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BIOTECHNOLOGY

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A new breed of genetically modified mosquitoes carries a gene that cripples its own offspring. They could crush native mosquito populations and block the spread of disease. And they are already in the air—though that's been a secret

By Bijal P. Trivedi

Buzz kill: The *Aedes aegypti* mosquito is the primary carrier of dengue fever.

Photograph by David Liittschwager

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OUTSIDE TAPACHULA, CHIAPAS, MEXICO—10 MILES FROM GUATEMALA.

To reach the cages, we follow the main highway out of town, driving past soy, cocoa, banana and lustrous dark-green mango plantations thriving in the rich volcanic soil. Past the tiny village of Rio Florido the road degenerates into an undulating dirt tract. We bump along on waves of baked mud until we reach a security checkpoint, guard at the ready. A sign posted on the barbed wire–enclosed compound pictures a mosquito flanked by a man and woman: *Estos mosquitos genéticamente modificados requieren un manejo especial,* it reads. *We play by the rules.*

Inside, cashew trees frame a cluster of gauzy mesh cages perched on a platform. The cages hold thousands of *Aedes aegypti* mosquitoes—the local species, smaller and quieter than the typical buzzing specimens found in the U.S. At 7 A.M., the scene looks ethereal: rays of sunlight filter through layers of mesh creating a glowing, yellow hue. Inside the cages, however, genetically modified mosquitoes are waging a death match against the locals, an attempted genocide-by-mating that has the potential to wipe out dengue fever, one of the world's most troublesome, aggressive diseases.

Throughout a swath of subtropical and tropical countries, four closely related dengue viruses infect about 100 million people annually, causing a spectrum of illness—from flu-like aches to internal hemorrhaging, shock and death. No vaccine or cure exists. As with other mosquito-borne diseases, the primary public health strategy is to prevent people from being bitten. To that end, authorities attempt to rid neighborhoods of standing water where the insects breed, spray with insecticides, and distribute bed nets and other low-tech mosquito blockers. They pursue containment, not conquest.

Anthony James, however, is mounting an offensive. James, a molecular biologist at the University of California, Irvine, and his colleagues have added genes to *A. aegypti* that block the development of flight muscles in females. When a genetically modified male mosquito mates with a wild female, he passes his engineered genes to the offspring. The females—the biters—don't survive long. When they emerge from the pupal stage, they sit motionless on the water. They won't fly, mate or spread disease. The male progeny, in contrast, will live to spread their filicidal seed. In time, the absence of female offspring should lead to a population crash, which James's collaborator has already demonstrated in the controlled environment of an indoor laboratory in Colorado. Now he has brought his bugs south.

The technology marks the first time scientists have genetically engineered an organism to specifically wipe out a native population to block disease transmission. If the modified mosquitoes

IN BRIEF

Scientists have genetically engineered mosquitoes with a self-destruct mechanism, an advance that could slow the spread of mosquito-borne diseases.

One team of scientists has been conducting tests of the mosquitoes in cages in southern Mexico. Another has been releasing mosquitoes out into the wild.

The intentional release of genetically modified insects has sparked international controversy, especially because the first releases were conducted in secret.

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triumph, then releasing them in dengue-endemic zones worldwide could prevent tens of millions of people from suffering. Yet opponents of the plan warn of unintended consequences—even if mosquitoes are the intended victims.

Researchers also struggle with how to test their creations. No international laws or agencies exist to police trials of new transgenic organisms. For the most part, scientists and biotech companies can do what they want—even performing uncontrolled releases of test organisms in developing countries, neither warning the residents that their backyards are about to become a de facto biocolonialist field laboratory nor gaining their consent.

James has spent years attempting to play it straight. He has worked with community leaders in Tapachula, acquiring property through the traditional land-sharing program and building a secure test facility—all arduous, time-consuming, careful work. But he is not the only researcher testing modified mosquitoes outside the lab. James's colleague Luke Alphey, founder of the U.K.-based biotechnology company Oxitec, has quietly pursued a more aggressive test strategy. In 2009 and 2010 his organization took advantage of the minimal regulations in the Caribbean's Grand Cayman island to release millions of genetically modified mosquitoes into the wild. James first learned of the experiments when Alphey described them publicly at a conference in Atlanta in 2010—14 months after the fact. Since then, Oxitec has continued the trials, releasing modified mosquitoes in Malaysia and Brazil.

