Vision Research 48 (2008) 1449–1455

Contents lists available at ScienceDirect

Vision Research

journal homepage: www.elsevier.com/locate/visres

Polarized light and oviposition site selection in the yellow fever mosquito: No evidence for positive polarotaxis in *Aedes aegypti*

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ARTICLE INFO

Article history:
Received 23 January 2008
Received in revised form 1 April 2008

Keywords:
Aedes aegypti
Yellow fever mosquito
Egg-laying
Oviposition site selection
Polarized light
Polarization vision
Polarization pattern
Water detection

ABSTRACT

Aquatic insects and insects associated with water use horizontally polarized light (i.e., positive polarotaxis) to detect potential aquatic or moist oviposition sites. Mosquitoes lay their eggs onto wet substrata, in water, water-filled tree/rock holes, or man-made small containers/bottles/old tyres containing water. Until now it has remained unknown whether mosquitoes are polarotactic or not. The knowledge how mosquitoes locate water would be important to develop new control measures against them. Thus, we studied in dual-choice laboratory experiments the role of horizontally polarized light in the selection of oviposition sites in blood-fed, gravid females of the yellow fever mosquito, *Aedes aegypti*. On the basis of our results we propose that *Ae. aegypti* is not polarotactic. Thus the yellow fever mosquito is the first known water-associated insect species that does not detect water by means of the horizontally polarized water-reflected light. This can be explained by the reflection–polarization characteristics of small-volume water-filled cavities/containers preferred by *Ae. aegypti* as oviposition sites.

1. Introduction

The yellow fever mosquito, *Aedes aegypti* is found throughout subtropical and tropical areas of the world and considered the major vector for the transmission of dengue and yellow fever. It is a largely diurnal-biting species (Chadee, 1988) that apparently uses chemical and visual cues to locate its host (Kawada, Takemura, Arikawa, & Takagi, 2005). Gravid mosquito females, generally, need to locate suitable bodies of water into which they can lay their eggs, so that their aquatic larvae can develop normally (Clements, 1963), and in this regard female *Ae. aegypti* are no exception. However, they are known to accept small and inconspicuous containers like tree holes and, in urban areas, flower vases, discarded tyres, cans, bottles, and paper cups as breeding sites (Seng & Jute, 1994). How they find water is still not fully understood.

Hygroreception is known to play some role in the oviposition of mosquitoes (Clements, 1999), and hygroreceptors have, indeed, been described from the mosquito antenna (Yokohari, 1999). However, these receptors can operate only over relatively short distances. Another possibility therefore is that they use visual cues, since mosquitoes in flight are known to depend on optical inputs for orientation (Allan, Day, & Edman, 1987). Reflected light from water surfaces has been reported to influence oviposition site location by mosquitoes in the field (Belton, 1967). Moreover, Kennedy (1941) observed that gravid mosquitoes prepared for oviposition when flying over a mirror and some gravid mosquitoes were even seen to respond to the sight of water (Muirhead-Thompson, 1940) and the movement of mosquito larvae within it (McCrae, 1984).

In a series of observations Schwind (1985, 1991, 1995) discovered that several species of aquatic bugs and beetles are polarotactic, i.e. find water by means of the horizontal polarization of light reflected from the water surface. Later studies (Bernáth, Szedenics, Molnár, Kriska, & Horváth, 2001; Csabai, Boda, Bernáth, Kriska, & Horváth, 2006; Horváth, Bernáth, & Molnár, 1998; Horváth, Malik, Kriska, & Wildermuth, 2007; Horváth & Varjú, 2004; Kriska, Bernáth, & Horváth, 2007; Kriska, Csabai, Boda, Malik, & Horváth, 2006; Kriska, Horváth, & Andrikovics, 1998; Kriska, Malik, Szivák, & Horváth, 2008; Wildermuth, 1998; Wildermuth & Horváth, 2005) showed that beside numerous aquatic bugs and beetles also many other insect species associated with water (i.e. as larvae developing in water, but

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doi:10.1016/j.visres.2008.04.007

as adults being terrestrial), like dragonflies, mayflies and caddisflies, exhibit positive polarotaxis when searching for water. Until now no aquatic insect species has been found that would not select its aquatic habitat by positive polarotaxis.

