

Natural Variation in Juvenile Hormone Mimic induced Melanotic Tumors in *Drosophila*

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ABSTRACT

When *Drosophila melanogaster* undergoes metamorphosis their blood cells transform in shape and become lamellocytes, which encapsulate tissue and produce melanotic tumors. This occurs as a response to infection or overgrowth of a tissue; however, the effect of hormone regulation in this process is relatively unknown. Metamorphosis in *Drosophila* is controlled by two hormones, 20-hydroxyecdysone and juvenile hormone (JH), which regulate changes in gene expression during metamorphosis, and inhibit ecdysone from making these changes earlier during development, respectively. The formation and development of blood cells occurs in the lymph gland, which differentiates throughout the larval period and its metamorphosis is triggered by a signal produced when ecdysone has reached peak levels. Larval exposure to juvenile hormone mimics (JHM) leads to melanotic tumors. The purpose of this experiment is to characterize variation in lymph gland sensitivity to JHM across a collection of natural caught variants and map the changes in sensitivity to differences in their genotypes. In this experiment approximately 40 *Drosophila* Genetic Reference Panel (DGRP) lines were treated with JHM, methoprene. The production of melanotic tumors among the different genotypes was observed after the treatment. There was significant variation in the incidence and type of tumors produced across the lines examined. We divided the tumors into at least three categories. The first category contains individuals that portray several (more than 1) very small tumors, possibly melanized crystal cells. The second category includes those with a single, big tumor (bigger than a segment). In the third category the shape of the tumors is distinguishably different, the tumors seem to be a chain starting with a big tumor followed by smaller ones trailing down the body of the fly. It is hypothesized that because of this particular shape, these tumors may be a result of melanization of the lymph gland, itself.

OBJECTIVES

- Characterize variation in melanotic tumor formation in the DGRP lines.
- Categorize the different kinds of melanotic tumors based on their size and shape.
- Determine what tissue the melanotic tumors may be originating from.
- Perform a genome wide association study (GWAS) to find polymorphisms associated with tumor formation.
- Use genetic mapping to narrow down the associated polymorphisms from the GWAS analysis to determine whether the mutation is on the 2nd or 3rd chromosome.

