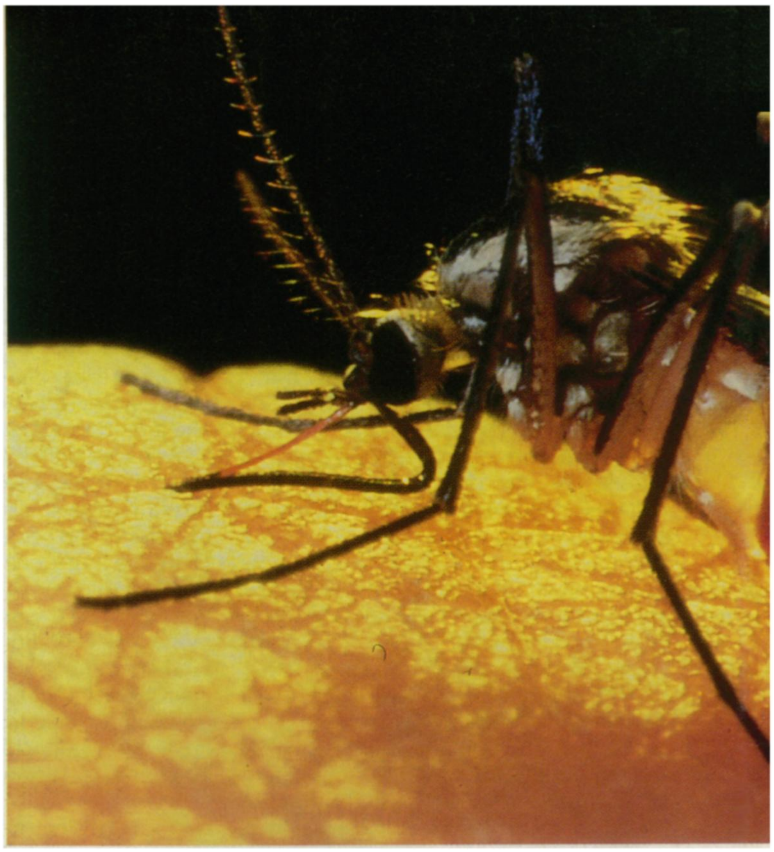
The Swat Team



Gene-altered mosquitoes appear on the horizon

By RICK WEISS

With so much attention focused nowadays on gene-altered mice — the promising new research tool of modern genetics — it seems few researchers remember the lowly fruit fly, which for decades has bared its genes to inquiring biologists.

That the scientific spotlight has wandered somewhat from these insects may appear justified at first. After all, the attention geneticists lavished upon the fruit-bowl pest had more to do with its rapid reproductive rate and ease of handling than with its relevance to human health. But as geneticists leaped from fruit fly to mouse, did they overlook another six-legged candidate? Might the fruit fly's close cousin, the mosquito, deserve more scrutiny from molecular biologists?

A handful of researchers, working in mosquito-netted chambers called insectaries, believe so. Though their substantial discoveries over the past three years have not made headlines, the work may eventually yield a powerful weapon in the global war against malaria and other mosquito-borne diseases — major causes of illness and death in much of the developing world.

Already, these scientists have created specific, gene-altered versions of *Anopheles gambiae*, the world's number-one malaria transmitter, responsible for 150 million cases of malaria in tropical Africa annually. They have accomplished similar feats with *Aedes triseriatus*, carrier of the most common U.S. form of mosquito-borne human encephalitis, and with *Aedes aegypti*, the primary carrier of yellow fever, dengue fever and dengue hemorrhagic fever. These successes suggest that genetically engineered insects, if released in large enough numbers to dilute or overwhelm native insect populations, ultimately may prove as useful as pesticides and vaccines in preventing insect-borne diseases.

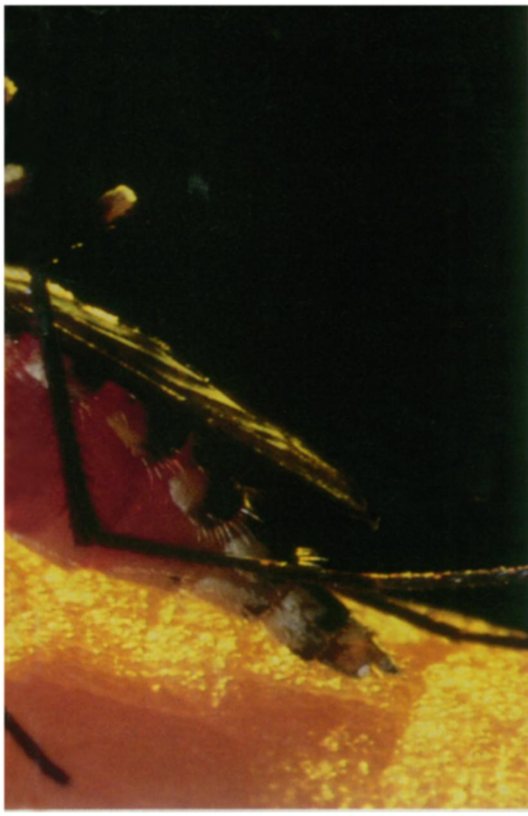
An effort to map all the genes on the three chromosomes of *Aedes aegypti* is gaining momentum in several U.S. laboratories. Researchers have already zeroed in on several genes responsible for mosquito "vector competence," or the ability to transmit pathogenic organisms. The work hints of a future in which scientists may alter the inherited traits of entire mosquito populations in targeted regions, rendering them incapable of spreading disease. In short, while most people view this insect as a worthless pest, "we're turning the mosquito into a genetic animal," says George B. Craig, an entomologist at the University of Notre Dame (Ind.).