Deadly mates: Inside the cages in southern Mexico, scientists introduce genetically modified mosquitoes into a group of locals. The intruders should crash the native population.

COURTESY OF BIJAL P. TRIVEDI

Experts fear Oxitec's actions could trigger a backlash against all genetically modified insects reminiscent of Europe's rejection of GM crops, a move that could snuff out the technology before scientists can fully understand both its promise and its potential consequences.

That would be a shame because the technology has such promise. The Colorado test demonstrated that the modified mosquitoes work in a controlled environment, although a few indoor cages are not the wilds of Central America, Brazil or Malaysia. To fight the sickness and death that ride inside the mosquito, the scientists' creations must conquer the jungle.

FORCED STERILIZATION

IN 2001 James was already a pioneer of modern molecular mosquito genetics—the first researcher to genetically alter a mosquito and the first to clone a mosquito gene. That year he decided to apply his knowledge to the problems of disease transmission. He wondered if he could use a strategy designed to control agricultural pests on mosquitoes instead.

A year before, Alphey, then at the University of Oxford, had developed a technique for generating fruit flies harboring genes that selectively killed females. The population-control strategy is just a postgenomic riff on sterile insect technology (SIT), which has successfully controlled crop pests for 60 years. Technicians rear vast numbers of insects, sterilizing the males with blasts of radiation. When they mate with females in local fields, the union produces no offspring. The strategy is insecticide-free, targets only the pest species and has been successfully applied many times—including a large-scale Mediterranean fruit fly (medfly) eradication program in 1977 in Tapachula.

Unfortunately, sterile insect technology has never worked with mosquitoes. Radiation severely weakens adult males, and the processes of sorting and transport kill them before they can mate. Extending Alphey's new fruit fly technique to mosquitoes, however, would enable researchers to design effectively sterile male mosquitoes from the genome up.

To kill female mosquitoes—the ones that suck blood and spread disease—James needed to hijack a genetic region that only females make use of. In 2002 James and Alphey identified a naturally occurring switch that controls flight-muscle development in females. Turn it off, and flight muscles won't develop. Female mosquitoes emerging from the pupal stage just squat on the water's surface, flightless, unable to attract mates. It was the perfect target.

Alphey founded Oxitec in 2002 to capitalize on the technology. In 2005 the Foundation for the National Institutes of Health, funded in large part by the Bill & Melinda Gates Foundation,

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granted James $20 million to test genetic strategies against dengue. James gave Oxitec $5 million to build the mosquitoes.

The collaborators designed a stretch of DNA that included a handful of genes and the regulatory switches needed to turn them on and off at the correct time. The system works like a relay team. During the mosquito's metamorphosis from larva to adult, the female-specific switch flips on, activating the first gene, which produces a protein. This protein activates a second switch that kicks on gene number two, which then manufactures a toxin that destroys the female's flight muscles. The researchers also added genes for fluorescent proteins that make modified larvae glow red and green, allowing them to monitor the spread of the genes through the population.

To breed large populations of a mosquito that they had explicitly programmed to die, Alphey and James needed a way to protect the females from the toxic gene cassette until after they reproduced. The trick was lacing the water with an antidote—the antibiotic tetracycline, which blocks production of the flight muscle–destroying protein. This design is also an emergency fail-safe: if a few of these genetically modified mosquitoes escape, they cannot reproduce without the drug.

The first tests of the new breed came in 2008 and 2009, when Megan Wise de Valdez, a colleague of James's who at the time was based at Colorado State University, introduced modified males to a population of ordinary *A. aegypti* mosquitoes in the laboratory. Within five months the population crashed. The kill switch worked. The next step was to bring the modified mosquitoes into the field.

BREAKBONE FEVER

IN TAPACHULA, where James has set up his netted laboratory, dengue has long been a problem, as it has been in much of Mexico. "Dengue is my most important concern on a day-to-day basis," said Hermilo Domínguez Zárate, undersecretary of health for Chiapas, when I visited the region last year. Dengue spreads explosively, causing the most hardship in densely populated areas.