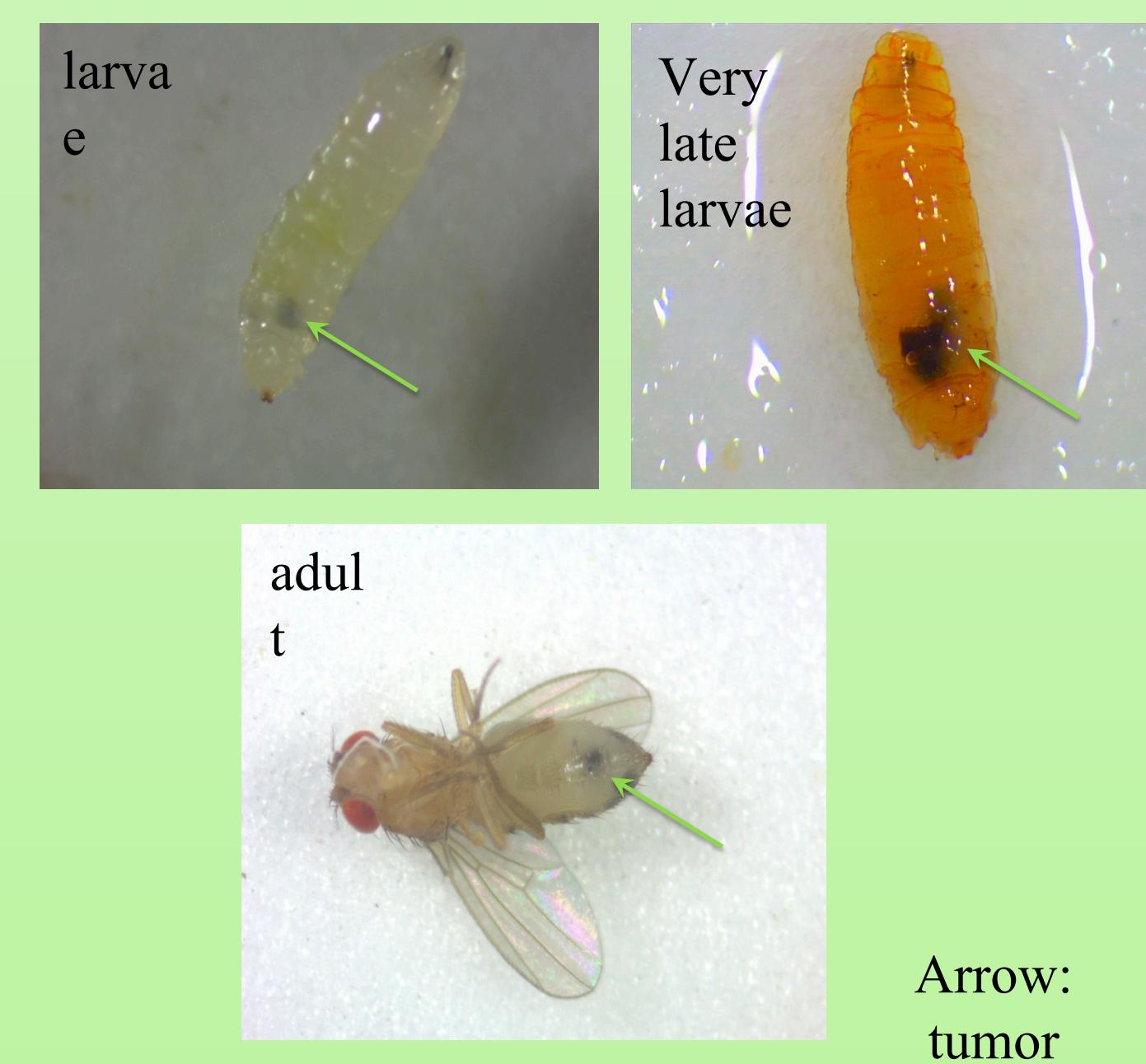
HYPOTHESES

- We predict that there is natural variation in JH induced tumor type.
- We predict that the different tumor types have different developmental origins.
- We predict that sensitivity to produce tumors is controlled by a single mutation of large effect.

BACKGROUND INFORMATION: Melanotic tumors

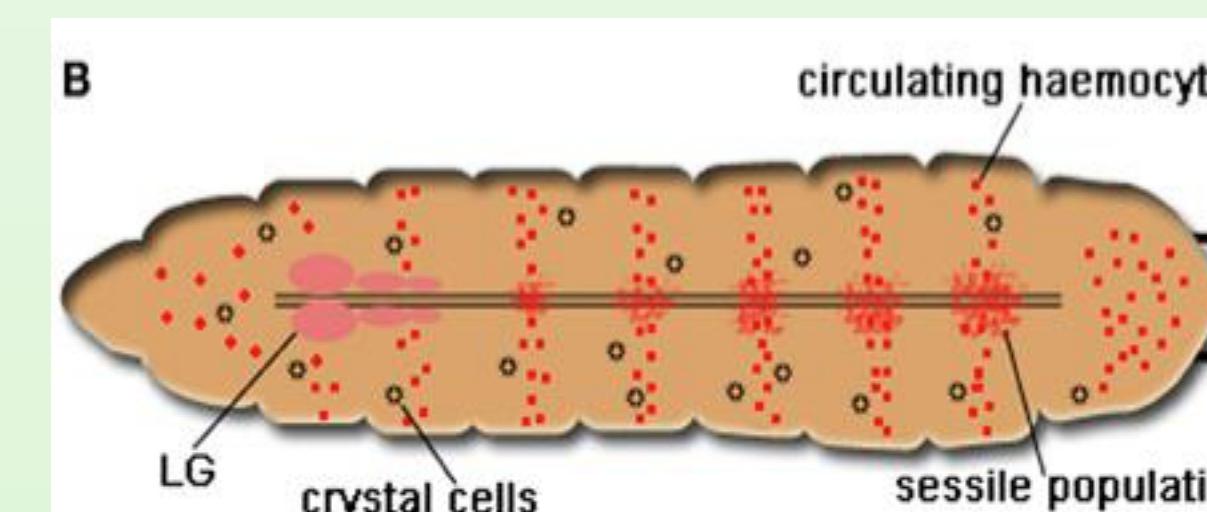
- There have been many reported cases of hereditary melanotic tumors in *Drosophila*, however not much is known about the role of hormones in their formation. (Rizki 1960)
- These tumors represent accumulations of transformed blood cells, a variant called lamellocyte. These cells encapsulate tissues and can result in melanized tissue masses. (Rizki 1960)
- The process of transformation these blood cells go through has been tied to the processes of pupation and metamorphosis. (Rizki 1960)
- Larval fat body development is regulated by two hormones: Ecdysone and Juvenile Hormone. (Rizki 1960)

