Building the Better Bug

Inserting new genes into a few specific insect species could stop some infectious diseases, benefit agriculture and produce innovative materials

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TRANSGENIC INSECTS can be given new characteristics, as illustrated by these five *Aedes aegypti* mosquitoes. Normal individuals have what appear to be black eyes, the result of large amounts of red pigment. A mutant version of *Ae. aegypti* has white eyes because of the lack of an enzyme, kynurenine hydroxylase, required to synthesize the red pigment. This white-eyed condition can be altered via the insertion of the gene for the enzyme. The resultant mosquitoes produce enough pigment to have visibly pink eyes. Such eye-color changes merely point out the potential of transgenic technology for producing a strain incapable of transmitting yellow fever or dengue.

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An extraterrestrial visitor would surely acknowledge humanity to be the dominant species on the earth. Should that visitor move past individual species and up the levels of taxonomic classification, however, the alien's field report might well give the class Insecta top billing. More than one million insect species have been identified, accounting for five sixths of all species of animals. Each U.S. acre averages 400 pounds of insects, compared with only 14 pounds of people. Where humans and insects interact, vast economic interests hang in the balance. Even more profoundly, the clash of humans and insects that carry diseases is often a matter of life and death.

A few insect species, most notably those that feed on blood, are still responsible for spreading major human diseases, such as malaria, yellow fever, trypanosomiasis and dengue, as well as some conditions affecting livestock. Malaria alone accounts for between 300 million and 500 million clinical cases annually and some 1.5 million to 2.7 million deaths. About 200,000 people come down with yellow fever annually, and 30,000 die. Some 50 million people contract dengue every year; mortality can reach 15 percent without treatment. In many developing countries, nonfatal but debilitating conditions, such as dysentery, can be transmitted by insects, including the common housefly.

Public health efforts against insect-borne diseases have been limited to three basic strategies: rid the area entirely of the insect, use pesticides and physical barriers such as bed nets to keep at least some of the insects away, or develop a vaccine. The first undertaking has worked, in some areas [see "The Philadelphia Yellow Fever Epidemic of 1793," by Kenneth R. Foster, Mary F. Jenkins and Anna Coxe Toogood; SCIENTIFIC AMERICAN, August]. Lowering the exposure to insects has had limited success. Vaccine development remains spotty; for example, the world still awaits an effective and affordable malaria vaccine.

An additional approach could cut this Gordian knot: simply make the insect unable to transmit disease. Insect bites themselves have little health consequence for most people; the pathogenic viruses, protozoa and filarial worms they transmit are the culprits. In the 1960s Chris F. Curtis of the London School of Hygiene and Tropical Medicine proposed that malaria could be stopped in its tracks if a way could be found genetically to convert its carrier, the *Anopheles* mosquito, to a form incapable of transmitting the *Plasmodium* protozoan actually responsible for the disease. Some mosquitoes are in fact naturally "refractory," or unable to transmit *Plasmodium*.

Curtis's proposal was impossible to implement for decades. But it soon may be realistic, thanks to modern genetic technologies. Genetic material from one species can be permanently integrated into the DNA of individuals from another species, conferring new traits. The resultant plant or animal that carries the new DNA is called transgenic.

Finding ways to engineer refractoriness into disease-carrying mosquitoes and other insect vectors now drives an extremely active area of research. The benefits of developing transgenic insects are not limited to medicine, however. The insertion of genes for useful products into the genomes of cows and goats has already created animals that produce pharmaceuticals in their milk. Applied to insects, transgenics could fundamentally change agriculture and the synthesis of some materials.

Jumping Genes Open Doors

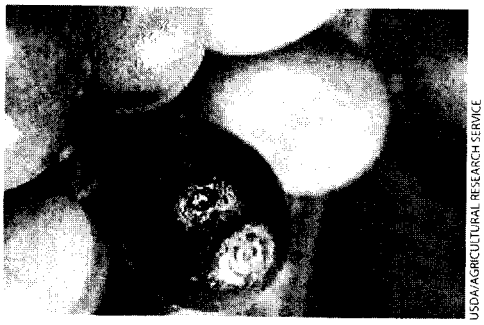
The first glimmers of transgenic insect research date back to the 1960s, with the motivation being improved gene analysis rather than any direct applications outside the lab. Most of the early efforts to alter a genome consisted of injecting an insect egg with, or even simply bathing it in, DNA. Neither technique ever developed into a reliable method for producing transgenic insects.

