THE AUSSIE MOZZIE POSSE

A UNIQUE EXPERIMENT NOW UNDER WAY IN NORTH QUEENSLAND MAY SPELL THE END OF DENGUE FEVER AND OTHER MOSQUITO-BORNE DISEASES. ASHLEY HAY REPORTS FROM THE INSECT WARFARE FRONT LINE.

THEY FLUTTER AGAINST THE FINE MESH THAT traps them in a small white bucket; tiny shimmers of darkness with white stripes on their six spindly legs and a white lyre shape on their thorax. These delicate creatures are *Aedes aegypti*, the mosquitoes responsible for the spread of yellow fever, the Chikungunya virus and dengue fever, a disease that annually affects more than 100 million people worldwide.

Crouched in a welcome patch of shade, away from the Cairns summer sun, I peel back the silky white gauze, blow across the open mouth of the container and shake it, stepping back as the 40 captives make their way into the air.

Cairns is Australia's dengue fever capital; the 2009-2010 dengue season set a new record when 28 people imported the disease from overseas. By early January, this season's number had passed 50. Yet here we are, releasing more *Aedes* – more dengue carriers, or vectors – into the world.

But these are no ordinary mosquitoes. They're pioneers in a world-first population-replacement program, set free to interbreed with local *Aedes* and pass on their Trojan-horse bacterial infection. *Aedes aegypti* mosquitoes infected with the *Wolbachia* bacterium cannot transmit dengue. Which, given that dengue has no known vaccine and no specific treatment (and that mosquitoes can develop resistance to most insecticides used against them), makes this an unprecedented and potentially revolutionary scientific experiment.

A white van crawls along the kerb followed by a white car. Both are emblazoned with the words "Eliminate Dengue: Our Challenge" and a huge picture of the stripy-legged *Aedes*. It's 9.30am and the team moves from site to site, releasing a batch of mosquitoes at each – behind a fence, in a carport or on the edge of a footpath. The numbers work out at about 10 mosquitoes per household in the two trial sites near Cairns.

In the car sits the man who is leading the project, Professor Scott O'Neill, based at the University of Queensland and the progenitor of the idea that the tiny *Wolbachia* microbe – which measures only two-thousandths of a millimetre at most – might be a big enough weapon to take on the scourge of dengue. The 48-year-old combines affability with a certain immediacy and precision, and has an occasional American lilt to his voice, the result of a decade spent at Yale and the University of Illinois.

"I wake up at three in the morning and I think, 'We could crash and burn any day,'" O'Neill confessed two months before the release. "Science is like that. We could have a bad result, and that would kill the whole thing. We're realists about that. But while it's going well, we're enjoying the world."

Now, in January, we peel the gauze back from another bucket, blow across it to tempt the mosquitoes out with the carbon dioxide on our breath, and watch these tiny organisms launch themselves, perhaps into scientific history.

For a discipline stereotyped in terms of individual research, science often has a fabulously accumulative architecture. It's about ingredients, says one of this project's members, Professor Ary Hoffmann from the University of Melbourne. And it's about stories that intersect and fly off on tangents – sometimes towards dead ends, sometimes towards spectacular breakthroughs. It's about persistence, creativity and, quite often, serendipity that might be decades – even centuries – in the making.

This story really starts in the late 18th century with the expansion of global maritime traffic that probably brought *Aedes aegypti* to this part of the world. In the 1920s and '30s, the parasitic microbe *Wolbachia* was discovered and named. "At first, researchers wanted to see if it was a pathogen [infectious agent]," says O'Neill. "They injected it into mice and things, discovered it wasn't and so everyone lost interest in it."

In 1971, an American PhD student, Janice Yen, realised that a *Wolbachia*-infected male mosquito crossed with an uninfected female yielded no viable eggs. She submitted her PhD and disappeared from the world of science. Her research sat, unused, waiting.

