Mosquito Modifications: New Approaches to Controlling Malaria

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Mosquito Modifications: New Approaches to Controlling Malaria

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Malaria kills about one million people each year, but efforts to destroy disease-carrying mosquitoes have succeeded only in breeding tougher bugs. Researchers have begun to look for ways to create malaria-resistant mosquitoes. One approach is to bioengineer transgenic mosquitoes that, when released into the wild, would lead to a new race of malaria-proof young. Another approach uses mosquitoes' natural resistance to *Plasmodium* infection.

Most of the people waiting at the Outpatient Department of Apac Hospital in Northern Uganda are mothers and their malaria-stricken children under five years old. Malaria, carried by mosquito vectors, kills at least one million people worldwide each year. The majority of fatalities are young children living in sub-Saharan Africa, who die of malaria-induced anemia because their undeveloped immune systems cannot fight off the malaria parasite as effectively as an adult's. Photograph: Toshihiro Horii, University of Osaka, from PLoS Biology *(doi:10.1371/journal.pbio.0000039).*

As daylight wanes on the island of São Tomé, a team of biologists heads out to spy on one of the most important, but least studied, bits of natural history in Africa: the sex life of the mosquito *Anopheles gambiae,* the most widespread vector of malaria on the continent. The researchers, led by J. D. Charlwood of the Danish Bilharziasis Laboratory, have scoped out likely spots scattered on the outskirts of a village, places where a footpath intersects grassland or a swath of dark soil meets the bleached wood of a tree stump. At dusk, male mosquitoes gather over these areas of color contrast, form swarms, and await the arrival of potential mates.

Looking into the columns of hovering insects in the fading light, the researchers watch and count as more and more mosquitoes begin to couple, their hind ends interlocking as they fall out of the swarm. The mating process peaks and drops off within 15 to 20 minutes, and then, in the darkness, females fly to nearby homes to bite the villagers. Each female needs a series of blood meals for sustenance as she incubates the eggs that will form a new generation.

Malaria, a parasitic disease transmitted by infected mosquitoes, threatens an estimated three billion people in 106 nations. Most of the fatalities caused by

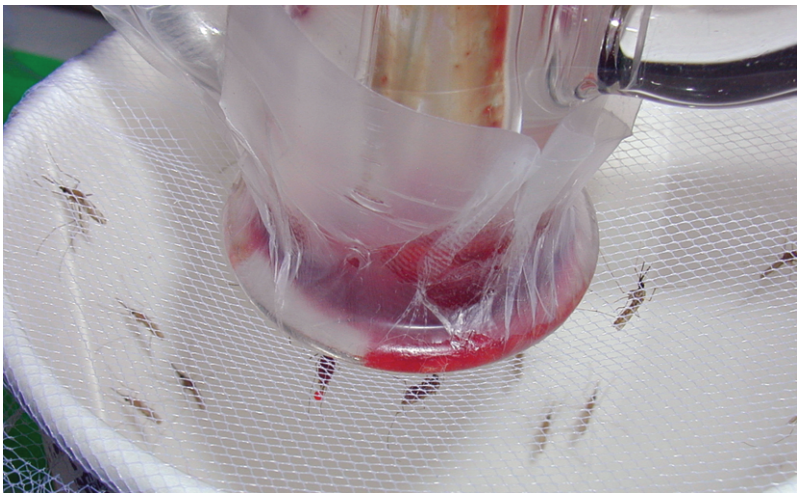
malaria are young African children. In recent years, a new global effort to control the disease has risen from the ashes of a failed campaign that once tried to eradicate it.

Failed attempts

That first major antimalaria drive was based on two chemicals that seemed to hold the promise of destroying both *Plasmodium falciparum,* the most virulent of the protozoan parasites that can attack human liver and red blood cells during malaria infection, and the mosquito vectors that carry the disease. Chloroquine, a cheap, effective equivalent of the plant extract quinine, long the most successful antimalaria drug in the world, was first synthesized in the 1940s.

