Gene drive research: why it matters

THE ROYAL SOCIETY

Cover image
Anopheles gambiae

Gene drive research: why it matters

Summary

Gene drives are systems that bias the inheritance of a particular DNA sequence. Many such systems occur naturally and scientists are now investigating the potential to develop new ones using synthetic biology techniques. Synthetic gene drives are being developed for a range of purposes that would benefit humanity, but it is not possible at this stage to know whether the benefits outweigh the risks. Further research will help to reduce uncertainties and characterise potential risks and benefits. It is important that any research that does proceed is appropriately governed and combined with public debate. The Royal Society therefore recommends that the UN Convention on Biological Diversity avoid the adoption of any position that would support an international moratorium on gene drive research, including experimental field trials.

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Synthetic biology and gene drives

Synthetic biology builds on established technologies for changing DNA in existing organisms by using engineering principles to design and assemble new biological components and systems. The ability to develop synthetic gene drives has become increasingly feasible in recent years thanks to the emergence of genome editing technologies. Unlike many synthetic biology applications, gene drives are intended for use in wild populations, such as insects that carry diseases. So organisms containing gene drives are intended for deliberate release into the environment. Some gene drives are also designed to spread within specific wild populations in a self-sustaining way. These characteristics have led to considerable international debate around the technology[1].

1. *Sackler Forum 2015: Trends in synthetic biology and gain of function and regulatory implications*. The Royal Society (2016).

Scientists are also working on ways of assessing and mitigating some of the risks posed by gene drives.

Gene drive applications

Gene drives are being developed for a range of purposes and sectors. Perhaps the most prominent of these is to reduce the populations of malaria-transmitting mosquitoes[2]. In this context, gene drives have been proposed as providing a new means of tackling a disease that still infects 200 million people and causes half a million deaths each year[3].

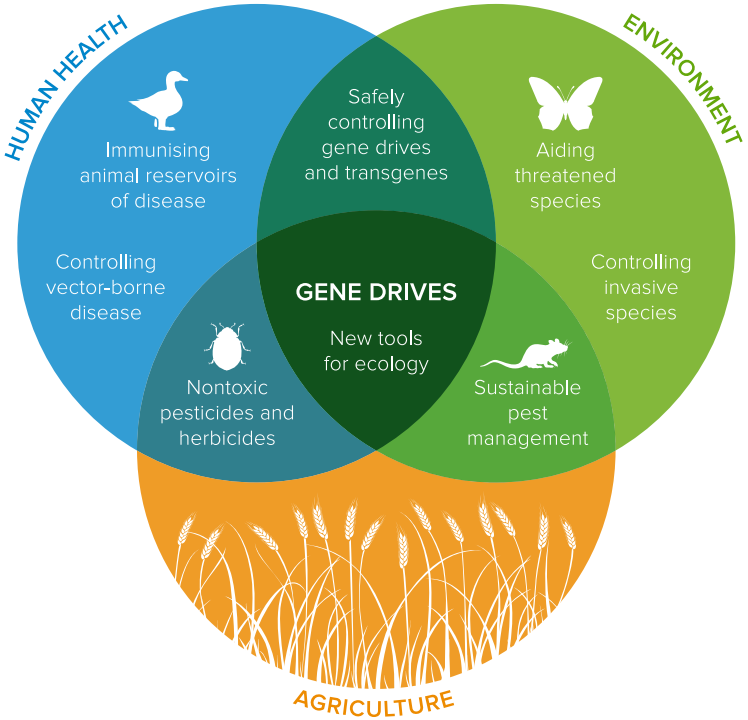
Other applications under development include providing a targeted means of controlling or eradicating invasive species[4]. Figure 1 summarises some of the proposed applications for gene drives.

Managing risks and benefits

Despite their possible benefits, gene drives also carry risks since the broader ecological consequences of reducing or eliminating a population can be uncertain. Confinement strategies, safeguards and appropriate governance for their use will be critically important[5,6]. Researchers working on gene drives are mindful of these risks and have called for a close assessment of the technology's potential impacts; not just scientifically but in a much broader sense, encompassing societal impacts, public perception and acceptance, ecological and health impacts, biosafety and biosecurity issues, as well as how regulation and other forms of governance might manage the risks and promote the benefits[7].

