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Vector-control research centres raise millions of male mosquitoes to infect with *Wolbachia* strains.

Wolbachia goes to work in the war on mosquitoes

The bacterium has helped combat dengue, but can it be used to purge other mosquito-borne diseases? **By Sandy Ong**

Because of the diseases they carry, mosquitoes are responsible for the loss of more human lives each year than any other species, including humans. Their bites transmit a range of parasites and viruses that are responsible for up to 17% of all infectious diseases, including malaria, dengue and chikungunya, according to the World Health Organization.

Humans have wielded numerous weapons and strategies against their bites: bed nets, insecticides, oil over standing water and, in the past decade, a bacterium called *Wolbachia*.

Wolbachia is present in more than 60% of all insects, including dragonflies, butterflies and moths. But the *Aedes aegypti* mosquito – the vector responsible for majority of the 96 million annual dengue cases leading to 40,000 deaths – is an anomaly, with no naturally occurring *Wolbachia*.

When the bacterium is introduced into *Ae. aegypti* eggs, the dengue virus is unable to replicate in the modified mosquitoes that hatch. The exact mechanisms for this are unclear, but some experts suggest that *Wolbachia* outcompetes the virus for resources such as lipids, or turbocharges the host's immune response. Regardless, the *Wolbachia*-modified mosquito is prevented from spreading dengue through future bites.

There are two approaches to tackling dengue with *Wolbachia*. The first involves releasing only modified male mosquitoes. Since 2015, this strategy has been successfully adopted in Singapore and Guangzhou, China, and in parts of the United States, such as Miami, Texas and California. Because eggs produced from unmodified females that mate with modified males do not hatch, the number of mosquitoes in the community is greatly reduced.

The second approach, used by some cities in Vietnam, Indonesia, Malaysia, Brazil and Australia, among others, involves releasing modified mosquitoes of both sexes. The infected females pass the bacteria to their offspring.

Over time (several months to years, depending on characteristics of the release site), the modified mosquitoes replace the native population.

"The use of *Wolbachia* as a tool for reducing the capacity of mosquitoes to transmit dengue is a proven technology," says Leo Braack, a vector-control specialist at the University of Pretoria in South Africa. "Its efficacy has been demonstrated in large-scale studies in multiple countries."

For instance, when modified mosquitoes were released in Singapore in 2018, dengue incidence was observed to have been reduced by 88% after a year. In Brazil, there was a 69% reduction in reported dengue cases over three years compared with a control area, and in Yogyakarta, Indonesia, a 77% reduction in cases was observed over 27 months following the deployment of infected mosquitoes from March to December 2017.

Testing wider applications

Success with dengue has prompted epidemiologists to ask whether *Wolbachia* can be used to control other mosquito-borne diseases. For Zika, chikungunya and yellow fever — the other major viruses spread by *Ae. aegypti* mosquitoes — the answer, in theory, is yes.

"There's a whole lot of laboratory work that's been done showing that mosquitoes become resistant [to these diseases]," says Scott O'Neill, who researches vector-borne diseases at Monash University in Melbourne, Australia, and who pioneered *Wolbachia* research for dengue control (L. A. Moreira *et al. Cell* **139**, 1268–1278; 2009). O'Neill is also the founder of the non-profit organization World Mosquito Program, which works with governments and communities on *Wolbachia*-release programmes, currently in 11 countries, from bases in Melbourne, Ho Chi Minh City, Vietnam, and Panama City in central America.

But demonstrating *Wolbachia*'s effectiveness against these other viruses in the field is tricky. Zika, yellow fever and chikungunya crop up much more sporadically than dengue, which is more predictable based on previous hotspots, temperature and climate conditions. "The challenge is timing a trial in a particular area to coincide with when there is an outbreak," says O'Neill. "That requires a bit of luck to have both of those things together."

Guessing where the next outbreak might be and setting up a trial in anticipation risks wasted effort if no outbreak happens, says O'Neill. But, waiting for an outbreak to occur and then starting the trial is often not feasible, "given that trials are very complex and challenging to set up", he says. For one thing, gaining community trust and acceptance of a mosquito-release programme takes time.

This might explain why no one has attempted to organize such field trials for diseases other than dengue so far. Some dengue studies, however, have been able to observe *Wolbachia* effectiveness against other arboviruses incidentally. For instance, when O'Neill and his collaborators released *Wolbachia*-infected mosquitoes to control dengue in the Brazilian city of Niterói from 2017 to 2019, they simultaneously observed a 56% and 37% reduction in chikungunya and Zika incidence, respectively (S. B. Pinto *et al. PLoS. Negl. Trop. Dis.* **15**, e0009556; 2021).

