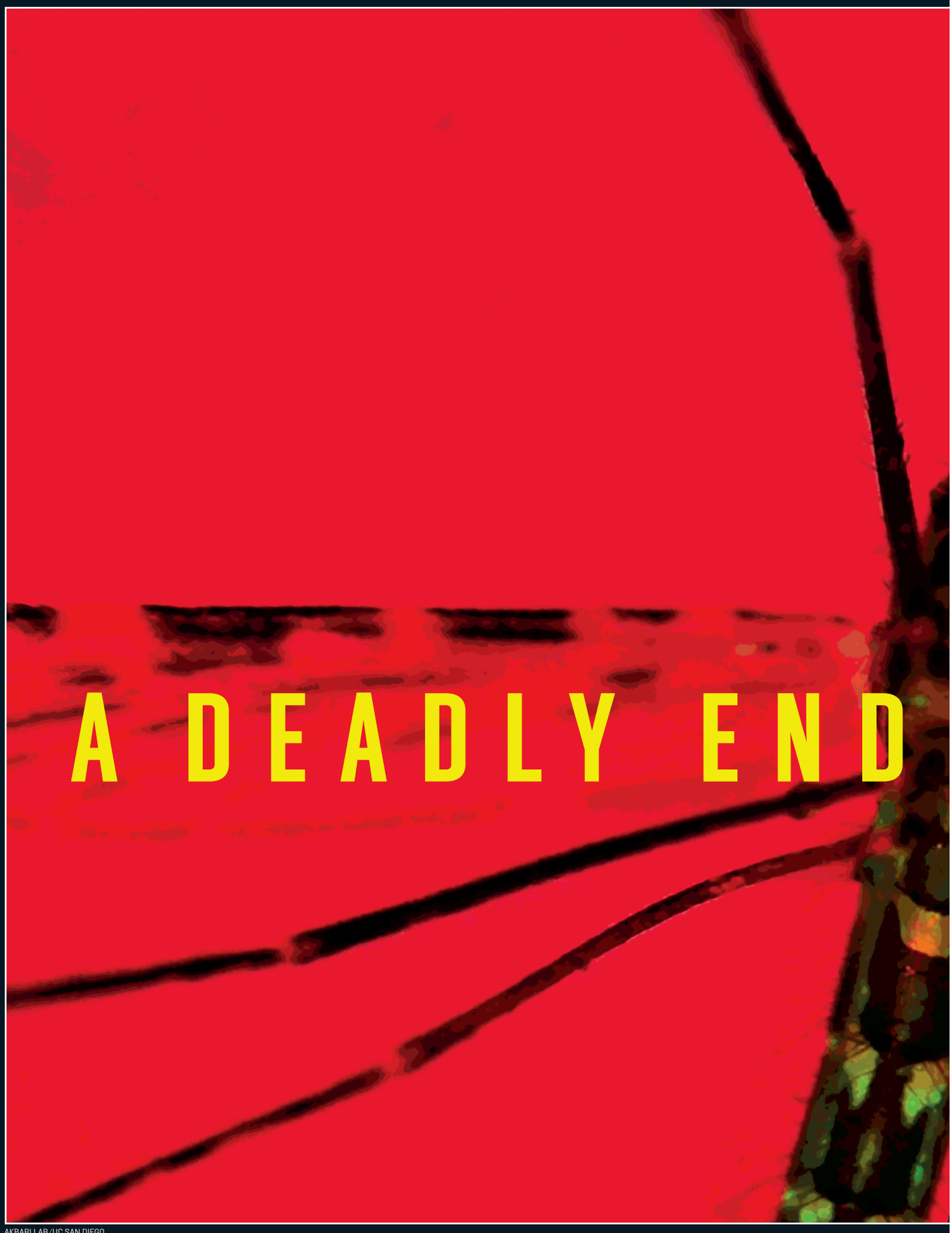
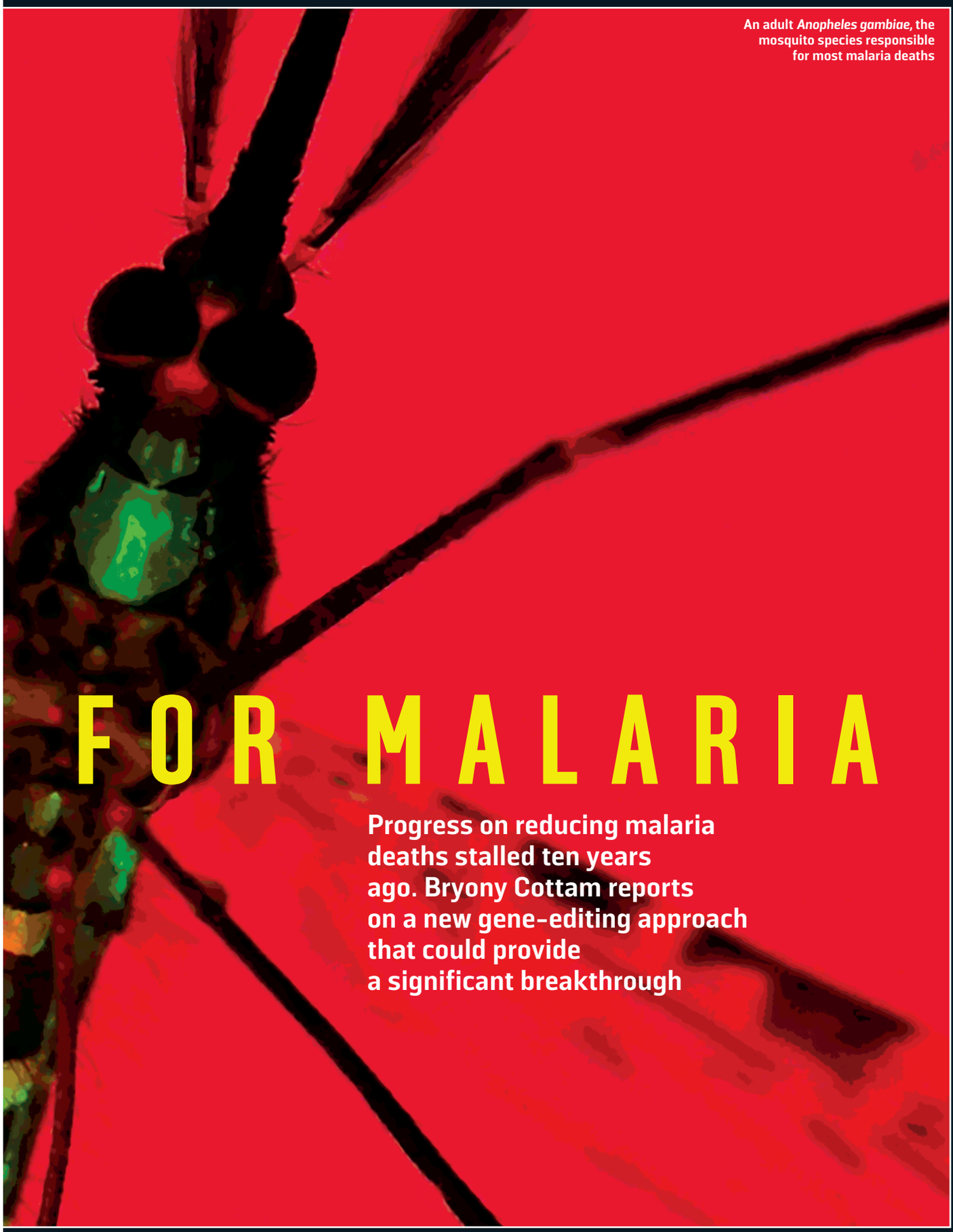
A DEADLY END



