New Hope for Malaria Control

SHARON LEVY

From gene drives to chemical lures, researchers continue the battle against a major killer.

Abdoulaye Diabaté holds a vivid memory of his first bout of malaria, when he was 5 years old. He lay on sweat-soaked sheets, burning up with fever in his family's home in a small village in Burkina Faso. The fear in his parents' faces was clear. They knew he might die—the disease was, and still is, a common killer of African children.

Diabaté—now head of medical entomology at the Institut de Recherche en Sciences de la Santé/Centre Muraz in Bobo-Dioulasso, Burkina Faso—works to understand the behavioral ecology of the mosquitoes that spread malaria infection in Africa. This knowledge is critical to a range of new strategies in malaria control. Low-tech tactics include luring mosquitoes into traps, where they die before they can bite anyone. At the other extreme, sophisticated biotechnology holds the promise of engineering mosquitoes resistant to malaria infection and capable of spreading immunity to the wild population.

Over the past 15 years, an international effort has made remarkable progress in the fight against malaria. The crucial tools have been insecticide-treated bed nets and insecticides sprayed on the inside walls of houses. These prevention methods target mosquitoes in peoples' homes, because *Anopheles gambiae*, the dominant vector of malaria in Africa, has evolved to live among and feed on people. Each female requires several blood meals in order to produce her eggs, and larvae can grow in a puddle as small as the hoofprint of a passing cow.

Issa Lylmo, PhD, collects malaria vectors from homes in rural Tanzania. Photograph: Heather Ferguson.

The rise of bed nets and indoor spraying, along with the use of a new drug combination to treat infected patients, created a 66 percent drop in the malaria death rate in Africa between 2000 and 2015. In the same period, infection rates dropped by 37 percent globally and 42 percent in Africa.

"The insecticide-treated bed net is a simple, humble, and highly effective intervention," says Heather Ferguson, an infectious disease ecologist at the University of Glasgow who studies malaria-bearing mosquitoes in Tanzania. "Bed nets have saved more lives than most vaccines."

Still, more than 3 billion people worldwide remain at risk of contracting malaria. According to the most recent estimates from the World Health Organization, there were 214 million cases last year and 438,000 deaths. Most of the victims lived in sub-Saharan Africa, where 88 percent of malaria infections and 90 percent of malaria deaths occurred in 2015. The vast majority of those killed were children under the age of 5 years.

BioScience 66: 439–445.
doi:10.1093/biosci/biw057

Now, *Anopheles gambiae* mosquitoes are starting to change their behavior, biting people outside their houses. Mosquitoes are also evolving resistance to pyrethroids, the only insecticides approved for use on bed nets. There are signs that the malaria parasite is developing resistance to artemisinin, the key ingredient in drug combinations used to treat infected patients. Despite significant progress, the battle against malaria is far from over.

Anthony James, a molecular biologist at the University of California, Irvine, has been working for years to engineer a mosquito immune to malaria. Using cells from the mouse immune system, his team developed genes for antibodies directed at the malaria parasite. By 2012, they had shown that lab-reared mosquitoes carrying the synthetic genes spliced into their DNA could resist infection.

Spreading the trait into wild populations remained a daunting prospect, however. Any kind of bioengineered trait tends to lower a creature's odds of surviving and mating in the wild. Even without the genetic baggage imposed by researchers, lab-reared mosquitoes tend to be weak competitors for wild mates.

In 2003, Austin Burt, an evolutionary geneticist at Imperial College London, published a paper outlining the potential to harness selfish genetic elements, which bias the odds in favor of their own inheritance, although they provide no fitness advantage. Burt saw that these particularly selfish genes, which have evolved in many species, could be engineered to drive a desired trait into a wild population. He focused on homing endonuclease genes (HEGs). An HEG codes for an enzyme that recognizes and cuts a sequence on chromosomes that lack a copy of the HEG. The broken chromosome is then repaired using the HEG as a template. Thus, the HEG transcribes itself onto any chromosomes that lack the trait. This means that the HEG trait can be passed on to more than 90 percent of the offspring, instead of the 50:50 ratio of typical Mendelian inheritance. Systems such as this one, capable of spreading a trait rapidly through a population even though it may decrease an individual's fitness, are called *gene drives*.

