HIJACKING EVOLUTION

Gene-drive technology could alter the genome of an entire species. Researchers need to answer these key questions before deploying it in the wild.

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Austin Burt and Andrea Crisanti had been trying for eight years to hijack the mosquito genome. They wanted to bypass natural selection and plug in a gene that would mushroom through the population faster than a mutation handed down by the usual process of inheritance. In the back of their minds was a way to prevent malaria by spreading a gene to knock out mosquito populations so that they cannot transmit the disease.

Crisanti remembers failing over and over. But finally, in 2011, the two geneticists at Imperial College London got back the DNA results they'd been hoping for: a gene they had inserted into the mosquito genome had radiated through the population, reaching more than 85% of the insects' descendants[1].

It was the first engineered 'gene drive': a genetic modification designed to spread through a population at higher-than-normal rates of inheritance. Gene drives have rapidly become a routine technology in some laboratories; scientists can now whip up a drive in months. The technique relies on the gene-editing tool CRISPR and some bits of RNA to alter or silence a specific gene, or insert a new one. In the next generation, the whole drive copies itself onto its partner chromosome so that the genome no longer has the natural version of the chosen gene, and instead has two copies of the gene drive. In this way, the change is passed on to up to 100% of offspring, rather than around 50% (see 'How gene drives work').

Since 2014, scientists have engineered CRISPR-based gene-drive systems in mosquitoes, fruit flies and fungi, and are currently developing them in mice. But that's just the beginning of the story. Questions about whether a gene drive is possible have been supplanted by other unknowns: how well they will work, how to test them and who should regulate the technology. Gene drives have been proposed as a way to reduce or eliminate insect-borne diseases, control invasive species and even reverse insecticide resistance in pests. No engineered gene drive has yet been released into the wild, but the technology could in principle be ready as soon as three years from now, says Crisanti. He collaborates with Target Malaria, a non-profit international research consortium seeking to use gene-drive mosquitoes for malaria control in Africa. On 1 July, the group released a test batch of mosquitoes — genetically engineered but not yet equipped with gene drives — in a village in Burkina Faso.

Gene drives are unlike any ecological fix ever tested before, says

Fredros Okumu, director of science at the Ifakara Health Institute in Dar es Salaam, Tanzania. "Gene drives will spread by themselves," he says. "We've got to prepare people and share information openly with all the countries concerned."

The technical challenges are not as daunting as the social and diplomatic ones, says bioengineer Kevin Esvelt at the Massachusetts Institute of Technology (MIT) Media Lab in Cambridge, who was among the first to build a CRISPR-based gene drive. "Technologies like this have real-world consequences for people's lives that can be nearly immediate."

Given the potential concerns about gene drives, *Nature* explores five key questions about the technology and its applications.

WILL GENE DRIVES EVEN WORK?

Building a gene drive to manipulate or eradicate a population is like picking a fight with natural selection, and that fight might not be easy to win.

As soon as researchers began to make gene drives regularly in labs, animals developed resistance against them — accumulating mutations that prevented the drives from spreading. In tests of two drives inserted into fruit flies, for example, genetic variants conferring resistance formed frequently[2]. Most commonly, mutations alter a sequence that CRISPR is set to recognize, preventing the gene from being edited. In experiments with caged mosquitoes, Crisanti and Target Malaria researcher Tony Nolan watched a gene drive gradually decrease in frequency over multiple generations owing to resistant mutations at the target gene[3]. The results rocked the field. Would resistance render gene drives impotent?

Not necessarily — if researchers select the right target. Some genes are highly conserved, meaning that any change is likely to kill their owners. Picking these genes as a drive target means fewer mutations and less resistance. In September 2018, Crisanti and his team crashed a population of caged *Anopheles gambiae* mosquitoes with 100% efficiency[4] by making a drive that disrupts a fertility gene called *doublesex*. With the drive in place, female mosquitoes cannot bite and do not lay eggs; within 8–12 generations, the caged populations produced no eggs at all. And because it is crucial for procreation, *doublesex* is resistant to mutations, including those that would confer resistance to a drive construct.

