λ_0 is the smaller of the two zeros of $K(\lambda) = 0$ as given in (29). This remarkable result is important both theoretically and computationally. It will make it unnecessary to solve the Dirichlet eigenvalue problem (20) and (27).

We first make the following observation:

LEMMA 6. *The smaller zero of $K_1(\lambda, \eta) = \pi^2$ is a decreasing function of the parameter η provided that λ is in the interval $[0, g_0)$.*

Proof: Differentiate both sides of (35) with respect to η to get

$$\left\{ 1 + \frac{h_0[(f_0 + g_0)\bar{\beta}\eta + f_0]}{(1 + \bar{\beta}\eta)[\lambda_\pi(\eta) - \lambda]^2} \right\} \frac{d\lambda}{d\eta} = \frac{h_0\bar{\beta}(\lambda - g_0)[2\lambda_\pi(\eta) - \lambda]}{(1 + \bar{\beta}\eta)^2[\lambda_\pi(\eta) - \lambda]^2}. \qquad (42)$$

The coefficient of $d\lambda/d\eta$ on the left is positive for $0 \leq \lambda < \lambda_\pi(\eta)$. The right-hand side of (42) is negative for all $0 \leq \lambda < g_0 < \lambda_\pi(\eta)$. Hence, we have $d\lambda/d\eta < 0$ for $0 \leq \lambda < g_0$. ∎

THEOREM 3. $\lambda_0 < \lambda_s$ *for every $\beta \in [0, 1)$.*

Proof: To establish the desired result, we consider the two cases $\pi^2 \geq g_0$ and $\pi^2 < g_0$ separately.

Case (1) $g_0 \leq \pi^2$: For this case the proof is reduced to showing (1a) $\lambda_0 < g_0$, and (1b) $g_0 \leq \lambda_s$.
For (1a), $\lambda_0 < g_0$, we note

$$K(\lambda_0) = 0 < K(g_0) = K_1(g_0, A_{\lambda_s}) = g_0 \leq \pi^2 \leq K_1(\lambda_s, A_{\lambda_s}), \qquad (43)$$

and $0 < g_0 < \lambda_c$ (see (28)). With $dK_1/d\lambda > 0$ in the interval $0 \leq \lambda < \lambda_c$ (by Lemma (4)) and $0 < \lambda_0, g_0 < \lambda_c$, we have $\lambda_0 < g_0$ proving (1a).
For (1b), $g_0 \leq \lambda_s$, we need to consider two possibilities. If $\lambda_s \geq \lambda_\pi(A_{\lambda_s})$, we have the desired result because $\lambda_\pi(A_{\lambda_s}) \geq g_0$. If on the other hand $\lambda_s < \lambda_\pi(A_{\lambda_s})$, the desired result follows from Lemma (4), the second half of (43), and $0 < \lambda_0, g_0, < \lambda_\pi(A_{\lambda_s})$.

Case (2) $\pi^2 < g_0$: For this case, the proof is reduced by Lemma (5) to showing (2a) $\lambda^* > \lambda_0$, and (2b) $\lambda^* < \tilde{\lambda}_s$, where λ^* is the smaller root of $K_1(\lambda^*, 1) = \pi^2$.
For (2a), $\lambda^* > \lambda_0$, we note $K_1(\lambda^*, 1) = K(\lambda^*)$ so that λ^* is also a root of $K(\lambda^*) = \pi^2$. Hence, we have $K(\lambda_0) = 0 < \pi^2 = K(\lambda^*)$ and therewith $\lambda_0 < \lambda^*$ by Lemma (1).
For (2b), $\lambda^* < \tilde{\lambda}_s$, we have from $K_1(\tilde{\lambda}_s, A_{\lambda_s}) = \pi^2 < g_0 = K_1(g_0, A_{\lambda_s})$ and $0 < \tilde{\lambda}_s, g_0 < \lambda_\pi(A_{\lambda_s})$ the inequality $\tilde{\lambda}_s < g_0$ by Lemma (4). As $\tilde{\lambda}_s < g_0$ and $\lambda^* < g_0$ (because $\pi^2 < g_0$), we have from Lemma (6) $\lambda_s > \tilde{\lambda}_s > \lambda^*$ giving (2b). ∎

Altogether, the results above indicate that the decay rate of perturbations from steady state (or of any transient solution) of System B corresponds to the

Table 1
Decay Rate of Transients for System B

v/R_0	β	$\beta/(1-\beta)$	t_s	λ_t	λ_0
2×10^{-5}	0.10	1/9	34.7	0.19907	0.19947
5×10^{-5}	0.25	1/3	34.9	0.19793	0.19816
10^{-4}	0.50	1	35.8	0.19295	0.19194
1.5×10^{-4}	0.75	3	40.7	0.16972	0.15896
1.8×10^{-4}	0.90	9	103.3	0.06687	0.06798

smaller root λ_0 of $K(\lambda) = 0$ given in (29); it is therefore not necessary to solve the more difficult Dirichlet eigenvalue problem (20) and (27). Keeping in mind that the steady state solution $\bar{a}(x)$ can only be obtained numerically [5] and $\bar{a}(x)$ appears in the coefficients of the differential Equation (20), only a numerical solution can be expected of the Dirichlet eigenvalue problem. Not having to solve that eigenvalue problem numerically to determine the decay rate of the transients is a huge simplification.

To illustrate the conclusion that the decay rate of transients and the time to steady state are given by λ_0, the smaller root of $K(\lambda) = 0$ (with $K(\lambda)$ given in (23)), we calculate λ_0 from (29) for a range of $\beta = (v/R_0)/k_{\text{deg}}$ by varying the morphogen production rate v/R_0 while keeping all other parameters fixed (with $D_L = 10^{-7}$ cm^2/sec, $X_{\max} = 0.01$ cm, $k_{\text{deg}} = 2 \times 10^{-4}$/sec, $k_{\text{off}} = 10^{-6}$/sec, and $k_{\text{on}}R_0 = 0.01$/sec). The results are compared with the corresponding estimates λ_t obtained from the time t_s needed for the solution of the initial boundary value problem defined by (1)–(4) to reach steady state. This time is defined as the value t_s of t, for which $1 - b(0, t)/\beta/\lesssim \epsilon$ with $\lambda_t = -lu(\epsilon)/t_s$. In Table 1, the comparison is given for five values of v/R_0 with ϵ taken to be 10^{-3}. Other cases have also been investigated with similarly good agreement between λ_t and λ_s.

3. Receptor synthesis and degradation

The system (1)–(4) is understood to be a simplified model of the actual morphogen activities in the biological host (such as the wing imaginal disc of a Drosophila fruit fly). Among the simplifications made to arrive at this model, designated as System B in [10], one feature is particularly difficult to justify. The concentration of cell surface receptors, whether or not occupied by morphogens, is taken to be fixed and uniform across the span of the wing disc in this system. In reality, morphogen-receptor complexes in a wing disc eventually degrade and the receptors involved are lost. Concurrently,

new receptors are synthesized to replenish the receptor concentration possibly resulting in a spatially nonuniform distribution of receptor concentration. In this section, we will formulate a more realistic wing disc model that allows for receptor degradation and synthesis explicitly and designate it as System R. One principal result for this system is the successful reduction of the BVP for the steady state behavior to one of the same form as the corresponding BVP for System B. The reduction allows us to use simpler systems with fixed receptor concentration (without receptor synthesis and degradation analogous to System B) to investigate the effects of other morphogen activities such as feedback mechanism and morphogens binding to nonreceptors (e.g., proteoglygans). In Subsection 3.5, we will establish for System R a result on the decay rate of transients analogous to that of System B proved in Subsection 2.4. More specifically, we will prove that it is also not necessary to solve the nonlinear eigenvalue problem for the differential equations in the linear stability analysis for the steady state solution of System R.