The* Anopheles gambiae *mosquito is evolving resistance to pyrethroids, the only approved insecticides for use on bed nets. Photograph: Muhammad Mahdi Karim.

For a decade, researchers struggled to build an effective gene drive that could propagate through a population of wild insects. Burt and his colleagues worked on a drive that would disrupt genes required for fertility in female *Anopheles gambiae*. If successful, this trait could cause the rapid collapse of targeted mosquito populations in the wild. Major technical problems remained, however.

CRISPR to the rescue?

A breakthrough came with the recent discovery of CRISPR, an elegant form of acquired immunity used by bacteria to combat viral infections. The attacking virus inserts its own genetic code into the bacterium's genome. The bacterium fights back, producing an endonuclease called Cas9. Guided by an RNA sequence that fits the viral DNA, Cas9 neatly trims the viral genes from the chromosome. Using engineered guide RNAs, which are easy to make with any desired sequence, the Cas9 enzyme can be designed to edit the genome of any organism. It has been used to edit the DNA of human cells in the laboratory.

"CRISPR/Cas9 is a molecular scalpel that can be easily programmed to cut just about any DNA sequence," explains Kevin Esvelt, an evolutionary engineer who leads the Sculpting Evolution Group at the Massachusetts Institute of Technology (MIT) Media Lab. "Before CRISPR, we really didn't have a good way of targeting any given DNA sequence in a way that was accessible to most laboratories." The technique has been tested in a wide range of species, from yeast and nematodes to fruit flies and zebrafish, and can probably be harnessed to drive genes into wild populations of any fast-breeding, sexually reproducing creature.

CRISPR's ease and flexibility is especially important for malaria research, because, Esvelt explains, "Working with mosquitoes is a tremendous pain." Mosquitoes, particularly anopheline mosquitoes, are difficult to rear in a lab, and the adult females are finicky eaters who may decline to feed on anything other than a human arm or ankle. The existing genetic techniques being used to reduce local populations of *Aedes aegypti*, the mosquito that vectors dengue and Zika virus

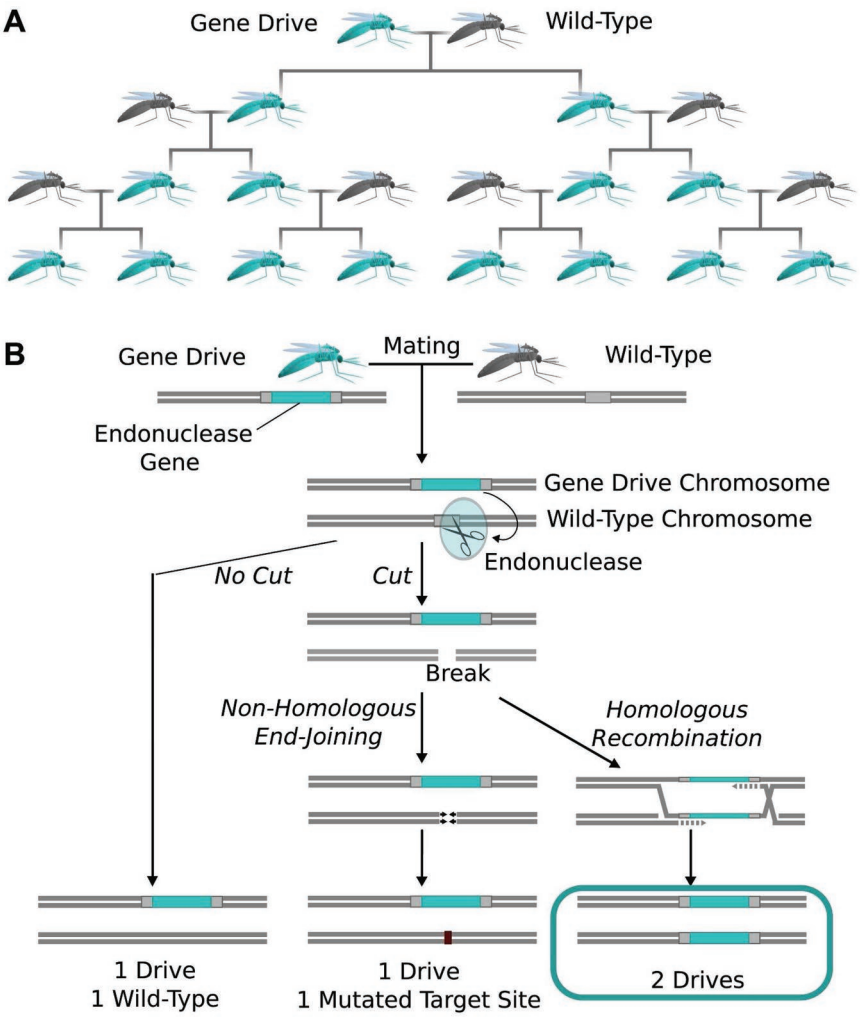
in the Americas, require the constant production of large numbers of engineered males. If successful gene drives can be created, a mere handful of engineered mosquitoes could, in theory, launch a new trait capable of spreading quickly through an entire wild population.

