Aggregation of a Distributed Source in Morphogen Gradient Formation

By A. D. Lander, Q. Nie, B. Vargas, and F. Y. M. Wan

In the development of a biological entity, ligands (such as Decapentaplegic (Dpp) along the anterior–posterior axis of the Drosophila wing imaginal disc) are synthesized at a localized source and transported away from the source for binding with cell surface receptors to form concentration gradients of ligand–receptor complexes for cell signaling. Generally speaking, activities such as diffusion and reversible binding with degradable receptors also take place in the region of ligand production. The effects of such morphogen activities in the region of localized distributed ligand source on the ligand–receptor concentration gradient in the entire biological entity have been modeled and analyzed as System F in [1]. In this paper, we deduce from System F, a related end source model (System A) in which the effects of the distributed ligand source is replaced by an idealized point stimulus at the border between the (posterior) chamber and the ligand production region that simulates the average effects of the ligand activities in the production zone. This aggregated end source model is shown to adequately reproduce the significant implications of System F and to contain the corresponding ad hoc point source model, System R of [2], as a special case. Because of its simpler mathematical structure and the absence of any limitation on the ligand synthesis rate for the existence of steady-state gradients, System A type models are expected to be used widely. An example of such application is the recent study of the inhibiting effects of the formation of nonsignaling ligand–nonreceptor complexes [3].

Address for correspondence: A. D. Lander, Department of Developmental and Cell Bilogy, University of California, Irvine, CA 92697-3875; e-mail: adlander@uci.edu

STUDIES IN APPLIED MATHEMATICS 114:343–374

Published by Blackwell Publishing, 350 Main Street, Malden, MA 02148, USA, and 9600 Garsington Road, Oxford, OX4 2DQ, UK.

1. Introduction

Morphogens (or ligands) are molecular substances that bind to cell surface receptors and other kinds of (nonreceptor) molecules. The gradients of morphogen-receptor complex concentrations are known to be responsible for cell signaling and tissue patterning during the developmental phase of the biological host. For a number of morphogen families (including Decapentaplegic (Dpp) along the anterior–posterior axis in the wing imaginal disc of Drosophila fruit flies), it is well established that the concentration gradients are formed by morphogens transported away from a localized production site and, in the process, reversibly bound to the surface receptors of cells, some are near and others further away from the production site (see [4–6], [7] and other references cited in [8]). Recently, the mechanism of morphogen transport has been reexamined by both theoreticians and experimentalists to resolve the uncertainty regarding the role of diffusion in transporting morphogens (see [8] and references therein). Appropriate mathematical models of different complexity were formulated and analyzed in [8] and [2] to study the diffusive transport of morphogens. Each consists of a system of partial differential equations and auxiliary conditions (defining an initial-boundary value problem, abbreviated as IBVP) reflecting a relevant selection of known morphogen activities in the wing disc. The first group of results from our quantitative investigation was reported in [8] with the mathematical underpinning of the results given in [2] (see also [9]). These results show that *diffusive models* of morphogen transport can account for much of the known experimental data 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, they favor it. At the same time, they suggest that models that allow for additional morphogen activities are needed to reproduce and explain other known experimental data such as robustness or to remove unexpected restrictions such as that imposed on the ligand synthesis rate for the existence of steady-state behavior in these models. Efforts of this nature can be found in [10] and [3] and the references therein.

The one-dimensional models (Systems B, R, and C) of [8] and [2] all idealized the narrow region between the anterior and posterior chamber of the wing disc as a point. In reality, Dpp is synthesized in a production site of finite extent between the two chambers of the wing disc in which morphogen activities such as diffusion and reversible binding with renewable receptors also take place. A subsequent investigation [1] analyzed an extracellular model of the wing disc corresponding to System R in [2], but now with a spatially distributed ligand synthesis rate over a (narrow) region between the two chambers, henceforth designated as System F. One significant feature of this distributed source model is that unlike System R (and Systems B and C), there is no longer a restriction on the morphogen production rate for the existence

of a steady-state concentration of ligand–receptor complexes. In this paper, we will deduce from System F an appropriate aggregated source model to reduce the complexity of the mathematical model and the attendant analysis and computations. This derived aggregated point source model, designated as System A, is shown to reproduce all the significant consequences of System F on the one hand and to contain System R as a special case on the other hand, delimiting the range of applicability of the latter (and related ad hoc point source models) in the process. Because of their relative mathematical simplicity along with their effective characterization of the relevant biological activities, aggregate source type models are expected to be more attractive for the purpose of analysis and therefore more widely used in the study of morphogen gradients. One example of such applications in [3] provides an explanation for the apparent inconsistency between the experimental results of [11] and [12].

2. Spatially distributed synthesis of morphogens and receptors

2.1. An extracellular formulation

In this paper, we derive from a spatially distributed morphogen source model of the Drosophila wing disc of [1] (System F), a simpler model with an aggregated source at the border between the anterior and posterior chamber. As in [8], we simplify the development by working with a one-dimensional model for the posterior chamber of the wing disc ignoring variations in the ventral–dorsal direction and the apical-basal direction; extensions of the one-dimensional model to account for developments in these other directions are straightforward (see [9] for example). We will work with an extracellular formulation similar to System R in [2] where we have shown that the results for such a model may be reinterpreted as the corresponding results for a model where morphogen–receptor complexes internalize (through endocytosis) before degradation (see also [13]). Features and results of System F [1] relevant to the development of a related aggregated end source model will be summarized in this section. In Section 3, we will aggregate the effects of the activities in the ligand synthesis region of this model to result in a corresponding end source model. The corresponding ad hoc point source model (System R) previously investigated in [2] is then seen to be a limiting case of the aggregated end source model (System A).

Let $[L(X, T)]$ be the concentration of a diffusing ligand such as Dpp at time T and location X in the span from the midpoint of the morphogen production region $X = -X_{\min}$ to the edge of the posterior chamber of the wing disc at $X = X_{\max}$ with morphogen produced only in $-X_{\min} < X < 0$. Let $[R(X, T)]$ and $[LR(X, T)]$ be the concentration of unoccupied receptors and ligand occupied receptors, respectively. For the developmental processes

described in [8], we add to Fick's second law for diffusive ligand transport ($\partial[L]/\partial T = D_L \partial^2[L]/\partial X^2$, D_L being the diffusion coefficient) terms that incorporate the rate of receptor binding, $-k_{\text{on}}[L][R]$, and dissociation, $k_{\text{off}}[LR]$, with k_{on} and k_{off} being the binding rate constant and dissociation rate constant, respectively. In living tissues, molecules that bind receptors do not simply stay bound or dissociate; they also (endocytose and) degrade [7]. In accounting for the time rate of change of the ligand–receptor complexes, we allow for constitutive degradation of $[LR]$ by introducing a degradation rate term with a rate constant k_{deg}. There is also a separate accounting of the time rate of change of the concentration of unoccupied receptors as they are being synthesized and degrade continuously in time (with a degradation rate constant k_g as in [8] ($=k'_{\text{deg}}$ in [2])). In this way, we obtain the following reaction-diffusion system for the evolution of the three concentrations $[L]$, $[LR]$, and $[R]$ (see [1]):

$$\frac{\partial[L]}{\partial T} = D_L \frac{\partial^2[L]}{\partial^2 X} - k_{\text{on}}[L][R] + k_{\text{off}}[LR] + V_L(X, T), \tag{1}$$

$$\frac{\partial[LR]}{\partial T} = k_{\text{on}}[L][R] - (k_{\text{off}} + k_{\text{deg}})[LR], \tag{2}$$

$$\frac{\partial[R]}{\partial T} = V_R(X, T) - k_{\text{on}}[L][R] + k_{\text{off}}[LR] - k_g[R]. \tag{3}$$

for $-X_{\min} < X < X_{\max}$ and $T > 0$, where $V_L(X, T)$ and $V_R(X, T)$ are the rates at which ligand molecules and receptors are synthesized, respectively. In [2], we were interested only in the portion of the wing disc corresponding to $X > 0$ where there is no morphogen production (so that $V_L(X, T) = 0$ for $X > 0$) with ligand introduced into the region $0 < X < X_{\max}$ through a point source at the end $X = 0$. We will discuss in later sections the relation between such an ad hoc point source model (System R) and the present distributed source model (designated as System F henceforth), which considers explicitly the activities in the region $-X_{\min} < X < 0$ where morphogens are produced.

With $-X_{\min}$ being the midpoint of the ligand production region, we have by symmetry of the anterior and posterior chamber of the wing disc

$$X = -X_{\min} : \frac{\partial[L]}{\partial X} = 0 \quad (T > 0). \tag{4}$$

The edge of the posterior chamber at the far end of the wing disc is taken to be absorbing so that

$$X = X_{\max} : [L] = 0 \quad (T > 0). \tag{5}$$

At $T = 0$, we have the initial conditions

$$[L] = [LR] = 0, \quad [R] = [R_0(X)] \quad (-X_{\min} < X < X_{\max}), \tag{6}$$

where $R_0(x)$ is the distribution of steady-state unoccupied receptor concentration prior to the introduction of ligand.

To reduce the number of parameters in the problem, we let $\bar{R}_0$ be a reference unoccupied receptor concentration level (to be specified later) and introduce the normalized quantities

$$t = \frac{D_L}{X_{\max}^2} T, \quad x = \frac{X}{X_{\max}}, \quad x_m = \frac{X_{\min}}{X_{\max}}, \tag{7}$$

$$v_L(x,t) = \frac{V_L/\bar{R}_0}{D_L/X_{\max}^2}, \quad v_R(x,t) = \frac{V_R/\bar{R}_0}{D_L/X_{\max}^2},$$

$$\{a, b, r, r_0\} = \frac{1}{\bar{R}_0}\{[L], [LR], [R], [R_0]\}, \tag{8}$$

$$\{f_0, g_0, g_r, h_0\} = \frac{1}{D_L/X_{\max}^2}\{k_{\text{off}}, k_{\text{deg}}, k_g, k_{\text{on}}\bar{R}_0\}. \tag{9}$$

In terms of these new quantities, we rewrite the IBVP for System F above in the following normalized form

$$\frac{\partial a}{\partial t} = \frac{\partial^2 a}{\partial x^2} - h_0 a r + f_0 b + v_L(x,t) \quad (-x_m < x < 1) \tag{10}$$

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

$$\frac{\partial r}{\partial t} = v_R(x,t) - h_0 a r - g_r r + f_0 b \quad (-x_m \le x \le 1) \tag{11}$$

$$x = -x_m : \frac{\partial a}{\partial x} = 0, \quad x = 1 : a = 0 \tag{12}$$

for $t > 0$ and

$$t = 0 : a = b = 0, \quad r = r_0(x) \quad (-x_m < x < 1). \tag{13}$$

2.2. Time-independent steady-state behavior

For the purpose of this paper, it suffices to limit consideration to ligand and receptor synthesis rates of the form

$$v_L(x,t) = v_L(x) = \bar{v}_L H(-x) = \begin{cases} \bar{v}_L \\ 0 \end{cases}, \quad \text{with } \bar{v}_L = \frac{\bar{V}_L/\bar{R}_0}{D_L/X_{\max}^2} \tag{14}$$

$$v_R(x,t) = v_R(x) = \bar{v}_p\{\rho^2 H(-X) + H(X)\} \equiv \bar{v}_p r_0(x), \quad \bar{v}_p = \frac{\bar{V}_p/\bar{R}_0}{D_L/X_{\max}^2} \tag{15}$$

for $t > 0$ and nonnegative constants $\bar{V}_L$, $\bar{V}_p$, and ρ^2. In (14) and (15), $H(\cdot)$ is the Heaviside unit step function. With the initial receptor concentration taken

to be the steady-state receptor distribution prior to the onset of morphogen production, $R_0(x) = V_R(X)/k_g$, we take

$$\bar{R}_0 = \frac{\bar{V}_p}{k_g}, \tag{16}$$

where $\bar{V}_p$ is the uniform receptor synthesis rate for $x > 0$ so that

$$\bar{v}_p = g_r, \quad R_0(x) = \bar{R}_0 r_0(x) = \bar{R}_0\{\rho^2 H(-X) + H(X)\}. \tag{17}$$

