Divergence in Chromatin Conformation between *Drosophila* Species Reflected in *white* gene Position Effect Variegation

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My summer research project focuses on the genetic basis of barriers that separate two lineages. Oftentimes, hybrids between two species suffer from reduced fitness, which is manifested in their reduced ability to reproduce and survive. Previous studies have concluded that the cause of greatly reduced viability and fertility in hybrids may be attributed to abnormal amounts of gene product, a result of irregular gene expression. My summer project plans to address the mechanism by which genes are either under- or over-expressed in hybrids.

Our hypothesis for the incorrect gene expression in hybrids is that a divergence in the way DNA is packaged in the two parent species contributes to the gene expression problem, such that DNA conformation in chromosomes of hybrids is flawed. The way in which chromatin, the DNA-protein complex in nuclei, is packaged largely influences gene expression. Depending on whether an area of chromatin is loosely or tightly packaged, genes may or may not be expressed and lead to proper appearance of the corresponding phenotype. One way to assess DNA packaging is to look at the expression of a transgene, a gene that has been engineered and inserted at a particular location (in our case, on the border of loosely and tightly packaged chromatin) of the genome. By looking at the levels of expression of the transgene purposefully inserted in hybrids we can assess whether the DNA conformation between two parent species is altered. My summer research applies this approach in the model system of a fruit fly, into which we have inserted a transgene (*white* gene) that controls eye pigmentation in the fourth chromosome telomere. If the transgene is expressed in a fly eye cell, it will be red, and if the transgene is silenced in a fly eye cell, the cell will be white. Thus, a fly eye can have a variegated phenotype, patches of red and white, for its eye. These varying levels of pigmentation will be compared between parent species and their hybrid progeny.

Our methods to test our hypothesis consist of a three-step approach. First, female *Drosophila melanogaster* virgins were collected. Then these virgin females were crossed with different stocks of another fruit fly specie's (*Drosophila simulans*) males. A one to one ratio of female virgins and males was used to perform crosses and forty of each gender were put into vials and incubated at 20 degrees Celsius. Hybrid flies were then collected every few days and frozen so that we could assess eye pigmentation. Once all hybrid flies were collected, pictures of their eyes were taken under a microscope and uploaded to a computer. IMAGEJ, a software program, allowed us to analyze average pixel value (a measurement of pigment) in the hybrid eyes. The darker the eye the lower the average pixel value, and the lighter the eye the higher average pixel value. These values were then compared to the average pixel values of the parent species.

Thus far, we have completed crosses and eye pigment analyses for Australia and Malawi background hybrids. These results indicate that hybrid fruit flies have consistently less expression of the *white* transgene than the pure species parents (figure 1). This supports the hypothesis that there is chromatin mispackaging in the hybrids that results in their reduced fitness.

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