GENETIC ENGINEERING

Piecing together the vector control puzzle

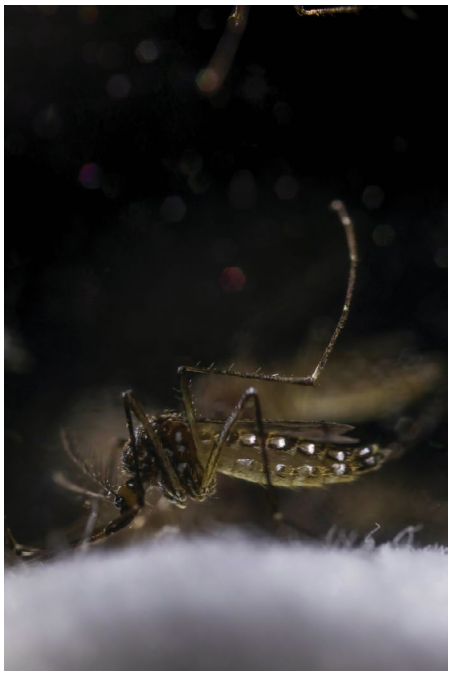
Buchman, A. et al. *PLoS Pathog* **16**, e1008103 (2020)
Li, M. et al. *eLife* **9**, e51701 (2020)

Working with mosquitoes isn't all that different or more difficult than working with other insects in the lab, says Omar Akbari, a researcher at the University of California, San Diego. The challenge is just getting to them. Reaching the mosquito room in La Jolla starts with an eye scan, after which one must don personal protective equipment and pass through several anterooms, corridors, doors, and air curtains. The biosafety precautions are necessary: a number of mosquito-borne contagious diseases are being studied in the insects.

The mosquito *Aedes aegypti* is a vector for such viruses as Dengue, yellow fever, chikungunya, and Zika. Control the mosquitoes and it might be possible to control these deadly and debilitating diseases but traditional means of mosquito management—such as environmental management and insecticide application—can be expensive and inefficient says Akbari. His lab's research has been focused on developing new technologies to better control mosquito populations. It's a puzzle to solve, but he and his colleagues recently tackled two important pieces.

One puzzle piece involved preventing the insects' ability to transmit disease in the first place. Writing in *PLoS Pathogens*, Akbari and an international team of collaborators demonstrate proof-of-concept of an anti-Dengue virus effector in *Ae. aegypti*. Eliminating Dengue has been complicated by the fact that there are four unique strains, or serotypes, of the virus that can co-circulate in endemic areas. "They're essentially different viruses," Akbari says. Past efforts to block the transmission of one serotype in mosquitoes have left insects still capable of transmitting the other three.

A few years ago however, researchers at Vanderbilt University in Tennessee isolated an antibody from humans that was broadly neutralizing against all four Dengue virus serotypes. Mosquitoes don't normally produce antibodies, but Akbari and his colleagues were able to engineer transgenic *Ae. aegypti* containing part of the genetic sequence that confers protection against the disease in people. They paired that with a promotor such that the antibody fragment would only be expressed in the midgut—the barrier through which the virus must pass into the mosquito—following a blood meal. Should such a transgenic mosquito happen to dine on a human infected with Dengue, the antibody would be released and the virus neutralized and prevented from disseminating into the insect for further transmission.



The mosquito *Aedes aegypti*. Credit: Erik Jepsen, UC San Diego Publications

The antibody approach proved potent—the mosquitoes they engineered could not be infected with nor transmit any of the Dengue serotypes, with minimal impact of the insects' fitness otherwise. But to combat Dengue in the field, that antibody-producing capability must be introduced into wild mosquito populations.

That's another piece of the puzzle. One could take lab-raised mosquitoes that are homozygous for the effector and attempt to inundate wild populations with them—some modeling suggests that *might* work, says Akbari—but he's been working on a more direct route.

In work published in *eLife*, Akbari and colleagues in California demonstrate proof-of-concept for a gene drive that could be used with *Ae. aegypti*. Gene drives are a form of genetic engineering that are intended to weight the genetic inheritance coin and force an allele into a population more quickly than should occur naturally. There are however concerns about the potential for unexpected impacts on wild populations.

Using a CRISPR/Cas9 approach, Akbari and his team decided to separate the components of the gene drive, engineering a two-part, 'split' system in which the necessary guide RNA is placed in a separate location in the mosquito genome from the Cas9 endonuclease. The drive components would thus be inherited separately and, eventually, disappear as one component or the other fails to make it to the next generation. The split drive approach should thus be safer, Akbari says, and prevent the unstoppable spread of the drive beyond the intended mosquito population.

In the paper, the team showed that the efficiency of their split drive was over 90%, with computer modeling suggesting that releasing males with the split drive and desired genetic cargo at 1:1 ratios with the wild population could spread the intended allele in a timeframe that could effectively reduce disease transmission.

Though this proof-of-concept drive was for a phenotypic readout rather than an attempt to control disease, it could be coupled with such an anti-disease genetic approach—say, a transgenic mosquito that's immune to Dengue virus?

With a proof-of-concept anti-virus effector sans gene drive, and a proof-of-concept gene drive sans effector, Akbari says the next step is to put the two pieces together. Pending positive results and regulatory approval to introduce the work into the field, the result would be mosquitoes incapable of transmitting Dengue virus that could be released into endemic areas and propagate that immunity into the mosquito population.

Theoretically, the approach could work beyond Dengue virus as well. Any virus that can be neutralized by a known human antibody could be targeted—the antibody need only to be sequenced and engineered into mosquitoes, coupled with a split gene drive. The work opens up the door to targeting other pathogens, says Akbari.

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