2. Further information in Box 2 on p.23 of *Sackler Forum 2015: Trends in synthetic biology and gain of function and regulatory implications.* The Royal Society (2016*)*
3. *World Malaria Report.* World Health Organisation (2015).
4. Esvelt, K.M., Smidler, A.L., Catteruccia, F., Church, G.M. Concerning RNA-guided gene drives for the alteration of wild populations. eLife. 2014; 3: e03401
5. *Sackler Forum 2015: Trends in synthetic biology and gain of function and regulatory implications*. The Royal Society (2016).
6. Hammond, A., Galizi, R., Kyrou, K., Simoni, A., Siniscalchi, C., Katsanos, D., Gribble, M., Baker, D., Marois, E., Russell, A., Burt, A., Windcihler, N., Crisanti, A., Nolan, T., 2016. A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector *Anopheles gambiae*. *Nature Biotechnology*. 34: 78 - 83.
7. Op. cit. note 5.

FIGURE 1



Speculative possible uses for gene drives. Of these, controlling vector-borne disease and controlling invasive species are the two most actively researched areas[8].

8. Figure reproduced from Figure 5 on p.22 of *Sackler Forum 2015: Trends in synthetic biology and gain of function and regulatory implications*. The Royal Society (2016).

To implement this close assessment, the scientific community has called for transparency from the outset of research, including discussion with the public preceding some research projects and certainly taking place before any release of modified organisms into the environment[9]. It will also be important to develop interdisciplinary research groups that bring together synthetic biologists, ecologists, evolutionary biologists, environmental scientists and social scientists amongst others, to examine potential risks from the outset. These approaches are well exemplified by Target Malaria, a not-for-profit research consortium that aims to develop and share technology for malaria control. The consortium includes scientists, stakeholder engagement teams, risk assessment specialists and regulatory experts from Africa, North America and Europe[10].

Scientists are also working on ways of assessing and mitigating some of the risks posed by gene drives. Organisms should be assessed on a case-by-case basis, based on lessons learned from the release of organisms that have been genetically modified but not with a gene drive[11]. One of the simplest barriers to unintended effects from gene drive research might be geographical or ecological confinement, with the use of offshore laboratories or offshore field trials. In the case of the malaria gene drive work, the initial research is being conducted in the UK in contained laboratories with *Anopheles gambiae* mosquitoes[12]. In the unlikely event of escape from the laboratory, these mosquitoes should not be able to survive due to the cool climate and there is no native wild population with which they could mate. In addition, experiments being considered in Australia to explore the use of gene drives to control

9. Op. cit. note 5.
10. targetmalaria.org/who-we-are/ (Retrieved 12/10/2018).
11. Op. cit. note 5.
12. Op. cit. note 5.

invasive rodent species would be conducted on islands. Assuming the satisfactory completion of geographically or ecologically confined trials, it will also be necessary to conduct field trials in intended release locations before any non-experimental releases in those areas. The extent to which a particular location provides safe and effective containment will need to be assessed on a case-by-case basis.

Once a gene drive is released, potential containment mechanisms include limiting the number of generations over which the gene drive operates in order to partially contain it[13], or developing a drive for a DNA sequence that is specific to the target population. It may also be possible to release a 'reversal drive' or other engineered trait which can remove the initially introduced trait[14].

RECOMMENDATION

Given the potential benefits of gene drives, it is important that research to develop this technology continues. It is equally important that research assessing the risks of releasing gene drive organisms into the environment continues in parallel. Any future use of gene drives should be preceded by public debate about the relative desirability of using gene drives compared with alternative social, economic or technological solutions. Given the early stage nature of much of this work, any move to prohibit research into gene drives would stifle this debate without a clear understanding of what the technology can do and whether it can be used safely. The Royal Society therefore recommends that the UN Convention on Biological Diversity avoid the adoption of any position that would support an international moratorium on gene drive research, including experimental field trials.

