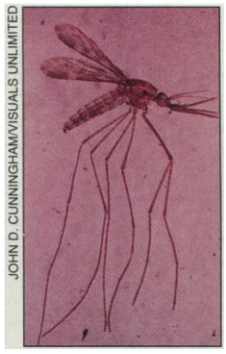
Malaria: Focus on Mosquito Genes

Researchers battling malaria and some other insect-borne diseases hope to engineer strains of insects that cannot carry the human pathogens they now transmit



Scourge of Africa. *Anopheles gambiae.*

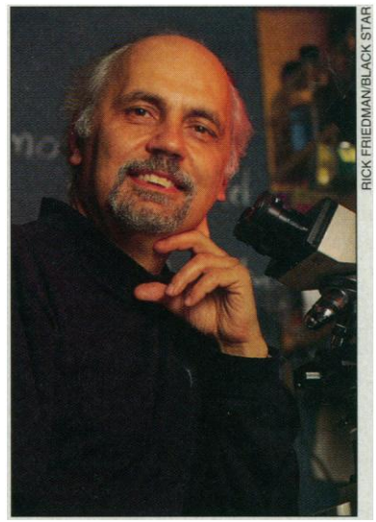
In the early 1960s, malaria seemed finally to be a disease on the run. Field workers armed with DDT were winning the battle against the disease by attacking its transmission routes, or vectors: malaria-carrying mosquitoes. But by the end of the decade, the tide of the battle had turned as the mosquitoes evolved resistance to DDT and other insecticides. Malaria again seemed unstoppable. Indeed, the mosquitoes even evaded another of science's wonder weapons, the technique of swamping insect populations with sterile males to slow down their rate of reproduction. The wild mosquitoes simply bred too fast to make the technique workable. It was "like bailing out the ocean with a sieve," says vector biologist Frank Collins of the Centers for Disease Control and Prevention (CDC) in Atlanta.

After these setbacks, most researchers all but abandoned hope of stamping out insect-borne diseases by focusing on the insects themselves and turned instead to developing drugs and vaccines—only to find themselves confronted by another wily foe, the malaria parasite, which developed drug resistance and evaded candidate vaccines (*Science*, 21 January 1990, p. 399). But now biologists are mounting new campaigns against malaria-carrying mosquitoes and other insect disease vectors. They have a powerful new weapon—genetic engineering—and a new strategy: Instead of trying to eradicate the insects, vector biologists hope to produce transgenic strains that are incapable of transmitting disease. Researchers are already searching for genes that can help insect vectors resist infection with human pathogens, and they are looking for ways to spread these genes through insect populations in the wild.

The stakes are high: Malaria alone kills more than a million people each year in Africa, where it is carried by the mosquito *Anopheles gambiae*, which has been notoriously difficult to control by conventional means. (Strategies involving insect control and antimalaria drugs have, however, been more effective elsewhere in the tropics, where malaria is transmitted by other types of mosquito.) "The [*An. gambiae*] mosquito itself is the problem," says malariologist Louis Miller of the National Institute of Allergy and Infectious Diseases (NIAID).

This new focus on insect vector genetics owes much to the MacArthur Foundation—and, in Britain, the Wellcome Trust—which backed this approach in the late 1980s, before other agencies recognized its potential. The MacArthur program, in particular, invigorated the field, bringing in heavyweights from neighboring disciplines, such as *Drosophila* geneticist Fotis Kafatos, now director-general of the European Molecular Biology Laboratory. And this week sees a major conquest for the program, with the publication on page 605 of a linkage map of the X chromosome of *An. gambiae*, produced by Kafatos' lab at Harvard University and CDC's Collins. When complete, the map may lead researchers to genes that influence the interaction between mosquitoes and the protozoan malaria parasite it carries—and perhaps to key genes that can be manipulated to reduce malaria transmission.

Within a year, Kafatos expects to have achieved another victory by extending the linkage map to the other two *An. gambiae* chromosomes. Armed with this and a physical genome map, completed in Kafatos' Harvard University lab in 1991, biologists will be "in a position to map [*An. gambiae*] traits very effectively," he says. And the malaria mosquito is not the only mosquito to have a genome project all its own: In the current issue of the *Journal of Heredity*, a team led by Dave Severson of the University of Wisconsin describes a linkage map for *Aedes aegypti*—which transmits the dengue and yellow fever viruses.



Mosquito mapper. Fotis Kafatos *(above)* and CDC's Frank Collins are mapping *An. gambiae*'s chromosomes.

