# Characterization of Genes Influencing the Age-specific Changes in Phagocytosis of *Drosophila melanogaster,* Using an *in vivo* Phagocytosis Assay

**Shonda Campbell,** Murielle Abissi, Dr. Jeff Leips
University of Maryland Baltimore County, Baltimore, MD
Biological Sciences Department

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## Introduction

- The immune response declines with age. However, the effect of age on immunity varies among individuals. Genetic differences among individuals contribute to this variation, but the genes responsible are largely unknown.
- A previous study [1] in our lab identified a number of candidate genes involved in clearing an *E. coli* infection, and the expression of these genes varied significantly between young and old flies.
- Interestingly, a small category of genes were associated with the ability of older flies to clear an infection. These were genes involved in cytoskeleton organization and endosomal/lysosomal/vesicle transport.
- **Because of that, we hypothesize that age-related changes in some aspects of phagocytosis are important for maintaining immune function in older flies.** To test this, we used an *in vivo* phagocytosis assay [2] that allows us to assess the age-specific effects of genes on different aspects of phagocytosis.

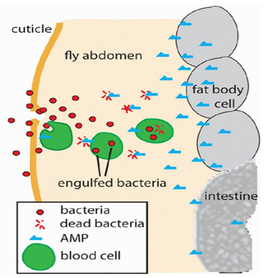


**Figure 1:** The innate immune response in *Drosophila* involves secretion of antimicrobial peptides and phagocytic hemocytes whose function is to engulf and destroy bacteria after infection. Figure courtesy of Dr. Starz-Gaiano.

## Materials and Methods

- Used RNAi lines to knock down candidate gene expression.
- One- and 5-week old females were injected with either Bioparticles or pHrodo.
- Hemocytes along the dissected dorsal were visualized and fluorescent events per hemocyte were counted.

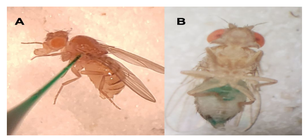


**Figure 2: Injection site (A) and visual verification (B).**

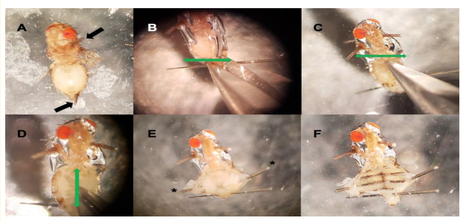


**Figure 3: Dorsal vessel dissection. (A)** Pin thorax and posterior end of abdomen (black arrows). **(B, C, D)** Make incisions (green arrows**). (E)** Filet open the abdominal cavity, and remove internal tissue **(F)**.

## Results

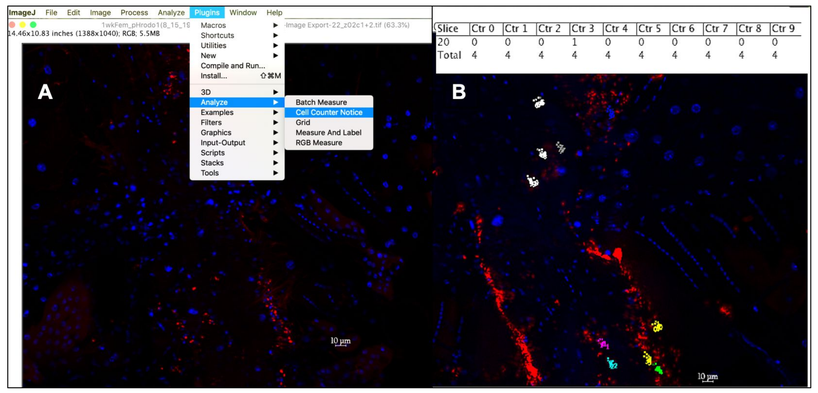


**Figure 4: Quantifying phagocytic events within a 10 µm hemocyte using the cell counter in ImageJ (A)** to keep track of phagocytic events per cell. **B)** This will assign a different color to each cell counted. Each dot corresponds to a fluorescent event within that cell.