We are interested in a time-independent steady-state solution $\bar{a}(x), \bar{b}(x)$, and $\bar{r}(x)$ for the system (10)–(12). For such a solution, we may set the time derivatives in these equations to zero to get

$$0 = \bar{a}'' - h_0\bar{a}\bar{r} + f_0\bar{b} + v_L(x) \quad (-x_m < x < 1) \tag{18}$$

$$0 = h_0\bar{a}\bar{r} - (f_0 + g_0)\bar{b}, \quad 0 = v_R(x) - h_0\bar{a}\bar{r} - g_r\bar{r} + f_0\bar{b} \quad (-x_m \le x \le 1), \tag{19}$$

where a prime indicates differentiation with respect to x, $(\,)' = d(\,)/dx$. The nonlinear system of ordinary differential equation (ODE) (18) and (19) is augmented by the boundary conditions

$$\bar{a}'(-x_m) = 0, \quad \bar{a}(1) = 0. \tag{20}$$

With $v_L(x)$ and $v_R(x)$ both being piecewise constant as given in (14) and (15), the form of the (18) and (19) requires that $\bar{a}(x)$ and its first derivative be continuous at $x = 0$.

As in the steady-state problem for System R in [2], the two equations in (19) may be solved for $\bar{b}$ and $\bar{r}$ in terms of $\bar{a}$ to obtain

$$\bar{r} = \frac{\alpha_0 r_0(x)}{\alpha_0 + \zeta\bar{a}}, \quad \bar{b} = \frac{r_0(x)\bar{a}}{\alpha_0 + \zeta\bar{a}}, \tag{21}$$

where

$$\zeta = \frac{g_0}{g_r} = \frac{k_{\text{deg}}}{k_g}, \quad \alpha_0 = \frac{g_0 + f_0}{h_0}. \tag{22}$$

The expressions in (21) are now used to eliminate $\bar{r}$ and $\bar{b}$ from (18) to get a second-order ODE for $\bar{a}$ alone:

$$\bar{a}'' - \frac{g_0 r_0(x)\bar{a}}{\alpha_0 + \zeta\bar{a}} + v_L(x) = 0 \quad (-x_m < x < 1). \tag{23}$$

This second-order ODE is supplemented by the two boundary conditions (20), keeping in mind also the continuity conditions on $\bar{a}$ and $\bar{a}'$ at $x = 0$.

For our choice of synthesis rates V_L and V_R, we have $v_L = 0$ and $r_0(x) = 1$ for the range $0 < x < 1$ so that

$$\bar{a}'' = \frac{g_0\bar{a}}{\alpha_0 + \zeta\bar{a}} = \frac{g_r\bar{a}}{\alpha_r + \bar{a}} \quad (0 < x < 1), \quad \alpha_r = \frac{g_r}{g_0}\alpha_0. \tag{24}$$

In the complementary range $-x_m < x < 0$, we have $v_L = \bar{v}_L$ and $r_0(x) = \rho^2$ so that

$$\bar{a}'' - \frac{\rho^2 g_0\bar{a}}{\alpha_0 + \zeta\bar{a}} + \bar{v}_L = 0 \quad (-x_m < x < 0).$$

for some prescribed value of $\rho^2 \geq 0$.

For the model with a distributed ligand synthesis rate in $(-x_m, 0)$ formulated above, $\bar{a}(0)$ is determined by the ligand activities within the production region $(-x_m, 0)$ and is therefore not known a priori. The coupling between the morphogen activities in the two regions $-x_m < x < 0$ and $0 < x < 1$ (with $\bar{a}(x)$ and $\bar{a}'(x)$ continuous at $x = 0$) makes it necessary to consider a single boundary value problem (BVP) for the entire solution domain $-x_m < x < 0$, which is structurally different from the corresponding BVP for the point source cases considered in [8, 2]. As such, the issues of existence, uniqueness, monotonicity, and stability of the steady-state concentration gradients were analyzed anew in [1]. We established there the existence and linear stability of a unique, monotone steady-state concentration of ligand–receptor complexes for System F without any restriction on the ratio of ligand synthesis rate to the receptor-mediated degradation rate. Asymptotic solutions various special cases were also obtained in [1] to delineate the dependence of the steady-state behavior on the biological parameters. For subsequent comparisons with results of the aggregated source model to be derived in Section 3, we will summarize some of these approximate solutions in the next subsection.

2.3. Approximate solutions

2.3.1. *No receptor synthesis in the morphogen production region.* For the extreme case where there is no *receptor* synthesis in the morphogen production region $-x_m < x < 0$ so that $\rho^2 = 0$ (and thereby no concentration of either occupied or unoccupied receptors in that interval), the exact solution of the ODE in that region and the reflecting end condition $a'(-x_m) = 0$ is

$$\bar{a}(x) = \bar{v}_L\left\{c_0 - x_m x - \frac{1}{2}x^2\right\} \quad (-x_m < x < 0).$$

The constant of integration c_0 is to be determined through the continuity conditions at $x = 0$. It turns out that we can in fact determine the solution in

the region $x > 0$ without knowing c_0 and then calculate c_0 from the solution obtained. This is because we have

$$\bar{a}'(0) = -\bar{v}_L x_m, \tag{25}$$

which is a known quantity. Because of the continuity of $\bar{a}'$ at the junction $x = 0$, the condition (25) serves as the second boundary condition (in addition to $\bar{a}(1) = 0$) for the ODE

$$\bar{a}'' = \frac{g_0 \bar{a}}{\alpha_0 + \zeta \bar{a}} \quad (0 < x < 1).$$

This two-point BVP determines $\bar{a}(x)$ in $0 < x < 1$. The continuity of $\bar{a}$ at $x = 0$ then determines c_0 to be

$$c_0 = \frac{\bar{a}(0)}{\bar{v}_L}.$$

For $\zeta \ll 1$, an explicit solution for the problem is

$$\bar{a}(x) \sim \bar{a}_0(x) = \begin{cases} \bar{v}_L \left[\frac{x_m}{\mu} \tanh \mu - x_m x - \frac{1}{2} x^2 \right] & (-x_m < x < 0) \\ \frac{\bar{v}_L x_m}{\mu \cosh \mu} \sinh(\mu(1 - x)) & (0 < x < 1), \end{cases} \tag{26}$$

where

$$\mu^2 = \frac{g_0}{\alpha_0} \equiv \psi, \tag{27}$$

is generally >1 for useful gradients. In particular, we have

$$\bar{a}(0) \sim \bar{a}_0(0) = \frac{\bar{v}_L x_m}{\mu} \tanh \mu. \tag{28}$$

For more general ζ, we may determine $\bar{a}(x)$ for $0 \leq x \leq 1$ numerically and then calculate c_0 from the result obtained.

2.3.2. *Perturbation solution for* $\zeta \ll 1 (\rho^2 > 0)$. For $\rho^2 > 0$ and $\zeta < 1$, we consider a perturbation solution in ζ:

$$\bar{a}(x; \zeta) = \sum_{k=0}^{\infty} \bar{a}_k(x) \zeta^k.$$

The leading term $\bar{a}_0$, determined by the BVP

$$\frac{d^2 \bar{a}_0}{dx^2} - \mu^2 r_0(x) \bar{a}_0 + v_L(x) = 0, \quad \bar{a}_0'(-x_m) = \bar{a}_0(1) = 0,$$

is an adequate approximation of the exact solution for a sufficiently small value of ζ so that $\zeta \bar{a} \ll \alpha_0$. Here, we have, in terms of the Heaviside unit step

function $H(\cdot)$, $r_0(x) = \{H(x) + \rho^2 H(-x)\}$, with $\bar{a}_0$ and $\bar{a}_0'$ continuous at $x = 0$. The exact solution for this linear BVP is

$$\bar{a}_0(x) = \begin{cases} \frac{\bar{v}_L}{\rho^2\mu^2}\left\{1 - \frac{\cosh(\mu)}{\Delta_m}\cosh(\rho\mu(x_m + x))\right\} & (-x_m < x < 0) \\ \frac{\bar{v}_L \sinh(\rho\mu x_m)}{\rho\mu^2\Delta_m}\sinh(\mu(1-x)) & (0 < x < 1) \end{cases} \tag{29}$$

where

$$\Delta_m = \cosh(\mu)\cosh(\rho\mu x_m) + \rho\sinh(\mu)\sinh(\rho\mu x_m),$$

with

$$\bar{a}(0) \sim \bar{a}_0(0) = \frac{\bar{v}_L}{\rho\mu^2\Delta_m}\sinh(\rho\mu x_m)\sinh(\mu). \tag{30}$$

Higher-order correction terms of the perturbation series can also be obtained.

Note as $\rho \longrightarrow 0$, the results reduce to those of the last section. In particular, we have from (30)

$$\bar{a}(0) \sim \bar{a}_0(0) \longrightarrow \frac{\bar{v}_L x_m}{\mu}\tanh\mu. \tag{31}$$

as in (28). On the other hand, with $x_m \to 0$ but keeping $v_0 = \bar{v}_L x_m$ fixed, (30) becomes

$$\bar{a}_0(0) = \frac{\bar{v}_0}{\mu^2\left[\rho^2 x_m + \frac{1}{\mu}\coth(\mu)\right]} \to \frac{\bar{v}_0}{\mu}\tanh(\mu). \tag{32}$$

Unless we keep $\bar{v}_L x_m = v_0$ fixed and finite as $x_m \to 0$, we would not maintain a finite aggregated ligand synthesis rate for an equivalent end source at $x = 0$. Also, we would need to keep $\rho^2 x_m = \rho_m^2$ fixed if a prescribed aggregated rate of ligand–receptor interaction should be maintained at the source end (as in the case of Systems B, C, and R).

