Protagonists dig deeper in their efforts to crush a complex foe

Close quarters: an electron micrograph image of the head of a female *Anopheles arabiensis* mosquito

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Mosquitoes Changes in the insect's behaviour have made it tougher for scientists engaged in the battle, says *Sarah Murray*

According to ancient Chinese warlord Sun Tzu, the key to success in combat is to know your enemy. This principle is being taken seriously in the war on malaria. While part of it involves studying how the behaviour of different mosquito species affects malaria transmission, scientists are digging deeper. They want to know their foe's genetic make up so that they can alter it.

The enemy is complex. About 500 malaria mosquito species are distributed around the world, with about 50 transmitting malaria. Why only some species or populations are efficient vectors, or carriers, of malaria remains unclear.

Understanding breeding patterns or whether a malaria-transmitting mosquito lives mainly inside or outside can help advance disease transmission and control techniques.

While most malaria transmission occurs in sub-tropical areas of Africa, the US and Asia, mosquitoes have different behavioural patterns. For a start, *Anopheles gambiae*, the species that transmits malaria, prefer to bite humans while *Anopheles quadriannulatus*, a non-vector species, stick to animals.

Variations are found in when and where mosquitoes bite, with *Anopheles gambiae* feeding and resting indoors while *Anopheles arabiensis* function outdoors. Worryingly, research has found that some of these behavioural patterns are changing in response to human interventions.

"The use of insecticides inside has caused changes in mosquito behaviour," explains Igor Sharakhov, an entomology professor at Virginia Polytechnic Institute and State University. "They have become more active outdoors and more active early in the day, not just at night."

This has implications for outdoor workers such as forest workers, fishermen and farm labourers, as well as victims of natural disasters or wars living in temporary shelters, and has prompted research into clothing impregnated with insecticide.

Evidence indicates that the range of mosquito forms is increasing. A team of researchers from the London School of Hygiene & Tropical Medicine has found that in parts of West Africa, different molecular types of *Anopheles gambiae* have been interbreeding, creating a more complex range of forms.

Interbreeding has the potential to accelerate the spread of insecticide resistance, says David Conway, the professor who led the research, "because the gene can enter the population without having to wait for a new mutation".

Prof Conway warns that the finding does not signal a major threat to malaria control. "But it's a warming that we need to understand these populations that we are trying to control because it could make insecticide use less effective," he says.

As well as tracking mosquitoes through field research, the study of genetics is seen as having great potential. Progress in understanding the biological blueprint of the insects is bringing researchers closer to the possibility of genetically editing mosquitoes.

Much of the data generated so far is on VectorBase, a website that makes available genomes and related information on five vectors, including mosquitoes. Frank Collins, one of the project's principal investigators, sees many uses for the data. Before even entering the realm of genetic modification, he argues that an understanding of the mosquito genome could help advance the development of new control products such as insecticides.

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"These could target features of mosquito genomes that are not represented in mammalian genomes," says Prof Collins, who is professor of biological sciences at Indiana's University of Notre Dame. "So you might be able to develop something that is less toxic to non-target organisms."

Understanding mosquito behaviour at the molecular level makes possible entirely new kinds of control techniques. Identifying the mechanism through which a mosquito finds a human host might allow the development of a false host that is in fact a toxic trap. Another approach would be to edit mosquitoes' genes to render them incapable of transmitting the disease. In one project demonstrating the feasibility of this approach, researchers at Virginia Polytechnic Institute and State University used gene disruption to change the eye colour of a mosquito.

At Johns Hopkins Malaria Research Institute in Baltimore researchers have genetically modified a bacterium commonly found in a mosquito's intestine so that it secretes proteins that are toxic to the malaria parasite.

Researchers from the University of Irvine and Oxitec, an Oxford university spin-out biotech company, have been developing flightless mosquitoes that could help control the spread of diseases such as malaria and dengue fever.

"Now it's getting more exciting because we are discovering all these genetic tools that we can apply to malaria," says Prof Sharakhov.

Researchers seek out man-made alternatives to natural remedies

Drugs

Long development process starts to pay, writes *Andrew Jack*

Many thousands of years after nature evolved the most effective current treatments for malaria, researchers aim to introduce man-made alternatives to help keep ahead of the parasite.

