Visualizing and characterizing cellularization and germ-band extension in *Bradysia coprophila* embryogenesis
Gabriel O'Brien, 2026

This project works to examine the processes of cellularization and germ-band extension in *Bradysia coprophila* embryonic development, which is not well understood. In comparison to *Bradysia*, embryogenesis has been extremely well characterized in the model organism *Drosophila melanogaster*, or the common fruit fly. In *Drosophila*, once an egg is fertilized, it undergoes eight rapid nuclear divisions within the cytoplasm. Next, the nuclei migrate to the periphery of the embryo, forming a syncytial blastoderm. The syncytial blastoderm is characterized by a monolayer of nuclei that lack individual cell membranes and are housed in a common cytoplasm. In *Drosophila*, cellularization occurs about ~2-3 hours post fertilization, resulting in a cellular blastoderm in which each nuclei now has its own cell membrane.

The expression of early embryonic patterning genes is responsible for defining where cell types and body structures will develop in an embryo. There are four types of patterning genes in *Drosophila*: maternal effect genes, gap genes, pair-rule genes, and segment polarity genes. All four of these gene types are expressed sequentially, establishing finer and finer segments. In *Drosophila*, the peak of expression takes place during the syncytial blastoderm stage before cellularization. In particular, *Bicoid* is one of the most well-studied patterning genes in *Drosophila*. It is a maternal effect gene that helps to set up the anterior-posterior axis. *Bicoid* is specific to a subsection of higher Dipteran flies, and lower Dipteran species, like *Bradysia*, lack *Bicoid*. This leads us to question how *Bradysia* patterns their embryos.

Understanding *Bradysia* embryonic patterning is one of the larger goals of the Bateman lab, and my project more specifically investigates the timing of cellularization, which is an important part of *Bradysia* embryogenesis. I also look at germ-band extension, which is a process where the germ-band nearly doubles in length along the anterior/posterior axis and develops into the segmented trunk of the embryo. It is mostly unknown whether *Bradysia* undergoes long- or short-germ segmentation. Long-germ segmentation is where the embryo segments all at once prior to germ-band extension, and short-germ segmentation is where the embryo segments sequentially during germ-band extension. *Engrailed* is a segment polarity gene that was identified in *Bradysia*, and it signals for germ-band extension to occur.

In order to collect embryos that were precisely timed, I had to stimulate egg lays in *Bradysia* females. To get the females to lay their eggs, I poked their wings into agar and squeezed their thoraxes with forceps. I removed the females from the agar one hour after initiating egg lays to decrease the range of timepoints within my sample. For fixation, I dechorionated the embryos in a bleach solution, and devitellinated the embryos in a heptane and formaldehyde solution.

I used an anti-actin antibody stain to characterize when cellularization (the transition from syncytial to cellular blastoderm) occurs, allowing me to visualize cell membrane formation. Sources state that cellularization occurs from 9 to 12 hours after egg laying in *Bradysia*. I found that cellularization can be spotted as early as 4-5 hours after egg laying (between cycles 6 and 7 of embryogenesis). I also used an anti-engrailed antibody stain to visualize when embryo segmentation occurs, allowing me to characterize germ-band extension. The previous researcher found that *B. coprophila* embryo morphology is consistent with short-germ segmentation, and she examined embryo morphology from as early as 14 hours after egg laying. One of my goals was to look at some additional time points of germ-band extension, and I found that segmentation can be spotted at 26-27 hours after egg laying. In the future, I would like to visualize expression patterns of *Engrailed* at more time points to better characterize germ-band extension. I would also like to stain for other membrane markers, such as integral membrane proteins, to further characterize *Bradysia* embryogenesis.

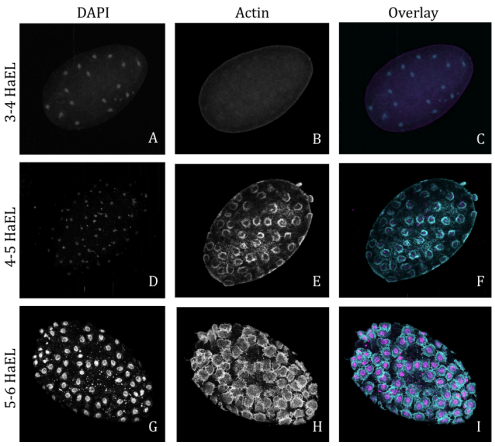

Figure 1: Visualization of actin expression in B. coprophila embryos through anti-actin antibody staining.

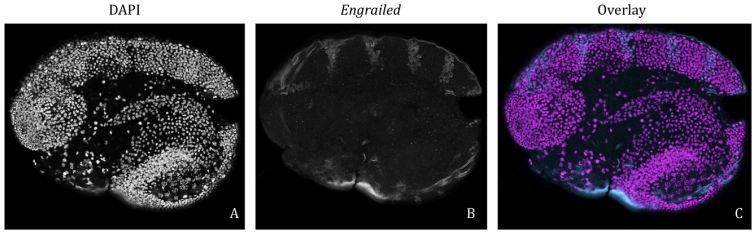

Figure 2: Visualization of *Engrailed* expression in *B. coprophila* embryos through anti-*engrailed* antibody staining 26-27 HaEL.

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