All genetic research leapt forward in the early 1980s with the insect work of Gerald M. Rubin and Alan C. Spradling, both then at the Carnegie Institute of Washington. Rubin and Spradling were investigating fascinating genetic entities known as transposable elements. These strange strings of DNA have the ability to cut and paste themselves repeatedly into different chromosomes. Formally called transposons, their acrobatics also earned them the nickname "jumping genes." Geneticist Barbara McClintock discovered transposons during research in the 1940s on corn. The importance of her findings finally won her the Nobel Prize in 1983.

The particular transposon that the Carnegie researchers investigated came from the genome of that workhorse of the laboratory, the fruit fly *Drosophila melanogaster*. This species has little importance as an agricultural pest but has been fundamental in genetics research for the past 70 years. Acknowledging the propensity of the transposon to integrate itself into chromosomes, Rubin and Spradling had a simple and clever notion: Why not attach to it the gene they wanted the fly to have? They introduced an altered transposon into a cell, where it indeed pasted itself into the chromosome, creating transgenic *D. melanogaster*. The success and simplicity of their technique revolutionized the way researchers study the genetics and biology of that species.

The *Drosophila* transposon is known as the P element. It was discovered in the 1970s when geneticists noted a puzzling phenomenon. When males from certain populations mated with females of other populations, their progeny had numerous genetic aberrations, such as mutations, broken chromosomes and developmental abnormalities. Because the genetic entities responsible were discovered to come from only the paternal lineage, they were dubbed P factors. Eventually shown to be a transposon, the P factor of most interest to geneticists was renamed the P element and has proved priceless to *Drosophila* geneticists, allowing analysis of isolated genes and their effects.

Unfortunately, in 1986 a set of experiments by one of us (O'Brochta) and Alfred M. Handler of the U.S. Department of Agriculture in Gainesville, Fla., came to a frustrating conclusion: the P element is of little practical value beyond basic genetics research involving *D. melanogaster*. It will not readily insert itself into the chromosomes of other species. Ultimately, however, these experiments led to a new path, by shifting experimenters' attention to other transposable elements



USDA/AGRICULTURAL RESEARCH SERVICE

MEDFLY ALIGHTS on coffee bean. Saving crops drives initial applications of transgenic insect technology. Genetic-control methods aimed at Medflies, the perpetrators of much agricultural damage, already save billions of dollars annually worldwide.

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and strategies. Recent work has begun to uncover methods for creating transgenic insect species of greater importance.

The realization that the P element would not prove useful outside of *D. melanogaster* sent biologists in search of more generally functional transposons. An obvious question became one of choice: Which transposable elements showed the most promise? Most researchers believed we should stick with a proved commodity and seek transposons that generally resembled the P element (called short inverted repeat-type transposable elements). Our lab developed techniques to determine quickly whether a particular transposon would successfully incorporate itself into the DNA of an insect species, which has helped speed the entire process of vector evaluation and development. Early efforts with transposons having structures similar to the P element have rewarded the choice to sail within sight of charted land.

In 1995 Charalambos Savakis and his colleagues at the Institute of Molecular Biology and Biotechnology on the Greek island of Crete succeeded in using a transposon called *Minos,* isolated from *D. hydei.* Using *Minos* to insert a novel gene into a Medfly, they created the first transgenic version of that animal. The transformation changed a fly with colorless eyes to one that expressed a gene for red-colored eyes (in a sense, effecting gene therapy). Subsequently, Handler and his co-workers successfully transformed Medflies with pigment-free eyes to ones having color, using a transposon called *piggyBac* that comes from the cabbage looper moth. Obviously, changing an insect's eye color is of little inherent interest; the importance of these groundbreaking successes is their illustration of the potential for creating transgenic insects that express truly valuable genes.

