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Mechanisms and Measurements of Scale Invariance of Morphogen Gradients

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Abstract

Morphogen gradients provide positional information to underlying cells that translate the information into differential gene expression and eventually different cell fates. Scale invariance is the property where the gradients of the morphogen adjust proportionately to the size of the domain. Scale invariance of morphogen gradients or patterns of differentiation is a common phenomenon observed between individuals within the same species and between homologous tissues or structures in different species. To determine whether or not a pattern is scale invariant, others and we have developed definitions and measurements of gradient scaling. These include point-wise and global scaling errors as well as global scaling power. Furthermore, there are a number of mathematical conditions for scale invariance of advection-diffusion-reaction models that inform mechanisms of scaling. Herein we provide a deeper perspective on modeling and measurement of scale invariance of morphogen gradients.

Keywords

Scale invariance; Morphogen gradient; Reaction-diffusion; Mathematical model; Morphogenesis

1 Introduction

1.1 Definition of Scale Invariance

A morphogen is a chemical component that forms a nonuniform spatial distribution over a field of cells that drives differential gene expression in relation to its concentration and ultimately patterns the underlying tissue or organ. In nature, many organisms and their tissues and organs vary substantially in size but differ little in morphology, appearing to be scaled versions of a common template or pattern [1]. This preservation of proportion is called scaling, or scale invariance. Pattern scale invariance is achieved by the scaling of morphogen gradients with system sizes.

1.2 Significance of Studying Scaling

It is only a slight overestimate to say that the most important attribute of an animal, both physiologically and ecologically, is its size. Size constrains virtually every

aspect of structure and function and strongly influences the nature of most inter- and intraspecific interactions. Body mass, which in any given taxon is a close correlate of size, is the most widely useful predictor of physiological rates.—G. A. Bartholomew 1981, *Insect thermoregulation*, p. 46.

Scaling achieves precise and robust patterning in proportion to perturbations of system size and is a very important feature for animals to survive [2–4]. Failure of scaling leads to developmental defects or even death. In *Xenopus* dorsal-ventral (DV) patterning, for example, the ventral half fails to scale with size perturbation after bisection at blastula stage and results in a mass of ventral tissues without DV axis structure, whereas the dorsal grows into proportionally patterned DV axis [5]. An intriguing aspect of scaling is that the reaction, diffusion, and advection equations that capture the biophysics of morphogen gradient pattern formation DO NOT automatically provide scaling and in fact the equations do not naturally have any information about the size of the system that is being patterned. Each process or balance of processes—diffusion, advection, and morphogen capture/decay—contains a biophysical scale that depends on the parameters of the process but not on the size of the system. Processes have evolved to tune the biophysical parameters to adjust them in some relation to the system's size. Studies of scale invariance often focus on how biology solved the problem by feedback that modifies the rates of diffusion or chemical reaction to adjust to the size of the system. Knowledge of scaling mechanisms provides information on how cells communicate over distances, sense their positions, and how morphogen gradients work. This has impact in our understanding of the regeneration of tissues and organs, and patterning at multiple levels of organization from the cellular to the organism to the ecosystem scale [6].

1.3 Types and Examples of Scaling

Pattern scale invariance among individuals of different species is called *interspecies scaling*. For example, as shown in Fig. 1a, the interspecies scaling of patterns of gap and pair-rule gene expression can be traced back to the interspecies scaling of Bcd gradients with blastoderm embryo size of *Lucilia sericata*, *Drosophila melanogaster*, and *Drosophila busckii*, reproduced from Gregor et al. with permission from PNAS [7]. The scaling of Bcd gradients most likely depends on the species-specific effective lifetime of Bcd. Pattern scale invariance among different individuals within a species is called *intraspecies scaling*. For example, intraspecies scaling of A-P axis development in *Drosophila melanogaster* is suggested as being achieved by scaling of anterior Bcd production rate with embryo volume [8].

