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The functional genomics of genitalia rotation in male *Anopheles funestus*

by

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Abstract

Malaria is at the forefront of diseases transmitted by mosquito vectors worldwide with approximately 409, 000 deaths and over 229 million cases in 2019. Malaria is caused by the *Plasmodium* parasite and is transferred between human hosts by mosquito vectors. The reproductive cycle of the *Plasmodium* parasite takes place within the human host and the *Anopheles* mosquito vector. The *Anopheles funestus* malaria vector is anthropophilic and endophilic (i.e., preferring indoor human feeding). In several African countries a concern for the sustainable control of *An. funestus* is problematic due to the rapid development of its insecticide resistance. A lack of research of this species' biology limits the development of novel control interventions and this fact forms the rationale to study the biology of the *An. funestus* male mosquito. This includes understanding the adult male mosquito's sexual characteristics, physiology, and swarming behaviour, such as genitalia rotation, antennal fibrillae erection and male accessory gland development. The *An. funestus* male genitalia rotation is an important step prior to mating. The genitalia of the newly emerged *An. funestus* male has to rotate between 135° and 180° for the male to become sexually mature. Since the inversion of the genitals is of great importance to the process of sexual maturation and reproduction, an understanding of the underlying genetic mechanisms of male genitalia rotation in this mosquito could be fundamental to vector biology studies.

The aim of this study was to add to the scientific body of knowledge on this complex physiological change in the male mosquito. This was achieved by characterising the physical state of inversion in males that actively participated in swarming in a wild *An. funestus* population in Zambia. Wild *An. funestus* swarms have never been molecularly identified to a species level by using multiplex PCR and molecular assays nor has the rotation status of their genitalia been reported.

It was found that immature male anophelines do not participate in swarming until their genitalia have rotated to Stage 4 (90 - 135°), which indicates that behavioural change occurs with the onset of male sexual maturation. This study identified and validated putative transcripts associated with the male *An. funestus* sexual maturation process with the use of RNA-sequencing, next generation sequencing technologies and real-time quantitative PCR. RNA-sequencing found that several cuticular transcripts that were down regulated in Stage 3,

and this was validated with qPCR. A hypothesis was developed that the down regulation of the cuticular transcripts allows for the cuticles to remain soft in order to allow for the genitalia rotation to take place without the cuticle breaking. RNA interference (traditional as well as novel chitosan nanoparticles) was subsequently used as a gene silencing tool to knock-down specific transcripts (RPL19 and AFUN000965). The phenotypic effects of this knock-down were observed with regards to male sexual maturation development of the genitalia rotation, accessory glands, spermatogenesis, and male palp erection.

The results obtained, identified a large number of transcripts associated with the biological sexual maturation in the male *An. funestus* mosquito and could lead to identifying potential targets for novel vector control interventions for malaria.