Malaria mosquito rearing – maintaining quality and quantity of laboratory-reared insects

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Entomologists often require large numbers of experimental insects for laboratory studies. The quality of these insects should be high and constant, otherwise the results of replicate experiments can be contradictive or results from subsequent fieldwork might be disappointing. The quality of experimental insects can be influenced by population density, nutrition, environmental conditions, etc. These factors can strongly influence the fitness of the insects and hence, the outcome of experiments and should therefore not be ignored. The rearing of malaria mosquitoes (Diptera, Culicidae, Anophelinae) is complex and demanding for several reasons. Anopheline larvae are affected by temperature, density and available nutrition; mating is not necessarily accomplished naturally and females need a blood meal to develop eggs. The climate chambers where the mosquitoes are kept are warm and sweaty. Because of these tropical conditions the larvae develop fast and need to be cared for daily. The Laboratory of Entomology in Wageningen has cultured different colonies of malaria vectors successfully for many years. In this paper we discuss different aspects off the rearing process which affect mosquito fitness and are of importance for the quality of fundamental and applied research.

Keywords: mosquito rearing, Anophelinae, mating, blood feeding, fitness

Malaria mosquitoes (Diptera, Culicidae, Anophelinae) are the vectors of malaria parasites (*Plasmodium*), which affect millions of people worldwide. Up to 90% of the fatal cases occur in sub-Sahara Africa with an estimated annual death toll of 0.5-3 million people (WHO 2000). Possibilities to reduce malaria incidence are studied worldwide. Vector control is one of the options, as interruption of transmission of malaria parasites is clearly the most effective disease control strategy. In laboratories, mosquito colonies are needed in order to conduct studies on vector biology, vector-parasite interactions, insecticide susceptibility, vaccine studies etc. These laboratory studies are often designed in such a way that the outcome can be linked to and/or implemented in a field situation at a later stage of the research project. For this reason it is important to maintain the original gene pool, and physiological- and behavioural characteristics of the insects under study as much as possible. A healthy insect colony does not only increase the possibilities for successful experiments in the field, it also reduces the chance of contradicting results. The quality of experimental insects can be influenced by population density, food availability, climatic conditions, etc. These factors can

strongly influence the outcome of experiments and should therefore not be ignored. For example in laboratory studies on the chemical ecology of insect vectors like malaria mosquitoes, temporal, environmental and physiological aspects should be considered (Lazzari *et al.* 2004; Takken 2005).

This paper discusses the potential bottlenecks that workers handling mosquito cultures are confronted with. For some aspects of the rearing process, the techniques used are reflected towards the situation in the field. The biology of malaria mosquitoes will not be discussed in detail, but can be found in Clements (1992, 1999) and Service (1993).

Rearing Conditions

The malaria mosquito rearing at Wageningen University takes place in culture rooms that simulate natural climatic conditions. Temperature is set at 27°C and relative humidity at 80%. *Anopheles gambiae* Giles, the world's most important malaria vector, is a nocturnal mosquito with peak biting times between 02:00 and 4:00 am (Haddow 1946, Gillies & Coetzee 1987). To study the chemical ecology of these insects and to avoid conflicts with the researchers working hours, the light and dark regimen of the culture rooms are shifted from 06:00-18:00 towards 00:00-12:00 so that behavioural and electrophysiological research can be conducted during peak activity of both mosquito and researcher.

Sugar

Adult mosquitoes are kept in 30-cm cubic metal cages covered with mesh gauze. The insects have continuously access to a 6% glucose solution. Sugar availability can influence the nutrition-seeking behaviour of both male and female mosquitoes (Foster 1995, Hancock & Foster 1997). Therefore, it can also reduce the blood-feeding frequency of female *An. gambiae* (Gary & Foster 2001, 2004). Since the culture cannot do without a carbohydrate source (*e.g.* essential for the survival of the males), but because of the relevance for blood-feeding behaviour of the females, experimental female mosquitoes are removed from the culture 14-18 h before the start of experiments and given access to water only.

Mating

In anopheline mosquitoes mating is initiated in flight and is associated with the swarming behaviour of the males. For *An. gambiae*, held under laboratory conditions, evidence has been found for swarm markers on the ground (Charlwood & Jones 1980). Marchand (1985) described the difficulties to elicit swarming behaviour in standard 30 cm cube cages with wild-caught *An. gambiae sensu stricto* and *An. arabiensis*. In our laboratory, the relatively small cubic cages of 30-cm do not seem to reduce mating success. The insemination rate is regularly checked and found to be mostly above 93% in 5-day old females. Conversely, females of *An.*

quadriannulatus, another member of the *An. gambiae* complex and also reared at our laboratory, expressed a reduced insemination rate when held in cubic 30-cm cages. At a sharp transition from light to dark the insemination rate was approx. 10%. When an artificial dusk and dawn lighting system was installed, allowing for a 30 min. transition from light to darkness and vice-versa, the insemination rate rose to 60%. The use of taller cages of 30 × 30 × 60 cm resulted in a mating success similar to that of *An. gambiae* s.s. (Takken & Knols 1990, Takken *et al.* 2002). This shows that environmental conditions can be critical for a successful mating of these anopheline mosquitoes. As a routine measure, all our mosquito colonies have been equipped with an artificial dusk and dawn lighting system.