In 1939, chemist Paul Muller discovered that an organochlorine compound known as DDT worked as a powerful insecticide. His achievement was widely celebrated, and DDT was used for disease control worldwide during and after World War II. Louse-infested refugees were doused with it, and the chemical was dropped in many areas where mosquitoes were thought to breed. When Muller was awarded the Nobel Prize for his work in 1948, many still cherished the hope that DDT would wipe out malaria-bearing mosquitoes forever. Yet the first wild mosquitoes to evolve resistance to DDT had already been identified two years earlier, in 1946. Excessive use of DDT in agriculture accelerated the evolution of insect resistance. By the early 1960s, about 400,000 metric tons of DDT were used annually, 70 to 80 percent of which was for control of crop pests.

Malaria has been effectively wiped out in the United States and many other developed nations, but both *Plasmodium* and its mosquito vectors still flourish in many poorer, hotter countries. The malaria parasite has evolved resistance to chloroquine and to subsequent generations of drugs. Today the only reliable malaria treatment is a cocktail of drugs that hit the parasite in several different ways at once. Likewise, mosquitoes and other insects have shown a great facility for detoxifying DDT and several forms of alternative insecticide. Recent studies of DDT resistance in the fruit fly *Drosophila melanogaster,* which is used as an experimental template by many insect researchers, show that a mutation at a single gene locus confers resistance to DDT and an array of other pesticides, and it is likely that a similar mutation occurs in DDT-resistant mosquitoes.



Mosquitoes, the offspring of wild-caught mothers, feed on a membrane containing blood from people in Mali infected with the malaria parasite, Plasmodium falciparum. *Recent studies of these wild lineages have shown that the majority of* Anopheles gambiae *mosquitoes possess natural immunity to malaria. Photograph: Ken Vernick.*

By 1972, when the United States banned DDT because of its long-lived toxic impacts on wildlife and human health, 19 species of malaria-transmitting mosquito were resistant to the chemical. When the World Health Organization recently reiterated its support for limited use of DDT inside the homes of rural people living in malaria-affected regions, there was a flurry of passionate responses in the North American press, including claims that environmentalists who supported the ban on DDT had the blood of millions of African malaria victims on their hands. Those claims ignore political facts—DDT has remained available in many countries—as well as a basic biological reality. "Genes for DDT resistance can persist in populations for decades," writes entomologist May Berenbaum, of the University of Illinois. "Spraying DDT in the interior walls of houses, the form of chemical use now advocated as the solution to Africa's malaria problem, led to the evolution of resistance 40 years ago, and will almost certainly lead to it again unless resistance monitoring and management strategies are put into place."

Berenbaum points out that modern-day pockets of mosquito resistance to DDT are already well documented in Africa. Mosquitoes can also quickly evolve resistance to alternative poisons: research on Bioko Island, off the coast of Cameroon, recently found that a new pyrethroid insecticide lost its punch in less than two years. For now, indoor spraying of DDT to help control the raging epidemic may be the best tool at hand in some parts of Africa, but the threat of mosquitoes developing resistance remains—and a less toxic alternative, pyrethroid-laden mosquito bed nets, can be just as effective.

Building a better mosquito

With the insecticide arms race doomed to fail, researchers have begun to explore an intriguing new strategy. Instead of wiping out winged vectors with poisons, they hope to build a better mosquito, one that is immune to *Plasmodium* infection. The goal is to someday neutralize the deadly threat of malaria by making mosquitoes healthier, leaving the victims of *Anopheles* bites at risk of nothing worse than an itchy bump.

Laboratory work on ways to manipulate the mosquito genome to confer malaria resistance is in some ways surprisingly advanced. Marcelo Jacobs-Lorena, of Johns Hopkins University, and his colleagues have inserted an extra gene into *Anopheles stephensi,* a mosquito that transmits malaria in India; the gene makes the insects resistant to mouse malaria, *Plasmodium berghei.* (*P. berghei* is the commonly used laboratory malaria model, because working with *P. falciparum* requires expensive, sophisticated biohazard facilities.) Several different research groups in the United States and Europe are working with different varieties of transgenic mosquitoes that have been made immune not only to malaria but also to dengue fever, another deadly mosquito-borne illness. Still, transferring such a trait into wild insect populations presents a formidable challenge.

