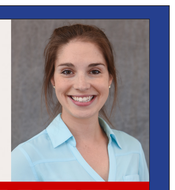
Gene Drive in the Zika Mosquito *Aedes aegypti*

A **Killer-Rescue** Gene Drive System for Mosquito Population Replacement

Sophia H. Webster (shwebste@ncsu.edu) and Max J. Scott
Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC
Genetic Engineering and Society (GES) Center, North Carolina State University, Raleigh, NC



Relevance

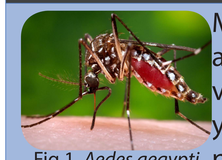


Fig 1. *Aedes aegypti*

Mosquitoes are some of the most dangerous and deadly animals in the world. *Aedes aegypti* vectors zika, dengue fever, chikungunya, and yellow fever that infect hundreds of millions of people each year. The zika epidemic is linked to severe fetal brain and birth defects including microcephaly. **Genetic pest management** using **genetic engineering** is an innovative form of mosquito control that can work in combination with current tools such as insecticides and mosquito habitat removal.

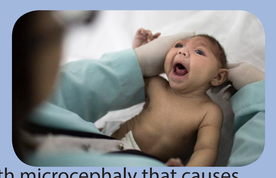


Fig 2 & 3. Newborn with microcephaly that causes severely reduced head size and brain defects

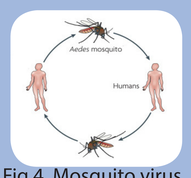


Fig 4. Mosquito virus transmission cycle

Background

Mosquito genetic pest management can be used for several goals, one of which is **population replacement**. To achieve this, mosquitoes are genetically engineered to carry anti-pathogen genes that will be spread through releases of transgenic mosquitoes carrying a gene drive system.

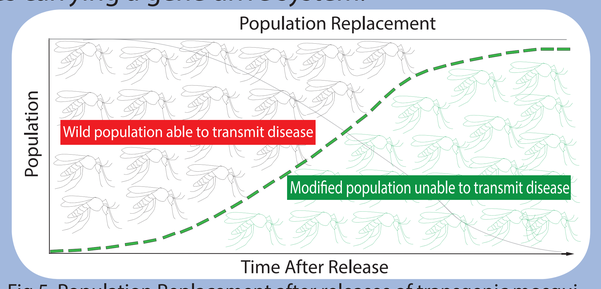
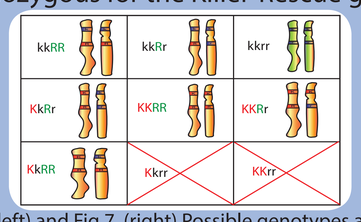


Fig 5. Population Replacement after releases of transgenic mosquitoes carrying an anti-pathogen gene.

Objective

Construct a gene drive system for mosquito population replacement in *Aedes aegypti* and *Drosophila melanogaster* (model organism used for inital testing of system).

This system, named **Killer-Rescue**, consists of two engineered constructs: (1) Killer and (2) Rescue that are present on independently segregating loci. The anti pathogen (when available) will be linked to the rescue. The system functions as a gene drive because only mosquitoes inherting the rescue, with anti-pathogen linked, contribute to the next generation of mosquitoes. There are 9 possible genotypes (Fig 6.), only some of which are viable. The releases of transgenic mosquitoes will be homozygous for the Killer-Rescue genes (Fig 7.).



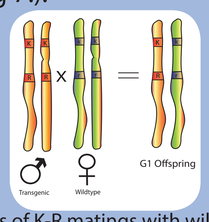


Fig 6. (left) and Fig 7. (right) Possible genotypes after releases of K-R matings with wildtype population. Offspring that inherit only K alleles will not survive and those inherting K&R or R alone will survive and drive the anti-pathogen through the target population.

Outline of Killer-Rescue System

The killer and rescue alleles will be inserted at locations that segregate independently. When transgenics are mated to wildtype mosquitoes their offspring will either inherit killer, rescue, or both. Those inheriting only the killer will die, and those carrying the rescue anti-pathogen will contribute to the next generation of mosquitoes.

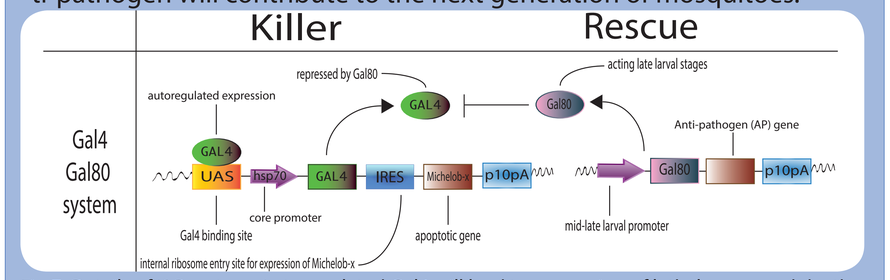


Fig 7. Details of K-R system. Autoregulated Gal4 will lead to expression of lethal genes and death unless rescue is also present in which Gal80 will repress Gal4 and lethality. When mosquitoes inherit both transgenes they also pass on the anti-pathogen gene. When they inherit only the killer, they die.

Materials and Methods

Killer and Rescue constructs (Fig 7.) were designed in MacVector and built with traditional cloning, genomic DNA extraction, promoter isolation from *Aedes aegypti*, PCR, and gel electrophoresis. The constructs were built in the transposable element vectors *PiggyBac* and *pMos*.

