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Polarotaxis in egg-laying yellow fever mosquitoes *Aedes (Stegomyia) aegypti* is masked due to infochemicals

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ABSTRACT

Aquatic and water-associated insects need to locate suitable bodies of water to lay their eggs in and allow their aquatic larvae to develop. More than 300 species are known to solve this task by positive polarotaxis, relying primarily on the horizontally polarized light reflected from the water surface. The yellow fever mosquito *Aedes (Stegomyia) aegypti* has been thought to be an exception, locating its breeding habitats by chemical cues like odour of conspecifics, their eggs, or water vapour. We now demonstrate through dual-choice experiments that horizontally polarized light can also attract ovipositing *Ae. aegypti* females when the latter are deprived of chemical cues: water-filled transparent egg-trays illuminated by horizontally polarized light from below gained a 94.2% higher total number of eggs than trays exposed to unpolarized light, but only when no chemical substances capable of functioning as cues were present. *Ae. aegypti* is the first known water-associated insect in which polarotaxis exists, but does not play a dominant role in locating water bodies and can be constrained in the presence of chemical cues.

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1. Introduction

Mosquitoes are being extensively studied worldwide, because they serve as vectors of several diseases and are considered a nuisance. Gravid female mosquitoes need to find suitable bodies of water in which their aquatic larvae can develop. Responses elicited by visual, chemical and tactile stimuli and involved in the location and selection of oviposition sites have repeatedly been studied in the hope of developing efficient egg-traps to monitor mosquito populations and increasing efficacy of control methods. Although no universal oviposition-attractant cocktail is known in *Aedes (Stegomyia) aegypti*, various compounds, e.g. pentatonic and tridecanoic carboxylic acids have been reported to influence this insect's ability to locate water bodies (Bentley and Day, 1989; Clements, 1999; Mendki et al., 2000; Ganesan et al., 2006; Navarro-Silva et al., 2009). Even water vapour *per se* is known to be an attractant (Clements, 1999; Yokohari, 1999).

Yet, chemical cues are reliable only over relatively short distances, and mosquitoes in flight are known to depend also on visual inputs for orientation, taking advantage of their well-developed compound eyes (Allan et al., 1987; Land et al., 1999).

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Optical characteristics of water surfaces have been reported to influence oviposition site location by mosquitoes in the field. Belton (1967) experienced increased numbers of *Culex restuans* T. egg-rafts in black-lined shallow ponds in the field. Gravid mosquitoes were seen to turn towards the sight of water (Muirhead-Thompson, 1940) and the movement of mosquito larvae within it (McCrae, 1984). Kennedy (1942) examined the attractiveness of bright and dark surfaces with and without glass or water overlays, thus producing mirror-like (specular) or diffuse reflection, respectively. Besides the attractiveness of dark targets against a white background, he also observed increased pre-oviposition activities in *Ae. aegypti*, *Anopheles atroparvus* v. T. and *Culex pipiens* L. over glass-covered black test surfaces, but admitted that he could not understand why this was so. Attractiveness of visual cues not typical of larval habitats were also reported. McCrae (1984), for example, found *Anopheles gambiae* G. females to lay more eggs in black-bottomed pots, containing clear tap water instead of the turbid, muddy water from the larval habitat.

Light reflected from smooth (shiny) and dark dielectric surfaces possesses a high degree of polarization with a direction of polarization parallel to the surface and perpendicular to the plane of reflection, unless the degree of polarization is reduced by the light component backscattered from the material. Numerous aquatic and water-associated insect species, e.g. dragonflies, mayflies, aquatic beetles and bugs (Schwind, 1991, 1995; Wildermuth,

1998; Kriska et al., 1998, 2007; Horváth et al., 1998, 2008; Bernáth et al., 2001; Horváth and Varjú, 2004; Horváth and Kriska, 2008) have been shown to possess positive polarotaxis, which means they are attracted by the horizontal polarization of light reflected from the water surface when locating potential aquatic or moist oviposition sites irrespective of the chemical composition of the water body. All the above observations on mosquitoes and anomalies could be explained by hypothesizing polarization sensitivity or positive polarotaxis, capabilities which to date have not been demonstrated in this dipteran family.