During my trip to Chiapas I toured Pobres Unidos—Poor United—an impoverished neighborhood on Tapachula's outskirts that suffered the most dengue cases in 2009 and 2010, along with Janine Ramsey, a parasitologist on James's team who leads day-to-day work at the field site, and Rogelio Danis-Lozano, a medical epidemiologist.

One home we visited belonged to Maria, who asked that I not use her last name. As with most homes in Pobres Unidos, Maria's house has only three walls, like a house on a movie set, so she has no way to keep mosquitoes out. The moist dirt floor creates a humid environment that lures the insects close. Piles of trash and dozens of containers collect rainwater, providing countless locations for mosquitoes to deposit eggs.

Danis-Lozano directed our attention to a large yellow tub brimming with freshwater and pointed to hundreds of skinny, black, threadlike mosquito larvae swimming vigorously in erratic zigzag patterns. Maria knows about dengue, of course, but Danis-Lozano discovered she had no idea that the larvae in her washtub morph into disease-spreading mosquitoes.

It is a scene that is mirrored in poor, crowded neighborhoods worldwide. More than 100 countries suffer from dengue, from Asia to Africa to the Americas. Symptoms of dengue's mild form—"breakbone fever"—mimic the flu: fever, joint and muscle pain, and crippling headaches that last about a week. A second infection can trigger potentially deadly dengue hemorrhagic fever, which induces vomiting, severe abdominal cramps, and internal hemorrhaging. Blood streams from the eyes, nose, mouth and vagina. Without treatment, hemorrhagic dengue kills up to 20 percent of its victims; with costly expert care, mortality drops to 1 percent. The annual worldwide death toll exceeds all other viral hemorrhagic fevers—including Ebola and Marburg—combined.

In 2008 epidemiologist David M. Morens and Anthony S. Fauci, director of the National Institute of Allergy and Infectious Diseases, warned that dengue is "one of the world's most aggressive reemerging infections." The frequency and magnitude of outbreaks have been rising, spread by growing international travel and the exodus of people into cities. Caseloads have doubled every decade since the 1970s. In 2009 Florida public health officials reported the first dengue cases there in more than seven decades, raising fears among epidemiologists that the disease would soon take root in the continental U.S.

One reason James decided to apply his genetic technology to the fight against dengue fever—instead of, say, malaria—is that the virus is primarily transmitted by a single species of mosquito. (Between 30 and 40 species of mosquito carry malaria.) *A. aegypti,* the world's main dengue vector, is an invasive tree-dwelling African species that hitched a ride on slave ships some 400 years ago. It is now an urbanite, breeding beside homes in anything that holds a few tablespoons of clean water. The mosquito bites during the day, so bed nets provide no protection. And it bites humans almost exclusively, drawing the nutrients that give it a life span of up to a month—plenty of time to bite and spread disease.

A. aegypti is stealthy, lacking the sharp, unnerving buzz that provokes a swift swat or panicked wave. Inside the secure insectary at the Regional Center for Public Health Research in Tapachula, I could barely hear a swarm of transgenic mosquitoes in a small cage. Laura Valerio, an entomologist at U.C. Davis, stuck in her gloved hand to point out a female. The intrusion scared the males, which took flight and zoomed around the cage. Females, however, just sat there or hopped away clumsily.

Modified mosquito larvae would later be moved to James's field site, which consists of five pairs of cages, each with a control cage housing a population of wild mosquitoes and a treatment cage where modified mosquitoes mix with locals. Each cage is guarded by multiple layers of mesh—protection against escapees—which researchers must carefully navigate through as they add new test subjects to the experiment.

The strict protocol is an attempt to avoid past errors. Developing countries have long made a convenient location for First World field trials, but a cavalier attitude toward the local environment has led to backlash that derailed entire research programs. Perhaps no field is more fraught with abuses—both real and perceived—than genetically modified organisms.