A major problem is that lab-reared mosquitoes are likely to have trouble competing with their wild relatives. Many details of mosquito life histories remain mysterious, and it's unlikely that humans can manufacture mosquitoes whose immune responses have been engineered to thwart malaria without inadvertently changing other important traits along the way. In the wild, mosquitoes must adapt to local conditions, which are sometimes harsh. In some parts of the world, they must survive a long dry spell each year. In others, mosquitoes can breed year-round in continuously wet habitats but are targeted by a multitude of predators. The great majority of wild larvae—more than 90 percent—don't survive.

For those that do live, size can be a critical factor. Often, the smallest adults die before they have a chance to mate or eat. If adults make it to a sunset swarm to hunt for a mate, subtle factors can affect their success. Males use a complex sensory organ to track and amplify the sound of a female's whine. Both a female's ability to produce the right tones and a male's capacity to track them are crucial to successful pairing.

Despite these complexities, wild mosquito populations are often exceedingly dense. Millions of mosquitoes can hatch daily in a single village. Pushing a bioengineered trait into such a vast wild population would be an uphill struggle. In a recent review of the existing studies of mosquito reproduction and survival, Charlwood estimates that, assuming a highly fit malaria-resistant mosquito can be produced, it would take many decades for the resistance trait to come to dominate a wild population through normal genetic inheritance. And complete population replacement is the goal: if even a small proportion of vector mosquitoes live, they'll continue to spread malaria to people.

In any human-designed mosquito hatchery, the insects are bound to be subject to adaptive pressures that differ from those in the outside world. Any tweaks to their innate timing systems could render transgenic mosquitoes useless in the wild. In nature, mating takes place during a precise 20-minute window at dusk. Colonies that breed indoors, where the lights are either on or off, are likely to undergo selection for insects that will mate after dark. Even a slight delay in the biological clocks of human-reared mosquitoes could leave them unable to find wild mates.

Antimalarial transgenes themselves make mosquitoes less likely to survive in the wild. "Bioengineered *Plasmodium*-resistant mosquitoes have so far all had a fitness disadvantage compared to wild strains," says Willem Takken, an entomologist with Wageningen University in the Netherlands. In order to replace malaria carriers in nature, "genetically modified mosquitoes would need to overcome that handicap and demonstrate strong behavioral and biological advantages over wild mosquitoes."

Selfish transgenes

Molecular biologists are aware of the problem, and for several years they have been talking about possible ways to force a transgene into a wild insect population at rates much faster than those produced by normal Mendelian inheritance. In a paper published in *Science* in April 2007, Bruce Hay, Chun-Hong Chen, and their colleagues at the California Institute of Technology describe a genetic trick that accomplishes this task in *Drosophila,* and they hope it will translate with relative ease into the mosquito genome.

Hay's group has designed a genetic element they've dubbed *Medea,* a set of genes that spreads quickly through a population not because it makes individuals more fit, but because it kills the competition. "You can think of it as someone running a race," says Hay. "One way to win is to be better than your competitor. The other way is to whack them in the knees. That's the way *Medea* works, through everyone's favorite behavior: spite."

The inspiration for this new twist in bioengineering came from the discovery by Richard Beeman, an entomologist at Kansas State University, of a naturally occurring gene in the flour beetle that dominated populations by killing competing genotypes rather than by conferring a survival or reproductive advantage. Beeman hypothesized that this trait was actually a pair of genes, one a toxin that poisoned every egg cell a mother beetle produced, the other an antidote that rescued only the offspring carrying the selfish gene set. Hay and his group read Beeman's studies and began to brainstorm ways to design a system in *Drosophila* that could replicate this pattern. In theory, such a set of selfish genes, once linked to a disease-resistance trait, could be an invaluable "gene driver" able to rapidly push engineered characteristics into wild insect populations.