3.1. An extracellular formulation with receptor synthesis

Instead of the simple system (1)–(4), we will investigate here a more complex system where receptor synthesis and degradation are accounted for explicitly. Let $r(x, t)$ be the unoccupied receptor concentration at the dimensionless location x and dimensionless time t normalized by the *uniform* initial receptor concentration R_0. The binding term in the rate of change equation for both free and bound morphogen concentrations now takes the form $h_0 a r$ (instead of $h_0 a (1 - b)$). In addition, there is now a third differential equation governing the rate of change of the unoccupied receptor concentration $r(x, t)$. In terms of the normalized concentrations, we have the following system of three differential equations:

$$\frac{\partial a}{\partial t} = \frac{\partial^2 a}{\partial x^2} - h_0 a r + f_0 b \quad (0 < x < 1), \tag{44}$$

$$\frac{\partial b}{\partial t} = h_0 a r - (f_0 + g_0) b,$$

$$\frac{\partial r}{\partial t} = \Omega_0 w_r(x, t) - h_0 a r - g_r r + f_0 b \quad (0 \le x \le 1) \tag{45}$$

for $t > 0$ where $w_r(x, t)$ is the nonnegative *receptor synthesis rate* normalized by its own maximum value, $\omega_{\max}$ (so that $w_r(x, t) \le 1$), g_r is the receptor degradation rate constant $k'_{\deg}$ normalized by $D_L / X_{\max}^2$ and

$$\Omega_0 = \frac{\omega_{\max} / R_0}{D_L / X_{\max}^2}.$$

The nonlinear reaction-diffusion system (44) and (45) is augmented by the boundary conditions

$$x = 0 : \frac{\partial a}{\partial t} = v_0 - h_0 a r + f_0 b, \quad x = 1 : a = 0 \tag{46}$$

for $t > 0$ and the *inhomogeneous* initial conditions

$$t = 0: \quad a = b = 0, \quad r = r_0(x), \quad (0 \le x \le 1). \tag{47}$$

For the existence of a time-independent steady-state behavior, the morphogen production rate at the source end must be uniform in time as well (so that v_0 is a constant) and the prescribed receptor synthesis rate must also be uniform in time so that $w_r(x, t) = w_r(x)$. (We will defer a discussion of feedback effects on receptor synthesis to a future article.) Though it is not necessary to do so, we will for simplicity limit ourselves to a uniform synthesis rate $\omega_{\max}$ so that $w_r(x) = 1$. With the initial receptor concentration taken to be the steady state receptor distribution prior to the onset of morphogen production, we have

$$R_0 = \frac{\omega_{\max}}{k'_{\deg}} = \frac{\omega_{\max}}{g_r D_L / X_{\max}^2} \tag{48}$$

so that $\Omega_0 = g_r$.

3.2. Time-independent steady-state behavior

We seek a time-independent steady-state solution for the reaction-diffusion system (44)–(46) by setting all time derivatives to zero to get

$$0 = \bar{a}'' - h_0 \bar{a} \bar{r} + f_0 \bar{b} \quad (0 < x < 1) \tag{49}$$

$$0 = h_0 \bar{a} \bar{r} - (f_0 + g_0) \bar{b}, \quad 0 = g_r - h_0 \bar{a} \bar{r} - g_r \bar{r} + f_0 \bar{b} \quad (0 \le x \le 1), \tag{50}$$

where a prime indicates differentiation with respect to x and where we have taken $w_r(x) = 1$ in this article. The nonlinear system of ODE (49) and (50) is augmented by the boundary conditions

$$x = 0: \quad 0 = v_0 - h_0 \bar{a} \bar{r} + f_0 \bar{b}, \quad x = 1: \quad \bar{a} = 0 \tag{51}$$

obtained from (46) by setting the time derivative to zero there.

Similar to the steady-state problem for System B, the two equations in (50) may be solved for $\bar{b}$ and $\bar{r}$ in terms of $\bar{a}$ to obtain

$$\bar{r} = \frac{\alpha_r}{\alpha_r + \bar{a}}, \quad \bar{b} = \frac{g_r \bar{a}}{g_0(\alpha_r + \bar{a})}, \quad \text{with} \quad \alpha_r = \frac{g_r}{g_0} \alpha_0. \tag{52}$$

These expressions are used to eliminate $\bar{r}$ and $\bar{b}$ from (49) to get a second-order ODE for $\bar{a}$:

$$\bar{a}'' = \frac{g_r \bar{a}}{\alpha_r + \bar{a}} \quad (0 < x < 1).$$

Similarly, upon elimination of $\bar{r}$ and $\bar{b}$ from (51), we obtain the following boundary conditions for $\bar{a}(x)$:

$$\bar{a}(0) = a_0 \equiv \alpha_r \bar{\beta}_r, \quad \bar{a}(1) = 0, \tag{53a}$$

where

$$\beta_r = \frac{v_0}{g_r} = \frac{g_0}{g_r}\beta, \quad \bar{\beta}_r \equiv \frac{\beta_r}{1 - \beta_r}. \tag{54}$$

Remarkably, the boundary value problem for $\bar{a}(x)$ for the present more complex system that accounts explicitly for receptor degradation and synthesis is identical in form to the corresponding BVP for System B with the parameters g_0 and β in the simpler system replaced by g_r and β_r, respectively. Results similar to those for the simpler system can therefore be obtained by the same methods used for System B. We summarize the main results for the steady-state concentrations in the following theorem and its corollary:

THEOREM 4. *The condition $\beta_r < 1$ is a necessary and sufficient condition for the existence of unique steady state concentrations $\bar{a}(x)$, $\bar{r}(x)$, and $\bar{b}(x)$.*

COROLLARY 1. *The steady state concentration $\bar{a}(x)$ does not attain a maximum or minimum at an interior point of $[0, 1]$ and hence monotone decreasing from $\bar{a}(0) = a_0$ to $\bar{a}(1) = 0$.*

With $\beta_r = (g_0/g_r)\beta$ and generally $g_0/g_r < 1$, steady morphogen gradients exist for a wide range of normalized production rate β, including $\beta > 1$, as long as $\beta_r < 1$ (see Table 2 at the end of subsection (3.6)).

Theorem (4) also holds for any time independent normalized nonnegative receptor synthesis rate $w_r(x)$ which needs not be uniform in space. Corollary (1) also holds for any positive spatially nonuniform $w_r(x)$.

Let

$$\bar{a}(x) = a_0 A(x) = \alpha_r \bar{\beta}_r A(x). \tag{55}$$

We can rewrite the BVP for $\bar{a}(x)$ in terms of $A(x)$:

$$A'' = \frac{\psi_r A}{1 + \bar{\beta}_r A}, \quad A(0) = 1, \quad A(1) = 0, \tag{56}$$

where

$$\psi_r = \frac{g_r}{\alpha_r} \equiv \mu_r^2 = \mu^2 = \psi. \tag{57}$$

In the form of (56), a perturbation solution for $A(x)$ may be obtained for $\bar{\beta}_r \ll 1$ and $(1 - \beta_r) \ll 1$ similar to what was done in System B [12].

