# A new scaling measure quantifies the conservation of proportions of gene expression profiles in developing organic shapes

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**Abstract.** Most organisms and organs go through a developmental phase before they reach their adult size and proportions. We studied the scaling properties of the fruit fly embryo and its wing. Even in batches of genetically identical embryos their length fluctuates about 10% around its mean value of 0.5mm. Yet, expression domains of certain genes that define the adult body plan of the fly extend over areas proportional to embryo length. This kind of scaling property makes sure that while flies may have different sizes, the proportionality of their respective body parts are extremely well conserved, thus ensuring their functionality. A similar phenomenon is observed for the wing, which evolves from a growing tissue known as the imaginal wing disk. Again expression patterns of many genes responsible for the later cell differentiation scale with the growing total size of the wing disk. Here we focus on how scaling of graded expression profiles can be quantified properly in growing tissues. This extends our previous work for the quantification of scaling of discrete expression domains (Mol. Syst. Biol.: 2010, 6;351).

## 1 Introduction

Developmental biology addresses the fundamental question of how a fertilized egg gives rise to an adult organism. This includes in particular the establishment and transformation of the *shape* of the organism. When the egg is fertilized, the embryo is made up of only one cell, that eventually divides and gives rise to a large number of genetically identical cells. One may ask how cells containing the same DNA can give rise to an adult body containing different organs and limbs, as well as hundreds of different cell types. This is because development relies essentially on the *control of gene expression* [18]. However, since all cells are initially identical, the control of gene regulation needs to be started by the mother, which breaks the initial symmetry very early in development with the use of maternal cues [5, 6]. These cues, also called *morphogens*, allow to spatially *pattern* the embryo into compartments of differentially expressed genes that will eventually define the adult body plan [30, 19]. Similarly, in a developing tissue, patterning preludes *differentiation*, which is the process by which cells

acquire a well-defined fate, contributing to the generation of cellular diversity. Because these differentiated cells are not randomly distributed, they can organize into intricate tissues and organs during a process called *morphogenesis*. Another important aspect of developmental biology is growth and size regulation. Indeed, it is intriguing how cell division can be so tightly regulated, allowing e.g. to have both arms of the same size, meaning that a growing tissue actually "knows" when it has reached its final shape.

## 2 Morphogens and their interpretation

Here we focus on the *formation* and *interpretation* of morphogen gradients acting at different stages of *Drosophila* development. Morphogen gradient formation are shaped by production, degradation and transport. Production of the morphogen is generally assumed to be localized in a small region of the tissue and constant over time, though this domain can extend during growth (e.g. Dpp production region in *Drosophila* wing imaginal disc [29]). Other studies also argue that the morphogen is produced from a dynamic gradient of mRNA (e.g. *bcd* mRNA gradient in *Drosophila* embryo [24], *hoxd13* in the vertebrate developing limb bud [12] or *fgf8* in the vertebrate embryo [7]). Degradation of the morphogen (or its interaction with some other molecules, e.g. receptors) can also help shaping the gradient, in particular if it is controlled by the morphogen signal itself, where it was shown to increase robustness to gene dosage changes[10]. Lastly, morphogen transport (either active or passive) is essential to promote the formation of a long-range gradient from a localized source.

In 1970, Francis Crick proposed that gradients could form over a field of 50 cells within a few hours [2]. Since then, diffusion has often been proposed as a mechanism for morphogen transport, in particular when morphogen movement is reported to be non-directional, which is in agreement with passive diffusion. However, since measured diffusion constants are much smaller than those measured in aqueous media, it is now common to talk about *effective* or *restricted* diffusion, when referring to non-directional passive transport in the crowded extracellular matrix [26, 15, 27].