As a tissue or organ develops over time, the morphogen concentration changes until it reaches quasi-steady state. There are two types of scale invariance depending on the dynamics of this process: *steady-state scaling* and *dynamic scaling*. Steady-state scaling is the scaling of morphogen gradients of different individuals when morphogen concentration no longer appreciably changes with time. Dynamic scaling is the scaling of a morphogen gradient of the same individual with respect to tissue/organ size as the organ grows. Dynamic scaling also includes the scaling of morphogen gradients of different individuals developing in a population such as sibling embryos in a single clutch at different time points before steady state. One example of dynamic scaling reports that the Decapentaplegic (Dpp) gradient of *Drosophila* during the growth of the wing imaginal disc depends on the

regulation of Dpp capture and decay to ensure dynamic scaling [9]. That is, the gradient of Dpp appears to change in direct proportion to the imaginal disc size.

Numerous examples of interspecies and intraspecies scaling have contributed to our understanding of how scaling is achieved and how to measure systems to test for scaling. The first experimental evidence of scaling in development was observed by Hans Spemann back in 1923. Spemann bisected amphibian embryos into dorsal and ventral halves and found that the dorsal half of the embryo developed normally up through the tadpole stage [10]. This simple experiment demonstrated the intrinsic robustness to size perturbation. Scaling is also observed in *Drosophila* DV patterning, skin pattern formation in fish pigmentation, and under bisection in goldfish embryo development [5,11–14]. Interspecies scaling of neural tube patterning between the zebra finch and the chick has also been reported to be regulated by activating and repressive transcription factors of the morphogen Sonic Hedgehog (SHH) (Fig. 1b), reproduced from Uygur et al. with permission from Developmental Cell [15]. Measuring scale invariance and determining the mechanisms that lead to scaling have provided a number of useful metrics to quantify scaling. In this paper, we take a theoretical view of the advection-diffusion-reaction model for morphogen patterning and conditions that inform mechanisms of scaling. Then we offer perspectives on point-wise and global measurement approaches of morphogen gradient scaling that others and we have developed.

2 Methods

2.1 Mechanisms of Scale Invariance for Morphogen-Mediated Patterning of Advection–Diffusion–Reaction Model

Theoretical studies on morphogen-mediated patterning provide important inspiration on possible mechanisms of scale invariance and measures of scaling. In order to identify mechanisms of scaling, mathematical equations are formulated for how morphogen concentration changes throughout space as a function of time. In so doing, we can understand how different processes interact, how system size is involved in these processes and find mathematical conditions on how scaling is achieved. A brief introduction for advection, diffusion, reaction equations is provided below.

The dispersal of a morphogen involves reaction and physical transport by diffusion and advection. Suppose the molecular diffusion rate is independent of space; then in a one-dimensional system of length L the reaction transport equation for a morphogen system in Cartesian coordinates is as follows [1]:

$$\frac{\partial m}{\partial t} = D_m \frac{\partial^2 m}{\partial x^2} - v_x \frac{\partial m}{\partial x} - k_m R(m) \text{ where } m = m(x, t) \quad (1)$$

$$q_{\text{in}} = -D_m \frac{\partial m(0, t)}{\partial x} + v_x m(0, t) \text{ or } m(0, t) = m_0 \quad (2)$$

$$q_{\text{out}} = -D_m \frac{\partial m(L, t)}{\partial x} + v_x m(L, t) \text{ or } m(L, t) = m_1 \quad (3)$$

$$m(x, 0) = f(x) \quad (4)$$

Here m is the concentration of the morphogen, t is time, x is the vector of the Cartesian coordinate, D_m is the diffusion coefficient of the morphogen, v_x is the velocity of cytoplasm or growing tissue that contributes to advection in the x direction, k_m is the first-order decay rate of the morphogen, $R(m)$ contains all the reaction steps that affect m , q_{in} is the input molecular flux of the morphogen, and q_{out} is the output molecular flux of the morphogen.

