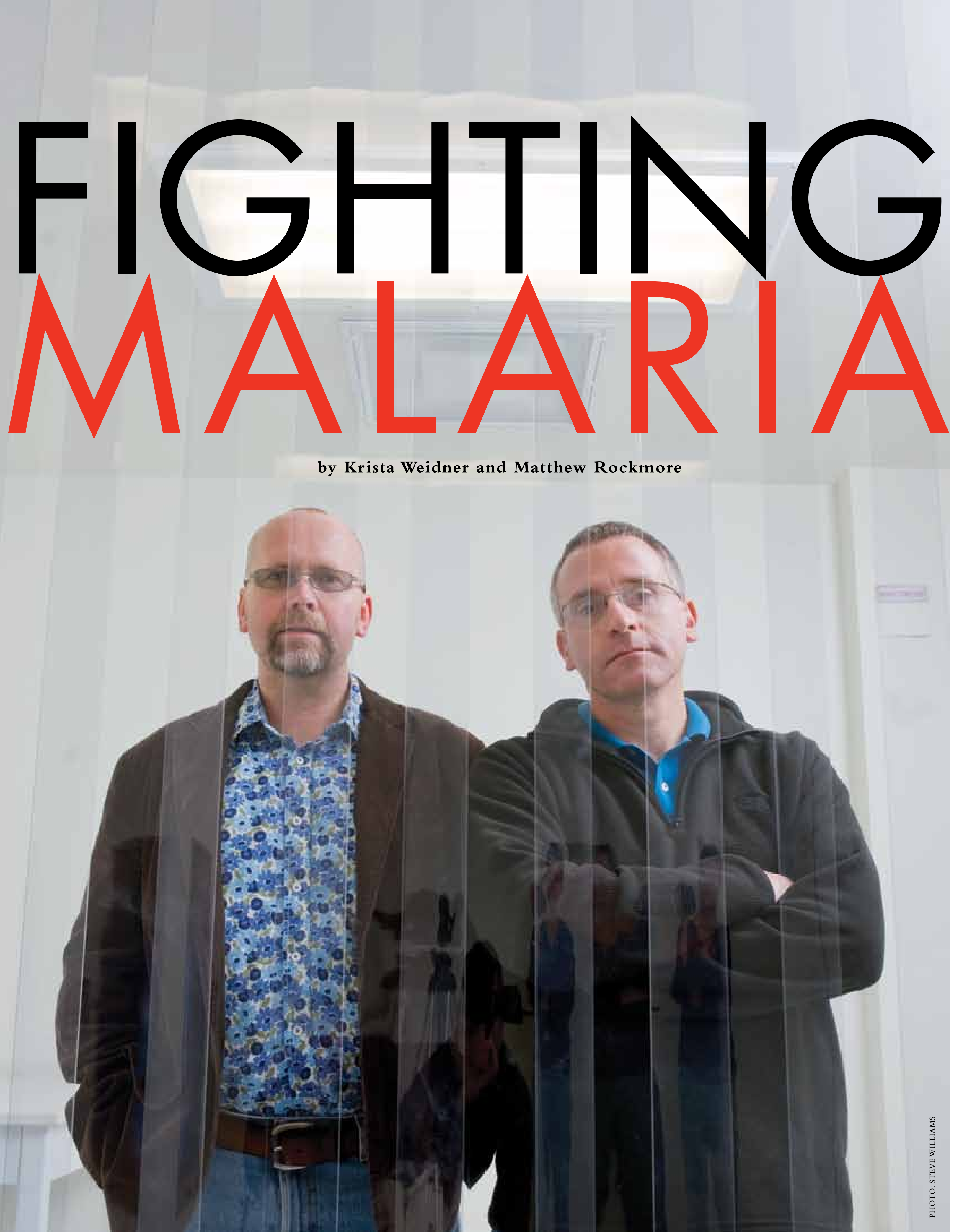
FIGHTING MALARIA

by Krista Weidner and Matthew Rockmore



Entomologists Matthew Thomas and Andrew Read stand in a secure section of the newly constructed $3 million insectary. This particular workspace will house small, self-contained incubators used for research on malaria vectors.

PHOTO: STEVE WILLIAMS

Researchers in the college attack the problem of malaria.