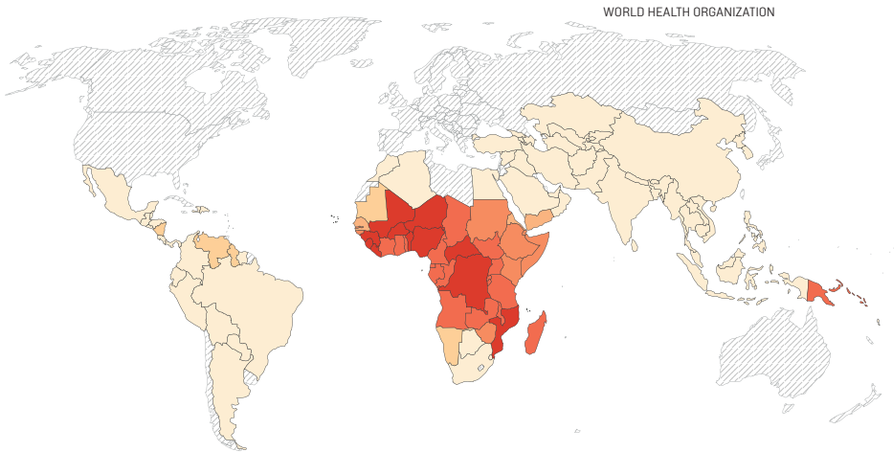
AKBARI LAB/UC SAN DIEGO



An adult *Anopheles gambiae*, the mosquito species responsible for most malaria deaths