Earlier this year investigators reported two discrete transformations of the *Aedes aegypti* mosquito, which transmits yellow fever and dengue. The successful manipulations of this inadvertently malevolent creature have led to greater optimism that geneticists will soon be able to convert it into a noncombatant in the disease wars. First, Anthony A. James and his colleagues at the University of California at Irvine altered *Ae. aegypti* via a housefly transposon called *Hermes,* which was originally isolated in our laboratory. (In contrast to the P element, *Hermes* appears to be an efficient vehicle for the creation of transgenic insects ranging from moths to mosquitoes. Work with it is helping to further the understanding of the biochemistry of transposon movement and regulation.) James and his research group then succeeded in incorporating into *Ae. aegypti* a transposon called *mariner,* isolated from the fruit fly species *D. mauritiana.* Again, the effect was simply to change eye color.

In research that dovetails with these two transgenic developments, Barry J. Beaty and his co-workers at Colorado State University demonstrated the feasibility of engineering refractoriness into *Ae. aegypti.* One way to get a host to express a gene it does not ordinarily have is to infect it with a virus carrying that novel DNA sequence. Beaty's team infected the mosquitoes with a nonpathogenic virus that included a gene that prevented the dengue virus from replicating in its host's salivary glands. The infection stops subsequent transmission.

Beaty's research shows that it is possible to create a refractory insect, and therefore no theoretical barriers exist to impede creation of such a creature via genetic insertion. An overarching problem remains, however. Merely waiting for a transgenic insect to pass on its new gene to huge numbers of descendants in

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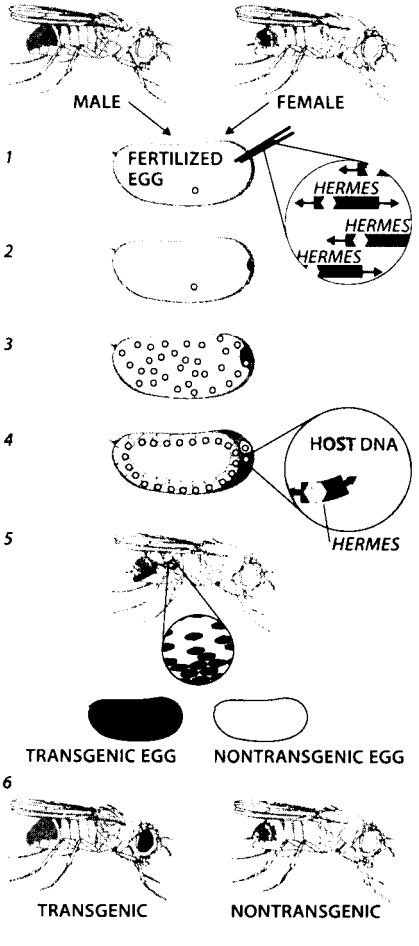
TRANSGENIC MEDFLY has its natural eye color restored. White-eyed mutants produce red pigment but cannot transport the pigment to the eyes. The red-eyed Medfly on the left is a transgenic that has been given the transposable element *piggyBac,* which is carrying a normal copy of the gene enabling pigment transport to the eye.



DMITRY KRASNY; SOURCE: PETER LAWRENCE

MAKING TRANSGENIC INSECTS requires the insertion of a gene (*blue*), carried by a transposable element such as *Hermes* (*red*), into a fertilized egg (*1*). The new genetic material is strategically placed at the polar plasm (*2*), that section of the egg destined to become the still nascent insect's own egg cells when it reaches maturity. After numerous divisions of the egg's nuclear material (*3*), most of it segregates to the periphery, where it will become the nuclei of the cells of the insect's body; two nuclei, however, will migrate to the pole to become the insect's egg cells (*4*) when it reaches maturity (*5*). Should those cells have incorporated the transgene, progeny will be transgenic (*6*).

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a strictly Mendelian fashion—in which a parent possessing one copy of the gene contributes it to only half of his or her descendants—can be a lengthy process.

A more practical plan would spread the genetic change through large numbers of insects much more quickly. Fortunately, the basic components for quickly creating an entire insect population all carrying the key gene appear to be available. Once again, the transposon makes things possible.