It's a warm day, but suddenly you begin to shake uncontrollably. Minutes later, beads of sweat appear on your face. When next you begin to vomit, you decide to go to the hospital. A nurse asks if you've recently eaten or done anything out of the ordinary. "Just last week I returned home from a trip to Nepal," you say. "Ah, that may explain it," says the nurse. "I bet you have malaria."

Luckily, as an American, you have access to powerful drugs that can eliminate the malaria parasite from your body. Lucky you.

Each year malaria infects up to 500 million people worldwide, and as many as one million people die from the disease. Those who die often do so because they cannnot afford to be treated. "In many cases, malaria is not difficult to treat," says Liwang Cui, a Penn State professor of entomology, "but we are talking about the poorest of the poor regions." These places, he adds, are where malaria is most rampant.

Cui is one of several researchers in the college who are studying malaria, a disease caused by *Plasmodium* parasites that are carried by *Anopheles* mosquitoes. He also is one of three researchers in the college to be involved in a recent $14 million grant funded by the U.S. National Institutes of Health (NIH) to address the problem of malaria by creating 10 malaria research centers around the world. Cui will serve as the principal investigator for the Southeast Asia Malaria Research Center, while entomologists Matthew Thomas and Andrew Read will serve as co-investigators for the Center for the Study of Complex Malaria in India.

