

Why it can be useful to attract the enemy: leading mosquitoes around by the nose

Mosquitoes as disease vectors

A little more than a hundred years have passed since mosquitoes were found not just to be an irritating pest – they can actually be deadly. In India in 1897, a British army doctor, Ronald Ross, confirmed the hypothesis that malaria was borne by the *Anopheles* mosquito. Three years later, in October 1900, a working group led by his American colleague, Walter Reed, confirmed the role played by another mosquito species – *Aedes aegypti* (fig. 1), in transmitting the yellow fever pathogen.

But things did not stop there: as time went by mosquitoes turned out to be vectors of a broad spectrum of diseases. Tab. 1 shows the most important mosquito-borne diseases worldwide. Fig. 2 shows the approximate distribution



Fig. 1: *Aedes aegypti*, vector of yellow fever and dengue. (Source: E A Goeldi (1905) *Os Mosquitos no Pará.*) ■



Fig. 2: The distribution of the main mosquito-borne viral diseases affecting man. DEN: Dengue Fever, EEE: Eastern Equine Encephalitis, JE: Japanese Encephalitis, LAC: La Crosse Encephalitis, MVE: Murray Valley Encephalitis, SLE: St Louis Encephalitis, VEE: Venezuelan Equine Encephalitis, WEE: Western Equine Encephalitis, WNV: West Nile Virus, YF: Yellow Fever. Derived from various sources ■

on of a selection of viruses transmitted to humans by mosquitoes. For some of the pathogens transmitted by mosquitoes, such as malaria or dengue fever, we have still yet to find an effective vaccine or method for warding off disease. All too often, the only things standing in the way of widespread vaccination programs are poverty and over-population. Sudden outbreaks can even catch well-prepared health programs off guard. Sometimes mosquitoes wreak havoc in places where people otherwise only consider them to be an irritating pest, as was the case in the US when it was struck by West Nile fever. This particular disease is also transmitted by mosquitoes, and is most common in birds. But by changing its host it can also transfer itself to

Disease	Cases / Year	Deaths / Year
<i>Malaria</i>	300.000.000	1.000.000
<i>Yellow Fever</i>	200.000	30.000
<i>Dengue Fever/ Dengue Hemorrhagic Fever</i>	20.000.000	24.000
<i>Japanese Encephalitis</i>	50.000	15.000
<i>Lymphatic Filariasis (Elephantiasis)</i>	120.000.000	-

Tab. 1: The currently most important diseases transmitted by mosquitoes. Sources: World Health Organization (www.who.int) and the Centers for Disease Control and Prevention in the US (www.cdc.gov) ■

people. Once considered only an „old-world“ virus, West Nile first appeared in America in 1999, when it was discovered in the corpses of dead birds. But soon it claimed its first human victims. In its first year, the epidemic boasted 62 seriously ill and 7 deaths. In the following 2 years, serious infections numbered 21 and 66 respectively, there were 2 deaths and then 9. In 2002 the number of reported cases rose to 4156, with 284 deaths. By 2003 as many as 9858 instances of the disease were counted in 40 States, with 262 deaths. (Source: Centers for Disease Control and Prevention, USA, www.cdc.gov.)

Whether a mosquito has the potential to transmit disease, and which diseases it can transmit, depend primarily on its preferred host. Some mosquito species do not really care who their host is, they tap into the first vertebrate they can find. These are the ones most likely to transfer diseases from one type of host to another, as was the case with the West Nile fever virus. We face more serious problems with the mosquito species that prefer humans or will only seek out humans for a tasty top-up of blood – even if they could actually turn to other hosts. It is mainly this selective type of behaviour that causes the rapid proliferation of epidemics. Some of the best known cases of this were with *Anopheles gambiae*, the mosquito transmitting malaria, or *Aedes aegypti*, the transmitter of yellow fever and

dengue; despite intensive efforts to treat and eradicate these diseases, infection levels continue to rise. Worldwide, more than 500 million people are infected every year by diseases passed on by mosquitoes. The damage this inflicts on countries' economies is put in the tens of billions. ■

The role of efficient monitoring systems

There are a number of ways to wage wholesale war on mosquitoes. The most common technique is to use chemical and biological insecticides, or destroy the waters in which they lay their eggs. But the ecological side-effects, the often astronomical costs involved and the danger of the mosquito developing insecticide



Fig. 3: Monitoring of mosquito populations: the hunt in Brazil for water receptacles infested by eggs, larvae or pupae (photo: Á.E. Eiras, University of Minas Gerais, Belo Horizonte, Brazil.) ■

resistance make it increasingly essential for us to simply become more efficient at what we do.