3.3. Linear stability

To investigate the stability of the steady-state solution obtained in the last subsection, we consider small perturbations from steady state in the form

$$\{a(x, t), b(x, t), r(x, t)\} = \{\bar{a}(x), \bar{b}(x), \bar{r}(x)\} + e^{-\lambda t}\{\hat{a}(x), \hat{b}(x), \hat{r}(x)\}. \quad (58)$$

After linearization, the partial differential equations of System R become

$$-\lambda\hat{a} = \hat{a}'' - h_0(\bar{r}\hat{a} + \bar{a}\hat{r}) + f_0\hat{b}, \quad (59)$$

$$-\lambda\hat{b} = h_0(\bar{r}\hat{a} + \bar{a}\hat{r}) - (f_0 + g_0)\hat{b}, \quad (60)$$

$$-\lambda\hat{r} = -h_0(\bar{r}\hat{a} + \bar{a}\hat{r}) - g_r\hat{r} + f_0\hat{b}. \quad (61)$$

The relations (60) and (61) are then solved for $\hat{b}$ and $\hat{r}$ in terms of $\hat{a}$ making use of $\bar{b} = g_r\bar{a}/[g_0(\bar{a} + \alpha_r)]$ to get

$$\hat{r} = \frac{h_0(\lambda - g_0)\bar{r}(x)\hat{a}}{(g_r - \lambda)(f_0 + g_0 - \lambda) + h_0\bar{a}(x)(g_0 - \lambda)}$$

$$= \frac{g_0 h_0(\lambda - g_0)\hat{a}}{(1 + \bar{\beta}_r A)[g_0(g_r - \lambda)(f_0 + g_0 - \lambda) + g_r\bar{\beta}_r A(f_0 + g_0)(g_0 - \lambda)]}, \quad (62)$$

$$\hat{b} = \frac{h_0(g_r - \lambda)\bar{r}(x)\hat{a}}{(g_r - \lambda)(f_0 + g_0 - \lambda) + h_0\bar{a}(x)(g_0 - \lambda)}$$

$$= \frac{g_0 h_0(g_r - \lambda)\hat{a}}{(1 + \bar{\beta}_r A)[g_0(g_r - \lambda)(f_0 + g_0 - \lambda) + g_r\bar{\beta}_r A(f_0 + g_0)(g_0 - \lambda)]}. \quad (63)$$

Upon using (63) and (62) to eliminate $\hat{b}$ and $\hat{r}$ from (59), we obtain

$$\hat{a}'' + [\lambda - q_r(x; \lambda)]\,\hat{a} = 0, \quad (64)$$

where

$$q_r(x; \lambda) \equiv \frac{N_r(x; \lambda)}{D_r(x; \lambda)} = \frac{h_0\bar{r}(x)(g_r - \lambda)(g_0 - \lambda)}{(g_r - \lambda)(g_0 + f_0 - \lambda) + h_0\bar{a}(x)(g_0 - \lambda)}$$

$$= \frac{g_0 h_0(g_0 - \lambda)(g_r - \lambda)}{(1 + \bar{\beta}_r A)[g_0(g_r - \lambda)(g_0 + f_0 - \lambda) + g_r\bar{\beta}_r A(f_0 + g_0)(g_0 - \lambda)]}.$$

$$(65)$$

A corresponding elimination of $\hat{b}$ and $\hat{r}$ from the linearized boundary conditions

$$x = 0: \quad -\lambda\hat{a} = -(h_0\hat{a}\bar{r} + \bar{a}\hat{r}) + f_0\hat{b}, \quad x = 1: \quad \bar{a} = 0$$

leads to the following two homogeneous boundary conditions for $\hat{a}(x)$:

$$K_r(\lambda)\hat{a}(0) = 0, \quad \hat{a}(1) = 0, \tag{66}$$

where

$$K_r(\lambda) = \lambda - \frac{N_r(0;\lambda)}{D_r(0;\lambda)} = \lambda - \frac{h_0\bar{r}(0)(g_r - \lambda)(g_0 - \lambda)}{(g_r - \lambda)(g_0 + f_0 - \lambda) + h_0\bar{a}(0)(g_0 - \lambda)}$$

$$= \lambda - \frac{g_0 h_0(g_0 - \lambda)(g_r - \lambda)}{(1 + \bar{\beta}_r)[g_0(g_r - \lambda)(g_0 + f_0 - \lambda) + g_r\bar{\beta}_r(f_0 + g_0)(g_0 - \lambda)]}.$$
$$\tag{67}$$

The first condition in (66) requires that either

$$\hat{a}(0) = 0, \quad \text{or} \quad K_r(\lambda) = 0. \tag{68}$$

LEMMA 7. *All three zeros of $K_r(\lambda) = 0$ are positive.*

Proof: If λ is a complex zero, then so is its complex conjugate λ^*. Form $K_r(\lambda) - K_r(\lambda^*)$ to get

$$K_r(\lambda) - K_r(\lambda^*) = (\lambda - \lambda^*) - \frac{N_r(0;\lambda)D_r(0;\lambda^*) - N_r(0;\lambda^*)D_r(0;\lambda)}{D_r(0;\lambda)D_r(0;\lambda^*)} \tag{69}$$

$$= (\lambda - \lambda^*)\left\{1 + \frac{F(\lambda,\lambda^*)}{|D(0,\lambda)|^2}\right\} = 0 \tag{70}$$

where

$$F(\lambda,\lambda^*) = [f_0 + h_0\bar{a}(0)][\text{Re}^2(\lambda) + \text{Im}^2(\lambda)] - 2\text{Re}(\lambda)[g_r f_0 + g_0 h_0\bar{a}(0)]$$

$$+ [g_r^2 f_0 + g_0^2 h_0\bar{a}(0)]$$

$$= [F_r(\lambda)]^2 - \frac{[g_r f_0 + g_0 h_0\bar{a}(0)]^2}{f_0 + h_0\bar{a}(0)} + [g_r^2 f_0 + g_0^2 h_0\bar{a}(0)]$$

$$+ [f_0 + h_0\bar{a}(0)]\,\text{Im}^2(\lambda)$$

$$= [F_r(\lambda)]^2 + [f_0 + h_0\bar{a}(0)]\,\text{Im}^2(\lambda) + \frac{f_0 h_0\bar{a}(0)[g_r - g_0]^2}{f_0 + h_0\bar{a}(0)} \tag{71}$$

with

$$[F_r(\lambda)]^2 = \left[\sqrt{f_0 + h_0\bar{a}(0)}\text{Re}(\lambda) - \frac{g_r f_0 + g_0 h_0\bar{a}(0)}{\sqrt{f_0 + h_0\bar{a}(0)}}\right]^2 > 0. \tag{72}$$

As $F(\lambda,\lambda^*) > 0$, the relation (70) requires $\lambda - \lambda^* = 0$ so that λ must be real.

Given $K_r(0) < 0$, $\lambda = 0$ is not a zero. Suppose λ is negative with $\lambda = -|\lambda| < 0$. Then we have

$$K_r(-|\lambda|) = -\left\{|\lambda| + \frac{h_0\bar{r}(0)(|\lambda| + g_0)(|\lambda| + g_r)}{(|\lambda| + g_r)(g_0 + f_0 + |\lambda|) + h_0\bar{a}(0)(|\lambda| + g_0)}\right\} < 0.$$

Hence, the zeros of $K_r(\lambda) = 0$ must be positive. ∎

We next show that the eigenvalues of the homogeneous BVP defined by the differential Equation (64) and the homogeneous Dirichlet boundary conditions

$$\hat{a}(0) = 0, \quad \hat{a}(1) = 0 \tag{73}$$

must also be positive.

LEMMA 8. *All the eigenvalues of* (64) *and* (73) *are real.*

Proof: Suppose λ is complex with eigenfunction $a_\lambda(x)$, then λ^* is also an eigenvalue with eigenfunction $a_\lambda^*(x)$ where $(\cdot)^*$ is the complex conjugate of $(\cdot)$. The bilinear relation

$$\int_0^1 [(a^*)a'' - (a^*)''a]\,dx = 0$$

requires

$$\int_0^1 \left\{(\lambda - \lambda^*) - \left[q_r(x;\lambda) - q_r(x;\lambda^*)\right]\right\}(a^*a)\,dx = 0, \tag{74}$$

where we have made use of the fact that $K_r(\lambda) \neq 0$ for a complex λ (see Lemma 7) so that the boundary conditions in (73) apply. It is straightforward to verify $q_r(x;\lambda) - q_r(x;\lambda^*) = -(\lambda - \lambda^*)\Phi(x;\lambda\lambda^*)$ where