The morphogen gradient is meant to be interpreted by the cells in the patterning field. To be defined as such, the morphogen should form a gradient capable of activating or repressing target genes in a concentration-dependent manner. This means that the cells in the tissue read out the morphogen concentration and activate some target genes if the levels of morphogen are high enough. Because different genes can have different thresholds of activation, the cells in the tissue start to express different combinations of genes, depending on where they lie along the morphogen gradient (Figure 1). This model is known as the *French flag* [30], where the flag is used to demonstrate the effect of a morphogen on cell fate determination. High concentrations activate some gene $a$, lower concentrations induce another gene $b$, and at even lower concentrations the default gene $c$ is transcribed. As a consequence, three cell fates emerge: "blue" for the cells expressing genes $a$, $b$ and $c$, "white" for those expressing only genes $b$ and $c$,

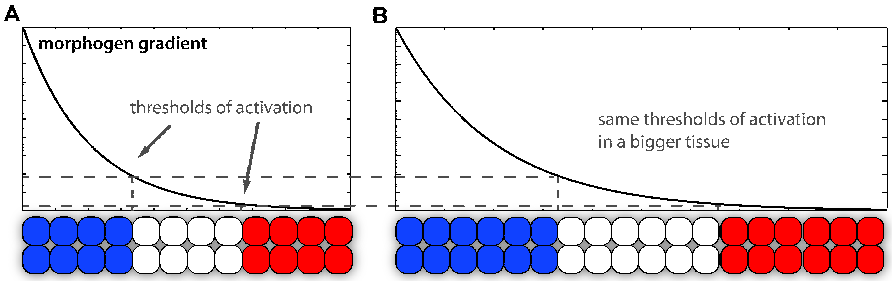


**Fig. 1.** *The* French flag *model for morphogen gradient interpretation and scaling* [30]. **(A***)* A morphogen gradient is established in the target field. The cells read out the morphogen concentrations and activate some target genes depending on the morphogen levels. Here, high concentrations activate a "blue" gene, lower concentrations induce a "white" gene, and at even lower concentrations the default "red" state is established. **(B***)* In a bigger tissue where the morphogen gradient scales, the pattern will keep the same proportions (1/3 of blue, 1/3 of white and 1/3 of red cells, compare with *(*A)) and thus scale perfectly, since the target genes remain activated at the same threshold concentrations.

and "red" for the remaining cells. These gene expression domains get more and more refined during development, eventually defining the segments of the adult body and specifying cell fates upon differentiation.

The cascade of activation or inhibition can be combined with several morphogens, further extended at the level of the target genes which can e.g. mutually repress each other [14], or even be feedback-driven [9], hence extending the static and hierarchical French flag view of patterning [13]. Other studies also suggest that pattern formation is a temporally dynamic process, coupled with oscillators [3] or raise questions about the influence of time on morphogen activity and desensitization to it [16, 4]. Finally, although many studies consider that morphogen gradients start to be decoded once they have reached steady state, increasing evidence suggests that in some cases pre-steady state decoding is important for robustness [1] and differential gene expression [20, 21]. All together, it appears that morphogen gradients are essential to organize and coordinate *temporal* and *spatial* development, assigning to each cell a role depending on its position within the embryo or tissue, in a dynamic and yet to be refined manner.

## 3 Scaling and its quantification

Despite noteworthy variances in the height and weight of various individuals from the same species (for example humans), the proportions between different body parts are much less variable. This scaling relationship between the size of body parts or organs with the overall body size is called allometry, a phenomenon that has long fascinated biologists [25, 23, 11, 28]. Stern and Emlen described three kinds of allometry: ontogenetic, static and evolutionary. The growth trajectory

of an organ relative to body size during the development of a single individual was coined ontogenetic allometry. Evolutionary allometry, by contrast, deals with the size relationship between organs across species. Lastly, static allometry refers to the scaling relationship between one body part and the total body size, when several individuals are compared at a single developmental stage. For example, the relative volumes of endoderm and ectoderm in a sea urchin embryo are constant in the face of an eight-fold size range of the embryos [30], and starved fly larvae and mice embryos form smaller adults with proportionally smaller body parts [8]. With few exceptions [22], the mechanisms underlying these allometries remain unknown.