2.3.3. *High morphogen production rate.* For very high morphogen production rate so that $\zeta\bar{v}_L \gg \alpha_0$, we let $\bar{a}(x) = \bar{v}_L A_h(x)$. The BVP for $\bar{a}$ may be written in terms of $A_h(x)$ as

$$A_h'' = \frac{g_0 r_0(x) A_h}{\alpha_0 + \zeta\bar{v}_L A_h} - H(-x), \quad A_h'(-x_m) = 0, \quad A_h(1) = 0$$

with A_h' and A_h continuous at $x = 0$. A leading term approximate solution $A_0(x)$ for $\zeta\bar{v}_L \gg \alpha_0$ is determined by the linear BVP

$$A_0'' = \frac{g_0}{\zeta\bar{v}_L} r_0(x) - H(-x) \sim -H(-x), \quad A_0'(-x_m) = 0, \quad A_0(1) = 0$$

and A_0' and A_0 continuous at $x = 0$. If in addition, we have $\zeta \bar{v}_L \gg \max\{g_0, g_0\rho^2\}$, the solution of this problem is

$$\bar{a}(x) \sim \begin{cases} \bar{v}_L\left\{x_m - \left(x_m x + \frac{1}{2}x^2\right)\right\} & (-x_m < x < 0) \\ \bar{v}_L x_m(1-x) & (0 < x < 1) \end{cases} \tag{33}$$

with

$$\bar{a}(0) \sim \bar{v}_L x_m \quad \text{and} \quad \bar{a}'(0) \sim -\bar{v}_L x_m. \tag{34}$$

3. The aggregated source formulation

The theoretical results of [1] for System F with a distributed ligand source in $-x_m < x < 0$ provide us with the assurance that we can meaningfully compute the steady-state gradients of interest for any ligand synthesis rate $\bar{V}_L$. However, the presence of the two distinct regions, $-x_m < x < 0$ and $0 < x < 1$, with different morphogen activities poses unwelcome tedium to the solution process except for a small region of the parameter space. It is therefore desirable to find an appropriate simplification of this model. One possible approach is to reduce the problem to one for a single solution domain with morphogens produced and infused at an end point. In fact, ad hoc end source models were first developed and analyzed (as Systems B, R, and C) in [8], [13], and [2] principally because the mathematical problems involved were simpler than their distributed source counterpart. We now use System F to show how these ad hoc point source systems may be related to the corresponding more realistic distributed source models. We do this by aggregating the ligand activities in $-x_m \leq x < 0$ and suitably approximating the aggregated results for small x_m. In doing so, we reduce the effects of the distributed source to a point source at $x = 0$ that simulates an (approximate) average effect of the distributed source. However, unlike the previous ad hoc formulations, the aggregated end source problem developed below is a direct and appropriate consequence of the distributed source model with all approximations made explicitly in the derivation.

For an appropriate reduction of the steady-state problem of System F to an aggregate source problem, we recall the following differential equation for the steady-state free ligand concentration in the range $-X_{\min} < X < 0$ before normalization obtained from (1) and (2) after setting the time derivative to zero:

$$0 = D_L \frac{d^2 L}{dX^2} - k_{\text{deg}}[LR] + \bar{V}_L \quad (-X_{\min} < X < 0). \tag{35}$$

To capture the overall effect of the morphogen activities in the region of ligand synthesis, we integrate (35) over the interval $-X_{\min} \leq X \leq 0$ to get

$$D_L \left[\frac{dL}{dX}\right]_{X=0} - k_{\text{deg}} \int_{-X_{\min}}^{0} [LR]\, dX + \bar{V}_L X_{\min} = 0,$$

or

$$\frac{D_L}{X_{\min}} \left[\frac{dL}{dX}\right]_{X=0} - k_{\text{deg}}[LR]_{X=\tilde{X}} + \bar{V}_L = 0. \tag{36}$$

for some $\tilde{X}$ in the interval $(-X_{\min}, 0)$. In deducing (36), we have used the reflecting boundary condition at the end $X = -X_{\min}$ to eliminate a term involving dL/dX at that end. The change of $[LR]$ over the interval $[-X_{\min}, 0]$ is generally expected to be relatively small compared to the drop from $X = 0$ to $X = X_{\max}$ (see subsection 2.3 above and Section 3 in [1]). In that case, we may approximate $[LR]_{X=\tilde{X}}$ by $[LR]_{X=0-}$ with $0-$ indicating a point slightly less than 0. This gives the following approximation of the exact relation (36):

$$\left[\frac{D_L}{X_{\min}} \frac{dL}{dX} - k_{\text{deg}}[LR] + \bar{V}_L\right]_{X=0-} \simeq 0$$

or, in terms of the dimensionless variables,

$$\frac{X_{\max}}{X_{\min}} \bar{a}'(0-) - g_0 \bar{b}(0-) + \bar{\nu}_L = 0, \tag{37}$$

where we indicated the acceptance of the approximation by using $=$ instead of $\simeq$ henceforth. With $\bar{b}(x)$ and $\bar{r}(x)$ given in terms of $\bar{a}(x)$ by (21),

$$\bar{r} = \frac{\alpha_0 \rho^2}{\alpha_0 + \zeta \bar{a}}, \quad \bar{b} = \frac{\rho^2 \bar{a}}{\alpha_0 + \zeta \bar{a}} \quad (-x_m \le x < 0), \tag{38}$$

we may use the second relation in (38) to eliminate $\bar{b}(0-)$ in (37) to get a boundary condition at $x = 0$ on $\bar{a}(x)$ alone as we did previously:

$$\sigma_0 \bar{a}'(0) - \frac{g_0 \rho^2 \bar{a}(0)}{\alpha_0 + \zeta \bar{a}(0)} + \bar{\nu}_L = 0, \quad \sigma_0 = \frac{X_{\max}}{X_{\min}}. \tag{39}$$

Note that we have replaced $0-$ by 0 since both $\bar{a}$ and $\bar{a}'$ are continuous at $x = 0$.

The relation (39) gives an average effect of the distributed ligand synthesis rate in $x < 0$ on the morphogen concentration at the border between the ligand production region and the posterior chamber. It serves as a boundary condition to augment the physical condition $\bar{a}(1) = 0$ of a sink at the far edge $X = X_{\max}$ so that the ODE (24) and these two boundary conditions define a BVP for ligand activities in the wing disc chamber $0 \le x \le 1$ with an aggregated source at $x = 0$. Once we know $\bar{a}(x)$, the two other concentration gradients $\bar{b}(x)$ and $\bar{r}(x)$ are obtained from (21) with $r_0(x) = 1$ for $x > 0$. As such, we have derived from System F an *aggregated end source model*, which we will designate as *System A* henceforth. The effects of a distributed source in $-X_{\min} \le X < 0$ are captured by an end flux at $X = 0$ given in terms of the

parameters $\sigma_0 = X_{\max}/X_{\min}$ and ρ^2 in the boundary condition (39), where ρ^2 is the ratio of receptor synthesis rate in the distributed source region $X < 0$ to that of the wing disc chamber $X > 0$.

In view of (39), the ad hoc point source model, System R, may be considered as limiting the special case of the aggregated end source model System A in two different ways. For $X_{\min} \gg X_{\max}$ so that $\sigma_0 \ll 1$, it is reasonable to neglect the term multiplied by σ_0 (which corresponds to the limiting case of $\sigma_0 = 0$). System A is then reduced to System R if the receptor synthesis rates in $x < 0$ and $x > 0$ are the same so that $\rho^2 = 1$.

At the other extreme with $X_{\min} \ll X_{\max}$ (as in the case of the Drosophila wing disc), we have $\sigma_0 = 1/x_m \gg 1$ so that the flux term appears to dominate the left-hand side of (39), and we would have $\bar{a}'(0) = 0$ in the limit as $x_m \to 0$. However, by holding the morphogen synthesis rate $\bar{V}_L$ fixed as $X_{\min}$ tends to zero; the total concentration of morphogen produced over the entire interval $-X_{\min} < X < 0$ would tend to zero, resulting in no ligand production (and therefore no net ligand flux across $X = 0$). An alternative formulation of a point source model would be to keep $\bar{V}_L X_{\min}/X_{\max} = V_0$ (and hence $\bar{v}_L x_m = v_0$) fixed so that we have the same ligand synthesis rate at $X = 0$ as $X_{\min} \to 0$. In that case, we see later that System R becomes a first approximation of System A if we have $\rho^2 x_m = 1$ and $\psi = \mu^2 = g_0/\alpha_0$ is sufficiently large so that $\sigma_0/\psi = (\mu^2 x_m)^{-1} \ll 1$.

In either case, the present reduction of the System F to an aggregated end source formulation has made it possible to delineate the limitation of System R and its range of applicability. We will comment further on the numerical significance or insignificance of the flux term in (39) later in this paper. However, independent of its effect on the magnitude of the various concentrations, the presence of this flux term results in a significant qualitative change in the existence theory of the steady state solution. From the analysis of the next section, we will see that the retention of the flux term eliminates any restriction on the range of the synthesis rate relative to the degradation rate as required by System R (and other ad hoc point source models in [2]). But before we proceed with this analysis, we note that a similar development for the IBVP for the time-dependent concentrations leads to the following condition point source condition at $X = 0$:

$$X = 0-: \quad \frac{\partial [L]}{\partial T} = \sigma_0 \frac{D_L}{X_{\max}^2} \frac{\partial [L]}{\partial X} - k_{\text{on}}[R][L] + k_{\text{off}}[LR] + \bar{V}_L \tag{40}$$

or

$$x = 0-: \quad \frac{\partial a}{\partial t} = \sigma_0 \frac{\partial a}{\partial x} - h_0 r a + f_0 b + \bar{v}_L. \tag{41}$$

where we have approximated $a(\bar{x}, t), b(\bar{x}, t)$, and $r(\bar{x}, t)$ for some $\bar{x}$ in $(-x_m, 0)$ by their values at $x = 0-$. It should be be kept in mind that the PDE (10) requires both $a(x, t)$ and $\partial a(x, t)/\partial x$ to be continuous at $x = 0$.

4. Existence, uniqueness, and monotonicity

The existence of a steady-state solution of System R (as well as of Systems B and C) is proved simply by identifying an upper and a lower solution for the monotone method of Amann [14] and Sattinger [15] (see also [16]). However, because of the form of the new boundary condition (39) at $x = 0$, the same method is not directly applicable to the corresponding BVP for System A:

$$\bar{a}'' - \frac{g_0 \bar{a}}{\alpha_0 + \zeta \bar{a}} = 0 \quad (0 < x < 1), \tag{42}$$

$$\sigma_0 \bar{a}'(0) - \frac{g_0 \rho^2 \bar{a}(0)}{\alpha_0 + \zeta \bar{a}(0)} + \bar{\nu}_L = 0, \quad \bar{a}(1) = 0, \tag{43}$$

where $\sigma_0 = X_{\max}/X_{\min} = 1/x_m$. On the other hand, it does provide a basis for an existence proof for the new problem.

THEOREM 1. *For positive values of the parameters* σ_0, g_0, α_0, ζ, *and* $\bar{\nu}_L$ *and for* $\rho^2 \geq 0$, *there exists a regular solution* $\bar{a}(x) \geq 0$ *of the BVP* (42) *and* (43).

Proof: For any $a_0 \geq 0$, the BVP defined by (42) and the Dirichlet conditions $\bar{a}(0) = a_0$ and $\bar{a}(1) = 0$ is known to have a unique, nonnegative, monotone decreasing (analytic) solution in $0 < x < 1$ [2] with $\bar{a}(x) \equiv 0$ for $a_0 = 0$. Let $s(a_0)$ be the resulting since $\bar{a}'(0)$ depends on a_0, we set $\bar{a}'(0) \equiv s(a_0)$ to denote this dependence. It is known from [2] that $s(a_0)$ is negative for positive a_0. Let $B[a_0] \equiv \sigma_0 s(a_0) + \bar{\nu}_L - g_0 \rho^2 a_0/(\alpha_0 + \zeta a_0)$. Evidently, we have $B[0] > 0$. If $\bar{\beta}_f = \beta/(\rho^2 - \zeta\beta) > 0$ with $\beta = \bar{\nu}_L/g_0$, then we can complete the proof simply by noting $B[\alpha_0 \bar{\beta}_f] = \sigma_0 s(a_0) < 0$. Because $\bar{a}'(x)$ and $\bar{a}(x)$ depend continuously on a_0, we have by the intermediate value theorem that there is a value $\tilde{a}_0$ for which $B[\tilde{a}_0] = 0$. The solution of the Dirichlet BVP with $a_0 = \tilde{a}_0 > 0$ is then a solution of the BVP (42)–(43).