Earlier sporadic studies on polarization sensitivity of mosquitoes failed to prove reactions to polarization signals while excluding reactions to light intensity and chemical cues, or used insufficient numbers of mosquito individuals, and thus led to unconvincing conclusions (Kalmus, 1958; Kovrov and Monchadskiy, 1963; Wellington, 1974; Clements, 1999; Bernáth et al., 2008). However, positive polarotaxis was recently demonstrated in tabanid flies (Horváth et al., 2008, 2010; Kriska et al., 2009) and non-biting midges (Lerner et al., 2008; Horváth et al., 2011). Relying on reflection-polarization in detecting water bodies is considered to be a likely asset of all flying aquatic insect species. Mosquitoes possess compound eyes specialized to function in bright as well as dark environments (Meyer-Rochow and Waldvogel, 1979; Land et al., 1999) and would represent a remarkable exception, if they were not to perceive and utilize the horizontal polarization of light reflected from water surfaces.

We now show that *Ae. aegypti* indeed possesses polarotaxis. In addition, it is the first known water-associated insect species in which polarization sensitivity does not play a dominant role in oviposition site selection and can be constrained in the presence of chemical cues. This finding may help to interpret some contradictory results in mosquito ecology.

2. Materials and methods

We performed dual-choice experiments in the laboratory to test the attractiveness of horizontally polarized light to blood-fed, gravid *Ae. aegypti* females. We used an experimental design that allowed testing the attractiveness of egg-trays solely on the basis of light polarization. The only difference between the egg-trays was the state of polarization of the emitted light, while their emission spectra and intensities were identical. Any diluted infochemicals in the egg-trays originating from eggs or females were either removed through continuous rinsing, or allowed to accumulate.

The dual-choice experiments were carried out in a closed two-compartment cage (length = 50 cm, width = 40 cm) placed in a windowless, darkened room (Fig. 1). The upper part (height = 40 cm) served as test-cage, the lower part (height = 30 cm) contained the light sources. A small access window (10 cm × 10 cm) on the vertical front wall was used for introducing and removing gravid, blood-fed female mosquitoes. Ventilation of the cage was provided by a horizontal top window (27 cm × 34 cm) covered with a white mosquito net.

The horizontal base plate, separating the two compartments, was equipped with two square transilluminated glass windows (10 cm × 10 cm, 1 mm thick, 8 cm apart) in the immediate vicinity of the vertical back wall of the test cage (Fig. 1A and B). Combinations of a 15 W fluorescent blacklight tube (Philips Cleo 15 W S-R, emitting ultraviolet light with a spectrum ranging between 300 nm and 410 nm and having a peak wavelength at 350 nm) and two identical 20 W incandescent halogen reflector lamps were used as light sources (Fig. 1B) to offer linearly polarized light stimuli also in the UV range perceivable by *Ae. aegypti* (Muir et al., 1992). Heat generated by the lamps was dissipated by a fan continuously driving air through the lower compartment, maintaining a

temperature of 24.2 ± 2.1 °C and a relative humidity of $56 \pm 19\%$ in the test cage, close to those maintained in the breeding chamber. Both windows were continuously transilluminated by the black-light tube and one of the incandescent lamps through an inset sheet polarizer (KÅSEMAN B + W P-W64, transmitting light of wavelengths longer than 365 nm) and 5 sheets of white wax paper, which later functioned as a 5-layer depolarizer (Fig. 1B). This arrangement resulted in a dim illumination with wavelengths not shorter than 365 nm. UV receptors of *Ae. aegypti* can be excited at 370 nm, thus the UV content was perceivable by mosquitoes. However, the UV component was only about 1% of the total irradiation of the egg-trays. Polarizers were placed in such a way that they transmitted light with a horizontal direction of polarization perpendicular to the long axis of the test cage. Changing the order of polarizers and depolarizers, provided totally linearly polarized or unpolarized light of identical intensities and spectral compositions (Figs. 1B and 2). To avoid unwanted reflection-polarization-induced intensity patterns and polarization of light reflected from the inner surfaces of the test cage, all surfaces in the upper compartment were painted matte white or wrapped by matte white filter paper, which later was always replaced before any choice experiment with a new mosquito group started.

Rinsable egg-trays of glass (10 cm × 10 cm × 2.5 cm) were placed onto both windows. Walls of the egg-trays were painted matte white on the outer side to avoid unwanted reflection-polarization or refraction-polarization phenomena. Strips of paper with a width of 2.5 cm placed onto the inner surfaces of the walls of egg-trays had similar optical functions. Since their lower section was immersed into the water they also served as a constantly moist substrate for egg-laying mosquitoes and supported diffusion of chemicals released by the eggs. The bases of the trays, formed by 1 mm thick glass panes, were left transparent. Filled with dechlorinated tap water to a depth of 1.5 cm, the two egg-trays acted as combined ultraviolet-visible (UV-VIS) light sources with equal total radiances of 1.005 ± 0.002 W/m² (measured by a TEK Lumacolor II J18 photometer equipped with a J1812 radiation head) differing only in the degree of linear polarization of emanating light (Fig. 2). No other light source was applied in the test cage.