Artemisinin, derived from the Chinese sweet wormwood plant, remains the ingredient of choice in drugs to cure the disease but comes with a problem. Its complex nature means that manufacturers have until now struggled to find cheaper and more consistent synthetic equivalents.

Global production over the past decade since the widespread introduction of artemisinin combination therapies (ACTs) has been hampered by weather, land for cultivation and other uncertain growing conditions, as well as market manipulations to further restrict supply and push up the price.

That is beginning to change. In one of a number of such collaborations under way, this month the French drug company Sanofi unveiled plans for large-scale semi-synthetic production of artemisinin, following a painfully long and complex process of development.

Building on work by the US non-profit business One-WorldHealth, the company Amyris and the University of California, Berkeley, Sanofi has finalised plans to produce 35 tonnes of artemisinin this year, rising to 50-60 tonnes a year from 2014 – enough for 80-150m treatments.

The elaborate work involves fermentation of artemisinic acid in Bulgaria, photochemistry to transform it into artemisinin in Italy and processing and mixing with other drugs such as amodiaquine, one of the company's existing antimalarial combinations. Sanofi has pledged to produce it on a no-profit, no-loss basis to help keep treatments affordable.

A farmer with the artemesia crop

"Promoting a steady and affordable supply of high-quality artemisinin is a critical part of our efforts to eradicate malaria," says Steve Davis, president of PATH, the Seattle-based non profit group that helped support the work.

Artificial artemisinin is not the only example of a public-private partnership beginning to show results. David Reddy, head of the Medicines for Malaria Venture (MMV), describes a new "challenge" model to accelerate traditional drug development techniques.

Designed in conjunction with the Queensland Institute of Medical Research, it permits healthy volunteers to be injected with small quantities of malaria-infected red blood cells. The evolution of the parasite in the body is observed using sophisticated Polymerase Chain Reaction tests. That minimises the dangers and speeds up traditional drug testing, which was conducted in patients exposed to malaria naturally in endemic regions.

Mr Reddy cites MMV's work in developing common pharmacokinetic tests and making widely available libraries of experimental compounds owned by normally intensely competitive and secretive companies. That has so far allowed it to identify and circulate a "malaria box" of 400 potential drugs to more than 100 research teams around the world.

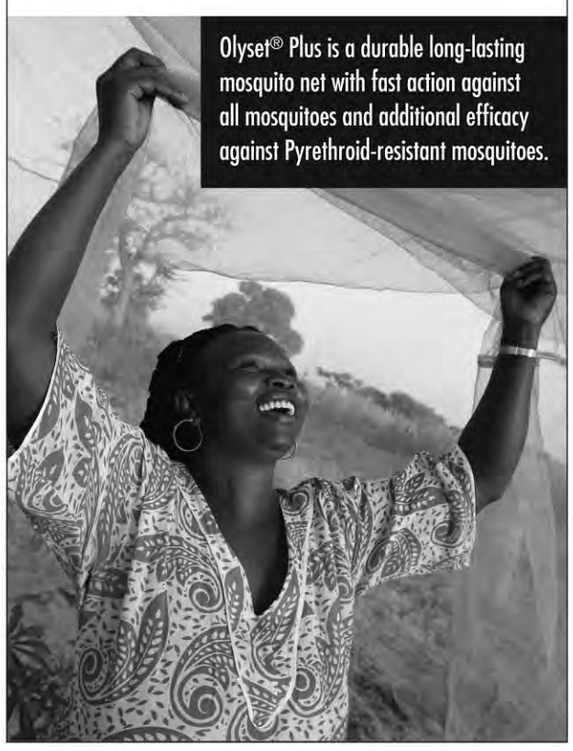
"There's not a lot of profit to be made in malaria," he says. "The last thing companies want to do is waste their money on drugs that will not measure up. If you can begin a common understanding of what drugs are needed and the criteria for selection, and not reinvent the wheel, that creates savings and de-risks the work for industry." Promising pre-clinical discoveries have been made by university researchers in countries including South Africa, as well as in companies in more developed and emerging economies alike.

