

## Mosquito mating behaviour

Willem Takken<sup>1</sup>, Carlo Costantini<sup>2</sup>, Guimogo Dolo<sup>3</sup>, Ahmed Hassanali<sup>4</sup>,  
N'Fale Sagnon<sup>2</sup> and Eli Osir<sup>4</sup>

### Abstract

Mating is one aspect of behaviour that has been much ignored in mosquito biology. Yet, the success of a transgenic release strategy depends on normal, competitive mating between introduced and wild individuals. An overview is presented of current knowledge of mating behaviour in *Culicidae*, including timing of mating, means of sperm transfer, refractory behaviour and multiple mating. Most lacunae were found in mate finding: it is known that some species use swarming while other mate on or near the vertebrate host. At short range males locate females by acoustic signals, but there is no knowledge how the sexes locate each other from a distance. It is argued that mass rearing of mosquitoes for sterile-insect release or transgenic release should include steps to safeguard male fitness. A series of challenges for future studies are discussed, including cues that control swarming behaviour, mate-finding behaviour and identification of genes that control mating behaviour.

**Keywords:** *Culicidae*; fitness; swarming; multiple mating; sperm; gene

### Introduction

Of the critical behaviours that characterize the mosquito life strategy, mating is probably the least understood and most understudied. Yet, as mosquitoes depend on sexual reproduction for species maintenance, this aspect of mosquito biology should receive the highest attention when seeking new avenues for mosquito control and interventions for mosquito-borne disease. Which behavioural steps need to be considered when mating is concerned? As a rule, newly emerged male mosquitoes are unfit for coupling with a female, as the external genitalia require a morphological change. This is accomplished by inversion of the terminalia within the first 24 hr following emergence. In many species, male accessory glands mature during the first few days of adult life, and this is needed before sperm can be successfully transferred (Clements 1999). Thus, males of many mosquito species require several days to mature before a first successful mating can take place. In *Anopheles gambiae* Giles *sensu stricto* and *An. arabiensis* Patton optimal mating occurs with 5–7-day-old males

<sup>1</sup> Laboratory of Entomology, Wageningen University and Research Centre, PO Box 8031, 6700 EH Wageningen, The Netherlands. E-mail: Willem.Takken@wur.nl

<sup>2</sup> Centre National de Recherche et Formation sur le Paludisme (CNRFP), 01 B.P. 2208, Ouagadougou, Burkina Faso. E-mail: costantini.cnlp@fasonet.bf

<sup>3</sup> Malaria Research and Training Center, Faculté de Médecine de Pharmacie et d'Odonto-stomatologie, B.P. 1805, Bamako, Mali. E-mail: dolo@mrtcbko.org

<sup>4</sup> International Centre of Insect Physiology and Ecology (ICIPE), PO Box 30772, Nairobi, Kenya. E-mail: ahassanali@icipe.org ; eosir@icipe.org

(Reisen 2003; Verhoek and Takken 1994). In other species, males may mate at an earlier age, but sperm may then not be successfully transferred due to immaturity. Females, by contrast, are ready to mate almost as soon as they emerge from the pupal cases. As an extreme example, in some species females are inseminated immediately following emergence by males who sit and wait next to the emergence site to pounce on any female as they are unfolding their wings, or they even grab the female pupa shortly before emergence (Provost and Haeger 1967). In most species, though, there is a 24–48-hour time lag between emergence and mating. Mating is not needed for egg development and maturation, but in most species eggs can only be deposited when insemination has occurred (Clements 1999). As a rule, female mosquitoes mate before taking a first blood meal, but in several anophelines a large proportion of virgins may blood-feed prior to mating. Such a blood meal is essential for the development of a metabolic energy reservoir (Gillies 1954; Lyimo and Takken 1993; Takken, Klowden and Chambers 1998). Many females may imbibe nectar or other carbohydrate sources prior to mating, presumably again to acquire an energy reservoir for flying and mate-finding (Foster 1995; Foster and Takken 2004). In *Aedes aegypti* L. mating is accompanied by a change in behaviour, caused by the transfer of ‘matrone’, a male hormone, which makes the female refractory to successive matings and induces host-seeking behaviour (Craig Jr 1967). A similar hormonal effect was also reported from *Culex tarsalis* Coquillett. Such behavioural physiology does not occur in *An. gambiae* s.s., where male accessory-gland substances do not induce a change in female behaviour (Klowden 2001). The success of male mating is determined by fitness, and this may have consequences for the number of times a male can mate. Obviously, this is determined by male size and feeding behaviour, and the efficiency of finding nectar sources. Aspects governing male fitness are poorly understood and appear to be difficult to estimate, in particular in the field (Charlwood 2003).

