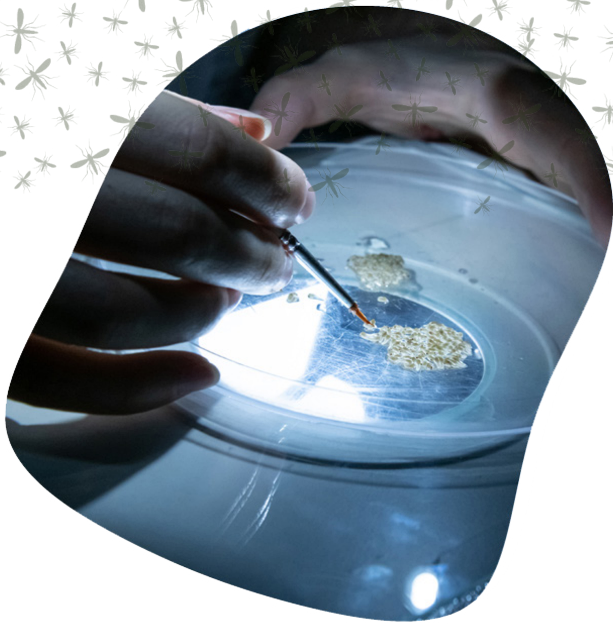
Non gene drive genetically modified male bias mosquitoes



In 2021, we concluded our work on the non gene drive genetically modified sterile male mosquitoes, following the small-scale release that took place in 2019, and subsequent monitoring and sharing of results. Our current phase of research is on a strain of mosquitoes that we call "male bias". It is a fertile, genetically modified mosquito, without a gene drive. This strain would only persist for some generations alongside a wild population in nature, which gives it limited ability to spread.

The purpose of this phase is to study this new strain in our insectaries in Africa, gather data and train our teams. We are engaging with stakeholders and developing transparent and robust relationships with regulatory authorities.

Describing the male bias mosquitoes

The male bias mosquito is fertile, so it can mate and have viable offspring. It is genetically modified to produce mainly male offspring (approximately 95% in the laboratory). Male mosquitoes do not bite and therefore do not transmit disease. Because it does not carry the gene drive technology (50% of the offspring carry the transgene through normal inheritance), in the context of an approved field release, the modification would only be passed on through a limited number of generations before fading out of the population, probably within two wet seasons. The male bias is paternal, this means that the males with the modification will produce a majority male offspring, whereas modified females that mate with males and carry the modification will have a normal sex ratio of 50% female and 50% male.

Characteristics of the non gene drive genetically modified male bias mosquitoes:

- No gene drive (the transgene is inherited by 50% of the offspring)
- Both females and males are fertile
- The male bias is paternal (males have mainly male offspring, females produce an equal number of males and females)
- In an approved release, the transgene would disappear from the mosquito population within a limited number of generations

The male bias mosquitoes were initially developed at the Crisanti Lab at the lab at Imperial College London (Galizi et al., 2014[1]), then bred and tested at our partner institutions Polo d'Innovazione di Genomica, Genetica e Biologia (PoloGGB) in Italy and the Centers for Disease Control and Prevention (CDC) in the US. Additional safety studies were also conducted by specialised independent research organisations.

The mosquitoes are used for contained use studies in some of our Arthropod Containment Level 2 (ACL2) insectaries in Africa.

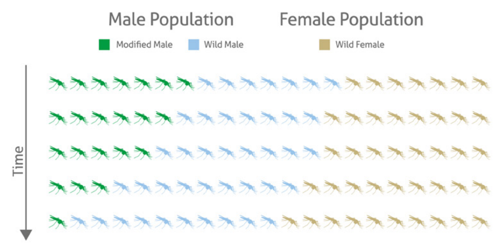
1 Galizi, R., Doyle, L., Menichelli, M. et al. A synthetic sex ratio distortion system for the control of the human malaria mosquito. Nat Commun 5, 3977 (2014). *https://doi.org/10.1038/ncomms4977*

The genetic modification to create male bias mosquitoes

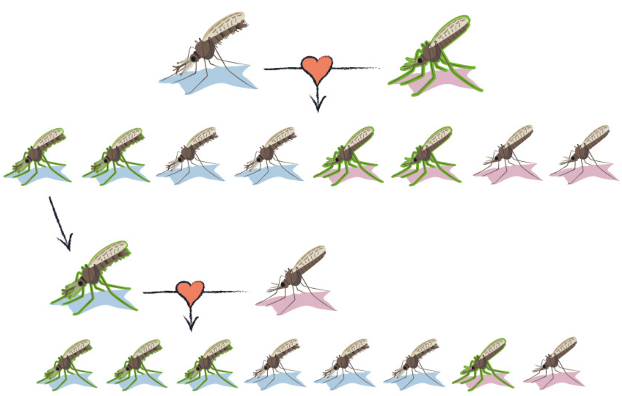
In mosquitoes, as for many animals, sex is determined by a pair of sex chromosomes. Females have two copies of the X chromosome, while males have one X and one Y chromosome. Mosquitoes also have two pairs of autosomes.