Because of their propensity for jumping to new chromosomes, as well as making multiple copies of themselves, transposons burst free of the constraints of strict Mendelian heredity. A transformation event achieved by a scientist may have placed a single transposon, carrying a single key gene, into the genome of the target animal. But that transposon then may act as a free agent and spread itself throughout the genome. When that happens, more than half the offspring inherit the transgene. Within the relatively short time of a very few insect generations, most of the population expresses the trait.

This type of gene dispersal has been seen in nature. In fact, the P element itself appears to be a recent addition to *D. melanogaster*'s genome, most likely jumping over from *D. willistoni* no more than a century ago.

Other techniques may be effective at creating transgenic species. Human gene therapy is based in part on unique systems (derived from retroviruses but no longer infectious themselves) that are able to integrate genes into a new host's genome. (This is a true transgenic transformation, as opposed to Beaty's use of viruses to simply infect an individual organism that will henceforth produce the viral gene product.) These stripped-down viral vectors, known as pantropic pseudotyped retroviruses, can interact with virtually any cell from any organism. They have recently been used to create transgenic fish and clams and have successfully integrated genetic material into cultured mosquito cells. These viral vectors may yet prove their worth in efforts with whole insects.

Because the objective is to alter an insect's phenotype—the outward expression of its genetic makeup, or genotype—most workers have logically concentrated on ways to integrate foreign genetic material into the chromosomes of the host insect itself. But Frank F. Richards and his colleagues at Yale University are developing a clever approach that sabotages the cargo rather than the ship. Because virtually all insects harbor microbes, either as passive hitchhikers or active colonists, Richards is counting on transforming them rather than their hosts. Insects that carry engineered microbes are called paratransgenic, as the insect genome itself is untouched.

Richards and Charles B. Beard of the Centers for Disease Control and Prevention have produced paratransgenic, blood-sucking kissing bugs that can no longer transmit the trypanosome microbe responsible for Chagas' disease, which afflicts some 18 million South Americans with cardiovascular, gastrointestinal and neurological problems. The researchers isolated a bacterial symbiont from the kissing bugs and genetically modified it to secrete a protein that killed its trypanosome fellow travelers. The researchers placed the bacteria back in the insects, which then no longer served as hosts for the disease-causing protozoa. The genetically engineered symbionts successfully spread through a caged population of insects, converting them from vectors of a horrific disease to mere pests.

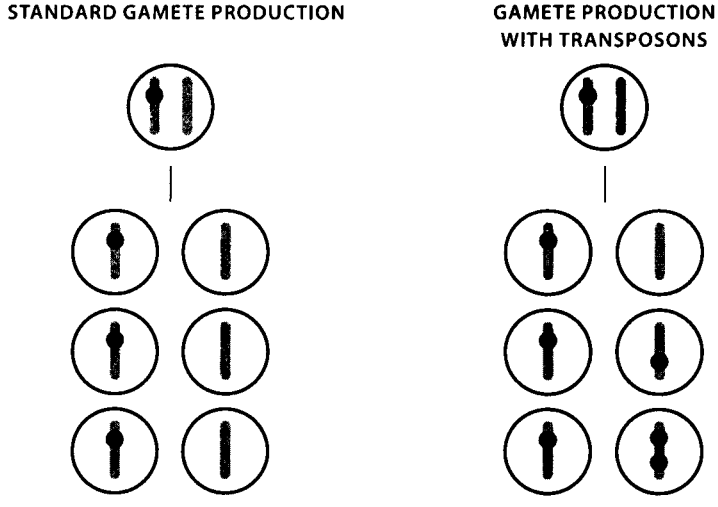
Agricultural Applications

Obviously, insects play crucial roles, both positive and negative, in agriculture. Most species are, in fact, beneficial or even essential. For example, honeybees are responsible for the pollination of $10 billion worth of produce in the U.S. Countless other species take part in nutrient recycling and help to maintain high soil quality. A small minority, however, compete with us for food. Since the development of agriculture, these pests have continually threatened our capacity to grow, harvest and store crops.