According to the above, one could have expected mosquitoes to be also polarotactic, i.e., able to find appropriate aquatic or moist oviposition sites by means of the horizontal polarization of light reflected from the water surface or wet substrata (Horváth & Varjú, 2004). Although over the last decades the study of the biology of mosquitoes was intense (e.g. Clements, 1963, 1999) due to their offensive biting habits and role in spreading various dangerous diseases, it has remained unknown whether they are polarotactic or not. To know this could be of importance in developing new effective measures against them, since more and more mosquito populations become resistant to the pesticides used to control them (DARP, 2007; Kang et al., 1995).

Although some field and laboratory experiments had earlier been carried out to investigate colour preference and effect of substrate brightness on egg-laying in mosquitoes (e.g. Belton, 1967; Dhileepan, 1997; Jones & Schreiber, 1994; McCrae, 1984; Muir, Kay, & Thorne, 1992a; Muir, Thorne, & Kay, 1992b; Wen, Muir, & Kay, 1997; Williams, 1962), mosquito polarization sensitivity was only sporadically studied (Kalmus, 1958; Kovrov & Monchadskiy, 1963; Wellington, 1974). Thus, we investigated in double-choice laboratory experiments the role of horizontally polarized light in the selection of the oviposition site in blood-fed, gravid females of the yellow fever mosquito, *Aedes aegypti*, one of the most dangerous and wide-spread mosquito species in the world. Earlier studies on the eyes of mosquitoes have not given any indication of the existence of polarization sensitivity in the ventral eye region (e.g., Brammer, 1970; Brammer, Stein, & Anderson, 1978; Clements, 1963, 1999; Land, Gibson, Horwood, & Zeil, 1999; Muir et al., 1992b; Sato, 1959).

2. Materials and methods

Ae. aegypti imagos were obtained from a laboratory colony kept in the Plant Protection Institute of the Hungarian Academy of Sciences, Budapest. Larvae in rearing dishes were fed with powdered cat food. Emerging adults copulated in a separated cage, in which they were maintained at 28 ± 5 °C and 30–40% relative humidity under a day:night regimen of 16:8 h light:dark. Females were blood-fed on caged mice twice a week.

One double-choice and two pilot experiments were carried out in the laboratory to reveal the possible role of linearly polarized light in the egg laying of *Ae. Aegypti*. Groups of adult gravid female mosquitoes were placed into a dark test cage immediately after blood feeding. They were kept there for 72 h before being returned to the breeding cage. Resting, blood-fed, female mosquitoes needed about 48–60 h to mature their eggs (Clements, 1999). Although some eggs were deposited during the first 48 h, most were laid during the third 24-h period.

All experiments were carried out in the same test cage (length = 50 cm, width = 40 cm, height = 40 cm) placed in a windowless darkened room. A small access window (10 cm × 10 cm) on one of the sidewalls was used for introducing gravid, blood-fed female mosquitoes. The cage was equipped with two square illuminating windows (10 cm × 10 cm, 8 cm apart) in the immediate vicinity of the back wall of the test cage (Fig. 1A). Both windows were transilluminated through a combination of sheet polarizers and depolarizers (Fig. 1C). The test cage was arranged in such a way that the illuminating windows were vertical (Fig. 1A). During the experiments the windows provided a moderate, 40 lux illumination of the chamber (measured by a Gossen Starlite multifunctional light detecting instrument) that allowed mosquitoes to fly and oviposit actively. To ensure a sufficiently high ambient light intensity was important, because *Ae. aegypti* is diurnal and thus needs some light to be active during daytime. To avoid unwanted polarization of light reflected from the inner surfaces of the test cage, all these surfaces were wrapped by white, matt dry filter paper, which was always replaced before every choice experiment with a new mosquito group.

In all experiments two Petri dishes filled with dechlorinated clear tap water were offered for egg laying. An open-surface dish supplied with distilled water from a small tank (both covered outside by matt white dry filter paper) was used to replenish the evaporated water through communicating vessels, which also maintained constant water level in the Petri dishes during the tests. The replenishing open-surface dish was placed near the access window on the opposite side of the test cage (Fig. 1A) to keep relative air humidity homogeneous throughout the cage. The homogeneous distribution of air humidity eliminated the possibility that positive polarotaxis could be overridden by positive hygrotaxis in the test chamber.

