Using Reporter Transgenes to Verify Polymorphic Enhancers in *Drosophila* flies

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The study I participated in this summer was part of an ongoing project with the goal of determining whether regulatory genetic variation contributes significantly to observed phenotypic variation within and between species. This is important because it would help determine what proportion of phenotypic variation is driven by mutations in the amino acid sequences of proteins vs. mutations in regulatory DNA that alter gene expression.

Enhancers are short stretches of DNA that regulate the time and place of gene transcription. To assess whether the same enhancers are active in different *Drosophila* species and populations, the Palopoli lab measured chromatin conformation genome-wide, since a stretch of noncoding DNA that is in an open chromatin conformation is generally an active enhancer. Previous work identified thousands of putative enhancers that were active in the brains of flies from some populations but not others.

This summer, the goal was to verify that the identified stretches of noncoding DNA, which were found to be in an open chromatin conformation, are indeed enhancers. First, we scanned the chromatin conformation results for the *Drosophila* genome using the UCSC Genome Browser to identify putative enhancers. Second, we carried out molecular biology protocols to generate transgenic reporter constructs for putative enhancers.

The criteria used to select the five enhancer candidates were 1) Are the predicted enhancer sequences intronic or intergenic? 2) Are the likely enhancer sequences polymorphic (active in some *Drosophila* populations and species, but not others)? and 3) Are the enhancer sequences active in *Drosophila melanogaster* flies from Oregon, since those are the flies that will be used to form transgenic flies? Of the five enhancer candidates, only two were successfully inserted upstream of the GFP gene in PJFC28 plasmids via Gibson Assembly. A third enhancer that was identified the previous spring as being an excellent enhancer candidate was also successfully inserted into the pJFC28 plasmid. The enhancer-containing plasmids and the enhancer sequences were confirmed via DNA sequencing before sending to a lab to form transgenic flies.

In the fall, the Palopoli lab will identify additional enhancer sequences and insert them into plasmids to form more transgenic flies. In addition, the transgenic flies formed from the three reporter constructs made this summer will have their brain tissue analyzed for GFP. If the flies express GFP in their brains, this will suggest that regulatory genetic variation significantly contributes to phenotypic variation between different species and populations.

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