A key issue in combating mosquito-borne disease is therefore how quickly we identify rapidly developing mosquito hot-spots. Pockets of dangerous mosquitoes can be moni-

tored in a number of ways. One is to examine the biotopes and waters populated by larvae, pupae and eggs (fig. 3). One drawback with this method is that you already have to know where the breeding grounds are (or look for them), and the analysis requires specialist knowledge. Another approach is to try and catch adult mosquitoes. But using capturing devices in large areas is costly and findings are not representative: so far, you can only use generic baits such as lights and carbon dioxide. For this reason, to gather sufficiently representative samples of the overall mosquito population for health assessment reasons, scientists often still turn to volunteers – human guinea-pigs, who catch approaching mosquitoes and count them. The ethics of this technique are somewhat dubious due to the risk of infection. It is also unreliable (mosquito catcher motivation varies widely). And it is expensive. Much better solutions would be to find simple, inexpensive, highly targeted traps for adult mosquitoes which are as appealing as human hosts or the natural breeding grounds. Another significant advantage associated with the capturing of adult mosquitoes is that these can then be examined using molecular biological testing to ascertain whether they are already infected with pathogens. ■

Tempting aroma cocktails

Scientists at the Institute of Zoology at the University of Regensburg (Germany) have now joined forces with the University of Minas Gerais in Belo Horizonte (Brazil) to pool research findings and develop new types of monitoring systems. The aim is to fight disease-transmitting mosquitoes more efficiently. The Regensburg researchers identified the types of aroma mosquitoes recognise on human beings and how they actually zoom in on them. Their colleagues in Brazil investigated the aromas used by pregnant mosquitoes to find a suitable place to lay their eggs.

