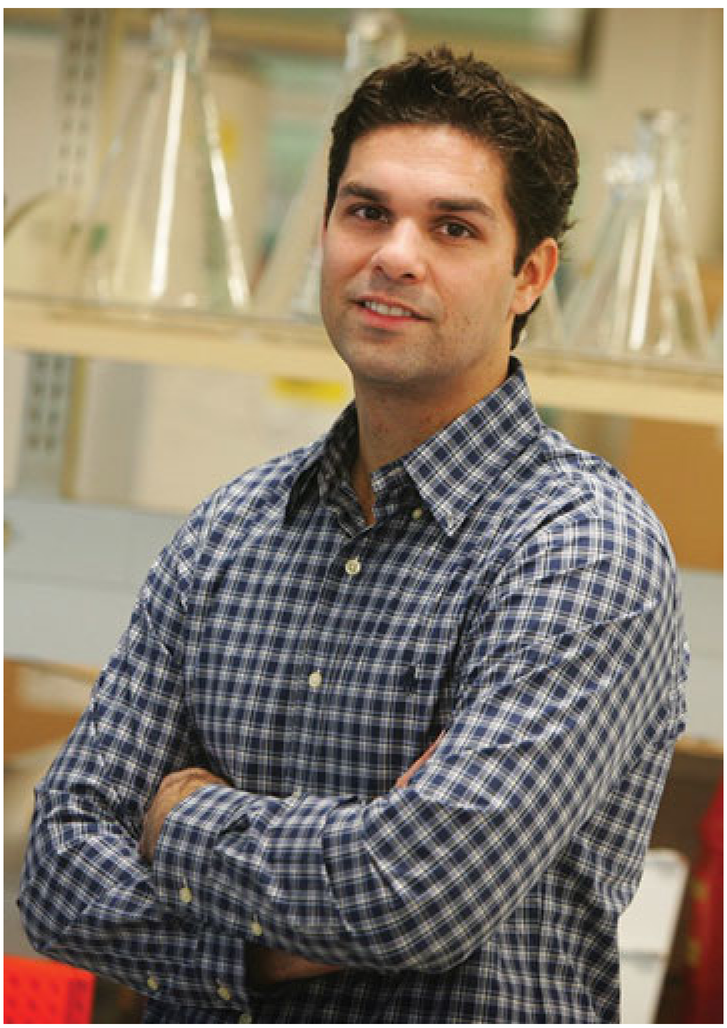
COVER STORY

Can gene drives end mosquito-borne disease?

Infectious Disease News, February 2018

When **Omar S. Akbari, PhD,** moved his lab of genetically modified mosquitoes from the University of California, Riverside, to the University of California, San Diego, he took only eggs, collecting some from each strain and sealing them in containers for the 90-mile trip south.

“Just the eggs. Not the adults — that’s how they’d escape, if something were to happen,” Akbari, a biologist and assistant professor at UC-San Diego, told *Infectious Disease News*.



Source: Kurt Miller, Riverside Press Enterprise/SCNG.

Akbari’s lab contains 260 cages filled with 130 strains of genetically modified mosquitoes plus additional cages for more experiments. The lab is designed to keep mosquitoes inside. Four doors separate the insectary from the outside world. Even if a mosquito were to escape from its cage, an air blower triggered by the innermost door should keep it from getting out of the room. For good measure, there are mosquito traps positioned from the insectary to the hallway outside the main lab.

Even if they did somehow escape, Akbari said many of his mosquitoes could not survive in the wild. Some could not even survive in the lab. Using a technology that allows scientists to make desirable edits in an organism’s genetic material, his lab has engineered what he called “interesting mutants.” These included mosquitoes with three

Omar S. Akbari, PhD, biologist and assistant professor at the University of California, San Diego, said the world will have to decide if it wants to use gene drives. “I hope that we do,” he said.

eyes, three mouth parts, no wings, notched wings, eyes that were white instead of black and tiny eyes that presumably rendered the insects blind. This was not mad science, Akbari said, because each of the experiments had an applied reason for driving certain genes into a population. For instance, a system that produced striking yellow mosquitoes could be used to optically sort the insects and release only males into the wild.

The experiments were conducted using CRISPR-Cas9, a gene-editing mechanism that scientists can use to drive a self-destructive gene — like one that produces offspring that cannot survive — through a population of insects. In just a short time, CRISPR-Cas9 has made a world without mosquito-borne disease seem more possible.

