Improving RNAi-mediated sex-separation of mosquitoes for the Sterile Insect Technique

Kousha Kamal, Steve Whyard
Department of Biological Sciences, University of Manitoba, Winnipeg, MB, Canada

University of Manitoba

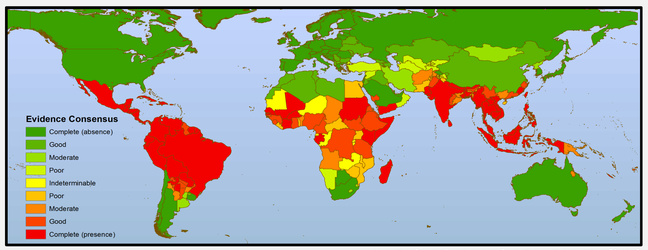
BACKGROUND

- **Sterile Insect Technique (SIT)** involves releasing mass-reared sterile males into an environment to suppress an insect population over time.
- **RNA interference (RNAi)** can be used to knockdown both female-specific and male fertility genes in mosquito larvae to create a cohort of sterilized males for release in the SIT.
- **Problem:** sex-sorting *Aedes aegypti* mosquitoes through RNAi knockdown has not been entirely effective (Whyard et al. 2015).

James Gathany, CDC

AEDES AEGYPTI

- Female *Aedes aegypti* mosquitoes are a major transmitter of Dengue across the world.
- The incidence of this viral infection has increased 30-fold over the past half-century, now affecting up to 100 million people each year (WHO, 2019).



Dengue across the world

O. J. Brady *et al.*, *PLoS Neglect. Trop. D.* **6**, e1760 (2012).

OBJECTIVE

Identify new female-specific genes in *Aedes aegypti* mosquitoes that could be knocked down via RNAi and used to effectively sex-sort them in mass-rearing facilities.

HYPOTHESES & PREDICTIONS

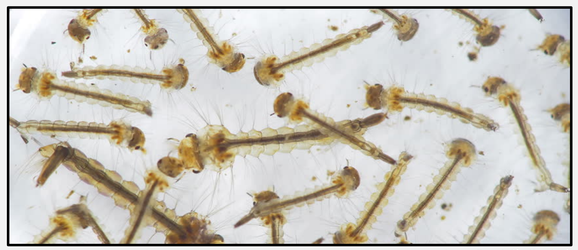
H_A: a subset of the tested genes are either essential or required for female development.
If this hypothesis is true, there will be one of two desirable outcomes:

1. Female lethality
2. Halting of female larval development

METHODS

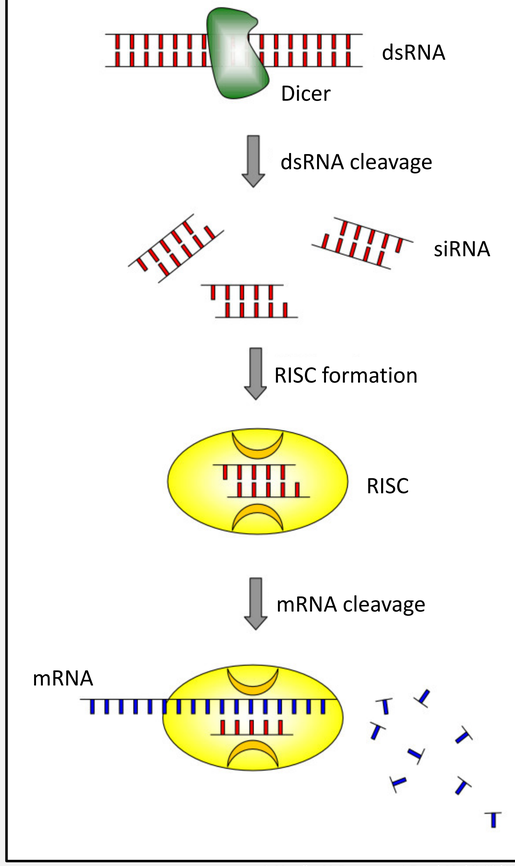
~10 candidate genes will be knocked down using a typical RNAi feeding method. Larvae will be grown in water vials and fed 5mm cubes of agar that contain *E. coli* expressing the desired double stranded RNA (dsRNA) molecule.
Three sets of treatments:

1. **Negative control**: plasmid with no dsRNA.
2. **Gene knockdown**: plasmid with dsRNA homologous to target gene.
3. **Reverse transcription PCR**: to assess the level of knockdown in each treatment. RNA contents will be compared to that of the negative control.



***Aedes aegypti* larvae**

James Benet, Shutterstock.

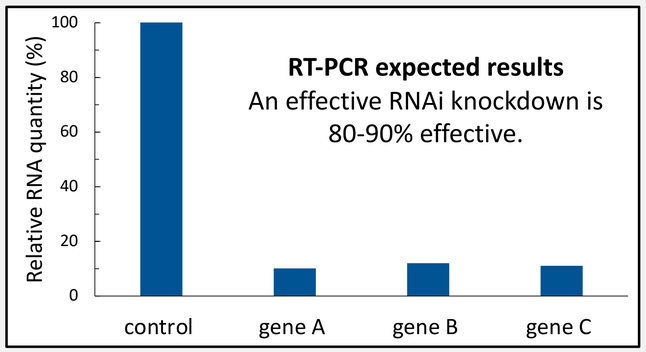


RNA interference

S. Mocellin, M. Provenzano, *J. Transl. Med.* **2,** 39 (2004).

EXPECTED RESULTS

- **Female lethality**: sex ratios of the resulting cohorts will be recorded. Knocking down an essential gene will eliminate close to 100% of the females.
- **Halting female development**: significantly delaying pupation in female larvae could allow for mechanical separation of the sexes. Females typically pupate one day later than males. However, knocking down a gene involved in female pupation will extend this window substantially.



CONCLUSIONS

Developing a robust sex-sorting protocol for *Aedes aegypti* could have serious implications on the prospects of RNAi-mediated vector control in the field.

ACKNOWLEDGEMENTS

I'd like to thank Dr. Steve Whyard, Dr. Jane Waterman, and my fellow BIOL 3100 students for their support and advice.
Work cited: S. Whyard *et al.*, *Parasites Vectors*, **8**, 1 (2015).