$\Phi(x;\lambda\lambda^*)$

$$= \frac{g_0 h_0\left\{g_0 f_0([g_r - \mathrm{Re}(\lambda)]^2 + [\mathrm{Im}(\lambda)]^2) + g_r \bar{\beta}_r A(x)([g_0 - \mathrm{Re}(\lambda)]^2 + [\mathrm{Im}(\lambda)]^2)\right\}}{D_r(x;\lambda)D_r(x;\lambda^*)}$$

is a positive real value function. In that case, the bilinear form (74) becomes

$$(\lambda - \lambda^*)\int_0^1 aa^*[1 + \Phi(x;\lambda\lambda^*)]\,dx = 0. \tag{75}$$

Since the integral is positive for any nontrivial function $a(x;\lambda)$, we must have $\lambda - \lambda^* = 0$. Hence, λ does not have an imaginary part. ∎

THEOREM 5. *All eigenvalues of the eigenvalue problem* (59)–(61) *and* (66) *are positive and the unique steady state concentration* $\bar{a}(x)$ *is asymptotically stable (and therefore so are* $\bar{b}(x)$ *and* $\bar{r}(x)$*).*

Proof: We already know that λ must be real and that the zeros of $K_r(\lambda) = 0$ are positive. Suppose $\lambda \leq 0$ so that the boundary conditions (73) apply. Let $\hat{a}_\lambda(x)$ be a corresponding nontrivial eigenfunction of the homogeneous BVP (64) and (73) for the eigenvalue λ. Multiply (64) by $\hat{a}_\lambda$ and integrate over the solution domain to get

$$\int_0^1 \left\{ \hat{a}_\lambda \hat{a}_\lambda'' - q_r(x; \lambda)(\hat{a}_\lambda)^2 \right\} dx = -\lambda \int_0^1 (\hat{a}_\lambda)^2 dx.$$

After integration by parts and applying the homogeneous boundary conditions (73), we obtain

$$\lambda \int_0^1 (\hat{a}_\lambda)^2 dx = \int_0^1 (\hat{a}_\lambda')^2 dx + \int_0^1 q_r(x; \lambda)(\hat{a}_\lambda)^2 dx. \tag{76}$$

With $\lambda = -|\lambda| \leq 0$, we have

$$q_r(x; -|\lambda|)$$
$$= \frac{g_0 h_0 (g_0 + |\lambda|)(g_r + |\lambda|)}{(1 + \bar{\beta}_r A)[g_0(g_r + |\lambda|)(g_0 + f_0 + |\lambda|) + \bar{\beta}_r A g_r (f_0 + g_0)(g_0 + |\lambda|)]} > 0.$$

For any nontrivial solution of the eigenvalue problem under the assumption $\lambda \leq 0$, the right-hand side of (76) is positive, which contradicts the assumption $\lambda = -|\lambda| \leq 0$. Hence the eigenvalues of the eigenvalue problem (64) and (73) must be positive and the theorem is proved. ∎

3.4. The smallest eigenvalue of the Dirichlet eigenvalue problem

Similar to System B, we want to know the order of magnitude of the smallest eigenvalue of (59)–(61) and (66). It would give us some idea of how quickly any transients dies out. However, for System R, we do not have an explicit solution for either the smallest zero of $K_r(\lambda)$ or the smallest eigenvalue of (64)–(66); hence relevant bounds are needed for both quantities. We consider first the smallest zero for $K_r(\lambda)$ by noting that this function has only two singularities; both being simple poles at the two zeros of the denominator of (67):

$$\Gamma_1(\lambda) \equiv g_0(g_r - \lambda)(g_0 + f_0 - \lambda) + g_r \bar{\beta}_r (g_0 + f_0)(g_0 - \lambda) = 0. \tag{77}$$

With $\Gamma_1(g_0 + f_0) < 0$ and $\Gamma_1(\lambda) \to \infty$ as $\lambda \to \infty$, there is at least one root of $\Gamma_1(\lambda) = 0$ in the interval $(g_0 + f_0, \infty)$. Let λ_m be the smaller root of (77). We have the following result on the location of this root:

LEMMA 9.

(a) Let λ_m be the smaller of the two roots of (77), $g_m = \min\{g_0, g_r\}$ and $g_M = \max\{g_0, g_r\}$. The location of λ_m is given by

$$\begin{cases} g_m < \lambda_m < g_M & \text{if } g_r < g_0 + f_0 \\ g_0 < \lambda_m < g_0 + f_0 & \text{if } g_r > g_0 + f_0. \end{cases}$$

(b) There is at least one zero of $K_r(\lambda) = 0$ in the interval $0 \leq \lambda < g_m < \lambda_m$.

Proof:

(a) The proof is simply a matter of checking the signs of $\Gamma_1(g_0)$, $\Gamma_1(g_r)$, and $\Gamma_1(g_0 + f_0)$ for the two ranges of g_r.

(b) As a consequence of part (a), we have $K_r(\lambda)$ bounded in $0 \leq \lambda < \lambda_m$, and $K_r(\lambda) \to \infty$ as $\lambda \uparrow \lambda_m$. The desired result follows from $K_r(0) < 0$ and $K_r(g_m) = g_m > 0$. ∎

The following stronger statement can be made about the smallest zero λ_0 of $K_r(\lambda) = 0$:

LEMMA 10. *$K_r(\lambda)$ is a monotone increasing function of λ in $0 \leq \lambda < \lambda_m$; hence it has only one (and therefore the smallest) root λ_0 in that interval.*

Proof: It suffices to compute the derivative of K_r with respect to λ to obtain

$$\frac{dK_r}{d\lambda} = 1 + g_0 h_0 \frac{\{g_r \bar{\beta}_r (g_0 + f_0)(\lambda - g_0)^2 + g_0 f_0 (\lambda - g_r)^2\}}{(1 + \bar{\beta}_r)[\Gamma_1(\lambda)]^2} > 0. \qquad ∎$$

The smallest eigenvalue of the homogeneous BVP defined by the differential Equation (64) and the homogeneous boundary conditions (66) is the smaller of two positive numbers: the smallest zero λ_0 of $K_r(\lambda) = 0$ and the smallest eigenvalue λ_s of the nonlinear Dirichlet eigenvalue problem (64) and (73). While we have from Lemma (10) some qualitative estimate of the location of λ_0 (but not an explicit solution as we did for System B), the solution of the smallest eigenvalue of (64) and (73) can only be obtained by numerical methods. Fortunately as in System B, it is not necessary to determine any of the eigenvalues of (64) and (73) because, as we prove in the next subsection, the eigenvalues of this Dirichlet eigenvalue problem must be greater than λ_0. As a first step toward this result, we will again obtain an explicit lower bound for the minimum eigenvalue of (64) and (73).

Let $\lambda(>0)$ be an eigenvalue of (64) and (73) and $\hat{a}(x)$ the corresponding eigenfunction. Upon multiplying ODE (64) for this eigen-pair by $\hat{a}(x)$ and

integrating by parts, we obtain the following Rayleigh quotient-like relation for λ after observing the boundary conditions (73) which apply to $\hat{a}(x)$:

$$\lambda \int_0^1 (\hat{a})^2 \, dx = \int_0^1 (\hat{a}')^2 \, dx + \int_0^1 q_r(x; \lambda)(\hat{a})^2 \, dx. \qquad (78)$$

The following key result follows from (78) and the first mean value theorem for integrals (see p. 179, [13]):

LEMMA 11. *There exists some $\xi = \xi(\lambda)$ in (0,1) for which*

$$\lambda \int_0^1 (\hat{a})^2 \, dx = \int_0^1 (\hat{a}')^2 \, dx + q_r(\xi; \lambda) \int_0^1 (\hat{a})^2 \, dx. \qquad (79)$$

Proof: The proof is analogous to that for the corresponding lemma for System B and will not be repeated here.   ∎

We now rewrite (79) as

$$K_{1r}(\lambda, A_\lambda) \int_0^1 (\hat{a})^2 \, dx = \int_0^1 (\hat{a}')^2 \, dx, \qquad (80)$$

where $A_\lambda = A(\xi(\lambda))$,

$$K_{1r}(\lambda, \eta) = \lambda - \frac{g_0 h_0 (g_0 - \lambda)(g_r - \lambda)}{(1 + \bar{\beta}_r \eta)\Gamma_\eta(\lambda, \eta)}, \qquad (81)$$

and

$$\Gamma_\eta(\lambda, \eta) = g_0(g_r - \lambda)(g_0 + f_0 - \lambda) + g_r \bar{\beta}_r \eta (f_0 + g_0)(g_0 - \lambda). \qquad (82)$$