Regulatory innovation is under way, such as the pioneering authorisation last year by the European Medicines Agency of Pyramax, a drug developed with Shin Poong of South Korea and MMV, under its "article 58" rule.

That offers approval of a medicine by a well-respected organisation but for use in developing countries rather than within the EU, where the risks and benefits would be different.

Nature has not been standing still, either. The emergence of malaria strains resistant to artemisinin in southeast Asia is pushing researchers to seek entirely new classes of compounds. Paul Herrling, chairman of the Novartis institute for tropical diseases, which developed Coartem, the first ACT, cites promising advances with several experimental medicines.

"We want to continue to be a major player in malaria," he says. While sharing much of its expertise with MMV, the company is developing on its own the most advanced compound – codenamed 609 – partly because "MMV is not particularly swimming in money" and it hopes to generate a modest return on the drug.



Push required in fight against killer

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led to calls from specialists such as Professor Nick White at Oxford university for an intensive campaign to saturate the region with the latest drugs in an effort to wipe out the malaria parasite entirely.

It points to the need to continue investing in research and development. The malaria drug pipeline looks healthy, with a range of experimental medicines under test. These offer the prospects of shorter cures, a shift away from reliance on ACTs and alternative treatments for *Plasmodium vivax*, the second most common type of malaria in Asia.

Work on developing a malaria vaccine is continuing, though interim findings of the most advanced product – GlaxoSmith Kline's RTSS – suggest only modest protection in children.

A sharp debate might be expected, therefore, over the costs of adding another product to the "armatorium" and the stretching of fragile health systems with the purchase and distribution of RTSS.

There are many competing demands ahead as far as the use of existing tools is concerned. Intermittent preventive treatment for pregnant mothers has a powerful effect in reducing the risk of infection in the newly born. Seasonal malaria chemoprevention for children aged three to 59 months has been shown to reduce illness. The former

Rob Newman, of the WHO, says data collection is vital

tool has much scope for improved uptake; the latter has barely been used to date.

Dr Fatoumata Nafo-Traoré, head of the Roll Back Malaria partnership of public and private organisations, praises African leaders' efforts against the disease but warns that "the inadequate dissemination, uptake and application of research results within African countries themselves have created a needless separation of research and policy setting."

Scope exists for greater efficiencies, partly by breaking down barriers between "vertical" diseases. Malaria has boosted funding for bed nets that also tackle lymphatic filariasis (elephantiasis). The latter's community drug workers, however, could be used more to help with bednet distribution. The product they give out – ivermectin – also kills mosquitoes. The Global Fund to Fight Aids, TB and Malaria, the largest conduit of multilateral donor support, is seeking a fresh injection of funds this year after a restructuring designed to target spending more effectively.

Nigeria and the Democratic Republic of Congo alone are estimated to account for 40 per cent of malaria deaths globally. With India, they are host to 40 per cent of all infections. This suggests the need for greater focus in the fight against the disease if the greatest short-term impact is to be achieved.

> 'Every suspected case should get a diagnostic, be treated and tracked'

Yet Prof Sir Richard Feachem from the University of California San Francisco argues that excessive attention on high-incidence countries comes at the expense of 34 lower incidence ones that have good prospects of eliminating malaria. He calls for more concentration on "hotspots and hot pops" – the latter being the population of migrant adult males, who are becoming the most important infected group.

Tighter targeting of malaria will be essential, such as when protecting the rubber tappers in Myanmar who work at night, rendering bednets useless. The Malaria Consortium is studying the use of insecticide-impregnated wristbands to keep mosquitoes at bay.

Despite the closure by the Global Fund of its unit supporting an affordable medicines facility, more partnership with unregulated private vendors of drugs seems inevitable while widespread lack of stock exists in public clinics.

More generally, the "three Ts" – test, treat and track – need greater resources. Without more monitoring, and support from health workers, it is impossible fully to understand malaria or tackle it. "In the very highest burden countries we don't have the hard, real data to say whether we are on track," says Rob Newman, head of the WHO's global malaria programme. "Every suspected case should get a diagnostic, be treated and tracked in a surveillance system. That data to make informed decisions is going to be critical."