POISON IN THE WELLS

IN 1969, FOR EXAMPLE, the World Health Organization and the Indian government teamed up to study genetic control of three mosquito species: *Culex fatigans,* which spreads filariae (parasites that cause elephantitis); *A. aegypti,* which spreads dengue and yellow fever; and malaria-spreading *Anopheles ste-*

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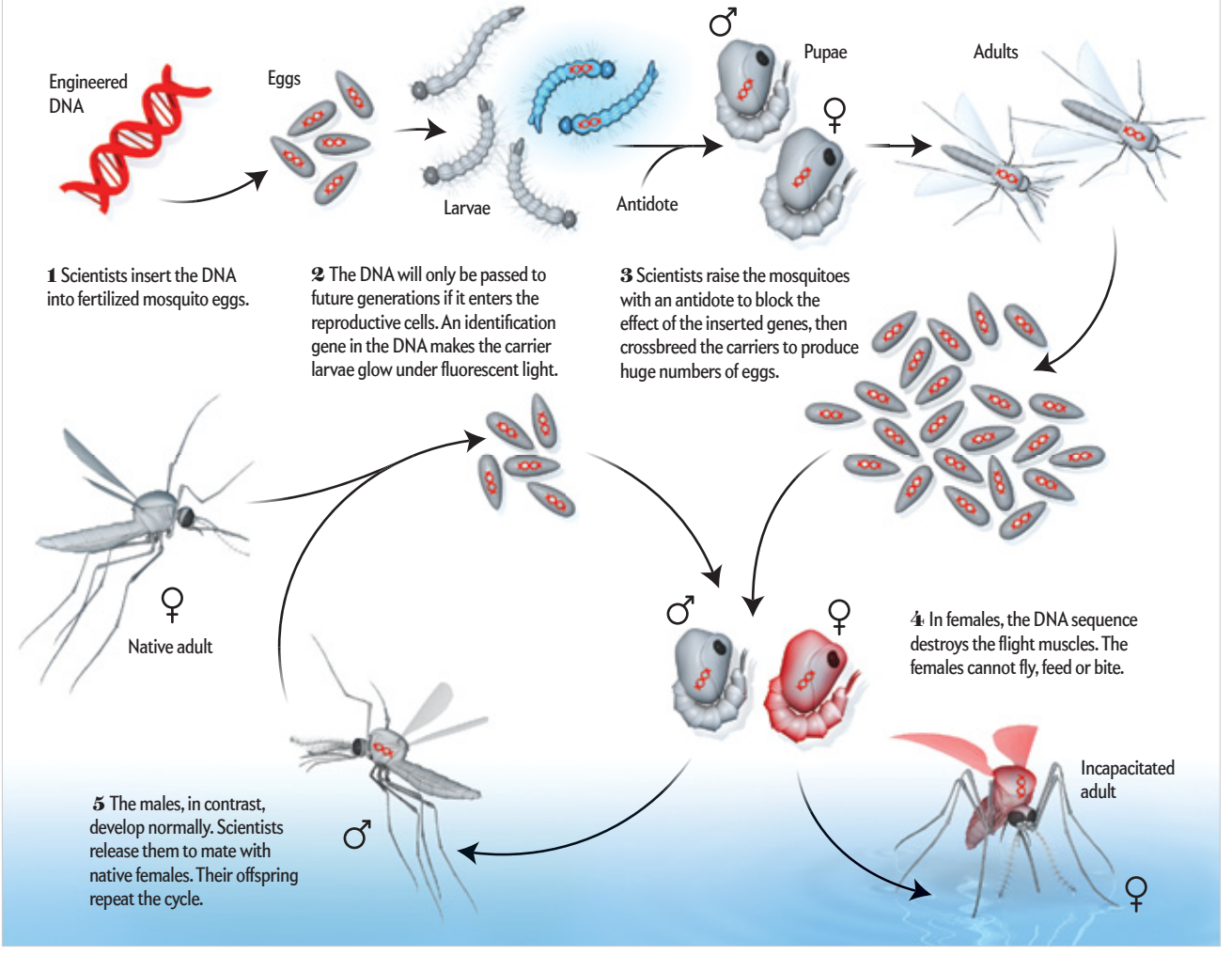
The Female Kill Switch

The genetically modified mosquitoes in Mexico have been designed to decimate local mosquito populations. Scientists insert a genetic sequence into mosquito eggs that destroys the flight muscles of females. Male mosquitoes (which do not bite) are left to spread through the native ecosystem and pass on the crippling genes. In time, the lack of females leads to a population crash.



phensi. The U.S. government funded some of the research.