In recent months, two pioneering studies (see the "Further reading" box) that used Cas9 to engineer a gene drive in malaria-bearing mosquitoes have been published. Working in James's lab in Irvine, researchers installed two synthetic genes for malaria resistance in the DNA of *Anopheles stephensi*, a vector mosquito native to the Indian subcontinent. The drive worked at high efficiency, at least at the start: About 99 percent of the offspring of crosses between a bioengineered male and a wild-type female carried the malaria-resistant trait. By the fourth generation, however, transmission of the trait had shrunk to 50 percent—the same as normal Mendelian inheritance.

The problem, explains James, was the expression of guide RNAs and Cas9 throughout the eggs of females that inherited the trait. The enzyme chopped up male chromosomes before they had paired with their female counterparts. The DNA repairs that followed failed to transcribe the malaria resistance sequence. The lab is now working to limit expression of Cas9 to the male germ line. This should avoid the unwanted snipping of chromosomes at the wrong moment.

Burt and his colleagues used Cas9 to disrupt three genes needed for female fertility in *A. gambiae* to test the idea that a gene drive could cause a crash in a wild mosquito population. The drive was designed so that females carrying a single copy would be fertile, whereas those carrying two copies would be sterile. Inheritance of even one gene drive copy dropped female fertility in lab mosquitoes by more than 90 percent, however, preventing the trait from being passed on to offspring. As in the James study, this was caused by Cas9 expression at the wrong place and time. The drive copied itself into somatic as well as germline cells, rendering even heterozygous females infertile. Researchers are now working on a fix for this problem.

In any nuclease-based gene drive, homologous DNA repair, the process that transcribes the engineered gene onto a new chromosome, must compete with nonhomologous end joining, in which the cell patches up broken strands of DNA without using a template. If Cas9-based gene drives are to succeed, "it's critical to ensure that the cell copies over the



The spread of endonuclease gene drives. ***(A) When an organism carrying an endonuclease gene drive (blue) mates with a wild-type organism (grey), the gene drive is preferentially inherited by all offspring. This can enable the drive to spread until it is present in all members of the population—even if it is mildly deleterious to the organism. (B) Endonuclease gene drives are preferentially inherited because the endonuclease cuts the homologous wild-type chromosome. When the cell repairs the break using homologous recombination, it must use the gene-drive chromosome as a repair template, thereby copying the drive onto the wild-type chromosome. If the endonuclease fails to cut or the cell uses the competing nonhomologous end-joining repair pathway, the drive is not copied, so efficient gene drives must reliably cut when homology-directed repair is most likely. Image and caption reprinted from: Esvelt, K, Smidler AL, Catteruccia F, Church GM. 2014. Concerning RNA-guided gene drives for the alteration of wild populations. eLIFE (art. e03401).***

drive system to the other chromosome efficiently," says Omar Akbari, a molecular biologist at the University of California, Riverside. When broken strands of DNA are repaired via nonhomologous joining instead, alleles resistant to the gene drive are created. The two published studies on mosquito gene drives represent significant advances, but neither, says Akbari, is "ready for the planet."

