# Colorful gene marks mosquito manipulation

Vampires may star in movies and television shows, but mosquitoes are the deadliest bloodsuckers around. While feeding, mosquitoes infect millions of people with the microorganisms that cause killer diseases such as dengue and yellow fevers and malaria.

Eradicating these insects has proved impractical, and vaccines for the illnesses are unavailable, so some researchers are exploring another option: genetically engineering mosquitoes so they can no longer pass on the pathogens.

Investigators have now taken a major step toward that goal by showing that they can add to one mosquito species a working gene that is inherited by future generations.

The method used to transform the insects, developed in concert by research groups led by Anthony A. James of the University of California, Irvine and Frank H. Collins of Notre Dame University in South Bend, Ind., appears in the March 31 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES.

In these initial studies, the added gene merely provides a visible marker—a change in eye color—that registers the technique's success. The scientists envision eventually inserting genes that would make mosquitoes unable to transmit viruses or the parasite that causes malaria.

"This achievement opens a wide vista of possibilities of introducing and testing foreign DNA sequences in the mosquito germ line for both basic research and the development of a wide array of biological control methods," note Margaret G. Kidwell and Alice R. Wattam, both of the University of Arizona in Tucson, in an accompanying commentary.

The investigators worked with *Aedes aegypti*, a species that spreads the viruses causing dengue and yellow fevers. A natural mutant strain, which has white eyes instead of dark purple ones, offered a clear test of their manipulations. If they could alter the mutant's eye color, say by inserting the fruit fly gene that gives those flies' eyes a reddish tint, the investigators would have visible proof of their ability to add genes.

To deliver the genetic cargo, they turned to transposable elements, unusual DNA sequences that can cut themselves out of chromosomes and insert themselves elsewhere in DNA, even in a different chromosome. Collins' group spliced the gene for eye color into two different transposable elements, one from fruit flies and one from house flies, and James' team injected copies of this DNA construct into *Aedes aegypti* embryos.

Several of the manipulated insects were born with a reddish eye color uncharacteristic of the species. Moreover, in some cases, this coloring has persisted for 10 generations, attesting to the added gene's stable integration into the mosquito genome.

Other scientists have shown that they can infect mosquitoes with viruses engineered to carry genes that thwart human pathogens (SN: 5/11/96, p. 295). That protection isn't heritable, however, so investigators are planning to take such protective genes and use transposable elements to install them in the mosquito genome.

By introducing resistant insects into nature, the scientists hope ultimately to spread the protective genes to most mosquitoes in the wild. To combat malaria, researchers must duplicate their genetic engineering feat in *Anopheles gambiae*, the mosquito primarily responsible for spreading that disease. —*J. Travis*


*Eyes of common, naturally mutant, and genetically engineered mosquitoes.*

# Ritalin may work better as purer compound

Half of every dose of the drug Ritalin, taken by an estimated 2 million children with attention deficit–hyperactivity disorder (ADHD), may contribute nothing to its therapeutic effect, while possibly adding to its side effects, say researchers at Brookhaven National Laboratory in Upton, N.Y.

Ritalin contains equal amounts of two molecular forms, or enantiomers, of the compound methylphenidate. They possess most of the same chemical properties, but structurally they are mirror images of each other. According to the Brookhaven analysis, one enantiomer is much more potent than the other and is responsible for Ritalin's beneficial effects of improving attention and reducing impulsivity.

Yu-Shin Ding, Joanna S. Fowler, and Nora Volkow tested the two forms separately to see how well each binds to receptor molecules in the brain. They labeled methylphenidate with a radioactive tracer, carbon-11, and injected small doses into two volunteers. Using positron emission tomography (PET), the researchers could take images of the brain and see where the drug accumulated.

The scans revealed a "dramatic difference," says Ding. They showed that the form of methylphenidate known as the *d-threo* enantiomer targets the parts of the brain—the basal ganglia—involved in the drug's therapeutic effect. In contrast, the mirror image *l-threo* enantiomer distributes itself nonspecifically over the entire brain.

Ding presented the group's findings at a meeting of the American Chemical Society in Dallas this week and proposed using the *d-threo* enantiomer alone in treating ADHD. The less effective enantiomer may contribute to Ritalin's side effects, including insomnia or loss of appetite, or to long-term complications such as liver damage, speculates Ding. Researchers would need to perform additional studies to determine whether it plays a detrimental role.

Previous studies have indicated that the two enantiomers of methylphenidate cause different behavioral responses in patients, but the Brookhaven study visually establishes where the drug acts in the human brain, says William F. Trager of the University of Washington in Seattle.

Methylphenidate acts by increasing the amount of the neurotransmitter dopamine in the brain. Nerve cells release dopamine, then control its abundance by reabsorbing it and breaking it down. Methylphenidate blocks the reuptake of dopamine, making more of the neurotransmitter available to bind to receptors and exert its effects on behavior. The *d-threo* form appears to block dopamine uptake better than the *l-threo* form.

One of the most infamous examples of the mirror image property, or chirality, is the drug thalidomide. Widely prescribed for pregnant women in Britain as an antinausea treatment in the late 1950s, only one enantiomer was beneficial; the other caused birth defects. In general, Trager says, "it's much better to give just one enantiomer because you've eliminated all other variables." Even if one form is inactive, it may interfere with the working form.

Many issues must be considered when designing a drug. Administering Ritalin as a single enantiomer may not be necessary, says Trager, since the body seems to metabolize the mirror-image molecules differently, thereby enriching the blood with the more potent form. Also, the pharmaceutical company may choose not to separate enantiomers since the process adds to manufacturing costs.

On the other hand, because metabolism varies among people, "you're always sure of giving the right dose to that individual by giving the pure enantiomer," Trager adds. Today, many new drugs are produced as pure enantiomers from the start because companies have available advanced chemical synthesis and separation techniques. —*C. Wu*