FOR MALARIA

Progress on reducing malaria deaths stalled ten years ago. Bryony Cottam reports on a new gene-editing approach that could provide a significant breakthrough



The number of new cases of malaria in a year per 1,000 population at risk in 2020

Every mosquito laboratory has a different method for feeding its mosquitoes. At the Catteruccia Lab at Harvard University, where Andie Smidler spent several hours a day tending to the insects as a PhD student, mosquitoes are fed on human donor blood served through a membrane feeding system called a Hemotek. 'But here,' she says, 'we feed them on anaesthetised mice.'

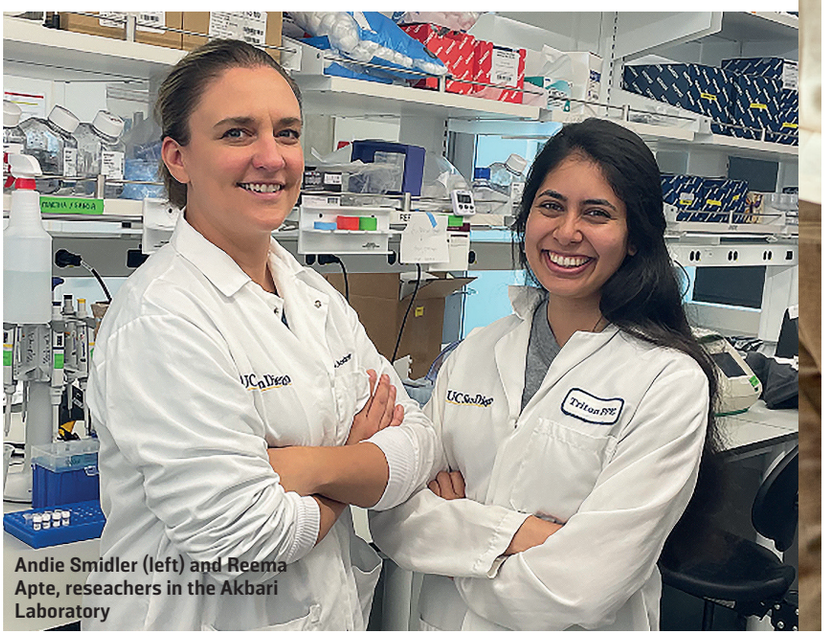
When Smidler moved to the Akbari Lab at the University of California, San Diego (UCSD), where she's now a postdoctoral scholar, she brought *Anopheles gambiae* – a species more commonly known as the African malaria mosquito – with her from Harvard. She's now responsible for what she estimates is around 20,000 *A. gambiae,* one of five species that she and her colleagues currently study, but at any one time there might be as many as 100,000 mosquitoes in the UCSD lab.

Omar Akbari, principal investigator at the Akbari Lab, explains that access to the chambers where the mosquitoes are housed is restricted by a card-reader door lock followed by an iris scanner. Beyond these first two doors, a curtain of air blocks the movement of any flying insects. 'It's an ultra-secure facility,' he says. 'You don't want the mosquitoes to get out.'

Of the more than 3,500 species of mosquito on Earth, only a handful are responsible for transmitting malaria. Of these, *A. gambiae*, which is endemic to sub-Saharan Africa, is the most efficient at spreading the most lethal of the malaria parasites, *Plasmodium falciparum*. The World Health Organization (WHO) estimates that in 2021, malaria caused 619,000 deaths globally, 96 per cent of which (593,000 deaths) occurred in Africa. *P. falciparum* was responsible for most of these.