Unintended consequences versus child mortality

Many biologists and social scientists agree that the planet is not yet ready for even a technically perfect gene drive. Genetically modified (GM) crops have caused controversy worldwide and sometimes have had impacts on nontarget species, although the engineered changes are meant to affect only a particular strain of domesticated plant limited to farm fields. A gene drive designed to spread through a wild population could trigger unpredictable impacts on ecology and human health—effects that could quickly cross international borders. Scientists have suggested using Cas9 to engineer gene drives that can wipe out an invasive species or alter the population of an insect pest to protect a crop. Such gene drives can be seen as environmentally friendly, because they avoid the use of toxic pesticides that can affect whole ecosystems. However, the release of a gene drive may unleash an unprecedented force for widespread ecological change.

"Any field trial of a gene drive in the environment where the target species exists amounts to global deployment," says Esvelt. He thinks that the engineering glitches in early versions of mosquito gene drives could be worked out in a year or two. The actual release of drive-bearing mosquitoes is much further off, because a long process of public debate and policymaking must come first. "We have a moral obligation to develop this technology very differently by being transparent from the outset and ensuring a level of community guidance that has really not been seen before," he says.

Women wait by their sick babies while they receive treatment for malaria, in the municipal hospital of M'banza Congo, Zaíre Province. Pregnant women and children under age 5 are among the groups most vulnerable to the scourge of malaria. Photograph: Alison Bird/USAID.

Further reading.

Esvelt, K, Smidler AL, Catteruccia F, Church GM. 2014. Concerning RNA-guided gene drives for the alteration of wild populations. eLIFE (art. e03401).

Champer J, Buchman A, Akbari OS. 2016. Cheating evolution: Engineering gene drives to manipulate the fate of wild populations. Nature Reviews Genetics 17: 146–159. doi:10.1038/nrg.2015.34

Diabate A, Tripet F. 2015. Targeting male mosquito mating behaviour for malaria control. Parasites and Vectors 8 (art. 347). doi:10.1186/s13071-015-0961-8

Gantz V, et al. 2015. Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito *Anopheles stephensi*. Proceedings of the National Academy of Sciences 112: E6736–E6743. doi:10.1073/pnas.1521077112

Hammond A, et al. 2016. A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*. Nature Biotechnology 34: 78–83. doi:10.1038/nbt.3439

Malaria's impact on African children is so devastating that the control of vector mosquitoes is seen as the planet's most compelling argument for the use of gene drives. Esvelt points out that of 3500 mosquito species on Earth, only 40 transmit human malaria, and the great majority of malaria deaths are tied to just four species of anopheline mosquito in Africa. "I have yet to meet an ecologist who is worried that anything we might do to malarial mosquitoes will cause the ecosystem to do more than hiccup," he says.

Ferguson notes that when she traps adult mosquitoes in Tanzania, *A. gambiae* typically make up less than 10 percent of the total. Many other mosquito species coexist with *A. gambiae*, and most do not transmit malaria. If a gene drive succeeded in eliminating *A. gambiae*, these other mosquito species would probably

continue to fill their ecological niche, acting as a food source for fish, birds, and bats. There is some risk that the malaria parasite would evolve to use other mosquito species as vectors—a possibility that can be tested only by unleashing a gene drive.

"I lived for 3 years in a small village in Tanzania and saw so many lovely young kids die, killed by this one mosquito," says Ferguson. "I'd find it very hard to put forward the moral argument that we shouldn't attempt to do anything about *A. gambiae* because there might be some unintended consequences." When her Tanzanian colleagues travel to remote villages, they often use their field vehicle as an emergency ambulance to get infected kids to medical attention. Recently, they transported a 2-year-old girl who died in her mother's arms just before they reached a hospital.

Before gene drives can be deployed against malaria, however, Africa's 46 nations would need to agree on a way to regulate bioengineered mosquitoes. "None of the rest of us should get a say, because we don't live with those mosquitoes and Africans do," Esvelt says. He believes that scientists should not build gene-drive systems unless they are open about what they are doing, use safeguards to prevent the accidental escape of engineered organisms, and solicit constant feedback from the people whom the drive is intended to help. He is trying to follow his own advice as his lab works to develop a gene drive that would render white-footed mice immune to Lyme disease, thereby breaking the cycle that infects ticks, which transmit the bacterium when they bite people. If approved, the initial tests of bioengineered mice would take place on an island off the New England coast.