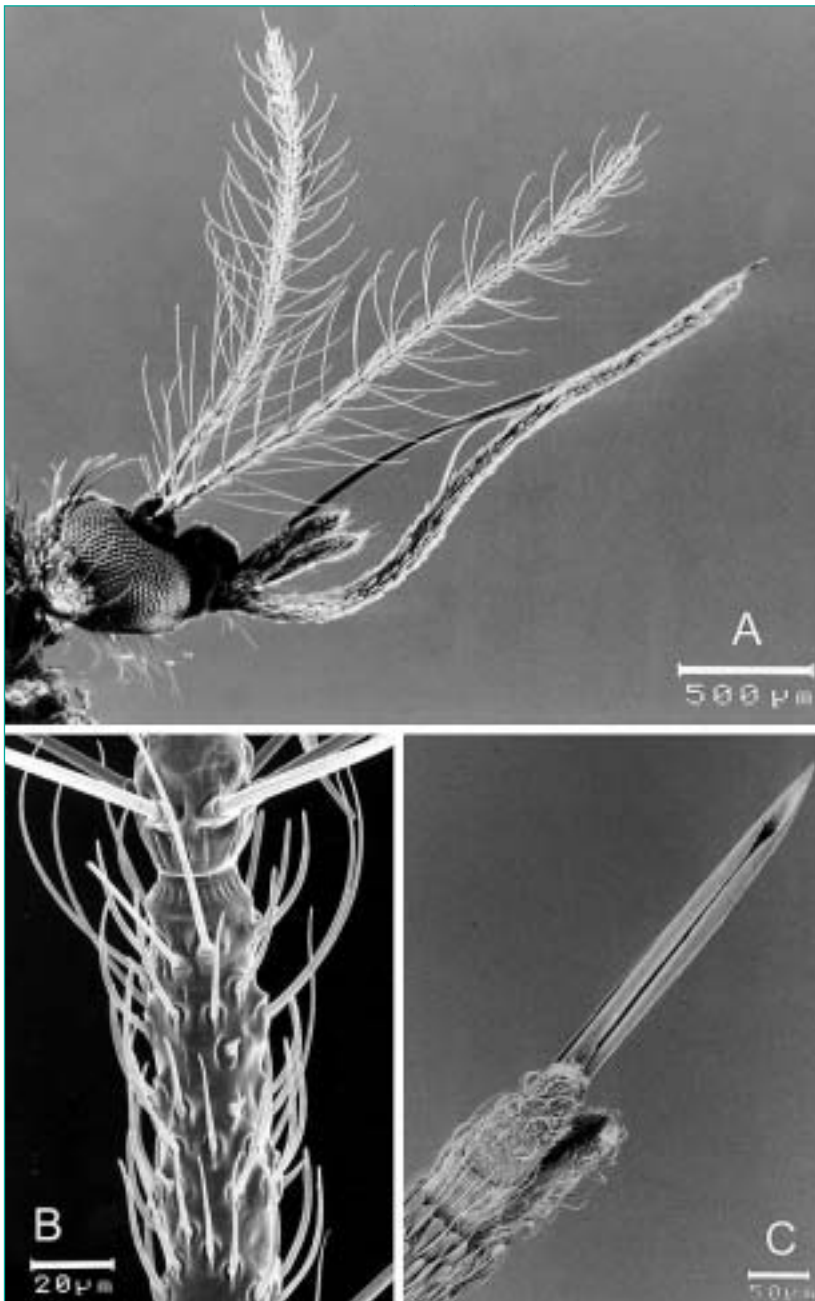


Fig. 4: Images of a yellow fever mosquito (*Aedes aegypti*) taken with a scanning electron microscope. A) head with antennae, proboscis with stylet and sheath, and two palps (paired extensions at the base of the proboscis). B) A segment of the antenna with olfactory hairs containing the olfactory cells. C) Tip of the stylet which is injected into the skin of the host to feed on blood. (Images: A. Kühn, University of Regensburg) ■

Both baited cocktails are now being introduced to improved monitor systems, making it easier to track and influence mosquito population developments quickly, economically and accurately.

[How mosquitoes spot their hosts](#)

Workers in the Regensburg laboratory focussed primarily on odours emitted by the human body. These are attractive to female mosquitoes

who roam around looking for human hosts.

As with all insects, mosquitoes mainly pick up smells with their antennae. Under the microscope you can make out elongated mechano-sensitive bristles and short olfactory hairs (sensillae) covering the whole antenna (fig. 4). Tiny pores on the outside of each hair allow scents to pass through to the sensory cells inside. There are

different types of hairs, distinguished by morphological features. Generally, each contains characteristic sets of sensory cells. In total, each antenna boast more than 2000 olfactory cells on around 1000 scent-detecting hairs. You can record the neuronal activity of individual hairs by using electrophysiological techniques, measuring how they react to scents. Depending on the stimulus, the olfactory cells respond by increasing or inhibiting neuronal activity. It is not until insects start recognising patterns of activity across many cells that they can piece together the properties of individual scent stimuli, as well as their concentration and duration.

To break down the parts of the human bouquet with the most appeal, scientists isolated aromatic components on human skin and measured their electrophysiological effects, as well their effect in attracting mosquitoes in behavioural assays. According to their findings, only certain aromas are needed to identify the right host. But when they examined each component in isolation they hardly had any effect. It is only when they are brought together in certain combinations that the attractiveness of each aroma unfolds, tempting the mosquito in a similar fashion to their natural host. So mosquitoes recognise their favourite hosts by their characteristic aroma patterns. This aroma cocktail has been registered for patent. The substances involved have no adverse effects on health, are easy to handle and economical, side-stepping expensive carbon dioxides used to date. Laboratory and field testing shows that they can entice several species of mosquito attracted to humans.