But for diseases spread by other mosquitoes — such as malaria, which is carried by mosquitoes in the *Anopheles* genus, and West Nile and Japanese encephalitis, which is carried mainly by *Culex* mosquitoes — *Wolbachia* use is less clear-cut. For a start, these diseases are transmitted by numerous species, unlike dengue, which has only one primary vector (*Ae. aegypti*). In Thailand, for example, there are between 10 and 16 *Anopheles* species that carry malaria, says Jetsumon Sattabongkot, director of Mahidol University's Virax Research Unit, Bangkok. "And that's not even in the whole country, it's just in some locations," she says.

Similarly, for Japanese encephalitis, "there can be three or four *Culex* vectors in the same location", says Sattabongkot.

Different mosquito systems have different physiological systems, so a *Wolbachia* strain that can be introduced to one species may not work for another. To complicate things further, both *Anopheles* and *Culex* mosquitoes contain naturally occurring *Wolbachia*. "So, you'd have to find a strain of *Wolbachia* that would effectively compete against the natural strains and that also reduces the transmission efficiency," says Scott Weaver, a virologist at the University of Texas at Galveston.

Chasing strains

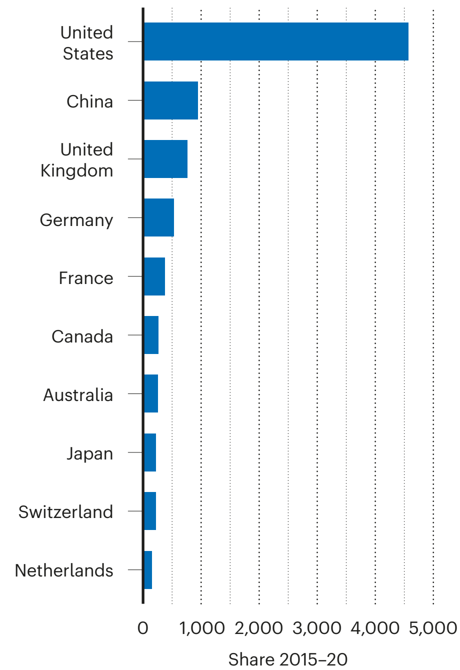
Finding a suitable *Wolbachia* strain–species match, or 'system', is no mean feat. Scientists have to select contenders from thousands of possibilities, then assess their impact on the mosquito host. A 'good' strain should be able to effectively block transmission of the pathogen in question, form a stable symbiosis with a minimum or low fitness cost to its host, and be passed to future mosquito generations, among other desirable traits, says Zhiyong Xi, a medical entomologist at Michigan State University in East Lansing, Michigan.

"We need to build multiple *Wolbachia* systems to target different species based on the need of a particular location," he says. "By comparison, if you build a system for the *Aedes* vector, you basically can cover nearly all the regions in the world for disease control."

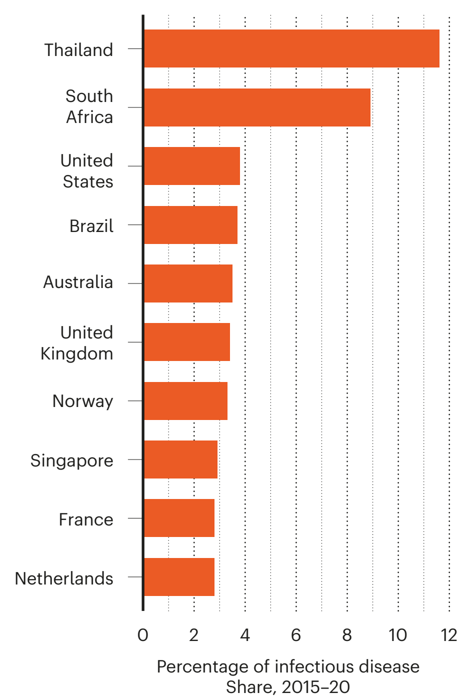
GLOBAL STANDOUTS

The United States dominates infectious-diseases research output in the Nature Index, with nearly five times the Share of China. Among the leading 25 countries for infectious-diseases output, Thailand and South Africa have the highest proportion of output related to the topic, however, their total Share is low compared with countries such as the United States and United Kingdom.