The idea of altering natural insect populations to human advantage is not new, but has rarely proved successful. In the 1970s, scientists repeatedly failed to reduce fertility rates among malaria mosquitoes and crop-damaging Mediterranean fruit flies through large-scale releases of males sterilized by irradiation. The goal was to have these males mate with wild females, tricking the females into thinking they had performed their reproductive duty when in fact they would bear no offspring at all.

These well-made plans went awry.

For one thing, the radiation doses were too large, notes Craig. The sterilized insects "could barely walk away, much less get up and spread their sperm around," he says. In addition, these individuals — the products of generations of laboratory-reared insects — appeared ill-adapted to life in the wild. Craig recalls one release of 20 million sterile *Anopheles* males in Florida that had no apparent effect on the wild population there. In the laboratory, he says, "they were used to just rolling over on their side and finding a female." But outdoors, "they just sat on this rock and watched females fly by. They weren't adapted to going out and going after these females."

More recent irradiation experiments, especially those involving Mediterranean fruit flies in California, appear somewhat more promising. But for disease-carrying insects, many ecologists would prefer an approach that specifically targets the machinery of vector



Photos: Leonard Munstermann/Fran Heyl Assoc.

Aedes aegypti, the primary carrier of yellow fever and dengue fever, has become widely resistant to pesticides but may succumb to genetic engineering.

competence, leaving other traits intact.

The first clues that specific flaws in this machinery occur naturally – and thus might prove amenable to genetic manipulation – cropped up in the 1930s with the observation that certain populations of insects appeared incapable of transmitting diseases commonly spread by their kind. For example, accumulating evidence indicated that some strains of *Anopheles gambiae* did not transmit the malaria-causing protozoan.

In the Oct. 31, 1986 SCIENCE, a research team led by Frank H. Collins and Robert W. Gwadz of the National Institute of Allergy and Infectious Diseases (NIAID) explained why. They reported discovering that mosquitoes of these strains quickly encapsulated any protozoans they ingested, preventing the microscopic organisms from infecting any human subsequently attacked by the insect. A series of selective-breeding experiments suggested that the encapsulation trait depends upon one or a very few mosquito genes.

Scientists understand little about the mechanics behind this and other forms of vector incompetence. But research in several laboratories now indicates such phenomena can arise in several ways, says Notre Dame biologist Paul Grimstad. For instance, a disease organism might fail to establish itself inside the insect, perhaps because of an immunologic attack encountered in the insect's gut. Or, once entrenched, it might fail to develop properly. In other cases, the organism might fail to migrate to the insect's salivary glands or to pass with insect saliva into a new human host.

"The ideal thing would be to have a mosquito that's incapable of becoming infected," Grimstad says. But he also expresses optimism about a mosquito variety he and others are investigating that becomes infected with an encephalitis-causing virus but does not pass the virus in its saliva.

That mosquito, *Aedes hendersoni*, is a close relative of *Aedes triseriatus*, which spreads La Crosse fever throughout the central northern United States. This serious, difficult-to-diagnose form of encephalitis infects hundreds and perhaps thousands of U.S. children annually, according to the Centers for Disease Control. But while both mosquito species become infected with the La Crosse virus, *Aedes hendersoni* fails to transmit the virus to humans.

Recent studies by Grimstad and others suggest that the salivary glands in *Aedes hendersoni*, compared with those in *Aedes triseriatus*, have smaller pores, effectively sieving out the virus before the mosquito injects its saliva into a human host. Moreover, scientists have narrowed the search for the genes that influence these salivary traits, and they now suspect that only one or two genes may account for the critical difference. "It looks like a good possibility" that scientists will be able to move this trait into *Aedes triseriatus*, Craig reported at December's annual meeting of the Entomological Society of America in San Antonio, Tex.

