Bayer's research scientists want to make people invisible to malaria-spreading mosquitoes

Stopping mosquitoes from **smelling**

Every time an infected mosquito bites someone it can transfer deadly parasites to its victim: the malaria pathogen can be fatal to humans. Research scientists working for Bayer CropScience now want to use an entirely new strategy to get to grips with these dangerous blood-suckers: they are looking for chemicals that block specific odor receptors in the mosquitoes and so make them odor-blind. This would stop the insects finding either victims or sexual partners, and would dramatically reduce the spread of this tropical fever.

Mosquitoes can find their victims even in the dark. They head straight for their meal of blood, magically attracted by the odorous cocktail of exhaled breath and sweat. They are mainly found in tropical regions. Insects rely almost entirely on their sense of smell to find their way around, in contrast to humans for whom sight is the most important sense for orientation purposes.

The Anopheles mosquito, which can transmit malaria, "follows its nose" as it flies around looking for blood. But these swarms of disease-transmitting creatures do not have scenting organs like those of humans. Instead, they use highly sensitive antennae to identify odors. These antennae are covered with fine scenting hairs called sensilla, which can pick up scent molecules passing by. The odor molecules pass through tiny pores in the sensilla and reach the mosquitoes' sensory cells. Each scenting hair is like a miniature nose.

Every bite can leave behind a deadly cargo

"If the antennae of the mosquitoes detect carbon dioxide and lactic acid, they know for sure that people are nearby and what direction they have to follow to reach the blood they need to reproduce," explains Dr. Klaus Raming, Head of Physiology and Biochemistry in Insecticide Research at Bayer CropScience. "Female mosquitoes have a particular taste for protein-rich human blood, because it is vital to help mosquito eggs mature and provides them with nutrients," continues Raming.

But whenever an infected mosquito bites someone, it can also leave a deadly cargo in its victim's bloodstream: parasites that can cause malaria. The consequences are life-threatening: almost a million people die from this tropical disease every year. But there are ways that people can be protected against mosquitoes: for example nets placed over the bed like the new LifeNet™ mosquito net developed by Bayer CropScience (see *research* 21, "Combating malaria

Focus on blood-suckers: mosquitoes (photo, right) rely mainly on their sense of smell when searching for protein sources. If they can smell exhaled breath and sweat, human skin cannot be far away. Only female mosquitoes bite, as they need nutrient-rich blood for their eggs to mature. Dr. Klaus Raming, Sabine Hartig and Dr. Katharina Wölfel of Bayer CropScience's Insecticide Research unit (photo, left, left to right) are systematically looking for molecules that block the mosquitoes' odor receptors. They want to stop mosquitoes transmitting malaria or other tropical diseases.

Photos: Sabine Bungert (5), Peter Ginter (1)/Bayer AG, Eye of Science/Agentur Focus (1), Bayer AG (1)

Cell cultures with a sense of smell: tests on live mosquitoes are very costly and time-consuming. This is why Dr. Maike Forstner (photo, left) is growing cells in the laboratory (photo, center) that can perceive odors almost as well as insects. In order to identify and test promising substances from Bayer's substance library, a Bayer research team headed by Dr. Arnd Voerste and Dr. Klaus Raming (photo, right, left to right) uses automated test systems and robot lines.

with special meshes"). The fibers of these nets are impregnated with the insecticide deltamethrin, which kills the dangerous insects. The nets have already been recommended by the World Health Organization (WHO) for controlling and preventing malaria.

Now research scientists working for Bayer CropScience want to offer people living in tropical regions protection against these deadly blood-suckers with a completely new strategy: one that literally leads them astray. The scientists are placing a kind of invisible veil over the mosquitoes' scent antennae. As a result, the insects lose their keen sense of smell and mill around aimlessly, unable to find either humans to slake their thirst for blood or their way home to the swamps where they live. Bayer's scientists are currently engaged in a research project in which they are looking for compounds that stop mosquitoes from being able to smell - making them odor-blind, which will stop them biting.

The key impulses for this work came from a Nobel Laureate

The renowned scientist and recipient of the Nobel Prize in Medicine, Professor Richard Axel, was behind this new approach. In 2004 U.S.-based Axel was awarded the Nobel Prize in Medicine together with Dr. Linda Buck for their work elucidating odor perception in mammals. He then moved on to study the odor mechanism of insects. On the basis of his findings, the time was right to bring together expertise in the field of odor research, and to work with industry to turn academic knowledge into innovations. A meeting of experts was arranged by Bayer CropScience in Monheim, and this was the impetus for a project launched in 2010 involving outstanding scientists and other partners. Raming recalls: "Great progress was being made at the time on our understanding of insect olfactory mechanisms on the basis of research into the sense of smell in insects. Before Professor Axel started his work, very little was known."