Hay's group tinkered with the installation of various toxin genes but could not find a workable system. Either the toxins were so powerful they killed every oocyte before it could be fertilized, or so weak they had no significant effect at all. While trying to formulate an alternative approach, says Hay, "We realized there's an entirely different way to make a toxin. Instead of adding the expression of a poisonous protein, we could silence a gene needed for normal embryonic development." His lab has since been able to produce a selfish gene set, coding for RNA sequences that silence the expression of *Myd88,* a gene essential for the normal early development of fly embryos. This is linked with an "antidote" gene, a spare copy of *Myd88* that is expressed by the embryo soon after fer-

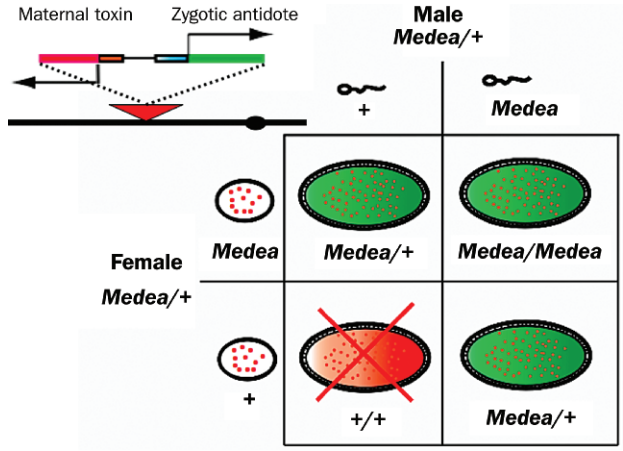
tilization, just in time to rescue the newly conceived fly. So a female with the *Medea* trait—"Medea" stands for "maternal effect dominant embryo arrest"—dooms every one of her own oocytes by failing to express *Myd88* herself, and only the embryos that have inherited the *Medea* genes have the ability to save themselves.

The next step is to apply this work from *Drosophila* to *A. gambiae*. The mosquito's entire genome was recently sequenced, but it remains little known compared with *Drosophila*'s genetic code, which has been explored and tinkered with by a multitude of researchers. Still, early oogenesis and embryogenesis in the mosquito is similar to that in *Drosophila*. The hope is that bioengineers will be able to use the counterparts of the genes identified in *Drosophila* to build a *Medea* element in mosquitoes, then link it to malaria-resistance genes.

"Building *Medea* in *Drosophila* is the equivalent of building a model airplane as a test for seeing how you'd build a real plane," says Hay. "We know we can build something that flies in a lab, in a model insect. Now we need to go do it in a real insect, the mosquito, and we want it to fly not only in a nice simple lab environment but [also] in the wild, where it's exposed to uncontrolled temperature, humidity, predators, and genetic diversity."

Hay envisions factories that will someday churn out disease-resistant, *Medea*-bearing male mosquitoes for release to areas of endemic malaria. Any releases of transgenic mosquitoes are expected to be limited to males, which don't feed on blood and so have no direct contact with people. If the plan succeeds, the right gene driver could replace an entire population of wild mosquitoes with offspring bearing the antimalaria transgene in the course of a year or two.

He acknowledges that any factory environment would probably select for traits that reduce mosquitoes' fitness in the wild, but he believes an effective gene driver can overcome the problem. "Some proportion of them are going to make it," he says. "Once you produce a first generation of hybrid mosquitoes in the wild, the selfish genetic element can go do its thing."

