Female-Specific Larval Lethality in the Yellow Fever Mosquito *Aedes aegypti*

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**Abstract**

The mosquito, Aedes aegypti, is the primary vector of dengue, yellow fever, and Zika viruses. Dengue alone threatens over 390 million people worldwide, causing over 300,000 deaths annually. Chemical pesticides are the main method of disease suppression, but new, environmentally friendly methods of mosquito control are needed. The Sterile Insect Technique (SIT) is a pesticide-free method of locally controlling pest insects by releasing large numbers of sterile males, to out-compete wild males for female mates. For this method to work effectively, few or no females should be released with sterile males as sterile females can still spread diseases. Thus, efficient sex-sorting is needed, and to date, no large-scale sex-sorting methods for mosquitoes have been sufficiently effective for use in sterile insect technique.
Introduction

The mosquito *Aedes aegypti* is the primary vector of dengue, yellow fever, and Zika viruses. Dengue alone threatens over 390 million people worldwide (Figure 1), causing over 300,000 deaths annually. Chemical pesticides are the main method of disease suppression, but new, environmentally friendly methods of mosquito control are needed.

The Sterile Insect Technique (SIT) is a pesticide-free method of locally controlling pest insects by releasing large numbers of sterile males, to outcompete wild males for female mates. For this method to work effectively, few or no females should be released with sterile males as sterile females can still spread diseases. Thus, efficient sex-sorting is needed, and to date, no large-scale sex-sorting methods for mosquitoes have been sufficiently effective for use in sterile insect technique.

Methods

1. Sex-sort larvae by microscopic inspection
2. Long-read RNA sequencing
3. Computer algorithm design to search for male vs. female specific transcripts
4. Prepare bacteria expressing hairpin dsRNAs
5. Feed larvae dsRNA-expressing bacteria
6. Assess if RNAi-mediated knockdown occurred using qRT-PCR
7. Assess impacts of female development

Results

- RNA sequencing identified 114 female-specific transcripts (Figure 4).
- RNA sequencing also identified over 100 new female-specific splice variants (Figure 5).
- qRT-PCR confirms that RNAseq search algorithm is accurate – female specific transcripts are expressed only in females (Table 1).
- Larvae fed on female-specific dsRNAs show delayed growth rates or increased mortality.

Research Objectives

To improve the SIT programs for mosquito control, we will use RNA interference (RNAs) technologies to selectively kill female larvae before they become biting adults (Figure 2).

1. Identify genes that are uniquely expressed or processed (spliced) within female larvae.
2. Identify which female genes, when targeted by RNAi, can prevent female development.

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Table 1: A value of 1 signifies a 100% female-specificity, a value of 0 indicates no sex bias, and a value of -1 signifies a 100% male-specificity. This table only illustrates a small subset of the genes analyzed.

Conclusion

- RNAseq search algorithm has identified 114 new female-specific genes expressed in larvae (confirmed by qRT-PCR).
- RNAi-bacterial feeding mosquito larvae is slowing female development, but the search continues for a gene that prevents female development entirely.
- Slowing female development may still work for SIT – if males develop faster, they can be collected from the lagging females more easily.

References


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