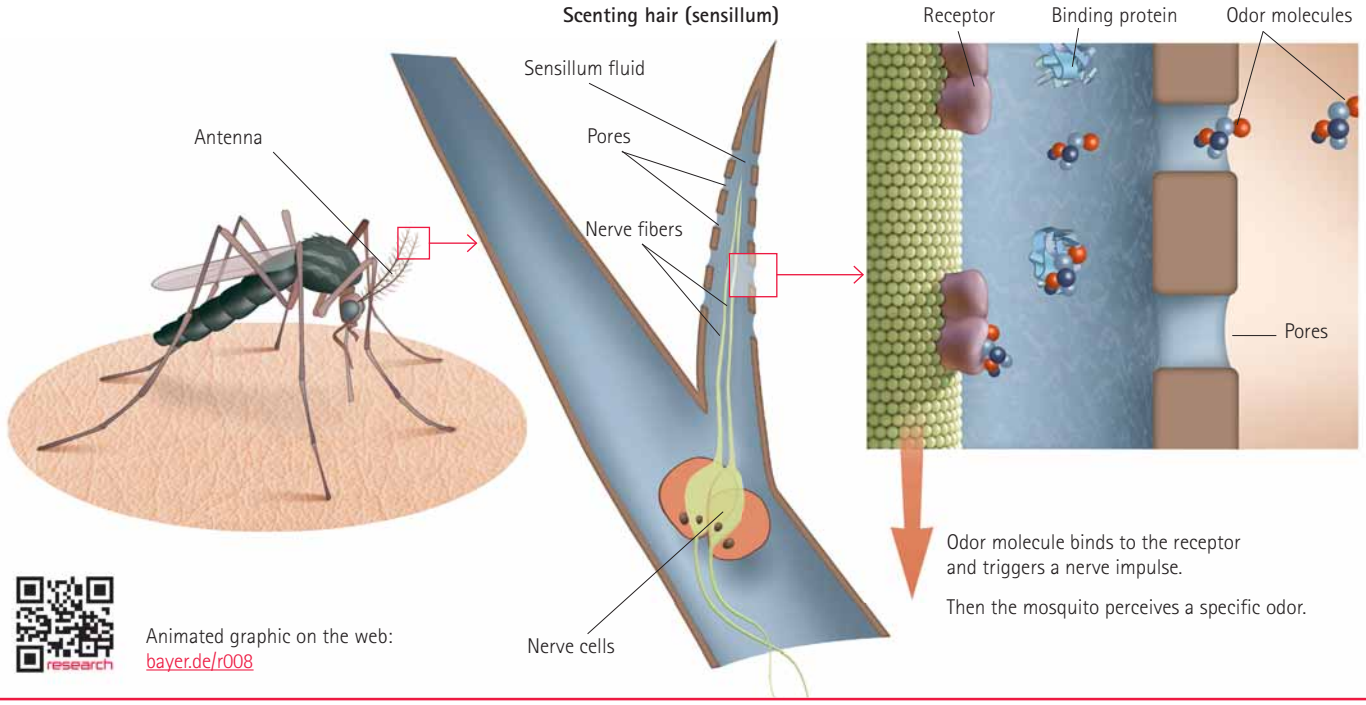
The project is funded via the "Grand Challenges in Global Health" initiative with resources from the U.S. Foundation for the National Institutes of Health. The aim is to find molecules that make mosquitoes "blind" to odors. The scientists use Bayer CropScience's vast substance library in their work. This facility contains over two million chemical compounds that could potentially have this characteristic. Dr. Markus Dollinger, Head of Insecticide Research at Bayer CropScience, explains: "Testing every single substance on live mosquitoes would be not only very expensive but also an extremely long process. Fortunately we can speed things up considerably nowadays by carrying out biochemical screening in the laboratory." Bayer's research scientists use special cells for this purpose. The cells divide continuously and are also used in pharmaceutical research and other applications. The special advantage of this, as Dr. Katharina Wölfel, head of the project at Bayer CropScience, explains, is that "these cells have a sense of smell which is almost as acute as that of insects."

Insects recognize hundreds of different odors

The cell cultures were developed by neurobiologist Professor Leslie Vosshall, a research scientist working at Rockefeller University, New York, who is also taking part in the project. Each of these manipulated cells also contains odor receptors. Insects have about 60 different types of these stimulus receptors in their antennae. They consist of protein structures onto which only certain molecules can dock – just like a special lock that will only accept one key. "The right molecular

From scent to nerve impulse: how mosquitoes smell

Insects have highly sensitive antennae instead of noses. Fine scenting hairs called sensilla capture passing odor molecules from substances such as sweat and exhaled breath, and convert them into nerve signals: if an odor molecule passes through one of the tiny pores , a binding protein transports it to a specific receptor. An ion channel opens and a nerve impulse is generated. Then the mosquito can smell the odor.