In 1972 a scientist anonymously published an article in India's *National Herald* alleging that researchers had been placing mosquitoes treated with thiotepa—described as a mustard gas derivative that causes birth defects and cancer in animals—in village drinking wells. The scientists in charge of the project issued a timid rebuttal and rebuffed subsequent interview requests from the press. Then, in 1974, the Press Trust of India ran a story with the incendiary headline "WHO Works for U.S. Secret Research in India." The article alleged that the mosquito project was being used to test the practicality of using *A. aegypti* as a biowarfare agent. India was being used to test "chemicals or methods not permitted in sponsoring countries," the account ran, also charging that *A. aegypti* was being studied because "its eggs (unlike those of other mosquitoes) can be dried, put on a piece of paper in an envelope and mailed to any part of the country where they can hatch." Although the investigators strenuously denied the allegations, the public relations debacle prompted the WHO to abandon the program.

Since then, investigators have been terrified of conducting field trials of genetically modified (GM) organisms, says Stephanie James (no relation to Anthony), director of the Grand Challenges in Global Health initiative at the Foundation for the National Institutes of Health. "There was a real psychological barrier. They knew they couldn't afford to mess up."

"All my career I've been told you'll never get people to agree to do this," Anthony James told me. At the 2005 inaugural dinner for Grand Challenges grant recipients, he consulted Jim Lavery, who specializes in the science of community engagement at Toronto's Center for Global Health Research at St. Michael's Hospital. "GM freaks people out," James said. "So how do you involve the community?"

Illustration by Emily Cooper

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Lavery suggested choosing a location where dengue was a significant public health issue and control methods were failing, in a country with a stringent, sophisticated regulatory structure capable of assessing the risks and benefits of a genetically modified, dengue-fighting mosquito. That way locals would be comfortable that the effort would not endanger or exploit them. He and mosquito field-trial veteran Thomas Scott of U.C. Davis helped Anthony James assemble an international team of mosquito ecologists, anthropologists and ethicists long before he had enough mosquitoes to test.

By 2006 Tapachula was the front-runner for these trials. Mexico had national laws on genetically modified organisms and had signed the Cartagena Protocol on Biosafety—the international framework for importing them. Experience with the medfly meant the Tapachula community wasn't "freaked out" by the idea of modifying an insect, Lavery says.

"At first the request for land sounded strange," said Martimiano Barrios Matute, leader of the farming community where the experiment is based. Why would anyone want to build large cages and fill them with man-made mosquitoes? The community was also confused about what transgenic mosquitoes could do. Could escapees hurt them or their fields? Would their sterility be transferred to other insects?

James and his group addressed the community's concerns and purchased the land to build the cages through the traditional communal land-ownership program in the area. And they continue to engage the locals as the experiment continues.

In a weekly town hall gathering in the Casa de la Cultura on Tapachula's historic main square, Ramsey, the project's field site manager, described the project to an audience of community leaders, 30 men and five women. It was hard to tell she is an American expatriate as she held the room transfixed; she was animated, gesturing and joking.

When she concluded, the audience cautiously asked questions. One man asked if he could visit the mosquito cages. Another wanted to know what happens if mosquitoes escape. A young woman asked why people are against transgenics. An elderly man from a mountain village asked whether malaria and dengue are different. Ramsey answered them all, then smiled and shook hands as she left.

"Now that we understand, even more so do we like the project," said Barrios Matute, a slender soy farmer with gold-capped teeth. "It will benefit not only Rio Florido but all around Rio Florido and Mexico and other parts of the world."

THE GREAT ESCAPE

WHILE ALL THIS slow scientific and community work was going on in Mexico, Alphey was quietly taking a dramatically different approach. Last November he arrived at the annual meeting of the American Society of Tropical Medicine and Hygiene with a surprising story to tell. Beginning in September 2009, Alphey said, Oxitec had been releasing genetically modified mosquitoes on Grand Cayman island in the Caribbean. (The mosquitoes are similar to the ones being tested in Tapachula, but not identical—in the Cayman strain, both male and female mosquitoes die as larvae.) Between May and October of 2010 Oxitec released more than three million male mosquitoes, he revealed, which cut the indigenous *A. aegypti* population by 80 percent. The data have been submitted for publication.