Leading 10 countries by infectious-disease output, 2015–20



Leading 10 countries by proportion of infectious-disease output, 2015–20



SOURCE: NATURE INDEX

Once scientists have selected a suitable *Wolbachia* strain and successfully injected it into mosquito eggs in the lab, they then need to mass-breed the insects. Mosquitoes from the *Culex* genus, like *Aedes*, are relatively easy to rear. The malaria-carrying *Anopheles* is another matter, however.

"They like to form mating swarms, so they need large cages," says Stephen Dobson, an entomologist at the University of Kentucky in Lexington. Dobson is the founder of MosquitoMate, a biotechnology start-up company that offers commercially available modified male *Aedes* mosquitoes for homeowners, hotels and other private players looking to combat their dengue problem. MosquitoMate has been approved by the US Food and Drug Administration for use in 20 states and Washington DC.

Anopheles mosquitoes also prefer damp conditions, requiring moist papers to lay their eggs on, says Indra Vythilingam, an entomologist at the University of Malaya in Kuala Lumpur. *Aedes* mosquitoes, by comparison, are content with dry substrates.

Setting up the breeding facilities isn't cheap or easy. They are typically staffed by 20 to 40 people; strict biosafety measures have to be in place to prevent any mosquitoes from escaping, as well as to remove dust and scales shed by adult mosquitoes, which can cause serious respiratory symptoms for people exposed to them. There are currently nine such facilities in southeast Asia, Latin America, China, the United States and Australia; the world's largest one, in Guangzhou, China, can produce more than 30 million *Wolbachia* mosquitoes a week.

"Building a mass-rearing facility is really a challenge for some areas," says Dobson. Additionally, securing grants can be difficult. "It's much simpler to get funding to do work on dengue because it's a much more important pathogen overall than something like West Nile," says Weaver, referring to dengue's high infection rates. Estimates suggest that four in every ten people worldwide live in dengue-prone areas.

But, given how increased travel and trade, as well as global warming, are causing these diseases to spread wider and faster, "*Wolbachia* should be welcomed as a very effective tool", says Braack.

Duane Gubler, an infectious-diseases researcher at the Duke-NUS Medical School, Singapore, agrees. "It's not going to be a panacea but it's going to provide a tremendous boon to public-health agencies that need to control these diseases," he says.

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SOLVIN ZANKL/NATUREPL.COM

The *Culex pipiens* mosquito transmits West Nile virus, among others.

A BREEDING GROUND FOR MALARIA CONTROL

***Wolbachia* research shows great promise for malaria control, but finding the right bacterial strain is crucial.**

In 2013, Zhiyong Xi's team at Michigan State University announced a world first: they had created a stable line of *Wolbachia*-infected *Anopheles stephensi* mosquitoes, paving the way for a new malaria-control method (G. Bian *et al. Science* **340**, 748–751; 2013).

A. stephensi is a major malaria vector in south Asia and, more recently, in Africa. In 2019, there were an estimated 229 million cases of malaria worldwide, and 410,000 related deaths, with Africa bearing the brunt of the disease. It was the fourth most-researched infectious disease or disease group by article Count in the Nature Index for 2015–20, after HIV/AIDS, coronavirus and influenza.

Although the technique for infecting *Aedes* mosquitoes with the *Wolbachia* bacterium was established by 2013, with field trials proving the system's effectiveness at reducing dengue transmission, research involving the malaria-transmitting *Anopheles* genus was less advanced.

Anopheles mosquitoes are notoriously difficult to rear, but *A. stephensi* is "one of the culturable species" under lab conditions, says Ary Hoffmann, an entomologist at the University of Melbourne in Australia.

According to Xi, his lab's achievement in successfully transinfecting the mosquitoes and breeding them in the lab "showed their potential to reduce malaria transmission".

It's a good start, but there is scope for improvement in the *Wolbachia*-infected mosquito line they developed. For example, it makes the mosquitoes too weak to breed optimally, says Steven Sinkins, an entomologist at the University of Glasgow, UK. "The big issue is that the fitness cost is too high to allow it to spread through *A. stephensi* populations."

This doesn't mean the bacterium cannot be used to control malaria, says Sinkins. Rather, "it could be about finding the right *Wolbachia* strain to use".

That is something that Xi and his team have continued to work on since publishing their 2013 paper in *Science*. In results yet to be written up, Xi says his team has identified another potential *Wolbachia* strain for use against malaria. In further preliminary work, he says they have also introduced the bacterium into two other *Anopheles* species that are major vectors of malaria in Africa (*A. arabiensis* and *A. gambiae*).

Progressing to field trials with the modified mosquitoes would be the next step, he says. **Sandy Ong**