One of the most critical issues in mosquito mating is our lack of understanding of mate-finding. Many culicine species, characteristically, mate in swarms, when males aggregate in sometimes large numbers, forming nearly-cylindrical swarms of several metres height. This has been observed most notably in the genera *Anopheles*, *Culex* and *Ochlerotatus*, but species of other genera may also exhibit swarming (Clements 1999). Such swarms are often found in characteristic sites, presumably guided by a visual marker (Marchand 1984; Charlwood et al. 2002b; Yuval and Bouskila 1993; Charlwood, Thompson and Madsen 2003; Yuval, Wekesa and Washino 1993). It is unknown how males aggregate or what factors influence the sustenance of swarms. Even more intriguing is the fact that we do not know how females locate male swarms. Single females fly into the swarm and are detected by their lower wing-beat frequency (Belton 1994; Clements 1999). Several males may arrive near the female, which departs with one of them from the swarm *in copula*. Larger males were reported more successful in mating than smaller ones (Yuval and Bouskila 1993; Yuval, Wekesa and Washino 1993) although Charlwood et al. (2002a) showed that in *An. gambiae* Giles s.s. there was no effect of male body size on mating success. Intriguingly female body size has also an advantage in mate selection, larger females of *An. gambiae* s.s. being preferentially selected for mating (Okanda et al. 2002). It has been suggested that female swarm finding is directed by olfactory cues (Takken 1999; Takken and Knols 1999), perhaps, in addition, aided by the same visual cues that guide males to swarming sites. Many culicines mate near the vertebrate host, males of *Mansonia* spp. being attracted to host odours (McIver, Wilkes and Gillies 1980) and therefore being able to locate females in search of a blood meal. In

conclusion, other than proof of acoustic communication between the sexes, the behavioural process governing mating in mosquitoes remains a black box.

Whereas hybridization between closely related species has frequently been observed in the laboratory (Davidson 1964), such encounters are relatively rare in the wild (White 1971; Tripet et al. 2001). Apparently mating barriers exist, which serve to prevent coupling between related species and, hence, waste of resources. Nevertheless, Tripet et al. (2001) reported 1.2% cross-mating between two molecular forms of *An. gambiae s.s.* in Mali, demonstrating that cross-form hybridizations are not entirely excluded. As a rule female mosquitoes become refractory to male encounters following insemination (see above), but from laboratory studies it is well-known that female *An. gambiae* can mate several times (Charlwood and Jones 1979; Gomulski 1990). Field studies concerning this aspect are rare, but molecular techniques using genetic fingerprinting have now been developed that allow detailed study of this phenomenon. For instance, it was reported that in *An. gambiae s.s.* up to 2.5% of field-collected females had been inseminated by at least 2 different males, of which two-thirds had mated with males of the same chromosomal form (Tripet et al. 2003). As studies on genetic exchange between mosquito populations are important with regard to population genetics and behaviour, the extent of multiple matings needs to be considered as well.

## Genetic control and mating behaviour

Past efforts for the genetic control of mosquitoes using the sterile-insect technique (SIT) have been less successful than expected, partially because of low degree of competitiveness between sterile and wild males (Lounibos 2003; Reisen 2003). Many mosquito species can be cultured in large numbers under controlled conditions, but due to genetic selection and loss of natural traits, such insects may behave differently from their wild siblings. Newly developed tools for genetic manipulation of mosquitoes rendering them refractory to human pathogens or altering host preference appear promising, in theory, as effective solutions for disease control (Ito et al. 2002; Besansky, Hill and Costantini 2004). However, the required establishment of laboratory cultures and subsequent genetic transformation of target mosquito species may result in insects with widely different mating behaviours compared to their wild siblings. Unless competitive ability and mating behaviour are adequately understood, the release of transgenic or sterilized mosquitoes may result in failures akin to those observed in several former SIT studies.