2.1.1 Diffusion-Dominated Transport—In many cases, the velocity of cytoplasm and the growing tissue is negligible, and the transport of morphogen is diffusion-dominated. In these situations, the advection term can be neglected and Eq. 1 can be simplified to:

$$\frac{\partial m}{\partial t} = \left(D_m \frac{\partial^2 m}{\partial x^2} \right) - k_m R(m) \quad (5)$$

Setting $x = 0$ as the source and $x = L$ as a no flux boundary leads to Eqs. 6 and 7

$$-D_m \frac{\partial m(x, t)}{\partial x} = q, \quad x = 0 \quad (6)$$

$$\frac{\partial m(x, t)}{\partial x} = 0, \quad x = L \quad (7)$$

Assume an initial spatial distribution of zero morphogen, Eq. 4 is simplified to:

$$m(x, 0) = 0 \quad (8)$$

In order to identify general mechanisms that lead to scale invariance, we introduce system size into the equations by defining a dimensionless space variable $\xi = \frac{x}{L}$. A dimensionless time variable $\tau = \frac{t}{T}$ is also defined for time scale. Eqs. 5–7 are then rewritten in terms of the two dimensionless variables:

$$\left(\frac{1}{k_m T}\right) \frac{\partial m}{\partial \tau} = \left(\frac{D_m}{k_m L^2}\right) \frac{\partial^2 m}{\partial \xi^2} - R(m) \quad (9)$$

$$-\frac{\partial m(\xi, \tau)}{\partial \xi} = \frac{q_{\text{in}} L}{D_m}, \quad \xi = 0 \quad (10)$$

$$\frac{\partial m(\xi, \tau)}{\partial \xi} = 0, \quad \xi = 1 \quad (11)$$

Scaling will be achieved if and only if there is no explicit dependence on L in Eqs. 9–11, i.e., the system length terms in the diffusion, morphogen reaction as well as morphogen flux processes can be canceled out. Here, scale invariance is achieved if these three terms

$k_m T$, $\frac{D_m}{k_m L^2}$ and $\frac{q_{\text{in}} L}{D_m}$ are independent of L .

In the scenario of steady-state or quasi-steady state ($\frac{\partial m}{\partial \tau} = 0$), assume linear decay, Eqs. 9–11 can be written as:

$$0 = \left(\frac{D_m}{L^2}\right) \frac{d^2 m}{d\xi^2} - k_m m \quad (12)$$

$$-\frac{dm(\xi)}{d\xi} = \frac{q_{\text{in}} L}{D_m}, \quad \xi = 0 \quad (13)$$

$$\frac{dm(\xi)}{d\xi} = 0, \quad \xi = 1 \quad (14)$$

For large k_m , small D_m , the solution is approximately:

$$m(\xi) \approx \frac{q_{\text{in}}}{\sqrt{k_m D_m}} e^{\left(-\sqrt{\frac{k_m L^2}{D_m}} \xi\right)} \quad (15)$$

The system scales if $\frac{k_m}{D_m} \propto L^{-2}$, and $q_{\text{in}} \propto \sqrt{k_m D_m}$. Thus scaling is achieved by changes in the ratio of the effective morphogen removal rate k_m or the effective morphogen diffusion rate D_m .

2.1.2 Advection-Dominated Transport—Advection is dominated when the morphogen is not diffusive and mainly transported by the growing tissue. This is the case if the morphogen or morphogen-ligand complex is bound to the surface on cells or resides inside cells [16–18]. For example, advection in a cell-bound ligand has been reported to contribute to ensure dynamic scaling of the Dpp gradient in the *Drosophila* wing imaginal disc [19], and if the dispersal of morphogen is dominated by advection, and the decay is linear to morphogen concentration, Eqs. 1–4 can be rewritten as follows, introducing variable $\xi = \frac{x}{L}$ and variable $\tau = \frac{t}{T}$ yields:

$$\frac{1}{T} \frac{\partial m}{\partial \tau} = - \frac{v_x}{L} \frac{\partial m}{\partial \xi} - k_m m \quad (16)$$

$$q_{\text{in}} = v_x m(0, \tau) \quad (17)$$

$$q_{\text{out}} \approx 0 \quad (18)$$

Consider steady state ($\frac{\partial m}{\partial \tau} = 0$):

$$\frac{dm}{d\xi} = - \frac{k_m L}{v_x} m \quad (19)$$

$$q_{\text{in}} = v_x m(0) \quad (20)$$

$$q_{\text{out}} \approx 0 \quad (21)$$

This has the solution:

$$m(\xi) = \frac{q_{\text{in}}}{\nu_x} e^{\left(-\frac{k_m L}{\nu_x} \xi\right)} \quad (22)$$