The proof for the case $\bar{\beta}_f \leq 0$ is slightly more complicated. Let $y(x; a_0) \equiv \partial \bar{a}/\partial a_0$; it follows from the BVP for $\bar{a}(x; a_0)$ that $y(x; a_0)$ is the solution of the BVP:

$$y' = \frac{g_0 \alpha_0 y}{(\alpha_0 + \zeta \bar{a})^2}, \quad y(0) = 1, \quad y(1) = 0.$$

Evidently, $y_u(x) = 1$ and $y_\ell(x) = 0$ are, respectively, an upper and lower solutions of the problem above for $\bar{a}(x)$ resulting from $a_0 > 0$. By the monotone method of Amann and Sattinger, there is a unique, nonnegative, and monotone decreasing solution $y(x; a_0)$ for this problem with $y'(x; a_0) < 0$. In particular, we have $y'(0; a_0) = \partial[a'(0; a_0)]/\partial a_0 < 0$; hence $B[a_0]$ is decreasing function of a_0. With $B[0] > 0$, there exists some $\tilde{a}_0 > 0$ for which $B[\tilde{a}_0] = 0$. Again, the solution of the Dirichlet BVP with $a_0 = \tilde{a}_0 > 0$ is a solution of the BVP (42)–(43). ■

Note that the ligand synthesis rate was restricted by $\beta < \bar{\nu}_L/g_0$ in [2]; otherwise we would have $a_0 < 0$ which is biologically inadmissible. In the proof above, the solution of our problem naturally satisfies the nonnegativity requirement and hence imposes no restriction on the synthesis (or $\bar{\nu}_L/g_0$).

THEOREM 2. *The nonnegative solution of Theorem* (1) *is unique.*

Proof: The proof is essentially the same as that for System R (see [2]). Suppose there are two solutions $\bar{a}_1(x)$ and $\bar{a}_2(x)$. Let $a(x) = \bar{a}_1 - \bar{a}_2$, then the BVP for $\bar{a}_k(x)$ implies

$$a'' = \frac{g_0\bar{a}_1}{\zeta\bar{a}_1 + \alpha_0} - \frac{g_0\bar{a}_2}{\zeta\bar{a}_2 + \alpha_0} = \frac{g_0\alpha_0 a}{(\zeta\bar{a}_1 + \alpha_0)(\zeta\bar{a}_2 + \alpha_0)},$$

$$a(1) = 0, \quad \sigma_0 a(0) = \frac{\rho^2 g_0\alpha_0 a(0)}{(\zeta\bar{a}_1(0) + \alpha_0)(\zeta\bar{a}_2(0) + \alpha_0)}.$$

Multiply both sides of the differential equation above by $a(x)$ and integrate the resulting relation over [0, 1]. After integrating by parts and applying the boundary conditions for $a(x)$, we obtain

$$\frac{\rho^2 g_0\alpha_0 a^2(0)}{\sigma_0(\zeta\bar{a}_1(0) + \alpha_0)(\zeta\bar{a}_2(0) + \alpha_0)} + \int_0^1 (a')^2\,dx + \int_0^1 \frac{\rho^2 g_0\alpha_0 a^2}{(\zeta\bar{a}_1 + \alpha_0)(\zeta\bar{a}_2 + \alpha_0)}\,dx = 0. \tag{44}$$

Because $\bar{a}_1 \geq 0$ and $\bar{a}_2 \geq 0$, the condition (44) requires $a(x) \equiv 0$ and hence uniqueness. ■

THEOREM 3. *The nonnegative solution of Theorem* (1) *is a monotone-decreasing function.*

Proof: Suppose there is a local maximum of $\bar{a}$ at an interior point x_0; then we have $\bar{a}''(x_0) \leq 0$. At the same time, we have from (42)

$$\bar{a}''(x_0) = \frac{g_0\bar{a}(x_0)}{\zeta\bar{a}(x_0) + \alpha_0} \geq 0,$$

because morphogen concentration has already been shown to be nonnegative. Together they require $\bar{a}(x_0) = 0$. But $\bar{a}(x)$ is nonnegative (and $\bar{a}(x_0)$ is a maximum); therefore we must have $\bar{a}(x) \equiv 0$, which violates the boundary condition at $x = 0$. Hence, $\bar{a}(x)$ does not have an interior maximum.

The ODE (42) requires $\bar{a}(x)$ to be continuous and smooth. It follows that the steady-state concentration $\bar{a}(x)$ also cannot have a local minimum $\bar{a}(x_0) = 0$ at an interior point x_0. Otherwise, we would have $\bar{a}(x) = 0$ for $x \geq x_0$ and, by the continuity of $\bar{a}(x)$ and $\bar{a}'(x)$, $\bar{a}(x) \equiv 0$ for $0 \leq x \leq x_0$ as well. Hence, $\bar{a}(x)$

must be monotone, and, given the boundary conditions at the two ends, it must be monotone decreasing. ■

5. Steady-state solutions for special cases

5.1. No receptor synthesis in the morphogen production region

For the extreme case where there is no receptor synthesis in the morphogen production region so that $\rho^2 = \bar{V}_n/\bar{V}_p = 0$ (and hence no concentration of either occupied or unoccupied receptors in that region), the end condition (39) simplifies to

$$\bar{a}'(0) = -\bar{v}_L x_m, \tag{45}$$

where the right-hand side is a known quantity. The two-point BVP defined by the ODE (42) and the end conditions (43) with $\rho^2 = 0$ determines $\bar{a}(x)$. The ODE is autonomous; hence the BVP can be solved exactly except for a numerical solution of a nonlinear equation for the initial amplitude $a_0 \equiv \bar{a}(0)$. For $\zeta \ll 1$, it is straightforward to obtain the following leading term perturbation solution in ζ for $\bar{a}(x)$:

THEOREM 4. *The leading term perturbation solution in the small parameter ζ for $\bar{a}(x)$ is*

$$\bar{a}(x) \sim \frac{\bar{v}_L x_m}{\mu \cosh \mu} \sinh(\mu(1-x)), \tag{46}$$

with

$$\bar{a}(0) \sim \frac{\bar{v}_L x_m}{\mu} \tanh \mu. \tag{47}$$

Remark 1. The expressions (46) and (47) are identical to the corresponding results for System F for $\rho^2 = 0$ (see (29) and (31)). Hence, the present aggregated source model (System A) correctly replicates the value $\bar{a}(0)$ of the more realistic distributed source model for this extreme case of $\rho^2 = 0$, at least for $\zeta \ll 1$. Moreover, the ODE for the range $0 < x < 1$ as well as the end condition at $x = 1$ for Systems F and A are identical; it follows that the distributions of morphogen concentrations must be the same for both models at least for $\zeta \ll 1$.

For more general values of ζ, we integrate the ODE (42) once to get

$$\frac{1}{2}\left\{[\bar{a}'(x)]^2 - s_1^2\right\} = g_r \bar{a}(x) - \left(\frac{g_r}{\mu}\right)^2 \ell n \left(\frac{\alpha_0 + \zeta \bar{a}(x)}{\alpha_0}\right), \tag{48}$$

where we have made use of the fact that $\bar{a}(1) = 0$ and where $s_1 = \bar{a}'(1)$ is an unknown constant. The boundary condition (45) is then applied to get s_1^2 in terms of $a_0 = \bar{a}(0)$:

$$\left(\frac{\mu}{g_r}s_1\right)^2 = (\mu\beta_m)^2 - 2z_0 + 2\ell n(1+z_0),$$

where $\beta_m = \bar{v}_L x_m/g_r = \zeta\beta x_m$, $z(x) = \zeta\bar{a}(x)/\alpha_0$, and $z_0 = \zeta a_0/\alpha_0$, so that (48) becomes

$$z' = -\mu\sqrt{(\mu\beta_m)^2 - 2(z_0 - z) + 2\ln\left(\frac{1+z_0}{1+z}\right)}. \tag{49}$$

Given $\mu\beta_m$, the ODE (49) and the end condition $z(0) = z_0$ (corresponding to $\bar{a}(0) = a_0$) determines $\bar{a}(x; a_0)$ with an unknown parameter a_0. The condition $\bar{a}(1; a_0) = 0$ then fixes a_0 (in terms of the known parameter $\mu\beta_m = \mu\zeta\beta x_m$). The dependence of a_0 on $\beta = \bar{v}_L/g_0$, a critical amplitude parameter in point source models (see [2, 8]), for a typical set of other biological parameter values is illustrated in column 2 of Table 1 below. The corresponding values of a_0 by the approximate solution (45) are also given in column 4 there. It is seen from the results in that table that the leading-term perturbation solution for a_0 is very accurate for $\beta \leq 1$ and is still within 10% of the accurate (to 10^{-5}) numerical solution for $\beta \leq 5$ (with $\zeta = 0.2$ and $\zeta\beta = 1$ for the set of parameter values used for these results). More significantly, the accurate numerical solutions in Table 1 agree with the corresponding solutions for Systems F in ([1]) at least to three significant figures for $\rho^2 = 0$.

5.2. Perturbation solutions for small ζ

For $\rho^2 > 0$ but $\zeta \ll 1$ (corresponding to $k_{\text{deg}} \ll k_g$ and $g_0 \ll g_r$), we may seek an appropriate parametric expansion of the solution in the parameter ζ. For

Table 1

$a_0 = \bar{a}(0)$ vs. $\beta = \bar{v}_L/g_0 (g_0 = 0.2, g_r = 1.0, h_0 = 10, f_0 = 0.001, x_m = 0.1)$

β	a_0 $(\rho^2 = 0)$	$a_0\|_{X_{\max}=\infty}$ $(\rho^2 = 0)$	$a_0\|_{\zeta=0}$ $(\rho^2 = 0)$	$a_0\|_{\zeta=0}$ $(\rho^2 = 1)$	$a_0\|_{X_{\max}=\infty}$ $(\rho^2 = 1)$	a_0 $(\rho^2 = 1)$
0.25	0.001588	0.001593	0.001579	0.001202	0.001212	0.001209
0.50	0.003191	0.003204	0.003159	0.002403	0.002439	0.002432
1.00	0.006448	0.006474	0.006317	0.004807	0.004935	0.004920
5.00	0.034913	0.035119	0.031587	0.024033	0.027082	0.026965
10.00	0.076665	0.077381	0.063173	0.048066	0.060314	0.060209
25.00	0.243073	0.250764	0.157933	0.120165	0.205606	0.200968

a moderate morphogen production rate $\bar{v}_0 \equiv \bar{v}_L x_m$ so that $\zeta \bar{a}(x) \ll \alpha_0$, it is appropriate to take

$$\bar{a}(x;\zeta) = \sum_{k=0}^{\infty} a_k(x)\zeta^k.$$

The leading-term solution is determined by the linear BVP:

$$a_0'' - \mu^2 a_0 = 0, \quad \mu^2 = \frac{g_0}{\alpha_0} = \psi$$

subject to the boundary conditions

$$\sigma_0 \bar{a}_0'(0) - \mu^2 \rho^2 a_0(0) + \bar{v}_L = 0, \quad a_0(1) = 0.$$

It is straightforward to obtain the exact solution of this linear BVP.

