TARGET MALARIA
A Vector Control Research Alliance

Information note

Male bias: Background strain and transgene insertion site

Background

As part of our step-by-step development pathway, Target Malaria concluded in 2021 its work in Africa on the sterile male strain and is now focusing its efforts on the "male bias" strain [1]. Currently, this strain is being assessed under a Contained Use Permit by our team at Institut de recherche en Sciences de la Santé (IRSS) in Burkina Faso. We are planning future studies with this strain at the Uganda Virus Research Institute (UVRI) in Uganda as well. This is in addition to the work already performed by our teams in London (Imperial College), Italy (PoloGGB), and the US (CDCF).

For more information: Factsheet on the male bias mosquito

As part of our engagement activities, stakeholders have indicated an interest in understanding in more detail the male bias strain, including the genetic background and the insertion site of the genetic modification. This information note aims at providing more detailed and technical information on these topics.

Mosquito strains in Africa

Worldwide there are more than 3500 species of mosquitoes, with 837 of those species in Africa. Malaria is spread by the bite of female *Anopheles* mosquitoes. More than 500 *Anopheles* species have been described worldwide, and more than 30 are considered a public health problem. In sub-Saharan Africa only four species are responsible for the majority of malaria transmission; the closely related *An. gambiae*, *An. coluzzii* and *An. arabiensis*, and *An. funestus*.

Background species of the male bias strain

The male bias strain founding line was developed by Target Malaria in the mosquito strain called "G3". This is a lab-adapted background strain that was originally collected from the field in 1975 and **identified as *An. gambiae*** from the *An. gambiae sensu lato (s.l.)* species complex.[1] This is a strain routinely used by many laboratories investigating *Anopheles* biology and malaria research so there is a lot of information available on this strain.

[1] https://www.beiresources.org/Catalog/BEIVectors/MRA-112.aspx

When the G3 mosquito species was established in the laboratory in 1975, *An. gambiae* was recognised as a single species. Since that time, extensive genetic analysis of "fresh" wild-caught *An. gambiae* has led to its re-classification in 2013 into two distinct species[2]: [2]

- *An. gambiae sensu stricto (s.s.)*
- *An. coluzzii* [1]

The two species *An. coluzzii* and *An. gambiae s.s.* are extremely closely related and are both part of the *An. gambiae s.l.* species complex (which contains at least nine sibling species). Because of the close evolutionary relationship between *An. gambiae s.*s. and *An. coluzzii*, **interspecific hybrids are still routinely found in the wild**, albeit at low frequencies [2-5]. The phenomenon of "**porous species boundaries**" has been well-established for many years in the ***An. gambiae s.l.*** species complex, as well as other insects [6,7].

To generate a genetically modified strain with a genetic background like the local mosquito population, the male bias strain was repeatedly backcrossed in the lab to a wild-type (WT) colony derived from local field populations of *Anopheles* mosquitoes, a process known as **introgression**:

- In the case of Burkina Faso, this consisted of a local *An. coluzzii* population [8]
- In the case of Uganda, this consisted of a local *An. gambiae* population

These local WT laboratory colonies are also periodically refreshed with mosquito larvae collected from the field to ensure the genetic background remains close to that of the mosquitoes found in the local natural environment.

Location of the male bias transgene

The process of genetically modifying *An. gambiae* mosquitoes starts with injection of plasmid DNA containing the transgene into embryos from the G3 lab-strain. The adults that emerge from those injections are mated to wild type G3 mosquitoes. The larvae produced are screened for the fluorescent marker that is part of the transgene using a fluorescence microscope; those that fluoresce are genetically modified.

In the case of the male bias strain, the transgene is found on **Chromosome 2R 19D (Figure 1)**. The location was determined using a combination of **DNA sequence analysis** and ***in-situ* hybridisation** in the laboratory.

The location of the male bias transgene on Chromosome 2R 19D is in a poorly annotated centromeric region. In general, reference genome data consists of a combination of DNA sequences plus annotation, which includes information about these sequences, such as location of genes, whether they code for proteins, regulate genetic processes, or have no known function. Repetitive and unannotated DNA sequences pose a challenge to the characterisation of genomes. [9]

[2] tps://pubmed.ncbi.nlm.nih.gov/26131476/

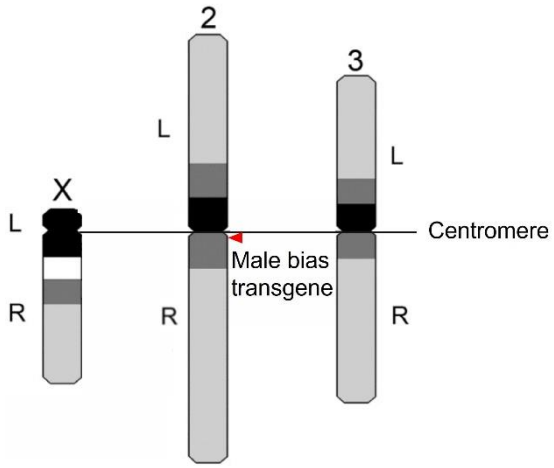

Figure 1: A schematic overview of the *An. gambiae s.s.* and *An. coluzzii* chromosomes X, 2 and 3 adapted from Sharma *et al.*, 2020 [10]. The transgene is located on the right arm (R) of chromosome 2, shown as a red triangle. The various dark shades and the white shade represent areas in the Chromosome that are densely packed and more difficult to sequence. The centromere connects the left (L) and right (R) arm of the chromosomes.

Initial assessments of the male bias transgenic strain, along with numerous other strains, were based on preliminary data using a common molecular technique known as inverse PCR, which characterizes the DNA either side of the genetic insert. If the DNA flanking the insert is annotated in the genome, that tells you where the transgene has integrated. When the male bias laboratory studies were published in 2014, this limited data set suggested that the transgene was at chromosome location 3R 36D in the genome [1]. Aware of the need for additional investigations, Target Malaria researchers continued to study the location of the transgene using an expanded array of different molecular and cytological methods, such as Whole Genome Sequencing, Southern analysis and Fluorescent *In situ* Hybridisation (FISH) of polytene chromosomes [9].

Combined with improved *Anopheles* reference genome sequences available through the work of the 1000 *Anopheles* Genomes Consortium [11], the additional research carried out by the Target Malaria team in London pinpointed the specific location of the transgene to a highly repetitive area of the *Anopheles* genome on Chromosome 2R 19D [9]. This means that the sequence of mosquito DNA surrounding the transgene can be found at many locations of the genome, which is why **the initial observation of its location was reallocated, based on more detailed and accurate analyses.**

These developments are positive and represent scientific progress. These later results represent the output from more detailed scientific investigations and are a classic example of the scientific process in action: to continuously update and refine interpretations of reality based on the latest data and evidence. Target Malaria researchers published these results, subjecting them to peer review and the transparency of the publication process [9].

Conclusion

It is important to note that the new information on the location of the male bias transgene does not change the risk assessment. The activity of the transgene and its effect to induce male bias is unchanged. The transgene is stable and is inherited in a Mendelian fashion to 50% of its offspring [12]. Thus, the newly gained information on the location of the transgene has not changed the risk profile of this transgenic strain in the field [9, 12, 13].

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