Blood feeding

Most laboratory cultures of anopheline mosquitoes are being blood fed through membranes or on laboratory animals (mice, guinea pigs). In Wageningen the anthropophilic *An. gambiae s.s.* is being fed on a human arm. This mosquito is the most anthropophilic member of the *An. gambiae* complex and is highly responsive to and attracted by human odour offered in laboratory setups (Takken & Knols 1990, Braks & Takken 1999). It is necessary to maintain this anthropophilic nature of cultured mosquitoes in order to link laboratory data to field studies. Laarman (1958) demonstrated that attenuation to the blood host might cause a change in host preference. For this reason blood feeding on a human arm is preferred above the alternative method of membrane feeding. A team of three volunteer workers handling the mosquito cultures offer their underarm to adult female mosquitoes twice a week for 10 minutes on a rotation schedule. The volunteers are wearing a surgical glove during feeding to prevent biting on highly sensitive fingertips. The blood feeding takes place *ca.* 4 hours prior to dawn. Most adult females need only one blood meal to complete an oviposition cycle, but some individuals, especially the smaller ones, may require more than one blood meal before they will complete their first gonotrophic cycle (Briegel 1990, Briegel & Horler 1993, Lyimo & Takken 1993, Takken *et al.* 1998). Since there is only a small group of workers feeding the cultures, there is a chance that the culture becomes habituated to the specific odour mixture of the volunteers. If this would be the case, the mosquitoes will still show anthropophilic behaviour but they may express preferential behaviour to host-associated cues.

The other *An. gambiae* s.l. colonies and colonies of other mosquito species in Wageningen are fed on bovine blood through a membrane feeding system (Discovery Workshops, Accrington Lancs, UK). The blood is collected weekly from the university's experimental animal farm, and placed immediately in sealed 10 cc heparin-treated vacutainers. All blood collection procedures have been approved by the Committee on Ethics of Animal Experimentation of Wageningen University and Research Centre.

Eggs and larvae

One day after blood feeding the mosquitoes are offered a damp filter paper for oviposition. The paper is folded into a cone and put in a small plastic cup filled with tap water. For *An. quadriannulatus* the water is enriched with a hay infusion acting as an oviposition stimulant. Eggs are laid in the standard mosquito holding cage, and after collection gently placed in larval trays for emergence (see below). The role of certain semiochemicals acting as possible oviposition stimuli for *An. gambiae* seems negligible for laboratory reared cultures. It would require more attention when laboratory studies were conducted on oviposition site behaviour (Sumba *et al*. 2004)

A crucial part of the mosquito rearing process is looking after the larvae. Larval densities should not be too high, but aiming for the densities that have been reported from the field seems not feasible with respect to available space and time. We use plastic larval trays of 10 × 25 cm and 8 cm high, filled with 1 L of tap water. The tap water has been placed in plastic jerry cans a few days before use to allow for evaporation of chlorine substances and to adjust to the temperature in the rearing room. The densities within the laboratory are around 0.3 larvae/cm^2. This is considerably higher compared to estimated densities in the field varying from 0.0013 to 0.072 larvae/cm^2 (Koenraadt & Takken 2003, Koenraadt *et al.* 2004). However, the distribution of larvae in the field tends to be clustered, so also here interactions between siblings are likely to occur (Service 1971, Koenraadt *et al.* 2004). Although cannibalism within laboratory larvae has been observed (Koenraadt & Takken 2003), its overall impact on the mosquito culture is negligible. Competition for food is being avoided by providing the larvae an optimum quantity of Tetramin® fishfood which is 0.1 mg/larva for the first instars and 0.3 mg/larva for the other three larval stages (Lyimo & Takken 1993, Smallegange, unpublished data). Overfeeding will cause imminent contamination of the water and can result in suffocation of the larvae.

GENERAL DISCUSSION

This paper describes only a few examples of measures taken during the rearing process to guard the fitness of the mosquitoes. Colleagues working in different disciplines and with different organisms may, however, encounter comparable trade-offs during the design of their culture. Laboratory conditions are usually different from field conditions. For this reason, ecologists who conduct laboratory experiments with living organisms should be cautious in the application of their results to a field situation. Degeneration of the gene pool, loss or changes within its behavioural repertoire, shifts in circadian rhythm and related responsiveness patterns are likely to occur as a result of specific laboratory conditions. It is assumed that each culture passes a bottleneck when established in the laboratory and we have little knowledge about the loss of essential traits. If natural conditions are taken into account during the design of culturing conditions, the

factors mentioned above can be kept to a minimum. However, it would often be searching for the right balance between what is desirable and what is attainable.

Acknowledgements We would like to express our gratitude to Frans K.M. van Aggelen, André J. Gidding and Leo Koopman who faithfully look after the mosquito cultures and act as voluntary blood donors.

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