Then, in the mid-1980s, a young Australian researcher – Ary Hoffmann – arrived at the University of California in Davis to work with a scientist called Michael Turelli. "I was interested in whether fruit flies that bred on oranges were different from flies that bred on apples," says Hoffmann. "So I went to southern California and collected flies from oranges, and I went to northern California and collected flies from apples, and I did some crosses. And I observed, to my absolute horror, that the crosses were fine in one direction – with males from the north and females from the south you got plenty of offspring. But from the opposite cross, there was none at all. I thought, 'What's going on here? This is crazy.'"

Going back to "some very old literature", Hoffmann saw that the same thing had been observed in mosquitoes. "That's when I first thought, 'It's this weird thing called *Wolbachia*', which wasn't supposed to be anywhere but in mosquitoes."

Over the following years, he watched *Wolbachia* spread itself through the native Californian fruit flies, *Drosophila simulans*, moving about 100 kilometres a year. It was the first time anyone had watched an organism drive itself into the genetic fabric of another in a natural setting.

Scott O'Neill's trajectory in the story began as a young boy meeting an uncle who was an academic. The man had "this room of books, his study, and it had this beautiful smell", he remembers. "I was entranced." He chose to study science, and after completing his PhD in entomology at the University of Queensland (UQ), he moved to the United States, where he tried to find a way to modify *Wolbachia* to carry new genetic material into mosquito populations that would prevent them spreading disease.

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GREAT AUSTRALIAN BITE: (from top) an *Aedes aegypti* mosquito, responsible for the spread of dengue fever and other diseases; Professor Scott O'Neill; O'Neill and Petrina Johnson inspect *Wolbachia*-infected *Aedes* mosquitoes at James Cook University in Cairns; releasing the mosquitoes in Gordonvale, south of Cairns.

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Then, in 1997, researchers from the California Institute of Technology discovered that one strain of *Wolbachia* shortened the life span of the organisms it infected. "Immediately," says O'Neill, "the penny dropped."

After someone contracts dengue, they will pass the virus to any *Aedes aegypti* that bites them three to four days later. After another eight to 10 days, that mosquito will relay the dengue virus on to anyone it bites. The dengue life cycle, therefore, takes about a fortnight, so if an *Aedes aegypti* mosquito contracting dengue couldn't live the next eight or 10 days to bite someone else, it would break the cycle of transmission.

"If we could take this bacteria that shortens life span and put it into a mosquito, we wouldn't have to do anything else," O'Neill explains. He won funding to investigate this approach, but couldn't make it work.

Then the last ingredient came into play. In January 2003, the Bill & Melinda Gates Foundation donated $US200 million to a series of "Grand Challenges in Global Health". Two-and-a-half years later, Scott O'Neill, back in Brisbane at UQ, won funding under the Gateses' initiative to "modify mosquito population age structure to eliminate dengue transmission".

"We had a bit of a chest-tightening moment, thinking, 'They've given us all this money; now we have to make it work – this thing we haven't been able to do for six years,'" says O'Neill. "Still, for the first time, we could think about what we really needed to try to do. For the first time in my career, I had enough money to actually give it a go."

He had close to $US7 million, five years and a team spanning five countries. *Wolbachia* occurs naturally in a wide range of insects – perhaps as many as 70 per cent – from pantry moths and fruit flies to the beautiful green-and-yellow Cairns birdwing butterfly. It also occurs naturally in other species of *Aedes* mosquitoes. How hard could it be to get it into *aegypti*?

When Conor McMeniman joined the O'Neill lab at UQ as a PhD student in 2004, people had been trying – and failing – to get one promisingly life-shortening strain of *Wolbachia*, called wMelPop, into *Aedes aegypti* for more than five years.

Over more than 18 months, McMeniman injected about 10,000 mosquito embryos. For his mosquitoes to lay eggs, they needed a blood meal – they had to bite a human. "I used to feed them on myself," he says, matter-of-factly. "Five days before we needed eggs, I stuck my arm in the cage." The mosquitoes fed, he'd put them in test tubes in the dark "for about an hour, and they'd dump their eggs". The eggs needed to be injected within 90 minutes.