“I think it’s fair to say it surprised a lot of people with how well it works,” **Anthony A. James, PhD,** professor of microbiology and molecular genetics in the School of Medicine and professor of molecular biology and biochemistry in the Ayala School of Biological Sciences at UC-Irvine, said in an interview.

Infectious Disease News spoke with several experts about the promise of using gene drive technology, including CRISPR-Cas9, as a tool for mosquito control and its potential to eradicate mosquito-borne diseases.

‘We need better tools’

Mosquitoes kill millions of people worldwide each year. According to WHO, malaria infected at least 216 million people worldwide in 2016, killing 445,000, most of them children in Africa. The Zika virus epidemic has caused thousands of cases of microcephaly in infants, mainly in Brazil, which also experienced a large yellow fever outbreak last year that prompted mass vaccination campaigns and raised fears that the disease would spread to the country’s largest cities. About half the world’s population now lives in an area at risk for dengue, which can develop into a serious and sometimes fatal disease. The first indigenous outbreak of chikungunya in the Americas began in 2013, sickening millions of people, according to the Pan American Health Organization.

Efforts to control these diseases have historically focused on using pesticides to rapidly reduce populations of mosquitoes, like the aerial spraying that took place around Miami in 2016 to kill *Aedes aegypti* mosquitoes that carry Zika. Officials urge people to protect themselves by using insect repellent, covering exposed skin, fixing broken window screens and removing standing water in their yards.

For decades, scientists have been trying to develop better ways to combat mosquito-borne diseases. Previous methods seem primitive compared with gene drives, whose promise has grown since the introduction of CRISPR-Cas9, which has made the process more efficient and straightforward.

“Years of using insecticides and pesticides have shown us that it’s not sustainable,” said James, who began exploring genetic solutions to mosquito-borne diseases in 1986. “We don’t want to continue to put tons of potentially toxic chemicals for off-target organisms into the field. If we have something that’s better and cleaner and highly specific to the target organism, why not use it? We need better tools that are better targeted. Even though this is a new technology that hasn’t been in the field yet, it’s got to be better than pesticides.”

After years of research without results, gene drive technology has progressed rapidly in the last several years since the introduction of CRISPR-Cas9. The system is seen as being critical to the future of mosquito control and the eradication of mosquito-borne diseases, but no gene drive has ever been approved for use in the wild, and the technology represents just one of many new areas of exploration.

“There is no silver bullet,” **Marcelo Jacobs-Lorena, PhD,** professor of molecular microbiology and immunology in the John Hopkins Bloomberg School of Public Health, said in an interview. “If a gene drive is approved and implemented, I don’t think it can, by itself, eliminate a disease. We have to combine all the resources we have. That’s the only way we will conquer malaria or any other disease.”

CRISPR is a helpful acronym for clustered regularly interspaced short palindromic repeats, and Cas9 stands for CRISPR-associated protein 9. Using CRISPR technology, scientists can modify genomic sequences by cutting and editing targeted sections of DNA. CRISPR-Cas9 is not the first gene drive technology to be tested by experts, but it has produced the most encouraging results.

“I personally don’t believe that any other gene drive mechanism will work,” Jacobs-Lorena said.

Key findings

Scientists are exploring other methods for mosquito control, including interventions that do not necessarily require a gene drive to implement.

Marcelo Jacobs-Lorena

Recently, the EPA approved the use of a strain of *Wolbachia* for mosquito control. Passed to females during mating, the bacterium ensures that offspring do not survive. It works in both *A. aegypti* and *A. albopictus* mosquitoes, which transmit Zika, dengue, chikungunya and yellow fever. But *Wolbachia* is not effective against the many species of *Anopheles* mosquitoes that carry the *Plasmodium* malaria-causing parasite.

Results from two studies conducted at the Johns Hopkins Malaria Research Institute and published in *Science* last year raised the possibility of using other genetic methods to control malaria. In one, researchers altered the gene activity of several strains of *A. stephensi* mosquitoes to boost their immunity to the *P. falciparum* parasite. They found the process also altered the insects’ mating preferences so that genetically modified males preferred unmodified wild females and wild males preferred genetically modified females. This helped spread the genetic modification to successive generations of mosquitoes — an unexpected finding. It took only five generations, or around 10 to 12 weeks, for the modification to dominate the population. The mosquitoes have maintained a high level of resistance to the parasite for more than 7 years.