THEOREM 5. *For $\zeta \ll 1$, a leading-term perturbation solution for $\bar{a}(x)$ in ζ is given by*

$$\begin{aligned} a_0(x) &= \frac{\bar{v}_L}{\mu^2} \frac{\sinh(\mu(1-x))}{\sinh(\mu)\left[\rho^2 + \frac{\sigma_0}{\mu}\coth(\mu)\right]} \\ &= \frac{\beta\alpha_0 \sinh(\mu(1-x))}{\sinh(\mu)\left[\rho^2 + \frac{\sigma_0}{\mu}\coth(\mu)\right]}, \quad \beta = \bar{v}_L/g_0. \end{aligned} \tag{50}$$

Remark 2. For relatively high binding rate, the parameter $\mu^2 = g_0 h_0/(f_0 + g_0)$ is generally large compared to 1. Hence, if $\sigma_0 = 1/x_m$ is $O(1)$ or smaller, the contribution from the flux term is negligible. This observation provided the motivation for the omission of the flux term in System R (as well as Systems B and C in [2, 8]). The omission of the flux term is attractive as it leads to simpler theoretical and computational treatments of the problem. However, with the aggregated source model (System A) derived from System F, the flux coefficient σ_0 is now seen to be $X_{\max}/X_{\min} = 1/x_m$ which may well be $\gg 1$ (and is typically the case for a Drosophila wing disc). Unless μ is sufficiently large so that $\sigma_0/\mu = (\mu x_m)^{-1}$ is negligibly small, the contribution of the flux term generally cannot be omitted. For the typical set of parameter values for the Drosophila wing disc used in Table 1, we have $\mu x_m \simeq 0.32$ so that the condition for omitting the flux term is not satisfied. With the flux term, results given in the fifth column of Table 1 for $\rho^2 = 1$ show that the leading term perturbation solution is very accurate for $\beta < 5$ and has only about a 12% error for $\beta = 5$ relative to the accurate numerical solution of column 7.

For the extreme case of $x_m = X_{\min}/X_{\max} \to 0$, care must be taken so that there is a finite amount of ligand in the system. This is done by leaving $\bar{v}_L x_m = v_0$ fixed and finite as $x_m \to 0$. In that case, we have

$$a_0(0) = \frac{v_0}{\mu^2\left[\rho^2 x_m + \frac{1}{\mu}\coth(\mu)\right]} \to \frac{v_0}{\mu}\tanh(\mu), \tag{51}$$

which is in agreement with (32). Thus for $\zeta \ll 1$, the aggregated source model (System A) replicates the characteristics features of System F for $\rho^2 > 0$ as well.

5.3. Approximate solution for high ligand synthesis rates

With all biological parameters other than $\bar{v}_L$ fixed, it is expected that the maximum steady-state free ligand concentration would increase with $\bar{v}_L$. Let $\bar{a}(x) = \bar{v}_L x_m A(x)$ and write the BVP for $\bar{a}(x)$ in terms of $A(x)$:

$$A'' - \frac{1}{\beta_m}\frac{A}{\varepsilon + A} = 0, \quad \varepsilon = \frac{g_r\alpha_0}{g_0\bar{v}_L x_m} = \frac{1}{\zeta\beta x_m \mu^2} = \frac{1}{\beta_m\mu^2}$$

$$A(1) = 0, \quad A'(0) - \frac{\rho^2 x_m}{\beta_m}\frac{A(0)}{\varepsilon + A(0)} + 1 = 0.$$

For a sufficiently high ligand synthesis rate $\bar{v}_L$ so that $\varepsilon \ll 1$, we may seek a perturbation solution of $A(x)$ in ε with its leading term determined by

$$A_0'' = \frac{1}{\beta_m}, \quad A_0'(0) - \frac{\rho^2 x_m}{\beta_m} + 1 = 0, \quad A_0(1) = 0.$$

The condition $\varepsilon \ll 1$ requires $\beta_m = \zeta\beta x_m = \bar{v}_L x_m / g_r \gg 1/\mu^2$; it is certainly satisfied by $\bar{v}_L x_m / g_r \leq 1$ since $\mu^2 = g_0/\alpha_0 = O(h_0)$ is the effective normalized binding rate and is usually large compared to unity. The factor x_m is often small (as in a Drosophila wing disc) so that the second term of the end condition at $x = 0$ may be omitted sometimes; however, we retain the term here to allow for moderate values of x_m.

THEOREM 6: *For $\varepsilon \ll 1$, we have the following leading-term perturbation solution in ε*

$$\bar{a}(x) \sim \bar{v}_L x_m \left\{\left(1 - \frac{\rho^2 g_r}{\bar{v}_L}\right)(1 - x) - \frac{g_r}{2\bar{v}_L x_m}(1 - x^2)\right\} \tag{52}$$

with

$$a_0 = \bar{a}(0) \sim \bar{v}_L x_m \left(1 - \frac{\rho^2 g_r}{\bar{v}_L} - \frac{g_r}{2\bar{v}_L x_m}\right). \tag{53}$$

Remark 3. For very large values of $\bar{v}_L$, we may further simplifiy the above result to get

$$\bar{a}(0) \sim \bar{v}_L x_m \quad \text{and} \quad \bar{a}'(0) \sim -\bar{v}_L x_m.$$

The asymptotic behavior of $\bar{a}(0)$ and $\bar{a}'(0)$ is therefore identical to that of System F given in (34) [1]. As such, the aggregated source model (System A)

Table 2
Asymptotic vs. Numerical Solution for High Ligand Synthesis Rates
($g_0 = 0.2$, $g_r = 1.0$, $h_0 = 10$, $f_0 = 0.001$, $x_m = 0.1$)

$\bar{v}_L$	β	ε	a_0 ($\rho^2 = 1$)	a_0 (53) ($\rho^2 = 1$)	a_0 ($\rho^2 = 0$)	a_0 (53) ($\rho^2 = 0$)
10	50	0.10050	0.5568	0.4000	0.6287	0.5000
20	100	0.05025	1.4683	1.4000	1.5586	1.5000
40	200	0.02513	3.4306	3.4000	3.5271	3.5000
80	400	0.01256	7.4145	7.4000	7.5130	7.5000
160	800	0.00628	15.4071	15.4000	15.5064	15.5000

reproduces the behavior of the distributed source model (System F) for the higher range of morphogen production rate as well. At the same time, the results of this section indicate that the flux term in the end condition at $x = 0$ is indispensable in obtaining the correct asymptotic behavior for high $\bar{v}_L$ beyond the limit $\beta_r = \bar{v}_L/g_r < 1$ imposed by the ad hoc point source model System R on the existence of steady-state concentrations.

With $g_0 = 0.2$, $g_r = 1.0$, $h_0 = 10$, $f_0 = 0.001$, and $x_m = 0.1$, we compare in Table 2 the approximate solution in (53) for a range of $\bar{v}_L$ values with the corresponding accurate numerical solution. We see from the results that the asymptotic solutions are accurate to within 5% for $\bar{v}_L = 20$ (with $\beta = 100$ and $\varepsilon = 0.05025$) and with negligible relative error for larger $\bar{v}_L$. The range of $\beta(=\bar{v}_L/g_0)$ values is significant in that we have not only $\beta \gg 1$ but also $\zeta\beta = \bar{v}_L/g_r > 1$ in all cases, confirming the existence of stable state concentration gradients for values of β well beyond the restricted range of $\beta = v_0/g_0 < 1$ required by System R in [2].

5.4. Approximate solutions for large $X_{\max}$

In this subsection, we consider the solution for the limiting case of $X_{\max} = \infty$ with $\bar{a}(x) \to 0$ and $\bar{a}'(x) \to 0$ as $x \to \infty$. (Note that x is now X normalized by some reference length X_0.) For example, we may take $X_0 = X_{\min}$, the width of the ligand production zone. The approximation is expected to be appropriate for the case of a very large $X_{\max}$, say $X_{\max} \gg X_{\min}$. For this limiting case, the governing ODE (23) can be integrated once to give

$$\frac{1}{2}[\bar{a}'(x)]^2 = g_r\bar{a}(x) - \left(\frac{g_r}{\mu}\right)^2 \ell n\left(\frac{\alpha_0 + \zeta\bar{a}(x)}{\alpha_0}\right), \tag{54}$$

where μ^2 is as previously defined in (27) and where we have made use of the conditions that $\bar{a}(x) \to 0$ and $\bar{a}'(x) \to 0$ as $x \to \infty$. At $x = 0$, we have from (54)

$$\frac{1}{2}[\bar{a}'(0)]^2 = g_r a_0 - \left(\frac{g_r}{\mu}\right)^2 \ell n\left(\frac{\alpha_0 + \zeta a_0}{\alpha_0}\right), \tag{55}$$

where $a_0 = \bar{a}(0)$ is still to be determined. In the relation (55), $\bar{a}'(0)$ can be expressed in terms of a_0 by way of (39) to give $\bar{v}_L x_m / g_r = \beta_m$ as a function of $z_0 = \zeta a_0/\alpha_0$ with μ and $\rho_m^2 \equiv \rho^2 x_m$ as parameters:

$$\beta_m = \frac{\rho^2 x_m z_0}{1 + z_0} + \frac{\sqrt{2}}{\mu}\sqrt{z_0 - \ln(1 + z_0)}. \tag{56}$$

For a given positive value of β_m, it is not difficult to show that the relation (56) determines a unique positive solution for the unknown z_0.

For $\rho^2 = 0$, the relation (56) reduces to

$$\frac{\mu}{\sqrt{2}}\beta_m = \sqrt{z_0 - \ln(1 + z_0)}. \tag{57}$$

The quantity $\mu\beta_m/\sqrt{2}(=\mu\bar{v}_L x_m/\sqrt{2}g_r = \mu\zeta x_m\beta/\sqrt{2})$ defined by (57) is a monotone increasing, concave function of nonnegative z_0. Hence, there is a unique solution for the BVP for $\bar{a}(x)$,

$$z' = -\sqrt{2}\mu\sqrt{z - \ln(1 + z)}, \quad z(0) = z_0, \tag{58}$$

as assured by the existence theorem. Note that in terms of the biological parameters, the quantity $\mu\beta_m$ is independent of the choice of X_0. Values of a_0 for different values of $\beta = \bar{v}_L/g_0$ are given in column 3 of Table 1 for the previously selected set of other biological parameter values relevant to Drosophila wing imaginal discs. The agreement with the corresponding exact numerical solution in column 2 is to a relative error of less than half of a percent for the range of $\bar{v}_L$ calculated. It is important to observe that the solution of the initial value problem (58) is of the form $z = Z(\xi; z_0(\mu\beta_m))$ (so that $\bar{a}(x) = \alpha_0 Z(\xi; z_0(\mu\beta_m))/\zeta$), with $\xi = \mu x$ and $z_0(\mu\beta_m)$ being the unique positive solution of (57). Hence, we know all about the structure of the solution in this limiting case without solving any differential equation.

