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The anti-Methuselah bug: A plague on plagues; A cunning new strategy promises to eliminate killers like dengue and malaria, but it's not without risk

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I am standing underneath a house. Like many traditional raised houses here in Queensland, Australia, the space under this one is an extension of the living area, with old furniture, laundry airing in the corner, stubby holders on the coffee table - and mosquitoes. Hundreds of them.

I swat at my bare ankles and arms. At this house, though, guests are not usually allowed to kill mozzies. Quite the opposite, in fact. Here, most visitors come to feed the mosquitoes - fresh blood, 10 minutes a day, every day.

For these are no ordinary mosquitoes, and this is no ordinary house. Instead, it's a mock-up of the underbelly of an old "Queenslander", complete with its own backyard mimicking typical conditions under the shade of a mango tree, all enclosed by a mosquito-proof, stainless-steel mesh cage. Welcome to the state-of-the-art Tropical Medicine Mosquito Research Facility on the Cairns campus of James Cook University.

The mosquitoes and the mock house are part of an ingenious strategy for combating dengue fever, a mosquito-borne disease that has reached pandemic proportions in a matter of decades. And if the approach works against dengue fever it should also work against other insect-borne diseases, including the biggest killer, malaria. That possibility has won the team developing this strategy funding through the Bill and Melinda Gates Foundation.

The potential benefits are huge, but before it can go ahead the team will have to convince people the strategy is safe. If things go wrong, it might make dengue spread even faster.

Still, the situation is already pretty bad. In south-east Asia, long plagued by dengue, outbreaks are becoming bigger, more frequent and longer lasting. In Australia, the Pacific and most of the Americas, where dengue was once rare, the disease is taking hold with a vengeance. An outbreak earlier this year that affected at least 60,000 people in Brazil and Argentina reached Buenos Aires for the first time.

Break bone fever

The symptoms range from mild aches and fatigue to severe joint and muscle pain - hence the name "break bone fever" -

but standard dengue fever is rarely fatal. The severest form of the disease, dengue haemorrhagic fever, on the other hand, can kill 1 in 20, with children most likely to die. Dengue haemorrhagic fever usually occurs when more than one of the four different viral strains that cause dengue are present in an area, and has spread from nine countries in 1970 to 60 today. There is no vaccine and no cure - doctors can only treat the symptoms.

Here in Cairns, the city is battling its worst ever outbreak of dengue fever. "We're sitting on 900 cases, 910, 915. The exact number doesn't matter because you can be sure that there will be more tomorrow," Scott Ritchie, head of dengue control at Queensland Health in Cairns, told me earlier this month. The big fear is that dengue will become endemic, he says, which could devastate the state's tourist trade.

Dengue's recent rise is usually blamed on increasing international travel, urbanisation and population growth, along with a failure to invest in long-term prevention. But none of these would have made a difference without what Ritchie calls the cockroach of mosquitoes, *Aedes aegypti* (left).

Female *A. aegypti* feed almost exclusively on human blood, and unlike most other mosquito species, it thrives in human habitats. Their larvae grow readily in water-filled plant pot plates and discarded plastic containers. Some even claim that it is more adept at avoiding well-aimed swats.

When an *A. aegypti* mosquito bites someone who has dengue, the virus begins replicating in the mosquito's gut. From there it spreads to the salivary gland and, 10 to 14 days after being infected, the mosquito's saliva contains enough virus to infect anyone it bites.

The conventional strategy for tackling dengue is to kill the mosquitoes. In this small city alone, for instance, Ritchie has a team of 40 people working non-stop to wipe out mosquitoes with traps and sprays, as well as getting rid of any pools of water in which their larvae could grow.

There is another approach, however: modify the mosquitoes so they cannot pass on the disease. This could be done in a number of ways (see "Modifying mozzies"), but the simplest is to make them die young, before infected mosquitoes become infectious. "The incubation period is long relative to the insect's lifespan, so if we can get them to die early, we can eliminate their ability to transmit diseases without having to eradicate them," says Scott O'Neill of the University of Queensland in Brisbane, who heads the international team developing the scheme.