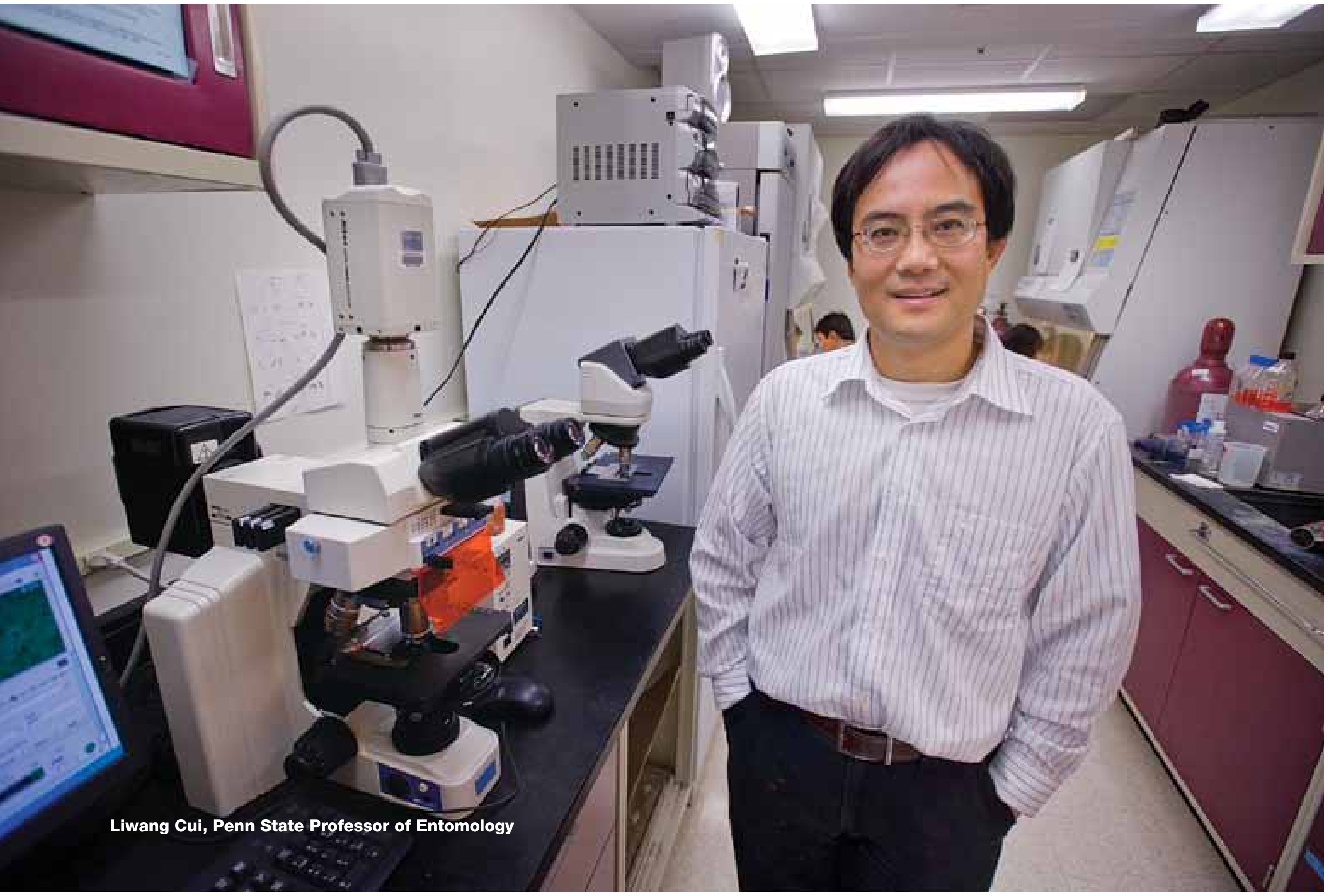
Killing the Parasite

Efforts to eradicate malaria most often target either the parasite or the mosquito. Cui focuses on the parasite. In particular, he is using molecular techniques to identify the species of *Plasmodium* parasites that occur in different regions of Southeast Asia. "Southeast Asia accounts for 30 percent of the world's malaria infections and 8 percent of the world's deaths from malaria," he says. "Part of the problem is that in Southeast Asia four different species of mosquito carry a variety of forms of the malaria parasite, and each form of the parasite requires a different treatment." To treat malaria effectively with the correct drugs, he adds, it's important to start with an accurate diagnosis.

Cui also is investigating how various species of parasites respond to drugs. In Southeast Asia, *Plasmodium falciparum* causes the most serious form of malaria. Although drugs to treat this form of the parasite are available, the microorganism is evolving resistance to some of them. Cui uses molecular techniques to examine how the parasites respond to drugs. "If we find a resistant parasite, we look at its genome," he says. "Once we know its genetic background, we can see how it changed genetically to become resistant to the drug, and this helps us figure out why drug resistance happens."

Finally, Cui is addressing the problem of counterfeit drugs. "Many of the drugs circulating in these regions are fake," he says. "There's no government regulation, so people buy whatever they can get, and these fake drugs do no good at all. As we develop improved diagnostic methods and strategies to control drug resistance, we also want to find ways to identify counterfeit drugs."

malaria parasites during 30-minute intervals while temperatures fluctuate, Thomas, Read, and postdoctoral fellow Krijn Paaijmans have found that short-term variations in temperature are important in determining how long a mosquito lives and how well it can transmit the parasite. "Short-term temperature variations can have a big impact on the malaria parasite," says Thomas, adding that the recent completion of



Liwang Cui, Penn State Professor of Entomology

PHOTO: STEVE WILLIAMS

a $3 million insectary on campus has improved the team's ability to do this type of work.

While Cui is focusing on drug resistance among various forms of *Plasmodium* parasites and on counterfeit drugs, Thomas, a professor of entomology, and Read, a professor of biology and of entomology, are investigating how environmental factors influence the life cycle of *Plasmodium* and, therefore, the intensity of malaria transmission.

According to Thomas, entomologists have long known that *Plasmodium*'s life cycle depends on a variety of climatic factors, including rainfall, humidity, and especially temperature. Below certain temperatures, the parasite cannot complete its life cycle fast enough to be transmitted to humans. Since few mosquitoes survive beyond two weeks, even minor delays in the parasite's life cycle can have important effects on transmission rates.

Using thermodynamic models to estimate the growth of

Killing the Mosquito

In addition to studying *Plasmodium* parasites, Thomas is also investigating mosquitoes. He is particularly interested in learning how the insects evolve resistance to insecticides. "Global malaria intervention efforts rely heavily on insecticides, but even though using bed nets with insecticides and spraying insecticides indoors work to some extent, the methods can encourage mosquitoes to become resistant," he says.

According to Thomas, a female mosquito needs human blood to mature her eggs. When she comes into contact with an insecticide, she either dies or is repelled. "The

Frederick W. Knipe

Pioneer in Malaria Research

Frederick W. Knipe ('17 agronomy) was not afraid of hard work. He drained waterlogged fields with picks and shovels, and he gouged out ditches with dynamite—all with the goal of removing the breeding grounds of mosquitoes.

PHOTOS: COURTESY OF KNIPE FAMILY

Knipe (left foreground) on the bank of a canal in Cyprus, 1949.