In the research we conducted over the last years, we have considered gene expression domains as a molecular pre-cursor for tissue patterning and asked whether their boundaries scale with embryo size or wing pouch size during growth. For the wing imaginal disc, we also considered the morphogen activity gradient and tested whether it adapts to organ size. In the case of perfect scaling, the pattern of gene expression domains (e.g. the *French flag* in Figure 1A) should scale with tissue size. Assuming a *French flag* decoding model, this means that the morphogen gradient should spread further in bigger tissues, to ensure that the boundaries of the gene expression domains shift and therefore preserve the correct proportions of the pattern (see Figure 1B).

As we discussed above scaling ensures to keep a proportional body/organ plan under the unavoidable fluctuations in embryo/tissue size. We define scaling as the relative response in gene expression domain boundary $x$ due to variations in tissue (or embryo) size $L$ [17]:

$$S \equiv \frac{dx}{dL} \cdot \frac{L}{x} = \frac{dlog(x)}{dlog(L)} \tag{1}$$

Here, perfect scaling corresponds to $S = 1$. In this case, fluctuations in embryo length, $dL/L$, are exactly compensated by fluctuations in position, $dx/x$, implying perfectly conserved proportions (Figure 2A). We use the terms hypo- and hyper-scaling to refer to $S < 1$ and $S > 1$, respectively. A position that hypo-scales does not compensate enough for a change in tissue size, meaning that in bigger tissues, the absolute domain boundary position is not shifted enough to keep the correct proportions (Figure 2B). In contrast, hyper-scaling is the tendency to overcompensate for changes in tissue size (Figure 2C).

In our analysis, we examined both morphogen gradients as well as several of their target genes, which are expressed in rather sharp domains where we assume that the boundary position matters more than the levels of the protein. In the case of sharp domains, we extracted the protein expression profiles (as deduced from fluorescent intensities) for each gene of interest in tissues/embryos of different sizes and asked whether the position of the boundary scales with tissue/embryo size (Figure 3). The boundary of the domain was defined as the position with the steepest drop in protein concentration or was obtained by fitting a Hill function to the protein profile. We then estimated scaling by weighted linear regression (error bars on the domain boundaries coming from the fitting procedure):

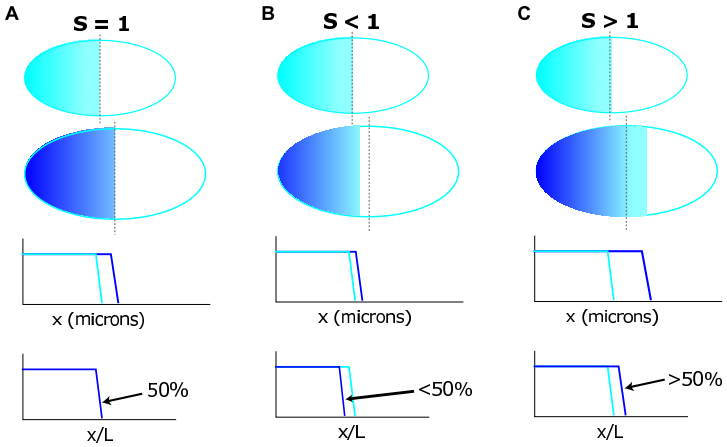


**Fig. 2.** *Meaning of scaling.* **(A)** Perfect scaling, $S = 1$. A domain that spans 50% of a small embryo (top) will also span 50% of a bigger embryo (below, dotted lines at 50% embryo size). The protein profiles collapse on relative units $x/L$, but not in absolute positions, $x$. Proportions are well preserved. **(B)** Hypo-scaling, $S < 1$. A domain that spans 50% of a small embryo does not expand enough in a bigger embryo, spanning less than 50% (dotted lines at 50% embryo size). **(C)** Hyper-scaling, $S > 1$. A domain that spans 50% in a small embryo expands too much in a bigger embryo, spanning more than 50% (dotted lines at 50% embryo size).