[How mosquitoes decide where to lay their eggs](#)

On the other side of the Atlantic, colleagues in Brazil tackled the problem from a different angle, putting the particularly malicious

dengue and yellow fever mosquitoes under the microscope. They were interested in female mosquitoes after they had sucked blood from humans. After an incubation period lasting several days, the female mosquito goes on the hunt for a suitable place to lay its eggs.

We mainly still have the *Aedes aegypti* mosquito to thank for passing on yellow fever and dengue. But we have also had to start looking out for its close relative, the Asian Tiger Mosquito, *Aedes albopictus*, which has joined its cousin in spreading these diseases worldwide – and not only in tropical and sub-tropical regions, since they can also survive at cooler latitudes: in

ded by humans to bring their young into the world; they are quite happy to use even small quantities of water, in discarded plastic beakers, bottles, broken drainpipes, tyres left out in the open, plants using hydroponic (soil-less) growing media, watering cans, rainwater collection tubs, etc.

Mosquitoes go about identifying good oviposition sites the same way they look for a host – by using their sense of smell. Water rich in the nutrients needed by mosquito larvae has a distinctive smell, so this is what mosquitoes look for to lay their eggs in. In the laboratory in Belo Horizonte, scientists therefore started by

Innovative monitoring systems for combating disease

If used in the right trap, both aroma cocktails can significantly improve the efficiency of monitoring systems. The synthetic host aroma works particularly well in a special ventilator trap (also developed in Regensburg and currently being patented) which optimises the emission of odours. By way of contrast, the oviposition attractant requires a plastic container filled with water, coated on the inside with a special adhesive which grabs onto the mosquitoes. This capturing device is also registered for patent and is highly economical to produce. Outdoor testing in Brazil shows that the best strategy to monitor mosquitoes is to use both systems together.

The ventilator trap uses a synthetic human odour. It is a highly sensitive monitoring system and can capture up to ten times more mosquitoes per day than the oviposition trap. The latter offers significant cost benefits and, unlike the ventilator trap, does not need power. The straightforward oviposition trap can be used for on-going monitoring purposes across wide areas. The ventilator trap can be used on a more targeted basis in mosquito hot spots when you need to find out the precise density of the mosquito population quickly.

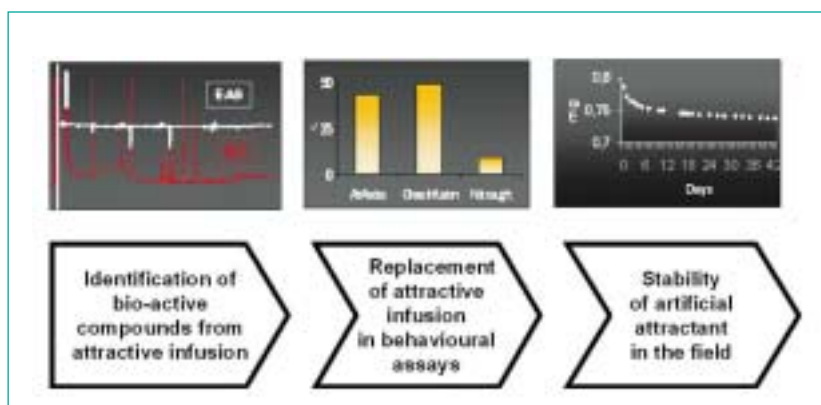


Fig. 5: Development of a synthetic oviposition attractant. Left: The schematic output of a combined gas chromatography and electrophysiological measurement device. Electrical nerve signals are taken from the antenna of a pregnant yellow fever mosquito positioned directly at exit of a gas chromatograph. The gas chromatogram is shown in red (GC), the electrophysiological responses are in white (EAG). Peaks can be seen on the EAG curve only when certain substances are emitted from the GC. Middle: A comparison of synthetic and natural attractants in release and capture testing. Right: The stability of the synthetic oviposition attractant. (Á E Eiras, University of Minas Gerais, Belo Horizonte, Brazil) ■

Europe, the Asian Tiger Mosquito has already reached Greece, the Balkans, northern Italy and southern France. In the US, this species was first documented in Texas in 1985 and is currently established in more than 25 states.

