# Reproduction and immunity trade-offs in *Aedes aegypti* mosquitoes

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## Abstract

Reproduction and immunity are metabolically expensive systems; therefore, organisms with a limited amount of resources have to invest carefully (Schwenke et al. 2016). With the goal of producing offspring, organisms must invest resources into reproduction, yet also reserve resources for protecting themselves. In many cases reproduction and immunity are not directly linked. However, it has been shown in *Aedes aegypti* mosquitoes that, in addition to inducing egg production, a blood meal also increases the number of circulating immune cells called hemocytes (Castillo et all. 2011; Castillo et al. 2006). These findings elicit the question, does reproduction (blood-feeding) result in lower immunity for the mosquito? Through a variety of bacterial injections into blood-fed and non-blood-fed mosquitoes, this project worked to understand the possible tradeoffs between immunity and reproduction. We found an inverse relationship between immunity and reproduction, in that mosquitoes laid fewer and smaller eggs when injected with both live and heat-killed bacteria, but only for the more virulent species and higher doses. Interestingly, the results also showed that blood-fed mosquitoes were more successful in clearing or tolerating less virulent bacterial infections, suggesting resources gained from a blood meal are used to produce an anticipatory immune response. These preliminary findings are essential for continuing research and strengthening our understanding of the *A. aegypti* immune system with hopes of controlling or preventing diseases propagated by *A. aegypti* in the future.

## Introduction

### Disease Transmission

*Aedes aegypti* is capable of transmitting

- Yellow fever
- Dengue fever
- Zika fever
- Chikungunya
- Mayaro

**Figure 1:** *A. aegypti* non-blood-fed female (left) and blood-fed female (right).

Photo: Jena Johnson

### Reproduction

*A. aegypti* females require a blood meal to obtain proteins for egg production.

One blood meal returns one egg clutch. A female can lay two to four clutches over her lifetime.

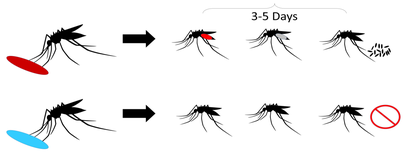


**Figure 2:** A blood meal is required each time before *A. aegypti* can lay a clutch of eggs. It takes 3-5 days after feeding to digest the nutrients and reproduce. A diet of water and nectar will not suffice.

### Trade-off

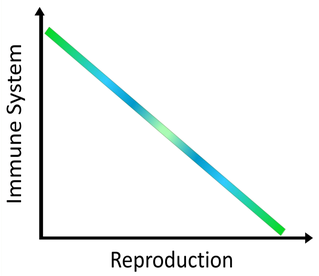


**Figure 3:** Hypothesized inverse relationship between the immune system and reproduction.

### Immune System

*A. aegypti* immune system is comprised of multiple defense reactions

- Physical barriers
- Local immune response
- Systemic immune response

Antimicrobial peptides (AMP), reactive oxygen species, phenoloxidase (PO), and specialized hemocytes all defend the organism from infection.

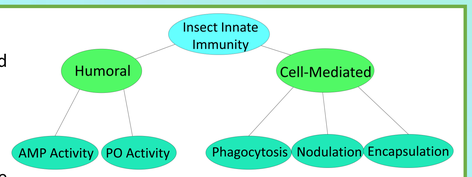


**Figure 4:** Conceptual map of the immune system of *A. aegypti.*

Image Idea: Kimberly Katie Booth, North Dakota State University

## Methods

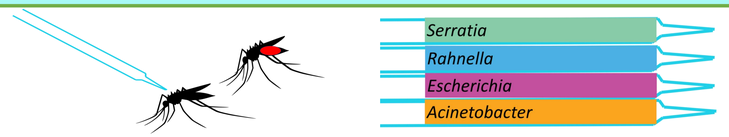


**Figure 5:** Blood-fed (BF) and non-blood-fed (NBF) mosquitoes were tested in each experiment via injection of four naturally occurring bacteria that are resistant to particular antibiotics and that were transformed to express green fluorescent protein (GFP). Mosquitoes were injected with living or heat-killed bacteria of each species at two doses ($10^3$ and $10^5$ cells). Uninjected mosquitoes served as a control.

## Results and Conclusions

### Survival Assay

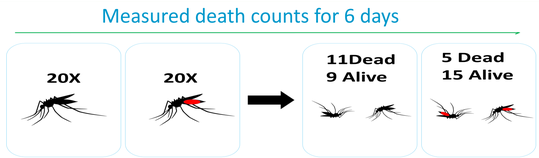


**Figure 7:** Over 6 days, groups of 20 NBF and 20 BF mosquitoes were monitored for survival following injection of living or heat-killed bacteria in three independent replicates.

### Survival Assay

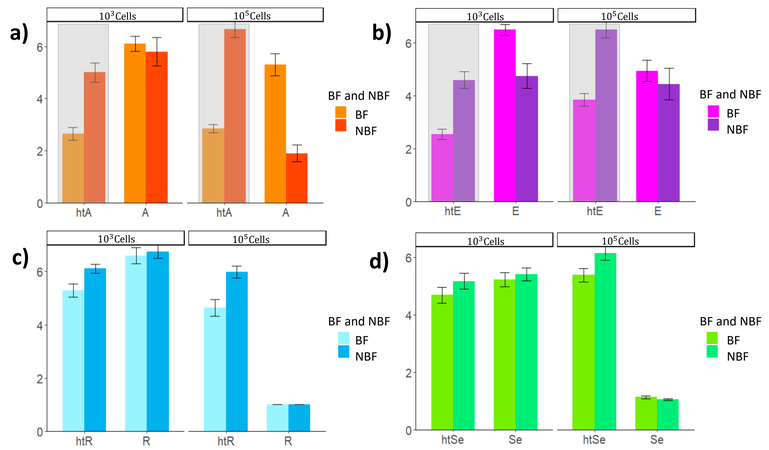


**Figure 10:** Survival (days) for a) *Acinetobacter* b) *Escherichia* c) *Rahnella* and d) *Serratia* grouped by feeding type after injection with $10^3$or $10^5$ bacterial cells ± standard error.