In his first six experiments, the *Wolbachia* survived injection into the egg, but somewhere between the first and the third generation of the mosquitoes he bred from those eggs, the infection would be lost. "This was a fairly frustrating but tantalising sort of thing," he says. "After a while, it became a bit obsessive." Having lost nearly all six colonies, he thought, "'Well, I'll give it one last shot' – and that just happened to be the one that took hold." It might have been a refinement in his technique, a slight change in the *Wolbachia* or, as he suggests, "just luck". The results were published in 2009 in the academic journal *Science*. McMeniman was awarded his doctorate and a fellowship at Rockefeller University in New York.

In Cairns, at James Cook University, two 20-metre-long purpose-built cages were decked out to re-create the dark, dank *Aedes* habitat found under any elevated Queenslander house to allow the mosquitoes to be tested in an environment that was slightly more realistic than a lab cage.

It had always been assumed that, once released, the infected mosquitoes would perpetuate themselves in the field. There was just one problem: in Melbourne, Ary Hoffmann's team discovered that while these short-lived wMelPop mosquitoes "could spread pretty well in the wet season, in the dry season, they came to a grinding halt". For the infection to be perpetuated, releases would have to be on-going. "It was one of those moments," says Hoffmann, "where we thought, 'Gosh, this project is about to hit some serious trouble…'"

And then, another turning point. When the team began injecting *Wolbachia* into mosquitoes that were already carrying dengue, they found they didn't transmit dengue at all. "That was my serendipity moment," says O'Neill. "If we put *Wolbachia* into *aegypti*, they couldn't transmit pathogens to humans."

The life-shortening effect was irrelevant; *Wolbachia* effectively immunised the mosquitoes.

And there was more: "When you look at all the different mosquitoes, it's only the ones that don't have very strong *Wolbachia* infections that transmit disease," says O'Neill. "The nuisance mosquitoes that just bite you, many of them have *Wolbachia*. Perhaps *Wolbachia* had been determining which mosquitoes were vectors and which weren't. Perhaps putting *Wolbachia* into a mosquito that is a vector was quite a natural thing, and we were just helping it along."

Fortuitously, there was indeed a type of *Wolbachia* that blocked dengue without shortening the mosquitoes' lives and affecting their ability to breed through the dry. The strain, called wMel, had been discovered by Ary Hoffmann in 1988, when he tried crossing fruit flies from Townsville with fruit flies from Melbourne. "It's an Aussie," Hoffmann laughs, "and it came out of your fruit bowl. That's where we sourced the flies." Thomas Walker, a post-doctoral researcher in Scott O'Neill's lab, established it in *Aedes aegypti* mosquitoes "in about a month", he reckons, "from four or five days' injections".

And so McMeniman's thousands of injections weren't ultimately part of the solution. He's pragmatic, albeit slightly wistful: "It's bitter-sweet, I guess. I spent four or five years of my life working towards the project's aim: these experiments are fairly labour-intensive, they're very technically demanding, and they're not very good for your social life. But we're a team in the end – and what both Tom and I did was really, really cool."

DRIVING OUT FROM THE ELIMINATE DENGUE shopfront in central Cairns towards his office at James Cook University, Professor Scott Ritchie is talking about those two huge field cages, 20 metres long, purpose-built for the *Wolbachia* project. "If our old cages were three-star accommodation for mozzies," he says, "then these are the Taj Mahal."

An ebullient American and a self-described "quarterback" against mosquito-borne disease in this part of the world, Ritchie arrived in Australia in 1994 and took "a dengue-related job" in Cairns with Queensland Health. "It's real *aegypti* country," he says. Far North Queensland was fast becoming Australia's dengue hot spot, and by 1998, the region had a Dengue Action Response Team (DART) mandated to enter properties and treat *Aedes* breeding sites (anywhere that holds water, such as a pot-plant holder or the cupping leaves of a bromeliad; anywhere that's dark, such as under a bed) with insecticide.

While this approach has successfully stemmed more than 30 dengue outbreaks, "we're ticking the milestones to dengue becoming endemic in north Queensland unless an alternative strategy comes on board", says Brian Montgomery, Eliminate Dengue's field trial manager. It's just one reason, says Ritchie, that governments would find a control strategy like the *Wolbachia* project "very sexy".

