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## Introduction

- Pleiotropy is a case in which a single gene affects two or more otherwise unrelated traits. This may cause limitations on changes to some adaptations due to gene correlation with other traits, as well as result in decreased ability to predict how organisms will adapt to a given environment (2).
- This may be seen in the relationship between melanin-based coloration and aggression behaviors in *Drosophila melanogaster*. Differences in melanin-based coloration have been correlated with various differences in behaviors in several species (1).
- Dopamine is a precursor in the melanin biosynthesis pathway in *D. melanogaster*, so increased melanin utilization for coloration may result in less available free dopamine, which may cause more aggressive behaviors (3).

**Hypothesis:** We hypothesized that selecting for darker pigmentation would result in more aggressive behaviors while selecting for lighter pigmentation would result in less aggressive behaviors.

## Methods

- Light and dark color flies were selected with aggression tests being run every five generations up to generation 15.
- ImageJ was utilized to analyze the color of the trident section of the flies' thoraxes.
- Images were made grayscale and calibrated for black and white values (0 and 255) utilizing the black and white background of the images.
- The freeform selection tool was utilized to analyze the color of the trident after color calibration. The values were recorded in a spreadsheet.

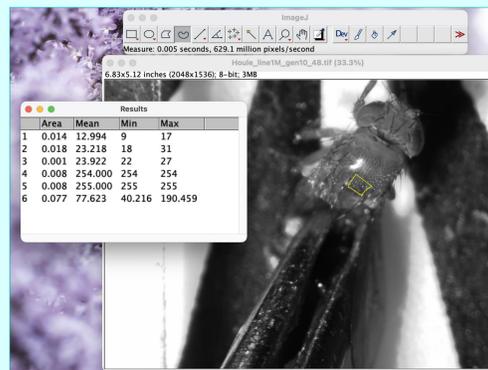


Figure 1: ImageJ software interface with black and white calibration values in the table and the trident area of the fly selected with the freeform tool.

## Results

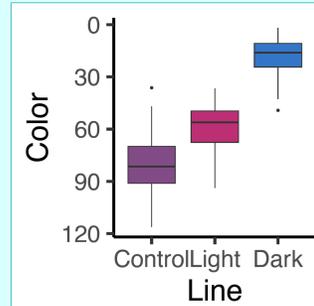


Figure 2: An example of the color data obtained from beta regression model. This graph was for replicate 1 generation 10 males. All other graphs showed similar distributions.

	Control vs. Light	Control vs. Dark	Light vs. Dark
Replicate 1 Females	p-value = 0.5568	p-value < 0.0001	p-value < 0.0001
Replicate 1 Males	p-value < 0.0001	p-value < 0.0001	p-value < 0.0001
Replicate 2 Females	p-value = 0.9998	p-value = 0.0355	p-value = 0.0343
Replicate 2 Males	p-value = 0.9823	p-value < 0.0001	p-value < 0.0001

Table 1: All color data analyzed via beta regression model.

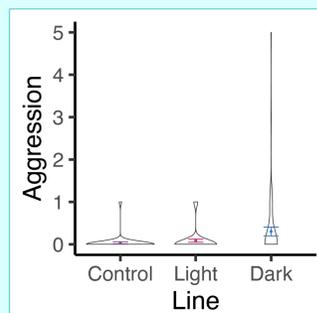


Figure 3: Aggression data using hurdle model for replicate 1 generation 15 males and females.

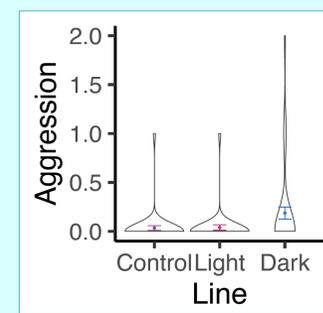


Figure 4: Aggression data using hurdle model for replicate 2 generation 15 males and females.

	Control vs. Light	Control vs. Dark	Light vs. Dark
Replicate 1	p-value = 0.5568	p-value < 0.0001	p-value < 0.0001
Replicate 2	p-value = 0.9998	p-value = 0.0355	p-value = 0.0343

Table 2: All aggression data analyzed via hurdle model.

## Results Cont.

- The color of flies considered to be light was shown to vary significantly from those considered dark in all groups as all p-values were below 0.05, with most under 0.0001
- Darker colored flies were shown to be significantly more aggressive than those of lighter color as all p-values for the difference of aggression between light and dark were below 0.05, with the first replicate being even lower.

## Conclusions and Future Directions

- Darker flies were seen to be significantly more aggressive than lighter colored flies when selecting for both light and dark colors across multiple generations.
- This supports our hypothesis that dark *D. melanogaster* individuals are more aggressive than their lighter counterparts.
- This provides more evidence for a possible pleiotropic effect between color and aggression in *D. melanogaster*.
- The next step in this research will be to determine the exact genes which are causing this pleiotropic effect.

## References

1. Ducrest, A.-L., Keller, L., & Roulin, A. (2008). Pleiotropy in the melanocortin system, coloration and behavioural syndromes. *Trends in Ecology & Evolution*, 23(9), 502–510.
2. San-Jose, L. M., & Roulin, A. (2018). Toward Understanding the Repeated Occurrence of Associations between Melanin-Based Coloration and Multiple Phenotypes. *The American Naturalist*, 192(2), 111–130.
3. Takahashi, A. (2013). Pigmentation and behavior: potential association through pleiotropic genes in *Drosophila*. *Genes & Genetic Systems*, 88(3), 165–174.

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