Since *Aedes* species are known to lay eggs individually only on moist substrata (Clements, 1999), the Petri dishes were supplied with 18 cm long and 2 cm high vertical annular strips of white filter paper on their perimeters (Fig. 1B). These paper strips were continuously wet during the experiments. In the first pilot experiment we confirmed that a water surface screened by dry matt/shiny, black/white reflecting surfaces, acting as an invisible source of high air humidity and dry test surfaces with different reflection–polarization characteristics, were insufficient to elicit egg laying, because *Ae. aegypti* females need direct contact with a wet surface for oviposition under any illumination condition, including total darkness. As a control, in our second pilot experiment we presented a water-filled Petri dish (with a wet paper strip) and an empty Petri dish (with a dry paper strip) transilluminated from below by unpolarized visible and ultraviolet light, and counted the eggs laid onto the paper strips. We found that gravid, blood-fed yellow fever mosquitoes oviposited exclusively onto the wet paper strip of the water-filled Petri dish, telling us that the mosquitoes could discriminate. This showed that our experimental conditions were appropriate.

The Petri dishes were put next to the side-windows (Fig. 1A). Since the photoreceptors of *Ae. aegypti* are either green-, or ultraviolet-sensitive (Snow, 1980), the test cage was equipped to operate with separate visible (400 nm < λ) and UV (λ < 400 nm) transilluminations of the windows. During the choice experiment and the videopolarimetric measurement of the polarization patterns of the stimuli (Fig. 2) the test cage was in darkness and light stimulus entered only through the two bottom-windows. The polarization patterns in Fig. 4 were also measured by videopolarimetry, the method of which has been described in detail elsewhere (Horváth & Varjú, 1997).

In the dual-choice experiment polystyrene Petri dishes with a diameter of 8 cm were placed next to the side-windows separated by a vertical wall under another horizontal sheltering wall, both wrapped by matt white dry filter paper (Fig 1A). This arrangement ensured that the water surface in the Petri dishes did not reflect vertically polarized light (being possibly neutral or repellent for female mosquitoes); it reflected only unpolarized or weakly horizontally polarized light according to the characteristics of the side illumination. All mosquitoes flying below the horizontal sheltering wall had to choose between the two separated, exactly horizontally polarized and unpolarized light sources. Although the illuminating light came from the side rather than from the water surface, this circumstance did not prevent the mosquitoes from oviposition. Polarized and unpolarized combined vis + UV transilluminations of the side-windows were produced by means of the combination of UV-transmitting polarizers and depolarizers (Fig. 1C).

In the dual-choice experiment test groups of 5–30 blood-fed female mosquitoes were placed into the test cage for 3 days. The number of eggs laid on the outer (convex) and inner (concave) sides of the wet paper strips in the Petri dishes (Fig. 1B) were counted under a microscope and divided by the number of females to reduce variance. Since *Ae. aegypti* females usually lay eggs on wet surfaces (Clements, 1999), eggs found in the water were considered to be washed off from the inner surface of the wet paper strips. The average number of eggs laid onto the wet paper strips by mosquitoes of any given group was analysed by two-way ANOVA (Sokal & Rohlf, 1981) to examine the possible preference of Petri dishes illuminated from the side by totally horizontally polarized light or unpolarized light, and the possible preference of left or right Petri dishes. Normality of the data was checked by Kolmogorov–Smirnov test (Sokal & Rohlf, 1981).

3. Results

In our dual-choice experiments with gravid females of *Ae. aegypti* egg-laying occurred 48–72 h after blood-feeding. Since the average number of eggs laid by females was highly variable (mean = 26.5, standard deviation = 16.3), we were forced to use relatively large groups of mosquitoes in the tests. Table 1 provides information on the number of females in the experiments, the number of mosquito groups, the total number of eggs laid, and the average number of eggs per female laid onto the wet paper strips in the Petri dishes illuminated from the side by totally horizontally polarized and unpolarized visible and ultraviolet light.

In the experiments blood-fed, gravid female mosquitoes preferred the concave inner surface of the wet paper strips. The spatial distribution of the eggs laid was always homogeneous and random on the wet paper strips. The degree of linear polarization d (≈0% or 100%) of light illuminating the two Petri dishes from the side had no influence on the number of eggs laid per female (Fig. 3A, Table 1). Neither the left, nor the right position of the Petri dish proved to be significantly more attractive to the mosquitoes in our experiments (Fig. 3B, Tables 1 and 2).