Team finds key mechanism of DDT resistance in malarial mosquitoes

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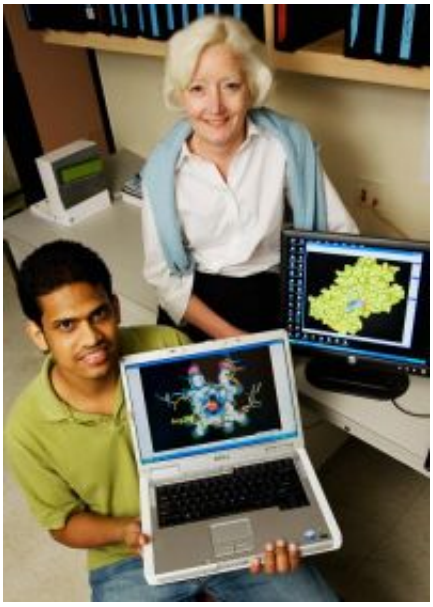

Biochemistry professor Mary Schuler and postdoctoral researcher Sanjeewa Rupasinghe used molecular modeling techniques to identify a protein that can metabolize DDT. Credit: Photo by L. Brian Stauffer, U. of I. News Bureau

University of Illinois researchers have identified a key detoxifying protein in Anopheles mosquitoes that metabolizes DDT, a synthetic insecticide used since World War II to control the mosquitoes that spread malaria.

The new findings, described this week in the *Proceedings of the National Academy of Sciences,* reveal that a protein produced at elevated levels in

DDT-resistant Anopheles gambiae mosquitoes actually metabolizes the insecticide.

Anopheles gambiae as a species includes many closely related mosquito strains that transmit the malarial parasite to humans and other animals. The A. gambiae genome, isolated from an insecticide-susceptible strain, was first published in 2002.

The protein that metabolized DDT, CYP6Z1, belongs to a class of cytochrome P450 monooxygenases (P450s) that are known to be important detoxifying agents in many species. Many studies in a variety of insect species have shown that P450s play key roles in insect defenses against plant toxins.

Using molecular modeling techniques based on the three-dimensional structure of a similar protein found in humans, principal investigator Mary A. Schuler and postdoctoral researchers Ting-Lan Chiu and Sanjeewa Rupasinghe were able to visualize the likely orientation of the molecules that allowed CYP6Z1 to bind to, and inactivate, DDT.

The researchers' model predicted that the active site of CYP6Z1 could accommodate a single molecule of DDT and inactivate it by adding oxygen to a chlorinated side group on the DDT molecule.

Their model of a similar protein, CYP6Z2, which is also produced at elevated levels in some DDT-resistant Anopheles mosquito strains, predicted that it was structurally incapable of binding – and hence inactivating – DDT.

Biochemical studies conducted by postdoctoral researcher Zhimou Wen confirmed that CYP6Z1 did in fact inactivate DDT while CYP6Z2 did not.

"To understand the relationship of different P450s, you really need to look at three-dimensional active site predictions in order to see what are critical variations between evolutionarily related P450s," Schuler said.

"The configuration of the CYP6Z1 active site is open enough so that DDT can come in close enough to the reactive center to be oxygenated and, therefore, disabled."

Schuler is a professor of cell and developmental biology, of biochemistry, of plant biology and of entomology and is affiliated with the Institute for Genomic Biology.

Malaria infects between 300 million and 500 million people a year, according to the World Health Organization, and is the leading cause of disease-related sickness and death in the world. Although banned in the United States, DDT is used in mosquito-control programs in many other parts of the world.

Schuler chose the CYP6Z1 protein for further study from a list of P450 genes that were transcriptionally elevated in resistant mosquitoes because its gene structure closely resembled other P450s that she and entomology department head May Berenbaum had studied in pest insects in the United States.

Much earlier work by Schuler, Berenbaum and their colleagues had identified the CYP6 family of related P450s as an important part of insects' defense against plant toxins and some insecticides. Efficient expression of these proteins allows insects to survive on host plants normally toxic to other species, and confers resistance to some insecticides.

"In the mosquito genome you've got somewhat over a hundred P450 genes, and if you can identify which ones are responsible for DDT

resistance, there are many things you can do to control this pest species," Schuler said. "And if you can effectively block the actions of proteins that metabolize DDT then you can prevent the resistance levels from becoming elevated in natural populations."

By comparing models developed for the CYP6Z1 proteins in "sensitive" and "resistant" strains of A. gambiae mosquitoes, the researchers found that, from a three-dimensional perspective, the CYP6Z1 proteins were not appreciably different from one another. Variations dID occur, but often these were on the surface of the protein in regions not important for DDT metabolism.

"With biochemical analysis showing that the CYP6Z1 protein can metabolize DDT quite efficiently, you have to ask: What's the difference between the sensitive strain and the resistant strain?" Schuler said. "It has to be that these transcripts and their proteins are over-expressed in the resistant strains and, as a consequence, are allowing them to exhibit this resistance."

It is probable that exposure to potent, naturally occurring plant toxins or to synthetic insecticides causes the insects to step up production of certain P450 proteins, such as CYP6Z1, that subsequently aid in the detoxification of these compounds, Schuler said. Other studies have shown that insects encountering high levels of plant toxins in their food sources have higher levels of detoxifying proteins in their bodies, allowing them to withstand exposure to a broad range of insecticides, she said.

"There's a lot out there that still has to be learned about mosquito populations in the wild," she said.

Source: University of Illinois at Urbana-Champaign