The relation (80) is the Rayleigh quotient for K_{1r}. With the auxiliary conditions (73), it is well known that the minimum value of K_{1r} is π^2 attained when $\hat{a}(x)$ is a multiple of $\sin(\pi x)$ [17] so that we have

$$K_{1r}(\lambda, \eta) \geq \pi^2. \qquad (83)$$

The following observation on $K_{1r}(\lambda, \eta)$ will be useful later:

LEMMA 12. *For any $\eta \in [0, 1]$, let $\lambda_{\pi r}(\eta)$ be the smaller of the only two (positive simple pole) singularities of the $K_{1r}(\lambda, \eta)$. Then $K_{1r}(\lambda, \eta)$ is a monotone increasing function of λ in $0 \leq \lambda < \lambda_{\pi r}(\eta)$. Hence, $K_{1r}(\lambda, \eta) = \pi^2$ has only one zero in that interval.*

Proof: Note that $K_r(\lambda) = K_{1r}(\lambda, 1)$ and we can compute $dK_{1r}/d\lambda$ to show that it is positive for $\eta \in [0, 1]$ as we did for $K_r(\lambda)$. As $K_{1r}(0, \eta) < 0$ and

$K_{1r}(\lambda, \eta) \uparrow \infty$ as $\lambda \uparrow \lambda_{\pi r}(\eta)$ for $\eta \in [0, 1]$, there is only one zero of $K_{1r}(\lambda, \eta) = \pi^2$ in $0 < \lambda < \lambda_{\pi r}(\eta)$. ∎

Let λ_s denote the smallest eigenvalue of the Dirichlet eigenvalue problem (64) and (73). By Lemma 12 and $K_{1r}(0, A_{\lambda_s}) < 0$, the equation

$$K_{1r}(\lambda, A_{\lambda_s}) = \pi^2 \tag{84}$$

has a unique zero, denoted by $\tilde{\lambda}_s$, in the interval $[0, \lambda_{\pi r}(A_{\lambda_s}))$. The following lemma on the relative magnitude of $\tilde{\lambda}_s$ and λ_s gives an approximate location for λ_s useful in subsequent developments:

LEMMA 13. $\tilde{\lambda}_s \leq \lambda_s$.

Proof: With $0 < \tilde{\lambda}_s < \lambda_{\pi r}(A_{\lambda_s})$, we have from (83)

$$K_{1r}(\lambda_s, A_{\lambda_s}) \geq \pi^2 = K_{1r}(\tilde{\lambda}_s, A_{\lambda_s}). \tag{85}$$

The results to be proved follows from Lemma 12 with $\eta = A_{\lambda_s} \in [0, 1]$. ∎

3.5. The decay rate of the transients

To show $\lambda_s \geq \lambda_0$, where λ_0 is the smallest of the three zeros of $K_r(\lambda) = 0$, we first make the following observation:

LEMMA 14. *The smallest zero of $K_{1r}(\lambda, \eta) = \pi^2$ is a decreasing function of the parameter η provided $\lambda \in [0, g_m)$ (with $g_m = \min\{g_0, g_r\}$ as previously defined).*

Proof: Differentiate both sides of $K_{1r}(\lambda, \eta) = \pi^2$ with respect to η to get

$$\left\{ 1 + \frac{h_0 g_0 \zeta(\lambda, \eta)}{(1 + \bar{\beta}_r \eta)[\Gamma(\lambda, \eta)]^2} \right\} \frac{d\lambda}{d\eta} = -\frac{g_0 h_0 \bar{\beta}_r (g_0 - \lambda)(g_r - \lambda)\Delta(\lambda, \eta)}{(1 + \bar{\beta}\eta)^2 [\Gamma(\lambda, \eta)]^2}, \tag{86}$$

where $\Gamma(\lambda, \eta)$ are as previously defined in (82),

$$\Delta(\lambda, \eta) = g_0(g_r - \lambda)(f_0 + g_0 - \lambda) + g_r(f_0 + g_0)(g_0 - \lambda)(1 + 2\bar{\beta}_r \eta). \tag{87}$$

and

$$\zeta(\lambda, \eta) = g_0 f_0 (g_r - \lambda)^2 + g_r \bar{\beta}_r \eta (f_0 + g_0)(g_0 - \lambda)^2.$$

As $\zeta(\lambda, \eta) > 0$, the coefficient of $d\lambda/d\eta$ on the left side of (86) is positive. With $\lambda < g_m$, the right-hand side of (86) is negative. ∎

THEOREM 6. *For every $\beta_r \in [0, 1)$, we have $\lambda_0 < \lambda_s$.*

Proof: We have for $g_m \leq \pi^2$,

$$K_r(\lambda_0) = 0 < g_m = K_r(g_m) = K_{1r}(g_m, A_{\lambda_s}) \leq \pi^2 \leq K_{1r}(\lambda_s, A_{\lambda_s}).$$

With $\lambda_0 < \lambda_m$ and $dK_r/d\lambda > 0$ for $\lambda < \lambda_m$, it follows that $g_m > \lambda_0$. On the other hand, with $K_{1r}(\lambda, A_{\lambda_s})$ monotone increasing in $\lambda \in [0, \lambda_{\pi r}(A_{\lambda_s})]$ and $g_m < \lambda_{\pi r}(A_{\lambda_s})$, we have $\lambda_s \geq g_m$. Together, these two results imply

$$\lambda_s \geq g_m > \lambda_0.$$

For $g_m > \pi^2$, let the smallest zero of $K_r(\lambda) = \pi^2$ be denoted by λ_r^* so that $K_r(\lambda_r^*) = \pi^2$. We first show $0 < \lambda_r^*, \tilde{\lambda}_s < g_m$ (in order for the relevant lemmas to be applicable). But this follows from $K_r(\lambda_r^*) = \pi^2 < g_m = K_r(g_m)$ and $K_{1r}(\tilde{\lambda}_s, A_{\lambda_s}) = \pi^2 < g_m = K_{1r}(g_m, A_{\lambda_s})$. Note that

$$K_{r1}(\lambda_r^*, 1) = K_r(\lambda_r^*) = \pi^2 = K_{1r}(\tilde{\lambda}_s, A_{\lambda_s}).$$

Therefore, we have by Lemma 14 $\tilde{\lambda}_s > \lambda_r^*$. But $K_r(\lambda)$ is a monotone increasing function of λ; hence we have $\lambda_0 < \lambda_r^*$ and the desired result $\lambda_0 < \lambda_s$ follows by Lemma 13. ∎

As a consequence of this main result, the decay rate of any small perturbation from steady state (or of any transient solution) of System R is given by the smallest zero λ_0 of $K_r(\lambda) = 0$. It is not necessary to solve the more difficult Dirichlet eigenvalue problem (64) and (73).

