Gene Vectors

Agents of Transformation

A genetic element called *piggyBac,* which has a propensity for jumping into other genes and riding along in their chromosomes, can be used to transform insects.

Agricultural Research Service insect physiologist Paul D. Shirk and geneticist Alfred M. Handler want to use *piggyBac* to change the characteristics of insect pests. They are with the ARS Center for Medical, Agricultural, and Veterinary Entomology (CMAVE) in Gainesville, Florida,

Shirk, who is in CMAVE's Postharvest and Bioregulation Research Unit, and research associate O.P. Perrera modified the original *piggyBac* gene to create what's called a gene vector. This is a special type of DNA that can move foreign genes from one place to another among chromosomes, or strands of DNA, the genetic material that holds the code for living things.

Now Shirk is testing *piggyBac* in the Indianmeal moth, *Plodia interpunctella,* the number-one stored-product pest, and in two other pests that infest stored foods—Mediterranean flour moth, *Anagasta kuehniella,* and red flour beetle, *Tribolium castaneum.*

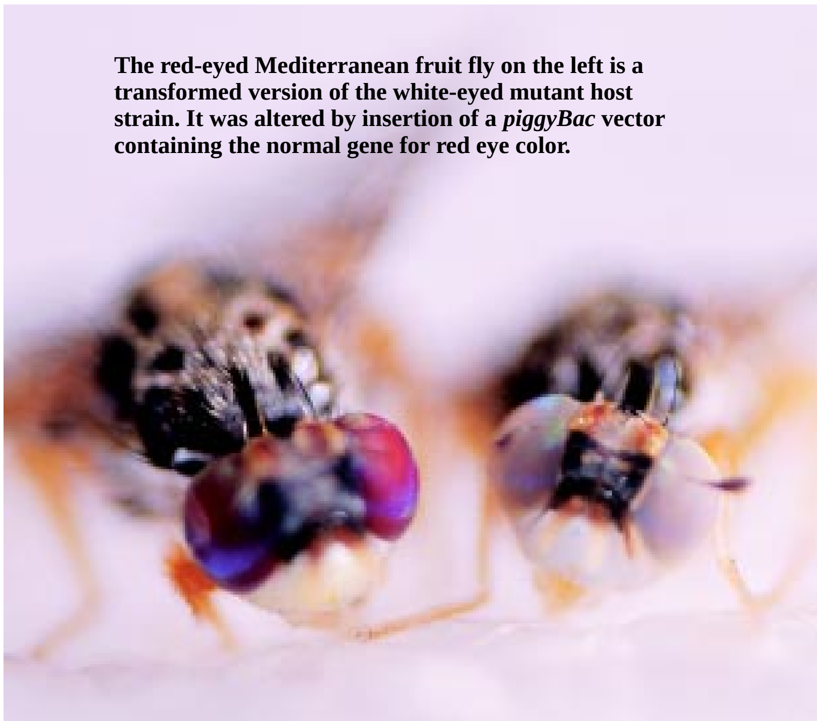
Initially, Shirk says, they'll use the *piggyBac* vector to mark laboratory-grown insects for use in sterile release programs. This control method involves growing pest insects in the lab, sterilizing the adults, and then releasing them to breed with wild populations. Nonfertile matings eventually reduce the pest insect populations, and the genetically altered insects do not affect humans or wildlife.

"*PiggyBac* can also be used to provide a genetic analysis of agricultural pest insects that is not possible now," Shirk says.

In the Beginning

So where did *piggyBac* come from? In 1983, Malcolm Fraser, Jr., associate professor in the University of Notre Dame's Biological Sciences Department in Notre Dame, Indiana, discovered *piggyBac* while looking at baculoviruses in cabbage looper moths. Baculoviruses are strains of viruses that infect insects.

AL HANDLER (K8393-20)



The red-eyed Mediterranean fruit fly on the left is a transformed version of the white-eyed mutant host strain. It was altered by insertion of a *piggyBac* vector containing the normal gene for red eye color.

"I found that mutations of the virus were occurring from a mobile piece of DNA within the cell," says Fraser. "This DNA essentially piggybacked into the baculovirus. The transformation efficiency appeared higher by far than by using other similar elements."

Shirk and Handler have successfully demonstrated the effectiveness of *piggyBac* as a vector by using eye-color transformations to signal genetic changes. Some abnormal moths are born with red eyes, when they should have black ones. Red-eyed moths lack an enzyme that keeps them from producing the normal eye color.

"Perera inserted a normal gene that produces the black eye color into *piggyBac,* to carry that trait into Mediterranean flour moths," says Shirk. "A new gene was permanently introduced into the host and changed the eye color of its offspring." The progeny have carried the genetic modification for black eyes over 12 generations.

In using the eye-color mutant of the Mediterranean flour moth, Shirk says, "The neat thing is that these moths are from a strain originally isolated in the 1920s and used in experiments that led to today's idea of what a gene really is. That's real use of genetic diversity and return on the investment in long-term research."

What's All This Portend?

Three important and possible future uses of *piggyBac,* Shirk says, will be introducing genes to mark a population so scientists can track and learn about it, developing a system that can spread certain genes into an insect population, and introducing genes to create sterile insects for use in sterile-release pest control programs.