Animated graphic on the web:
bayer.de/r008

key triggers a signal in the receptor which is passed on via the sensory cells to nerve cells down the line. As a result, the mosquito perceives a particular odor," explains Raming. The deadly blood-suckers can recognize several hundred different odors, as most odors activate more than one receptor in the cells at the same time. The brain then combines a specific aroma from this information. It is thanks mainly to the work of Professor Axel that these scent-detecting cells can be produced and bred, as he decoded the DNA code, or genetic blueprint, of odor receptors. Bayer's research scientists use gene transfer to insert the relevant sections of genetic material into the cells, which then produce odor-sensitive protein structures.

This test system allows scientists to investigate a very large number of substances in a short time: as soon as the cell cultures have been prepared, they pass through a line of robots in which biochemical screening takes place automatically. This is where the chemical compounds in Bayer's substance library encounter the odor-sensitive cell cultures. "In this way we can test the entire archive of molecules in just a few months," says Dollinger. If one of the receptors reacts to a chemical, the cell culture starts to fluoresce.

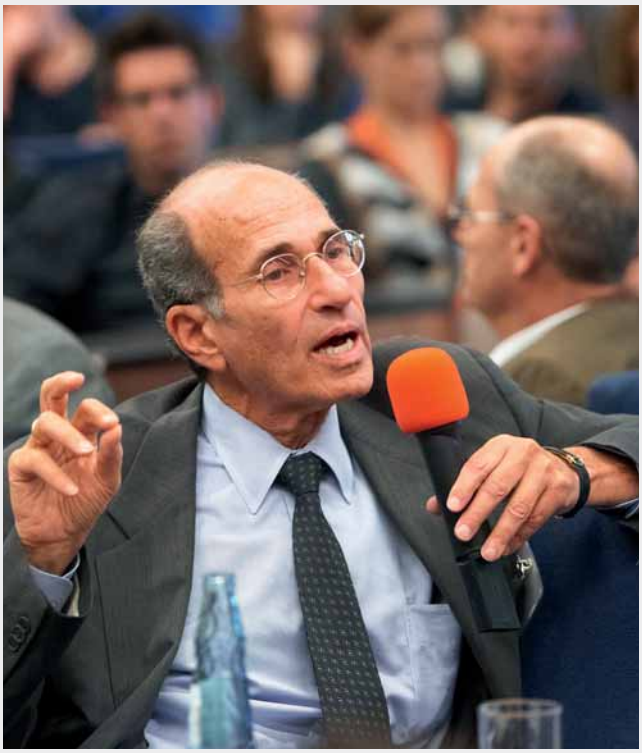
"Molecular clothes-pegs" block odor receptors

The scientists use this fluorescence test to pick out positive candidates and examine them more closely. "This is when the detailed work starts, because we precisely analyze each molecule," says Raming. At this stage, the questions addressed include: what effect does the substance have in the cells? How does it influence the receptors? Is a high concentration of the compound needed to trigger a signal, or is a small number of molecules enough? It is not until these questions have been answered that Bayer's research scientists test their effective compounds on living insects to find out whether their behavior really does change. "We want to find out whether mosquitoes still react to the scents that guide them to their meal of blood," says the biologist. If the odor receptors are blocked, humans are practically invisible to mosquitoes. The potential victim is off their scent radar, and consequently protected against malarial infection. But the "molecular clothes-peg" for scent receptors has other benefits too: the mosquitoes cannot find a mate, or

Professor Richard Axel, holder of the Nobel Prize in Medicine

"The sense of smell is essential for insects"

Professor Richard Axel was awarded the Nobel Prize in Medicine in 2004 for his work elucidating odor perception in mammals. research *talked to the U.S. scientist about how the sense of smell works in insects and about the research collaboration with Bayer CropScience.*



How does olfactory perception differ between humans and insects?
Despite the differences in the anatomy of the olfactory system in humans and insects and between human and insect brains, the logic of odor recognition and olfactory processing appear quite similar. In humans, smell is often viewed as an aesthetic sense, but for most animals smell is the primal sense. It allows animals to recognize food, predators, and mates.

How sensitive is the olfactory sense of insects?
Insects exhibit a remarkably sensitive olfactory sensory system that can allow the detection of odor molecules over great distances. Pheromones, odor molecules given off by one animal that elicit highly stereotyped behaviors in a second animal, are among the most robust sensory stimuli. The pheromone bombykol synthesized by the female silkworm moth can attract males at a distance of several kilometers. A single female pine sawfly expresses a pheromone that is able to attract over 10,000 males from the field in just a few days.