Graphs of $\bar{v}_L x_m/g_r(=\beta_m)$ versus $\zeta a_0/\alpha_0 = z_0$ for different values of the parameter μ are given in Figure 1 for the other extreme case of $\rho^2 = 1$ with $x_m = 0.1$. Similar to (57), the quantities $\mu\beta_m$ and μx_m in (56) do not vary with the choice of X_0. The monotone increasing graphs of β_m in Figure 1 ensure that a positive root $z_0 = \zeta a_0/\alpha_0$ is uniquely determined by a prescribed value of $\bar{v}_L$ (with $\beta = \bar{v}_L/g_0$ and $\bar{v}_L/g_r = \zeta\beta$). Having the unique positive solution z_0 of (56), the ODE (58) can be integrated exactly to give $\bar{a}(x)$. With $g_0 = 0.2$, $g_r = 1.0$, $h_0 = 10$, $f_0 = 0.001$, and $x_m = 0.1$, we obtain in column 6 of Table 1 the values of a_0 for a range of values of $\beta = \bar{v}_L/g_0$ and $\rho^2 = 1$. These values are in excellent agreement with the corresponding accurate numerical solution in column 7 (as well as the relevant numerical results for System F reported in [1]). Together, results for the two extreme values of ρ^2 suggest

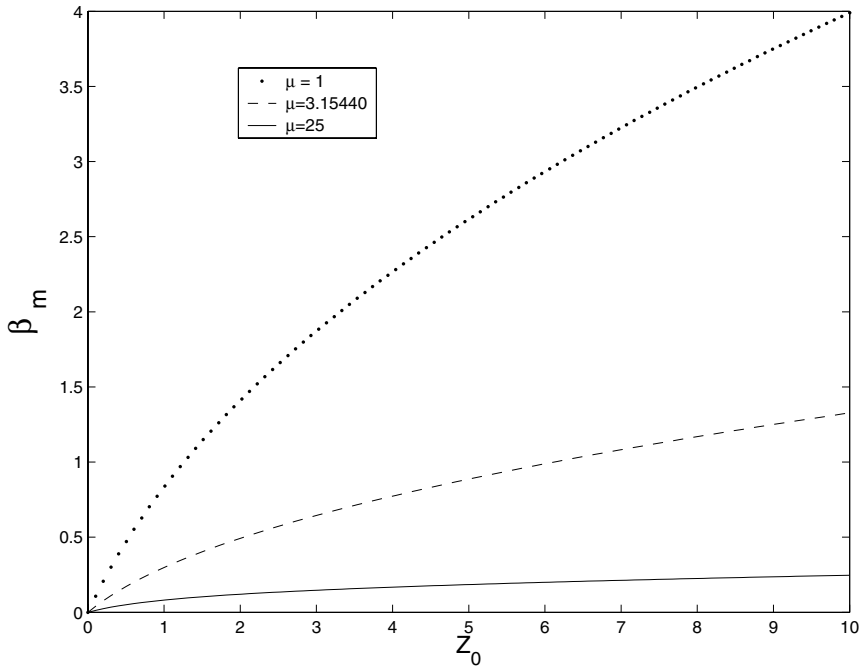

Figure 1. Normalized steady-state end free ligand concentration z_0 versus synthesis-to-degradation rate ratio $\beta_m(\rho^2 = 1$ and $x_m = 0.1)$.

that the limiting case of $X_{\max} = \infty$ is a useful simplification and adequate approximation for problems with $X_{\max} \gg X_{\min}$.

6. Linear stability analysis

6.1. A nonlinear eigenvalue problem

The stability of the steady-state solution of the aggregated source problem with respect to a small perturbation can also be examined by considering a time-dependent solution of the form

$$\{a(x,t), b(x,t), r(x,t)\} = \{\bar{a}(x), \bar{b}(x), \bar{r}(x)\} + e^{-\lambda t}\{\hat{a}(x), \hat{b}(x), \hat{r}(x)\}, \quad (59)$$

and linearizing the governing PDE and boundary conditions (10)–(12) to obtain the following eigenvalue problem for $\{\hat{a}(x), \hat{b}(x), \hat{r}(x)\}$ with λ as the eigenvalue parameter:

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

$$-\lambda\hat{b} = h_0(\bar{r}\hat{a} + \bar{a}\hat{r}) - (f_0 + g_0)\hat{b} \quad (61)$$

$$-\lambda\hat{r} = -h_0(\bar{r}\hat{a} + \bar{a}\hat{r}) - g_r\hat{r} + f_0\hat{b}. \tag{62}$$

$$\hat{a}'(-x_m) = 0, \quad \hat{a}(1) = 0. \tag{63}$$

The relations (61) and (62) are solved for $\hat{b}$ and $\hat{r}$ in terms of $\hat{a}$ making use of (21) to get

$$\hat{r} = \frac{h_0(\lambda - g_0)\bar{r}(x)\hat{a}}{(g_r - \lambda)(f_0 + g_0 - \lambda) + h_0\bar{a}(x)(g_0 - \lambda)} \tag{64}$$

$$\hat{b} = \frac{h_0(g_r - \lambda)\bar{r}(x)\hat{a}}{(g_r - \lambda)(f_0 + g_0 - \lambda) + h_0\bar{a}(x)(g_0 - \lambda)}. \tag{65}$$

The expressions (64) and (65) are then used to eliminate $\hat{b}$ and $\hat{r}$ from (60) to obtain

$$\hat{a}'' + [\lambda - q_r(x;\lambda)]\,\hat{a} = 0 \quad (0 < x < 1), \tag{66}$$

with

$$\begin{aligned} q_r(x;\lambda) &= \frac{1}{1 + \zeta\bar{\beta}_0 A(x)} \frac{h_0(g_r - \lambda)(g_0 - \lambda)}{(g_r - \lambda)(g_0 + f_0 - \lambda) + (g_0 + f_0)(g_0 - \lambda)\bar{\beta}_0 A(x)} \\ &\equiv \frac{1}{1 + \zeta\bar{\beta}_0 A(x)} \frac{N_r(x;\lambda)}{D_r(x;\lambda)}, \end{aligned} \tag{67}$$

where we have set

$$\bar{a}(x) = \alpha_0\bar{\beta}_0 A(x), \quad \text{with } A(0) = 1 \tag{68}$$

so that $\bar{a}(0) = \alpha_0\bar{\beta}_0$. Note that $\bar{\beta}_0$ is known to be positive from the solution of the steady-state problem. Let

$$\beta_0 = \frac{\bar{\beta}_0}{1 + \zeta\bar{\beta}_0}; \tag{69}$$

then $\beta_0 = \bar{b}(0+)$ is positive. (In contrast to Systems B, C, and R, there is no restriction on β_0 or the rate of morphogen synthesis in the present aggregated source model System A.)

The boundary conditions for the ODE (66) are

$$\sigma_0\hat{a}'(0) + \lambda\hat{a}(0) - (g_0 - \lambda)\hat{b}(0-) = 0, \quad \hat{a}(1) = 0 \tag{70a}$$

with $\hat{b}(0-)$ expressed in terms of $\hat{a}(0)$ and $\bar{a}(0)$ by (65) and (21) with $r_0(0-) = \rho^2$. The first end condition of (70a) is a consequence of (41) and (37) while the second follows from $a(1, t) = \bar{a}(1) = 0$. We now rewrite these end conditions in terms of $\hat{a}(x)$ alone to obtain

$$\sigma_0\hat{a}'(0) + [\lambda - q_\rho(\lambda)]\hat{a}(0) = 0, \quad \hat{a}(1) = 0, \tag{71}$$

where

$$q_\rho(\lambda) = \frac{\rho^2}{1+\zeta\bar{\beta}_0}\frac{h_0(g_r-\lambda)(g_0-\lambda)}{(g_r-\lambda)(g_0+f_0-\lambda)+h_0\bar{a}(0)(g_0-\lambda)} \equiv \frac{\rho^2}{1+\zeta\bar{\beta}_0}\frac{N_m(\lambda)}{D_m(\lambda)}. \tag{72}$$

Together, (66) and (71) define an eigenvalue problem with λ as the eigenvalue parameter. Though the ODE is linear in the unknown $\hat{a}(x)$, the eigenvalue problem is nonlinear since λ appears nonlinearly in $q_r(x;\lambda)$ and $q_\rho(\lambda)$ so that (66) and (71) is *not* a Sturm–Liouville problem.

6.2. Positive eigenvalues and asymptotic stability

In this subsection, we will show that the eigenvalues of the homogeneous boundary value problem (66) and (71) for $\hat{a}(x)$ must be positive. It follows then that the steady-state gradients are asymptotically stable according to linear stability theory.

LEMMA 1. *All the eigenvalues of the nonlinear eigenvalue problem* (66) *and* (71) *are real.*

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

$$\int_0^1 \left[(a_\lambda^*)a_\lambda'' - (a_\lambda^*)''a_\lambda\right]dx = \frac{|a_\lambda(0)|^2}{\sigma_0}\left[(\lambda-\lambda^*) - \left\{q_\rho(\lambda)-q_\rho(\lambda^*)\right\}\right],$$

(which can be established by integration by parts and applications of the boundary conditions in (71)) requires

$$\begin{aligned} 0 = &\int_0^1 \left\{(\lambda-\lambda^*) - \left[q_r(x;\lambda)-q_r(x;\lambda^*)\right]\right\}\left(a_\lambda^* a_\lambda\right)dx \\ &+ \frac{|a_\lambda(0)|^2}{\sigma_0}\left[(\lambda-\lambda^*) - \frac{\rho^2}{1+\zeta\bar{\beta}_0}\left\{\frac{N_m(\lambda)}{D_m(\lambda)} - \frac{N_m(\lambda^*)}{D_m(\lambda^*)}\right\}\right], \end{aligned} \tag{73}$$

where we have made use of the boundary conditions (71). It is straightforward to verify

$$\begin{aligned} q_\rho(\lambda)-q_\rho(\lambda^*) &= -(\lambda-\lambda^*)\frac{\rho^2 h_0\{f_0 Q(g_r,\lambda)+\bar{\beta}_0(g_0+f_0)Q(g_0,\lambda)\}}{(1+\zeta\bar{\beta}_0)D_m(\lambda)D_m(\lambda^*)} \\ &\equiv -(\lambda-\lambda^*)\Phi_m(\lambda,\lambda^*) \end{aligned}$$

with

$$Q(y, \lambda) = [y - \mathrm{Re}(\lambda)]^2 + [\mathrm{Im}(\lambda)]^2,$$

being a positive quantity for any λ so that $\Phi_m(\lambda\lambda^*) > 0$. Similarly, we have $q_r(x; \lambda) - q_r(x; \lambda^*) = -(\lambda - \lambda^*)\Phi(x; \lambda, \lambda^*)$ where

$$\Phi(x; \lambda\lambda^*) = \frac{h_0\{f_0 Q(g_r, \lambda) + (g_0 + f_0)\bar{\beta}_0 A(x) Q(g_0, \lambda)\}}{(1 + \zeta\bar{\beta}_0 A(x)) D_r(x; \lambda) D_r(x; \lambda^*)} > 0.$$

In that case, the condition (73) becomes

$$-(\lambda - \lambda^*)\left\{\int_0^1 a_\lambda a_\lambda^* [1 + \Phi(x; \lambda, \lambda^*)]\, dx + \frac{1}{\sigma_0}\left[1 + \Phi_m(\lambda, \lambda^*)\right] |a_\lambda(0)|^2\right\} = 0.$$