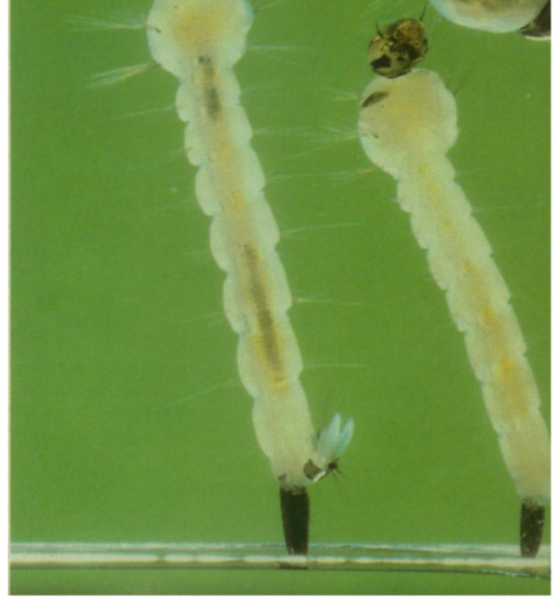
Researchers described the first successful injection of DNA into fruit fly eggs back in 1976, and the prospect of engineered mosquitoes has floated about in scientific discussions ever since. But in part because of peculiarities in the mosquito egg's outer coat, it took scientists another decade to develop a method for injecting DNA into mosquito eggs to create the world's first gene-altered mosquito. In the Aug. 14, 1987 SCIENCE, Louis H. Miller and his coworkers at the National Institutes of Health detailed the painstaking methodology that enabled them to insert a bacterial gene into *Anopheles gambiae* eggs, providing the long-awaited proof that mosquitoes are indeed malleable through genetic engineering.

Two years and more than 50 mosquito generations later, that marker gene persists in the laboratory-reared population, says Gwadz, who participated in the 1987 gene-splicing feat. In a new research report submitted for journal publication, he and his colleagues provide additional details about the chromosomal location of the integrated gene – information that should aid scientists in their quest to gain control over the mosquito genome.

Meanwhile, at least two other teams have reported creating genetically transformed, disease-spreading mosquitoes. Although some questions remain about the stability of the insertions, the work highlights a growing momentum in this area of research and reflects biologists' increased familiarity with the technique itself.

In 1988, for example, Vicki McGrane and Barry J. Beaty of Colorado State University in Fort Collins and their colleagues became the first to insert a bacterial marker gene into *Aedes triseriatus*. The work stirred excitement among entomologists because of a peculiar trait inherent in that species. Once infected with the La Crosse virus, *Aedes triseriatus* resists subsequent infection by this and other viruses – apparently because of a mechanism that blocks viral replication in the insect's midgut cells. Scientists hope to unravel the genetics behind this immunity-like mechanism and to use that knowledge to induce viral resistance in engineered mosquitoes *before* the insects have a chance to become infected and to pass the virus to humans.

"We know the area to go into," says Beaty, noting that the midgut-mediated



Larvae of the mosquito Aedes triseriatus thrive in used tires and other pockets of water. In the United States, the adults spread a childhood encephalitis.

resistance appears related to genes on the mosquito's second chromosome, with possible help from a few genes on chromosome three.

And just last year, Alison C. Morris, Paul Eggleston and Julian M. Crampton of the Liverpool (England) School of Tropical Medicine reported the first successful introduction of a bacterial gene into *Aedes aegypti*, a species that has developed widespread resistance to pesticides.

With the technique for inserting bacterial genes into *Aedes aegypti* now well established, the Liverpool researchers say they look forward to experimental insertions of various mosquito genes. "Such work should ultimately lead to an understanding and control of the molecular mechanisms involved in the transmission of pathogens by their insect vectors," they write in the January 1989 MEDICAL AND VETERINARY ENTOMOLOGY.

Large-scale disease control through engineered insects remains "far down the road," says Beaty. Other approaches, including safer pesticides and new drugs, will no doubt play important roles along the way. But in light of growing pesticide resistance among insect populations, environmental concerns about large-scale drainage of ecologically sensitive wetlands where mosquitoes breed, and the bleak prospects for rapid development of vaccines against such scourges as malaria and dengue, researchers say gene-altered mosquitoes start to look pretty good.

NIAID's Gwadz calls the replacement of entire insect populations a "very long-range goal" that will probably work best in specific circumstances, such as areas where targeted insect populations remain somewhat isolated or where an infestation is relatively new.

The genetic approach has some ecological advantages over a strategy of wiping out entire insect populations, Beaty adds. "Whenever you knock out something with pesticides, you create an ecological vacuum and something else moves in," he says. With improvements in genetic engineering techniques, "we may be able to enhance populations that are not good transmitters," without removing a significant piece of the ecological puzzle.

