**Project Proposal**

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Treehoppers develop intricate three-dimensional helmets from pronotum tissue, which is part of the insect’s body wall. The formation of this helmet is unique to this group, with related groups of insects having the ancestral condition of a flat body wall that lies flush with the rest of the body. Previous work found that during development of this helmet in the treehopper species *Entylia carinata*, there is expression of wing-patterning genes in the helmet region, an expression pattern that is not found in the leafhopper which retains the ancestral condition (Fisher et al. 2020). This study evaluated expression patterns in different body regions of treehoppers and leafhoppers at the fifth instar of development. While it is clear that in the fifth instar there is expression of wing-patterning genes in the helmet region of treehoppers, it is not clear when in development these genes begin to be expressed.

Our goal is to understand when in development the 3-D structure of treehopper helmets is constructed using expression data from different body regions of the treehopper and comparing between two stages of development, the fourth and fifth instar. In the fourth instar, the treehopper helmet is a single layer of epithelial tissue and by the fifth instar, the helmet becomes two layers of tissue that are folded intricately to create the three-dimensional structure (Adachi et al. 2020). We will compare expression patterns between body regions and fourth and fifth instar stages in development to begin to parse whether there are differences in expression between instars that can be connected to the differences in layers of epithelial tissue. We can also further understand where in development wing-patterning genes start to be expressed in the helmet body region, and whether the expression of wing genes is integral to the folding of epithelial tissue we see in the three-dimensional helmet on the fifth instar and adult.

To address this question, we will be analyzing fourth and fifth instar body region RNAseq data collected by Cera R. Fisher. This data will be uploaded on the Xanadu Cluster for us to access. Our analysis process will closely follow that which was done on previously collected fifth instar data described in Fisher et al. (2020). We will begin by conducting quality trimming and adaptor removal using the package Trimmomatic. Then we will analyze differential expression between body regions using Perl and R scripts in Trinity, and will map the reads to the reference assembly already created in Fisher et al. (2020) using Bowtie. We will then have to process these alignments through RSEM to get the number of transcripts in the library, and then analyze this data for differential expression using DESeq2. We will use DESeq2 to perform pairwise comparisons of every combination of instar and body region and create heatmap visualizations. To parse which sets of genes are upregulated in certain body regions over others, and upregulated in common between two body regions, we will use the R package GoSeq.

**Citations**

Adachi, H., Matsuda, K., Nishida, K. *et al.* (2020). Structure and development of the complex helmet of treehoppers (Insecta: Hemiptera: Membracidae). *Zoological Lett*, *6*(3). https://doi.org/10.1186/s40851-020-00155-7

Fisher, C. R., Wegrzyn, J. L., & Jockusch, E. L. (2020). Co-option of wing-patterning genes underlies the evolution of the treehopper helmet. *Nature Ecology & Evolution*, *4*(2), 250–260.