During the first decade of this century, the world made unprecedented progress towards the global eradication of malaria. It's a period that Pedro Alonso, who retired from the role of WHO Global Malaria Programme director in March last year, refers to as the 'golden era' of malaria intervention. New drugs, the widespread roll-out of insecticide-treated bed nets, the use of residual indoor pesticides and a lot of newly allocated funding contributed to making a significant dent in the number of new cases. Between 2000 and 2013, the WHO reported a 30 per cent reduction in case incidence, and mortality rates decreased by 47 per cent worldwide. Then, progress stalled.

Jaishree Raman heads the Laboratory for Antimalarial Resistance Monitoring and Malaria Operational Research at the South African National Institute for Communicable Diseases in Johannesburg. She says there are many reasons why progress has plateaued: funding has flatlined, the warm, wet seasons preferred by mosquitoes are growing longer and their range has expanded. But one important factor is the rise in antimalarial and insecticide resistance. The WHO reports that between 2010 and 2020, 78 of the 88 malaria-endemic countries detected mosquito resistance to at least one insecticide. 'In some areas,' says Raman, 'mosquitoes have even changed their behaviour.'



Andie Smidler (left) and Reema Apte, reseachers in the Akbari Laboratory

AKBARI LAB/UC SAN DIEGO

What's more, Raman says that *P. falciparum* has developed a resistance to the main rapid diagnostic test used for malaria in sub-Saharan Africa. 'So you can test a whole lot of people and they come up as malaria negative, while they're actually malaria positive,' she says. 'The malaria parasite is finding many ways to evade being detected and being effectively treated. And it is becoming a little bit scary.'

In the opening statement of the WHO World Malaria Report 2022, director-general Tedros Adhanom Ghebreyesus writes that despite mounting challenges – including the arrival of Covid-19 – most countries have managed to hold the line against malaria. However, the effectiveness of our main tools against

A Target Malaria team member engages with community members in the village of Abutia Amegame, Ghana

TARGET MALARIA

A HEAVY BURDEN TO BEAR

● Almost half of all malaria cases occur in four African countries (Nigeria, the Democratic Republic of the Congo, Uganda and Mozambique), but Olukemi Amodu, director of the Institute of Health at the University of Ibadan, says that the burden of malaria is particularly high in Nigeria; the country accounts for more than a quarter of all malaria cases globally. '*Anopheles gambiae* is very common in Nigeria – the weather conditions here are favourable for this mosquito,' she says, explaining that while some countries experience seasonal malaria transmission, where most cases occur during the rainy season, transmission in Nigeria occurs throughout the year. 'That puts about 97 per cent of the population at risk.' To make matters worse, *A. stephensi*, an invasive malaria-transmitting species from Asia, recently spread into sub-Saharan Africa and is now in Nigeria.

malaria is in decline. 'I think there is a major concern that we will have a drug-resistant outbreak, which would have serious consequences for any elimination, let alone eradication efforts,' adds Raman. As such, global healthcare leaders are on the lookout for new approaches, one of which is currently sitting in a cage in a chamber of the Akbari Lab.

At the moment, Smidler is feeding the female *A. gambiae* in her care with live mice roughly once a week. 'We're gearing up to inject embryos,' she says, 'so I need a fresh cage of mosquitoes every week.' Only female mosquitoes drink blood – making them the only transmitters, or 'vectors', of malaria – which provides them with the protein they need to produce their eggs. These *A. gambiae* embryos will be injected with CRISPR, a gene-editing tool designed, in this case, to target two genes: one that's important for female development and one that determines male fertility. So far, tests have resulted in only sterile males being born, while all the females die.

The idea is that, since female mosquitoes typically only mate once, the mass release of the sterile male

A mother puts her child to sleep inside a malaria net in Ghana

ARNE HOEL/THE WORLD BANK

A NEW VACCINE

● In April 2023, Nigeria provisionally approved the newest malaria vaccine (R21), which has been developed for use in children aged five and under – the group at the highest risk of severe malaria. The latest trials have demonstrated a high vaccine efficacy of between 70–80 per cent following the fourth dose, but some researchers are sceptical about whether children will complete the full course.'Will the new vaccine have an immediate impact in Nigeria? I think first of all there needs to be a greater awareness among the public of the benefits,' says Olukemi Amodu, director of the Institute of Health at the University of Ibadan. 'We've seen vaccine hesitancy around the routine vaccines that we already have, due to religious beliefs or misinformation, so we're not going to be able to prevent malaria with vaccines unless we do more to educate and support local communities.'

mosquitoes should prevent wild females from producing future generations. Insect populations can and have already been successfully suppressed by the release of sterilised males that have been irradiated with gamma or x-rays, a technique that was originally trialled in the USA as a way to control agricultural pests such as fruit flies and screwworms. But that method of sterilisation has a detrimental impact on the fitness of male mosquitoes, which then struggle to compete for mates with the wild males. That's why sterilisation needs to be done genetically, says Smidler.