3.6. Approximate decay rates

For $\bar{\beta}_r \ll 1$, a leading term perturbation solution for λ_0, denoted by $\bar{\lambda}_0$, is determined by $C_r(\bar{\lambda}_0) = 0$ where $C_r(\cdot)$ is $K_r(\cdot)$ (as defined by (67)) with all terms multiplied by $\bar{\beta}_r$ omitted. This leaves us with the cubic polynomial:

$$C_r(\bar{\lambda}_0) \equiv g_0(g_r - \bar{\lambda}_0)\{\bar{\lambda}_0^2 - (f_0 + g_0 + h_0)\bar{\lambda}_0 + g_0h_0\} = 0.$$

Thus, $\bar{\lambda}_0$ is the smaller of g_r and the smaller root of the quadratic equation

$$\bar{\lambda}_0^2 - (f_0 + g_0 + h_0)\bar{\lambda}_0 + g_0h_0 = 0.$$

For

$$\frac{4g_0h_0}{(f_0 + g_0 + h_0)^2} \ll 1$$

which is the case for useful biological gradients, we have

$$\lambda_0 \sim \bar{\lambda}_0 = \begin{cases} \frac{g_0}{1 + (g_0 + f_0)/h_0} & (g_0 < g_r) \\ g_r & (g_r < g_0). \end{cases} \tag{88}$$

On the other hand, the first approximation of λ_0 for $\bar{\beta}_r \gg 1$ is found to be $\tilde{\lambda}_0(1 - \beta_r)^2$ with

$$\lambda_0 \sim \tilde{\lambda}_0(1 - \beta_r)^2 = \frac{g_0 h_0}{f_0 + g_0}(1 - \beta_r)^2. \tag{89}$$

To illustrate the conclusion that the decay rate of transients and the time to steady state are governed by λ_0, the smallest root of $K_r(\lambda) = 0$ (see (67)), we calculate λ_0 for a few typical values of k_{deg} while keeping all other parameters fixed (with $D = 10^{-7}$ cm^2/sec, $X_{\text{max}} = 0.01$ cm, $v/R_0 = 8 \times 10^{-5}$/sec, $k_{\text{off}} = 10^{-6}$/sec, $k'_{\text{deg}} = 10^{-3}$/sec, and $k_{\text{on}}R_0 = 0.012$/sec). The results are compared with the corresponding estimates $\lambda_t = -lu(\epsilon)/t_s$ obtained from the (normalized) time t_s required for the solution of the initial boundary value problem defined by (44)–(47) to reach steady state. For our numerical solutions, this time is defined as the first value t_s of t for which $1 - b(0, t)/\beta \lesssim \epsilon$. In Table 2, comparisons are shown for four values of k_{deg} in the biologically meaning range $g_0 < g_r < \pi^2$ with ϵ taken to be 10^{-3}. The corresponding leading term perturbation solutions given by (88) are also included to show the accuracy of the asymptotic approximation.

We know from Lemma (9) that λ_0 is necessarily less than g_0 for these cases (with $g_0 < g_r$). For $\beta_r \ll 1$ and $g_0/h_0 \ll 1$ (which applies to for all four cases in Table 2), λ_0 is remarkably close to $g_0 = k_{\text{deg}}/(D/X_{\text{max}}^2)$ and accurately predicted by (88).

Table 2
Decay Rate of Transients for System R

k_{deg}	g_0	β	β_r	t_s	λ_t	λ_0	$\tilde{\lambda}_0$
10^{-5}	0.010	8.000	0.08	690.929	0.009998	0.009999	0.009991
3.3×10^{-5}	0.033	2.424	0.08	209.436	0.032983	0.032997	0.032907
8×10^{-5}	0.080	1.000	0.08	86.4464	0.079908	0.079993	0.079464
3.3×10^{-4}	0.330	0.242	0.08	21.0305	0.328465	0.329969	0.321142

4. Endocytosis

In reality, the receptor synthesis and degradation of the (normalized) morphogen-receptor complexes $b(x, t)$ and unoccupied receptors $r(x, t)$ take place in the interior of the cell compartments. A mathematical model more accurately accounting for this development would include the biological process of internalization, an informal reference to the biological processes of endocytosis and exocytosis. Such a model was formulated in [10] (designated as System C therein) and analyzed in [11] to investigate the effect of

internalization. For steady state behavior, we will be mainly interested in a receptor synthesis rate independent of time so that $\omega(X, T) = \omega(X) = \omega_0 w(x)$ and take $\omega_0 = \omega(X = 0)$ so that $w(0) = 1$. For System C, $b(x, t)$ is designated as the normalized morphogen-receptor concentration in the extracellular space while a new quantity $c(x, t)$ is the corresponding normalized concentration inside the posterior chamber. Also, we have to distinguish the receptor concentration outside and inside the cell compartment, denoted by $d(x, t)$ and $e(x, t)$, respectively, replacing the single normalized receptor concentration $r(x, t)$. Our choice of reference receptor concentration $\bar{R}_0$ is the steady state receptor concentration in the extracellular space in the absence of morphogens and for $\omega(X) = \omega_0$ so that $\omega(X) = 1$. In that case, the initial-boundary value problem (IBVP) for the various concentrations can now be formulated in the following normalized form:

$$\frac{\partial a}{\partial t} = \frac{\partial^2 a}{\partial x^2} - h_0 ad + f_0 b, \tag{90}$$

$$\frac{\partial b}{\partial t} = h_0 ad - (f_0 + j_0)b + k_0 c, \quad \frac{\partial c}{\partial t} = j_0 b - (k_0 + g_0)c, \tag{91}$$

$$\frac{\partial d}{\partial t} = -h_0 ad + f_0 b - j_1 d + k_1 e, \quad \frac{\partial e}{\partial t} = \frac{j_1 g_1}{k_1} w - (k_1 + g_1)e + j_1 d \tag{92}$$

all for $t > 0$, $0 \leq x \leq 1$, except for the first equation, which holds for the open interval $0 < x < 1$. In the notation of [10], j_0, k_0, and g_0 in these equations are the in-, out-, and degradation rate constants, k_{in}, k_{out}, and k_{deg}, for the bound morphogen, normalized by D_L/X_{max}^2, j_1, k_1, and g_1 are the in-, out-, and degradation rate constants for the unoccupied receptors, k_p, k_q, and k_g, normalized by D_L/X_{max}^2. Correspondingly, the boundary conditions become

$$x = 0: \quad \frac{\partial a}{\partial t} = v_0 - h_0 ad + f_0 b \quad (t > 0) \tag{93}$$

$$x = 1: \quad a = 0 \quad (t > 0) \tag{94}$$

and the initial conditions for $\omega(X) = 1$

$$t = 0 : a = b = c = 0, \quad d = 1, \quad e = j_1/k_1 \quad (0 \leq x \leq 1). \tag{95}$$

4.1. Time-independent solution

Our primary concern with this five-component system is the existence, uniqueness, and monotonicity of a steady state concentrations $\bar{a}(x)$, $\bar{b}(x)$, etc. More specifically, we consider the case of a nonnegative, time-independent receptor synthesis rate $\omega(x)$ and a positive morphogen production rate v and investigate the condition(s) under which steady state concentration gradients can be sustained and how the shape of the steady state gradients depends on the biological parameters. For reasons similar to that for System R, the steady

state problem can be reduced to a BVP identical in form to that for $\bar{a}(x)$ in Systems B and R (see [11]).

The reduction is accomplished by solving the four algebraic relations obtained from setting in (91) and (92) the time derivatives of $\bar{b}$, $\bar{c}$, $\bar{d}$, and $\bar{e}$ to zero and express these four quantities in terms of $\bar{a}$. The results are then used to express the remaining relations in terms of $\bar{a}(x)$ alone to get:

$$\frac{d^2 A}{dx^2} = \frac{\psi_\omega w(x) A}{1 + \bar{\beta}_\omega A}, \quad \text{with} \quad \bar{a}(x) \equiv \bar{a}_0 A(x) = \bar{\beta}_\omega \alpha_\omega A(x) \tag{96}$$

$$A(0) = 1, \quad A(1) = 0. \tag{97}$$

with

$$g_\omega = \frac{j_1 g_1}{k_1 + g_1}, \quad \alpha_\omega = \frac{g_\omega(k_0 f_0 + f_0 g_0 + j_0 g_0)}{j_0 g_0 h_0},$$

$$\psi_\omega = \frac{g_\omega}{\alpha_\omega} = \frac{j_0 g_0 h_0}{j_0 g_0 + f_0 g_0 + k_0 f_0}, \tag{98}$$

$$\bar{\beta}_\omega = \frac{\beta_\omega}{1 - \beta_\omega}, \quad \beta_\omega = \frac{v_0}{g_\omega} = \frac{v}{\bar{R}_0 k_{g,\text{obs}}}, \quad k_{g,\text{obs}} = \frac{k_p}{k_g + k_q} k_g \tag{99}$$