The team has conducted nine cage experiments of more than one million drive insertions targeting *doublesex* and has not seen any resistance, says Crisanti. Now the team is adapting the drive to cut not one but two loci on the *doublesex* gene, like treating an illness with a combination of drugs. "I want to make sure that the likelihood of developing resistance is very, very remote before saying the technology is ready for the field," says Crisanti.

In mammals, scientists have much more basic challenges than resistance to deal with. Last year, Kim Cooper and her colleagues at the University of California, San Diego (UCSD), engineered the beginnings of a gene drive in a mammal — a drive that interrupts a mouse gene, *Tyr*, and turns the animals' coats white[5]. The drive was only 72% efficient at copying itself in the genome and did not work well in the male germ line, says Cooper. She suspects this is because cell division happens at different times in the formation of eggs and sperm, which seems to affect the ability of the drive to copy successfully from one chromosome to another.

In that experiment, the drive did not self-propagate and Cooper did not follow the trait over multiple generations, so she emphasizes that it technically cannot be considered a gene drive. "There's still so much work to be done to show that something like this is even feasible," she adds.

WHAT ELSE ARE GENE DRIVES GOOD FOR?

Although mosquito applications dominate the field, proposed uses of gene drives also include conserving delicate ecosystems and speeding up lab work.

Some organisms have genomes that are challenging to manipulate, but doing so could help researchers to study them. Take *Candida albicans*, an often drug-resistant human fungal pathogen. As a postdoctoral researcher at the Broad Institute and MIT in Cambridge, Massachusetts, Rebecca Shapiro developed a system[6] to drive mutations into the fungus with close to 100% efficiency. She can now breed the fungus to silence two independent genes and bequeath those mutations to offspring. "It works insanely efficiently," says Shapiro, now at the University of Guelph in Canada. At UCSD, Cooper is using gene drives for a similar purpose, to create and study complex traits in mice.

The Genetic Biocontrol of Invasive Rodents (GBIRd) programme wants to do more with gene-drive mice than study them in a lab. GBIRd, a partnership of universities, governments and non-governmental organizations managed by the non-profit group Island Conservation, wants to use the technology to eliminate invasive rodents from islands, where they wreak havoc on native wildlife. Pesticides are currently used for this purpose, but they are expensive and difficult to use on larger islands with human populations. They are feasible on only about 15% of islands, says Royden Saah, GBIRd's programme manager. "We are trying to look at technologies that would take care of the other 85%."

GBIRd members David Threadgill at Texas A&M University in College Station and Paul Thomas at the University of Adelaide in Australia are developing gene-drive technologies in mice, although Saah estimates it will be several years before those drives are working successfully.

Meanwhile, some mosquito researchers hope to try something more subtle than completely wiping out insect populations as a means of preventing disease. In a May preprint[7], Omar Akbari and his colleagues at UCSD engineered *Aedes aegypti* mosquitoes to express an antibody that protected the insects against all four major strains of dengue. They are now attaching that antibody to a drive to see whether it will spread. Akbari is also building an all-purpose gene drive that activates a toxin when any virus, not just dengue, infects *A. aegypti*. "We want to build a Trojan horse in the mosquito," says Akbari. "When a mosquito is infected by a virus — whether it's dengue, Zika, chikungunya, yellow fever, whatever — it activates our system, which kills the mosquito."

CAN GENE DRIVES BE CONTROLLED?

Before Kevin Esvelt ever built a single CRISPR-based gene drive, he'd wake up in cold sweats thinking about the ramifications. "I realized, oh hey, this isn't just going to be about malaria, this is potentially going to be something any individual who can make a transgenic fruit fly could build to edit all the fruit flies."

It's no surprise, then, that in 2014, when Esvelt and geneticist George Church built their first gene drive at Harvard Medical School in Boston, Massachusetts, they simultaneously built a reversal drive to overwrite the original drive on command[8].