But how do you make insects die young? A bizarre group of bacteria, called *Wolbachia* , that live inside the cells of insects could help. Unlike most infectious bacteria, *Wolbachia* don't spread through contact. Instead, they are passed on through their host's eggs. As a result, *Wolbachia* have evolved devious ways of maximising the number of infected eggs laid. Some strains make females reproduce without mating. Others turn males into fully functional, egg-laying females. Yet others alter the sperm of infected males so that when they fertilise uninfected female eggs, the eggs die - so-called cytoplasmic incompatibility. As infected females continue to mate happily with both uninfected and infected males to produce infected offspring, the infection spreads through populations quickly despite any ill effects it may have.

So if you infect wild mosquitoes with *Wolbachia* the bacteria should, in theory, spread rapidly through the entire mosquito population. Do it with a strain of *Wolbachia* that shortens mosquitoes' lifespans and it should stop dengue in its tracks. "It's a novel, potentially very effective strategy. In theory you could eliminate disease transmission 100 per cent," says Jason Rasgon, a molecular entomologist at Johns Hopkins University in Baltimore, Maryland.

There is a strain of *Wolbachia* that shortens lifespan, called the "popcorn" strain because of the appearance of an infected insect's messed-up brain. The trouble is, it normally infects captive fruit flies rather than wild mosquitoes. So O'Neill and his team set about trying to create a strain that infects *A. aegypti* .

For three years, they injected popcorn *Wolbachia* into thousands of mosquito embryos and selected the bacteria that survived. As the team reported earlier this year, the painstaking work paid off. Female mosquitoes infected with the new

strain survive for just half of their usual 50 to 60 day laboratory lifespan (*Science* , vol 323, p 141).

Lab tests have not revealed any problems that would prevent the new *Wolbachia* strain from spreading. The new strain still triggers cytoplasmic incompatibility. Now the team is checking that popcorn mosquitoes can hold their own against normal mosquitoes, which is where the Queenslander mock-up comes in.

In a series of experiments, the team collected *A. aegypti* from the wild, infected half of them with popcorn *Wolbachia* and released 4000 at a time into the mesh cage. Then the numbers of infected and uninfected mosquitoes were monitored over the following weeks. The results are still being analysed, but the raw numbers look good, says O'Neill. If that is confirmed, the team will move on to the next step - monitoring mosquitoes over several generations to see if the *Wolbachia* strain spreads as expected.

It's already clear that releasing it isn't going to be as easy as opening the cage door. Because of the popcorn strain's effect on lifespan, its spread is not guaranteed. Computer models and lab studies predict that to establish *Wolbachia* in the wild, infected insects have to make up at least 36 per cent of a population, which would mean releasing vast numbers of mosquitoes. "We'd probably dribble them out rather than release clouds of mosquitoes, and we'd treat with insecticides before to bring down numbers so you don't see a surge in the mosquito population," says O'Neill.

Before the team gets to this point, however, they will have to convince the authorities that the approach is safe. For starters, could the mosquitoes pass *Wolbachia* on to humans? Given that *Wolbachia* is common in arthropods - it may lurk in a fifth or more of insects - yet has never been found in any vertebrates, this seems extremely unlikely.

Nevertheless, the team has run additional experiments to rule this out. They have shown that the bacteria are not found in mosquito saliva, so people will not even be exposed to them. They are also setting up experiments to see if spiders, lizards and other species that feed on mosquitoes can become infected.

A more plausible worry is that wild mosquitoes might evolve resistance to *Wolbachia* and start living longer again. A recent modelling study suggests this is unlikely, however, because when mosquitoes are killed later in life after they have laid the majority of their eggs, selection pressure is greatly reduced. And even if the strategy does only work for two or three decades, points out O'Neill, that buys you precious time to develop other preventive measures.

There is a far more worrying scenario: shorter-living mosquitoes could result in the evolution of a faster-replicating, faster-transmitting "hot" dengue strain. "If it fails, you want things to be the same as they were before, but this has the potential to make things worse because there is an exponential relationship between the time it takes [infectious agents] to develop in the mosquito, and the dynamics of disease transmission," says Rasgon. In other words, a faster-replicating dengue virus could lead to explosive outbreaks.

O'Neill suspects the pressure to replicate rapidly is so intense that the dengue virus already replicates as fast as possible. Others suggest the current rate might instead reflect a balance between replicating quickly to spread faster, and not replicating so quickly that the mosquito host is harmed. If so, shortening the lifespan of the host could alter this balance.