The concept of employing a genetic approach to deal with insect pests was first proposed in the 1940s by an American and a Russian. Edward F. Knipling of the USDA and Aleksandr S. Serebrovsky proposed similar schemes: inundate a pest population with sterile members of the same species. The majority of matings then become ineffectual. Serebrovsky's contribution was unknown in the West until 1968, when scientists, in particular Curtis, rediscovered it. Today this strategy is known as the sterile insect technique (SIT). Large numbers of insects can be sterilized, usually by ionizing radiation, and repeated infusions of such sterile organisms can wipe out a pest population [*see illustration at bottom on next page*]. The altered insect itself is, in effect, the weapon.

SIT is currently protecting parts of the agricultural economies of the U.S., Mexico, Guatemala, Chile, Argentina, Japan and Zanzibar, among other places. The strategy is ideal in locations as divergent as urban Los Angeles and tropical Zanzibar, where the aim is to eradicate a specific insect pest without harming the surrounding environment with chemical insecticides.

One of SIT's crowning achievements



DMITRY KRASNY; SOURCE: MARGARET KIDWELL AND JOSÉ RIBEIRO

NON-MENDELIAN HEREDITY is one of the added benefits of working with transposable elements. In ordinary gamete production (*left*), a gene of interest will appear in one half of gametes and will be transmitted, on average, to one half of progeny. Transposable elements, however, reproduce themselves and jump to other chromosomes. They can therefore wind up on more than one half of gametes and far more than one half of the members of the next generation.

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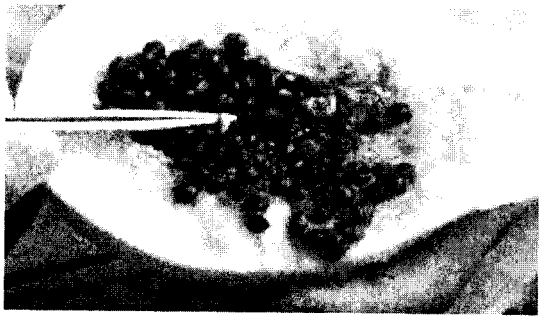
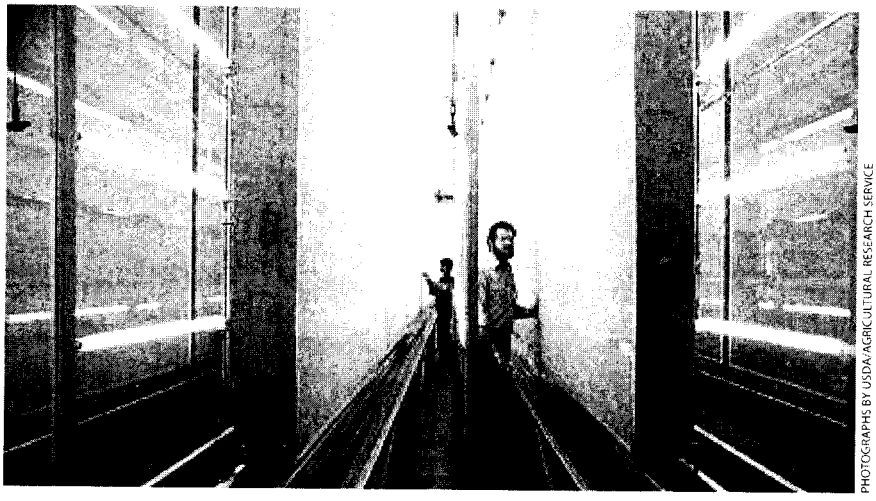


MEDFLY MAGGOTS, one of which is being plucked from this papaya, can destroy valuable crops. The edible pulp of the neck of the papaya, to the right of the black seeds, has been visibly damaged by the feeding of the insect larvae. Genetic controls can prevent such agricultural losses.



PHOTOGRAPHS BY USDA/AGRICULTURAL RESEARCH SERVICE

ENORMOUS CAGES hold the hundreds of thousands of Mediterranean fruit flies that are needed to implement genetic-control strategies.

was the eradication of the New World screwworm, first from the southeastern U.S. and eventually from Mexico, by the 1970s. (The screwworm will receive little sympathy from most onlookers. It lays eggs in open wounds in livestock, which then hatch into larvae that consume the animals' flesh. In effect, the screwworm eats its victims alive.) The technique has recently been used successfully against the dreaded Medfly in the Los Angeles area, with greater economic benefit: a permanent Medfly infestation would cost California approximately $1.5 billion annually.