The egg-trays were shielded by plates forming cubes of 20 cm × 15 cm × 15 cm, open only from the side facing the front wall (Figs. 1 and 2). Unpolarized light reflected from the walls provided a dim background illumination with a total radiance of 340–510 mW/m² within the shielding and of 110–160 mW/m² in the cage. Since water surfaces typically reflect horizontally polarized light, aquatic insects only find horizontally polarized light attractive (Horváth and Varjú, 2004). It is evident that insects approaching a horizontally positioned linear polarizer perpendicularly to its transmission direction will perceive horizontally polarized light, but if their approach is in parallel with the transmission direction of the polarizer, they will perceive vertically polarized light. Appropriate shielding ensured that mosquitoes flying in the choice-box and choosing to enter the cube of a transilluminated egg-tray perceived only horizontally polarized or unpolarized light, rather than vertically or obliquely polarized light (Fig. 1C).

The reflection-polarization characteristics of the stimuli in the test cage (Fig. 2) were measured by imaging polarimetry in the red (650 ± 40 nm), green (550 ± 40 nm) and blue (450 ± 40 nm) parts of the spectrum using a Kodak Z812IS digital camera and an ITOS IP-38-G rotateable linear polarizer. The method of imaging polarimetry has been described in detail elsewhere (Horváth and Varjú, 1997, 2004).

Egg-trays were equipped with side-mounted inflow-pipes and waste-pipes and flow-directing plates of glass. These permitted a continuous rinse supplied by an outer tank of 20 l containing dechlorinated tap water, placed 80 cm above the base plate of the test cage. Water was driven by gravitation into the egg-trays