13. Noble, C., Min, J., Loejarz, J., Buchthal, J., Chavez, A., Smidler, A.L., DeBeedictis, E.A., Church, G.M., Nowak, M.A., Esvelt, K.M. 2016. Daisy-chain gene drives for the alteration of local populations. BioRxiv.

14. Oye, J., Esvelt, K., Appleton, E., Catteruccia, F., Church, G., Kuiken, T., Lightfoot, S.B.Y., McNamara, J., Smidler, A., Collins, J.P. 2014. Regulating gene drives. Science. 345(6197): 626 – 628

Gene drive questions and answers

QUESTION ONE

What are gene drives?

Gene drives are systems that bias the inheritance of a particular DNA sequence. They can be used to increase the persistence of an introduced trait that would otherwise disappear from a population very rapidly because the introduced trait puts the organism at a disadvantage. They can also spread a desired trait through a population. Many such systems occur naturally, and these are inspiring the development of new gene drives using synthetic biology techniques. Synthetic gene drives use genome editing technologies, such as CRISPR/Cas9, to increase the probability that a particular gene is inherited from 50% to up to 100%.

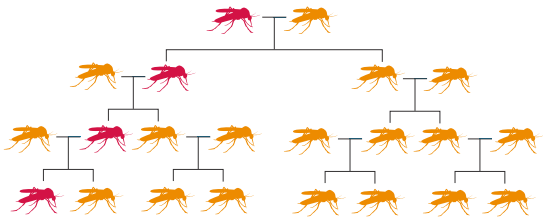
Figure 2 demonstrates the difference between the usual pattern of genetic inheritance (known as Mendelian inheritance after Gregor Mendel who identified it) and inheritance under a gene drive that has been designed to spread at the maximum possible rate through a population, known as a 'homing drive'. Even when a drive has been designed to spread at the maximum possible rate, experimental evidence from laboratory research shows this pattern of inheritance is hard to achieve due to the fact that some individuals in the population will have small variations in the genetic code that the drive has been designed to target[15]. These individuals are not susceptible to the drive and because they are more likely to survive in an environment where a gene drive to suppress a population has been released, they are more likely to pass on their genetic resistance to the gene drive to the next generation. This is known as 'evolved resistance' and is discussed further in answer to Question 4.

15. Champer, J., Buchman, A., Akbari, O. S. 2016. Cheating evolution: engineering gene drives to manipulate the fate of wild populations. *Nature Reviews Genetics*. 17(3): 146 – 159.

FIGURE 2

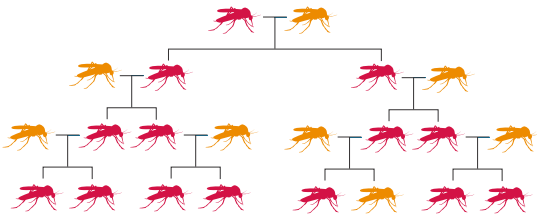
Mendelian inheritance versus gene drive inheritance

Mendelian inheritance



Altered gene (red) does not spread

Homing gene drive inheritance



Altered gene is almost always inherited

Whilst homing drives are the most frequently discussed type of gene drives, scientists are working on others that are designed to spread in a more limited way.

These deliberately limited gene drives are discussed further in answer to Question 3. Whether a homing drive or a more limited drive is appropriate will depend on the purpose that it is being developed for.

QUESTION TWO

What purposes are gene drives being developed for?

Many proposed applications for gene drives involve reducing the numbers of a particular animal population, such as the mosquitoes that transmit malaria. These are known as 'population suppression drives'. Rather than directly causing animals to die, population suppression drives would work by limiting reproduction. For example, gene drives could ensure that all offspring of an organism were male so that over time there were no females to mate with, leading to a population crash. Gene drives could also be used to increase the proportion of a population with a particular property, such as mice that are immune to Lyme disease. These are known as 'population conversion drives'.

The potential to suppress particular animal populations or to add or remove a particular trait is being explored to help address challenges in human health, biodiversity conservation and agriculture.

Human health

Gene drives to improve human health would target the animals that transmit disease. Much of the work in this sector has focused on the potential of gene drives to help reduce the number of people infected with malaria. In this context, scientists are working on both ways of reducing the number of malaria-transmitting mosquitoes and changing genes in mosquitoes to prevent them from carrying the malaria parasite (and so prevent them from passing it on to people).