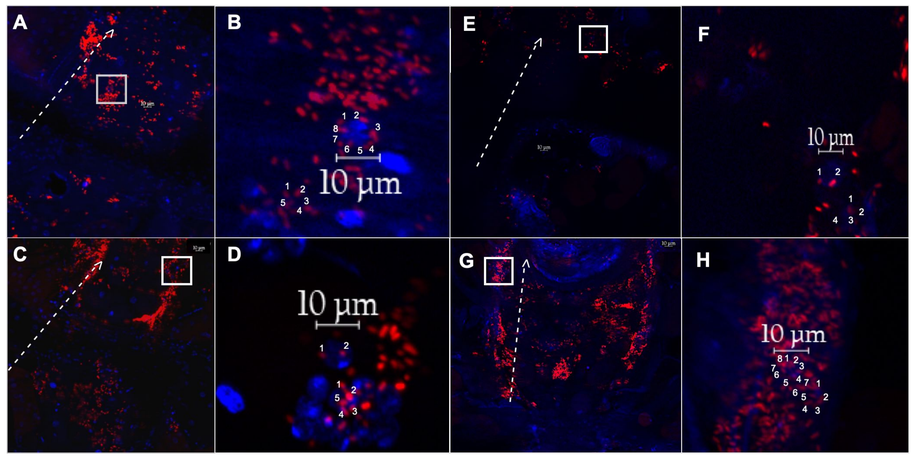


**Figure 5:** The dorsal vessel and associated hemocytes with engulfed *E. coli* pHrodo particles **(A and C)** or BioParticles **(E and G)** (red), from a 1-week and 5-week old fly, respectively, after recovering for 60 mins. **(B, D, F, and G)** Magnified inset of A, C, E and G (white box), respectively, showing two individual hemocytes with countable events. Dotted white line outlines the lateral side of the dorsal vessel, with arrow pointing towards the anterior region. Nuclei stained with DAPI (blue).

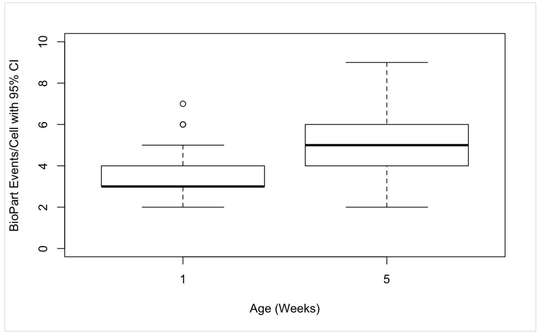


**Figure 6:** Box plots of phagocytic ability of 1-week and 5-week old virgin females, measured as the number of fluorescent *E. coli* Bioparticles per hemocyte at 60 min post injection. Boxes represent the middle two quartiles, separated by a line representing the median, and the whiskers show the 10th and 90th percentiles. N = 7 flies (70 cells) per age. Dots indicate outliers.
P < 0.001.

## Results Continued

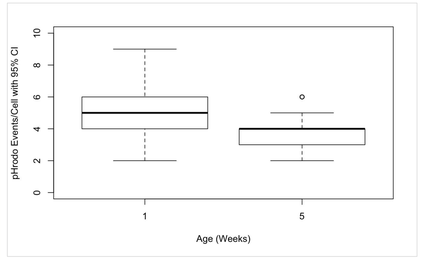


**Figure 7:** Box plots of phagocytic ability of 1-week and 5-week old virgin females, measured as the number of fluorescent pHrodo *E. coli* particles per hemocyte at 60 min post injection. Boxes represent the middle two quartiles, separated by a line representing the median, and the whiskers show the 10th and 90th percentiles. N = 7 flies (70 cells) per age. Dots indicate outliers.
P < 0.001.

## Discussion

- Here, *Hemese* (w*; P{w[+mC] = He-Gal4.Z}85, P{w[+mC] = UAS-GFP.nls}8) flies were used.
- When injected with Bioparticles, aged flies contained significantly more fluorescent events per cell compared to young flies.
  - ➢ This is consistent with results from a previous study done in our lab [3].
- When injected with pHrodo, aged flies contained significantly fewer fluorescent events per cell compared to young flies.
  - ➢ pHrodo is pH sensitive, so observing more fluorescent events indicates phagosome-lysosome fusion, required to efficiently degrade bacterial particles.
  - ➢ This suggests that aged flies have a reduced ability to degrade an infection once engulfed.
- These results support our hypothesis, and indicate that age-related changes in phagocytosis do occur, reducing the ability of older flies to efficiently clear an infection.
  - ➢ Changes likely occur during phagosome maturation, reducing or inhibiting phagosome-lysosome fusion.

## Future Directions

- Characterize select candidate genes for their role in age-specific declines in phagocytosis, using RNAi lines.
- Determine if these age-related changes are also sex-specific.

### Acknowledgements

- Dr. Michelle Starz-Gaiano for collaborating on this project.
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### References

1. Felix, T., et al. (2012). Age-specific Variation in Immune Response in *Drosophila melanogaster* Has a Genetic Basis. Genetics, 191 (3), 989-1002.
2. Campbell, S., Starz-Gaiano, M. & Leips, J. (2019). Assessing the Age-specific Phagocytic Ability of Adult Drosophila melanogaster Hemocytes Using An In vivo assay. JoVE
3. Horn, L., Leips, J. & Starz-Gaiano, M. (2014) Phagocytic Ability declines with age in adult Drosophila hemocytes. Aging Cell, 13 (4), 719-728