Effect of Mitosis on Chromosome Pairing in *Drosophila melanogaster*

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In *Drosophila melanogaster* chromosomes are paired in most cells. In their 2020 paper, Child made a model that would predict the trend of how chromosomal pairing increases over embryonic development; however pairing levels of real, live embryos increased much more rapidly than the model predicted. I hypothesized that mitosis might contribute to pairing increasing rapidly in the live embryos because the model did not account for it. To determine this, I bred flies that have fluorescent markers on their chromosomes. I took images of their cells directly before and after mitosis and measured the distances between the markers on each chromosome. I did not find that mitosis caused a significant increase in homologous pairing, so now I know further work into mitosis does not need to be done.

Organization of chromosomes in the nucleus is important for gene expression. In *Drosophila melanogaster,* chromosomes are paired in most cells. Their chromosomes do not start out paired however and as a fly embryo develops the homologous chromosomes find each other. This project could give more information about homologous pairing like what triggers them to come together. The information from this study could also help us to better understand an epigenetic phenomenon known as transvection. Transvection is a process during which regulatory proteins on one homolog start to regulate gene expression on the other homolog, and we know that the more closely the homologs are paired the more likely transvection is to occur. With these larger questions in mind I looked into homologous pairing over developmental time. In their 2020 paper, Child et al. produced a biophysical model for the trend in how homolog pairing increases over embryonic development; however pairing levels of real live embryos increased much more rapidly than the model predicted (see fig.1). Child's paper left off with the question of what could contribute to the more extreme trend we see in live embryos that the model does not consider. I predicted that mitosis might provide the newly divided cells a good opportunity to find their homologous pairs and that I would see a steeper increase in pairing directly after cell division.

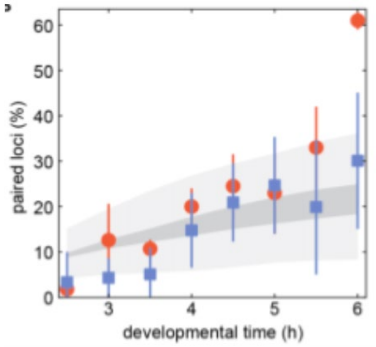
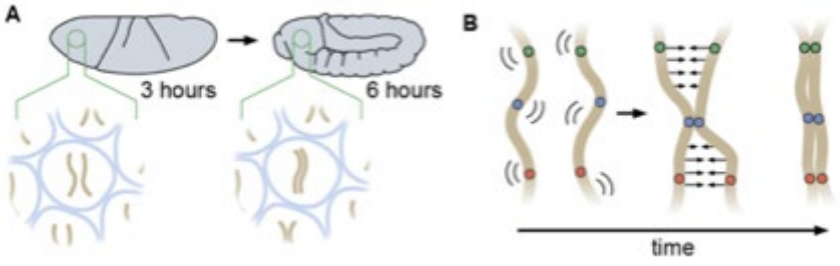


Fig. 1: Child et. al. 2020. model prediction of pairing over time (gray) compared to actual trend in live embryos (red and blue



Child et. Al. 2020

In order to determine if mitosis led to an increase in homolog pairing, I first had to breed embryos that would contain the necessary components of the fluorescent markers that would allow us to visualize homologous chromosomes. I made a genetic cross of UAS, DSCP, MS2; E,11 virgin females and Nullo gal4; UAS PP7 males and collected stage 5 (or cycle 14) embryos. The UAS, DSCP, MS2; E,11; Nullo gal4; UAS PP7 embryos had the genes for RNA stem loops which are made by RNA polymerase, and portions that bound to both the RNA stem loops and either red or green fluorescent proteins. these appeared on both homologs of polytene position 38F, which is a known pairing site (see figure 2). I then observed these live embryos using the confocal microscope and took videos of their early development from 130 minutes (about 2 hours) old to 230-250 minutes (about 4 hours) old. Because the fluorescent signals are attached to RNA polymerase, and RNA polymerase does not transcribe during mitosis, the signals go dark, so we know when a cell is undergoing mitosis.

