Precise spatial scaling in the early fly embryo

Victoria Antonetti,1,3 William Bialek,1,2,4 Thomas Gregor,1,2,5
Gentian Muhaxheri,1,3 Mariela Petkova,1,2,6 and Martin Scheeler1
1Joseph Henry Laboratories of Physics and 2Lewis–Sigler Institute for
Integrative Genomics, Princeton University, Princeton, NJ 08544
3Department of Physics, Lehman College, City University of New York, Bronx, NY 10468
4Initiative for the Theoretical Sciences, The Graduate Center,
City University of New York, 365 Fifth Ave., New York, NY 10016
5Department of Developmental and Stem Cell Biology UMR3738, Institut Pasteur, 75015 Paris, France
6Program in Biophysics, Harvard University, Cambridge MA 02138
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The early fly embryo offers a relatively pure version of the problem of spatial scaling in biological
pattern formation. Within three hours, a “blueprint” for the final segmented body plan of the
animal is visible in striped patterns of gene expression. We measure the positions of these stripes in
an ensemble of 100+ embryos from a laboratory strain of Drosophila melanogaster, under controlled
conditions. These embryos vary in length by only 4% (rms), yet stripes are positioned with 1%
accuracy; precision and scaling of the pattern are intertwined. We can see directly the variation of
absolute stripe positions with length, and the precision is so high as to exclude alternatives, such as
combinations of unscaled signals from the two ends of the embryo.

It is a common observation that animals vary more
in size than in proportions. A possible quantitative ver-
sion of this observation would be the claim that organ-
isms exhibit scaling, so that the dimensions of different
body segments all vary in proportion to the overall size
of the organism. If this were true, then pattern forma-
tion in biological systems would be qualitatively different
from that in the non–biological pattern forming systems
that we understand, such as Rayleigh–Bénard convection
or directional solidification [1–4]. In these systems the
length scales of pattern elements are set by microscopic
parameters, and if we change the size of the system we
see more repetitions of the pattern rather than expansion
or contraction of the original pattern.

The question of scaling in organisms mixes many as-
pects of development and growth. A possibly purer ver-
sion of the question is accessible in the early develop-
ment of insect embryos, such as the well studied fruit fly
Drosophila melanogaster [5]. In this system, a blueprint
for the final segmented body plan is visible in striped
patterns of gene expression, as shown in Fig 1. There are
seven of these “pair–rule” genes, which were identified
by the fact that mutations in any one of them result in
distortions of the final body plan [6]. Importantly, the
striped patterns of molecular concentrations vs. position
along the length of the embryo are visible two to three
hours after the egg is laid, even before there are complete
membranes separating all the cells. During this period
the size of the egg is constant, and there are no large
scale cellular movements. While we should be cautious
about oversimplification, the early fly embryo is close to
the physicist’s idealization of a box in which a complex
network of chemical reactions generates a spatial pattern.

Experiments on fruit fly development typically are
done with inbred laboratory stocks, which have vastly
less genetic diversity than found in natural populations.
Nonetheless, even under controlled conditions eggs vary
in length, with a standard deviation of ~4%; in a popu-
lation of ~100 embryos we find eggs that differ by ±10%
from the mean (examples below). The question of scaling
then is whether the positions of the stripes vary in pro-
portion to egg length, so that they are at fixed relative
positions in the embryo, independent of size.

Stripe positions are reproducible, from embryo to em-

FIG. 1: Striped pattern of eve expression in the fly embryo. (bottom) Raw image of the embryo, stained with fluorescent antibodies against the Eve protein. Focus is in the mid–sagittal plane, scale bar is 100 µm, and the dorsal side is at the top. (top) Fluorescence intensity vs position along the dorsal edge of the embryo, from which we extract the positions of the seven peaks. Inset shows the averaging window (white square) that we use in measuring the intensities; the width is 0.01L, comparable in size to the individual nuclei.
bryo, with a precision of \(\sim 1\%\) in scaled coordinates \[7\]. This might seem to necessitate scaling, to compensate for the \(\sim 4\%\) variations in embryo length, but this is not quite true since a stripe \(1/4\) of the way along the length of embryo would vary by only \(1\%\) in relative position even if it were fixed in absolute position, and stripes could be anchored to either end of the embryo. In the middle of the embryo, then, reproducible absolute positions would correspond to \(\sim 2\%\) fluctuations in relative position. But if cells have access to independent signals from both ends of the egg \[8–11\], perhaps these could be combined to generate a positional signal that fluctuates by only \(\sim \sqrt{2}\%\). Convincing evidence for scaling in response to the natural variations of embryo length in laboratory fly stocks thus hinges on extreme precision.