because $w(0) = 1$. The four steady state concentrations are given in terms of $\bar{a}(x)$ by

$$\bar{b}(x) = \frac{\alpha_b(x)\bar{a}(x)}{\bar{a}(x) + \alpha_\omega}, \quad \bar{c}(x) = \frac{j_0 \bar{b}(x)}{k_0 + g_0} = \frac{\alpha_c(x)\bar{a}(x)}{\bar{a}(x) + \alpha_\omega}, \tag{100}$$

$$\bar{d}(x) = \frac{(j_0 g_0 + f_0 g_0 + f_0 k_0)\bar{b}(x)}{(k_0 + g_0)h_0 \bar{a}(x)} = \frac{\alpha_\omega w(x)}{\bar{a}(x) + \alpha_\omega}, \tag{101}$$

$$\bar{e}(x) = \frac{1}{k_1 + g_1}\left\{\frac{j_1 g_1}{k_1}w(x) + \frac{j_1(j_0 g_0 + f_0 g_0 + f_0 k_0)}{(k_0 + g_0)h_0 \bar{a}(x)}\bar{b}(x)\right\}$$

$$= \frac{j_1 g_1 w(x)}{k_1(k_1 + g_1)}\left\{1 + \frac{k_1}{g_1}\frac{\alpha_\omega}{\bar{a}(x) + \alpha_\omega}\right\} \tag{102}$$

where

$$\alpha_b(x) = \frac{j_1 g_1(k_0 + g_0)w(x)}{j_0 g_0(k_1 + g_1)}, \quad \alpha_c(x) = \frac{j_1 g_1 w(x)}{g_0(k_1 + g_1)}. \tag{103}$$

Compared to System B (or R) the only difference in the BVP for $\bar{a}(x)$ is the appearance of the parameters $\bar{\beta}_\omega$ and ψ_ω instead of $\bar{\beta}$ and ψ (or $\bar{\beta}_r$ and ψ_r). As such, all the results for Systems B (and R) apply to this new system after we make the appropriate changes in the parameters. Additional comments on the similar form of the steady state BVP for Systems B and C can be found in [11].

4.2. Linear stability analysis of the steady state

A linear stability analysis similar to that for Systems B and R done in the previous sections can be carried out. We will only record here the final form of the relevant eigenvalue problem that determines linear stability:

$$\hat{a}'' + \{\lambda - q_\omega(x; \lambda)\}\hat{a} = 0, \tag{104}$$

$$\kappa(\lambda)\hat{a}(0) = 0, \quad \hat{a}(1) = 0 \tag{105}$$

where

$$q_\omega(x; \lambda) = \frac{\varphi_0(\lambda)h_0\bar{d}(x)}{\delta(x; \lambda)}, \tag{106}$$

$$\kappa(\lambda) = \lambda - q_\omega(0; \lambda) = \lambda - \varphi_0(\lambda)\frac{h_0\bar{d}(0)}{\delta(0; \lambda)}$$

$$= \lambda - \frac{h_0\bar{d}(0)\varphi_0(\lambda)}{\Delta_{21}(\lambda)\Delta_{20}(\lambda) - \Delta_{2a}(\lambda)\Delta_{1a}(\lambda)h_0\bar{a}(0)}. \tag{107}$$

with

$$\varphi_0(\lambda) = \left[\lambda^2 - (g_0 + j_0 + k_0)\lambda + j_0g_0\right]\left[\lambda^2 - (g_1 + j_1 + k_1)\lambda + j_1g_1\right], \tag{108}$$

$$\delta(x; \lambda) = \lambda^4 - \delta_3(x)\lambda^3 + \delta_2(x)\lambda^2 - \delta_1(x)\lambda + \delta_0(x)$$

$$\equiv \Delta_4(\lambda) - \Delta_3(\lambda)h_0\bar{a}(x)$$

$$\equiv \Delta_{21}(\lambda)\Delta_{20}(\lambda) - \Delta_{2a}(\lambda)\Delta_{1a}(\lambda)h_0\bar{a}(x), \tag{109}$$

$$\delta_3(x) = h_0\bar{a}(x) + (g_1 + j_1 + k_1) + (g_0 + f_0 + k_0 + j_0) \equiv h_0\bar{a}(x) + \delta_{30}, \tag{110}$$

$$\delta_2(x) = [(g_0 + k_0 + j_0) + (g_1 + k_1)]h_0\bar{a}(x)$$

$$+ (k_1 + g_1 + j_1)(k_0 + g_0 + f_0 + j_0) + (f_0g_0 + f_0k_0 + j_0g_0) + j_1g_1$$

$$\equiv \delta_{2a}h_0\bar{a}(x) + \delta_{20,} \tag{111}$$

$$\delta_1(x) = [(g_0 + k_0 + j_0)(g_1 + k_1) + j_0g_0]h_0\bar{a}(x)$$

$$+ j_1g_1(f_0 + g_0 + j_0 + k_0) + (f_0g_0 + f_0k_0 + j_0g_0)(g_1 + j_1 + k_1)$$

$$\equiv \delta_{1a}h_0\bar{a}(x) + \delta_{10,} \tag{112}$$

$$\delta_0(x) = j_0 g_0(g_1 + k_1)h_0 \bar{a}(x) + j_1 g_1(f_0 k_0 + j_0 g_0 + f_0 g_0)$$

$$\equiv \delta_{0a} h_0 \bar{a}(x) + \delta_{00.}$$ (113)

so that

$$\Delta_4(\lambda) = \lambda^4 - \delta_{30}\lambda^3 + \delta_{20}\lambda^2 - \delta_{10}\lambda + \delta_{00} = \Delta_{21}(\lambda)\Delta_{20}(\lambda),$$ (114)

$$\Delta_3(\lambda) = \lambda^3 - \delta_{2a}\lambda^2 + \delta_{1a}\lambda - \delta_{0a} = \Delta_{2a}(\lambda)\Delta_{1a}(\lambda),$$ (115)

where

$$\Delta_{21}(\lambda) = \lambda^2 - (g_1 + j_1 + k_1)\lambda + j_1 g_1,$$ (116)

$$\Delta_{20}(\lambda) = \lambda^2 - (g_0 + j_0 + k_0 + f_0)\lambda + (f_0 g_0 + f_0 k_0 + j_0 g_0),$$ (117)

$$\Delta_{2a}(\lambda) = \lambda^2 - (g_0 + j_0 + k_0)\lambda + j_0 g_0, \quad \Delta_{1a}(\lambda) = \lambda - (g_1 + k_1).$$ (118)

If λ is not a zero of $\kappa(\lambda) = 0$, the two boundary conditions in (105) reduce to the homogeneous Dirichlet conditions

$$\hat{a}(0) = 0, \quad \hat{a}(1) = 0.$$ (119)

For the eigenvalue problem (104)–(105), we again have (see [11]) the following results:

LEMMA 15. $\kappa(\lambda) = 0$ *has only positive (real) zeros and all the eigenvalues of* (104) *and* (119) *are positive.*

THEOREM 7. *The unique steady state concentration gradients $\bar{a}, \bar{b}, \bar{c}, \bar{d}$, and $\bar{e}$ for System C are asymptotically stable.*

4.3. The decay rate of the transients

As with Systems B and R, knowing the eigenvalues being positive is sufficient to ensure the (linear) stability of the steady state morphogen concentration gradients. But just as in the other systems, it is also important to know the dependence of the eigenvalues on the biological parameters to gain more insight to the time needed to get to steady state. By the techniques used for the two simpler systems, we can also prove that the decay rate of transients for System C comes from the eigenvalue corresponding to the smallest zero of $\kappa(\lambda) = 0$ (and not from the smallest eigenvalue of the eigenvalue problem defined by (104) and (119)). It is seen from (107) that the eigenvalues associated with $\kappa(\lambda) = 0$ correspond to the roots of the following fifth-degree polynomial:

$$\lambda[\Delta_{21}(\lambda)\Delta_{20}(\lambda) - h_0 \alpha_\omega \bar{\beta}_\omega \Delta_{2a}(\lambda)\Delta_{1a}(\lambda)] - h_0(1 - \beta_\omega)\varphi_0(\lambda) = 0,$$ (120)

where $\beta_\omega, g_\omega, \varphi_0(\lambda)$, and $\Delta_{mn}(\lambda)$ are as previously defined in terms of the various biological parameters and $\bar{\beta}_\omega = \beta_\omega/(1 - \beta_\omega)$. While there is no explicit

solution for the roots of (120), a numerical solution of the five positive roots of this fifth-degree polynomial for a given set of biological parameter values to prescribed accuracy is straightforward. Perturbation solutions for these roots for $\bar{\beta}_\omega \ll 1$ and $\bar{\beta}_\omega \gg 1$ are also similar to those for System B (see [11]).