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Biodiversity conservation

In the conservation sector, gene drives are being researched as a means of controlling or removing invasive species. They have also been proposed as a means of increasing the resistance of endangered species to disease and other threats, for example by targeting the mosquitoes that spread avian malaria to birds[16].

Much of the work on invasive species has focused on removing rodents from islands where they threaten ground nesting birds. This approach is being supported by not-for-profit organisations like Island Conservation. In this context, gene drives are being investigated as a potentially more effective and more humane way of eradicating rodents than existing trapping, hunting or poisoning programmes. Gene drives are also being considered as a way of controlling other invasive species, including wasps in New Zealand[17]. and cane toads in Australia[18].

Agriculture

Scientists have demonstrated a proof-of-concept population conversion drive that could form the basis of various population suppression strategies in a species of fruit fly whose larvae feed on soft fruits such as cherries[19]. There has also been discussion of using population conversion drives to make crop pests more susceptible to pesticides and herbicides[20].

16. *Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values*. National Academies of Sciences, Engineering, and Medicine (2016).
17. Lester, P.J., Beggs, J.R., Brown, R.L., Edwards E.D., Groenteman R., Toft, R.J., Twidle, A.M., Ward, D.F. 2013. The outlook for control of New Zealand's most abundant, widespread and damaging invertebrate pests: social wasps. *New Zealand Science Review*. 70(4): 56 – 62
18. *Gene Drives in Australia*. Australian Academy of Science. (2016).
19. Buchman, A., Marshall, J.M., Ostrovski, D., Yang, T., Akbari, O.S. Synthetically engineered Medea gene drive system in the worldwide crop pest Drosophila suzukii. 2018. *Proceedings of the National Academy of Sciences*. 115(18): 4735 – 4730
20. Medina, R.F. 2018. Gene drives and the management of agricultural pests. *Journal of Responsible Innovation*. 5(Sup1), S255 – S262.

QUESTION THREE

How can the spread of a gene drive be limited?

As discussed in answer to Question 1, scientists are working on drives that are designed to spread over a limited period of time or within a limited area. These are well suited to targeting a particular population, such as rats on a single island where they act as an invasive species, rather than all the populations of that rat species.

One method for limiting the area over which a gene drive spreads is to design it so that it only spreads if it is present in a particular proportion of the population. This introduces a threshold below which the drive does not spread, which is why these drives are known as 'threshold dependent drives'[21,22]. These drives would work by releasing a large number of gene drive organisms into a particular population so that they exceed the threshold in that population. If any of these gene drive organisms spread into a neighbouring population, they would be outnumbered by wild organisms, and so the drive would not spread in that population.

It would also be possible to stop the spread of the drive in the population where the gene drive organisms were originally released by releasing more organisms without the gene drive and so taking the proportion of gene drive organisms below the threshold necessary for it to spread. There are several possible molecular designs for achieving threshold dependent drives, with at least two demonstrated in laboratory populations of fruit flies[23,24].

Scientists are working on drives that are designed to spread over a limited period of time or within a limited area.

21. *Alphey, L. 2014. Genetic Control of Mosquitoes*. Annual Review of Entomology. 59(1): 205 – 224
22. Marshall, J.M., Akbari, O.S. 2018. Can CRISPR-Based Gene Drive Be Confined in the Wild? A Question for Molecular and Population Biology. ACS Chemical Biology.13 (2): 424-430
23. Buchman, A., Ivy, T., Marshall, J., Akbari, O.S., Hay, B.A. 2018. Engineered reciprocal chromosome translocations drive high threshold, reversible population replacement in Drosophila. *ACS Synthetic Biology*. 7(5): 1359 – 1370
24. Reeves, R. G., Bryk, J., Altrock, P.M., Denton, J.A., Reed, F.A. 2014. First Steps towards Underdominant Genetic Transformation of Insect Populations. PLoS ONE. 9(5): e97557

Image
Brown rat © Andrew_Howe.