## Challenges for future research

Mating in mosquitoes remains a poorly understood process. Yet, successful mating is critical for the success of proposed strategies for vector-borne-disease control using SIT or genetically modified mosquitoes (GMM). Some progress with studies on mating behaviour under field conditions has recently been reported with anophelines in São Tomé and Mozambique (Chambers and MacAvoy 2000; Charlwood et al. 2002a; 2002b; Charlwood, Thompson and Madsen 2003). However, such studies are few, and do not answer the question of how mating is accomplished and by which factors it is regulated. As insemination of wild female mosquitoes by released transgenic or sterile males is obviously a requirement for any genetic-control

programme, it is proposed that research focuses on the following aspects of mating behaviour:

- Cues that control male swarming
- Male feeding behaviour and fitness
- Female mate-location behaviour
- Pre- and post-mating behaviour
- Frequency of multiple-species swarming
- Genes that affect and/or regulate mating behaviour
- Factors that prevent hybridization of closely related species
- Factors that control multiple mating.

These aspects appear critical for a proper understanding of mosquito population biology and genetics. For instance, in population modelling of the behaviour of gene transfer between GMM and wild populations, the frequency of wild versus GMM matings should be well understood in order to predict the number of released individuals required for effective results. Also, SIT programmes require a constant monitoring of wild versus sterile matings to adjust the release rate over time. Finally, any driving mechanism of foreign DNA into wild populations requires a normal mating behaviour, and can only be evaluated once this behaviour is properly understood.

## References

- Belton, P., 1994. Attraction of male mosquitoes to sound. *Journal of the American Mosquito Control Association*, 10 (2 Part 2), 297-301.
- Besansky, N.J., Hill, C.A. and Costantini, C., 2004. No accounting for taste: host preference in malaria vectors. *Trends in Parasitology*, 20 (6), 249-251.
- Chambers, G.K. and MacAvoy, E.S., 2000. Microsatellites: consensus and controversy. *Comparative Biochemistry and Physiology. Part B. Biochemistry and Molecular Biology*, 126 (4), 455-476.
- Charlwood, J.D., 2003. May the force be with you: measuring mosquito fitness in the field. In: Takken, W. and Scott, T.W. eds. *Ecological aspects for application of genetically modified mosquitoes*. Kluwer Academic Publishers, Dordrecht, 47-62. Wageningen UR Frontis Series no. 2.
- Charlwood, J.D. and Jones, M.D.R., 1979. Mating behaviour in the mosquito, *Anopheles gambiae s.l.* I. Close range and contact behaviour. *Physiological Entomology*, 4 (2), 111-120.
- Charlwood, J.D., Pinto, J., Sousa, C.A., et al., 2002a. Male size does not affect mating success (of *Anopheles gambiae* in São Tomé). *Medical and Veterinary Entomology*, 16 (1), 109-111.
- Charlwood, J.D., Pinto, J., Sousa, C.A., et al., 2002b. The swarming and mating behaviour of *Anopheles gambiae s.s.* (Diptera : Culicidae) from São Tomé Island. *Journal of Vector Ecology*, 27 (2), 178-183.
- Charlwood, J.D., Thompson, R. and Madsen, H., 2003. Observations on the swarming and mating behaviour of *Anopheles funestus* from southern Mozambique. *Malaria Journal*, 2 (1), 10 p. [<http://www.malariajournal.com/content/2/1/2>]
- Clements, A.N., 1999. *The biology of mosquitoes. Vol. II. Sensory reception and behaviour*. Cabi, Wallingford.