SIT depends on genetics, in the form of traditional breeding programs. For example, a strain of Medfly has been created that allows scientists to kill all the female embryos with a pulse of high temperature. This strain permits entomologists to mass-produce, sterilize and release only males. By eliminating the females, the entire SIT program becomes more effective. Producing such strains, however, is difficult and time-consuming.

With transgenic technology, it should be possible to create insect strains in which only the females carry lethal genes. These deadly DNA segments would get expressed under specific conditions, such as the high-temperature pulse used in the traditional approach.

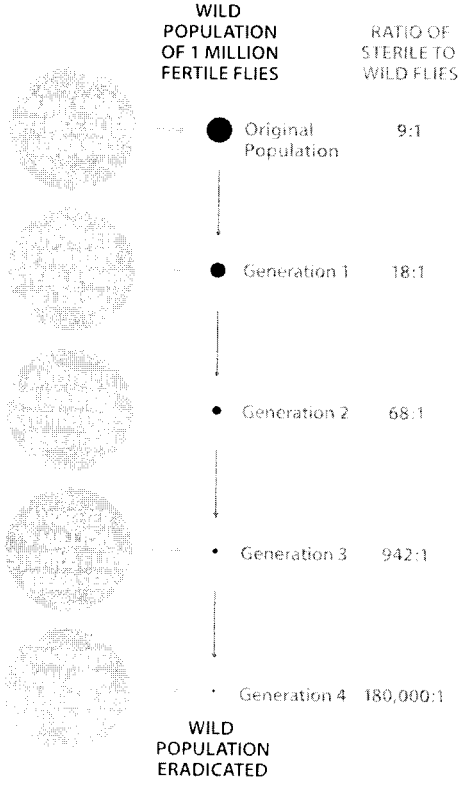
Transgenic technology also has the potential to reduce dependence on chemical pesticides. More than 900,000 American farms currently use insecticides, a reliance that can be counterproductive. Literally bathing insects in pesticides has actually driven the development of resistance to those killing agents. At least 183 species of insect and arachnid pests have developed resistance to one or more insecticides in the U.S. Furthermore, accumulation of chemical insecticides and their toxic breakdown products in our food, water, soil and textiles present a serious health issue. Biologists are therefore looking toward transgenics for the next wave of insect-control weapons.

One major problem with insecticides is that they kill many nontarget, beneficial species. They may wipe out useful predators and parasites, giving secondary pest species a chance to emerge. Transgenic technology may allow farmers to curtail pesticide use dramatically.

Currently a field can be sprayed with a wide array of different chemicals, each able to kill one or more harmful species. Unfortunately, the chemical controls against negative species often harm positive species as well. For example, mites attack California almond trees. One solution is to use an insecticide against those mites. Another is to release a species of predatory mite that kills the harmful mites. The trees, however, also fall victim to beetles and moths, so they are sprayed with other insecticides against those pests. And one of these insecticides kills the predatory mite that would free the tree from attack by the other mite species. A transgenic beneficial mite that could withstand the insecticides aimed at beetles and moths would allow farmers to spray fewer chemicals overall on their crops.

Of course, artificial selection for pesticide-resistant natural enemies is routine in labs, through conventional breeding practices. Brian A. Croft and his colleagues at the University of California at Riverside introduced insecticide-resistant predatory mites obtained from Washington State into orchards in southern California with good effect. Marjorie A. Hoy, now at the University of Florida, subsequently developed techniques that enabled the artificial selection of chemical resistance in natural predatory arthropods. Hoy and her coworkers developed an insecticide-resistant predatory mite that took part in the almond tree scenario just described.

This kind of effort, however, usually takes many insect generations and can



DMITRY KRASNY; SOURCE: GERALD FRANZ

STERILE INSECT TECHNIQUE (SIT) can be an effective weapon against pests. Wave after wave of sterile insects, mostly males when possible, far outnumber the fertile members of the same species, and cause most matings to be fruitless. Within a few generations, the pest population is decimated. Traditional breeding programs have made for successful SIT interventions, but transgenic technology has the potential to streamline these procedures.

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