Alphey defended his gung-ho approach, saying that Oxitec leaves outreach largely to the governments because they understand the cultural sensitivities. In Grand Cayman, outreach involved one five-minute spot on the local nightly news broadcast and a pamphlet that described the mosquitoes as sterile, avoiding any mention of genetic modification. There were no public meetings or opportunities for residents to voice concerns.

Alphey justified his actions at the Atlanta meeting. "In terms of publicity, we were only doing it in the Cayman Islands," he said. "We only need the community, people on the island, to know about it."

Mark Q. Benedict, a molecular biologist at the University of Perugia in Italy and consultant to the Gates Foundation, says Oxitec has broken no laws and calls the Cayman trials "courageous" for testing technology bound to attract "attention, both good and bad." Benedict says confused and conflicting media reports created the impression of "the lone scientist who rushes out with his bucket of mosquitoes and throws them into the environment without any oversight. That is not happening." Oxitec works with both local and national governments to gain approval before any field test.

Still, the Cayman release has provoked strong emotions—distrust, disappointment and frustration—from many of Alphey's colleagues, environmental groups and the public. "The international community was taken by surprise that this release had happened," says Bart Knols, a medical entomologist at University of Amsterdam and managing director of MalariaWorld. "Now the outside world perceives Oxitec as secretive, which makes the public wonder why. It breeds suspicion."

This is promising technology, Knols says. "If some party messes up badly and misinforms the public, the risk is that other GM trials will suffer." Now, because of Oxitec, he adds, "we have the same problems as the WHO had in India in 1976."

Other experts say the company is preying on countries with minimal bureaucracy and regulations. In the Cayman Islands, Oxitec conducted its trials in a place with a "streamlined regulatory structure," says Stephanie James, where the ink was barely dry on a biosafety bill that has yet to become law.

Malaysia was next. Amid protests from 20-plus nonprofit organizations, Oxitec launched a trial in an uninhabited area last December. A follow-up in a nearby village is pending. Even with a newly minted National Biosafety Board that monitors modified organisms and 2009 Malaysian Biosafety Act regulations, many feel that Malaysia lacks the experience to monitor the experiment, says Gurmit Singh, chair of the nonprofit Center for Environment, Technology and Development, Malaysia.

Anthony James slumped in a chair as we discussed the situation but, always diplomatic, said flatly, "That's the difficulty of working with corporations. I can't control corporate partners." He added, "If it blows up, I told you so. If not, you got lucky." James said that Oxitec's approach would be impossible in Mexico, adding that he is confident his team's community engagement activities have "set a standard for testing genetically modified organisms."

Alphey is undeterred. Earlier this year Oxitec launched a six-month trial in a poor suburb of Juazeiro, Bahia, in northern Brazil, which is plagued by mosquitoes and dengue year-round. Later this year Alphey plans to return to Grand Cayman to pit the Tapachula and Cayman strains of transgenic mosquitoes

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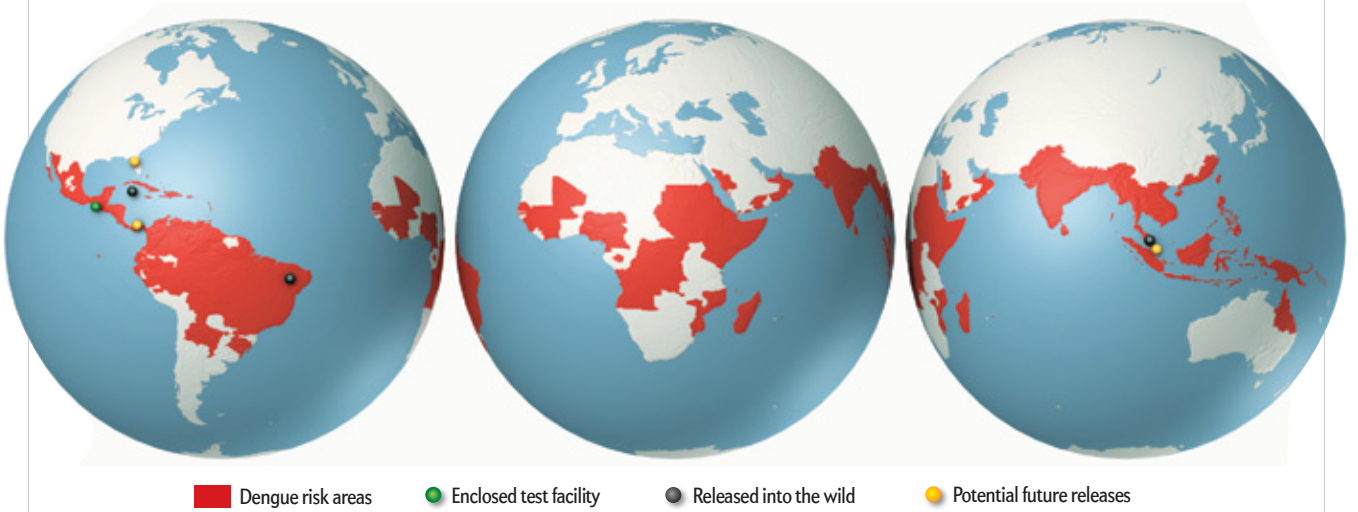
Where Dengue Lives