$$w \cdot log(\frac{x}{\bar{x}}) = S \cdot log(\frac{L}{\bar{L}}). \tag{2}$$

where $w$ are the normalized weights for each boundary position and $\bar{x}$, $\bar{L}$ are the average domain boundary and average tissue/embryo size, respectively (we divide by the means to center the data around zero in the plots, but this does not affect our definition of scaling since $dlog(x/\bar{x}) = dlog(x)$). The linear regression assumes that $log(x/\bar{x})$ and $log(L/\bar{L})$ are correlated.

Note that for small fluctuations in boundary position and embryo/tissue size (which is valid for fluctuations in embryo size which are rather small), $log(x/\bar{x}) \simeq \frac{\Delta x}{\bar{x}}$ and $log(L/\bar{L}) \simeq \frac{\Delta L}{\bar{L}}$. Thus, we can estimate scaling by performing a linear regression of the domain positions $x$ onto the embryo sizes $L$:

$$S \equiv \hat{\beta} \cdot \frac{\bar{L}}{\bar{x}} = \frac{cov(x, L)}{var(L)} \cdot \frac{\bar{L}}{\bar{x}} \tag{3}$$

where $\hat{\beta}$ is the *estimated* slope from a linear regression $x = x_0 + \beta L$ of the domain positions $x_i$ onto their respective embryo sizes $L_i$.

In the case where we consider scaling across several developmental stages (like for the wing disk that we studied throughout 40h of larval development), scaling might change with time. For example, the pattern could start hypo-scaling and then hyper-scaling, so that one should regress each time period separately to

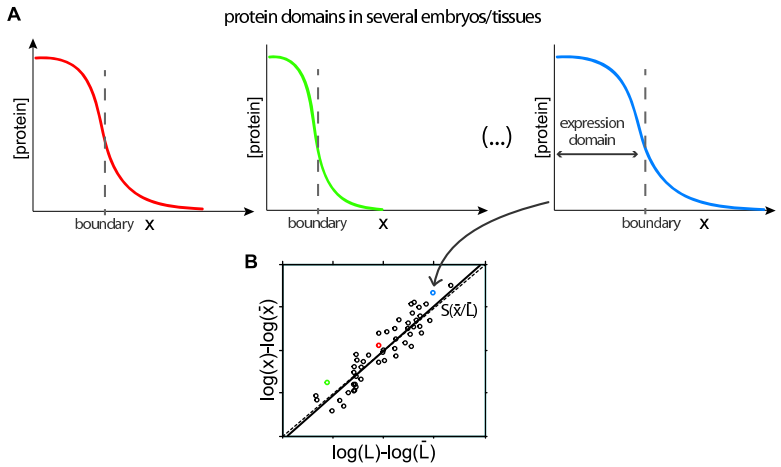


**Fig. 3.** *Scaling of gene expression domains.* **(A)** For a collection of embryos (or tissues), we determine the boundary position $x$ of some protein domain of interest as well as the embryo/tissue size $L$. **(B)** We plot the positions as a function of the sizes and determine scaling by linear regression (solid line; the dashed line is the diagonal which has a slope of one and thus corresponds to $S = 1$). In this example, scaling is close to perfect, with slight hyper-scaling. The colored circles refer to the protein profiles in A. Note that for small fluctuations, $log(x) - log(\bar{x}) \simeq \frac{\Delta x}{\bar{x}}$ and $log(L) - log(\bar{L}) \simeq \frac{\Delta L}{\bar{L}}$.

ensure that the data points are correlated, yielding a different scaling coefficient for each period.

