Doublesex construct

Target Malaria's vision is a world free of malaria. To support this vision, we are developing modified mosquitoes that can be released into a target population to reduce the number of mosquitoes that can transmit malaria over time. This would become a new and complementary method for malaria control. Target Malaria is adapting a natural concept called gene drive, a technology that holds the promise of being a self-sustaining and cost-effective method to achieve population control of malaria mosquitoes – malaria control by mosquito control.

The technology that Target Malaria is developing focuses on genetic modifications that affect the reproduction of mosquitoes, by targeting genes involved in fertility, such as the *doublesex (dsx)* gene. The *dsx* gene is responsible for determining the sex of mosquitoes, functioning as a switch that establishes whether the mosquito cells become male or female. Disruption of the *dsx* gene can thus interfere with the sexual development of the adult insects and their reproduction.

Gene drive in the *doublesex (dsx)* locus

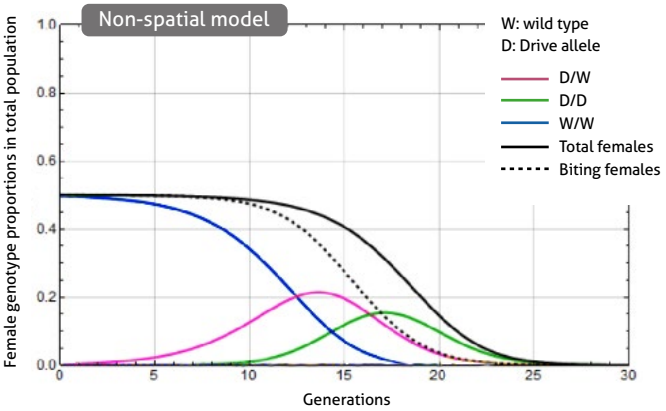
Gene drive refers to a phenomenon, whereby a particular heritable element biases inheritance in its favor, resulting in the gene becoming more prevalent in the population over successive generations.

Because our long-term goal is to reduce the number of malaria mosquitoes in the environment, we are using gene drive to impact the mosquitoes' ability to reproduce.

We are using very specific DNA-cutting enzymes (or nucleases), such as CRISPR/Cas, that recognize and cut specific sequences of DNA in the mosquito genome. Specifically, we are programming our nucleases to cut within the dsx gene to affect reproduction.

This means that modified mosquitoes that inherit the gene drive from just one parent remain fertile and can pass on to their progeny the gene drive in a self-sustaining manner. As the gene drive increases in frequency in the target population over time, mosquitoes are increasingly likely to inherit the genetic modification from both parents. Possession of two copies of the gene drive leads to sterility in females, causing a reduction in the mosquito population.

Heterozygous transgenic first wave (magenta), followed by homozygous transgenic second wave (green), after transgene release into a wild type population



Deterministic small cage model, 1% initial transgenic $dsxF^{CRISPRh}$ males. Parameter estimates from Kyrou *et al.*, 2018.

A. Beaghton, unpublished.

Target Malaria's *doublesex* mosquitoes

2018 Nature Biotechnology Publication: "A CRISPR–Cas9 gene drive targeting *doublesex* causes complete population suppression in caged *Anopheles gambiae* mosquitoes" [1]

This publication demonstrated that using CRISPR-Cas9 gene drive can successfully suppress a caged population of *Anopheles gambiae* mosquitoes by targeting a region of the *dsx* gene expressed only in females and essential for sex determination. When a small number of gene drive mosquitoes were introduced into a cage of wild type mosquitoes, the genetic modification spread through the population, reaching 100% frequency. Males who carried the gene drive did not show any overt changes, likewise females with just one copy of the modified gene were also unaffected. It was only when all females with two copies of the gene drive were born that they became completely sterile (they did not lay eggs or bite) and the population crashed.

It was the first time we were able to show complete suppression of a caged population of *Anopheles gambiae* mosquitoes, and it occurred within 7-11 generations (approx. 21-33 weeks) without signs of immediate resistance emerging. This study also marked the first time that scientists were able to use a genetic modification to stop the reproductive capacity of complex organisms in a laboratory setting using gene drives leading to population suppression. Following this publication, the gene drive mosquitoes were shipped to Polo of Genomics, Genetics and Biology (PoloGGB) in Italy where they are undergoing further evaluation in large cages housed within the contained laboratory with environmental conditions that mimic more closely the natural ecological ones.

2020 Nature Biotechnology Publication: "A Male-biased Sex-Distorter Gene Drive for the Human Malaria Vector *Anopheles gambiae*" [2]

This study describes the development of a sex-distorter mosquito that can spread in a self-sustaining manner within a population of *Anopheles* using a gene drive targeting the *dsx* gene.

While it spreads, the sex-distorter converts the population to being male dominated and the gene drive component reduces the proportion of functional and fertile females, eventually leading to population collapse. Manipulation of the sex ratio toward a male-biased population is predicted to be extremely effective in controlling malaria transmission because only females bite people and transmit the disease.

The findings thus far represent a significant advance in efforts to develop a functional gene drive with male bias to control malaria-carrying mosquitoes.

A promising candidate to help control malaria vectors in Africa

As an innovative approach to the challenge of vector control, gene drive technology has the potential to add new tools and interventions in the fight against malaria. More work is needed to understand how the technology is affected by more complex environmental conditions. We are planning to further evaluate the latest mosquito strains in large cages in our partner institute Polo GGB in Italy, where we can simulate tropical environments and observe more complex behavior, such as mating swarms.

No field studies have yet been planned for gene drive mosquitoes. We are between five to ten years from being ready to ask a regulatory authority to consider a contained use permit. Additional laboratory studies to further characterize the gene drive mosquitoes for efficacy and safety will be needed before moving the technology to the field. Environmental Impact Assessments, as defined by national legislation, will be conducted. We will continue our extensive work of engaging with communities and other stakeholders at every level to seek support and acceptance for progression with this technology for vector control for malaria.

1 Kyrou, K., Hammond, A., Galizi, R. *et al.* A CRISPR–Cas9 gene drive targeting *doublesex* causes complete population suppression in caged *Anopheles gambiae* mosquitoes. *Nat Biotechnol* **36**, 1062–1066 (2018). *https://doi.org/10.1038/nbt.4245*

2 Simoni, A., Hammond, A.M., Beaghton, A.K. *et al.* A male-biased sex-distorter gene drive for the human malaria vector *Anopheles gambiae*. *Nat Biotechnol* **38**, 1054–1060 (2020). *https://doi.org/10.1038/s41587-020-0508-1*