The rest of the field has followed suit, developing gene drives with built-in controls, external overrides or both. Funding most of that effort is the US Defense Advanced Research Projects Agency (DARPA), the research arm of the US Department of Defense. In 2017, DARPA's Safe Genes programme announced it was spending US$65 million across seven US research teams studying how to control, counter and reverse gene drives. "We're mitigating the potential for misuse, whether it's accidental or nefarious," says Renee Wegrzyn, the programme manager for Safe Genes.

Esvelt, funded in the initial phase of the programme, devised a self-exhausting drive known as a daisy drive. The drive is engineered to lose a link at a time, like plucking one flower from a chain linked head to stem, until it runs out over several generations[9].

At UCSD, Akbari's DARPA-funded team is developing gene drives that should be unable to spread beyond a target population of mosquitoes or flies. One such drive requires continual release for many generations. When those releases stop, it becomes diluted with wild-type versions of the gene and wipes itself out within four years. That might be long enough to eliminate a virus such as Zika or dengue from a mosquito population, says Akbari. "It's something that is, in my opinion, a little safer and still pretty effective." The team has already produced several versions of these drives for *A. aegypti*, the major vector for dengue virus[10].

The Target Malaria team is also developing a countermeasure, funded by DARPA, to stop the spread of the *doublesex* drive in a population.

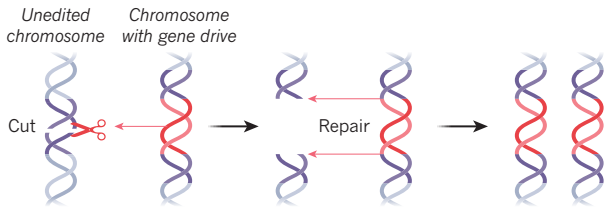
HOW CAN GENE DRIVES BE TRIALLED?

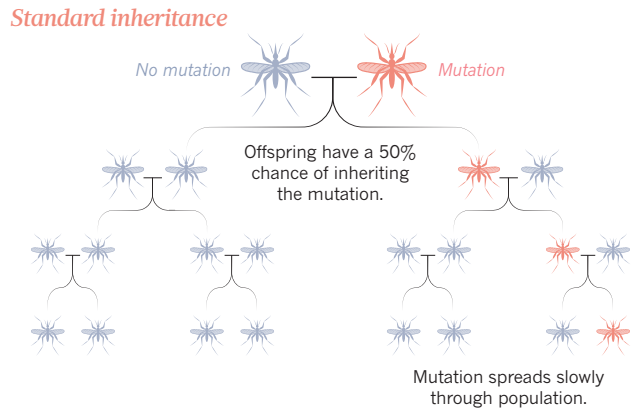
In lieu of a field test — which the DARPA Safe Genes contract expressly forbids and for which researchers agree the technology is not ready — teams are scaling up cage experiments and building ecological models

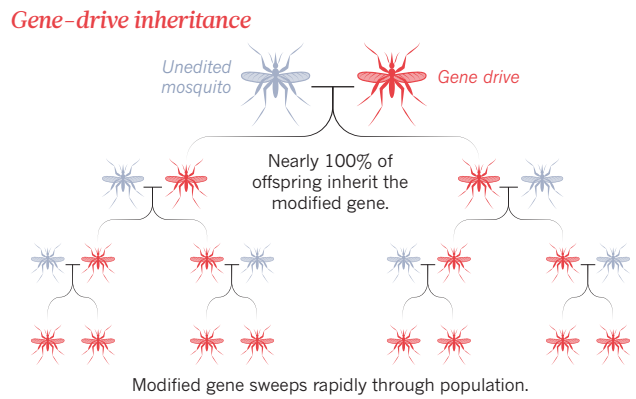
How gene drives work

Gene drives use CRISPR to insert and spread a genetic modification through a population at higher than normal rates of inheritance. Researchers plan to use drives to eradicate malaria-carrying mosquitoes and other pests.