Akbari's team isn't the only one developing new genetic technologies to put a stop to malaria-spreading mosquitoes. 'There are a number of different groups working on developing genetic bio-control technologies for mosquitoes, and a lot of different approaches have been tried,' says Akbari. 'But the main difference between these approaches and ours is that our technology doesn't rely on gene drives.'

In genetic engineering, gene drives are used to increase the probability that any offspring will inherit a particular genetic element. 'It's a technology that can spread easily into a population,' says Akbari, 'which can be a problem, because once you deploy it, it's going to be very difficult to stop it from spreading.' This raises a number of concerns about what might happen if there are any unintended consequences of the technology, including any wider ecological impacts it may have.

Smidler says that the Akbari Lab was built at a time when fears surrounding gene drives were at an all-time high – which explains, she adds, the slightly exaggerated safety precautions designed to prevent any mosquitoes escaping. That said, while an escaped *A. gambiae* would be unable to survive outdoors somewhere like the UK, it would be a very different situation in a place with a suitable climate – such as San Diego. 'But the whole point of our technology is that it's a dead end, right?' Smidler says. 'If a male mates with a female in the wild it doesn't matter, she'd be infertile.'

> 'We've essentially created a very evolutionarily stable system that can be used to suppress *Anopheles gambia* populations safely'

'Part of the motivation behind this research was to try to develop a technology that mitigates the limitations of gene drives,' says Akbari. 'We've essentially created a very evolutionarily stable system that can be used to suppress *A. gambiae* populations safely. That's not something that has previously been developed.'

Questions still remain about whether it's a good idea to entirely eliminate a mosquito species from an area, but Smidler believes that there's little evidence to suggest we shouldn't. 'Technically, that experiment has been done before,' she says. 'We had malaria in the USA before the 1940s and the way that we controlled it was to oil-slick the swamps and spray DDT everywhere. We eradicated the mosquito, as well as a lot of other insects.' By the time mosquitoes returned to

Only female mosquitoes suck blood and spread diseases such as malaria

DIGITAL IMAGES STUDIO/SHUTTERSTOCK

EVASIVE BEHAVIOUR

● The first recorded evidence that mosquitoes were adapting their behaviour to the use of insecticides came from the Solomon Islands, where a method called 'indoor residual spraying' had been used to deter mosquitoes from homes and buildings since the 1960s. Like many *Anopheles* mosquitoes, *A. farauti* – commonly found in the coastal areas of Papua New Guinea and the surrounding islands, including the Solomons – likes to feed indoors and at night. By the '90s, however, this preference had shifted to feeding outdoors in the early evening, before people turned indoors for the night.

the USA some 15 years later, the malaria parasite was long gone. 'Obviously, that was ecologically brutal and wouldn't pass muster today,' she adds. 'But the point is, if there were any ecological impacts at the time, then we don't recognise them today. There's nothing that serves as a warning lesson.'

Fred Aboagye-Antwi, principal investigator at Target Malaria Ghana, is inclined to agree. 'I will not conclusively say that there would be no impact because we are still investigating,' he says. 'But from what we have seen so far, a significant reduction in the number of *A. gambiae* is not going to impact the ecosystem.'