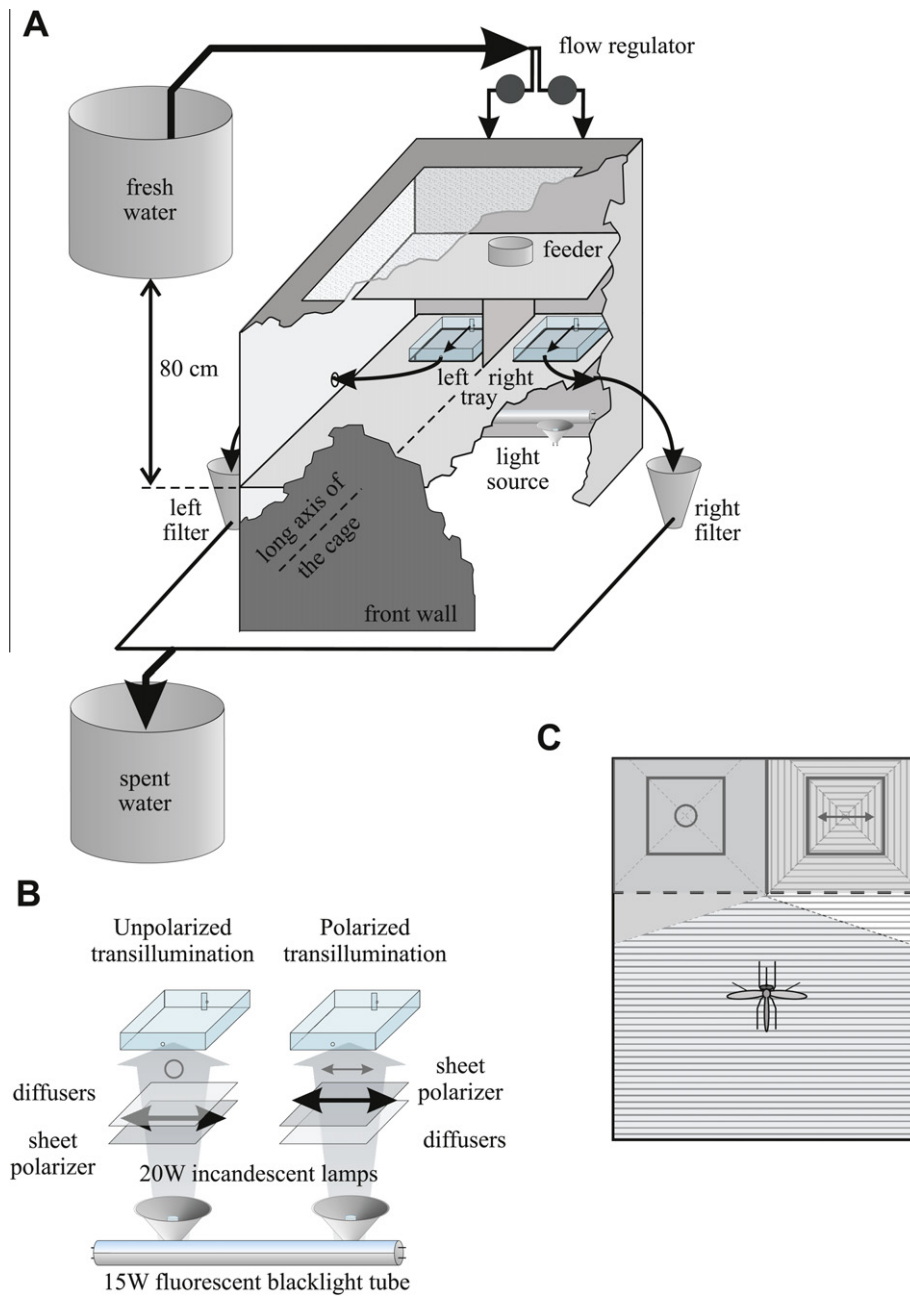


Fig. 1. Arrangement of the dual-choice experiment performed with gravid female *Ae. aegypti*. (A) Geometry of the test cage with rinsable egg-trays and transilluminating windows. (B) Production of unpolarized and totally linearly polarized transilluminations. (C) The egg-trays were shielded by plates forming cubes of 20 cm × 15 cm × 15 cm open only from the side facing the front. This arrangement ensured that mosquitoes flying in the cage (seen from above in the figure) perceived only unpolarized light (on the left) or horizontally polarized light (on the right), rather than vertically or obliquely polarized light.

through a flow regulator consisting of a Y-tube and separate taps. Effluent water was replenished by a pump controlled by a float switch placed into the freshwater tank; hydrostatic pressure was stabilized by a buffer tank of 20 l. This arrangement provided slow but very steady and identical water flows in the egg-trays during choice experiments. The surface current was commensurable to breeze-generated movements on still pond surfaces. Since both egg-trays were supplied from a single common Y-tube, slight periodic changes of the hydrostatic pressure affected both trays in the same way. Large-diameter waste-pipes ensured identical and stable water levels and constant water volumes of about 150 ml in the trays. Effluent rinse-water flowed through filters, which retained all solid particles (e.g. eggs, corpses) washed away accidentally.

Ae. aegypti mosquitoes originated from a laboratory colony kept in the Plant Protection Institute of the Hungarian Academy of Sciences, Budapest. Mosquitoes were maintained in a dedicated breeding chamber at 24 ± 3 °C and $55 \pm 20\%$ relative humidity under a day:night regimen of 17:7 h light:dark. Larvae in the rearing dishes were fed with powdered cat food. Adults emerged and copulated in separated net-walled colony cages, and were not mixed with earlier emerged individuals. Imagos were provided with sugar-feeders and were blood-fed on caged mice every second week.

Groups of 25 mated female *Ae. aegypti* were separated for dual-choice experiments in the preparation cages. After 24 h they were allowed to feed on caged mice for 1 h. Surviving blood-fed females