Antimalaria mosquitoes

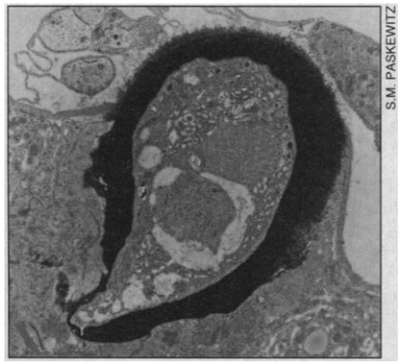
One of the first hints that the key to malaria control may lie in the mosquito genome came in the mid-1980s, when Collins bred a strain of *An. gambiae* that disables malaria ookinetes, the form of the parasite that crosses the mosquito gut wall. Once ookinetes get into the insect's body cavity, they attach to the gut and develop into the so-called oocysts, which in turn rupture after a couple of weeks to release large numbers of sporozoites, the stage of the malaria parasite that infects humans. Collins' malaria-resistant mosquitoes halt the process at an early stage by imprisoning ookinetes in a capsule made from the pigment melanin—a spectacular effect that is due to the enhancement of the mosquitoes' normal defenses against infection. Simple genetic modifications—such as attaching stronger promoter sequences to the genes responsible—could enhance this effect against malaria. But first Collins has to find those genes—and that is where the maps come in. He has already narrowed down the search to a region occupying about 10% of the *An. gambiae* genome and even has a candidate gene: a gene for a serine protease enzyme thought to be involved in the encapsulation response.

Collins and medical entomologist Susan Paskewitz of the University of Wisconsin have now cloned the serine protease gene from the malaria-resistant strain, and Collins aims to use the new genome maps to see if it maps to the same precise position as the enhanced encapsulation trait. Collins rates his chances of having stumbled across the correct gene on his first attempt as a "very long shot." But he is confident that the new maps will eventually lead him to the genes that underlie the strain's parasite resistance.

Other groups are plotting a different line of attack: Rather than searching for antimalaria genes in *An. gambiae* itself, they want to introduce foreign genes into the mosquito to make it resist the malaria parasite. Molecular geneticist Julian Crampton of the Liverpool School of Tropical Medicine and cell biologist Robert Sinden of London's Imperial College may have the ideal candidate: a mammalian immune system gene that pro-

duces an antibody against a malaria antigen carried by the ookinete. Crampton and Sinden's group are now cloning the gene for a fragment of the antibody that could be produced in insect cells, and which by itself disables the ookinete.

A particularly elegant part of the team's strategy is that they may be able to switch on the gene at just the right time and place. This possibility stems from work by a third arm of the collaboration, a group led by molecular biologist Andrea Crisanti of Rome's La Sapienza University. Crisanti's group has sequenced the genes for two of the mosquito's digestive enzymes, called trypsins, that are released into its midgut just as the malaria parasite is trying to cross the gut wall. The promoter sequences from these enzyme genes could act as a trigger if linked to the antibody gene, releasing large quantities of antibody into the gut when the parasite is most vulnerable. "If it works," says Crampton, "you could use the same general strategy against any insect-borne disease."



Locked up. Malaria ookinetes encapsulated in melanin in *An. gambiae* gut.

Crossing the species barrier

Before Crampton's strategy can be tested, however, he must find a reliable way to insert foreign genes into the mosquito—a barrier that will eventually confront all the groups hoping to create transgenic insects. Some tsetse fly biologists are attempting to sidestep the problem by ignoring the fly's genome and modifying its symbiotic bacteria instead (see box, p. 548). Others are taking a more conventional approach: Molecular geneticist Anthony James of the University of California, Irvine, says his group has "preliminary, but promising" data indicating that it may be possible to use retroviruses to smuggle genes into mosquito chromosomes.

Most vector biologists are, however, currently pinning their hopes on a genetic manipulation technique used by *Drosophila* geneticists, in which foreign genes are spliced into fragments of DNA known as transposable elements. Geneticists have found several of these elements that can readily be inserted into *Drosophila* chromosomes, and they can move around the genome of germline cells, multiplying as they do so—which means a transposable element can spread very rapidly through a population. For example, one such element, the *P* element, was first spotted in *Drosophila melanogaster* about 50 years ago, but it is now almost ubiquitous in the species. If a similar transposable element could be used to insert genes into *An. gambiae*, biologists could release relatively small numbers of the resulting transgenic insects, and the element would spread the genes through the wild population.

Given this potential, the race is on to find a transposable element that functions in *An. gambiae*. One leading candidate, called *minos*, was isolated from *Drosophila hydei* by geneticist Babis Savakis of the Institute for Molecular Biology and Biotechnology in Heraklion, Crete. *Minos* has already been introduced into *D. melanogaster* and Savakis is now collaborating with researchers at NIAID to see if it will function in *An. gambiae*.