- **Survival varied between the species of bacteria tested**
- **Survival varied with infection dose**
- **Some species differentially affected BF and NBF mosquitoes**
- ***Acinetobacter* and *Escherichia* were classified as less virulent while *Rahnella* and *Serratia* were classified as more virulent**
- **Issues with some of the heat-treated bacteria need to be further investigated**

## Acknowledgements and References

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Castillo, J, Brown, MR and Strand, MR (2011) Blood feeding and insulin-like peptide 3 stimulate proliferation of hemocytes in the mosquito Aedes aegypti. *PLoS pathogens* **7**: e1002274.
Castillo, J, Robertson, A and Strand, M (2006) Characterization of hemocytes from the mosquitoes Anopheles gambiae and Aedes aegypti. *Insect biochemistry and molecular biology* **36**: 891-903.
Schwenke, RA, Lazzaro, BP and Wolfner, MF (2016) Reproduction–immunity trade-offs in insects. *Annual Review of Entomology* **61**: 239-256.

### Egg Counts and Size

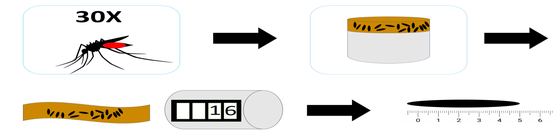


**Figure 6:** After bacterial injections, BF mosquitoes were placed into individual cages with paper to lay eggs. After 5 days, eggs were counted and measured for length.

### Egg Counts and Size

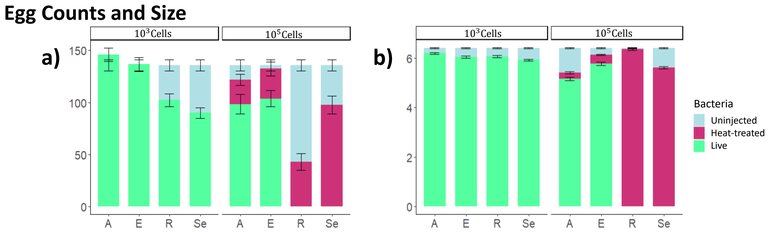


**Figure 9:** Effect of bacteria injection on a) egg count and b) egg size (mm). Each live bacterium treatment (green) was injected at $10^3$ and $10^5$ cells and compared to uninjected controls (blue). Heated-treated bacteria were also injected at $10^5$ cells (pink).

- **Mosquitoes injected with certain species of bacteria exhibited reductions in egg counts when compared to the control treatment**
- **More research needs to be conducted to fill in missing data points**

### Infection Clearance

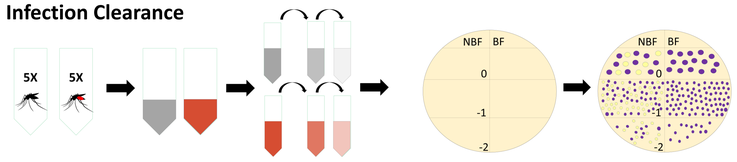


**Figure 8:** Five mosquitoes per treatment were individually homogenized at 0, 6, 24, and 72 hours and dilution series were pipetted onto antibiotic plates to determine the abundance of viable bacteria that were present.

### Infection Clearance

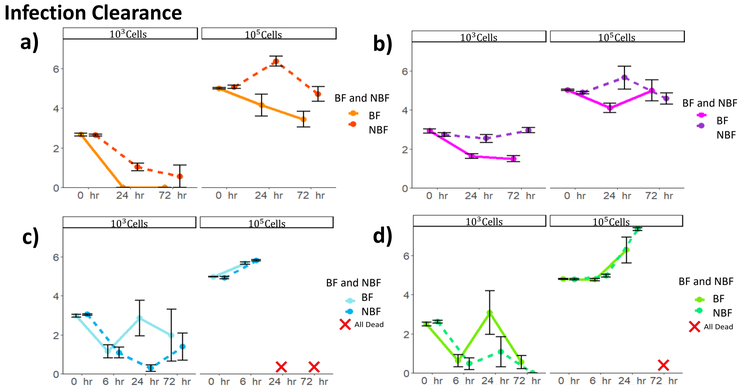


**Figure 11:** The ability of a) *Acinetobacter* b) *Escherichia* c) *Rahnella* and d) *Serratia* to clear a bacterial infection after being injected with $10^3$ or $10^5$ live, GFP-tagged bacterial cells in BF (solid) and NBF (dashed) mosquitoes at 0, 6, 24, and 72 hours.

- **Mosquitoes were less likely to clear virulent species and bacteria injected at higher doses**
- **Mosquitoes cleared *(Acinetobacter)* or tolerated *(Escherichia)* less virulent species**
- **In less virulent species, BF did better with clearance**
- **In more virulent species, NBF did better with clearance**