Once a gene drive is engineered into an animal's genome, the animal's offspring will inherit the drive on one chromosome and a normal gene from its other parent. During early development, the CRISPR portion of the drive cuts the other copy. The cut is then repaired using the drive as a template, leaving the offspring with two copies of the modification.







to explore the benefits and risks of a wild release safely.

In the town of Terni in central Italy, Crisanti and Nolan have enriched their mosquito cages with changing environmental conditions. "We want to scale up in order to test it across different genetic backgrounds, under more realistic scenarios," says Nolan, who now runs a lab at the Liverpool School of Tropical Medicine, UK. He and Crisanti want to replicate natural mating behaviour — such as males forming swarms to attract females — to see how it affects the spread of a gene drive.

The dynamics of the drive's spread in those cages so far is "promising", says Crisanti — the drive is being passed along efficiently without signs of resistance. If no concerns arise in the larger cage experiments, then the team will hand over the technology to independent groups for testing, with a view to gaining regulatory approval in roughly three years, he says.

The Target Malaria team is also building ecological models of prospective release locations to work out the on-the-ground dynamics. The most recent study[11] models mosquito populations at more than 40,000 settlements in Burkina Faso and surrounding countries. It takes into account rivers, lakes and rainfall, as well as field data on mosquito movement. The results[11] show that repeated introduction, rather than a single release, of modified mosquitoes over a few years across villages will be needed to reduce the insects' overall numbers.

"The theory says that, in principle, if you release once it would spread continent-wide. The reality is that would happen very slowly," says population biologist Charles Godfray at the University of Oxford, UK, a collaborator with Target Malaria and the study's lead researcher.

Another concern is that gene drives have the potential to alter entire populations and therefore entire ecosystems. They could also, in theory, negatively affect human health by causing the malaria parasite to evolve to be more virulent or to be carried by another host, says molecular biologist and bioethicist Natalie Kofler. She is the founding director of the Editing Nature group at Yale University in New Haven, Connecticut, which aims to address environmental genetic technologies worldwide. "This technology has the potential to be immensely powerful and to change the course of things that we may not be able to predict," says Kofler.

WHO DECIDES WHEN TO USE A GENE DRIVE?

For drug trials, a company can begin preparing for a field test just a year or two in advance. Gene drives will need more time, says Okumu. Last year, he was part of a 15-member scientific working group, organized by the Foundation for the National Institutes of Health, that put forward a series of recommendations[12] for using gene-drive mosquitoes in sub-Saharan Africa.

The report stresses that governments, communities and local scientists will need time to absorb the science and be empowered to regulate the technology. "I say this with all conviction — in the end, the best people to make these decisions are the countries themselves," says Okumu.

In 2017, Kofler gathered a group of scientists and ethicists to grapple with the societal questions surrounding gene drives[13]. "The main questions centre around justice," says Kofler. In discussions about releasing a genetically engineered organism into an African environment, groups that have historically been marginalized have a right to be part of the decision-making process, she says.

Okumu wants African scientists to develop and test gene-drive technology locally, which will require respect and willingness from funders to support such efforts. "People fear the unknown, and the unknown right now is being presented from a Western perspective," says Okumu. "I am looking forward to a day we can build these constructs in our own labs, and in this way build local trust."

In August 2018, the National Biosafety Agency of Burkina Faso authorized Target Malaria to release a strain of genetically modified sterile male mosquito, the first of its kind on the African continent. Last week, the team released about 6,400 mosquitoes that have been genetically engineered but do not harbour gene drives. The scientists hope that the release will improve perception of the research as well as provide data for future releases.

And although gene-drive mice are far from ready for release, GBIRd is already working with risk assessors, ethicists and ecologists to identify an island for an initial field trial. "We want to make sure we get this right," says Saah. "No matter how fast the technologies move, we can advance the social sciences and the ethics now." ■

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