Molecular entomologists Hugh Robertson and David Lampe of the University of Illinois, meanwhile, are focusing on a widespread family of transposable elements called *mariner*, which occur in the genome of many insects. *An. gambiae* possesses a *mariner* that is almost identical to those found in the horn fly and a species of lacewing, but these *mariners* all seem to have lost their ability to move. So Robertson and Lampe have taken the lacewing element and hooked it up to a more powerful promoter sequence, in the hope that this will kick it back into action.

The engineered *mariner* has yet to be put to the test, but Robertson is optimistic that it will function in a range of insect species—including *An. gambiae*. But even if this particular element does not live up to its promise, most insect geneticists believe that it is only a matter of time before researchers come up with elements that will function in *An. gambiae* and other disease-carrying insects. "My guess is that in virtually every organism you would be able to find elements that would work," says Kafatos.

Kafatos and his fellow vector biologists hope that by the time researchers have worked out systems to create transgenic insects, several candidate antimalaria genes will have been identified. The ultimate goal is to load several such genes together into a single strain to reduce the chances of the malaria parasite evolving resistance.

It will be a long campaign, however, and extensive safety testing will be required before large numbers of transgenic insects can be released into the wild. "I think we are talking about a 15- to 20-year timeframe," says Tore Godal, director of the World

Insect-Borne Viruses: Help From Plants

Biologists studying insect-borne viral diseases may have a head start over their colleagues working on diseases like malaria that are spread by protozoan parasites. The pathogenic human viruses transmitted by insects are similar genetically to those that infect plants. And because "a lot of work has been done on plant viruses," says virologist Stephen Higgs of Colorado State University, viral vector biologists are able to borrow ideas directly from genetic strategies that have been used successfully to protect plants from disease.

Higgs and his Colorado State colleagues are hoping to tackle viruses like dengue and yellow fever, which are transmitted by the mosquito *Aedes aegypti*. First, however, they are honing their techniques by working on the La Crosse virus, which causes an occasionally fatal form of encephalitis in the midwestern United States and is transmitted by the *Aedes triseriatus* mosquito. To introduce foreign genes in *Aedes* cells, the researchers use a modified Sindbis virus—a relatively harmless virus that infects a wide range of insects. The foreign genes are hooked up to a promoter sequence and spliced into a clone of Sindbis complementary DNA. Once inside *Aedes* cells, this cDNA produces infectious Sindbis virus and expresses the foreign genes.

Using this method, the Colorado State team has been studying suspected antiviral genes. "Antisense strategies seem to work very well," says molecular biologist Ken Olson. Taking a cue from studies with transgenic plants, the team has spliced a "back to front" version of the gene for the coat protein of the La Crosse virus into the Sindbis cDNA. The antisense RNA produced by the recombinant Sindbis, says Olson, seems to interfere with the corresponding La Crosse virus mRNA and almost completely prevents replication of the La Crosse virus inside *Aedes* cells. The next step is to feed blood containing the recombinant Sindbis cDNA to live *A. triseriatus* mosquitoes to see if this reduces their ability to transmit La Crosse encephalitis.

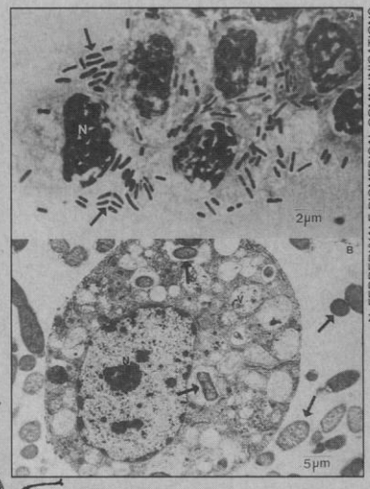
Unfortunately, the Sindbis virus system does not carry the genes into the mosquito genome. So, just as in malaria vector biology, there is no immediate prospect of making a transgenic insect capable of spreading antiviral genes through wild insect populations. But when the transgenic technology becomes available, the Colorado State researchers hope to have an armory of antiviral genes at the ready.

–P.A.

Bacteria May Provide Access to the Tsetse Fly

As geneticists search for ways to produce transgenic insects capable of spreading new genes through wild mosquito populations (see main text), tsetse fly biologists are trying another tactic. The tsetse fly *Glossina morsitans* not only transmits African trypanosomes, the protozoan parasites that cause sleeping sickness, but it also carries symbiotic bacteria, or symbionts, inside the cells of its gut and other tissues. This has led researchers to think about creating "pseudo-transgenic" tsetses by inserting genes into the symbionts rather than directly into the fly's own genome.

The possibility of using this approach was raised in 1987, when entomologist Ian Maudlin and biochemist Susan Welburn of the University of Bristol, England, cultured one of the species of bacterium found in the tsetse gut. And last year, researchers at Yale University brought it one step closer to reality when they genetically transformed the bacterial symbiont using a plasmid, a loop of DNA, carrying genes that confer resistance to antibiotics. The Bristol and Yale groups are now working to isolate genes that might confer resistance to trypanosomes. If such genes could be inserted into the symbiont, it may be possible to produce tsetses that are "immune" to trypanosomes.