While the importance of scaling as a conceptual problem has been appreciated for many years, there have been relatively few quantitative measurements on early embryos. In the fruit fly, early work indicated that positions of pair–rule stripes scale with embryo length \[12\], but the overall precision seen in those data was not as high as we now know to be characteristic of the stripes and of the positional information encoded in the gap gene network that provides input to the pair–rule genes \[7, 22\]. A subsequent series of papers focused on scaling in populations of fly embryos where length variations were enhanced by crossing closely related species \[13–14\], or by artificial selection \[15, 16\]. While these results make it very likely that pair–rule stripes exhibit scaling, the arguments above highlight the need for a very precise measurement within a single strain of flies.

In Figure 1 we show a single *Drosophila* embryo in nuclear cycle 14, stained for the protein encoded by the pair–rule gene *even-skipped* (*eve*). Staining procedures are described in Ref \[17\], and the image is taken in a scanning confocal microscope, with the focus in the mid-sagittal plane of the embryo. We measure fluorescence intensity in a sliding window along the dorsal edge of this image (inset to Fig 1), quantifying the seven stripes. Positions of the stripes are defined by the intensity peaks, which in this example are easy to identify; in other cases we use self–consistent templates to locate the peaks more accurately.

It is well known that pair–rule stripes move during development, even during nuclear cycle 14 \[18, 19\]. Our data are images of fixed embryos, but the extent of the cellularization membrane provides a measure of time in this nuclear cycle with a precision of one minute \[17\]. Results for the Eve stripe position vs time are shown in Fig 2 with position measured in units relative to the length of the embryo; we start 25 min into cycle 14, when all seven stripes are visible. We see that the dynamics are relatively smooth and systematic, as well as different for different stripes. In addition, variance around the systematic behavior is quite small.

In the presence of these dynamics, we have the choice of focusing on a small window of time, during which movements are small, or trying to exploit the observed systematic behavior to combine data from all time points. We will use the second approach; focusing on small time windows gives the same answers, but necessarily with larger error bars. On average, positions vary with time as

\[
x_i(t)/L = x_i(t_0)/L + s_i(t - t_0).
\]

Thus we can shift each measured position by an amount \(s_i(t - t_0)\), resulting in all data referred to the time \(t_0 = 45\) min. Similar results are obtained for Prd and Run stripes, although for these cases the complete set of seven stripes appears only a bit later in cycle 14.

Making use of data at all time points allows us to explore the absolute positions of stripes in embryos of varying size, as shown in Fig 3 for the Eve stripes. All of these measurements are made on embryos from the laboratory stock (OreR), kept at 25°C at all times before fixation. The mean length of the embryos (with no attempt to correct for shrinkage during fixation) is \(\langle L \rangle = 444 \pm 2 \mu m\), and the standard deviation across the population is \(\delta L_{rms}/\langle L \rangle = 0.04 \pm 0.003\), but with enough samples we see variations of more than 10% in \(L\) \[20, 21\]. Importantly, this is larger than the fractional distance between stripes. In most non–biological pattern forming systems, changing the system length by more than the spacing between pattern elements would result in the insertion of additional elements, which are never seen, either in the stripes of gene expression or the resulting segmented body plan of the fully developed embryo.

Figure 3 shows clearly that stripe positions vary with embryo length, and these positions cluster tightly around the lines corresponding to perfect scaling,

\[
x_i = \langle x_i/L \rangle L.
\]

![FIG. 2: Dynamics of Eve stripes during nuclear cycle 14, with positions measured as a fraction of the length of embryo. Lines from Eq [1].](image-url)
More quantitatively, we can measure the variance of fractional positions

\[ \sigma_f^2 = \left( \langle x_i / L \rangle - \langle (x_i / L)^2 \rangle \right), \]  

(3)

which is shown by the red points in Fig 4. For all but one of the 21 stripes, \( \sigma_f \approx 0.01 \), or less; error bars on these measurements are themselves very small. This is consistent with previous measurements on reproducibility of relative positions in small windows of time, and with the information content of the gap gene expression patterns that feed into the generation of pair–rule stripes \([13]\), and in the combined expression levels of different gap genes \([25]\).