Another proposed method for restricting the spread of gene drives is to design them so that their effectiveness decreases over time. Figure 3 shows one theoretical method for limiting the time that a gene drive works for, known as a 'daisy drive'. In this example, the action of the gene drive depends on the interaction of multiple sequences of DNA that are introduced into separate parts of the genome. As one of these sequences is not self-sustaining or 'driving', its incidence in the population fades over time and with it the effectiveness of the gene drive[25].

Finally, a mechanism that might be suitable for gene drives targeted at island populations is to look for variants of genes that are unique to those populations and then develop drives specifically for these variants.

25. Smidler, A., Noble, C., Olejarz, J., Buchthal, J., Min, J., Esvelt, K. 2017. Daisy-chain gene drives for the alteration of local populations. *Responsive Science*.

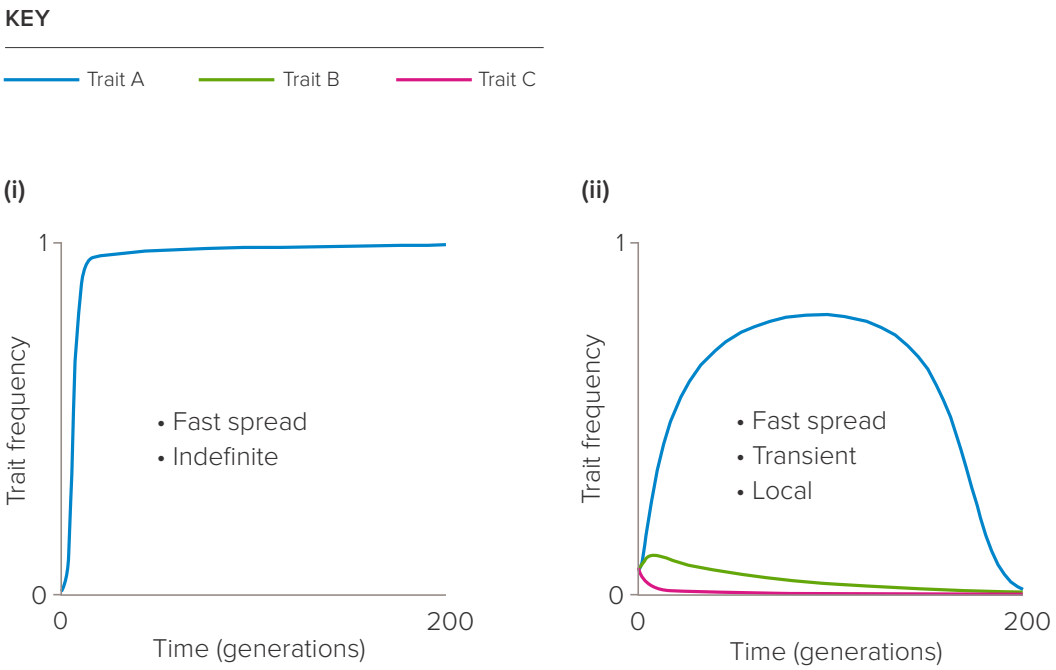
FIGURE 3

Self-sustaining gene drives (left) versus daisy drives (right)[26].

(i) Self-sustaining gene drives sustain themselves between generations by converting non-driving versions of a gene to driving versions of the gene. Assuming the drive has been designed to spread at the maximum rate possible, that it has no impact on a population's survival and reproduction rate, and that there is no evolved resistance to the drive, it can spread to 100% of the population, as demonstrated by the graph (i) opposite.

(ii) A daisy drive system involves introducing several genetic changes at different places in an organism's genome. In the example opposite, the blue line represents the genetic change that delivers the desired trait such as resistance to a disease (trait A), the green line (trait B) represents a gene drive that ensures the inheritance of trait A between generations, and the red line (trait C) is a gene drive that ensures the inheritance of trait B. Because there is nothing that ensures the inheritance of trait C over time, the proportion of organisms with this genetic change will reduce with each new generation. Without trait C, there is no way of sustaining trait B between generations and without trait B, there is no way of sustaining trait A. This pattern of inheritance is demonstrated by graph (ii) opposite.