The system scales if $k_m \propto L^{-1}$ or $(\nu_x \propto L) \wedge (q_{\text{in}} \propto L)$. This means that for scaling to occur, biology must have targeted k_m to decrease as the domain gets larger. In both of these derivations the requirements for scaling are captured by parameter groupings that dictate the amplitude and the shape of the gradient and this is useful in global measures of scale invariance discussed later herein.

2.2 Measures of Scaling

The most intuitive way to quickly estimate scaling is by plotting the morphogen gradients on absolute x and normalized x/L graphs and test whether or not the normalization of position of x to relative position on x/L plot improves the overlay of morphogen gradients around a mean morphogen profile [8, 20, 21]. Figure 2 shows an example of morphogen scaling in test and control systems. The French flag is often used as an illustration of pattern scale invariance in accordance with the scaled morphogen gradients between a test and control. The smaller French Flag is half the size of the larger French Flags in Fig. 2c, in correspondence to the relative length of test and control systems in Fig. 2a. The lengths of the control and the test after normalizing the position scale to an arbitrary unit of 1 are the same (see Note 1), as shown by the magenta and black lines in Fig. 2b. Compared to significant difference between morphogen gradients when plotted against absolute position x in Fig. 2a, the x/L graph in Fig. 2b shows that the two morphogen gradients converge. Consistent in Fig. 2d, the patterns of the two flags overlay one another after normalizing x to relative positions. Thus the two systems appear to scale.

2.2.1 Point-Wise Scaling Error—A few metrics have been developed to measure the scaling error in order to quantify scaling and compare the degree of scaling among different systems. One of the methods, point-wise scaling error, measures position shifts of the morphogen gradients of two systems at certain points in x . Suppose there are two one-dimensional systems, one is the wild-type system S of size L , and the other is the test system S' of size L' ($L' \leq L$). Figure 3a, b display two examples. In Fig. 3a, b, the figure in the center shows the morphogen gradients of S (in dashed line in blue) and S' (in dashed line in magenta) on x/L plot, whereas the figure at the upper right corner provides the morphogen gradients on unnormalized graph. Point-wise scaling error is measured by the position shift of the same morphogen level between S and S' at selected relative positions in the x/L plot. Here, we present the measurement of point-wise scaling error at the relative positions $x_1 = 0.25$, $x_2 = 0.5$, and $x_3 = 0.75$:

1. Use the x/L plot of morphogen gradient of S to measure the morphogen concentrations at three relative positions, x_1 , x_2 and x_3 and denote them as T_1 , T_2 , and T_3 accordingly. T_1 , T_2 , and T_3 are labeled on the left of Fig. 3a, b.

2. In the x/L plot of morphogen gradient of S' , find the relative positions corresponding to morphogen levels T_1 , T_2 , and T_3 , denoted as x'_1, x'_2 and x'_3 . The distance between the relative positions of the two systems is called the point-wise scaling error, denoted as $\delta_i = x_i - x'_i, i \in \{1, 2, 3\}$. δ_1, δ_2 and δ_3 are highlighted with double arrowheaded lines in black in both Fig. 3a and b. The point-wise scaling errors of the example in Fig. 3b are overall smaller than those of the example in Fig. 3a, and thus S and S' of Fig. 3b scale better than S and S' of Fig. 3a.
3. The average scaling error is measured by $\delta = \frac{1}{3} \sum_{i=1}^3 |\delta_i|$. In the case of perfect scaling, point-wise scaling errors and the average scaling error are all zero [22]. The average scaling error of Fig. 3b is smaller than that of Fig. 3a.

