

Development of a genetic sexing strain in *Anopheles stephensi*

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The Sterile Insect Technique (SIT) is an environmental friendly and species-specific method of pest control that relies on the release of large numbers of sterile males to achieve suppression or eradication of field populations. The feasibility of SIT was demonstrated by the eradication of the New World screwworm, *Cochliomyia hominivorax*, from North and Central America. Since then, SIT has been successfully applied to other insect species, including the agricultural pest *Ceratitis capitata*. Several releases of irradiated mosquito species have been carried out in the past to investigate SIT-related feasibility issues such as dispersal rates and mating competitiveness, and in some cases with the aim to reduce vector population. However, irrespective of the mosquito species and experimental conditions used, the lack of suitable and efficient genetic sexing methods to separate males from female individuals, has represented a major hurdle to the implementation of SIT in mosquitoes.

A successful SIT requires that large numbers of sexually active but sterile males must be released over large areas. The release of sterile females does not contribute to the population suppression, and in fact produces a negative effect. In a controlled SIT study performed on *C. capitata*, a release program was 3–5 times more effective in reducing the population size of a target field population when populations of male-only insects were released. Furthermore in SIT programs applied to mosquitoes, the release of both sexes must be carefully avoided, as female insects contribute to disease transmission. In previous mosquito releases, separation of the two sexes was achieved by complicated or inefficient procedures based on the size of the pupae and on pseudo-linkage of sex chromosomes to insecticide resistance and pupal color alleles. The success met in *C. capitata* on the other hand has highlighted the importance of developing effective and reliable genetic sexing strains for SIT programs.

Here we report on the development of the first genetic sexing strain in *Anopheles stephensi* mosquitoes. In this strain, male individuals are identified by their fluorescent gonads as early as at the 3rd instar larva stage, and can be efficiently separated from females using both manual methods and automated sorting machines. Importantly, transgenic males are not impaired in mating competitiveness and viable fluorescent spermatozoa are also detected in spermathecae of wild-type females mated with transgenic males. These lines combine most of the features desired for a safe assessment of the use of transgenic methodologies for malaria-control programs.