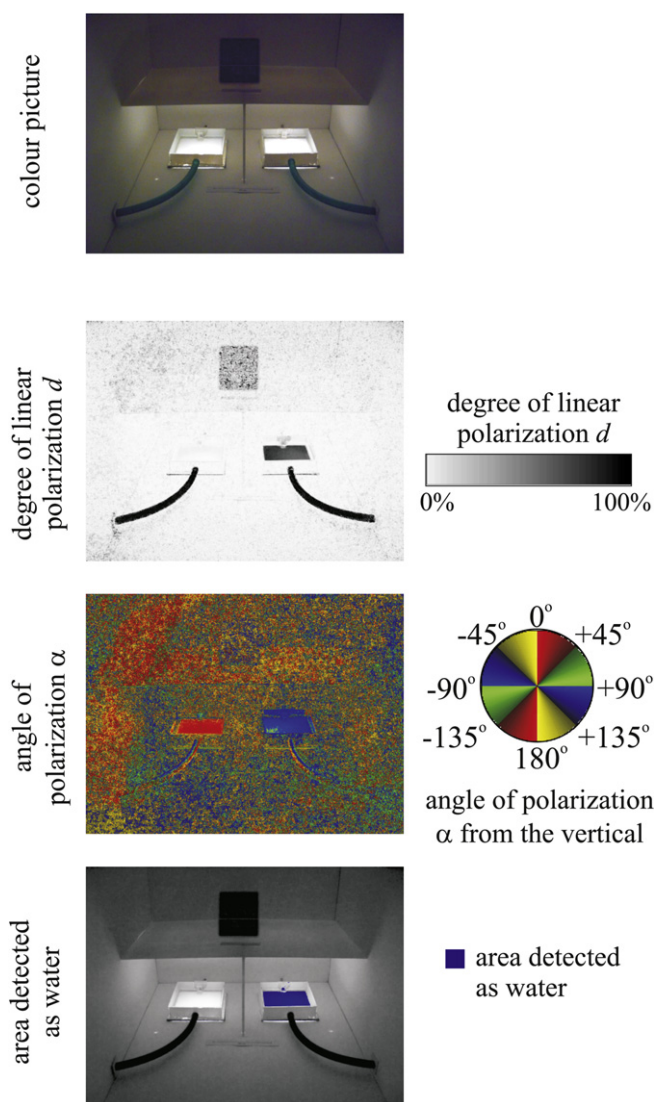


Fig. 2. Colour picture, patterns of the degree of linear polarization d and angle of polarization α (clockwise from the vertical), and area detected as water (for which the following two conditions are satisfied: $d > 20\%$ and $80^\circ < \alpha < 100^\circ$) for the transilluminated egg-trays in the test cage measured by imaging polarimetry in the green (550 ± 40 nm) part of the spectrum. The left/right window was transilluminated by unpolarized/totally horizontally polarized light. Left or right positions of linearly polarized and unpolarized transillumination were arranged randomly.

were kept in the preparation cages for an additional 48 h, before they were tested and transferred into the illuminated test cage (Fig. 1) for a 48-h dual-choice experiment. The number of individuals used in each test series depended on the number of available gravid females and varied between 5 and 20. A sugar-feeder placed on top of the shielding served as a food source during the choice

experiment (Fig. 1A). Unwanted reflection-polarization from the sugar solution were prevented by floating particles of white polystyrene foam. Blood-fed female mosquitoes needed about 48–60 h to mature their eggs at this temperature before starting to actively search for a place suitable for oviposition and laid most of their eggs in the egg-trays. After 48 h all females were removed from the test cage and killed.

Dual-choice experiments were carried out in two ways: (i) In rinsed tests the water flow was set to 0.7 ml/s in each egg-tray and kept up to continuously wash away any infochemicals originating from ovipositing females, eggs, or carcasses. Flow was slow enough not to prevent oviposition, but sufficiently fast to completely replace the water content of the egg-trays in less than 4 min. (ii) In non-rinsed tests, both egg trays were filled with clear dechlorinated tap water to a depth of 1.5 cm. Water flow was set to be lower than 0.01 ml/s, fast enough to replenish evaporated water, but nonetheless sufficiently slow to allow an accumulation of infochemicals. Transillumination and flow was switched on continuously during the whole duration of all tests. Rinsed and non-rinsed tests were evaluated as separate data series. However, the order of rinsed and non-rinsed experiments, left or right position of totally linearly polarized and unpolarized transillumination and combinations of egg-trays and polarizer sheets were arranged randomly.

The numbers of eggs laid onto the wet white paper strips, normalized with (divided by) the number of mosquitoes in any given group, was analysed by Wilcoxon's matched pairs signed-rank test (further on Wilcoxon MPSR test, Sokal and Rohlf, 1981) to examine the preference of egg-trays transilluminated by totally polarized light or unpolarized light, and possible preference of left or right position of the egg-trays. Eggs floating in the egg-trays or found in the filters were added to the eggs found on the corresponding paper strip. The numbers of carcasses found in the egg-trays were analysed correspondingly. Usage of parametric statistics was opposed by normality tests (Sokal and Rohlf, 1981). All statistical tests were performed with the STATISTICA 6.0 software.

3. Results

Gravid mosquitoes were introduced into the test cage during egg-rearing, in which phase they tend to rest on vertical surfaces and move only to avoid immediate danger. Females ready to lay eggs start a search-flight to locate suitable places. It is not synchronised when egg-rearing comes to end and motivation to oviposit occurs individually (not collectively). Constant lighting and temperature did not provide external synchronising signals, thus typically single females tried to lay eggs at any given time. Individual eggs frequently occur in groups, but most females lay their eggs during several rounds.

A striking difference occurred between rinsed and non-rinsed choice experiments (Table 1, Fig. 3). Once water-dissolved chemical substances were removed by rinsing, the egg-tray that emanated horizontally polarized light gained a 94.2% higher total

Table 1

Total number of *Ae. aegypti* females, number of mosquito groups in rinsed and non-rinsed dual-choice experiments, total number of eggs, average number and standard deviation of eggs per female laid onto the wet white paper strips, and total number of carcasses in the two egg-trays transilluminated by totally horizontally polarized and unpolarized visible + ultraviolet light.

