Life scientists streamline cutting-edge technique to edit mosquito genome

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An Aedes aegypti mosquito feeding in Dar es Salaam, Tanzania. Image credit: Muhammad Mahdi Karim, GNU FDL.

Life science researchers at Virginia Tech have accelerated a game-changing technology that's being used to study one of the planet's most lethal disease-carrying animals.

Writing in this week's *Proceedings of the National Academy of Sciences*, researchers revealed an improved way to study genes in mosquitoes using a genome-editing method known as CRISPR-Cas9, which exploded onto the life science scene in 2012.

Editing the genome of an organism allows scientists to study it by deleting certain genes to observe how the organism is affected, or even to add new genes. The new technique makes the editing process more efficient and may accelerate efforts to develop novel mosquito-control or disease-prevention strategies.

"We've cut the human capital it takes to evaluate genes in disease-carrying mosquitoes by a factor of 10," said Zach N. Adelman, an associate professor of entomology in the College of Agriculture and Life Sciences and a member of the Fralin Life Science Institute. "Not a lot of research groups have the resources to spend four months working with up to 5,000 mosquito embryos to investigate a gene that may ultimately have no bearing on their work. Now they can potentially do the same investigation in a week."

Mosquitoes transmit pathogens that cause malaria, dengue fever, and other high-impact diseases. In 2013, Malaria killed an estimated 584,000 people, most of them young children, according to the World Health Organization. Bill Gates, the co-founder of Microsoft and a philanthropist who supports social and health causes, has called the mosquito the world's deadliest animal.

"The mosquito is incredibly important as far as transmission of disease," said Kevin M. Myles, an associate professor of entomology in the College of Agriculture and Life Sciences and a member of the Fralin Life Science Institute. "With this model, any scientist asking a question about a mosquito phenotype can now find its genetic cause. That's important for basic research into mosquito biology and applied research to control disease-vector mosquitoes."

The scientific community has long rallied to fight mosquito-borne diseases.

"I am excited by the paper," said Laura C. Harrington, professor and chair of entomology at Cornell University, who was not involved in the research. "We are desperately in need of faster and more efficient ways to transform mosquitoes. This single hurdle is holding the entire field back from making the discoveries that will lead to novel and effective approaches to mosquito and, consequently, disease control."

When CRISPR arrived in 2012, it drastically reduced the time it took for researchers to rewrite an organism's DNA.

Medical and life scientists quickly saw the technique's potential to edit disease-causing genes in people or, in the case of mosquito research, strategically modify the genome of an animal known to carry parasites and viruses that cause malaria, dengue fever, and other high-impact diseases.

But natural repair mechanisms are triggered when damage occurs in the mosquito genome, reducing opportunities for new sequences to be inserted in to the genome to help scientists to evaluate the genes.

Adelman, Myles, and colleagues overcame this obstacle by developing a process that silences an important mosquito DNA repair mechanism, ensuring that the introduced changes are more likely to become a permanent part of the mosquito genome, and thus increasing opportunities to figure out the new gene's role in mosquito biology.

"I use genome editing to understand how mosquitoes find their hosts and ultimately to design new repellants and attractants to manipulate mosquito behavior," said Matthew DeGennaro, an assistant professor with the Biomolecular Sciences Institute at Florida International University, who was not involved in the research. "For me, this study shows that the CRISPR system is robust in mosquitoes. The researchers also improved the technique to increase the probability of precise insertion of DNA, making CRISPR more useful."

Postdoctoral associates Sanjay Basu, Azadeh Aryan, and Glady Samuel, research technician Michelle Anderson, and Justin Overcash, a Ph.D. student from Blacksburg, Virginia, all with the Department of Entomology at Virginia Tech, contributed to the research. Timothy Dahlem, director of the Mutation Generation and Detection Core at the University of Utah, provided expertise and DNA nucleases to target specific genes.

More information: Silencing of end-joining repair for efficient site-specific gene insertion after TALEN/CRISPR mutagenesis in Aedes aegypti , *Proceedings of the National Academy of Sciences*, www.pnas.org/cgi/doi/10.1073/pnas.1502370112

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