Box S1. Equations for morphogen patterning by reaction and transport

While many models are based on one dimension, patterning often occurs in two or three dimensions. Therefore, we present the equations in a general form starting from the basic physical processes of molecular flux by diffusion and advection, a process whereby molecules are swept along by the cytoplasm of a cell or extracellular flow as occurs to some degree in the syncytial blastoderm embryo for Drosophila melanogaster. For morphogen patterning mediated by reaction and transport, the continuity equation, combined with a constitutive equation for molecular flux take following form in Cartesian coordinates:

(S1.1) $$\frac{\partial c_i}{\partial t} + \nabla \bullet \mathbf{j}_i = R_i(\mathbf{x}, \mathbf{c}, \mathbf{p})$$ Continuity equation

(S1.2) $$\mathbf{j}_i = -D_i(\mathbf{x}, \mathbf{c}, \mathbf{p})\nabla c_i + \mathbf{v}c_i + B.C.s$$ Flux equation (Constitutive equation w/ convection)

(S1.3) $$\nabla = \hat{\mathbf{e}}_x \frac{\partial}{\partial x} + \hat{\mathbf{e}}_y \frac{\partial}{\partial y} + \hat{\mathbf{e}}_z \frac{\partial}{\partial z}$$ Definition of gradient

Here c_i is the concentration of species i, $\mathbf{j}$ is the molecular flux defined by a constitutive equation for diffusion based on Fick's law and advection, R_i is the reaction rate for species i, $\mathbf{x}$ is the vector for the spatial coordinate, $\mathbf{v}$ is the velocity of cytoplasm or growing tissue that contributes to advection, $\mathbf{c}$ is the vector of concentrations of all molecular species $i=1\ldots n$ that interact in the network (receptors, inhibitors, co-factors, etc.), and $\mathbf{p}$ is the vector of parameters and physical rate constants. Bold-face font indicates a vector quantity. B.C.'s denotes boundary conditions.

If we limit our analysis to one spatial dimension, assume D does not depend explicitly on position, but does depend on the concentration of molecular species (e.g. modulator molecules), the above equations can be simplified to the general reaction-transport equations for morphogen patterning by component i in 1D:

(S1.4) $$\frac{\partial c_i}{\partial t} + v_x \frac{\partial c_i}{\partial x} = \frac{\partial}{\partial x}\left(D_i(\mathbf{c}(x), \mathbf{p}) \frac{\partial c_i}{\partial x} \right) + R_i(\mathbf{x}, \mathbf{c}, \mathbf{p})$$

(S1.5) $$\frac{\partial c_i}{\partial t} + v_x \frac{\partial c_i}{\partial x} = D_i(\mathbf{c}(x), \mathbf{p}) \frac{\partial^2 c_i}{\partial x^2} + \left[\sum_j \frac{\partial D_i}{\partial c_j} \frac{\partial c_j}{\partial x} \right] \frac{\partial c_i}{\partial x} + R_i(\mathbf{x}, \mathbf{c}, \mathbf{p})$$

If molecular diffusion is independent of modulation and there is no advection, then the equation can be simplified further into the most common form of reaction-transport equation analyzed in morphogen patterning:

(S1.6) $$\frac{\partial c_i}{\partial t} = D_i \frac{\partial^2 c_i}{\partial x^2} + R_i(\mathbf{x}, \mathbf{c}, \mathbf{p})$$

In Box S3 example solutions to the steady-state form of this equation with different boundary conditions are shown and in Box S4 we consider the case when diffusion depends on modulators that may vary in space.

