Position effect variegation in the white gene as an assay for chromatin conformation in *Drosophila* hybrids

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This summer I sought to discover whether divergence in chromatin conformation between *Drosophila* fruit fly species contributes to gene expression problems in hybrids. Proper gene regulation is critical for an organism's fitness. Genes within DNA must be expressed in the proper locations, at the proper times, and in the proper amounts for an organism to survive and reproduce. Chromatin conformation is critical for gene regulation.

Hybrids (the offspring of two different species) are known to have decreased viability and fertility in comparison to pure species offspring. Our working hypothesis is that divergence in chromatin conformation between two parent species causes improper chromatin conformation in hybrids, and therefore improper gene expression.

Chromatin is DNA wrapped around special proteins called histones, while chromatin conformation refers to the structure and shape of chromatin. Chromatin conformation is important for gene regulation as this packaging of DNA controls much of gene expression. There are two main types of chromatin: euchromatin and heterochromatin. Euchromatin is more loosely packaged chromatin where the majority of genes are located. Because euchromatin is loosely packaged, the DNA is accessible to transcription factors that can "read" and express genes. Heterochromatin is tightly packaged, so transcription factors cannot access DNA or express genes in that region.

A phenomenon called position effect variegation (PEV) was central to our research. PEV is the reduced expression of a gene that is suppressed due to its proximity to heterochromatin. When a gene in euchromatin is located near a border with heterochromatin, the heterochromatin can "spread" and have a silencing effect on nearby genes in that cell. PEV has been shown to be associated with chromatin conformation, and chromatin structure is hypothesized to cause PEV.

Throughout the summer we crossed two species of *Drosophila* (*melanogaster* and *simulans*) to create hybrid flies. The *melanogaster* flies had a hsp70-white transgene insertion that was passed down to the hybrids. This transgene allowed us to observe PEV in fly eyes, and to use PEV as an assay for chromatin conformation. If the gene was expressed, the eye would appear red; if not, the eye would appear white. If PEV occurred, the gene would be mostly (but not entirely) suppressed, and the eye would appear mostly white with some patches of red. If levels of PEV were different between hybrids and their pure species parents, this would indicate that chromatin conformation was also different. By taking a picture of the hybrid fly eyes under a microscope, we were able to measure a pigment value for each eye, and thereby determine to what extent PEV occurred in each hybrid. A higher average pixel value indicated a whiter eye, and a lower average pixel value indicated a redder eye.

Our results for the summer show that PEV did occur in the hybrids, and that there is consistently less expression of the white gene in hybrids in comparison to in their pure species parents. These results support that there are differences in chromatin conformation between pure species and hybrids, and that divergence in chromatin conformation could play a role in faulty gene expression in hybrids.

Faculty Mentor: Professor Michael Palopoli

Funded by INBRE, IDeA Networks of Biomedical Research Excellence and NIH, National Institute of Health