“Our discovery is very important because it’s proof that spreading the gene doesn’t have to be based on a genetic mechanism. It could be something that changes the behavior of the insect,” **George Dimopoulos, PhD,** professor of molecular microbiology and immunology at the Johns Hopkins Bloomberg School of Public Health, told *Infectious Disease News*.

In another chance finding, Jacobs-Lorena and colleagues discovered a new strain of the *Serratia* bacterium engineered to kill the malaria parasite. The bacterium spreads efficiently, making it different from other mosquito-infecting bacteria. The strain, which was unexpectedly found in the insects’ ovaries, is transmitted easily from males to females during mating, then from females to 100% of their progeny, allowing it to move rapidly through mosquito populations.

Bringing all of this together, a study published in the *Proceedings of the National Academy of Sciences of the United States of America* (*PNAS*) in 2015 showed for the first time how gene drives can be used to spread antimalarial genes into a vector population. The study was the result of a collaboration between James, who had published findings on malaria-resistant genes in 2012, and two researchers from UC-San Diego, **Ethan Bier, PhD,** and **Valentino Gantz, PhD,** whose 2015 study in *Science* demonstrated that CRISPR-Cas9 could be used to spread gene mutations in fruit flies. Together, they adapted the technology quickly as a gene drive in mosquitoes.

In another milestone study published in *PNAS* in November 2017, Akbari and colleagues showed how they engineered Cas9 strains to be easily expressed through generations of *A. aegypti* mosquitoes in a set of experiments that produced the "mutants" in his lab. This is only one-half of what is called a split-gene drive. Akbari and colleagues are working on the other half, which involves crossing Cas9 strains with guide RNA that recognize DNA sequences and measuring the rates of inheritance.

Using CRISPR gene-editing technology in mosquitoes is just one application that is being studied by scientists. Numerous breakthroughs in other areas have occurred over the course of just a few years. Last year, researchers from Harvard showed they could use CRISPR-Cas systems to encode a short movie into the DNA of bacteria, a method that could be used to store data within living cells. The quick succession of these results has made researchers optimistic about the technology's future applications.

"The day before the discovery of antibiotics, the mildest infectious disease was life-threatening. After that, they at least became treatable. There are these major jumps that occur in science and medicine that have a profound effect," James said. "It's some scientist somewhere doing something that no one pays attention to and it turns out it's a big deal, like the CRISPR biology."

Regulatory barriers

When the first gene drive is introduced in the wild — perhaps not for another 5 to 10 years, according to Dimopoulos — it is unlikely to happen in the U.S., where James said getting the appropriate regulatory bodies on the same page is too much of an obstacle.

"You have to be in a country that has an integrated regulatory system where all interested parties have a vote and it's really straightforward. The United States is still so fragmented," he said. "The USDA, FDA, EPA, CDC — there's no single body that has a member of each one of those [agencies] that can work together."

George Dimopoulos

Still, James said a framework is in place for how to introduce a gene drive system in the wild. Under a four-phase trial plan devised by a working group at WHO, researchers would have to demonstrate that a gene drive is safe and effective. The next two phases would involve studies using large outdoor cages or the release of mosquitoes restricted to a certain geographic area or ecosystem, then an epidemiologic study showing that the drive has an impact on a disease. If all the criteria are met, a fourth phase would involve making it part of a standard disease control program.

James anticipates that the first mosquito gene drive in the wild will involve a small number of releases in a confined area. Researchers will monitor the insects over several seasons to determine the impact. But because no country has adopted guidelines that would specifically permit the release of a gene drive organism, he said it is too early to speculate where the first one might occur.

"There are efforts to bring regulatory structures up to speed. And we're happy with that," James said. "We work in public health. We're trying to save people from these diseases. We don't want to be known for doing something catastrophically stupid. That kind of defeats the whole purpose."

Ethical questions

There are other barriers to using gene drives in the wild, including ethical questions about changing an animal's biology.

The ethics of gene drives may be a matter of debate, but experts do not foresee them having serious ecological consequences, such as a drive jumping to another species or making mosquitoes a more competent vector for another pathogen — two concerns that have been raised. "This is science fiction," Dimopoulos said.

Still, to make gene drives a reality, Akbari said it would be important to demonstrate control over a system before a gene drive is released in the environment, to show that it can be reversed in case of any unintended consequences. According to him, this is easily done by designing another gene drive that can target the initial sequences. His lab was one of several in the University of California system that received $15 million in gene drive-related funding from the Defense Advanced Research Projects Agency

(DARPA), an agency in the U.S. Department of Defense that invests in breakthrough technologies. Akbari said one of the stipulations of the DARPA funding is that the researchers explore how a gene drive can be reversed.