Dengue fever is the most rapidly growing mosquito-borne viral disease in the world. Approximately 2.5 billion people live in countries where dengue is endemic (*below*), and the number of cases reported to the World Health Organization has been doubling each decade. Researchers have reported that releasing genetically modified mosquitoes into the wild has sharply reduced local mosquito populations.



SOURCE: *CDC HEALTH INFORMATION FOR INTERNATIONAL TRAVEL 2012*, BY THE CENTERS FOR DISEASE CONTROL AND PREVENTION, OXFORD UNIVERSITY PRESS, 2012 (*dengue risk areas*)

against the local mosquitoes to see which lives longer, flies farther and is better at mating with local females. Mosquito-control officials in Panama and the Philippines have shown interest, as have the authorities in Florida.

PERMANENT SPREAD

OF COURSE, MANY GROUPS oppose the release of any transgenic organisms, no matter how thoughtfully the scientists explain themselves beforehand. Janet Cotter, a senior scientist at Greenpeace Research Laboratories, warns that "Oxitec's release of GM mosquitoes is extremely risky. There's no such thing as 100 percent sterility, so there are going to be some fertile females that will be released, and we don't know the implications of that."

Some people wonder if it is ethical—or safe—to eliminate an organism, even in just a small geographic area. Proponents argue that *A. aegypti* is an invasive species that has evolved to exploit a solely human niche. "Urban *A. aegypti* is not part of any significant food chain," says Phil Lounibos, a mosquito ecologist at the Florida Medical Entomology Laboratory. Yet Lounibos doubts whether eliminating *A. aegypti* would stop dengue transmission permanently. "A previous campaign to eradicate this species from the Americas in the 1950s and 1960s, when it was the primary vector of urban yellow fever, failed miserably," he says. The invasive Asian tiger mosquito—another good dengue vector—readily occupies niches vacated by *A. aegypti*. Moreover, both the Cayman and Tapachula mosquito strains, even if successful, are not permanent. Migration of mosquitoes from neighboring regions into Tapachula could foil eradication attempts and mandate frequent releases of the modified males to keep the population in check.

James and his collaborators have been developing a self-sustaining but more controversial solution. It uses a "gene drive system," which promotes the spread of dengue resistance genes through a wild mosquito population, blocking the replication of at least one form of the dengue virus, known as type 2. Unlike the Tapachula mosquitoes, which die soon after release, mosquitoes outfitted with a gene drive will persist in the environment. James says field trials for gene drive systems are still a few years away.

"Something that spreads genes through populations is going to have much more difficult regulatory hurdles," James says, "so I'm happy to take something that is self-limiting, not sustainable, like [the Tapachula strain] and have that be our first shot."

Undersecretary of Health Domínguez Zárate views the genetically modified approach as "low cost and high creativity." "If dengue was something with less importance, then why modify something from nature?" he asks. "We need to respect nature as much as we can." Still, the costs of dengue outweigh the potential environmental risks, he says. "It's worth the gamble." SA

MORE TO EXPLORE

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SCIENTIFIC AMERICAN ONLINE

Should scientists be releasing genetically modified insects into the wild?
ScientificAmerican.com/nov2011/dengue

Maps by George Retseck

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