26. Adapted from Figure 1, Smidler, A., Noble, C., Olejarz, J., Buchthal, J., Min, J., Esvelt, K. 2017. Daisy-chain gene drives for the alteration of local populations. *Responsive Science*.


KEY
Trait A
Trait B
Trait C
(i)
1
Trait frequency
0
0
200
Time (generations)
• Fast spread
• Indefinite
(ii)
1
Trait frequency
0
0
200
Time (generations)
• Fast spread
• Transient
• Local

QUESTION FOUR

Could a single gene drive organism lead to the eradication of a whole species?

There are several factors that work against the spread of a synthetic gene drive.

Whilst it is theoretically possible that the release or escape of a single organism with a suppression drive could lead to a crash in that species, evidence from laboratory and modelling studies raises questions as to whether this is likely to happen in practice.

Firstly, the organism would have to be carrying a drive that has been designed to work in an entire species, as opposed to a single population of that species. As discussed in the answer to Question 3, scientists are working to develop gene drives that fade over time or only affect specific populations.

Secondly, there are several factors that work against the spread of a synthetic gene drive. One is small genetic differences between organisms in the gene that is targeted by the gene drive. Because the genome editing technique CRISPR/Cas9 works by recognising a specific sequence of DNA, if an organism has a variant of the gene targeted by the gene drive with a slightly different sequence, the gene drive will not work in that organism. This effect has been demonstrated in laboratory experiments. In one such experiment, the number of organisms carrying the gene drive increased rapidly for four generations after it was released, but the presence of the gene drive then decreased gradually until the experiment was stopped after 25 generations[27].

27. Hammond, A. M., Kyrou, K., Bruttini, M., North, A., Galizi, R., Karlsson, X., Kranjc, N., Carpi, F.M., D'Aurizio, R., Crisanti, A., Nolan, T. 2017. The creation and selection of mutations resistant to a gene drive over multiple generations in the malaria mosquito. *PLoS Genetics*. 13(10): e1007039.

In September 2018, scientists published research on a gene drive that did affect 100% of a population of caged laboratory mosquitoes. However, they also identified several reasons why this result might not be replicated if that gene drive was ever released into a wild population of mosquitoes[28]. Foremost amongst these was the observation from previous releases of genetically modified insects (not ones carrying gene drives) that these laboratory-developed organisms were less likely than their wild counterparts to survive in the wild and therefore less likely to have a chance to mate and pass on their modified genes.

Because no synthetic gene drive has ever been released into a wild population, we can only use models to try to predict what the consequences of this would be. Such models often assume several hundred gene drive organisms are released at once and even under these circumstances they do not always find that the result is the total elimination of a population, let alone a species[29]. However, other models looking at population conversion drives have suggested that whilst gene drives are unlikely to spread widely from the release of just one organism, they could spread widely from the release of two or more[30]. This highlights the need for detailed modelling research before the deliberate release of any gene drive.

28. Kyrou, K., Hammond, A.M., Galizi, R., Kranjc, N., Burt, A., Beaghton, A.K., Nolan, T., Crisanti, A. 2018. A CRISPR–Cas9 gene drive targeting doublesex causes complete population suppression in caged Anopheles gambiae mosquitoes. *Nature Biotechnology*. Published online.

29. Eckhoff, P.A., Wenger, E.A., Godfray, C.J., Burt, A. 2017. Mosquito gene drive impact on malaria elimination. *Proceedings of the National Academy of Sciences*. 114(2): E255 – E264

30. Noble, C., Adlam, B., Church, G.M., Esvelt, K.M. and Nowak, M.A. 2017. Current CRISPR gene drive systems are likely to be highly invasive in wild populations. *Elife*. 19(7): e33423

QUESTION FIVE

What will be the wider ecological consequences of using gene drives to control animal populations?

It will be important to assess what impact this population or species has in the wider ecosystem and what the consequences of removing it might be.

Where gene drives are used to reduce or eradicate a particular population or species it will be important to assess what impact this population or species has in the wider ecosystem and what the consequences of removing it might be. This is why research groups working on gene drives should include population biologists, ecologists and other environmental scientists.

