Background: There is an incredible morphological diversity in the animal kingdom. However, despite the number of differing morphological traits between animals, they all share a small set of highly conserved toolkit genes (developmental genes) that build their basic body plans [1, 2]. After development, some of these genes initiate the formation of other novel traits such as color patterns. Fruit flies are ideal candidates for studying these developmental genes because of their short lifespans, ease of genetic manipulation, and they exhibit complex color patterns on their wings and abdomen. Our lab chose *Drosophila guttifera* to study these toolkit genes because of the vivid, unmistakable color patterns that exist on the wings, the thorax, and the abdomen (Fig. 1). Furthermore, the genetic networks underlying pathways for fruit fly color patterns are similar to cancer and disease-causing pathways in humans [3].

Figure 1: Intricate spot patterns are displayed on *Drosophila guttifera*'s wings, thorax, and abdomen.

There are six longitudinal rows of melanin spots on the abdomen of *D. guttifera.* These melanin spots are arranged in sub-patterns, which combine to make up the entire pattern (Fig. 2). Production of the melanin spots is induced by multiple genes including the downstream pigmentation gene *tan* (*t*) [4]. The *t* gene is responsible for the production of brown pigment found in the spots. From 100 genes that are highly conserved between fruit flies and humans, our lab has produced extensive preliminary *in situ* hybridization data, which suggest that there are at least five developmental genes responsible for controlling expression of the *t* gene. These five genes are the morphogen genes *wingless* (*wg*), *decapentaplegic* (*dpp*), and *hedgehog* (*hh*), as well as the transcription factor genes *abdominal-A* (*abd-A*) and *zerknullt* (*zen*). These five genes are expressed in distinct sub-patterns on the developing abdomen upstream of *t* gene expression and foreshadow adult abdominal color patterns in *D. guttifera* (Fig 2). Preliminary data from our lab using a transgenic protocol published in my advisor's (Dr. Thomas Werner)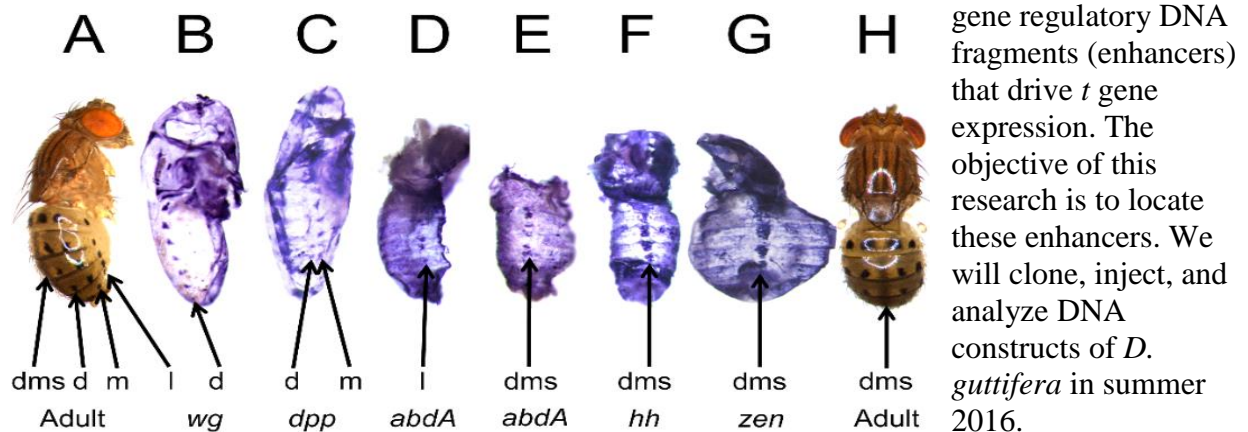 *Nature* article [2] suggest the location of several gene regulatory DNA fragments (enhancers) that drive *t* gene expression. The objective of this research is to locate these enhancers. We will clone, inject, and analyze DNA constructs of *D. guttifera* in summer 2016.

Figure 2: The entire abdominal color pattern of *Drosophila guttifera* may be induced by five developmental genes that regulate specific sub-patterns. The pattern consists of the dorsal midline shade (**dms**), and the dorsal (**d**), the median (**m**), and the lateral (**l**) pairs of spot rows.

Aim: The aim of this research is to locate the enhancers that control expression of the *t* gene on the abdomen of *D. guttifera.* Our lab already identified the enhancer sequences for the *yellow (y)* pigmentation gene last summer (a SURF project). Both the *y* and *t* genes contribute to the pigmentation patterns across insects, where they appear to be co-expressed in identical patterns that foreshadow pigmentation. How the co-expression of *y* and *t* is facilitated is unknown. The identification of the *t* enhancers of in this SURF project will thus allow us, for the first time, to answer the question of how two independent genetic loci end up being expressed in identical patterns. Notably, *D. guttifera* shows the most complex known co-expression pattern of *y* and *t*. Uncovering the genetic networks by which upstream-acting developmental genes control the spot expression pattern of the *t* gene in the abdomen of *D. guttifera* will enhance our understanding of how complex color patterns have evolved in animals.

Hypothesis: Based on our preliminary *in situ* hybridization data, I hypothesize the presence of four enhancers that control abdominal expression of the *t* gene and that each controls a specific sub-pattern of expression on the developing abdomen: 1) the dorsal midline shade (**dms**), 2) the dorsal pair of spot rows (**d**), 3) the median pair of spot rows (**m**), and 4) the lateral pair of spot rows (**l**) (Fig. 2) [2,4-7].

Methods: The location of the *t* gene enhancers driving color pattern expression on the developing *D. guttifera* abdomen will be identified by dividing the non-coding segments of the *t* locus into partially overlapping fragments of sizes ranging between 500 bp and 5000 bp. These fragments will be sub-cloned in front of the red-fluorescent protein (*DsRed*) gene. The reporter constructs will then be cloned into the *piggyBac* (*pBac*) transposon vector. I will work with graduate student Komal Raja to inject a construct into at least 1000 *D. guttifera* embryos for each reporter construct to produce transgenic lines. Transgenic individuals will be screened at the larva stage with the help of GFP expression in their eyes, using a fluorescent microscope. Once the transgenic larvae form pupae, they will be screened for the *DsRed* expression pattern on the developing abdominal epidermis. The *DsRed* expression pattern will be observed on the developing abdominal epidermis if the injected reporter construct contained an enhancer. Each enhancer is expected to be expressed in a specific sub-pattern, such as pairs of spot rows (see Fig. 2). After I have identified an enhancer, the DNA construct will be sub-divided into smaller fragments with the help of the graduate student. We will then use these fragments to identify the core enhancer sequences and to identify possible transcription factor binding sites that regulate *t* gene expression across the developing abdomen.

Predicted Outcome: I expect to identify between one and four enhancers of the *t* gene that regulate the spot pattern expressed on the *D. guttifera* abdomen.

Future Applications: The use of fruit flies as a model organism has long been responsible for promoting our understanding of genetics and genetic diseases, including many human diseases. The expression of the *t* gene (and the *y* gene) appears to be regulated by highly conserved developmental toolkit genes involved in animal development. Some of these genes, such as *wg*, *hh,* and *dpp*, are proto-oncogenes that can mutate into oncogenes and cause cancer in humans [3]. Thus, the study of genetic pigmentation pathways in *Drosophila* is likely to contribute to our knowledge of how human cancer genes and pathways function.

Works Cited

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