Box S2. Scale-invariance of reaction-diffusion equations for morphogen patterning

The scaling properties of a system of partial differential equations can be easily investigated by dimensional analysis. Consider equations S2.1-S2.3 for a secreted morphogen with boundary conditions, and assume an initial spatial distribution of zero morphogen, which gives:

(S2.1) $$\frac{\partial m}{\partial t} = \frac{\partial}{\partial x}\left(D_m \frac{\partial m(x,t)}{\partial x}\right) + k_m R(m)$$

(S2.2) $$-D_m \frac{\partial m(x,t)}{\partial x} = q, \qquad x = 0$$

(S2.3) $$\frac{\partial m(x,t)}{\partial x} = 0, \qquad x = L$$

To understand scaling of the solution, we choose a time scale T, we define the dimensionless time variable τ and a dimensionless space variable ξ as $\tau = t/T$ and $\xi = x/L$ and we rewrite the equations in terms of these variables. The resulting equations are:

(S2.4) $$\left(\frac{1}{k_m T}\right)\frac{\partial m}{\partial \tau} = \left(\frac{D_m}{k_m L^2}\right)\frac{\partial^2 m}{\partial \xi^2} + R(m).$$

(S2.5) $$-\frac{\partial m(\xi,\tau)}{\partial \xi} = \frac{qL}{D_m}, \qquad \xi = 0$$

(S2.6) $$\frac{\partial m(\xi,\tau)}{\partial \xi} = 0, \qquad \xi = 1$$

These equations contain dimensionless groups that affect the solution, and the length of the system appears in some of these groups. The solution $m(\xi, \tau)$ of the rescaled equations will be scale-invariant only if there is no explicit dependence on L in these equations. There are two cases that arise, depending on whether or not the input flux j vanishes.

1. If $q=0$, then there are two dimensionless groups: $k_m T$ and $D_m/k_m L^2$. The first is a dimensionless reaction time scale, and the second a dimensionless diffusion coefficient. Both the transient evolution and the steady-state morphogen pattern will be independent of the system size if these groups are independent of L, which can be achieved as follows:
   - Fix k_m, select $T=k_m^{-1}$ and modulate $D_m \propto L^2$
   - Choose $T=k_m^{-1}$, modulate $k_m \propto L^{-2}$ and fix D_m
   - Any combination of modulating D_m and k_m to make the dimensionless groups independent of L.

2. If $q \neq 0$ then there are three dimensionless groups: $k_m T$, $D_m/k_m L^2$, and $Q = qL/D_m$ and each of these must be modulated so that they are L-independent. This can be achieved by:
   - Fix k_m, select $T=k_m^{-1}$ and modulate $D_m \propto L^2$, $q \propto L$
   - Fix D_m, choose $T=k_m^{-1}$, modulate $k_m \propto L^{-2}$ and input flux $q \propto L^{-1}$
   - More generally, any combination of modulation that makes the dimensionless groups independent of L will lead to scale-invariance. A balanced modulation of transport and reaction, in which $D_m \propto L$ and $k_m \propto L^{-1}$ may be optimal, in that no scaling of the input flux is required.

Box S3. Mechanisms of scale-invariance for morphogen-mediated patterning

Starting with the 1D equivalent of continuity equation S1.1 and constitutive equation in S1.2 with simple linear decay and constant diffusion, the requirements for scale-invariance of morphogen patterning are readily apparent.

(S3.1) $$\frac{\partial m}{\partial t}+v_x\frac{\partial m}{\partial x}=D_m\frac{\partial^2 m}{\partial x^2}-k_m m \quad where \quad m=m\left(x,t\right)$$

(S3.2) $$q_{in}=-D_m\frac{\partial m}{\partial x}\left(0,t\right)+v_x m\left(0,t\right) \qquad or \qquad m\left(0,t\right)=m_0$$

(S3.3) $$q_{out}=-D_m\frac{\partial m}{\partial x}\left(L,t\right)+v_x m\left(L,t\right) \qquad or \qquad m\left(L,t\right)=m_1$$

(S3.4) $$m\left(x,0\right)=f\left(x\right)$$

Here q_{in} is the input molecular flux, v_x is the velocity in the x direction, and k_m is the decay rate of the morphogen. If we restrict ourselves to identifying conditions at steady-state or quasi-steady state, (S3.1) through (S3.3) provide easily identifiable conditions for scale invariance that vary depending on the contributions from boundary conditions and the type of molecular transport. First, (S3.1)-(S3.3) are scaled by the length. Defining $\xi = x/L$ gives the following:

(S3.5) $$0=\frac{D_m}{L^2}\frac{d^2m}{d\xi^2}-\frac{v_x}{L}\frac{dm}{d\xi}-k_m m$$

(S3.6) $$q_{in}=-\frac{D_m}{L}\frac{dm}{d\xi}\left(0\right)+v_x m\left(0\right) \qquad or \qquad m\left(0\right)=m_0$$

(S3.7) $$q_{out}=-\frac{D_m}{L}\frac{dm}{d\xi}\left(1\right)+v_x m\left(1\right) \qquad or \qquad m\left(1\right)=m_1$$

A Scaling of models with advective transport

Advection-dominated transport leads to the following equation:

$$\frac{dm}{d\xi}=-\frac{k_m L}{v_x}m$$
$$q_{in}=v_x m\left(0\right)$$

Note we drop boundary condition S3.7.
This has the solution:

$$m\left(\xi\right)=\frac{q_{in}}{v_x}\exp\left(-\frac{k_m L}{v_x}\xi\right)$$

which is scale invariant for:

$$k_m \propto L^{-1}$$
$$or \;\; v_x \propto L;\;\; j_{in} \propto L$$

B Boundary condition mediated scaling

In the absence of advection and decay, with fixed concentration endpoints, (S3.5)-(S3.7) simplify to:

$$\frac{d^2m}{d\xi^2}=0$$
$$m\left(0\right)=m_0$$
$$m\left(1\right)=m_1$$

This has the solution:

$$m\left(\xi\right)=\left(m_1-m_0\right)\xi+m_0$$

which is scale invariant automatically. Note that constant concentration endpoints are unlikely and haven't been observed.

C Scaling of diffusion-decay models of patterning

Reaction and diffusion with flux at the source and no flux elsewhere (S3.5)-(S3.7) simplify to:

$$\frac{d^2m}{d\xi^2}=\lambda^2 m;\quad \lambda^2=\frac{k_m L^2}{D_m}$$
$$-\frac{dm}{d\xi}\left(0\right)=Q;\;\; \frac{dm}{d\xi}\left(1\right)=0$$

This has the solution:

$$m\left(\xi\right)=\frac{Q}{\lambda}\left[\frac{e^{\lambda\left(2-\xi\right)}+e^{\lambda\xi}}{e^{2\lambda}-1}\right]$$

Or, for large λ (large k_m, small D_m):

$$m\left(\xi\right)\approx\frac{q}{\sqrt{k_m D_m}}\exp\left(-\sqrt{\frac{k_m L^2}{D_m}}\xi\right)$$

which is scale invariant for:

$$\left(k_m/D_m\right)\propto L^{-2} \;\; and \;\; q\propto\sqrt{k_m D_m}$$

Box S4. Modulation of morphogen scale.

One can envision many mechanisms of modulator activity that lead to morphogen scale invariance. Consider the special case of equation 8-9 in the text in which the modulator activity affects both reaction and diffusion as indicated below:

(S4.1) $$D_{m0}f\left(M\right)=D_m\left(M\right)\equiv D_m^M \quad and \quad \kappa R_m\left(m,M\right)=\kappa_M\left(M\right)r_m\left(m\right)\equiv\kappa_M r_m\left(m\right)$$

where D_{m0} is the intrinsic morphogen diffusion rate. Then equation 8 can be re-written as:

(S4.2) $$\frac{\partial m}{\partial t}=\frac{1}{L^2}\frac{\partial}{\partial\xi}\left(D_m^M\frac{\partial m}{\partial\xi}\right)+\kappa_M r_m\left(m\right)$$

If M is established by a boundary-sink mechanism, then D_m^M is space dependent and the level of M grows in proportion to L^2, ensuring scale-invariance (see Example 2, below). If M is spatially uniform and constant, then (S4.2) can be reduced to:

(S4.3) $$\frac{1}{\kappa_M}\frac{\partial m}{\partial t}=\left(\frac{D_m^M}{\kappa_M L^2}\right)\frac{\partial^2 m}{\partial\xi^2}+r_m\left(m\right)$$

The system will be scale-invariant if $D_m^M\kappa_M^{-1}$ is proportional to L^2. There are many mechanisms that ensure $D_m^M\kappa_M^{-1}\propto L^2$ and the specific molecular actions differ greatly between biological contexts. The following examples illustrate how M must vary for proper D_m^M and κ_M scaling.