To produce the male bias mosquito, we use a nuclease gene (a DNA-cutting enzyme) that is inserted into a specific part of one of the mosquito's autosomes. When this gene is activated during sperm production, it breaks the X chromosome in most of the sperm, resulting in a mosquito that is left with mainly the intact Y-bearing sperm and thus produces mostly male offspring.

When there are significantly more **males** *than* **females** *in the mosquito population, female offspring will have more breeding opportunities than* **male** *offspring. Modified* **males** *that produce predominantly* **male** *offspring are therefore at a* **competitive disadvantage** *and so the modification will be selected out of the population over time.*



We have identified two fitness costs associated with the transgene. First, transgenic adult males have reduced fertility and, second, their female progeny have reduced pupal survival rates[2].



Autosome: An autosome is any chromosome that is not a sex chromosome. *Anopheles gambiae* mosquitoes have two pairs of autosomes and one pair of sex chromosomes. Each autosome in a pair is inherited at a rate of 50%.

Chromosome: A chromosome is an organized package of DNA found in the nucleus of the cell. Genes are contained in chromosomes.

Nuclease: A nuclease is an enzyme capable of cleaving nucleic acids, thus acting like a pair of molecular scissors that actually cleave the target DNA.

Testing and assessing the new strain

We first developed our modified strain under contained laboratory conditions at Imperial College London.

We then assessed the modified strain to characterize the molecular nature of the modification, assess fertility, fitness and male bias.

The strain was then shipped to our partner institutions Polo d'Innovazione di Genomica, Genetica e Biologia (PoloGGB) in Italy and the Centers for Disease Control and Prevention (CDC) in the US to be studied further.

The genetic modification was transferred (introgressed) by crossing the lab strain where it was initially generated into the genetic background of *Anopheles coluzzii* mosquitoes, the predominant species in many West African countries.

2 *https://besjournals.onlinelibrary.wiley.com/doi/full/10.1111/1365-2664.13702*

Introgression is important to generate a mosquito strain that is as similar as possible to the local mosquito population (in terms of fitness, insecticide resistance, vector competence). The only difference is due to the genetic modification introduced (in this case the male bias phenotype).

New strains of genetically modified mosquitoes have novel characteristics that are always first examined in contained laboratories. Following an analysis of potential risks and through extensive discussions with external stakeholders, we conducted a number of safety and efficacy studies in small and large cages as well as literature reviews to determine:

- The ability to rear the modified mosquito and to compare its life cycle performance to unmodified mosquitoes
- The capacity of modified males to compete with unmodified males in larger populations in a more ecologically complex setting
- The persistence of the modified mosquitoes in the population to predict the time it would take after any approved release in the field for the modification to disappear from the wild population
- If the male bias strain can transmit malaria or other diseases more or less as effectively than similar unmodified mosquitoes
- How susceptible the modified mosquito is to insecticides
- If the modified mosquito could extend its usual geographic ranges, to live in new areas with more extreme environmental conditions
- If the modified mosquito could potentially behave differently from unmodified mosquitoes in a way that might disrupt ecosystem services or reduce the density of valued species

The regulatory process

Target Malaria partners in Africa must submit, as a minimum requirement, a regulatory dossier, which includes all required information needed to assess the characteristics and safety of the mosquito strain, to the competent national

These cage studies typically involve stable overlapping-generation populations of up to 1000 mosquitoes at the start and last for up to one year

authority for biosafety in their country and receive approval before they can import the strain and study it in their own research facilities. Different countries may have other required approval steps.

After import, further laboratory studies gather the relevant data that can be used to pursue further regulatory authorizations if those studies indicate that a small-scale release experiment would be relevant. The national authorities that grant the permissions for field work vary between different partner countries, but the national biosafety authority is always involved.

Engaging our stakeholders

Target Malaria started engaging with stakeholders in our partner countries in 2012, when our first African collaborating partners joined the consortium. Target Malaria's approach to stakeholder engagement prioritizes those directly affected by our activities. Therefore, in line with our research activities, engagement started in local communities where we were collecting mosquitoes for entomological studies. Since then, as our work has advanced and our technology has progressed, our engagement has covered the latest developments in our research.

Our stakeholder engagement teams are made up of stakeholder engagement practitioners and social scientists whose role is to open and maintain a dialogue with a wide variety of stakeholders at local, regional and national levels.

Recent developments

At the moment, our partner institutions in Burkina Faso, *Institut de Recherche en Sciences de la Santé* (IRSS) and in Uganda, Uganda Virus Research Institute (UVRI), are maintaining a colony of male bias mosquitoes at their insectaries.

Our work on the male bias strain is providing our teams with the skills and training necessary for the future phases of our research. We hope that it will take us closer to developing genetic technologies to control malaria in Africa.