	Total no. of females	No. of groups	Transillumination					
			Horizontally polarized			Unpolarized		
			Total no. of eggs	Aver. no. of egg/female	Total no. of carcasses	Total no. of eggs	Aver. no. of egg/female	Total no. of carcasses
Rinsed	196	16	3775	20.2 s.d. 14.6	21	1944	10.7 s.d. 8.3	14
Non-rinsed	187	16	2262	12.3 s.d. 10.4	9	2369	12.2 s.d. 9.4	10

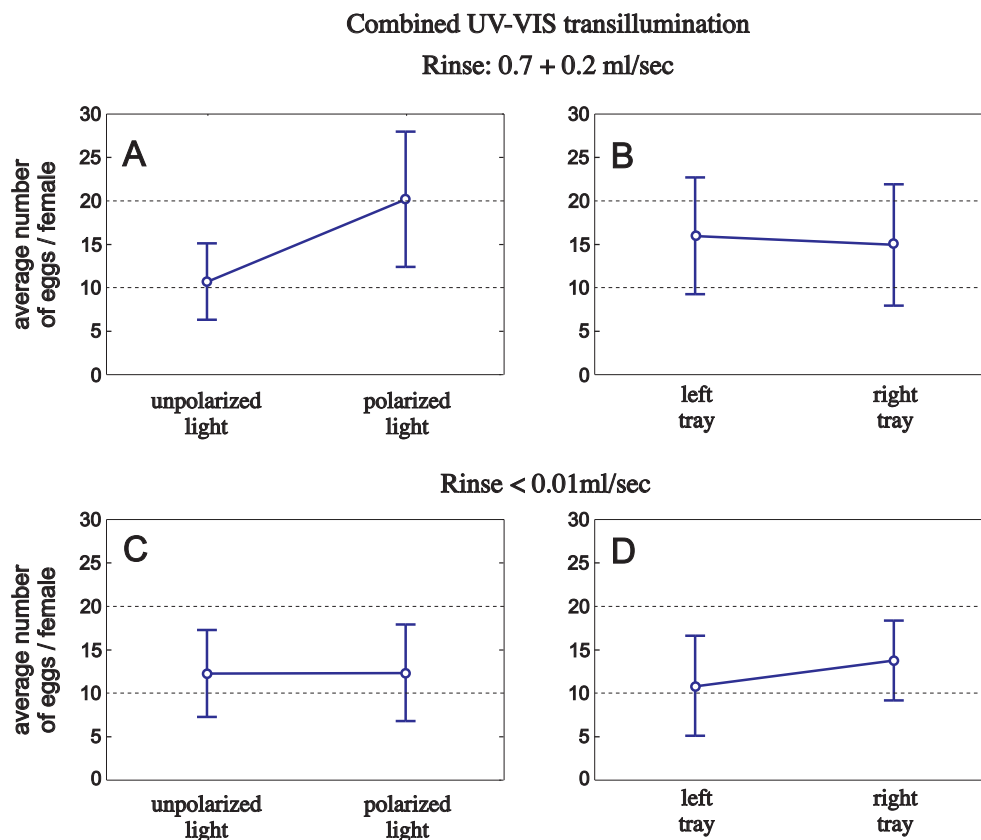


Fig. 3. (A) Average numbers of eggs per female laid by groups of *Ae. aegypti* onto the wet white paper strips in two continuously rinsed egg-trays transilluminated by unpolarized or totally horizontally polarized visible + ultraviolet light. (B) Average numbers of eggs per female in continuously rinsed left and right egg-trays. (C) Average numbers of eggs per female laid by groups of *Ae. aegypti* onto the wet white paper strips in the two egg-trays without rinse. (D) Average numbers of eggs per female in the left and right egg-trays without rinse. Dots show the average number of eggs per female and vertical bars indicate 95% confidence intervals.

number of eggs than the unpolarized light-emanating tray. In terms of eggs per female the tray transilluminated by horizontally polarized light gained a significantly higher score (Wilcoxon MPSR test: $p = 0.005$, $t = 14.0$, $Z = 2.792$, $df = 15$). However, if chemical substances were not removed the difference in the total number of eggs per females was statistically no longer significant (Wilcoxon MPSR test: $p = 0.877$, $t = 65.0$, $Z = 0.155$, $df = 15$), although the total number of eggs in the egg-tray transilluminated by horizontally polarized light was actually 4.5% lower (Fig. 3).