Example 1: Enhancer/Immobilizer

If the modulator's molecular function is to hinder diffusion and/or enhance reaction rates, then scaling can be ensured if M decreases in proportion to the tissue size by an appropriate amount. Suppose that the effect of M on the rates are as shown:

(S4.4) $$\frac{\partial m}{\partial t}=\frac{1}{L^2}\frac{\partial}{\partial\xi}\left(\underbrace{\frac{D_{m0}}{1+\alpha_1 M}}_{D_m^M}\frac{\partial m}{\partial\xi}\right)-\underbrace{\kappa\cdot\left(1+\alpha_2 M\right)}_{\kappa_M}\cdot r_m\left(m\right)$$

where the modulator can slow diffusion, enhance reactions, or a combination of both. General requirements to ensure scaling by modulation of D_m^M and κ_M, and parameters that provide the requirement in (S4.4) are below:

Table S4.1	*Example*		*General*		
Description	α_1	α_2	D_m^M	κ_M	M
RXN Enhancer	0	>0	const	$\propto M$	$\propto L^{-2}$
Immobilizer	>0	0	$\propto M^{-1}$	const	$\propto L^{-2}$
Combination	>0	>0	$\propto M^{-1}$	$\propto M$	$\propto L^{-1}$

Suppose a population of cells secretes a modulator M from a source $q(\mathbf{x})$, the modulator diffuses, decays linearly and no flux occurs across the boundaries. This gives:

(S4.5) $$\frac{\partial M}{\partial t}=D_M\nabla^2 M-k\cdot M+j\left(\mathbf{x}\right)$$

Then the average concentration of modulator at steady-state is given by:

(S4.6) $$M_{avg}=\frac{1}{k\theta}\int_\Omega j\left(\mathbf{x}\right)d\Omega \quad where\ \theta=\begin{cases}\propto L & 1D\\ \propto L^2 & 2D\\ \propto L^3 & 3D\end{cases}$$

Thus a line source in 2D yields $M_{avg}\propto L^{-1}$, whereas a finite number of secreting cells in 2D yields $M_{avg}\propto L^{-2}$. If diffusion is very rapid, then $M(x)\approx M_{avg}$ and scaling can be ensured by the "Combination" mechanism (see Table S4.1) for a line source of M, or by the RXN Enhancer or Immobilizer mechanism for a finite size source of M and isometric tissue expansion.

Example 2: Inhibitor/Mobilizer

If the modulator's molecular function is to enhance diffusion and/or hinder reaction rates, then scaling can be ensured if M increases in proportion to the tissue size by an appropriate amount. Consider the action of M on the morphogen by the following equation:

(S4.7) $$\frac{\partial m}{\partial t}=\frac{1}{L^2}\frac{\partial}{\partial\xi}\left(\underbrace{D_{m0}\cdot\left(1+\alpha_1 M\right)}_{D_m^M}\frac{\partial m}{\partial\xi}\right)-\underbrace{\frac{\kappa}{1+\alpha_2 M}}_{\kappa_M}r_m\left(m\right)$$

where the modulator can speed up diffusion, hinder reactions, or a combination of both. General requirements to ensure scaling by modulation of D_m^M and κ_M, and parameters that provide the requirement in (S4.7) are below:

Table S4.2	*Example*		*General*		
Description	α_1	α_2	D_m^M	κ_M	M
RXN Inhibitor	0	>0	const	$\propto M^{-1}$	$\propto L^2$
Mobilizer	>0	0	$\propto M$	const	$\propto L^2$
Combination	>0	>0	$\propto M$	$\propto M^{-1}$	$\propto L^1$

Suppose that all cells in a system are identical and produce M at a constant rate j. If the concentration is zero at the ends due to rapid leaking of M out of the domain or active degradation, then this gives:

(S4.8) $$\frac{\partial M}{\partial t}=\frac{D_M}{L^2}\frac{\partial^2 M}{\partial\xi^2}+j \quad where\begin{cases}M\left(0,t\right)=0\\ M\left(L,t\right)=0\end{cases}$$

which has the steady-state solution:

(S4.9) $$M\left(\xi\right)=\frac{qL^2}{2D_M}\left(\xi-\xi^2\right)$$

Thus $M(\xi)\propto L^2 f(\xi)$, and although the distribution of modulator is non-uniform in space, the local level adjusts in proportion to L^2, which would ensure scaling by either the RXN inhibitor or the Mobilizer mechanisms in Table S4.2 (Pate and Othmer, 1984).

Box S5. How amplitude modulation can lead to adequate scaling

There are numerous examples of tissue patterning between organisms within a species and between species where the patterns provide some degree of scale invariance but key differences arise upon closer inspection. Within populations of Drosophila that were artificially selected into groups based on their egg size, scaling is predominately mediated by the total flux of molecules into the system. While the specific mechanisms for Bcd-mediated transport are still being worked out, the profile has been frequently described by a reaction diffusion model that produces an exponentially decaying spatial distribution at "quasi" steady-state. This takes the form of equation (S5.1):

$$m_i(\xi) \approx \frac{q}{\sqrt{k_m D_m}} \exp\left(-\sqrt{\frac{k_m}{D_m}} L\xi\right) \quad \text{(S5.1)}$$

Intriguingly, the measured decay constant is roughly constant between the population of large embryos (~645 microns long) and small embryos (~518 microns long). Instead the principle difference between profiles is the amount of measured Bcd protein intensity throughout the Anterior of the embryo. Thus, even though the extent or range of the profiles are very different, the Bcd distribution is scaled enough by an increase in the total amount of Bcd in the system. This represents "flux" or "concentration" optimization. If the flux (concentration) is regulated to reduce error in positional information, approximate scaling can be achieved for a number of systems. If the interpretation of the pattern takes place at only one spatial position by a threshold, then (S5.1) can be regulated for exact scaling at that lone position. If multiple thresholds are interpreted, then this mechanism would lead to error in the placement of those boundaries. Intriguingly, Bcd patterning within Drosophila seems to scale by flux optimization. Suppose that there is a critical threshold in the gradient (e.g. the boundary of Hbk gene expression) that occurs at ξ_T. Then the flux increase required for scaling at ξ_T can be calculated by equation (S5.2):

$$q_L(L) = q_0 \exp\left(\sqrt{\frac{k_m}{D_m}}(L - L_0)\xi_T\right) \quad \text{(S5.2)}$$

Taking the data from Cheung et al. with $(D/k)^{1/2}$ = 99 microns, assuming ξ_T =0.4 as the critical threshold in the embryo (near the Hbk boundary), and using L=645 for the large embryo and L_0=518 for the small embryo, the calculated optimal flux q_L is 67% greater than the flux in the small embryo q_0. This is remarkably close to the increase in amplitude measured for Bcd scaling of 66.9%. Thus, while the amplitude correlates with embryo volume, it also correlates with a flux or concentration optimization process. The error at other spatial positions away from the critical threshold can be calculated by:

$$\Delta\xi \equiv \xi_L - \xi = \left(1 - \frac{L_0}{L}\right)(\xi_T - \xi) \quad \text{(S5.3)}$$

Depending on the allowable variations in the spatial positions of gene expression $\Delta\xi$, equation S5.3 can be used to estimate the range of lengths where flux optimization provides sufficient scaling.