A key figure in the college's long history of malaria research, Knipe, who graduated from Penn State in 1917 with a bachelor's degree in agronomy, worked as a malaria-control engineer for the Rockefeller Foundation during the 1920s. The foundation—known for its commitment to meeting social, economic, health, and environmental challenges—assigned him to the Balkans, where he was tasked with helping to identify and eradicate mosquito breeding grounds.

In the Balkan countries, including Bulgaria, Yugoslavia, Albania, and Greece, Knipe drained vast fields of stagnant water, which involved using dynamite to build levees, ditches, and canals. "It was tedious work, all done by hand with picks and shovels," says Fred's son Fritz Knipe. "After the dynamite blast, hand laborers cleaned up the passageways for drainage. Dad had workers construct a system of dams and floodgates to manage water flow."

After Knipe's work in the Balkans ended in the early 1930s, he was assigned to India to continue his work on malaria control. On the flatlands in the village of Pattukkottai, he once again focused on draining swamps and constructing canals. He also developed new spray equipment that could be used to kill both adult mosquitoes in homes and larvae in bodies of water. Knipe was known for his invention of more efficient spray nozzles for applying insecticides such as DDT and pyrethrum.

Fritz remembers his family's time in India. "Because the plains of Pattukkottai where Dad was working were extremely hot, most of the time my mother, my brother Dan, and I stayed in the cooler hills nearby. We lived in the beautiful hill station called Kodaikannal. I remember when we would visit Dad on the plains, the temperature was rarely under 100 degrees. Our home in Pattukkottai was very primitive, with sod walls and three or four coconut palms growing right through the house!"

During the 1940s, when malaria became a concern for U.S. troops, Knipe assisted with malaria-control efforts at the Pensacola Naval Air Station in Florida and, later, in Sardinia, Italy, where he set up a malaria abatement headquarters. "The incidence of malaria among U.S. troops and others in Italy was a serious problem during World War II," says Fritz. "My dad gained recognition from the Italian government for his work in vastly reducing the incidence of malaria in that country."

After the war, Knipe was reassigned to India and was instrumental in setting up the Malaria Institute of India. He also served on the World Health Organization's Expert Committee on Insecticides from 1948 until 1955. After that he returned to Penn State, where for several years he conducted research on the eradication of cockroaches and flies for the agronomy department.

Knipe died in 1983, at the age of 88, after an illustrious career in malaria control. His four sons—Dan, Fritz, Robert, and James—all live in California. "We're proud of the work my dad did," says Fritz. "He would be very pleased to know that malaria research continues in the college today."—*by Krista Weidner* ■

mosquitoes that die can't lay eggs or bite someone later and spread the disease," he says. "But the mosquitoes that survive quickly reproduce, and before long, you have a mosquito population dominated by insecticide resistance."

Thomas and Read are developing a biological insecticide, or biopesticide, that has the potential to be "evolution proof" because it reduces the selection pressure for resistance by killing the mosquito more slowly. Here's how it works: After a mosquito feeds on a person who is infected with malaria, it picks up the parasite. Roughly 12 days later, when the parasite has fully developed inside the mosquito, the mosquito can infect someone else. Biopesticides allow the mosquito to survive for those 12 days, during which it can continue to feed and lay eggs—essentially doing what it's programmed to do. Traditional insecticides, on the other hand, kill the mosquito soon after contact.

"With the biopesticide, instead of dying and having no reproductive output, a mosquito has the chance to lay a few batches of eggs," Thomas explains. "But if the mosquito doesn't die within 12 days or so, it's going to transmit malaria. So we need a balance of allowing a mosquito to breed as much as possible, but then stopping it before it can spread the disease. We believe the biopesticide we're working on can achieve that balance."

Thomas and his colleague Distinguished Professor of Entomology Tom Baker are further studying the biopesticide, which contains a fungus, to see how it affects the health of mosquitoes. "I had noticed that when a mosquito picks up the fungus from the biopesticide and becomes sick, it's less inclined to take a blood feed," says Thomas. "We wondered whether the fungus might be giving the mosquito a 'head cold' of sorts. When you get a head cold and you're all stuffed up and can't smell your food, you tend to lose your appetite. We think the same thing might happen with mosquitoes."

THOMAS AND READ ARE DEVELOPING A BIOPESTICIDE THAT HAS THE POTENTIAL TO BE EVOLUTION PROOF.