But any genetic traits scientists choose to enhance will have to work very efficiently in the altered mosquitoes. Gwadz notes that in Africa, mosquitoes typically inject 2,000 times more malaria-causing protozoans into humans than are needed to transmit the disease. Thus, even mosquitoes genetically engineered for a 99 percent reduction in their transmission efficiency would have little effect on the spread of malaria there, he says.

T. Litwak

On the brighter side, while scientists today know of only a few mechanisms by which insects become poor carriers of pathogens, other sources of vector incompetence – perhaps extremely efficient ones – probably await discovery, Gwadz asserts.

It will take time to understand these various biological mechanisms and to sort out the underlying genetics. Toward that end, entomologists and molecular biologists express excitement about a $1.1 million program initiated this year by the Chicago-based MacArthur Foundation. The money is earmarked to forge a marriage between modern genetics and vector biology at five U.S. research centers.

Researchers note that a thorough understanding of even one good mechanism of vector incompetence could lead to wholesale reductions in the number of people suffering from mosquito-borne diseases. "However it happens," says Gwadz, "if the mosquito can't transmit the disease, then that's okay." □

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and if such studies confirm these findings, it would appear that prudent public policy intended to address the educational disparity between poor and nonpoor children should include efforts to assure access to SBP for all low-income children."

Gill suggests children who tend to be absent and tardy also tend not to participate in SBP or to perform well in school, but there was no difference in absence and tardiness rates between SBP participants and nonparticipants in the year prior to SBP implementation.

Alan Meyers, Michael Weitzman
Boston University School of Medicine
Amy Sampson, Beatrice Rogers
Tufts University School of Nutrition
Boston, Mass.

Tasaday: Forked tongue?

It was misleading – ambiguous if not erroneous – to say in your report on the Tasaday that "the strange case . . . grows stranger still with new evidence presented at the annual meeting of the American Anthropological Association" ("Tasaday controversy grows more curious," SN: 11/25/89, p.343).

In fact, all new primary evidence at the meeting (chiefly genealogical and linguistic) supported the argument that the Tasaday were an authentic and distinct group of cave-dwelling food-gatherers who had resided in the Mindanao rain forest for at least several generations when researchers first contacted them in 1971.

There are disagreements on aspects of the Tasaday, but the "clash" you cite between linguists is not over whether Tasaday language is authentic but over when it split from a root language and to what degree it is uniquely Tasaday. All linguists who have done field work with the Tasaday say their speech is related to but distinct from that of neighboring peoples. In other words, the Tasaday are real, not phonies, which was the major question raised before the meeting.

In affirming Tasaday authenticity, four researchers who presented new data joined the 11 other anthropologists who have done field studies with the Tasaday. None of the anthropologists you quoted as saying the Tasaday are impostors have ever met the people, visited their habitat or gathered primary evidence. If *that* is what your report meant by a "strange case," then I hasten to agree.

John Nance
Portland, Ore.

Like cancer, AIDS and corruption, the Tasaday controversy refuses to die. The hoaxers, careless researchers and vested interests do not allow it. There are bucks to earn, reputations to protect, celebrity statuses to cultivate. The truth – well, it can wait.

Our paper argues that the Tasaday speak a distinct language and not a dialect of any Manobo language. Our analysis of Tasaday and representative Manobo languages reveals that Tasaday shares only 26 of its 100 basic vocabulary items with Cotabato, the Manobo language with which it was claimed to share 90 percent of its fundamental vocabulary at the meeting of the American Anthropological Association. A cognate sharing of 26 percent is very low, considering that the cutoff figure for dialects is about 70 percent.

We found that several aspects of Cotabato syntactic rules were violated in the Tasaday speech of 1972, including sentence formation, pronominalization, time reference and focus affixation patterns. Nor was there in several instances a consistency of patterning of the syntactic aberrations. To say the Tasaday speech taped and transcribed by C. Molony in 1972 is a dialect of Cotabato is to say the following sentences represent one dialect of English: "I is going yesterday I house"/"I will go yesterday mine house"/"I went my house yesterday"/"House yesterday."

A possible explanation could be that the Tasaday in the early '70s were not speaking their own language but were trying to learn and speak another, perhaps a language of prestige to them, spoken by an individual or group with superior technology which they wished to acquire.

Tasaday bilingualism – and the ignorance on the part of the Tasaday and their interpreters of what the linguists were truly after in 1971 and 1972 – partly accounts for the kind of linguistic data collected at the time. Thus, a huge chunk of those linguistic data is not Tasaday but Cotabato Manobo or a dialect of Cotabato such as Blit – the language spoken by Dafal, the man who reportedly introduced the Tasaday to trapping and hunting technology, and to Manuel Elizalde Jr., who introduced them to the outside world.

As the issues clarify themselves, it becomes clearer that the hoax is the hoax.

Araceli C. Hidalgo, Cesar A. Hidalgo
Linguists
University of Malaya
Kuala Lumpur, Malaya