The advantage of point-wise scaling error is that it is easy to understand and to calculate. It is very useful for evaluating scaling for smooth simulation results. However, point-wise scaling error cannot be measured if the morphogen level of S at a selected position does not exist on the morphogen gradient of S' . In addition, one condition required to make sure the point-wise scaling error method is accurate is that the points chosen to calculate scaling error can represent the general shape of the morphogen gradients. The value of scaling error highly depends on the relative positions chosen. The scaling error can appear to be small if the local positions are selected in such a way that the morphogen gradients are close to each other, i.e., δ is small.

2.2.2. Sensitivity Factor—Instead of focusing on several local positions like point-wise scaling error, global methods utilize the overall shape of the morphogen gradient. One of the global measurement methods calculates a parametric sensitivity with respect to the length L and is called the sensitivity factor. The sensitivity factor measures the position shifts continuously using the global function of morphogen gradients. The sensitivity error is a measure of the local sensitivity of pattern deformation corresponding to the changes of system size on continuous morphogen profiles. Assume that the relative plot of a morphogen profile can be depicted by functions $u(\xi, L_1)$ and $u(\xi, L_2)$. For example, in Fig. 4a, the control (black) and test (magenta) correspond to $u(\xi, L_1)$ and $u(\xi, L_2)$ respectively. Here $\xi = \frac{x}{L}$ is the dimensionless variable, L_1 is the length of system number 1 and L_2 is the length of system number 2. Given any $\xi_1 (0 \leq \xi_1 \leq 1)$, find the ξ_2 where $u(\xi_1, L_1) = u(\xi_2, L_2) = T$. As shown in Fig. 4a, find T using the red dashed line according to position ξ_1 and then find ξ_2 using the red perpendicular line starting from the intersection of the red dashed line and the magenta line. The relative position of the same morphogen level for perfect scaling is the same, i.e., $\xi_1 = \xi_2$. We measure the sensitivity of position shift in response to the changes of system size $\frac{\xi_2 - \xi_1}{L_2 - L_1}$ as the “sensitivity error,” SE. If we assume a small variation in L and in ξ , then we can apply a linear approximation: $u(\xi_2, L_2) = u(\xi_1, L_1) + u'_\xi(\xi_2 - \xi_1) + u'_L(L_2 - L_1)$. Thus, at positions where $u_0(\xi_1, L_1) = u_0(\xi_2, L_2)$, $SE = \frac{\xi_2 - \xi_1}{L_2 - L_1} = -\frac{u'_L}{u'_\xi}$. $SE \equiv 0$ for perfect scaling [23].

This sensitivity factor can be regarded as a correction of the scaling coefficient, which is given as $S_L = -\left(\frac{\partial u}{\partial L}\right)\left(\frac{\partial u}{\partial x}\right)^{-1}\frac{L}{x}$ [24, 25]. Figure 4b shows the corresponding sensitivity factor and scaling coefficient between the control and test morphogen gradients. As the relative position increases, the difference between the morphogen concentration of the control and the test go up, both the sensitivity factor and the scaling coefficient increase. This method extracts the information of the whole morphogen gradient profiles and is quantitative. However, this method only works when the three following conditions are satisfied:

1. The variations of system lengths are small.
2. The position shifts of the morphogen gradient profiles are small.
3. The morphogen gradient profiles can be modeled as a function $m = u(\xi, L)$.

2.2.3 Scaling Power—Another global method to determine scaling calculates a quantity called the scaling power. According to He et al. [26], when an exponential function is used to fit the Bcd morphogen gradient $m(\xi, L) = Ae^{-\Gamma\xi}$, where m is Bcd morphogen concentration, then scaling can be determined by examination of A (the amplitude parameter) and Γ (the slope parameter). The scaling power is defined for the amplitude parameter as $n_A = \frac{\partial A/A}{\partial L/L}$ and the scaling power of the slope parameter is defined as $n_\Gamma = \frac{\partial \Gamma/\Gamma}{\partial L/L}$. Then the analytical solution of Eq. 23

$$\frac{\partial m(L, \xi, t)}{\partial L} = 0 \quad (23)$$

Two cases are discussed. When scaling powers are 0, all positions satisfy Eq. 23. In this case scale invariance is achieved. When scaling powers are not 0, only one position satisfies Eq. 23 and this position is called the critical position.