- Craig Jr, G.B., 1967. Mosquitoes: female monogamy induced by male accessory gland substance. *Science*, 156 (781), 1499-1501.
- Davidson, G., 1964. The five mating types in the *Anopheles* complex. *Rivista di Malariologia*, 43, 167-183.
- Foster, W.A., 1995. Mosquito sugar feeding and reproductive energetics. *Annual Review of Entomology*, 40, 443-474.
- Foster, W.A. and Takken, W., 2004. Nectar-related vs. human-related volatiles: behavioural response and choice by female and male *Anopheles gambiae* (Diptera: Culicidae) between emergence and first feeding. *Bulletin of Entomological Research*, 94 (2), 145-157.
- Gillies, M.T., 1954. The recognition of age-groups within populations of *Anopheles gambiae* by the pre-gravid rate and the sporozoite rate. *Annals of Tropical Medicine and Parasitology*, 48, 58-74.
- Gomulski, L., 1990. Polyandry in nulliparous *Anopheles gambiae* mosquitoes (Diptera: Culicidae). *Bulletin of Entomological Research*, 80 (4), 393-396.
- Ito, J., Ghosh, A., Moreira, L.A., et al., 2002. Transgenic anopheline mosquitoes impaired in transmission of a malaria parasite. *Nature*, 417 (6887), 452-455.
- Klowden, M.J., 2001. Sexual receptivity in *Anopheles gambiae* mosquitoes: absence of control by male accessory gland substances. *Journal of Insect Physiology*, 47 (7), 661-666.
- Lounibos, L.P., 2003. Genetic-control trials and the ecology of *Aedes aegypti* at the Kenya coast. In: Takken, W. and Scott, T.W. eds. *Ecological aspects for application of genetically modified mosquitoes*. Kluwer Academic Publishers, Dordrecht, 33-43. Wageningen UR Frontis Series no. 2. [[http://library.wur.nl/frontis/malaria/04\\_lounibos.pdf](http://library.wur.nl/frontis/malaria/04_lounibos.pdf)]
- Lyimo, E.O. and Takken, W., 1993. Effects of adult body size on fecundity and pre-gravid rate of *Anopheles gambiae* females in Tanzania. *Medical and Veterinary Entomology*, 7, 328-332.
- Marchand, R.P., 1984. Field observations on swarming and mating in *Anopheles gambiae* mosquitoes in Tanzania. *Netherlands Journal of Zoology*, 34, 367-387.
- McIver, S.B., Wilkes, T.J. and Gillies, M.T., 1980. Attraction to mammals of male *Mansonia* (Mansonioides) (Diptera: Culicidae). *Bulletin of Entomological Research*, 70 (1), 11-16.
- Okanda, F., Dao, A., Njiru, B., et al., 2002. Behavioural determinants of gene flow in malaria vector populations: *Anopheles gambiae* males select large females as mates. *Malaria Journal*, 1 (10), 1-7. [<http://www.malariajournal.com/content/1/1/10>]
- Provost, M.W. and Haeger, J.S., 1967. Mating and pupal attendance in *Deinocerites cancer* and comparisons with *Opifex fuscus* (Diptera: Culicidae). *Annals of the Entomological Society of America*, 60, 565-574.
- Reisen, W.K., 2003. Lessons from the past: historical studies by the University of Maryland and the University of California, Berkeley. In: Takken, W. and Scott, T.W. eds. *Ecological aspects for application of genetically modified mosquitoes*. Kluwer Academic Publishers, Dordrecht, 25-32. Wageningen UR Frontis Series no. 2. [[http://library.wur.nl/frontis/malaria/03\\_reisen.pdf](http://library.wur.nl/frontis/malaria/03_reisen.pdf)]
- Takken, W., 1999. Chemical signals affecting mosquito behaviour. *Invertebrate Reproduction and Development*, 36 (1/3), 67-71.

- Takken, W., Klowden, M.J. and Chambers, G.M., 1998. Effect of body size on host seeking and blood meal utilization in *Anopheles gambiae sensu stricto* (Diptera: Culicidae): the disadvantage of being small. *Journal of Medical Entomology*, 35 (5), 639-645.
- Takken, W. and Knols, B.G., 1999. Odor-mediated behavior of Afrotropical malaria mosquitoes. *Annual Review of Entomology*, 44, 131-57.
- Tripet, F., Touré, Y.T., Dolo, G., et al., 2003. Frequency of multiple inseminations in field-collected *Anopheles gambiae* females revealed by DNA analysis of transferred sperm. *American Journal of Tropical Medicine and Hygiene*, 68 (1), 1-5.
- Tripet, F., Touré, Y.T., Taylor, C.E., et al., 2001. DNA analysis of transferred sperm reveals significant levels of gene flow between molecular forms of *Anopheles gambiae*. *Molecular Ecology*, 10 (7), 1725-1732.
- Verhoek, B.A. and Takken, W., 1994. Age effects on the insemination rate of *Anopheles gambiae* s.l. in the laboratory. *Entomologia Experimentalis et Applicata*, 72 (2), 167-172.
- White, G.B., 1971. Chromosomal evidence for natural interspecific hybridization by mosquitoes of the *Anopheles gambiae* complex. *Nature*, 231 (5299), 184-185.
- Yuval, B. and Bouskila, A., 1993. Temporal dynamics of mating and predation in mosquito swarms. *Oecologia*, 95 (1), 65-69.
- Yuval, B., Wekesa, J.W. and Washino, R.K., 1993. Effects of body size on swarming behaviour and mating success of male *Anopheles freeborni* (Diptera: Culicidae). *Journal of Insect Behaviour*, 6, 333-342.