The modern-day mozzie zapper

A research program led by the University of Queensland’s Scott O’Neill is developing a completely new way to fight dengue fever. Fiona Wylie reports on progress as this five-year program enters its third year, and ventures to north Queensland for some proof of the mozzie pudding.

IN JANUARY 2003, Bill and Melinda Gates announced a new initiative to fund research on disease that disproportionately affects people in the world’s poorest countries – the Grand Challenges in Global Health initiative. The Gates Foundation identified 14 major challenges to global health and established a competitive granting process to engage creative minds from diverse scientific disciplines in addressing these challenges.

In June 2005, a total of US$440 million was allocated to fund 43 projects over five years. One of the 14 Grand Challenges was to develop new control measures for insects that transmit human disease, including malaria and dengue fever.

A multinational collaboration led by Professor Scott O’Neill was successful in securing a US$6.7 million (A$10 million) grant from the Grand Challenges program. Together with other scientists and health experts from Thailand, Vietnam, Japan, Australia and the US, O’Neill is developing an innovative approach based on manipulating mosquito age to prevent the spread of dengue virus from mosquito to human.

The approach aims to reduce the need for current insecticide-based control approaches, which continue to have mixed success due to insect resistance, agent toxicity and both the financial and environmental cost of their large-scale application.

The success of O’Neill’s approach depends on the lifespan of a mosquito. An incubation time of around 12 days is needed for the dengue virus to pass from infected human via a mosquito bite to another human. Thus, only older mosquitoes can transmit the virus to a human host –mosquitoes live for up to 30 days in the wild – and removal of older individuals from a population would effectively block disease transmission without harming species survival or fitness.

Theoretical models suggest that this form of intervention could reduce dengue transmission by 90 to 100 per cent,” O’Neill says. This strategy also has potential to work in a much wider disease context including malaria, although dengue is the immediate focus.

Eternal youth

The key to this ‘eternal youth’ for dengue mosquitoes is an intracellular bacterial parasite called Wolbachia pipientis, first observed in the ovaries and testes of the mosquito Culex pipiens 70 years ago.

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Wolbachia naturally occur in 20 per cent of all insect species, including many mosquitoes native to Australia.

Two features of these bacteria are crucial in this context: Wolbachia are only transmitted vertically by females, in a process known as maternal inheritance, and are not transmitted Infectiously; and Wolbachia infection does not affect immature stages of the insect, but over-replicates in adults causing the early death of the host.

Therefore, Wolbachia's success is directly linked to the reproductive success of its insect host, and in fact, it has evolved to confer a reproductive advantage to infected females within a mosquito population. Importantly, Wolbachia does not infect vertebrates or induce direct pathology in mammals, including humans.

Almost half-way into the funding period, O'Neill and colleagues are pleased with their progress. "The program is really coming together nicely as a whole," he says. The team is ahead of schedule in all areas, from the science of producing suitable Wolbachia-infected strains of Aedes aegypti mosquitoes to establishing the necessary local and regulatory contacts and community support for the next field-based stages.

The first aim of the Grand Challenges project was to pre-adapt the bacteria to their new mosquito hosts in vitro prior to introduction in the field, and identify ways to optimise the maternal transmission efficiency.

The life-shortening strain of Wolbachia was originally isolated from the Drosophila melanogaster fly species. In achieving its initial aim, O'Neill's group at UQ successfully introduced this bacteria into Aedes aegypti mosquito populations collected from Cairns, optimised the spread of infection through a population, and developed a novel method for determining the age of mosquitoes, all under laboratory conditions.

**Bacterial infection**

The next aim of the program comprises "the really critical experiments of the entire project: to gain a biologically relevant estimate of what the bacterial infection will do to the age structure of a mosquito population in a 'real' setting," he says. Under the guidance of O'Neill and Dr Scott Ritchie of James Cook University, large insect cages measuring 20 x 8m are being built in a contained field setting in Cairns to measure the effects of Wolbachia on mosquito life expectancy and population dynamics.

"They are sort of like greenhouses for mosquitoes," O'Neill says. A Cairns-derived mosquito colony crossed with a laboratory-inbred colony and infected with the life-shortening Wolbachia bacteria will be tested in the cages.

"What we want to know is how well the infected mosquitoes perform outside of a laboratory; in a more natural and harsher environment with fluctuating temperatures and humidities, where everything is not given to them on a plate. Are the mosquitoes able to find hosts to feed on and how do they survive generally compared to wild-type mosquitoes?"

Once the cages are fully operational, there will be a range of experiments conducted.