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

THEOREM 7. *All eigenvalues of the nonlinear eigenvalue problem* (60)–(62) *and* (71) *are positive and the steady-state concentrations* $\bar{a}(x)$, $\bar{b}(x)$, *and* $\bar{r}(x)$ *are asymptotically stable by a linear stability analysis.*

Proof: Suppose $\lambda \leq 0$. Let $\hat{a}_\lambda(x)$ be a nontrivial eigenfunction of the homogeneous BVP (66) and (71) for the nonpositive eigenvalue λ. Multiply (66) 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 applications of the homogeneous boundary conditions (71), we obtain

$$\lambda \int_0^1 (\hat{a}_\lambda)^2\, dx = \int_0^1 (\hat{a}_\lambda')^2\, dx + \int_0^1 q_r(x; \lambda)(\hat{a}_\lambda)^2\, dx - \frac{1}{\sigma_0}(\hat{a}_\lambda(0))^2\left[\lambda - q_\rho(\lambda)\right]. \tag{74}$$

Because λ is not positive, we can write $\lambda = -|\lambda| \leq 0$ so that

$$q_r(x; -|\lambda|) = \frac{1}{1 + \zeta\bar{\beta}_0 A(x)} \frac{h_0(g_0 + |\lambda|)(g_r + |\lambda|)}{(g_r + |\lambda|)(g_0 + f_0 + |\lambda|) + h_0\bar{a}(x)(g_0 + |\lambda|)} > 0,$$

$$q_\rho(-|\lambda|) = \frac{\rho^2}{1 + \zeta\bar{\beta}_0} \frac{h_0(g_r + |\lambda|)(g_0 + |\lambda|)}{(g_r + |\lambda|)(g_0 + f_0 + |\lambda|) + (g_0 + f_0)\bar{\beta}_0(g_0 + |\lambda|)} > 0.$$

For any nontrivial solution of the eigenvalue problem under the assumption $\lambda \leq 0$, the right-hand side of (74) is positive which contradicts the assumption

$\lambda = -|\lambda| \leq 0$ (since the left-hand side of (74) is nonpositive for a nonpositive λ). Hence, the eigenvalues of the eigenvalue problem (66) and (71) must be positive and the theorem is proved. ■

7. Decay rate of transients

Although knowing the eigenvalues being positive is sufficient to ensure the (linear) asymptotic stability of the steady-state morphogen concentration gradients, we want to know the *smallest* eigenvalue (or an estimate of it) to get some idea of how quickly the system returns to a steady state after small perturbations. 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 thereby the time to reach a steady state.

The eigenvalue problem (66)–(71) whose solution is needed for the determination of decay rate of transients is nonlinear and the steady-state free Dpp concentration $\bar{a}(x)$ that appears in the coefficient $q_r(x; \lambda)$ of (66) is only known numerically in general. Hence, the smallest eigenvalue of (66)–(71), denoted by λ_s, generally can only be found by numerical methods. Accurate numerical solutions for the nonlinear eigenvalue problem is possible but tedious. In the subsections below, we will obtain (1) an explicit analytical solution for the case when the morphogen synthesis rate is relatively low, and (2) some tight upper and lower bounds for λ_s which would provide a good estimate of its actual value.

7.1. Approximate decay rates

We learned from (69) $\beta_0 = \bar{b}(0+)$ is a normalized amplitude factor for the bound ligand concentration, which is expected to be a decreasing function of the normalized synthesis rate $\bar{\nu}_L$. For sufficiently small $\bar{\nu}_L$, we should have $\bar{\beta}_0 \ll 1$. In that case, a perturbation solution for λ and $\hat{a}(x)$ may be obtained as parametric series in $\bar{\beta}_0$:

$$\{\hat{a}(x), \lambda\} \equiv \sum_{n=0}^{\infty} \{a_n(x), \lambda_n\} \bar{\beta}_0^n. \tag{75}$$

The leading-term solution is determined by the simpler linear eigenvalue problem

$$a_0'' + [\lambda_0 - q_{r0}(\lambda_0)] a_0 = 0 \quad (0 < x < 1), \tag{76}$$

$$\sigma_0 a_0'(0) + [\lambda_0 - q_{\rho 0}(\lambda_0)] a_0(0) = 0, \quad a_0(1) = 0, \tag{77}$$

with

$$q_{r0}(\lambda_0) = \frac{h_0(g_0 - \lambda_0)}{g_0 + f_0 - \lambda_0} = \frac{1}{\rho^2} q_{\rho 0}(\lambda_0). \tag{78}$$

Note that the leading term of the parametric series for $q_r(x; \lambda)$ does not depend on x. The exact solution for the eigenvalue problem (76) and (77) is

$$a_0(x) = c_0 \sin(\eta(1 - x)), \quad \eta^2 = \lambda_0 - \frac{h_0(g_0 - \lambda_0)}{g_0 + f_0 - \lambda_0}, \tag{79}$$

and λ_0 is a root of

$$\sigma_0 \eta = \left[\lambda_0 - \frac{\rho^2 h_0(g_0 - \lambda_0)}{g_0 + f_0 - \lambda_0}\right] \tan(\eta). \tag{80}$$

The slowest decay rate of the transients is given (approximately) by the smallest positive λ_0, denoted by $\lambda_0^{(0)}$, that satisfies (80) with η given in terms λ_0 by (79). The following two observations are helpful for further developments:

Remark 4. Though $\eta = 0$ also satisfies (80), it is not an admissible solution for the eigenvalue problem because it leads to a trivial solution for $a_0(x)$.

Remark 5. From the second equation of (79), we get

$$\left\{1 + \frac{h_0 f_0}{(g_0 + f_0 - \lambda_0)^2}\right\} \frac{d\lambda_0}{d(\eta^2)} = 1$$

so that λ_0 is an increasing function of η^2.

For $\rho^2 = 1$, (80) may be written as

$$\sigma_0 = \eta \tan(\eta). \tag{81}$$

It follows that $\lambda_0^{(0)}$ is the smaller of the two roots of

$$\lambda_0 - \frac{h_0(g_0 - \lambda_0)}{g_0 + f_0 - \lambda_0} = \eta_s^2,$$

for the smallest η_s that satisfies (80) with $\eta_s \leq \pi/2$. We are interested here for $x_m \ll 1$ (so that $\sigma_0 \gg 1$). In that case, we have $\eta_s \simeq \pi/2$ to a good first approximation and therewith

$$\lambda_0^2 - \left(g_0 + f_0 + h_0 + \pi^2/4\right)\lambda_0 + \left[\left(h_0 + \pi^2/4\right)g_0 + f_0\pi^2/4\right] = 0,$$

which is identical to the corresponding result of System F for a spatially distributed source and does not depend on the (normalized) ligand synthesis rate $\bar{v}_L$ (see [1]). For $g_0 = 0.2$, $f_0 = 0.001$, and $h_0 = 10$, the solution of the quadratic equation above gives $\lambda_0^{(0)} = 0.2001848$. For $f_0 = 0.01$ and $f_0 = 0.05$, the corresponding values for $\lambda_0^{(0)}$ are 0.2001847 and 0.2092109, respectively.

For sufficiently high synthesis rate so that $\zeta\bar{\beta}_0 \gg 1$, both q_ρ and q_r are $O((\zeta\bar{\beta}_0)^{-1})$. A leading-term perturbation solution in $1/(\zeta\bar{\beta}_0)$ also gives (81) for the determination of the (leading-term) eigenvalues but now with $\eta \sim \sqrt{\lambda}$. Hence, the present aggregated source model, System A, correctly reproduces another characteristic feature, the decay rate of transients, of the distributed source model System F for both low and high ligand synthesis rates.

The accuracy of these leading asymptotic solutions can be improved by obtaining higher-order correction terms in the relevant parametric series. Instead of doing that, we will obtain an upper bound and a lower bound for the smallest eigenvalue λ_s of the eigenvalue problem (66) and (71). It will be seen from these bounds and the numerical results for the three special cases how accurate the leading-term perturbation solution can be. For this purpose, we observe the following facts for sufficiently small $\bar{\nu}_L$:

(1) $\eta^2(\lambda_0 = g_0) = g_0$ with $g_0 < \pi^2/4 \approx \eta_s^2$ for Drosophila wing disc problems,
(2) $\eta^2(\lambda_0)$ has a simple pole at $g_0 + f_0$, and
(3) $\eta^2(\lambda_0)$ is an increasing function of λ_0 for $\lambda_0 < g_0 + f_0$.

With $g_0 < \eta_s^2 (\simeq \pi^2/4)$ for the particular set of parameter values considered above, it follows from the three observations above $g_0 < \lambda_0^{(0)} < g_0 + f_0$. This conclusion is consistent with the approximate solutions for $\lambda_0^{(0)}$ obtained above. For the three set of parameter values considered for actual solutions above, the upper and lower bounds narrowly delimit $\lambda_0^{(0)}$with $0.2 < \lambda_0^{(0)} < 0.201$ for the first case. In the next section, this method for finding bounds will be modified and applied to a broader range of values of $\bar{\nu}_L$ for which the perturbation method may not apply.

7.2. Bounds on the decay rate of transients

Recall that λ_s is the smallest eigenvalue of the (66) and (71). Let

$$\Lambda(\lambda) = [\lambda - q_\rho(\lambda)], \tag{82}$$

and

$$\Lambda_s = \lambda_s - \frac{\rho^2}{1 + \zeta\bar{\beta}_0} \frac{h_0(g_r - \lambda_s)(g_0 - \lambda_s)}{(g_r - \lambda_s)(g_0 + f_0 - \lambda_s) + \bar{\beta}_0(g_0 + f_0)(g_0 - \lambda_s)} \equiv \Lambda(\lambda_s). \tag{83}$$

The function $\Lambda(\lambda)$ has two simple poles which are the two roots of the quadratic equation

$$D_m(\lambda) \equiv (g_r - \lambda)(g_0 + f_0 - \lambda) + \bar{\beta}_0(g_0 + f_0)(g_0 - \lambda) = 0.$$

Let λ_c be the smaller of the two poles:

$$\lambda_c = \frac{1}{2}\left\{\gamma - \sqrt{\gamma^2 - 4(g_r + \bar{\beta}_0 g_0)(g_0 + f_0)}\right\}, \quad \gamma = (1 + \bar{\beta}_0)(g_0 + f_0) + g_r.$$

It is straightforward to prove the following key lemma:

LEMMA 2. *$\Lambda(\lambda)$ as given by (83) is a monotone-increasing function of λ in $0 \le \lambda < \lambda_c$ where λ_c is the smallest root of $D_m(\lambda)$ (or the smallest simple pole of $\Lambda(\lambda)$) with (i) $g_r < \lambda_c < g_0$ if $g_r < g_0$, or (ii) $g_0 < \lambda_c < \min\{g_0 + f_0, g_r)$ if $g_r > g_0$.*

Proof: We compute $d\Lambda/d\lambda$ to obtain

$$\frac{d\Lambda}{d\lambda} = 1 + \frac{h_0 \rho^2 Z(\lambda)}{(1 + \zeta \bar{\beta}_0)[D_m(\lambda)]^2}, \tag{84}$$

with

$$Z(\lambda) = \bar{\beta}_0(g_0 + f_0)(\lambda - g_0)^2 + f_0(\lambda - g_r)^2 \tag{85}$$

showing that $d\Lambda/d\lambda$ is positive because all the parameters involved are nonnegative. The inequalities on λ_c asserted by the lemma are immediate consequences of the form of the quadratic function $D_m(\lambda)$. ■

We know $\Lambda(\lambda) = \Lambda_s$ has a solution in $[0, \infty)$ because $\Lambda(\lambda_s) = \Lambda_s$ and λ_s being an eigenvalue of (66) and (71) must be positive. Our goal is to find λ_s or some bounds for it. We cannot simply solve $\Lambda(\lambda) = \Lambda_s$ for λ_s because we do not know Λ_s (which was defined in terms of the unknown λ_s by (83)). But we can now narrow down the range of λ_s with the help of Lemma 2.