Assessments could draw on research into the consequences of other interventions to reduce animal populations, such as spraying insecticides to kill mosquitoes or poisoning rodents on islands where there are native ground nesting birds. Applying this approach to *Anopheles gambiae*, one of the most significant of the roughly 40 species of malaria-transmitting mosquitoes (there are around 3,500 mosquito species in total), researchers have concluded that there is no other species that feeds solely on these mosquitoes and they are not likely to be a key component of the food web[31]. This research demonstrates the need to assess possible ecological consequences on a species-by-species basis in each location that a gene drive is released, whilst also taking into account the possible spread of the gene drive to locations beyond the release area.

31. Collins, C. M., Bonds, J. A., Quinlan, M. M. and Mumford, J. D. 2018. Effects of the removal or reduction in density of the malaria mosquito, Anopheles gambiae s.l., on interacting predators and competitors in local ecosystems. *Medical and Veterinary Entomology*. ePub ahead of print.

One of the concerns raised about the release of synthetic gene drives designed to suppress a species is the possibility that the gene drive accidentally spreads from the target species to an unrelated species, for example from a mosquito to a honey bee. The process of genetic material moving between unrelated species is known as horizontal gene transfer. Although evidence of historical horizontal gene transfer between animals is growing, the transfer of functional genetic sequences between animals is extremely rare. Moreover, for a gene drive to work in an unrelated species, that species would have to have exactly the same variant of the gene as the species that was originally targeted by the gene drive.

Although the one gene drive that has been shown to be 100% effective in mosquitoes in laboratory experiments targets a gene that is common to all insects, all but the most closely related species of mosquito have a different variant of this gene. Therefore, a gene drive targeting this gene in one mosquito species would not work in other insect species.

Despite the very low likelihood of a functional gene drive transferring between species, the US National Academy of Sciences report *Gene Drives on the Horizon: Advancing Science, Navigating Uncertainty, and Aligning Research with Public Values* recommended that the risk of horizontal gene transfer should be evaluated before any environmental release of a gene drive.

QUESTION SIX

Are gene drives being developed for people?

It would take centuries for a gene drive to spread a trait through a human population.

Whilst gene drives could be developed for any species that reproduces sexually, the way they work means they are best suited to species that reproduce quickly. As the generation time (the time from when an organism is born to when it has its own children) in humans is measured in decades, it would take centuries for a gene drive to spread a trait through a human population. By contrast, the generation time in mosquitoes is around four weeks, therefore a gene drive could change the genetics of a mosquito population within a few years.

QUESTION SEVEN

Will gene drives be released without local engagement?

Many of the scientists who are leading the development of gene drives are also pioneering methods of working with the communities where they propose to use those gene drives to ensure that they do not do anything that that community disagrees with. For example, Target Malaria (a not-for-profit research consortium working on gene drives in malaria-transmitting mosquitoes) has a team dedicated to working with the governments and communities where Target Malaria is carrying out research to ensure that those involved understand what Target Malaria is proposing to do and address any concerns that arise[32]. These teams include people with a variety of backgrounds, including in sociology, anthropology, political science, geography, and communications.

Their experiences are also diverse, with a mix of academic researchers, NGO workers, public programmes implementers, and corporate sector experts[33].

Similarly, a research project looking at a gene drive that would make mice immune to Lyme disease, and therefore disrupt the transmission of the disease to humans, has involved the communities where the gene drive would be released from the beginning of the project. This engagement has led the researchers to pause their research into gene drives and instead prioritise the development of mice that are immune to Lyme disease, but would spread this trait through Mendelian inheritance rather than gene drives[34].

32. targetmalaria.org/who-we-are/ (Accessed 12/10/2018)
33. targetmalaria.org/wp-content/uploads/pdf/target-malaria-response-to-NAS-recommendations.pdf (Accessed 12/10/2018)
34. nytimes.com/2016/06/08/science/ticks-lyme-disease-mice-nantucket.html (Accessed 12/10/2018)

The Royal Society

The Royal Society is a self-governing Fellowship of many of the world's most distinguished scientists drawn from all areas of science, engineering, and medicine. The Society's fundamental purpose, as it has been since its foundation in 1660, is to recognise, promote, and support excellence in science and to encourage the development and use of science for the benefit of humanity.

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