N. FERRERI/YALE BIOMEDICAL COMMUNICATIONS

Way in? Tsetse symbiotic bacteria *(arrows)* under light microscope *(top)* and electron microscope *(bottom)*.

Maudlin's group is focusing on a particular biochemical interaction among parasite, vector, and symbiont: Cells in the tsetse midgut secrete a lectin protein that can kill trypanosomes before they leave the gut. But this is inhibited by a sugar called *N*-acetyl-*D*-glucosamine, which is in turn produced when a chitinase enzyme secreted by the symbiont breaks down the tough chitin lining of the tsetse's gut wall. As a result, Maudlin has found that flies carrying large numbers of symbionts are more susceptible to trypanosomes. He reasons, however, that if he can find a way to remove the sugar or produce more lectin, he could block the trypanosome's life cycle. Maudlin's group is now working on ways to alter the symbiont, either by inserting the gene for the tsetse lectin or by adding a gene to make the symbiont mop up the lectin-inhibiting sugar.

The Yale tsetse group, led by molecular parasitologist Serap Aksoy, is taking a different tack. It is hoping to coax the symbiont into expressing a gene from a mammalian immune system. Angray Kang of the Scripps Research Institute in La Jolla, working in collaboration with Aksoy's group, aims to isolate the gene that produces an antibody fragment that attacks a trypanosome antigen called procyclin. The Yale group will then splice it into the symbiont to produce a transformed bacterium that should kill trypanosomes.

Even if both groups succeed in producing trypanosome-killing symbionts, however, they still face a tough problem: how to get the bacteria back into the fly. The normal symbionts would first have to be flushed out with an antibiotic, but this would also remove a second symbiotic bacterium carried inside specialized cells in the tsetse gut. This symbiont is believed to supply the tsetse with an essential nutrient—and because it has not yet been cultured there is currently no way to reintroduce it into the flies.

A second conundrum is how to spread engineered symbionts through wild populations. Tsetse symbionts are passed from female flies to their offspring, but this alone will not drive a transformed symbiont through a large wild population. The Yale researchers, however, believe that they may have found a solution. Using a probe that recognizes a specific genetic sequence, they have identified a third bacterium, called *Wolbachia pipientis*, in the ovaries of female tsetses. In other insect species, *Wolbachia* exerts an effect called cytoplasmic incompatibility: Female insects infected with *Wolbachia* can mate with both infected and uninfected males, but females lacking the bacterium produce viable offspring only with uninfected mates. As a result, *Wolbachia* can spread rapidly through an insect population—and some strains maximize their transmission at the expense of others, through similar incompatibility effects.

The Yale researchers do not yet know if *Wolbachia* will cause mating incompatibility in the tsetse. But if so, says Aksoy, it could be used to drive engineered symbionts through a population, as *Wolbachia* and an altered symbiont would be passed down together from females to their offspring. "You could couple the two bacterial systems to spread genes," she says.

That idea has sent a ripple of excitement through the malaria vector research community. Although the malaria mosquito *Anopheles gambiae* carries neither *Wolbachia* nor gut symbionts, entomologist Chris Curtis of the London School of Hygiene and Tropical Medicine is now collaborating with Scott O'Neill, the Yale group's *Wolbachia* expert, to see if *Wolbachia* can be introduced into *An. gambiae*. Curtis and O'Neill believe *Wolbachia* could be used to drive antimalaria genes through mosquito populations, if the genes were inserted into a vehicle that, like *Wolbachia*, is maternally inherited. "We're looking at small DNA viruses," says O'Neill. The leading candidate? A class of insect viruses called densoviruses, some of which are transmitted from mother to offspring.

–P.A.

Health Organization's Tropical Disease Research program. But if the remaining technical hurdles and safety questions can be overcome, this new approach "might even lead to the eradication of malaria," says NIAID's Miller.

–Peter Aldhous

Additional Reading

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H-M. Müller, *et al.*, "Members of a Trypsin Gene Family in *Anopheles gambiae* Are Induced in the Gut by Blood Meal," *EMBO Journal* **12**, 2891 (July 1993).

A. A. James, "Mosquito Molecular Genetics: The Hands That Feed Bite Back," *Science* **257**, 37 (3 July 1992).

H. M. Robertson, "The *Mariner* Transposable Element Is Widespread in Insects," *Nature* **362**, 241 (18 March 1993).

S. Higgs, *et al.*, "Alphavirus Expression Systems: Applications to Mosquito Vector Studies," *Parasitology Today* (in press).

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