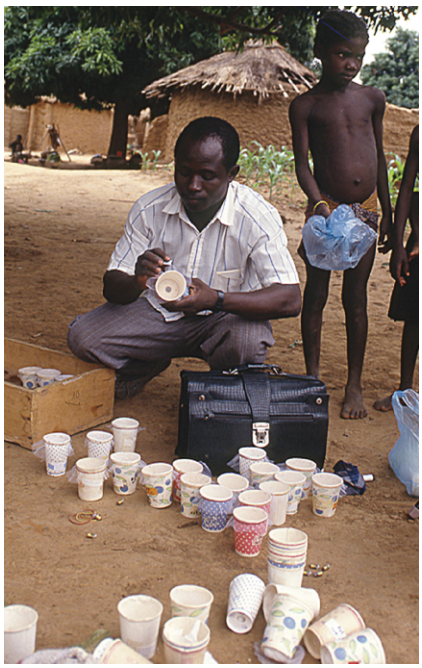


Medea *is a "spiteful" selfish genetic element that enhances its transmission from generation to generation by causing the death of offspring that fail to inherit it. Females that carry a* Medea *element express a toxin (shown here as red dots) that is inherited by all oocytes. Embryos (large ovals) that do not inherit* Medea *die because toxin activity (red background) is unimpeded. Embryos that inherit* Medea *from either parent (or both) survive because expression of an antidote early during embryogenesis (green background) neutralizes toxin activity.* Medea *comprises two closely linked genes (upper left): One is a maternal germ-line–specific promoter that drives the expression of gene-silencing RNA (ribonucleic acid) that is toxic to the embryo. The second is a zygotic (early embryo) promoter that drives the expression of an antidote. The diagram is by Bruce Hay.*

Naturally resistant mosquitoes

Wild mosquitoes may already have evolved the most effective immune defense against malaria, beating bioengineers to the punch. Recent work by geneticists Ken Vernick and Michelle Riehle, of the University of Minnesota, in collaboration with field researchers in Mali and Kenya, shows, for the first time, that the majority of *A. gambiae* in Africa possess innate resistance to the disease. Biologists in Mali captured wild female mosquitoes resting on the walls of huts in a malaria-affected village. The mosquitoes had mated in the wild, and their young were reared in a laboratory, then fed on blood from malaria-infected people in the same village. A few days later, the researchers dissected the mosquitoes and found that a majority had been able to kill the parasites they had ingested.

"The prevailing notion had been that it was a large proportion of the mosquito population that was transmitting malaria," says Riehle. "Now it seems the reverse is true: the majority of wild mosquitoes are resistant, and it's a minority



Guimogo Dolo, a researcher at the Malaria Research and Training Center in Bamako, Mali, processes field-caught Anopheles gambiae *females for use as mothers in generating pedigrees to be studied for innate malaria resistance. Photograph: Ken Vernick.*

that actually cause infection in humans." Sifting through the mosquito genome in the Minnesota lab, the Vernick group has identified a cluster of genes—which they've dubbed the *Plasmodium* resistance island, or PRI—responsible for innate immunity. They've homed in on a single gene locus coding for a leucine-rich protein, APL1, similar to molecules known to work in antipathogen responses of plants and mammals.

The study, published in *Science* in 2006, is unique in that it examines malaria resistance in mosquitoes that are the offspring of natural matings by wild parents in a malaria-endemic area. Most work on transgenic malaria resistance uses colonies of mosquitoes not only removed from the selective pressures of the wild but also tested by their response to rodent, rather than human, malaria. "I believe starting in nature is most important," says Riehle. "There the mosquitoes have to fend for themselves. Anything you develop in the lab, where all the insect's needs are taken care of, is very artificial."

In a study published in *Malaria Journal* in July 2007, the same researchers show that mosquitoes in Kenya, on the opposite side of Africa from Mali, possess innate malaria resistance that can be mapped to the same gene cluster. The same mechanism of immunity seems to exist in *A. gambiae* across Africa, suggesting it is an ancient, well-established trait that might be exploited to help stop the spread of the disease.

Riehle believes building a malaria control strategy around this natural immune response is a much safer bet than relying on transgenes. "We need to find a way to tip the balance in nature, to increase the fitness cost of being susceptible to malaria so that resistant mosquitoes outnumber and eventually wipe out the disease carriers," she says. The finding that resistant mosquitoes dominate wild populations is both encouraging and daunting: there must be some fitness benefits for malaria-susceptible insects, or they would naturally die out.