Examples of tumor development at different developmental stages



BACKGROUND INFORMATION: Lymph gland

- The formation and development of blood cells in *Drosophila* occurs in the lymph gland
- Lymph gland metamorphosis is triggered by a signal produced when ecdysone has reached peak levels.
- The lymph gland "is comprised of a large primary lobe and several smaller lobes (see top left of picture A). Differentiation into two types of hemocytes, plasmacytoid and crystal cells, is confined to the outer layer of the primary lobe; the center of the primary lobe and secondary lobes only contain proliferating prohemocytes."
- The blood cells differentiate throughout the larval period (Grigorian 2014)



Developing blood cells in larvae (adapter from (Wang, 2014)). LG=lymph gland, crystal cells = sessile population

B) Larval hematopoiesis. The LG (in pink) composes of the primary and secondary lobes and is located in the anterior end of the larva along the dorsal aorta. The sessile hemocyte population distributes diffusely along the segmental borders of the larva and consists of functional differentiated hemocytes and a few prohemocytes with an embryonic origin (shown in the same red color). Until the end of the third star, circulating hemocytes including plasmacytoid and crystal cells (small black circles) are derived from the embryonic hemocytes.

METHODOLOGY

Experiment #1: Determine if there is variation in hormonally induced tumor formation

- Expand, treat with methoprene (JH mimic), and score different genotypes (~40).
- Record and observe flies in different developmental stages and document production of tumors. For tumor generating lines, categorize tumors based on shape, size and abundance.

Experiment #2: Determine if tumors originated from the fat body

- Create a stable stock of homozygote flies with GFP (Green Fluorescent Protein) expressed in the fat body.
- Cross female virgins carrying the GFP driver to males of some of the melanotic lines with abundant and big tumors that could possibly be fat body derived.
- Treat the offspring of the crossed individuals with JH and observe under fluorescent light to determine if tumor is coming from the fat body.
- Dissect individuals in order to better determine the origin of the melanotic masses.

