Internship report- EDEN program summer of 2014:

tll function in axis specification in the fly Clogmia albipunctata-

Characterized by dsRNA silencing

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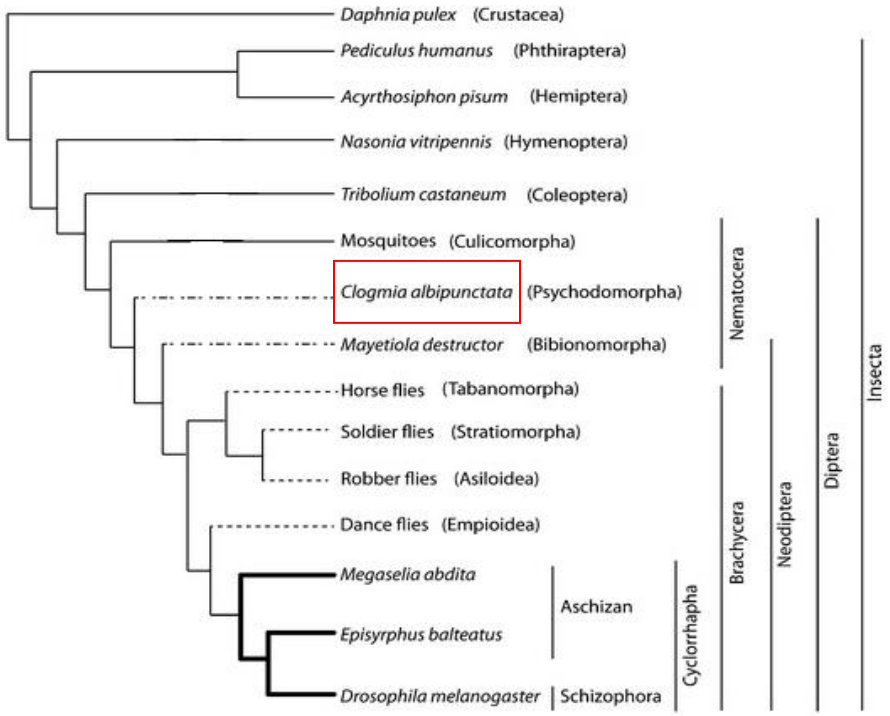
The question of axis specification is one of the most intriguing questions asked in early stages of embryonic development in bilaterians. The presence of axis controls the body plan and insures the organism will have two distinct ends. Dr.Schimdt Ott's lab addresses this question and compares different mechanisms and different "players" in the process of axis formation in dipterans.

During my internship, I focused on the gene *tailless* in the model organism *Clogmia albipunctata*, a fly in the Psychodidae family. This fly is of the lab's interest because of its location on the phylogenetic tree, which is close to the lab's main model organism taxa, the mosquito *Chironomus riparius (Figure 1)*. Another model organism studied in the lab is *Megaselia abdita*, this fly is studied in a different context. By looking at different branches we can gain an overview and perspective on the evolution of axis formation and of tll's role in this process. It has been shown in *drosophila melanogaster* that the absence of *tll* in early development stage leads to gaps in the developing embryos and to short larvae. In *Chironomus* the absence of tll resulted in double headed larvea, a mirror phenotype due to duplication of the anterior end. This result rises the question what would be phenotype in *Clogmia* who is closer to mosquitoes than to Drosophila.

To address this question I used the method of dsRNA silencing using microinjections. One of my main goals was to develop a system for Clogmia injections. The critical and most challenging part was to find a needle for the injections. The injection needles are pulled by a pulling machine and each parameter affects the size and the diameter of the tip. Reading the Micropipette Puller manual helped to determine and customize the parameters to the wanted properties. Another challenge was to inject through the chorion, removing the chorion within the first two hours after egg activation disrupt the embryos' development. I tried to remove the chorion by using different concentrations of bleach but the embryos didn't survive. Also, the chorion hardens as times passes, 1.5-2 hours after egg activation it is