One potential weapon for tipping the balance against malaria-bearing mosquitoes is a fungus, endemic to Africa, that attacks adult insects. The fungus weakens and kills more malaria-infected mosquitoes than malaria-free mosquitoes. Willem Takken is one of a group of researchers exploring the use of the fungus, already commercially produced for use against agricultural insect pests, as a way of knocking down mosquito populations in African villages. Fungal spores mixed in oil, sprayed on sheets of fabric, and then hung on the walls of homes killed a significant number of mosquitoes within days of their first blood meal. Even if the insects were susceptible to malaria, they died before they'd had time to incubate the contagious form of the *Plasmodium* parasite in their bodies. "We see a strong fitness effect of the fungus on the mosquitoes," says Takken. "The process is slow, which may slow down the eventual development of resistance mechanisms against the fungus. The advantage is that the fungus is naturally present in Africa, and mosquitoes may be regularly exposed to it in the wild."

A crucial part of the breakthrough study that found widespread innate immunity to malaria in African mosquitoes was the capture of wild, gravid Anopheles gambiae *females. They were collected from the walls of houses, where they rest after a blood meal. Photograph: Ken Vernick.*

One week after mosquitoes drank infected blood, researchers Oumou Niare (front) and Sekou Traore, both of the University of Bamako, Mali, dissected the insects to count the number of new malaria parasites they carried. Photograph: Ken Vernick.

Takken shares Riehle's belief that using innate immunity and naturally occurring pesticides is a better idea than attempting to bioengineer a solution. "Making use of natural resistance mechanisms has in principle a much brighter future, as the evolutionary selection processes have already taken place," he says. Resistance traits evolved in nature are less likely to cause unforeseen problems than completely novel, laboratory-built genes.

Other researchers disagree. "Most of the people I know who are trying to engineer resistance in mosquitoes are using human-designed genes, rather than trying to tweak the innate immune response," says entomologist Thomas Scott, of the University of California–Davis. "Natural immune systems might more quickly select for resistance in the malaria parasite, because it's something the organism is already dealing with."

Hay suggests an eventual combination of the two approaches: someday, bioengineers may be able to link a human-designed selfish gene driver to the naturally occurring malaria resistance genes identified by Vernick's lab. That kind of artificial boost may be able to wipe out the minority of the *A. gambiae* population that remain susceptible to malaria.

If transgenic mosquitoes are ever released into the wild, it will only be after a long series of experiments. Researchers will have to slowly move their bioengineered insects from the lab to carefully sealed outdoor cages to study the effects of natural temperature, humidity, and light regimes. They'll need to find ways to mass-produce designer insects. Bioengineered mosquitoes will face many legal and ethical hurdles: people might choose not to eat genetically modified foods, but no one in Africa will have a choice about living with genetically modified mosquitoes once they're released. Some scientists working in the field acknowledge that African health officials are wary of the idea of any genetically modified organism being set loose on their turf.

But as Scott points out, there are parts of Africa where families don't name their children until they're two years old because so many of them don't survive. Young children, whose immune systems are not fully developed, are more susceptible than adults to the loss of red blood cells caused by malaria, and they often die from it. "Around 30 percent of children between the ages of one and five were dying of malaria when I worked in western Kenya," he recalls. "How do the local people feel about this? They'd like it to stop. If you've got a good idea, they'll be open to it."

There's yet another layer of complication. While *A. gambiae* is the most populous malaria vector in Africa, other species of anopheline mosquitoes also carry the disease. Even if the entire population of *A. gambiae* was made malaria resistant, other species would continue to transmit malaria to people. "Taking *gambiae* out of the equation would be a fantastic contribution," says Scott, "but it won't eliminate the problem."

For now, practical malaria-control efforts continue to focus on distributing pyrethroid-laden bed nets, spraying DDT and other pesticides indoors, cleaning up mosquito breeding habitats near villages, and delivering effective antimalaria drug cocktails to the impoverished communities that need them most. Overcoming malaria will have to involve somehow controlling its passage through insect vectors, but the old vision of wiping mosquitoes off the face of the planet with mass pesticide spraying is history. Understanding mosquitoes and their complex relationship with the malaria parasite is now the critical challenge.

Visit these Web sites for more information:

www.who.int/tdr/publications/tdrnews/news65/resistance.htm

www.its.caltech.edu/~haylab/

www.ent.wur.nl/UK/Personnel/Research+Personnel/Willem+Takken

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