When do malarial mosquitoes need their sense of smell in particular?
The sense of smell is essential for the survival of all insects within their natural environment. Odors provide the language by which mosquitoes communicate with one another and the external world. As such, smell allows insects to identify food, predators, and mates. The chemicals expressed in human sweat, for example, along with exhaled carbon dioxide, attract female Anopheles mosquitoes to human hosts and elicit feeding behavior.

How did the collaboration with Bayer CropScience come about?
The elucidation of the molecular mechanisms of odor recognition immediately suggested the use of modern technologies to identify agents that might confound the olfactory system of the insect and disrupt odor-evoked behaviors, providing a novel approach to the control of both agricultural and human insect pests. Our laboratories do not have the technical expertise to approach this problem. Bayer CropScience has demonstrated a level of sophistication that has placed it at the pinnacle of this field. I visited Bayer and was astonished by the chemical and biological opportunities provided by vast chemical libraries and highly sophisticated high throughput screening technologies that could readily be adapted to this purpose.

What is the goal of this research project?
Our collaboration with Bayer CropScience exploits our understanding of odorant receptors and the neural circuits activated by specific odors to provide novel, safe and effective control of insect pests. The vast scientific resources of Bayer should permit the identification of a large number of molecules with the potential to alter the response of insects to odors, and these compounds may serve as repellants for both agricultural and human insect pests. We are also investigating the neural circuits that elicit these behaviors to identify other novel targets to control insect behavior. I view this collaboration with enthusiasm and excitement.

Looking for an answer: a simple blood test (photo, left) can show whether a patient has malaria. It takes about two hours to get the results. Dr. Klaus Raming and Dr. Katharina Wölfel (photo, right), scientists working for Bayer, are looking for substances that can block the odor receptors of malaria-carriers and other insects in order to prevent infection in the first place.

get back to their breeding grounds. This significantly weakens the population. Depriving these deadly pests of their sense of smell also makes it harder for them to find food.

All of the compounds in Bayer's substance library are currently going through a second set of tests, in which scientists are looking for new substances and mechanisms of action to control disease-carrying insects: "We are working with a British charity, the Innovative Vector Control Consortium (IVCC), to systematically test all our compounds for their potential use as mosquito insecticides," says Dr. Arnd Voerste, Head of Screening and Entomology in Insecticide Research at Bayer CropScience. The researchers are using a biological screening method in which tests are carried out directly on living insects. "The larva of the yellow fever mosquito is an initial indicator. It shows immediately whether or not a substance has insecticidal potential," he explains. This is the first opportunity that Bayer's research scientists have had to carry out tests directly on mosquitoes and sort through the substance library in the hunt for the criteria they want. Previously, substances have not been investigated in depth for the effects on malarial mosquitoes until they have undergone screening by scientists working in crop protection. This was a drawback, because "it was quite possible that many substances of potential interest to us were rejected at that stage because our criteria were not relevant," says Voerste. "If you're not looking for something, you don't find it."

Confusing insects could also be beneficial for crop protection

In preparation for the project with the IVCC, Bayer CropScience expanded its insecticide research platform, increased the capacities of the biological test laboratory at the Environmental Science unit in Monheim, and brought the high-throughput screening facility in Frankfurt into the project. Adds Voerste, "More scientists were recruited to the internal research network and have access to Bayer CropScience's crop protection and insecticidal expertise at all times." Finding new substances or mechanisms of action is a significant challenge: "Pyrethroids, a tried and tested substance class, are now more than 30 years old. Since then no new substances to combat these vectors and no new mechanisms of mosquito control have been discovered," he explains. But finding a new substance or mechanism would be a very important contribution to effective and safe malaria control and would help prevent the development of resistant strains of mosquitoes in the long term. Bayer's insect experts also hope that their findings will be beneficial to crop protection.

The innovative scent-based strategy also offers potential for agriculture, as researchers are looking not only for substances that act against mosquitoes but can also lead astray greedy crop pests. As Dollinger explains, "This could lead to very environmentally friendly insecticides for use in crop protection." And because insects' sense of smell works in a completely different way from that of humans, the compounds would be completely safe for people. Innovative solutions might also be found for animal parasites like ticks that can likewise transmit dangerous diseases. If Bayer's research scientists succeed with their insect-puzzling strategy, a cocktail of odors could in future make people, homes or entire fields of grain invisible to insect pests – thereby also securing harvests.

www.research.bayer.com/malaria
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