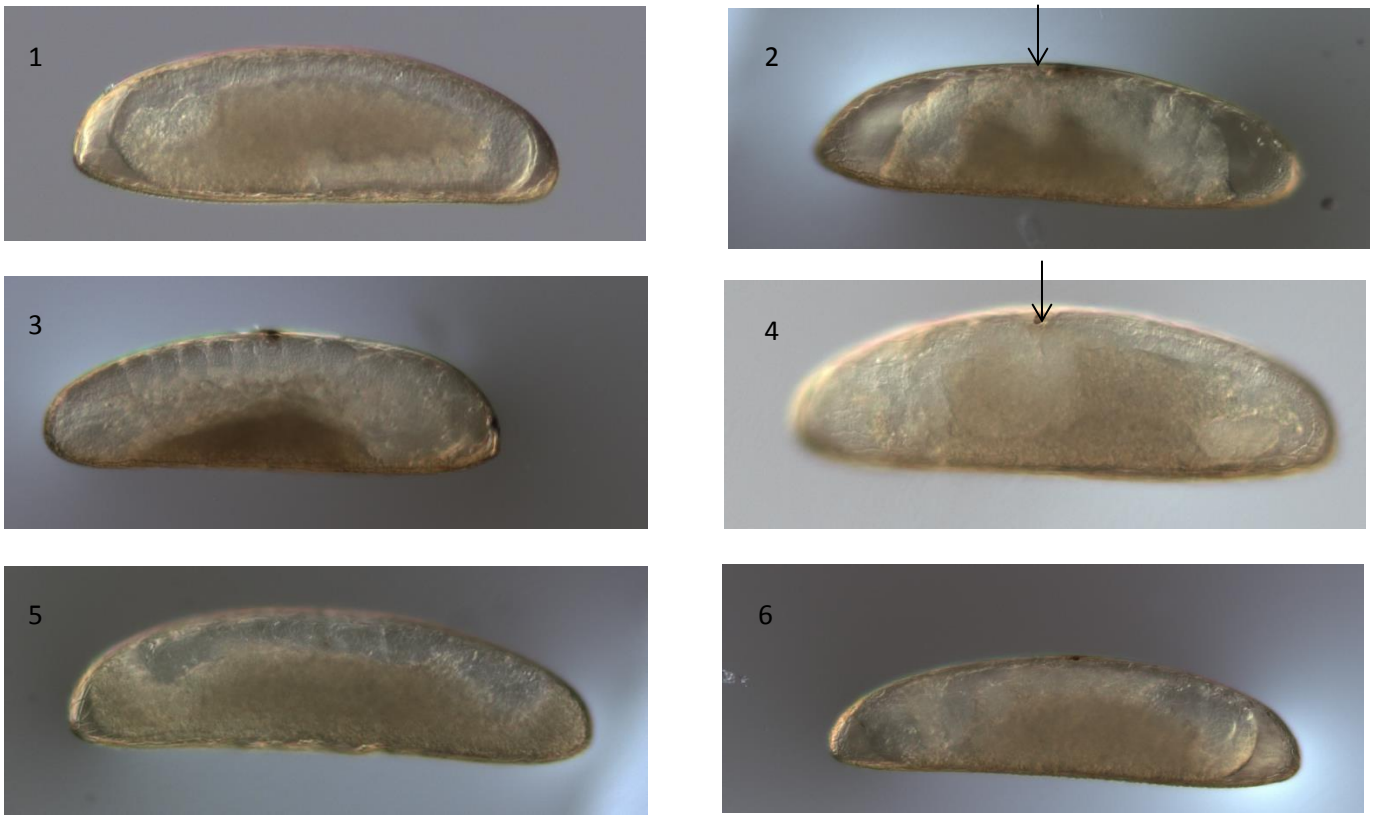
impossible to penetrate the chorion. Drying aligned embryos before injections helps avoiding capillary effect in which the cytoplasm goes up the needle. This parameter still needs to be optimized due to embryos not developing past germ band stage. Results show knockdown has occurred but at this stage of the experiment it is hard to tell how exactly it affects the embryos because none of the silenced embryos have hatched. In germ band stage it seems like there has been some sort of duplication (Figure 2) and shortening but no double head phenotypes were seen, even in later stages. Key goals for the future would be to improve rate of survival until cuticle or to use molecular probes for phenotypic analysis at an earlier stage.

Figure 1- Phylogenetic tree of flies



Source: Wotton, Karl R., et al. "Evolution and expression of BMP genes in flies."*Development genes and evolution* 223.5 (2013): 335-340

Figure 2- Embryos 48 hours after egg activation



1-WT. 2,4- suggested duplication. 3,5,6- silenced embryos, shorter than WT.

↓ Site of suggested duplication.

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Urs Schmidt-Ott's lab:

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