In the United States and around the globe, policy and regulatory frameworks lag behind the rapidly progressing science of gene drives, says Kenneth Oye, a political scientist at MIT. The Food and Drug Administration recently updated its guidance, which as of 2014 required that any genetic changes be safe for the target organism—a rule that makes no sense in the context of gene drives aimed to limit or modify populations of disease-bearing insects. Environmental Protection Agency standards require that engineered traits be stable. But designing traits that decay over time may become an important way of limiting the spread of gene drives.

The only existing international agreement on genetically modified organisms, the Cartagena Protocol, requires that GM organisms not cross national borders. That will be impossible to guarantee if gene drives are released into the wild. The United States and Canada, two of the countries doing advanced work on gene drives, are not signatories to the protocol.

GM mosquitoes in the fight against dengue and Zika.

A new factory is going up in the city of Piracicaba, Brazil. When it is up and running, the facility will produce 60 million genetically modified (GM) male mosquitoes every week.

The *Aedes aegypti* mosquito is a vector of dengue fever, chikungunya, and the Zika virus. The biotechnology firm Oxitec has developed a strain of *A. aegypti* that carries a dominant lethal trait, a self-destruct code. When the company's GM males mate with wild-type females, the offspring die before they reach adulthood. In field tests in Grand Cayman Island and northern Brazil, Oxitec says it has been able to reduce local mosquito populations by about 80 percent.

A. aegypti bite by day, so bed nets, a strategy successfully deployed against malaria-bearing *Anopheles* mosquitoes, won't work. *A. aegypti* are adapted to live among humans, and their larvae can flourish in any small puddle—even in a water-filled bottle cap.

Oxitec's method requires long-term production and the release of millions of bioengineered male mosquitoes. The idea is that the sustained release of the GM mosquitoes will lead to a crash in the numbers of *A. aegypti*. "We hope that we can deliver the city protection in a year's time," says Sofia Pinto, an Oxitec scientist in Brazil.

Oxitec reports that since it began releasing its GM mosquitoes in one Piracicaba neighborhood of 5000 people last April, wild-type mosquito larvae there have dropped by 82 percent. Amidst concern over the rapid spread of Zika, the company and city officials announced that they are expanding the program to cover an area of up to 60,000 people. Pinto says the company is in negotiations with other Brazilian cities.

"We have a public-engagement program which normally lasts for the first month or two," says Pinto. "We try to explain what we are going to do. It can be a hard concept that we're going to fight mosquitoes with more mosquitoes."

Oxitec emphasizes that the males they release do not bite—only female mosquitoes do that.

The company's proposal to test its GM mosquitoes in the Florida Keys has incited intense public opposition. Citizen activists point out that the information available on the Oxitec mosquito comes from the company itself, which stands to profit from a GM mosquito program. The Food and Drug Administration is evaluating the proposal, the first time the agency has confronted the complex issues of a potential release of GM insects for the purpose of disease control. In March, the agency issued a preliminary finding of no significant impact regarding the release of Oxitec mosquitoes.