"Primarily, we want to test theoretical models of infected mosquito dynamics in a native population," he says. The program's modellers, based at the University of California, can only go so far in silico and now need a few real numbers to run their models properly.

"We need to estimate parameters in a field setting such as egg production from the Wolbachia-infected mosquitoes as they age compared to uninfected specimens. Once such parameters have been established from the field cages, the numbers can be plugged back into the models to predict how the infection will spread."

Ultimately, the researchers want to know how many mosquitoes they need to release into an environment to get successful integration of the infection and have an effect on the age of the population, thus potentially blocking disease transmission.

"These predictions will in turn be tested still in the cage environment to see whether we get the life shortening predicted theoretically." This testing is a crucial prelude to establishing a release strategy.

O'Neill says that actually implementing a mosquito release strategy was never in the scope of the five-year Grand Challenges project. "But what I think we will be able to do with these new field-cage experiments is have a very clear idea of how likely the strategy is to work, and then it is really about preparing the groundwork for a future release - to make sure that people are comfortable with it, that we have good predictions of the efficacy and how much money and work would be needed..."
to implement each release.”

**No more Dorian Gray**

In practical terms, the Wolbachia life-shortening approach, if successful on a small scale, would be used in conjunction with other control and prevention methods, he says. “For example, to complement a vaccine strategy (when a vaccine comes along) because one of the problems with a vaccine for dengue is getting it out to all the people at risk, particularly in some of the poorer affected communities. Scott Ritchie talks about our strategy as a ‘safety net’ – if we get it to work, we would still have to undergo the same monitoring and evaluation of dengue cases.”

Most control measures are responses to outbreaks and not preventative; this would be a preventative approach that should allow the traditional control strategies to be less relied upon. “This is an important point because in Cairns for instance we have a very good control system in place that is easy to implement, but if you go to a place like Bangkok or Hanoi, it is a very different situation because it is that much harder in those developing country urban centres to control the mosquitoes well. It is almost impossible – there are so many places for them to breed and insecticide treatments often don’t work all that well.”

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**Wolbachia man**

Scott O’Neill joined the University of Queensland in 2001 and currently serves as head of the School of Integrative Biology. Prior to that, he led the vector biology section at Yale University’s Department of Epidemiology and Public Health. It was there that he started to think about a novel approach for controlling insect-transmitted disease via naturally occurring infections of vectors.

O’Neill is internationally recognised for his work on Wolbachia. His group recently published the first full genome sequence of Wolbachia in a collaboration between UQ and the Institute for Genomic Research (TIGR) in the USA.

O’Neill’s work is currently funded by multiple grants from the Australian Research Council, the World Health Organisation, the National Science Foundation USA, the McKnight Foundation and the Bill and Melinda Gates Foundation.
Man bites mosquito

Dengue fever is a mosquito-borne viral disease prevalent in over 100 countries. Up to 100 million cases of dengue are reported globally each year and over 2.5 billion people — two-fifths of the world’s population — are currently at risk of infection, making this a significant global disease burden. There are no specific treatments or effective vaccines currently available to fight dengue, and prevention relies on disease monitoring and vector-control programs.

Dengue was first established throughout the tropics with commercial shipping during the 18th century, and in Australia, reports of dengue epidemics date back to 1879. In 1905, during a large dengue outbreak in Brisbane, local medical practitioner Thomas Bancroft was the first to identify the urban-loving mosquito, *Aedes aegypti*, as a major dengue carrier. Currently, the disease is limited to north Queensland by the distribution of this mosquito vector.

The incidence of dengue worldwide has grown dramatically in recent decades, and this year’s outbreaks highlight the burden it places on many developing countries. Before 1970 only nine countries had experienced major dengue epidemics, but this number increased more than four-fold by 1995. As is the case globally, the problem of dengue fever in tropical Australia appears to be on the rise.

The 1990s saw large outbreaks in northern Queensland and the Torres Strait with five major epidemics and many smaller epidemics between 1992 and 2004. The increasing incidence of dengue is attributed mainly to rapid population growth, urbanisation and the huge increase in global travel. As Dr Paul Reiter from the US Centers for Disease Control and Prevention remarked at a dengue symposium in Cairns, “people are vectors of the dengue virus, travelling the world, infecting mosquitoes”.

According to the World Health Organisation, this year’s outbreaks of dengue fever are the worst in a decade, affecting most of the tropical regions of the world from affluent Singapore to poor countries like Cambodia and Vietnam. Already, two to three times as many people have died from dengue this year than in the whole of 2006.