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Analysis of the genitalia rotation in the male *Anopheles funestus* (Diptera: Culicidae)

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ABSTRACT

Anopheles funestus is a major malaria vector in Africa. Insecticide resistance has developed in populations of this species in several African countries, prompting the need to develop additional vector control methods such as the sterile insect technique (SIT). This technique requires an understanding of those underlying physiological events that lead to sexual maturity of *An. funestus* males, the rotation of their genitalia in particular. The aim of this study was to qualitatively and quantitatively describe genital rotation in *An. funestus* males as it is an essential function of sexual maturation. Genital rotation of all the males reached its final rotation stage (135–180° rotation) 36 h post emergence at 23 ± 1 °C in laboratory colonised *An. funestus* males. These males had a comparable rotation rate to wild caught *An. funestus* at the same temperature setting. A temperature change (either 18 ± 1 °C or 29 ± 1 °C versus 23 ± 1 °C) significantly influenced the genital rotation rate such that this rate increased with increasing temperature. This information enhances our knowledge of the *An. funestus* male biology. This is important in terms of applying the sterile insect technique as the understanding and manipulation of the rate of sexual maturation in males has implications for the timing of sterile male release.

1. Introduction

Malaria is one of the deadliest vector borne diseases, with an estimated 660,000 malaria deaths reported worldwide in 2010 of which the greatest proportion (90%) occurred in Africa (World Health Organisation, World Malaria Report 2012 FACT SHEET). *Anopheles funestus* is a major malaria vector in Africa and can be successfully controlled using indoor residual spraying (IRS) (Abilio et al., 2011; Chanda et al., 2011; Sinka et al., 2012). However, *An. funestus* populations have developed insecticide resistance in several countries including Benin, Burkina Faso, Ghana, Guinea, Kenya, Mali, Malawi, Mozambique, South Africa, Uganda and Zambia (Abilio et al., 2011; Brooke et al., 2001; Chanda et al., 2011; Coetzee and Koekemoer, 2013; Djouaka et al., 2011; Hargreaves et al., 2000; Hunt et al., 2011; Morgan et al., 2010).

Several recent studies have focussed on novel control interventions directed against the major African malaria vectors *Anopheles arabiensis* and *Anopheles gambiae* (Andreasen and Curtis, 2005; Helinski et al., 2009; Hood-Nowotny et al., 2006; Marois et al., 2012; Munhenga et al., 2011; Nolan et al., 2011; Oliva et al., 2011), yet there is a paucity of information concerning the adaptation of these techniques against *An. funestus* (Helinski et al., 2006). *Anopheles funestus* occurs in sympatry with *An. arabiensis* and *An. gambiae* in many African localities, and it is therefore important to consider all three species when novel or additional control interventions are considered or developed, including the renewed interest in the sterile insect technique (SIT) (Helinski et al., 2009; Hood-Nowotny et al., 2006; Nolan et al., 2011; Oliva et al., 2011; Sinka et al., 2012; Townson, 2009).

The SIT involves the mass release of sterile males that are fit enough to successfully compete for conspecific mates in the wild, ultimately leading to population suppression and even eradication (Catteruccia et al., 2009; Nolan et al., 2011). The application of SIT has successfully eradicated several populations of agricultural pests (reviewed by Dyck et al., 2005; Townson, 2009) such as the New World screwworm fly in Libya (Vargas-Terán et al., 1994), melon fly in Okinawa and the Mediterranean fruit fly from Chile and southern Peru (Hendrichs, 2000) as well as the population of the trypanosomiasis vector, the tsetse fly, in Zanzibar (Vreysen et al., 2000).

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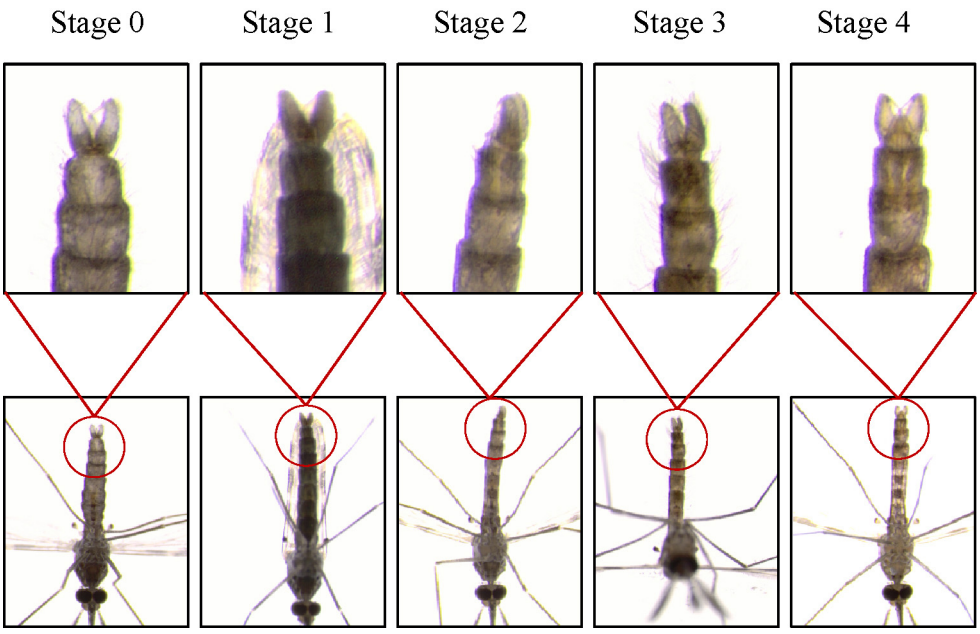