We found no statistically significant difference in the numbers of eggs per female laid into the right or the left egg-tray, neither in the rinsed choice experiment (Wilcoxon MPSR test: $p = 0.959$, $t = 67.0$, $Z = 0.052$, $df = 15$), nor in the non-rinsed one (Wilcoxon MPSR test: $p = 0.179$, $t = 42.0$, $Z = 1.344$, $df = 15$). The precise numbers of eggs found in the feeder could only be estimated due to the structure of the feeder. It was, however, free of eggs in most cases, and never received more than a few tens of eggs.

The number of mosquito carcasses found in the egg-trays could also be used to assess the attractiveness of the egg-trays to mosquitoes. Although trays emitting horizontally polarized light contained 50% more dead females in the rinsed experiments, the total number of carcasses was low (Table 1), suggesting that the chance of females to perish in the egg-trays was small. Differences in the numbers of carcasses per females were not significantly greater either in the trays emitting horizontally polarized light (Wilcoxon MPSR test: $p = 0.343$, $t = 14.5$, $Z = 0.948$, $df = 15$), or in the right or the left trays (Wilcoxon MPSR test: $p = 0.236$, $t = 12.500$, $Z = 1.185$, $df = 15$). The differences were much lower in the case of non-rinsed experiments either in comparisons between

the egg-trays transilluminated by polarized and unpolarized light (Wilcoxon MPSR test: $p = 0.407$, $t = 15.5$, $Z = 0.829$, $df = 15$), or between left and right trays (Wilcoxon MPSR test: $p = 0.800$, $t = 12.5$, $Z = 0.254$, $df = 15$).

4. Discussion

Long test-duration was necessary to allow mosquitoes to lay their eggs, but it also allowed them to explore the entire arena in various states of motivations. However, any factors possibly affecting the location of mosquito oviposition including polarization signals were present in all repetitions. If the presence of other females in the vicinity of egg-trays influences egg-laying females, then it always does in the same way. Interommatidial angles in the eye of *Ae. aegypti* are in the range of 6.3° – 6.5° (Kawada et al., 2006) resulting in very poor spatial resolution, which means that previously laid eggs are far too small to be seen by the mosquito. The only difference between rinsed and not-rinsed test, which resulted in strikingly different results, was the lack or the presence of dissolved materials in the egg trays.

Our results allow us to conclude the following: (a) *Ae. aegypti* can detect water visually by means of the horizontal polarization of light reflected from the water surface, but (b) vision is not the sense that dominates the search for water, the selection of oviposition sites and oviposition itself. *Ae. aegypti* females possess a polarotactic capacity that can facilitate remote water detection, but the sequels of polarotaxis are masked in the presence of stimulants indicative of conspecifics and/or their eggs or larvae in

water. This renders likely a strategy that removes the total reliance on one sensory modality and allows another to neutralize it when required.

When continuous rinsing was applied, female mosquitoes met egg-trays containing water with identical chemical compositions and could not make use of chemical cues in their choice of the better egg-laying site. Under this condition they had no information on earlier ovipositions. High and approximately zero degrees of polarization was the only difference between the egg-trays. Should polarized light have no effect, all individuals would choose randomly, thus no difference in the number of eggs laid would occur between the trays. Obviously females perceived polarization contrast and laid eggs predominantly into the tray emanating horizontally polarized light. Since it presumes the tendency of moving towards sources of polarized light, it proves the presence of polarotaxis in *Ae. aegypti*. However, polarization vision does not have the same decisive role in oviposition as in other aquatic insects. Females are able to divide their batch and lay portions of their eggs in different locations. The number of laid eggs is determined by several factors, and a few females even may produce relatively large numbers of eggs in generally non-preferred habitats.

When no rinsing was applied, chemical substances released from females and their eggs could accumulate in the egg-trays and serve as infochemicals to later arrivals in deciding where to lay their eggs. In this case the effect of polarization contrast was neutralized. Should chemical markers other than those diluted in the water determine the behaviour of mosquitoes, the effect of polarization would remain masked regardless of rinse. Thus when no rinsing was applied the total number of eggs laid in individual trays was influenced by the batch size left by earlier females and the length of time spent in the egg-trays. The minimum batch size able to influence later arrivals could be determined only if the acting chemicals are known. However, chemical stimulants may evoke behavioural responses in *Ae. aegypti* even in concentrations of merely 1 nmol/l (Geier et al., 1999), thus even small amounts of eggs could release sufficient amounts of stimulants. Note, that information on earlier ovipositions would not necessarily stimulate mosquitoes to oviposit, but can actually serve as a repellent as well. Later arrivals may (i) tend to follow the random choice of the first visiting female, or (ii) may choose the less populated tray, or (iii) may disregard chemical markers completely and choose randomly. If any of these three possibilities were realized, after several repetitions equal total numbers of eggs should be expected in the two egg trays. Since the number of eggs laid by individual females is highly variable, either egg-tray could turn out to be more attractive due to its chemical content. Reactions to these olfactory stimuli were strong enough to totally mask the influence of the polarization characteristics of light emitted by the egg-trays.