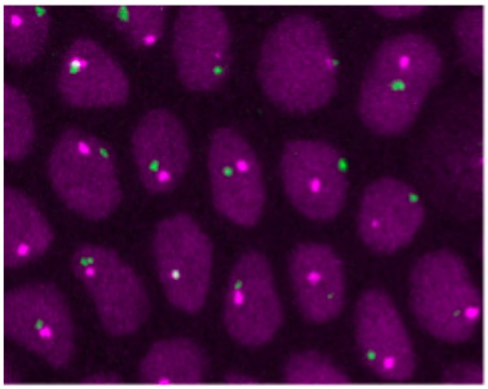
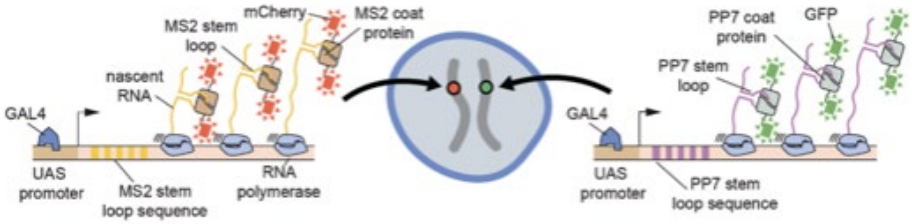
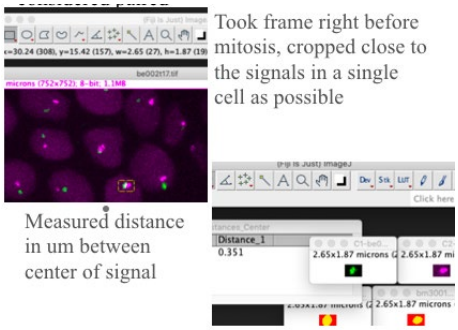


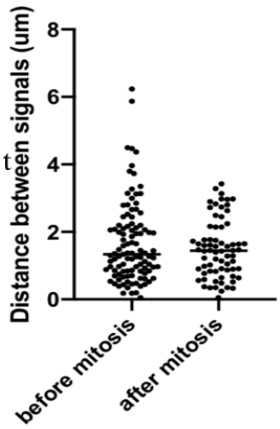
Fig 2: 130-140 minute-old UAS, DSCP, MS2; E,11; Nullo gal4; UAS PP7 embryo on confocal microscope



Child et. Al 2020

I took the frames that were directly before and after a mitotic event and analyzed the distances between each signal (and therefore the homologous chromosomes) using ImageJ software that was trained to distinguish signals from background and measure the center-to-center distance between the pixels in the image that were classified as “signal.” Any signals less than one micron apart were considered paired.





I did not find that mitosis led to any significant increase or decrease in homologous pairing. When nuclei were imaged before mitosis, they were 36.2% paired, but after mitosis they were 34.3% paired. This difference was not statistically significant when we did a T test. Now we know we do not need to look deeper into mitosis and can turn our attention to other potential causes of how homologous pairing increases over development. Future directions could include working out how to set up a similar visualization technique that would work in more mature tissues such as third instar wing discs or looking for other things that might impact the rate of increase in homolog pairing over development.

Thank you to my supervisor Professor Bateman; all the members of the Bateman lab; Michy, Aisha, and Derek; and NASA Maine Space Grant.

References:

Live imaging and biophysical modeling support a button-based mechanism of somatic homolog pairing in *Drosophila* Child et. al. 2021 eLife 2021;10:e64412 DOI: 10.7554/eLife.64412

Mitotic domains reveal early commitment of cells in Drosophila embryos VICTORIA E. FOE Friday Harbor Laboratories, University of Washington, Friday Harbor, WA 98250, USA

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