Resistance to gene drive

Many existing methods of malaria control are facing difficulties in maintaining effective performance, from insecticide and drug resistance to increasing costs of interventions. Controlling the malaria vector, the mosquito, remains the frontline for disease elimination.

Target Malaria aims to develop new genetic technologies in a strategy known as *gene drive* to reduce the malaria mosquito population and thus reduce malaria transmission.

Gene drive involves a genetic mechanism designed to spread through a population while at the same time reducing the mosquito population and decreasing malaria transmission. Gene drive is an especially appealing strategy as it could be deployed in remote locations, can work in parallel with the health care system while not being dependent on it, is potentially cost effective, can be used in complementarity with other vector control methods and has the potential for long term and sustainable impact.

The Target Malaria gene drive approach exploits a transgene encoding the nuclease CRISPR/Cas (a DNA-cutting enzyme). In our modified mosquitoes, the CRISPR/Cas enzyme does two things simultaneously:

- Firstly, CRISPR/Cas cuts and disrupts specific mosquito genes involved in fertility, so that females bearing it cannot reproduce normally.
- Secondly, it causes copies of its own transgene to be cut-and-pasted onto partner chromosomes at the same site as the female fertility gene.

This means that both the CRISPR/Cas transgene and female infertility spread through mosquito population at far greater rates than would occur by normal inheritance mechanisms, ultimately leading to reductions in the overall numbers of malaria vectors.

Resistance potential

Just as antibiotic use to treat bacterial infection can lead to drug-resistant bacteria, any method of mosquito population control - such as the use of insecticides - can lead to mosquitoes that are resistant to that method. Likewise, the risk exists that some form of resistance to the gene drive could emerge. Resistance arises through a common cause of natural selection, whatever the resistance is against.

It should be noted that resistance to gene drive and resistance to insecticides work by different and separate mechanisms. The genetic modification introduced in gene drive mosquitoes is not expected to affect insecticide resistance and, likewise, insecticide resistance does not interfere with gene drives. The mosquito strains developed by Target Malaria are checked to ensure the modification does not impact insecticide resistance.

Resistance to gene drive is most likely to arise by two mechanisms:

Resistance could arise from the action of the CRISPR/Cas transgene underpinning the gene drive system. The core mechanism of the gene drive involves the CRISPR/Cas nuclease, which recognizes and cuts specific sequences of DNA in the mosquito genome. The cell uses the CRISPR/Cas transgene as a template to repair the cut, copying it from chromosome to chromosome. Occasionally, however, cells sew the incision back together without copying the CRISPR/Cas transgene, leading to a new sequence that the CRISPR/Cas nuclease may no longer recognize, thus stopping the gene drive mechanism.

Resistance could arise from natural genetic variation in the wild mosquito population at the CRISPR/Cas target sequence that might prevent the gene drive from working efficiently. Mosquitoes with such variant sequences might be resistant to the drive and - as their fertility may be less affected than mosquitoes without the variant sequence - they could be favoured over time by natural selection. As a result, the spread of the gene drive would be slowed down or stopped altogether should such variants exist. Recent studies[1] analysed the genomes of 1142 wild *Anopheles* mosquitoes from across Africa. The study found extreme genetic diversity at many mosquito genes, which could limit the number of genomic sites that could be potential targets for gene drive. However, these analyses have also shed light on the fact that there are some genes that show little natural genetic variation, which will help researchers develop gene drives that may be less likely to develop resistance.

Resistance to gene drive can be detected and monitored by sequencing the DNA of target site in large numbers of mosquitoes. This is done routinely in specialized laboratories. Recent work[2] from the lab of Prof Andrea Crisanti (Imperial College London) confirmed that target site resistance against one gene drive mechanism evolved over several generations in caged mosquito populations: a gene drive designed to affect the fertility of mosquitoes was blocked by mutations at the target site that prevented CRISPR/Cas from cutting the DNA at that location, while at the same time rendering mosquitoes fertile. Additional work[3] on *Drosophila* fruit flies has led to a similar conclusion.

Strategies to address resistance

Understanding the nature of resistant mosquitoes and the speed with which they can arise will help gene drive researchers find ways to predict and manage resistance for future gene drive applications. Target Malaria strategies to delay the evolution of resistance include:

- Targeting multiple sequences in the same fertility gene
- Targeting multiple fertility genes
- Selecting target genes that show very little variation or that cannot tolerate changes to their sequences without harming the mosquito
- Developing more effective gene drives so that it overwhelms any counterpressure

All these approaches are complementary, and together they dramatically reduce the likelihood of resistance arising. Recent laboratory experiments[4] demonstrated that combining such approaches could lead to complete suppression of caged mosquito populations without detecting signs of resistance, as eradication is also the antidote to resistance.

Gene drive effectiveness

Some level of resistance to gene drives may be unavoidable. Researchers are addressing how to minimise any such effects long enough for a gene drive to spread throughout a population and have an impact on malaria mosquito population and disease transmission before resistance stops the drive from spreading.

We expect that, after we develop our first effective gene drive, the production of any additional variant gene drives that might be needed to overcome any resistance in the field will be significantly faster and less costly than the original version.

Target Malaria is engaging with malaria control experts to determine how long a gene drive intervention should be effective, for it to be a useful addition to the malaria control toolkit. There is a potential opportunity to get good levels of efficacy for many years before resistance occurs. During that time and before resistance arises, options could be developed for new gene drives and other controls to provide the next generation of vector control.

1 The Anopheles gambiae 1000 Genomes Consortium (2017): Ag1000G phase 2 AR1 data release. MalariaGEN. *http://www.malariagen.net/data/ag1000g-phase2-ar1*

2 Hammond M, Kyros K, Bruttini M, North A, Galizi R, Karlsson X, Carpi F, 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. bioRxiv doi: *https://doi.org/10.1101/149005*

3 Champer J, Reeves R, Oh SY, Liu C, Liu J, Clark AG, *et al.* (2017) Novel CRISPR/Cas9 gene drive constructs reveal insights into mechanisms of resistance allele formation and drive efficiency in genetically diverse populations. PLoS Genet 13(7): e1006796. *https://doi.org/10.1371/journal.pgen.1006796*

4 DOI: *https://doi.org/10.1038/nbt.4245*