In practice, we can derive scale invariance by showing that the scaling power of the amplitude and the slope parameter are zero:

1. Find a function that fits best the morphogen gradients in study.
2. Generate (A, L) and (Γ, L) for each sample, where A is the amplitude parameter and Γ is the slope parameter.
3. Derive the equations for $n_A=0$ by correlation between A and L . If A does not significantly correlates with L , then $n_A=0$.
4. Similarly, derive $n_\Gamma=0$.
5. Explore solutions for Eq. 23 and prove that (Eq. 23) is always satisfied for any position of ξ within $(0, 1)$ when $n_A=0$ and $n_\Gamma=0$. This result means the system is scale invariant.

The scaling power method is useful in evaluating scale invariance because it takes the overall gradient shape into account and is straightforward by mathematical derivation.

However, the method is harder to extend to general gradient shapes that do not achieve the same separation of amplitude and exponent that occurs for the classical exponential gradient shape used to develop the scaling power.

3. Note

1. Normalization of the morphogen gradient impacts the calculation of scaling. Using different normalization methods can lead to different conclusions. One of the normalization methods called the “anchor point normalization” pins the minimum and maximum of individual profiles within a population and this may lead to erroneous attributions of “scaling” as discussed by Brooks et al. [27]. Methods that integrate the area under the concentration curve for a gradient and use that integral value for scaling systematically alter scale invariance conclusions that are a byproduct of data normalization [28]. An alternative method for gradient data normalization, “control-normalization” can be applied to normalize samples stained and imaged on different days with different settings by multiplying each set of samples with a scalar value [28]. This scalar value is calculated using controls. For each set of samples, a group of controls should be imaged in conjunction with corresponding experimental condition. The scalar value for sample sets imaged on different days is determined by minimizing the sum of the error among the controls imaged on corresponding days. After control-normalization, the gradient range of the population average of each control group is rescaled to be within [0,1].

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Fig. 1.

Examples of three types of scale invariance. **(a)** Interspecies scaling of the Bcd protein distribution in blastoderm embryos of *Lucilia sericata*, *Drosophila melanogaster*, and *Drosophila busckii*, reproduced from Gregor et al. [7] with permission from PNAS, Copyright (2005) National Academy of Sciences, USA [7]; **(b)** interspecies scaling of neural tube development patterned by SHH in the zebra finch and the chick, reproduced from Uygur et al. [15] with permission from Developmental Cell [15]

Fig. 2. Scaling representation of the sample morphogen gradient from control (black) and test (magenta). The morphogen gradient profiles as a function of x (a) or X/L (b). m is the morphogen concentration. The system length of the test (magenta) is half of the control (black), shown in (a) and scaled to equal dimensionless length (b). The analogous French flags for scaling in absolute scale (c) and relative scale (d)

Fig. 3.

An example of measuring point-wise scaling error. **(a)** Morphogen profile of the control system S (blue) and a test system S' (magenta) system on x/L plot, with the profile on x plot on the right-top corner. **(b)**. Morphogen profile of the control (blue) and another test (red) system on x/L plot, with the profile on x plot on the right-top corner. The black horizontal arrow lines labeled δ_1 , δ_2 , δ_3 are the scaling error of the test morphogen gradient at the relative position 0.25, 0.5, and 0.75, respectively, in reference of the control. Taking the mean of these three scaling errors gives us the average scaling error. The average scaling error of **b** is smaller than that of **a**

Fig. 4.

An example of the sensitivity factor and scaling coefficient. The morphogen gradient profile on the x/L plot of the control is plotted in black line and that of the test is plotted in magenta. **(a).** Given $\xi_1 (0 \leq \xi_1 \leq 1)$, the morphogen concentration of the control at ξ_1 is T (red dashed line). Find ξ_2 where $u(\xi_1, L_1) = u(\xi_2, L_2) = T$. **(b)** The absolute values of the test morphogen profiles' sensitivity factor $|SE|$ is plotted in blue dashed line and the scaling coefficient $|SL|$ is plotted in red dashed line. The system length of the test is 80% of the control