Field entomology and laboratory work

The entomological work carried out by Target Malaria helps lay the foundations for every other aspect of the project's activities, from engagement, to modelling, to regulatory approval and beyond. Entomological studies typically represent the first stage in any in-country work, accompanied by stakeholder engagement with local communities at sites were field work is planned or carried out. Only if this work is accomplished successfully can in-country teams proceed to the next stages of the project.

Target Malaria's entomological work focuses on *Anopheles* mosquitoes, as they are the main vector of malaria in Sub-Saharan Africa. Within *Anopheles*, Target Malaria is currently most concerned with members of the *Anopheles gambiae* species complex, including *Anopheles coluzzii, Anopheles arabiensis*, and *Anopheles gambiae*.

Entomology work in Africa

Examples of the activities that the entomology teams in Africa are currently engaged in include field collection and analysis of local mosquito populations. Once the mosquitoes are collected, if dead specimens are what the field team targeted, they are taken to the laboratory to be examined and dissected. This may involve tests to determine what the insects last fed upon, whether they are carrying the *Plasmodium* parasite that causes malaria, and which species or sub-species they belong to. This can be done by examining the distinctive markings of individuals (for example when distinguishing between the *Anopheles, Culex,* and *Aedes* genera) or may involve microscopic or molecular analysis, as this is the only way to tell different members of the *Anopheles* genus apart. Data on the abundance of the different vectors of malaria collected inside and outside homes at different times of the year help Target Malaria establish detailed longitudinal studies of mosquito behaviour and population dynamics that also inform models of vector control interventions.

If live specimens are collected, they may be put to a very different set of uses. Entomological teams use live mosquitoes or larvae to establish and maintain wild mosquito colonies in contained insectaries. These colonies help researchers examine the behaviours and characteristics of local populations, to help answer questions around the spread of traits from generation to generation, and the ways in which resistance to various vector-control methods arises and spreads within wild populations. Data from these wild type mosquito colonies can also be compared with that collected from the populations of genetically-modified mosquitoes maintained in contained laboratories at our partner institution in Terni, Italy. It may eventually be compared with any gene-drive construct developed to provide valuable information on the relative effectiveness and fitness of the modified mosquito strains. Without this information, it would not be possible to model the potential benefits and/or risks of any proposed mosquito that Target Malaria is genetically modifying for population control.

Data from entomology activities is a key part of the information needed for submissions for regulatory approval that Target Malaria must make to national biosafety authorities. It is also important for outreach, education, and collaboration activities undertaken with local stakeholder communities, to ensure that any final technology addresses their needs and concerns, and is met with their full agreement.

"Mark , Release, Recapture" experiment (MRR)

Wild type live mosquitoes may also be used for "mark, release, recapture" experiments, in which male mosquitoes are marked with fluorescent powder and then released at village sites. Following the release, the entomology teams will then return daily to the same site to carry out field collections. The results of these collections are analysed to determine the number of previously captured and marked mosquitoes present. This provides valuable information on how long mosquitoes survive in nature, how large the local mosquito population is and how far released individuals can be expected to disperse geographically or persist in local populations over time. This information is a key determinant in modelling the potential environmental impacts of any release of genetically modified mosquitoes.

It is important to note that entomological collections and analyses have been carried out from the start of the project and will continue throughout its lifetime. This helps to guarantee that the project will have enough data to establish benchmarks and develop a full and accurate picture of mosquito population size and dynamics at different target sites over time, considering local seasonal variations.