To examine mosquitoes' physiological responses to different chemical cues, Thomas and Baker are "puffing" odors over a single, microscopic hair from a mosquito antenna. Each odor creates an electrical spark that travels to the mosquito's brain and causes a response. When the scientists puffed odor cues over both infected and healthy mosquitoes, they found that the infected mosquitoes were less responsive. "We think that the fungus can help stop malaria transmission not only by killing the mosquitoes slowly, thus preventing insecticide resistance, but also by interfering with mosquitoes' abilities to smell and to take blood feeds," says Thomas.

While biopesticides show promise for malaria control, another way to improve insecticide use is to apply existing

Matthew Thomas stands in the hallway that leads to large, walk-in environmental chambers. Three senior faculty members and their associated research teams will use this space to culture mosquito species. The new insectary is one of the most advanced in the United States.

PHOTOS: STEVE WILLIAMS

Will People Use It?

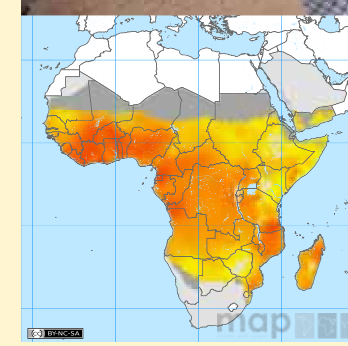
"There's a story that circulates in the scientific and funding communities," says Rachel Smith, assistant professor of communication arts and sciences (College of Liberal Arts) and member of the Huck Institutes of the Life Sciences. "In a few studies, researchers introduced bed nets for malaria control, and when they returned a month later they found that some nets were being used for fishing, some were covering plants, and one had been made into a wedding dress. So the mythology began: don't try to put anything out there in which humans are involved because they will resist it. We believe otherwise."

Smith and economist Jill Findeis, Distinguished Professor in the College of Agricultural Sciences, are working with Matt Thomas and Andrew Read to investigate methods for delivering their biopesticide in East Africa. "While the biggest biological challenge for malaria control is the development of mosquito resistance to insecticides, one of the biggest problems for delivering the technology is perceived user resistance," Findeis explains. "Matt and Andrew are developing the biopesticide in their lab, but for it to succeed in rural villages not typically targeted for mass spraying, user compliance must be high. How can we use social science to ensure that happens?"

A team of social scientists led by Findeis and Smith is working with researchers in Africa to understand economic, communication, and social forces influencing the uptake of two innovations under development: the biopesticide, and phosphorus-efficient legumes being developed by plant nutritionist Jonathan Lynch. For the biopesticide, Findeis and Smith want to know, for example, are households willing to dip a cloth into an insecticide mixture periodically to maintain its effectiveness? At what times of year can poor rural households afford the biopesticide (which, paradoxically, may not coincide with the malaria season)? Would they prefer certain colors or shapes for the cloth?

What they've observed is encouraging. "People will re-dip the cloths," says Findeis. "In rural Mozambique where malaria is endemic, there certainly is great interest. Local people ask, 'Will this control malaria here? When can we get it? Where can we get it?'

PHOTO: © TONY KARUMBA, GETTY IMAGES



SOURCE: THE MALARIA ATLAS PROJECT (MAP)

89 PERCENT OF MALARIA DEATHS WORLDWIDE OCCUR IN AFRICA.

As the development of the biological insecticide moves forward, we're working on the science to improve its widespread use."

"What's great about this work is that the science is still flexible at this stage," Smith adds. "Normally, social scientists are brought in after a product is finalized to develop strategies to encourage adoption of the product with all of its faults and challenges. But Matt and Andrew are open to adapting the technology during product development to increase chances that people will use it. For example, when Andrew learned that the biopesticide's smell could be important, he said they would search for fungal isolates that smell like cookies! Kidding aside, though, we're all learning what it will take.

"We have this great collaboration in which we're willing to consider multiple perspectives. That wouldn't happen if we were worried about keeping our science to ourselves." —*by Krista Weidner* insecticides differently. A traditional goal of malaria control efforts has been to kill all the mosquitoes (no mosquitoes, no malaria), but Thomas and Read believe that killing only old, infectious mosquitoes may prevent transmission. "Older mosquitoes—those that are at least 12 days old—are the only ones that are infectious because it takes at least 12 days for the *Plasmodium* parasite to develop," says Thomas. "We believe that by waiting to kill mosquitoes until after they've bred and laid eggs, we can wipe out malaria mosquitoes without triggering selection pressure for resistance."