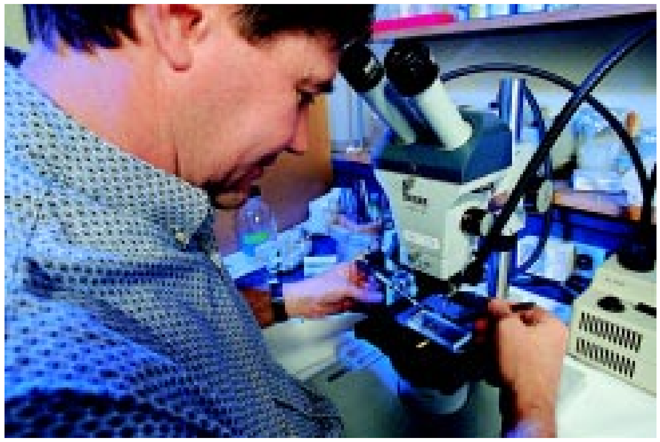
That's where Handler's research in CMAVE's Behavior and Biocontrol Research Unit is focused. He's looking at *piggyBac* as a way to transfer genes to improve sterile-release programs to control fruit flies—pests that cause major damage to citrus and other crops worldwide.

One of the most notorious of these is the Mediterranean fruit fly, *Ceratitis capitata*. It feeds on many fruits and vegetables and has most recently become a problem in parts of Florida. Handler is collaborating with Susan D. McCombs, an entomologist at the University of Hawaii, to genetically transform medflies.

He first conducted experiments using *piggyBac* marked with the medfly white gene, which restores red eye color to mutant white-eyed medfly strains. He wanted to see if gene transformation would be possible in this species. Since then, he has used *piggyBac* with green fluorescent protein (GFP) from a jellyfish to transform Caribbean fruit flies *(Anastrepha suspensa)* and *Drosophila*, as well as medflies. Under ultraviolet light, transgenic fruit flies modified with GFP glow like green lightbulbs.

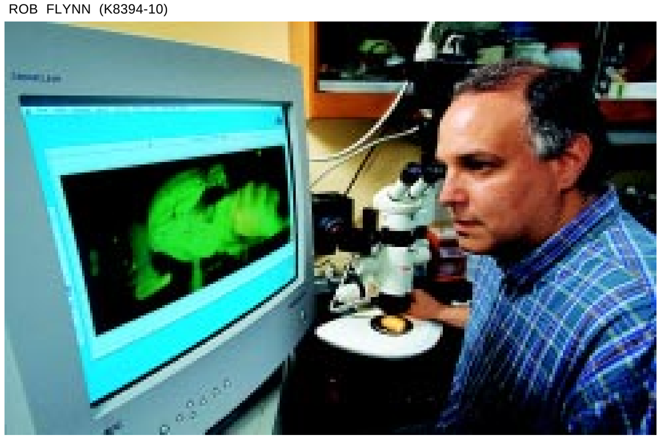
"The fact that a vector from a moth works so well in several fruit fly species is very encouraging for its use in many other insects," says Handler. "The success with GFP is equally important. This marker should also work in many insects, whereas eye-color markers are available for only a few."

ROB FLYNN (K8393-11)



Guided by a microscope, physiologist Paul Shirk injects a *piggyBac* gene vector containing a fluorescent marker into eggs of the Indianmeal moth and other stored product-insect pests.

ROB FLYNN (K8394-10)



Geneticist Al Handler examines digital photomicrographs of Caribbean fruit flies. The fly on the computer screen was transformed by using a *piggyBac* vector marked with green, fluorescent protein from jellyfish.

Another Measure of Success

"This is a major breakthrough," says Handler. "People have been trying to transform insect pests of agricultural and medical importance for nearly 14 years. In the past 2 years, our lab and others have had success with several species using only four vector systems. *PiggyBac* has been successful in the most insect species to date. Many exciting experiments for basic knowledge and field application are now possible."

Handler says this research will be useful in medfly and caribfly monitoring and sterile-release programs. Flies that are marked with GFP and released will be easily distinguished from the targeted wild flies in the field under ultraviolet light or by simple biochemical tests. This is critical to determining a release program's success and ensuring that wild flies have not infested fly-free zones.

Handler says that although the GFP marker may be used in the near future, the real benefit of this work relates to more sophisticated genetic manipulation of medflies that would allow genetic sexing and male sterilization.

Another promising gene vector the scientists are studying is *tagalong*, also discovered by Fraser. It's like *piggyBac*, but it can't move by itself.

While *piggyBac* relies on what's called a transposase enzyme to help it move, *tagalong* lacks this enzyme and relies on something else to help it travel. The scientists aren't sure what that something else is, but in the future they may be able to use *tagalong* as a gene carrier.

They agree that *piggyBac*'s potential is promising. They hope that they will soon use *piggyBac* to insert foreign genes that cause sterility or death in insects under certain conditions, such as low temperature. Such genes could be spread through an insect population in summer and have their effect in winter. This would allow the control of wild populations of pest insects without use of toxic chemicals.

Scientists in other states are also studying *piggyBac*'s effectiveness for transforming pink bollworms, boll weevils, codling moths, and mosquitos.—By **Tara Weaver-Missick,** ARS.

This research is part of Crop and Commodity Pest Biology, Control, and Quarantine, an ARS National Program described on the World Wide Web at http://www.nps.ars. usda.gov/programs/cppvs.htm.

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