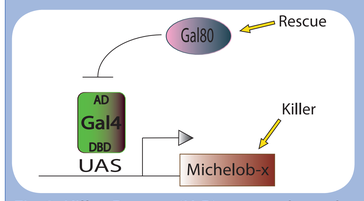


Fig 8. Killer Rescue (K-R) system based on the transcription activator Gal4 (killer) and repressor Gal80 (rescue).

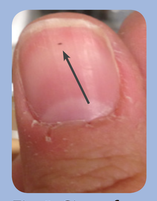


Fig 9. Size of mosquito egg.

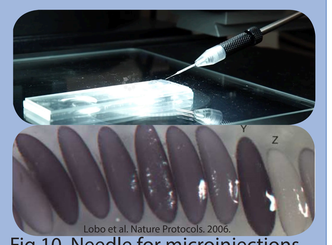


Fig 10. Needle for microinjections and embryos lined up for injecting.

After completing killer and rescue DNA constructs, *Aedes aegypti* embryo injections (Fig 10.) and screening for positive transgenics was accomplished at the Insect Transgenesis Facility at NCSU.

Results in mosquito *Aedes aegypti*

Genetically engineered mosquitoes carrying killer and rescue transgenes have been established based on the system described above. Both killer and rescue constructs contain the Hsp83 *Aedes aegypti* promoter marker (Fig. 10) that is brightly visible in full body from hatch to adult. Mosquitoes carrying the rescue are making Gal80 RNA, confirmed by RNA extraction and PCR (Fig 14.).

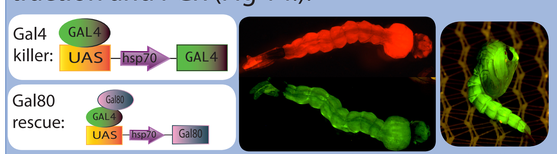


Fig 11. Positive transgenic mosquito lines established with Gal4-killer (red marker) and Gal80-rescue (green marker) constructs. Hsp83 promoter for full body marker expression is used for both killer and rescue constructs. The larvae with killer (red) is also carrying the rescue (green).

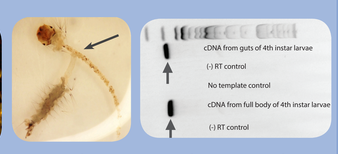


Fig 12. Left: Dissection of transgenic fourth instar larvae. Right: RT-PCR confirmation of rescue gene of interest (Gal80) expression in fourth instar larvae guts and full bodies.

Final experiment is to set up a cage trial for the gene drive. The goals of the experiment are to demonstrate:

(1) The rescue alone does not present fitness costs for the mosquito
(2) When the killer is present alone the mosquito does not survive
(3) When the killer and rescue are both present in the mosquito, the rescue nullifies the toxic effects of the killer.

Results in *Drosophila melanogaster*

Genetically engineered *D. melanogaster* carrying killer and rescue transgenes have been established based on the same system as described in mosquitos (Fig 7.).

The first step to testing K-R in *D. melanogaster* is to ensure Gal80 (rescue) is able to repress Gal4 production. To test this, a color repression experiment was conducted, using Gal4 driver lines and effector lines with a red marker. When the lines with a red marker are crossed to the Gal80 rescue line, the red color should be repressed if Gal80 is functioning (Fig 13.).

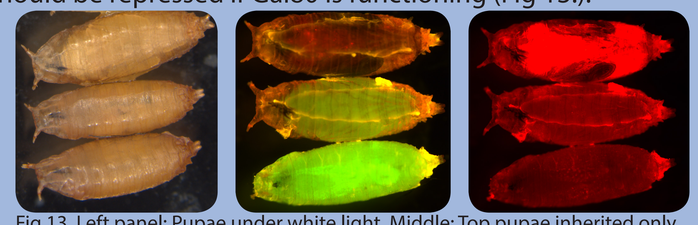


Fig 13. Left panel: Pupae under white light. Middle: Top pupae inherited only Gal4-red and bottom pupae inherited Gal4-red and Gal80-green. Right: Bottom two pupae Gal4-red production is repressed. Gal80 rescue (green) successfully represses the production of the red marker from the Gal4 driver line.

After confirming Gal80 repression of Gal4, the final K-R system was established together in the flies (Fig 14.). The Gal4 killer is so strong that the rescue by Gal80 is incomplete, thus some flies carrying K and R do not survive to adulthood. The flies are unable to make it out of their pupal case (Fig 15.). However, some flies carrying killer and rescue are healthy and viable, and produce offspring to contribute to the next generation.

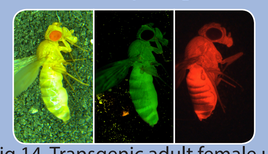


Fig 14. Transgenic adult female under white light and UV light. Female is carrying Gal80 rescue (green) and Gal4 killer (red).

Fig 15. Flies unable to completely eclose from pupal casing die while trying to emerge. These flies are positive for Gal4 killer (red) and Gal80 rescue (green not shown).

Summary & Conclusions

- Killer and Rescue lines have been established in *Ae. aegypti* and *D. melanogaster.*
- The production of Gal80 (rescue protein) has been confirmed
- Gal4 is lethal in *D. melanogaster* : Flies carrying only the killer don't hatch or die as young larvae
- Gal80 rescue is incomplete in *D. melanogaster,* but the system could still work as a gene drive.
- The results of the final gene drive experiment will determine:
  - If there are any fitness costs to carrying the rescue alone
  - When the killer is present alone if the mosquitoes/flies die
  - When the killer and rescue are both present the rescue nullifies the toxic effects of the killer and the insects survive
- The final goal is: once an anti-pathogen is available it can be linked to the rescue construct and spread through the population using the K-R system described here.

Acknowledgements

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