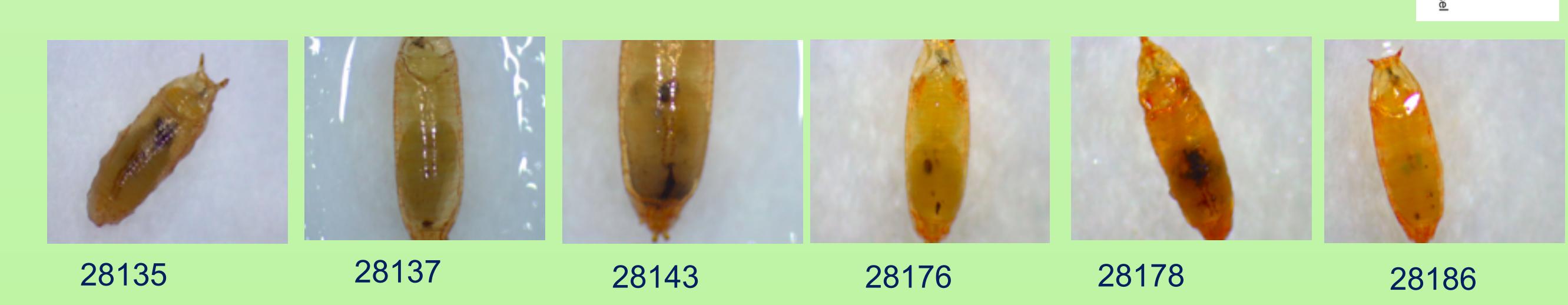


Prepupae expressing GFP in fat body cells

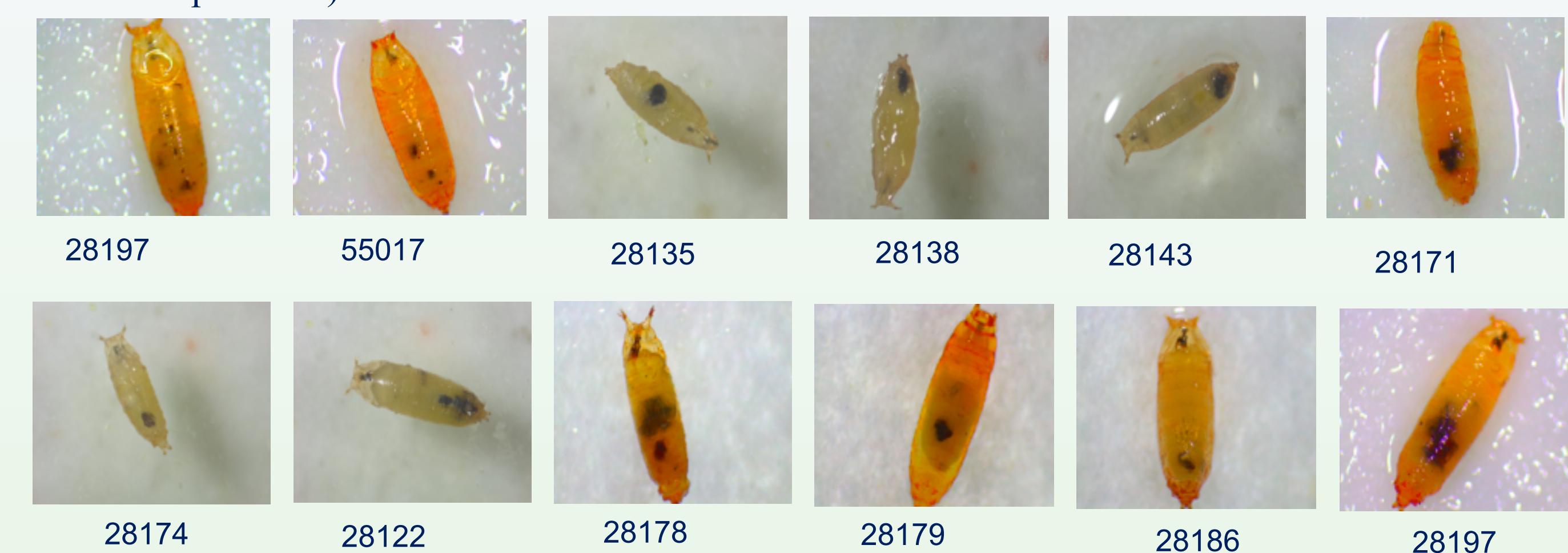
RESULTS

We found that there is natural variation in JH induced tumors. We split the phenotypes into 3 different categories.

Category #1: Big tumor followed by smaller ones trailing down the body (possibly lymph gland melanization).



Category #2: One Single Big tumor > a segment, possibly fat body encapsulation. (Usually towards posterior)



Category #3: Several very small tumors < a segment, possibly excessive crystal cell melanization. Scattered throughout the body.



CONCLUSIONS

Our original prediction that there would be variation in the types of tumor produced after JH treatment was indeed supported by the several categories that we were able to develop based on the differences in tumor appearance.

FUTURE DIRECTIONS

- Conduct similar experiments for another set of different genotypes and expand categories created if there is increased variation in the tumor production and appearance.
- Use genetic mapping to verify whether individuals have to be homozygotes in order for the tumors to be produced.
- Dissect larvae and observe the lymph gland separately in order to verify hypothesis that it is being melanized.

REFERENCES

- Wang, Lihui, Ilias Kounatidis, and Petros Ligoxygakis. "Drosophila as a Model to Study the Role of Blood Cells in Inflammation, Innate Immunity and Cancer." *Frontiers in Cellular and Infection Microbiology* 3 (2014): 113. Frontiers. Web. Oct. 2016.
- Hendrix, J. D. "Genetic Mapping in *Drosophila melanogaster*." *SpringReference* (N. D.): 1-4. Science Kennesaw State University. Web.
- Rizki, M. T. "Melanotic Tumor Formation in *Drosophila*." *Journal of Morphology* 106.2 (1960): 147-57. Middlebury College Interlibrary Loan. 30 Nov. 2015. Web. 30 June 2016.
- Grigorian, Melina, Lolitika Mandal, and Volker Hartenstein. "Hematopoiesis at the Onset of Metamorphosis: Terminal Differentiation and Dissociation of the *Drosophila* Lymph Gland." *Development genes and evolution* 221.3 (2011): 121–131. PMC. Web. Oct. 2016.