“This is definitely something that DARPA is interested in, mainly because if the gene drives got into the wrong hands and someone were to try to drive something as some kind of weapon, then how would you counter that weapon?” Akbari said. “We need countermeasures. Having countermeasures is definitely a high priority.”

Experts theorize that a gene drive could be used to make a species of mosquito extinct. Some see little reason not to eradicate a species like *A. aegypti*, an invasive insect in the Western Hemisphere that has evolved to become a human pest. But is it ethical? According to **Sahotra Sarkar, PhD,** professor of philosophy and integrative biology at the University of Texas, there may indeed be legitimate reasons to drive a species to extinction but not without first having a public discussion and developing policy guidelines.

“Until that happens, it would be unwise to recommend gene drives to drive any species to extinction,” Sarkar said.

According to Akbari, the science around gene drives is moving so fast that ethical questions about their use are likely going to be harder to address than scientific questions.

Source: Steve Zylius/UCI

Anthony A. James, PhD, professor and microbiologist at the University of California, Irvine, and colleagues showed for the first time how gene drives can be used to spread antimalarial genes into a vector population.

“We will reach a point where there is a drive system that can spread and can block the vector competence of malaria or dengue and we’ll have to decide whether or not to use it. I hope that we do,” he said.

Experts also said that engaging and educating the public will be a significant hurdle to eventually using gene drives in the wild — maybe the biggest one. There already have been examples of this, including a plan to release genetically modified mosquitoes in the Florida Keys that was initially voted down by residents but ultimately approved. Outdoor caged trials of the same mosquitoes, manufactured by the British company Oxitec, began in India last year.

Dimopoulos said one of the concerns is that the public will blame the release of a gene drive in the wild for an unrelated disease outbreak.

“We have to face that there is a huge resistance in the world for anything genetically modified. That is the biggest barrier,” Jacobs-Lorena said. “The advantage we have with mosquitoes is that the ultimate aim is to save lives.”

‘Scientists vs. evolution’

Whether using gene drives will achieve the ultimate goal of eradicating a mosquito-borne disease is unclear, but some experts are betting on the technology. UC-San Diego is building a new 2,000-square-foot insectary that is 10 times the size of the one in Akbari’s current lab. When it is finished later this year, Akbari will share the insectary with Bier.

The goal of a gene drive is to block a pathogen long enough that it is no longer carried by mosquitoes, humans or any other animals, Akbari said. After that, the question is whether nature will cooperate.

“I do think that evolution probably will find a way,” he said. “But given that we can engineer gene drives pretty fast, it would be an arms race: scientists vs. evolution.”

Dimopoulos said there is chance that something better than CRISPR-Cas9 will be discovered and make it even easier to alter the genes of mosquitoes on a large scale.

“It reminds me a little bit of when they sequenced the human genome,” he said. “If you would have told anyone 3 years earlier that they were going to [do that], no one would have believed you. People would have been laughing. Then, all of a sudden, there it was.” – *by Gerard Gallagher*

References:

Gantz VM, et al. *Proc Natl Acad Sci*. 2015;doi:10.1073/pnas.1521077112.

Gantz VM, et al. *Science*. 2015;doi:10.1126/science.aaa5945.

Isaacs AT, et al. *Proc Natl Acad Sci*. 2012;doi:10.1073/pnas.1207738109.

Ming L, et al. *Proc Natl Acad Sci*. 2017;doi:10.1073/pnas171153814.

Pan American Health Organization. Chikungunya. http://www.paho.org/hq/index.php?Itemid=40931. Accessed January 22, 2018.

Pike A, et al. *Science*. 2017;doi:10.1126/science.aak9691.

Shipman SL, et al. *Nature*. 2017;doi:0.1038/nature23017.

Wang S, et al. *Science*.2017;doi:10.1126/science.aan5478.

For more information:

Omar S. Akbari, PhD, can be reached at oakbari@ucsd.edu.

George Dimopoulos, PhD, can be reached at gdimopo1@jhu.edu.

Marcelo Jacobs-Lorena, PhD, can be reached at ljacob13@jhu.edu.

Anthony A. James, PhD, can be reached at aajames@uci.edu.

Sahotra Sarkar, PhD, can be reached at sarkar@austin.utexas.edu.

Disclosures: Akbari, Dimopoulos, Jacobs-Lorena, James and Sarkar report no relevant financial disclosures.