Thomas admits that this approach might be counter-intuitive. After all, allowing younger mosquitoes to live will result in more mosquitoes and more mosquito bites. But he and his colleagues argue that getting more mosquito bites is a price worth paying for not getting malaria. "If we can keep an insecticide working for the next 20 years because it doesn't impose selection pressure for resistance, we think it's worth the nuisance."

MALARIA FAST FACTS

1 **3.3 billion people (half the world's population) live in areas at risk of malaria transmission in 109 countries and territories.**

2 **35 countries (30 in Sub-Saharan Africa and 5 in Asia) account for 98 percent of global malaria deaths.**

3 **WHO estimates that in 2008 malaria caused 190—311 million clinical episodes and 708,000—1,003,000 deaths.**

4 **Malaria is the fifth cause of death from infectious diseases worldwide (after respiratory infections, HIV/AIDS, diarrheal diseases, and tuberculosis).**

5 **89 percent of the malaria deaths worldwide occur in Africa.**

6 **Malaria is the second leading cause of death from infectious diseases in Africa, after HIV/AIDS.**

Source: Centers for Disease Control, 2010

PHOTO: © PAULA BRONSTEIN, GETTY IMAGES

Fatal Attraction

While Thomas and Read are busy investigating mosquitoes' resistance to insecticides, Read also is working with Penn State Professor of Entomology Consuelo De Moraes and Assistant Professor of Entomology Mark Mescher to understand mosquito attraction. Scientists have long known that mosquitoes find their hosts through chemical cues—the carbon dioxide, warmth, and lactic acids that humans emit continuously—but Read, De Moraes, and Mescher wondered if there was more to it.

About five years ago, in a study done with school children in Kenya, researchers found a difference in how mosquitoes perceived children who had malaria. Specifically, they discovered that the children who had the transmissible, or infectious, stage of malaria were more attractive to mosquitoes. "Presumably, the *Plasmodium* parasite was changing the odors that affect mosquito attraction and disease transmission," says Mescher. "We think this difference in perception might be to encourage the mosquito to bite the infectious person so it can spread the parasite to someone else."

Consuelo De Moraes and Mark Mescher at one of the growth chambers used to determine whether mice infected with malaria emit volatiles that affect their attractiveness to mosquitoes.

PHOTO: STEVE WILLIAMS

WE HAVE SOME REALLY PRODUCTIVE SYNERGIES IN PLACE THAT PROMISE TO SIGNIFICANTLY ADVANCE MALARIA RESEARCH.

De Moraes and Mescher normally focus their research on plant volatiles—chemical cues that plants emit to communicate with other plants or insects. In their work with Read, they are drawing on their knowledge of plant volatiles to determine whether mice infected with malaria emit volatiles that affect their attractiveness to mosquitoes. In particular, they are monitoring the volatiles of both healthy mice and those that are infected with malaria. They follow the disease's progression, taking samples at regular intervals to determine the number of parasites and then analyzing the volatiles to see if they change as malaria progresses. "We want to see if we can relate volatile differences to attraction to mosquitoes," De Moraes says. "If we can do that—if we can identify particular signals that a mosquito might be using to identify an infectious person—it could lead to some exciting applications."

For example, researchers could develop a repellent that disrupts or masks the signal that's attracting the mosquito, thus preventing the mosquito from biting an infected person. Another potential application could be a diagnostic tool for malaria screening. Current screening techniques are invasive and impractical, requiring blood tests and lab analyses. A quick and simple test that could measure and detect the chemical compound that attracts mosquitoes—say a cotton swab rubbed onto the skin—could confirm the presence of the malaria parasite. "That would be a fantastic tool," says Mescher, "because people with low-level infections can be spreading malaria without even knowing they have it. If we want to eradicate malaria, it's really important to find the people who are asymptomatic but may be spreading the disease. Once we identify them, we can treat them and stop further transmission."

Whether by directly treating people who already carry the disease or by preventing them from being bitten in the first place, researchers at Penn State are doing their best to solve the problem of malaria. "This research on malaria is an example of something we do very well in the college, which is to bring together people with different skills to solve a problem," says Thomas. "We have some really productive synergies in place that promise to significantly advance malaria research." ■