It's quiet inside the cages, quiet and surprisingly still. Petrina Johnson, who oversees the mass production of the mosquitoes, shows me the cage where they fly free, feeding on volunteers to lay eggs for the releases.

"*Aegypti* have a really close association with humans," Johnson explains, "so for some earlier experiments, rather than sit here all the time, we made it smell human – we collected used gym towels and left them here. It smelt awesome, all dank and stinky…" In the second cage, mosquitoes are prepared for release, and every week Johnson delivers 12,000 mosquitoes, each four to six days old, to Brian Montgomery's release team (6000 for the northern Cairns suburb of Yorkeys Knob, 6000 for Gordonvale, south of Cairns). "We make another 6000 or so as a back-up. We're just a mosquito factory," she says, laughing.

For most people in Australia, dengue is an exotic disease with no bearing on day-to-day life. But for the residents of Far North Queensland, it's an immediate, alarming threat. "Many have had dengue before and are very fearful of having it again," says O'Neill. "Whatever they think of DART forcibly entering houses to spray insecticides under beds, in closets, major dengue outbreaks are still occurring. So people are very keen for something new to be done." More than half the residents in the two trial sites are happy for the team to release mosquitoes on their properties.

At a community engagement breakfast, representatives from those suburbs, and from Cairns's tourism industry – always heavily affected by reports of dengue – hear the latest about the trial.

"The story is simple," says the tourism industry rep. "Eliminate dengue and there's an economic benefit – that's what you're doing; that's it."

Along the table, Scott O'Neill demurs, "I'm looking for wood to touch. I don't want to over-promise and under-deliver. We're still a long way upstream."

Out in the field, the team distributes small black buckets lined with red felt in which they hope mosquitoes will lay eggs that show that the *Wolbachia* infection is beginning to spread. At the house of the project's first release, they weigh up locations for these traps – under a table, next to a door – while the owner jokes about it being *Wolbachia*'s ground zero. "There'll be a plaque on the fence," someone suggests. "There'll be tours."

"If it works," laughs the homeowner. "If it works."

Four weeks later, the eggs collected from those traps are analysed. There's *Wolbachia* in 20 per cent of them. "That's what our models had predicted it would be," says O'Neill, "so it's on the money." It's also the point from which the models – designed by Ary Hoffmann and Michael Turelli, working together again more than 20 years after they watched *Wolbachia* spread across California – suggest the bacteria will drive itself through the population.

"If it continues that way, we think the release should be successful," says O'Neill. "We're getting quietly confident." In March, the time of the last releases, the level had reached about 70 per cent.

AND NOW, WHAT NEXT? THE TRIAL IN CAIRNS IS funded for two years; a successful release this year may see another rolled out in less isolated areas in 2012. Regulatory approval is being sought for field releases in Thailand and Vietnam. And Scott O'Neill has just moved to Victoria's Monash University to become their dean of science, taking his lab, and this project, with him.

Plus there's the small matter of malaria. "The Gates people are really interested in malaria, more than they are in dengue," O'Neill confesses. "So they're saying, 'Fantastic, Scott, but can you do it for malaria?'" It's the million-dollar question – and the problem, again, is getting the infection into the mosquito. "*Aedes* mosquitoes have lots of close relatives with naturally occurring *Wolbachia*. But with *Anopheles* mosquitoes, which transmit malaria, no matter where you look in that genus, *Wolbachia* isn't there."

At UQ, Thomas Walker estimated he'd injected 20,000 *Anopheles* embryos "without success. It will require something novel, a novel technique or a breakthrough," he says. "It's gone past the point of hard work and perseverance." Around the world, several rival teams are on that hunt.

And up in Cairns, those dengue mosquitoes keep up their hard work of feeding and breeding in their comfortable cages, collaborating with the development of this subtle weapon to be used against their own kind. GW

MOZZIE BATTLERS: (from top) using a van to transport *Wolbachia*-infected mosquitoes in Gordonvale; the Bill & Melinda Gates Foundation funds research to eradicate dengue, as well as malaria; the mosquito breeding facility at James Cook University resembles the subfloor space of a typical Queenslander.

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