Figure 3 provides prima facie evidence for scaling of the Eve stripes, and we can make similar figures for Prd and Run. It nonetheless is useful, as noted at the outset, to think about scaling in relation to the precision of stripe placement. If we imagine a hypothetical embryo in which stripes were perfectly anchored in absolute position relative to the anterior pole of the embryo, then the relative positions \( f_i = x_i / L \) fluctuate only because the lengths of the embryos vary,

\[ \sigma_f^2(A) = \langle x_i \rangle^2 \left( \langle (1/L)^2 \rangle - \langle (1/L) \rangle^2 \right), \]  

(4)

and for anchoring at the posterior pole we have

\[ \sigma_f^2(P) = \langle (L - x_i) \rangle^2 \left( \langle (1/L)^2 \rangle - \langle (1/L) \rangle^2 \right). \]  

(5)

These results provide bounds on the reproducibility of relative positions, shown in blue in Fig 4. If we think of these fluctuations as random errors in the relative positional signals available to the mechanisms that generate each stripe, we can imagine combining these signals to reduce the error,

\[ \frac{1}{\sigma_f^2(A, P)} = \frac{1}{\sigma_f^2(A)} + \frac{1}{\sigma_f^2(P)}, \]  

(6)

with the results shown in cyan. For all but the most posterior Prd and Run stripes, the real embryos are significantly below these bounds (red points).

We have looked at scaling by asking if the length of the embryo influences the absolute positions of the pair–rule stripes. Conversely, scaling means that by measuring the distance from the anterior end of the embryo to (for example) the first Eve stripe, we can predict the length of the embryo. Indeed, if we do this, we can estimate the length of the embryo with \( \sim 2\% \) accuracy using just the first stripes of Eve, Run, or Prd. This information has to propagate \( \sim 300 \mu m \) from the posterior toward the anterior. This propagation of information over long distances is consistent with evidence for correlations among the fluctuations in stripe positions \([13]\), and in the combined expression levels of different gap genes \([25]\).

The pair–rule stripes emerge as the output of a cascade that leads from input signals provided by the mother to a network of gap genes and finally to the pair–rule genes. The best studied maternal input, Bicoid, exhibits scaling on average across different species of flies that have different length eggs \([24]\), and across the smaller range of length variations that can be achieved by artificial selection \([10]\); early work provided hints of scaling
from embryo to embryo within single species [25], but this remains unclear [26]. For the gap genes, the optimal readout of (relative) positional information reaches the 1% precision seen for the pair-rule stripes, and the functional form of this optimal readout make successful parameter–free predictions of the distortions of the pair-rule stripes in response to deletions of the maternal inputs [22]. These results, coupled with the scaling of pair-rule stripes demonstrated here, make it plausible that the gap gene expression profiles should scale, but this should be tested directly [27].

In summary, pair–rule stripes in the early fly embryo exhibit precise spatial scaling even in response to the small variations of egg length that occur within a single strain under controlled conditions. This suggests that biologically morphogenesis really belongs to a different class of problems than the widely studied examples of pattern formation in non–equilibrium, but non–biological systems [1–3]. To increase our statistical power, we have pooled data across nearly half an hour of nuclear cycle 14, which suggests strongly that scaling is present as soon as the full pattern of stripes is visible, rather than emerging gradually. Finally, in the fly the problems of scaling and precision are intertwined; any explanation for scaling would be incomplete if it did not account for the precision of the final pattern, and any effort to account for precision ultimately must address scaling.

We conclude with a cautionary note. We have studied a single strain of flies, within which there is relatively little genetic diversity. But it is difficult to exclude the possibility that most of embryo length variations that we see are genetically encoded, in which case it is possible that the same (or co-evolved) genetic differences influence the pair–rule stripes directly. In this scenario, evolutionary pressure would need to be so strong as to keep the molecular mechanisms of size control and stripe expression aligned with ~1% accuracy.

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