This overwhelming effect of infochemicals is the reason why until this study positive polarotaxis in *Ae. aegypti* had remained completely hidden. We do not reject earlier findings, but rather expand them and show the environmental limits of their validity.

Yellow fever mosquitoes can detect polarization contrast, which requires them to have polarization-sensitive photoreceptors. The presence and mechanisms of proper polarization vision should be affirmed by microanatomical investigations and electrophysiological measurements. A possible structural basis for polarization sensitivity in their eye, as with the eyes of other Diptera closely related to mosquitoes (Sato, 1959; Hardie, 1985; Meyer-Rochow and Waldvogel, 1979), is provided by the orthogonally directed microvilli of retinula cells 7 and 8, which are arranged in a tandem position in the centre of each ommatidium. These two cells have separate second order neuronal channels (Wolf and Ready, 1993), and thus could reliably relay information on the linear polarization to the brain of the insect. Polarization-sensitive ommatidia are

expected to be located in the ventral eye region of *Ae. aegypti* with neurons responsible for processing the polarization information.

Ae. aegypti typically breed in small and covered waters, whose detection can be difficult. Recognizing dark patches is useful in detecting entrances to cavities, but being sensitive to horizontal polarization of light in the lower visual field is helpful in identifying water surfaces either in cavities or in the open. These optical cues are perceptible for mosquitoes also under low light intensities, not requiring high spatial resolution. Mosquito species ovipositing on open water surfaces (e.g., *Anopheles* sp., *Culex* sp.) or dropping eggs while hovering over water (some species of the genera *Anopheles*, *Sabethes*, *Toxorhynchites* and *Wyeomyia*), would benefit even more from perceiving the horizontal polarization of light reflected from water surfaces (Clements, 1999). *Ae. aegypti* is probably not the only mosquito species, which may display polarization sensitivity.

Constrained polarotaxis of any mosquito species can influence the results of bioassays involving water-filled egg-trays and smooth black surfaces to test the effectiveness of chemical attractants or repellents in ovipositing females. Brightness contrasts are usually controlled to avoid artefacts originating from the preference of gravid female mosquitoes for dark oviposition sites and dark water surfaces against bright ones (Clements, 1999). However, polarization contrasts are usually not addressed, and the separation of intensity contrasts from polarization contrasts in choice experiments is not trivial (Horváth and Varjú, 2004, pp. 381–383). For example, more intense illumination would compensate for the lower reflectance of a darker surface compared to a brighter one, but it would not compensate for the higher degree of polarization of light reflected from it. Moreover, reflection-polarization phenomena may occur at widely used black cage walls producing unintended optical cues. Undetected polarization cues in bioassays may lead to ineffective molecules being regarded as active infochemicals (e.g., Bentley and Day, 1989). Thus, choice experiments to test the attractiveness of chemical agents in mosquitoes should either be carried out in total darkness, or designed to control both intensity and polarization cues, preferably completed with imaging polarimetric checks.

Polarization sensitivity in *Ae. aegypti* may conceivably serve also as a skylight-polarization compass. Early behavioural tests by Kalms (1958) and Wellington (1974) may support this hypothesis, although further information is required because of their methodological shortcomings. Skylight-navigation was demonstrated in several diurnal and also in some nocturnal insect species (Dacke et al., 2004; Horváth and Varjú, 2004), and it could assist dispersion of diurnal, crepuscular and even nocturnal mosquitoes.

The attractant role of horizontally polarized light in egg-laying mosquitoes highlights the importance of polarized light pollution. Large glass surfaces are common elements in modern architecture and are large water-imitating polarizing surfaces for polarotactic aquatic insects (Horváth and Kriska, 2008; Kriska et al., 2008; Malik et al., 2008; Horváth et al., 2009). Should urban mosquitoes also be attracted to such glass facades, then these buildings could serve as local foci of mosquito populations and must be given special attention by authorities involved in mosquito control measures and the prevention of the spread of arboviruses.

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