For the intermediate range of $\bar{\beta}_\omega$, we note that the normalized on-rate constant h_0 is typically large compared to all other normalized rate constants for useful biological gradients (including those in Drosophila). For positive $\bar{\beta}_\omega = O(1)$ so that

$$[h_0(1 - \beta_\omega)]^2 \gg h_0 \alpha_\omega \beta_\omega = \beta(k_0 f_0 + g_0 f_0 + j_0 g_0)/j_0,$$

a good first approximate solution for the smallest zero of $\kappa(\lambda) = 0$ is obtained from the following first approximation of (120):

$$\varphi_0(\lambda) = \Delta_{2a}(\lambda)\Delta_{21}(\lambda) \approx 0 \tag{121}$$

so that *the decay rate of transients corresponds to the smaller root of* $\Delta_{2a}(\lambda) = 0$ *or* $\Delta_{21}(\lambda) = 0$, *whichever is smaller*. In other words, except for terms of the order $[h_0(1 - \beta_\omega)]^{-2}$, we have

$$\lambda_0 \sim \min_{m \,\epsilon\, (0,1)} \frac{1}{2} \left\{ (g_m + j_m + k_m) - \sqrt{(g_m + j_m + k_m)^2 - 4 j_m g_m} \right\}. \tag{122}$$

If in addition

$$(g_i + k_i + j_i)^2 \gg 4 g_i j_i \quad (i = 0, 1), \tag{123}$$

we have the following approximate expressions for λ_0:

$$\lambda_0 \approx \bar{\lambda}_0 = \begin{cases} g_{0,\text{eff}} = \frac{g_0}{1 + (g_0 + k_0)/j_0} & (\text{if } g_{0,\text{eff}} < g_{1,\text{eff}}) \\ g_{1,\text{eff}} = \frac{g_1}{1 + (g_1 + k_1)/j_1} & (\text{if } g_{1,\text{eff}} < g_{0,\text{eff}}). \end{cases} \tag{124}$$

Instead of giving the proof of the main theorem regarding the decay rate of transients using the method already implemented for System B and System R, we compare in Table 3 λ_0 (obtained numerically from (120), with the estimate λ_N obtained from the time t_s needed for the numerical solution of the IBVP (91)–(95) to first satisfy the threshold condition $1 - b(0, t)/\beta/\leq \epsilon$ (taken to be 10^{-3} for the calculations done in Table 3). Both will be presented as a fraction of the approximate expression $\bar{\lambda}_0$ given above to show the adequacy of $\bar{\lambda}_0$ as a good first approximation. For the numerical solutions reported, the following set of biologically realistic parameter values was used throughout:

$$D_L = 10^{-7} \text{cm}^2/\text{sec}^{-1}, \quad X_{\max} = 0.01 \text{ cm}, \quad k_{\text{on}} \bar{R}_0 = 0.012/\text{sec}^{-1},$$
$$k_{\text{off}} = 10^{-6}/\text{sec}^{-1}, \quad k_{\text{out}} = 6.7 \times 10^{-5}/\text{sec}^{-1}, \quad k_g = 10^{-3}/\text{sec}^{-1},$$
$$k_q = 5 \times 10^{-5}/\text{sec}^{-1}.$$

Table 3

Decay Rate for Transients for System C

k_p/sec	k_{in}/sec	k_{deg}/sec	k_{deg}/k_g	β_ω	$\lambda_0/\bar{\lambda}_0$	$\lambda_N/\bar{\lambda}_0$
6×10^{-4}	6×10^{-4}	10^{-5}	0.01	0.14	1.0134	1.0133
6×10^{-4}	6×10^{-4}	3.3×10^{-5}	0.033	0.14	1.044	1.043
2×10^{-4}	2×10^{-4}	3.3×10^{-5}	0.033	0.42	1.087	1.084
2×10^{-5}	2×10^{-5}	3.3×10^{-4}	0.330	0.53	1.041[a]	1.039[a]
2×10^{-4}	2×10^{-5}	3.3×10^{-4}	0.330	0.42	1.041	1.040

[a] $v/\bar{R}_0 = 10^{-5}$/sec.

The normalized morphogen production rate parameter $v/\bar{R}_0$ is taken to be 8×10^{-5}/sec^{-1} except for one case. As indicated in Table 3, we reduce the magnitude of v/R_0 for that case to keep $\beta_\omega < 1$ to ensure the existence of a steady state solution. In the cases reported in Table 3 and other cases investigated, the difference between λ_0 and λ_N is much less than 1%. Given that the five sets of parameter values in Table 3 all satisfy the relation $4g_i j_i/(g_i + k_i + j_i)^2 < 10\%$, it is not surprising that percentage error incurred by the approximation $\bar{\lambda}_0$ is within the same range in all cases.

5. Conclusion

The two principal objectives of the present paper are to formulate and analyze System R, an extracellular model for Drosophila wing discs that allows for receptor synthesis and degradation, and to determine the slowest decay rate of transients for the free and bound Dpp gradients in three Drosophila systems (B, R, and C) of increasing complexity. For the decay rate problem, the usual linearization of the governing partial differential equations with respect to small perturbations from the steady state gradients led to linear system of differential equations, which can be further reduced to one second-order ordinary-differential equation for a single unknown in which the decay rate parameter λ appears nonlinearly. A similar linearization of the boundary conditions led to two homogeneous Dirichlet boundary conditions as one possible augmentation of the differential equation to form a conventional nonlinear eigenvalue problem. Nonlinear eigenvalue problems have occurred previously in many applications in science and engineering (see [6] for the earlier efforts in this area). They continue to be an important area of current research, e.g., [9].

In the present stability analyses for the different Drosophila wing disc models, the dynamic morphogen production condition at the source end offers an

alternative for one of the two augmenting conditions for the governing ODE of the stability problem. This alternative auxiliary condition provides an additional source for possible decay rates. Therefore, the eigenvalue problem has two sets of eigenvalues: those of the conventional homogeneous ODE with homogeneous Dirichlet boundary conditions and those from an auxiliary condition $\kappa(\lambda) = 0$ where $\kappa(\lambda)$ is rational function. The former requires numerical solutions of a conventional nonlinear eigenvalue problem in differential equations while the latter involves only finding the zeros of a rational function. It is proved in this paper that the (slowest) decay rate of transients corresponds to the smallest zero of $\kappa(\lambda) = 0$ thereby completing the developments in [11,12].

This remarkable result on the slowest decay rate is important both theoretically and computationally. Keeping in mind that even the steady state solutions needed for the stability analysis of the different models are known only numerically, not having to solve the nonlinear Dirichlet eigenvalue problem is both an attractive theoretical outcome and a significant computational simplification. The techniques for deducing this result will be useful for other linear or nonlinear eigenvalue problems. They certainly apply to stability problems for other Drosophila models similar to Systems B, R, and C for investigating the effects of inhibitors, nonreceptors, such as the HSGP family, ablation of receptors in small clones cells, and feedbacks as outlined in [10] (see also [7]).

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