Fig. 1. The different stages of genital rotation in *An. funestus* males when viewed from ventral side.

A popular method for the sterilisation of males for SIT involves the exposure of males to gamma radiation, which leads to chromosomal damage in the germ cells and ultimately the death of the early developing embryo after fertilisation (Catteruccia et al., 2009; Helinski et al., 2009; Nolan et al., 2011). In *Anopheles* species the females are monogamous, meaning that they are likely to mate only once (Baimai and Green, 1987; Charlwood et al., 2003; Magnusson et al., 2011). If the majority of matings occur with sterile males, and wild fertile males are essentially precluded from mating, the subsequent generation will be reduced.

Since the primary principle of SIT concerns copulation by sterile males, it is important to understand male biology in terms of sexual development. Upon emergence from the pupal case, rotation of the male genitalia has to occur before the male is able to mate (Howell and Knols, 2009; Ross and Roberts, 1943). Male genitalia are composed of abdominal segments 8–10 and include the claspers tipped with claws (Rees and Onishi, 1951; Ross and Roberts, 1943). These claspers are located on segment 10 and must rotate 180° post emergence in order to allow the male to grasp the female during copulation (Howell and Knols, 2009; Rees and Onishi, 1951; Ross and Roberts, 1943). Observations of genital rotation being influenced by laboratory conditions and ambient temperature have been made in *An. arabiensis* (Oliva et al., 2011) and *Aedes taeniorhynchus* (Provost et al., 1961), respectively.

The time needed for the claspers to complete their rotation appears to be species-specific and a limited number of studies have been done regarding genital rotation in Culicidae males (Bohart and Washino, 1978; Chevone and Richards, 1976; Khan and Reisen, 1977; Mahmood and Crans, 1999; Oliva et al., 2011, 2012; Provost et al., 1961; Rees and Onishi, 1951; Smith and Gadawski, 1994). The aim of this study was to qualitatively and quantitatively describe genital rotation in *An. funestus* males, as this is an essential function of sexual maturation.

2. Materials and methods

2.1. Laboratory An. funestus samples

Samples of adult males from *An. funestus* laboratory colony were used in this study. The colony was established in 2000 using wild-caught material from southern Mozambique (Hunt et al., 2005) and is housed in the Botha de Meillon insectary of the National Institute for Communicable Diseases (NICD), Johannesburg. This colony (FUMOZ-FUnestus from MOZambique) is maintained under standard insectary conditions of 23 ± 1 °C and 84% relative humidity with a 12:12 light/dark cycle and 45 min dusk/dawn simulation.

2.2. Wild An. funestus samples

An. funestus females were collected indoors from houses in the Kibali/Doko region, in the Democratic Republic of Congo (N03°07.929, E029°34.120, altitude 854 m) in July 2012. Live females were brought back to the NICD and induced to lay eggs. Eggs were reared through to F1 adults from which samples of males were drawn for use in this study. Rearing and insectary conditions were as described above.

2.3. Examination of genital rotation

Emerging males were collected at 30 min intervals and placed in 2 L cages supplemented with a 10% sucrose solution. Samples of 23–35 wild (F1) *An. funestus* males and 30–50 FUMOZ males were drawn at each time point during the experiment. The wild males were maintained at 23 ± 1 °C and 84% relative humidity. The FUMOZ males were maintained at either 18 ± 1 °C with 80% relative humidity, 23 ± 1 °C with 84% relative humidity or 29 ± 1 °C with 74% relative humidity.

Adult males (aged between 2 and 48 h) were removed from the cages at specific times and placed at −20 °C until the examination of their genitalia rotation, which in general was within 48 h. The degree of rotation of their genitalia was recorded and classified according to a scheme of different rotation stages (Oliva et al., 2011; Provost et al., 1961). At stage 0 there was no rotation of the genitalia, at stage 1 the genitalia were rotated between 0 and 45°, at stage 2 the genitalia were rotated between 45° and 90°, at stage 3 genitalia were rotated between 90° and 135° and by stage 4 genitalia had rotated between 135° and 180° (Fig. 1).