For the morphogen gradients, we need to take into account the protein levels and not restrict ourselves to some artificial domain boundary (e.g. the decay length describing the position at which the concentration levels have decreased by a factor $e \simeq 2.7$ in a exponentially decaying profile), since protein levels are important for mophogen decoding and the gradient may scale differently across positions. As a first indication of scaling, one could look at the profiles in absolute and relative positions, $x$ and $x/L$ respectively. As we show in Figure 5, scaling gradients from embryos/tissues of different sizes should collapse onto one single curve when plotted in relative units. In order to be more quantitative, we consider several thresholds of protein concentration read-out (*French flag* decoding [30]), though we could apply our scaling formalism to other decoding models (Figure 4A). For a given threshold of activation, we plot the corresponding positions against the embryo/tissue sizes for our collection of embryos/tissues (Figure 4B). Performing a linear regression (cf. Equation 2) yields a scaling coefficient for that particular threshold, which we can then associate to the average relative position $\bar{x}/\bar{L}$. By repeating this procedure for several thresholds of activation, we obtain a scaling coefficient for several relative positions in the patterning field (Figure 4C). When modeling morphogen gradients, we can also determine scaling analytically, provided we can solve the equation for the morphogen gra-

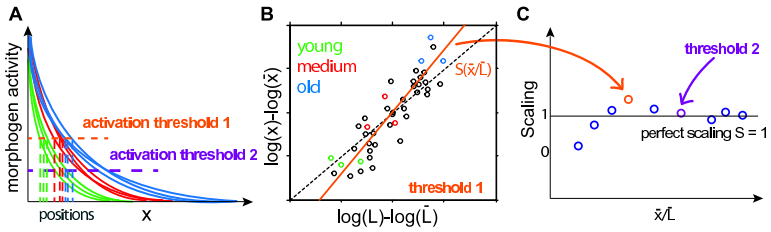


**Fig. 4.** *Scaling of morphogen gradients.* We consider morphogen activity gradient profiles in small (green), medium sized (red) and big (blue) embryos (or tissues). **(A)** For a given morphogen activation threshold (e.g. threshold 1 in orange), we retrieve the corresponding positions (French flag decoding model [30]). **(B)** For that particular threshold, we plot the positions against the embryo (or tissue) sizes. Linear regression yields a scaling coefficient for that particular threshold, which in this example hyper-scales (solid line; the dashed line is the diagonal which has a slope of one and thus corresponds to $S = 1$). We relate this scaling coefficient to the average relative position $\bar{x}/\bar{L}$. Colored circles refer to the profiles in A. **(C)** We repeat this procedure for several activation thresholds and obtain a position-dependent picture of scaling for several relative positions in the patterning field. The black solid line indicates perfect scaling, $S = 1$. The arrows indicate the scaling coefficient obtained from the linear regression in B, where we had hyper-scaling (orange), as well as the scaling coefficient obtained for another concentration threshold (purple).

dient profile $M(x,t)$. Assuming again that the threshold concentration is fixed (implying $dM = (\partial M/\partial x)dx + (\partial M/\partial L)dL = 0$), it follows from Equation 1 that the scaling coefficient is given by

$$S = -\left(\frac{\partial M}{\partial x}\right)^{-1} \cdot \frac{\partial M}{\partial L} \cdot \frac{L}{x} \tag{4}$$

Note that the above definition of scaling is generic and can be computed for any morphogen distribution $M(x,t)$ with explicit dependence on $L$.

## 4 Bicoid and Decapentaplegic

Though morphogens have been known for decades, it is not yet clear how these gradients form and are interpreted in order to yield highly robust patterns of gene expression. Over the last years we investigated the properties of Bicoid (Bcd) and Decapentaplegic (Dpp), two morphogens involved in the patterning of the anterior-posterior axis of *Drosophila* embryo and wing primordium, respectively.