The ideal scenario, of course, is that the dengue virus is wiped out before it has a chance to evolve. Ongoing lab experiments and, eventually, field trials should confirm which is the most likely outcome.

If things do go wrong, the need for around a third of a mosquito population to be infected for *Wolbachia* to spread could be an advantage. It means that intensive spraying campaigns, perhaps combined with the release of uninfected mosquitoes, might be able to eliminate *Wolbachia* .

Even if all the scientific issues can be resolved, though, past efforts at biocontrols have often gone wrong. Will the public accept the idea? Rasgon thinks people will be more open to the idea of manipulating nature when the aim is to wipe out a disease that could kill their children. "We already use bacteria to control pests, so you can think about it as just another biopesticide," he says.

There could also be legal hurdles. Country A may give the go-ahead and country B may not, but *Wolbachia* will not respect national borders. O'Neill doesn't see this as a huge problem as he thinks *Wolbachia* will only spread slowly without help, if at all. "It's not like throwing a match in a bush," he says.

The researchers are not waiting to see if these issues can be resolved for dengue before eyeing a bigger prize - malaria. This is a much tougher challenge, though, because while *A. aegypti* is responsible for 99 per cent of dengue transmission, 20 or so mosquito species transmit malaria.

O'Neill is attempting to introduce *Wolbachia* into the south-west Pacific malaria carrier, *Anopheles farauti* , and both Steven Sinkins's team at the University of Oxford and Rasgon's group are working on the major African malaria carrier, *Anopheles gambiae* .

No *Anopheles* species is infected with *Wolbachia* , so some thought this impossible. However, Rasgon has just managed to get the popcorn strain to infect the body cells of *A. gambiae* , though not yet the germ cells. "People said maybe *Anopheles* can't have *Wolbachia* . This shows it can," says Rasgon.

Meanwhile, in as little as two years' time, O'Neill hopes to do a pilot release of *Wolbachia* -infected *A. aegypti* in northern Queensland. "The big prize is Vietnam and Thailand, but we want to show that we are happy to do it in our own backyard before we do it in theirs," he says. Already, two collaborating teams in Thailand and Vietnam are crossing local dengue mosquitoes with *Wolbachia* -infected ones from Cairns to check that *Wolbachia* behaves in the same way.

If all goes well, the mosquitoes lurking under Queensland's houses could soon harbour the *Wolbachia* strain created by O'Neill's team. That won't stop me swatting them, though.

Modifying mozzies

Rachel Nowak

An alternative way to stop mosquitoes transmitting diseases is to genetically modify them. There are plenty of ways to do this. For instance, a kind of RNA called a ribozyme can cut up viral DNA and trigger cell suicide to ensure the infection does not spread. So giving mosquitoes genes for ribozymes specific to the dengue virus should prevent them becoming infected.

The tricky part is getting the extra genes into wild populations of mosquitoes. Although disease resistance might be an advantage for mosquitoes, and so would be selected for naturally, to ensure resistance genes spread rapidly in populations, researchers are developing various kinds of "gene drives".

One example is the MEDEA system, which consists of genes for a toxin and an antidote. Eggs lacking the antidote are killed by the mother's toxin, so instead of some offspring inheriting MEDEA, all of them do. The idea is to package resistance genes together with MEDEA and add the set to mosquitoes. The catch is that the resistance genes could become inactive or separated from the gene drive.

Massacring mozzies

Rachel Nowak

Dead mosquitoes don't carry diseases, and several high-tech new ways of zapping mozzies are in the offing. One is to release vast numbers of male mosquitoes carrying DNA that kills any offspring they have. The killer DNA, called OX513, can be switched off by adding a specific chemical to food, so it is easy to raise huge numbers of insects in captivity prior to their release. This is a new twist on the successful but more expensive "sterile male" approach.

Also on the horizon are improved insecticides that are far more toxic to mosquitoes than humans. Novel repellents

designed to block or overstimulate mosquito odour receptors, preventing them from finding humans to bite, are also being developed.

Then there is the weapon of mosquito destruction, or WMD, which will detect the wingbeat of female mosquitoes and zap them with a laser. "They don't burst into flames and fall out of the sky, but it's enough to damage them so they stop biting," says Jordin Kare of Intellectual Ventures in Bellevue, Washington, which is working on a lab prototype.

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