Homegrown alternatives

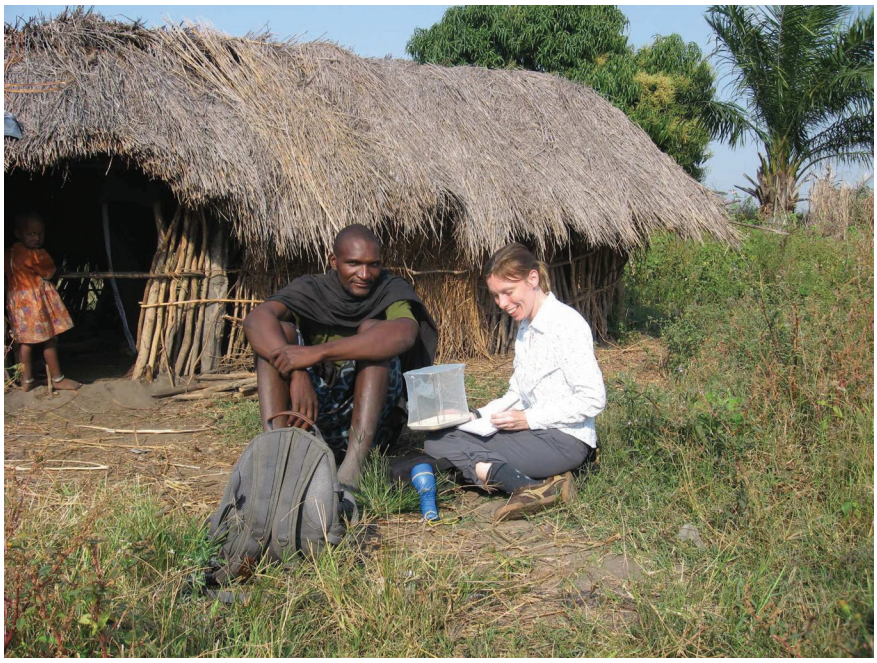
Meanwhile, a rising generation of African scientists is creating inventive ways to combat malaria. Fredros Okumu of the Ifakara Health Institute in Tanzania has developed mosquito lures, using chemicals found in human sweat and breath. Field

tested in Tanzanian villages, the lures attracted more mosquitoes than people did. Okumu is now working to establish decoy sites, where mosquitoes attracted to artificial lures can be trapped before they bite people.

Diabaté has pioneered the study of male mosquito biology—a topic traditionally ignored because only females bite people and transmit malaria. His research has shown that male *A. gambiae* form swarms above distinctive landmarks, where they court females. That information can be used to both decrease mosquito reproduction and contribute to estimates of malaria transmission rates.

Diabaté has also demonstrated the power of a simple, low-cost device, the Lehmann funnel entry trap, built into a mesh screen that can be mounted on windows. Mosquitoes attracted by the scent of people inside a house are caught in the trap, where they die of dehydration. The traps decreased mosquito density inside homes in Burkina Faso villages by 70 to 80 percent. Mosquitoes trapped in the study turned out to be highly resistant to pyrethroid insecticides—underlining the virtues of the funnel trap, which works without insecticide. Through a project called Target Malaria, Diabaté is also working on studies of genetic modifications that can limit the reproduction of *A. gambiae*.

"In the long run, we need a larger tool box for controlling malaria," says Calestous Juma, director of the Science, Technology, and Globalization project at Harvard's Kennedy School of Government. As a child growing up near Lake Victoria in Kenya, Juma helped fill in small puddles where mosquitoes might breed. These simple public health strategies have since fallen away, but he believes they should always be part of the solution. "We need to get away from the idea of a single silver bullet and recognize that the necessary tools will vary from place to place and depend on available resources," he says. "Mosquitoes are versatile, and they adapt faster than we are adapting our logic."



Heather Ferguson, of the University of Glasgow, with Markan, leader of Sagamanga, a village in southern Tanzania. Ferguson holds a cage of mosquitoes that were collected from the house shown here. Photograph: Issa Lyimo.

Bernadette Huho, PhD, (right) and Japhet Kihonda conduct laboratory analyses of mosquitoes caught in the wild in Tanzania. Photograph: Heather Ferguson.

Juma recently received a grant from the Gates Foundation to explore the political aspects of introducing gene drives in Africa. The key to acceptance of new technologies, he says, is local ownership. In the early years of mobile phones, there was much debate over their safety. When Africans started up their own phone businesses, the technology was quickly adopted. By

contrast, there has been intense ongoing resistance to the use of GM crops in Africa, which opponents fear would wipe out traditional farming methods and cause the loss of diverse crop strains bred by generations of local people.

It is critical that African scientists be involved in the development of any gene drives proposed for release on the continent. "Communities ravaged by malaria will respond quite differently to bioengineered mosquitoes than to genetically modified foods," Juma predicts. "Local capabilities and public engagement will have a great impact on whether the technologies are accepted."

Sharon Levy (levyscan@sbcglobal.net) is a science journalist based in northern California. She is at work on The Marsh Builders*, a book about wetland revival as a way to control water pollution.*