In order to tackle these questions, we analysed fluorescence images showing the pattern of gene expression domains in the early embryo and wing imaginal disc, that were generated in the Affolder lab at the Biozentrum of the University of Basel. After characterizing the extent of these domains in a quantitative

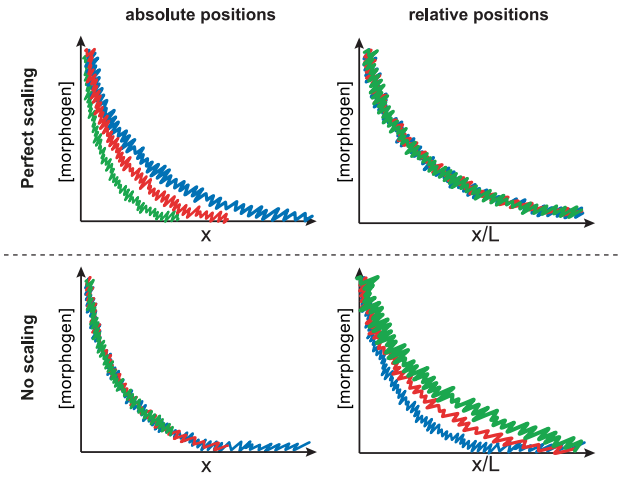


**Fig. 5.** *Morphogen gradients in relative and absolute positions.* We consider three morphogen gradient profiles in a small (green), medium sized (red) and big (blue) embryo (or tissue). In the absence of scaling, the three profiles overlap in absolute positions $x$ (top left). However, when plotting the profiles in relative positions $x/L$, they no longer collapse into one single curve (top right). In the case of perfect scaling, we observe the opposite scenario: profiles collapse in relative positions (bottom right) and not in absolute positions (bottom left).

and systematic manner, we introduced and applied a new *scaling measure* introduced above in order to assess how well proportions are maintained. we found that scaling emerged as a universal property both in early embryos (at least far away from the Bcd source) and in wing imaginal discs (across different developmental stages). Since we were also interested in understanding the mechanisms underlying scaling and how it is transmitted from the morphogen to the target genes down in the signaling cascade, we also quantified scaling in mutant flies where this property could be disrupted. While scaling is largely conserved in embryos with altered *bcd* dosage, we found that in the wing imaginal disc scaling is partly disrupted in *pentagone* mutants, which has been shown to be essential for long-range morphogen gradient formation. Our analyses also suggest that the target genes combine signals from two activity gradients downstream Dpp in a non-linear and gene-specific fashion in order to scale properly and ensure proper vein positioning in the wing. A detailed account of our analysis is currently under review in *PLoS Biology* (F. Hamaratoglu, A. Morton de Lachapelle at al., *Dpp signaling activity requires Pentagone to scale with tissue size in the Drosophila wing imaginal disc*).

In the early embryo, our modeling efforts suggest that Bcd trapping by the nuclei as well as pre-steady state decoding of the morphogen gradient are essential to ensure precise and scaled patterning of the Bcd signaling cascade [17].

## 5 Discussion

In summary, within a developing organism, cells need to know where they are in order to differentiate into the correct cell-type. Pattern formation is the process by which cells acquire their positional information and thus determine their fate. This can be achieved by the production and release of a diffusible signaling molecule, called a *morphogen*, which forms a concentration gradient: exposure to different morphogen levels leads to different cell fates. Though morphogens have been known for decades, it is not yet clear how these gradients form and yield such robust patterns. We have investigated the properties of Bicoid and Decapentaplegic, two morphogens involved in the patterning of the anterior-posterior axis of *Drosophila* embryo and wing primordium, respectively. In particular, we have been interested in understanding how the pattern proportions are maintained across embryos of different sizes or within a growing tissue, which is essential to yield a correctly proportioned organism or organ. Ultimately, the general understanding of how cells respond to signals and coordinate their actions could bring new insights into some diseases where these processes are disregulated and, theoretically, provide the ground to make artificial tissues.

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