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

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Introduction

The mosquito *Aedes aegypti* is the primary vector of dengue, yellow fever, and Zika viruses. Dengue alone threatens over 390 million people worldwide[1] (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 out-compete wild males for female mates[2]. 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.

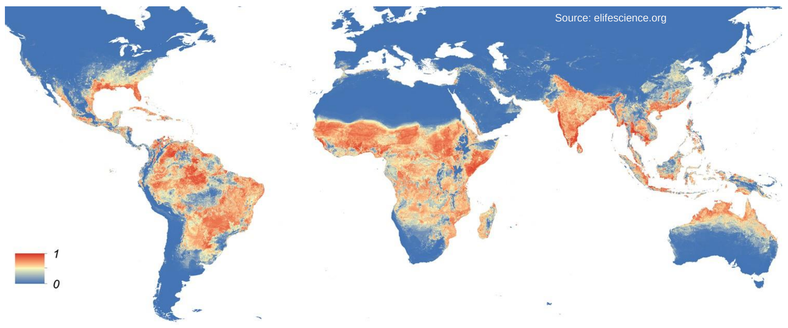


Figure 1: The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus.*

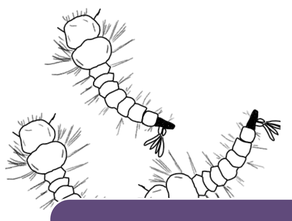
Figure 2: *Aedes aegypti* male (left) and female adults.

Research Objectives

To improve the SIT programs for mosquito control, we will use RNA interference (RNAi) technologies to selectively kill female larvae before they become biting adults (Figure 2). RNAi is a method of silencing a gene's expression by administering double-stranded RNA (dsRNA) to an organism, which results in destruction of the target gene's mRNA. For this project the following objectives must be achieved:

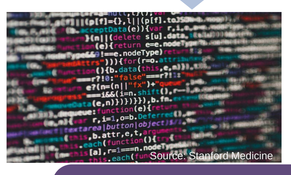
1. Identify genes that are uniquely expressed or processed (spliced) within female larvae.
2. Identify which female genes, when targeted by RNAi, can prevent female development.

Methods

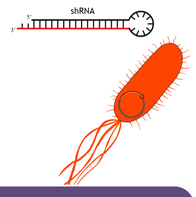


1. Sex-sort larvae by microscopic inspection

2. Long-read RNA sequencing

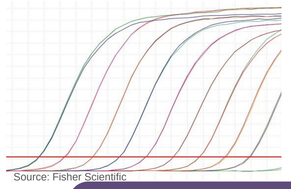


3. Computer algorithm design to search for male vs. female specific transcripts

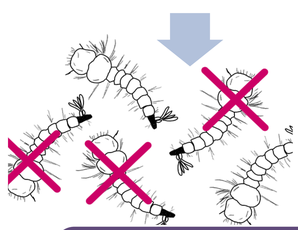


4. Prepare bacteria expressing hairpin dsRNAs

5. Feed larvae dsRNA-expressing bacteria



6. Assess if RNAi-mediated knockdown occurred using qRT-PCR



7. Assess impacts on female development

Gene	Value
4797	1.000
2728	0.691
0641	0.566
9313	0.169
5496	0.162
3217	0.018
4814	0.006
2233	0.000

Female-specific
Male-specific

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.

Results

- RNA sequencing identified 114 female-specific transcripts (Figure 4).

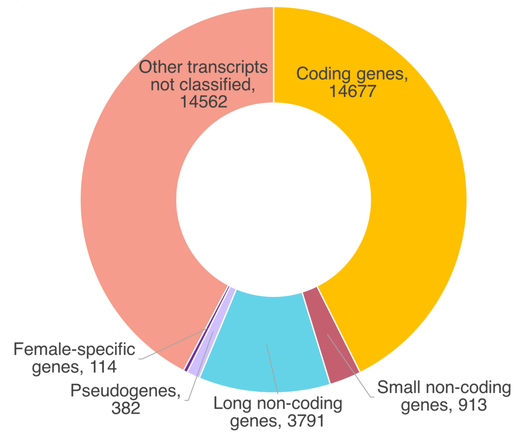


Figure 4: *Aedes aegypti* mosquito has 34 429 gene transcripts. RNA sequencing identified 114 of those to be female-specific in larvae.

- RNA sequencing also identified over 100 new female-specific splice variants (Figure 5).

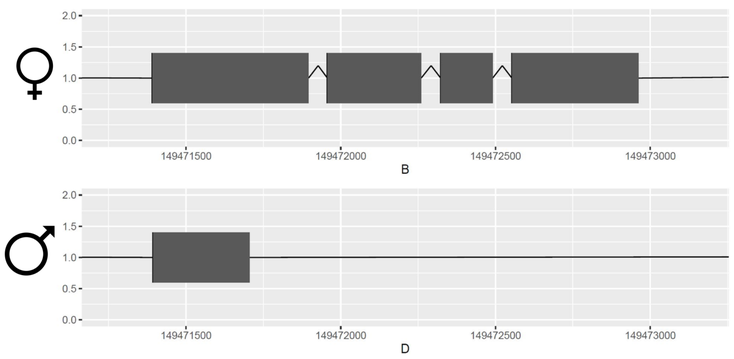


Figure 5. Female- (top) and male-specific (bottom) splice variants.

- 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.

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

1 World Health Organization. Available from https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue [accessed 2 September 2019] (2019).
2 Brady, O.J. *et al. PLoS Neglected Trop. Dis*. 6(8): e1760 (2012).

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