Overview:

The basic premise is that this particular selected line (2.49.3) has developed some sort of mutation over the 30 generations while this line was selected for increased egg size. Our initial experiments revealed that this embryo has interesting Bicoid (Bcd) morphogen gradient characteristics. The first is that the level of Bicoid protein at the anterior tip of the embryo is dramatically lower when compared to other similarly selected inbred lines. Second, the resulting gradient is significantly flatter and a necessarily increased length constant lambda (λ). Interestingly, this flatter gradient has allowed us to raise several questions: We compared that gradient to wildtype and found that as a result of the flatness, the converted positional error at the Hunchback threshold has been significantly increased. We confirmed this prediction with fluorescent in situ hybridization with full length DIG-labeled probes to Hunchback and Even-skipped; we find that the resultant precision of the boundary position has been decreased as predicted from the gradient data.

Further, we looked for the source of this obviously aberrant gradient; initial studies reveal that the total amount of Bicoid mRNA is consistent with previously reported results suggesting a correlation between egg size and amount. Interestingly, we noticed that the localization behavior of the mRNA was not as tightly clustered, in fact it can be said that the distribution of the Bicoid mRNA in this particular inbred line was rather diffused. We hypothesize that the selection process aimed at egg size has given rise to a mutation which affects the Bicoid mRNA distribution, which in turn produces a protein gradient with the characteristics which we observe; further, it can be shown that it results in greater positional errors downstream. This assertion is supported by that it has been reported that specific mutations which abolish the anterior localization of Bicoid mRNA, such as staufen, also give rise to a protein gradient which has a dramatically depressed Bicoid level at the anterior tip of the embryo (Dev Cell 2008). Secondly, it has also been shown that flatter gradient profiles also lead to greater defects in positional error than in their wildtype counterparts.

Results:

* Increased positional error from flatter Bicoid gradient generated in the absence of a specific mutation
  + Bicoid protein profiles (Large, wt)
  + Convert Intensity error to Positional error (rms)
  + Hb/Eve in situ confirming decrease in precision
* Flatter gradient is a result of poorly localized Bcd mRNA and not Bcd mRNA amount
  + 1x,2 x,3x Bcd mRNA FISH with Line 2.49.3
  + Contour line comparison showing Line 2.49.3 covers a larger area; infer larger volume.
* Specific mutation?
  + Localization mutant similar to Staufen?
  + Either anchoring or retrograde transport machinery
* Characterizing Line 2.49.3 as a mutant
  + Hatching rate differences at different temperatures
  + Membrane invagination in DIC live imaging study
* Sequencing data
  + Show a difference in mutation between Large and Small flies
  + Generate transgenic fly to validate

Materials and Methods:

1. Bcd Protein AB staining
2. Bcd mRNA FISH
3. Hb mRNA FISH
4. Cecelia Eve in situ
5. Statistical analysis via matlab

Results:

The Bicoid gradient remains scaled despite B0 being uncorrelated with egg length

In order to further our understanding of the phenomenon of scaling in the developing Drosophila embryo, we asked whether scaling can still be achieved in the absence of key components, and if so, what tradeoffs or compromises are made. It has been previously shown, in wildtype populations, that the anterior-most position of the Bcd gradient (B0) is positively correlated with the length of the egg (EL) ([He, Wen et al. 2008](#_ENREF_3)). This positive correlation persists even when the differences in the physical characteristics between embryos are maximized ([Cheung, Miles et al. 2011](#_ENREF_1)). In the present study, we examine inbred populations of embryos which have been selected based upon the size of their eggs, in the same manner as was published in our previous work. However, one particular line of embryos seems to have been maladapted with regard to the aforementioned B0-L correlation; and yet scaling remains steadfast.

In this study, two inbred lines, which have been artificially selected, were used ([Miles, Lott et al. 2011](#_ENREF_4)). To be clear, the present pair of lines is distinct from those which have been published previously.

To facilitate a valid comparison between these experimental data, the embryos were stained side-by-side. Quantitative fluorescence immunostaining was performed on these embryos; also included are embryos from *bcdE1* females, a null mutant to serve as a negative control for the purposes of background subtraction. All imaging data was captured within the linear range and the exposure time remained constant across all samples. Fluorescence intensity data was acquired as previously described ([He, Wen et al. 2008](#_ENREF_3)). Shown here in Fig. 1AB are midsagittal sections of representative embryos from their respective populations.

Here, we show that the anterior-most position of the Bicoid protein gradient (B0) in Line 2.49.3 (Large Egg) is dramatically lower than seen in Line 9.31.2 (Small Egg) (B0 = X and Y, respectively). This result is in stark contrast to previously published works emphasizing the relationship between the size of the embryo and different gradient features (Mean Egg Length = X and Y, respectively). Interestingly, when the two mean gradient profiles are plotted over relative egg length (x/L), their paths converge; we interpret this to mean that scaling has been achieved between these two lines(Fig. 1CD).

Precision is sacrificed for scaling (working title)

Both precision and scaling are necessary properties to ensure appropriate downstream patterning of the embryonic body plan. It has been previously demonstrated that a precise Bicoid protein gradient input is required for a precise output by Hunchback([He, Wen et al. 2008](#_ENREF_3)). Here we converted intensity errors into positional errors, as previously described ([Gregor, Tank et al. 2007](#_ENREF_2)) (Fig 2A).

Here we can observe that given similar intensity fluctuations in the Bcd protein gradient profile; as a result of a flatter gradient, Line 2.49.3 has a significantly larger converted positional error. The increase in positional error should result in a detectable increase in the positional error of the genes activated downstream. In order to compare these converted positional errors to experimentally obtained measured positional errors, we performed a fluorescent in situ hybridization to detect Eve mRNA.

from Eve boundary positions (Fig 2B)

The Bicoid protein gradient aberrations in Line 2.49.3 are not a result of reduced Bicoid mRNA amount

To investigate whether the significantly reduced amount of Bicoid protein at the anterior tip was a result of an insufficient amount of Bicoid mRNA, we performed a fluorescent in-situ hybridization of Lines 2.49.3 and 9.31.2 as previously described ([Cheung, Miles et al. 2011](#_ENREF_1)). Here, we show that the fluorescence intensities representing Bicoid mRNA in the larger Line 2.49.3 population is notably larger than those of the smaller Line 9.31.2 (Fig 3A). Also shown are representative images of their respective populations (Fig 3BC inset).

Refs:

Cheung, D., C. Miles, et al. (2011). "Scaling of the Bicoid morphogen gradient by a volume-dependent production rate." Development **138**(13): 2741-2749.

Gregor, T., D. W. Tank, et al. (2007). "Probing the limits to positional information." Cell **130**(1): 153-164.

He, F., Y. Wen, et al. (2008). "Probing intrinsic properties of a robust morphogen gradient in Drosophila." Dev Cell **15**(4): 558-567.

Miles, C. M., S. E. Lott, et al. (2011). "Artificial selection on egg size perturbs early pattern formation in Drosophila melanogaster." Evolution **65**(1): 33-42.