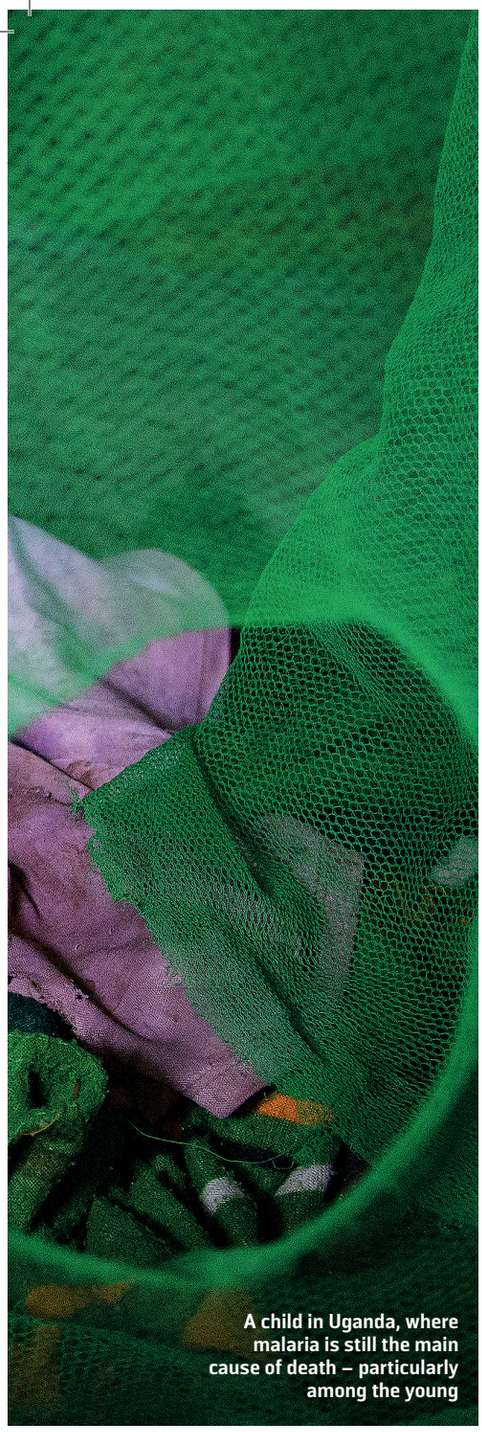
Aboagye-Antwi and his team at the University of Ghana are currently conducting a four-year ecological study to help predict the impact of locally suppressing

the mosquito species. 'If you do a species composition analysis, there might be a few *Anopheles*, but the majority of mosquitoes that we find are other species,' he says. 'So even if you take the *Anopheles* out of the picture, it's unlikely to affect the whole system significantly.' However, there's a chance that with *A. gambiae* gone, another vector could take its place. 'In ecology, species are always competing for a particular niche. This is something that needs more consideration and research before any genetic approach can be rolled out for malaria control.'

According to Aboagye-Antwi, one of the main challenges to eliminating malaria from Ghana is a lack of compliance. 'Talk to anyone, and they will tell you they know the importance of bednets, but I'd say it's only a very small percentage that use them. Most people have them at home, but they use them for growing vegetables or protecting their chickens.' Similarly, he says, the general Ghanaian public isn't well informed about the most effective uses of insecticide sprays, which is further contributing to insecticide resistance. 'If a genetic approach – which doesn't require much human involvement – is successful, then it could have a very, very big impact when it comes to malaria control within Africa.'