One thing both species have in common is the way they lay their eggs. Originally, both mosquitoes used natural water retaining cavities in tree holes and herbaceous plants as a breeding ground. But in an urban environment, there are also plenty of attractive locations provi-

examining the behaviour of pregnant mosquitoes to see which blends of water and nutrients they found most attractive. They then used a combined gas chromatography and electrophysiological measuring device to identify which substances set off a reaction in the antennae. In a sequence of experiments they then observed mosquito behaviour to work out the ideal mixture to attract their attention (fig. 5). In field testing, this mixture was found to be just as effective as their natural breeding grounds and it has now been registered for patents. ■

To process data gathered by the traps as quickly as possible, it is transferred to a so-called GIS (geographical information system) using a standard electronic data-gathering system. This makes it possible to identify high-risk areas quickly and accurately and make highly localised battle plans. You can then use the ventilator traps to assess how successful each campaign was. After a series of success studies in Belo Horizonte, scientists are currently carrying out broad-scale tests in Brazilian cities

on an early warning system designed to pinpoint dengue fever, using the same type of technology. Further testing is in the pipeline later in the year in North America, Europe, Africa and Asia. These tests will be looking at the first batch of a new type of monitoring trap.

To update, refine and market the new capturing devices efficiently, two university spin-off companies were set up at the beginning of 2003 – one in Belo Horizonte, the other in Regensburg, and each linked up commercially to the other. The Brazilian company, Ecovec Ltda focuses on the public health sector in South America; the German company, BioGents GmbH, manages Europe, North America, Asia and the remaining international markets. The combination of laboratory and outdoor testing, the complementary blend of expertise provided by the two research teams, and their tried-and-tested ability to work together closely (despite the long distances involved) point to a promising future for the joint venture.

It is also a good example of an attractive and inexpensive alternative to the types of preventative medicines used in vaccination programs, or the proposed release of genetically engineered vectors. For decades, bodies have channelled colossal research funds into the development of vaccinations to fight malaria and dengue. We have still yet to find one that works. In recent years, we have also witnessed intensified efforts to find a solution for combating these diseases with genetically engineered mosquitoes. This has been a similar drain on technology research funding, even though many experts have expressed their doubts about the technology. This contrasts to targeted research in the field of chemical ecology and the sensory ecology of mosquitoes, which – for

relatively small amounts of money – can help us move a significant way forward in the battle against disease. ■

New products in the fight against mosquitoes – in business and consumer markets

Highly effective lures and mosquito traps are not only useful instruments in the battle against disease. They can prove interesting to anybody trying to shoo away the irritating airborne pest without damaging the environment. In the United States in particular, there have been a variety of mosquito traps launched in recent years, targeted at the consumer market. Apart from a number of totally ineffective electrocution devices with light sources, most consumers plump for traps using propane gas to attract mosquitoes by generating carbon dioxide, heat and moisture. These traps cost anywhere between 300 and 1300 US dollars; last year the US market for such capturing devices was worth 650 million dollars. The consumer can clearly see the benefit of the methods used by these inventions: to catch the little bloodsuckers before they sting you, thus helping control the overall population.

As with the monitoring trap described above, these traps use carbon dioxide and other more generic lures, mainly because there are no alternatives. As a result, the initial outlay and running costs are high. They are also limited to areas outside the home because they use explosive and poisonous gases. In laboratory settings and outdoor testing, scientists have found that the mosquito capturing device from Regensburg with the human aroma frequently outperforms – or at least matches – propane gas driven traps. With the new lure, the manufacturing costs of capturing devices can be slashed to one tenth of current levels. They are also much easier to handle. Based on

the current capturing device and scientists' experience in the development process, a number of different types of trap are being developed for launching on consumer markets. Each will be targeted at different price segments and sub-markets. From straightforward adhesive traps with time-release aromas, to high performance devices for professional mosquito hunters in hotel grounds and parks, the new technology opens up completely new avenues in capturing unwanted mosquitoes – without damaging the environment, when and where it is needed. ■

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