THEOREM 8: *$\Lambda(\lambda) = \Lambda_s$ has only one root in $(0, \lambda_c)$.*

Proof: Because $\Lambda(0) < 0$ and $\Lambda(\lambda) \uparrow \infty$ as $\lambda \uparrow \lambda_c$, there is only one root of $\Lambda(\lambda) = \Lambda_s$ in $(0, \lambda_c)$. It must be λ_s with $0 < \lambda_s < \lambda_c$ because λ_s is the smallest eigenvalue and it is positive. ■

Theorem 8 above settles the existence and uniqueness of a positive λ_s. With the help of Lemma 2, we can obtain the following useful bounds for λ_s for the case of $\min\{g_0, g_r\} < \Lambda_s$ most relevant to the Dpp gradient in the Drosophila wing disc.

COROLLARY 1. *If $\min\{g_0, g_r\} < \Lambda_s$, we have $\lambda_s > \min\{g_0, g_r\}$ and hence $\min\{g_0, g_r\} < \lambda_s < \lambda_c$.*

Proof: The upper bound on λ_s is already known from Theorem 8. The lower bound is a direct consequence of Lemma 2 given $\Lambda(0) < 0$ and $0 < \Lambda(g_k) = g_k < \Lambda_s$, with g_k being g_0 or g_r, whichever is smaller. ■

Remark 6. Though we do not know Λ_s a priori, we have from the perturbation solution $\Lambda_s \simeq \pi^2/4$ for sufficiently small x_m and $\bar{\nu}_L$. For Dpp in the wing imaginal disc of Drosophilas, we have $g_0 < g_0 + f_0 < g_r < \pi^2/4$. It follows from Corollary 1 $g_0 < \lambda_s < \lambda_c < g_0 + f_0$ which gives a sharp upper and lower bound on the decay rate of transients. With $f_0 \ll g_0$ in some cases, the smallest eigenvalue is again limited to a very narrow range of values as illustrated by the approximate solutions for three sets of parameter values in the previous subsection. We summarize this observation in the following corollary:

COROLLARY 2. *If* $g_0 + f_0 \leq \min\{g_r, \Lambda_s\}$, *we have* $\lambda_\ell \equiv g_0 < \lambda_s < \lambda_c < g_0 + f_0 \equiv \lambda_u$. *If on the other hand* $g_r \leq \min\{g_0, \Lambda_s\}$, *then we have* $\lambda_\ell \equiv g_r < \lambda_s < \lambda_c < g_0 \equiv \lambda_u$.

In the complementary range $(\Lambda(0) < 0 <) \Lambda_s < \min\{g_r \cdot g_0\}$, we have the following corollary of Theorem 8:

COROLLARY 3. *For* $\Lambda_s < \min\{g_0, g_r\}$, *we have* $\lambda_\ell \equiv \Lambda_s < \lambda_s < \lambda_c < \max\{g_0, \min(g_0 + f_0, g_r)\} \equiv \lambda_u$.

Proof: The lower bound is a consequence of $\Lambda(\Lambda_s) < \Lambda_s$ and Lemma 2. The upper bound follows from the bounds on λ_c in same lemma. ■

Remark 7. The upper and lower bounds established above for System A are identical to the correspond bounds obtained for System F in [1]. Hence, the decay rates of the two systems are bounded by the same sharp bounds and therefore should be in close agreement (as confirmed by accurate numerical solutions). It is another indication that the aggregated source model developed herein successfully replicates the essential features of corresponding distributed source model. The simpler System A has the additional advantage that the sharper upper bound λ_c is known explicitly.

8. Conclusion

A system of partial differential equations and auxiliary conditions is formulated as System F in [1] for modeling the extracellular Dpp activities in *Drosophila* wing imaginal discs. It is analogous to System R of [2] but now allows for distributed morphogen production in a finite region between the (anterior and posterior) chambers of wing discs. In contrast to System R (and other ad hoc

point source models formulated and analyzed previously [2] and [8]), this new and more realistic model of the wing disc exhibits one new biologically significant feature: there is no restriction on the ligand synthesis rate for the existence of steady-state behavior. As concentrated end source model is more attractive for theoretical analysis and numerical simulations, we derived in this paper an appropriate end source model consistent with System F by aggregating the morphogen activities in the region where morphogens are synthesized. With some well-defined and biologically reasonable approximations, we deduced from System F an *aggregated source model* (designated as *System A*) that is seen to capture the principal features of System F and at the same time reduce to the corresponding ad hoc point source model System R under suitable circumstances.

The new System A has been shown to replicate the following essential features of System F when $X_{\min} \ll X_{\max}$ (which is the case for Dpp in a Drosophia wing disc):

1. It poses no limitation on the morphogen synthesis rate for the existence of a unique set of monotone decreasing and asymptotically stable steady-state free and bound ligand concentration gradients in the (posterior) chamber region of the wing disc.
2. It has the same analytical expression for the asymptotic steady-state free and bound ligand concentration gradients in the wing disc chamber for both low ($\zeta\beta \ll 1$) and high ($\zeta\beta \gg \sigma_0$) morphogen synthesis rates.
3. Numerical solutions for ligand synthesis rate are not covered by asymptotic solutions above are in good agreement with the corresponding results for System F.
4. It has the same leading-term perturbation solution for the decay rate of transient behavior for low and high ligand synthesis rates.
5. It shares the same relevant sharp upper and lower bounds on the decay rate for all synthesis rates.

As such, we may use a System A type model for investigating morphogen concentration gradients particularly when the morphogen synthesis region is narrow compared to the span of the region without morphogen production. As long as $X_{\min}/X_{\max} \ll 1$, numerical results of Subsection 5.4 suggest that we may further simplify the solution process by taking $X_{\max} = \infty$.

Having the aggregate source model, we can now see the factors responsible for the important qualitative difference between System F and the corresponding ad hoc point source model System R regarding the restriction on the morphogen synthesis rate for the existence of steady-state behavior. One essential difference between System A and System R is the presence of a flux term in the end condition at $X = 0$ (see (39) for the steady-state BVP and (41) for the IBVP) with a coefficient determined in terms of the parameters that appear in the model. Knowing this coefficient allows us to assess the significance of the

contribution from this previously omitted flux term. From (50) and (51), we can see that in the case $\zeta\beta = \zeta\bar{\nu}_L/g_0 = \bar{\nu}_L/g_r \ll 1$, we need

$$\frac{\sigma_0}{\mu} = \frac{X_{\max}}{X_{\min}}\sqrt{\frac{g_0 + f_0}{h_0 g_0}} = \sqrt{\frac{D/X_{\min}^2}{k_{\text{on}}R_0}}\sqrt{\frac{k_{\text{deg}} + k_{\text{off}}}{k_{\text{deg}}}} \ll 1$$

for the contribution of the flux term to the amplitude of the various morphogen gradients to be negligible. Thus System R may be used (instead of System A) if the binding rate constant $k_{\text{on}}R_0$ is sufficiently large compared to the diffusion rate $D/X_{\min}^2$, at least for $\zeta\beta = \bar{\nu}_L/g_r \ll 1$. However, no matter how small the ratio σ_0/μ may be, its presence appears to have been responsible for the removal of the restriction on the morphogen production rate υ_L.

To the extent that ad hoc point source models without an end flux term are more tractable analytically and computationally, System A type models may be (and has been) used instead of System F type distributed source models when $\sigma_0 \ll \mu$. Results of this paper show that it is generally prudent to include the flux term in the boundary condition (39) and (40) at the source end to allow for a broad range of ligand synthesis rate and effective binding rate of ligand with receptor. An aggregated source type model has been used in [3] to investigate morphogen gradient formation allowing for binding with nondiffusive nonreceptor sites such as HSPC proteoglygans. The results obtained there provide an explanation for the apparent inconsistency between the experimental results of [11] and [12].

Acknowledgment

The research is supported in part by NIH Grants R01GM67247 and P20GM066051 and NSF SCREMS Grant DMS0112416.

References

1. A. D. Lander, Q. Nie, and F. Y. M. Wan, Spatially distributed morphogen synthesis and morphogen gradient formation, *Math. Biosci. Eng*. (MBE) (submitted).
2. Y. Lou, Q. Nie, and F. Y. M. Wan, Nonlinear eigenvalue problems in the stability analysis of morphogen gradients, *Stud. Appl. Math.* 113:183–215 (2004).
3. A. D. Lander, Q. Nie, and F. Y. M. Wan, Membrane associated non-receptors and morphogen gradient formation (submitted).
4. E. V. Entchev, A. Schwabedissen, and M. Gonzalez-Gaitan, Gradient formation of the TGSF-beta homolog Dpp, *Cell* 103:981–991 (2000).
5. J. B. Gurdon and P. Y. Bourillot, Morphogen gradient interpretation, *Nature* 413:797–803 (2001).
6. M. Kerszberg and L. Wolpert, Mechanisms for positional signalling by morphogen transport: A theoretical study, *J. Theor. Biol.* 191:103–114 (1998).

7. A. A. Teleman and S. M. Cohen, Dpp gradient formation in the Drosophila wing imaginal disc, *Cell* 103:971–980 (2000).
8. A. D. Lander, Q. Nie, and F. Y. M. Wan, Do morphogen gradients arise by diffusion? *Dev. Cell* 2:785–796 (2002).
9. A. D. Lander, Q. Nie, F. Y. M. Wan, and J. Xu, Diffusion and Morphogen Gradient Formation—Part I: Extracellular Formulation (submitted).
10. A. Eldar, D. Rosin, B.-Z. Shilo, and N. Barlao, Self-enhanced ligand degradation underlies robustness of morphogen gradients, *Dev. Cell* 5:635–646 (2003).
11. K. M. Cadigan, M. P. Fish, E. J. Rulifson, and R. Nusse, Wingless repression of Drosophila frizzled 2 expression shapes the Wingless morphogen gradient in the wing, *Cell* 93:767–777 (1998).
12. T. Lecuit and S. M. Cohen, Dpp receptor levels contribute to shaping the Dpp morphogen gradient in the *Drosophila* wing imaginal disc, *Development* 125:4901–4907 (1998).
13. A. D. Lander, Q. Nie, and F. Y. M. Wan, Internalization and end flux in morphogen gradient formation, *Anal. Appl.* (submitted).
14. H. Amann, On the existence of positive solutions of nonlinear boundary value problems, *Indiana Univ. Math. J.* 21:125–146 (1971).
15. D. H. Sattinger, Monotone methods in nonlinear elliptic and parabolic boundary value problems, *Indiana Univ. Math. J.* 21:981–1000 (1972).
16. J. Smoller, *Shock Waves and Reaction-Diffusion Equations*, Springer Verlag, New York, 1982.

University of California, Irvine

(Received October 21, 2004)