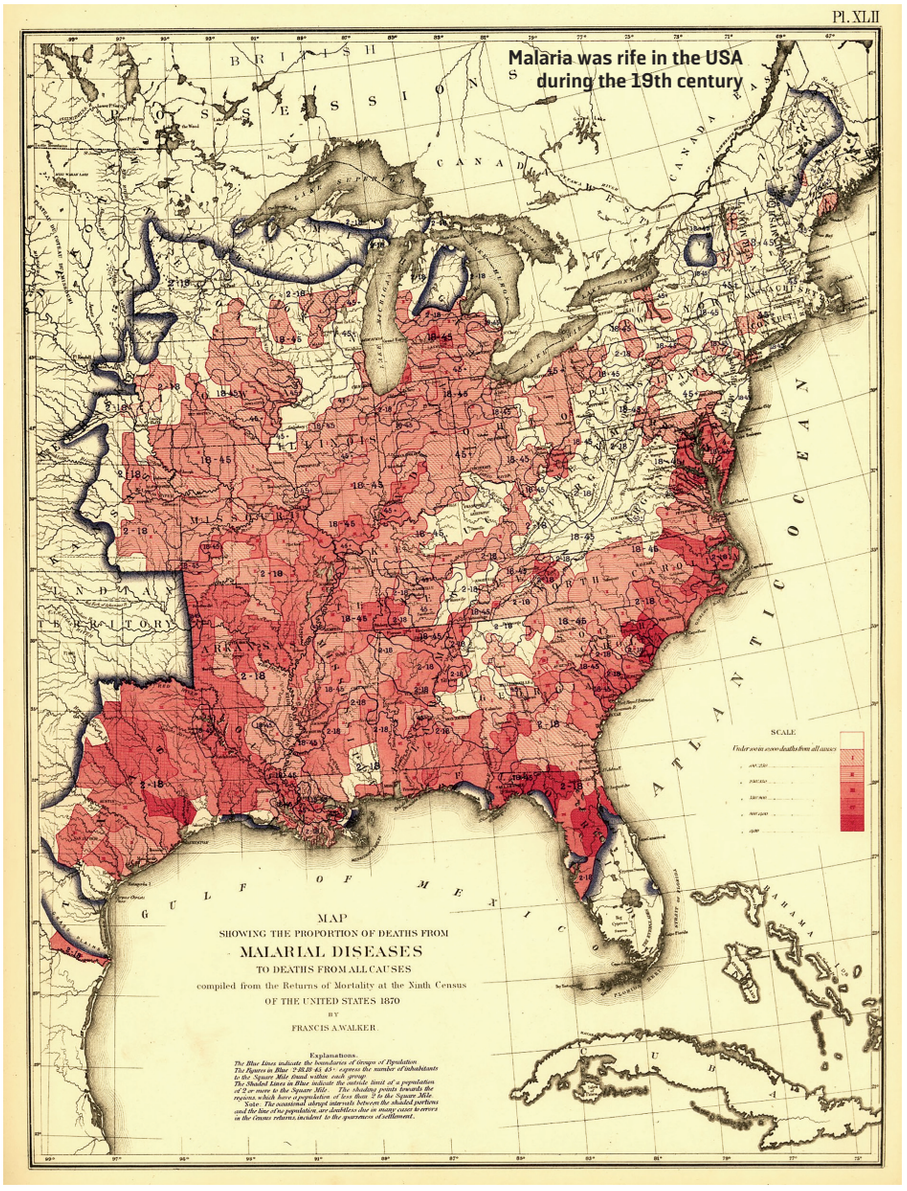
> If there's one thing to take away from our previous attempts to eradicate malaria, it's to not become complacent when presented with a promising new tool

If there's one thing to take away from our previous attempts to eradicate malaria, it's to not become complacent when presented with a new and promising tool. In the 1950s and '60s, many believed that the combination of chloroquine – the 'miracle drug' – and DDT would wipe out malaria parasites once and for all. More funding, resources and greater public education, as well as new technologies, are all desperately needed



RICHARD JUILLIART/SHUTTERSTOCK

A child in Uganda, where malaria is still the main cause of death – particularly among the young



1870 CENSUS: STATISTICAL ATLAS OF THE UNITED STATES

to ensure success. Nonetheless, says Aboagye-Antwi, the future is bright. 'Yes, the challenges are numerous, but elimination is possible.'

Smidler agrees that malaria eradication is fundamentally a team effort, but when asked whether her genetically modified mosquitoes could come the closest we've seen so far to a silver bullet, she admits she thinks they stand a pretty good chance.

'I don't want to call it a silver bullet,' she says. 'That's a little too cocky, especially for something that's going to be as labour intensive as this, that's going to need iterative releases of sterilised males over five, ten years or more.'

In a way, she adds, a gene drive fits that definition more closely, in the sense that the knock-on effects of releasing just ten mosquitoes could lead to malaria eradication. 'Maybe a better analogy for our technology would be a "silver buckshot", because it's not going to be a one-and-done. Yeah,' she says with a smile, 'maybe that's what it should be called. A silver buckshot.' ●

A SHORT HISTORY OF MALARIA

● As little as 100 years ago, malaria existed on every continent but Antarctica. In England, the last recorded outbreak of locally transmitted malaria occurred between 1917 and 1921, but in the centuries prior, it had plagued communities living near coastal marshland in areas such as the Somerset Levels, Essex, Sussex and Kent. Throughout the first half of the 20th century, the disease was successfully driven from much of Europe and North America and, in 1955, the newly formed WHO launched the first campaign for the worldwide eradication of malaria. A total of 35 countries were declared malaria-free. Then, realising it was too great a challenge to achieve within the timeframe that had been set, the WHO withdrew funding from the campaign. In many countries, malaria subsequently returned with a vengeance.