

# 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 two years of subsequent monitoring and sharing of results. Our next phase of research is on a new strain of mosquitoes that we call "male bias". It is a fertile genetically modified mosquito, without a gene drive. This strain will persist for some generations but with limited ability to spread further.

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

# Describing the male bias mosquitoes

The male bias mosquito is fertile, so it will mate and have viable offspring. It is genetically modified to produce mainly male offspring (up to 95% in the laboratory). Male mosquitoes do not bite and therefore do not transmit disease. Because it does not carry the gene drive technology, the modification will only be passed to a limited number of generations. The male bias is paternal, this means that while the males will have a majority male offspring, females will have a normal inheritance of 50% female and 50% male. Characteristics of the non gene drive genetically modified male bias mosquitoes:

- No gene drive
- Both females and males are fertile
- The male bias is paternal (males have male offspring, females have 50:50)
- Produce almost exclusively male offspring
- Modification lasts for up to two years

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

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



# 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.

To produce the male bias mosquito, we use a nuclease gene (a DNA-cutting enzyme) that is inserted into a specific part of the mosquito genome. When this gene is activated during sperm production, it breaks the X chromosome in many 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.

	Male Population Female Population
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Time	******
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We 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<sup>1</sup>.



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. It is one of the four classes of CRISPR–Cas-derived genome editing agents currently available for modifying genomes in experimental systems.

#### Testing and assessing the new strain

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

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 Center 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.

1 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 large cages to determine:

- The ability of the modified mosquito to develop and grow compared 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 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 the usual balance of ecosystems or increase the risk of disease

### The regulatory process

Target Malaria partners in Africa must submit, as a minimum requirement, a regulatory dossier to the competent national 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.

These contained studies will gather the relevant data that can be used to pursue further regulatory authorizations if the laboratory 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 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 development of 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 institution in Burkina Faso, Institut de Recherche en Sciences de la Santé (IRSS), will lead the research on the colony of male bias mosquitoes at its ACL2 insectary in Bobo-Dioulasso, Burkina Faso. The insectary was inspected and certified by the National Biosafety Agency in early 2022.

Following the approvals from the national competent authority for biosafety, the National Biosafety Agency (ANB), and the communities around the insectary in Burkina Faso, the non gene drive genetically modified male bias strain was shipped in March 2022 from Italy to the IRSS. It will be studied in contained use experiments for several years.

Our work on the male bias strain will provide 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.

