Another Step Forward in Genetic Engineering of Mosquitoes

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As a “proof of concept” of a new split gene-drive system developed by researchers at the University of California, Riverside, CRISPR/Cas9-mediated disruption of genes associated with cuticle pigment caused mosquitoes to turn from black to yellow, and disruption of genes associated with eye pigment caused eye color to change from black to white. The genetic modification was enabled by the researchers' development of *Aedes aegypti* mosquitoes that have the Cas9 enzyme “built-in,” inherited from their parents and passed down to their offspring. (Photo credit: University of California, Riverside)

“Yellow, three-eyed, wingless mosquitoes” have made headlines this week, but the researchers at the University of California, Riverside, behind the news call the disruption of mosquitoes’ cuticle, wing, and eye development a “proof of concept” for a new advance in the genetic engineering method known as CRISPR/Cas9. The method may accelerate scientists’ work toward identifying the genes to target for disruption of mosquitoes’ ability to carry and transmit human diseases.

Published Tuesday in the *Proceedings of the National Academy of Sciences* (PNAS), the study was led by Omar Akbari, Ph.D., an assistant professor of entomology in UCR's College of Natural and Agricultural Sciences and a member of the university's Institute for Integrative Genome Biology. Akbari and colleagues report their success in developing "transgenic mosquitoes that stably express the Cas9 enzyme in their germline."

In other words, the *Aedes aegypti* mosquitoes engineered in the lab have half of the CRISPR/Cas9 system built-in, inherited from their parents and passed down to their offspring.

*Wired*'s Megan Molteni explains it like so:

Cas9 is the DNA-chopping half of the Crispr gene editing system. So Akbari's team just had to inject the other half—a bit of guide RNA—into the embryos, for Cas9 to automatically execute its patented snipping action. ... In addition to advancing a new way to study mosquito physiology, these strains represent an important building block for efficient gene drives. Normally, the technology would require expressing both Cas9 and the guide RNA together in the same location. But that could make the drive system invasive and uncontrollable. One way to control them is to keep the components separated in the genome. And that's what Akbari is working on: a less virulent version...
Akbari tells *Entomology Today*, “Given that these strains allow for very high rates of CRISPR-induced mutagenesis (more then 90 percent in some cases), and double and triple mutants can now easily be generated as shown in the *PNAS* paper, in the future these strains can be used to develop genome-wide screens to disrupt and find genes that are important for vector competence, which may lead to new control measures to reduce the mosquitoes' ability to transmit disease to humans.”

Gene drives are a method for inserting genes in organisms that outplay the normal 50/50 odds of being passed on to offspring, and thus they've attracted great interest for their potential uses in suppressing populations of pest insects or disrupting their ability to vector disease. (For more, see the Entomological Society of America's infographic, “What is Gene Drive?” [PDF], and fact sheet, “Fighting Fire With Fire: How Science is Turning Mosquitoes Against Themselves” [PDF].) Indeed, the research Akbari and colleagues are conducting was funded by the National Institutes of Health—and also the U.S. Department of Defense's Defense Advanced Research Projects Agency. As Molteni writes, DARPA funding for gene drives is “going toward designing safer systems and developing tools to counter rogue gene drives that might get into the environment either by accident, or with malicious intent.”

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“Germline Cas9 expression yields highly efficient genome engineering in a major worldwide disease vector, *Aedes aegypti*”

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