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Reflected polarization guides chironomid females to oviposition sites

Amit Lerner[1,]*, Nikolay Meltser[2], Nir Sapir[3], Carynelisa Erlick[1], Nadav Shashar[4] and Meir Broza[2]

[1]Department of Atmospheric Sciences, The Hebrew University of Jerusalem, Jerusalem 91904, Israel, [2]Faculty of Science and Science Education, University of Haifa at Oranim, Tivon 36006, Israel, [3]Department of Evolution, Systematics and Ecology, The Hebrew University of Jerusalem, Jerusalem 91904, Israel and [4]Department of Life Sciences, Eilat Campus, Ben-Gurion University, Hatmarim St., Elat 88000, Israel

*Author for correspondence: amit.lerner@mail.huji.ac.il

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SUMMARY

Chironomids (Diptera: Chironomidae; non-biting midges) are known to be carriers of the *Vibrio cholerae* bacterium, responsible for the fatal cholera disease in humans. It was recently discovered that chironomid females choose their oviposition site by a visual cue. In this study, we test the hypothesis that this visual cue is the linear polarization of light reflected from the water surface. We conducted two multiple choice field experiments using egg traps with different light intensities and polarizations. With controlled illumination, a higher number of eggs was found under both high intensity and high polarization. Under natural illumination, no eggs were found in the unpolarized traps, and the egg number increased with the percentage polarization regardless of the light intensity. Field measurements showed that at sunset, when chironomids are active, the intensity of light reflected from their natural ponds decreases by 96%, while the percentage polarization remains stable and high at 60%. Furthermore, the percentage polarization is positively correlated with the total organic carbon (TOC) concentration in the water. Orthogonal alignment of the microvilli found in ommatidia from the ventral part of the female eye may provide the anatomical basis for polarization sensitivity. We conclude that the percentage polarization of reflected light is the cue by which chironomid females choose their oviposition site. It is a stable cue and can provide information on the amount of food available to the larvae in the water. Based on our results, we suggest that manipulating the polarization of reflected light is a viable way to control chironomid populations and mitigate cholera dispersion.

Key words: polarization vision, chironomids, oviposition, habitat selection, water turbidity.

INTRODUCTION

Chironomids (Diptera: Chironomidae; non-biting midges) are distributed worldwide, occupying a range of freshwater habitats such as streams, lakes and ponds. Mating occurs in the air during twilight (Armitage et al., 1995). After mating, the male dies, and the female descends to oviposit on the water surface. The eggs are laid in the form of egg batches (EB), each containing thousands of eggs wrapped in a gelatinous layer. Since the adults do not feed and are short lived, most females lay only once or twice and die soon after. Females usually oviposit in two stages, at the start of the evening and around sunrise. After emerging from the egg, the larva descends to the bottom of the water, where it feeds on the substrate and on suspended matter in the water. Then, 2–3 weeks later, the larva pupates and once the metamorphosis is complete, the pupa swims to the water surface, where the adult emerges into the air and is ready to mate (Pinder, 1986). Mating takes place within a couple of hours.

Chironomids are a nuisance to humans physically, commercially and healthwise (Armitage et al., 1995). Recently, the EB of chironomids were identified to host the *Vibrio cholerae* bacterium, a human pathogen responsible for the severe diarreal cholera disease (Broza and Halpern, 2001; Halpern et al., 2004). The bacterium shows successive host-pathogen population dynamics (Halpern et al., 2006), while exploiting the eggs as a carbon source (Broza and Halpern, 2001). The adult midges that emerge from the water surface are suggested to carry the bacterium between bodies of water (Broza et al., 2005; Paz and Broza, 2007), assisting the bacterium's dispersion and population stability.

Schwind (Schwind, 1991) was the first to suggest that chironomids prefer to oviposit in highly polarized patches. Meltser et al. (Meltser et al., 2008) showed that females do not oviposit randomly but prefer certain water bodies over the others, and that the cue which directs this preference is not olfactory but visual. Similar to the observations on blackflies (Diptera: Simullidae) by Golini and Davies (Golini and Davies, 1975), Meltser et al. observed that more EB are laid in dark *versus* bright reflecting patches. As dark surfaces reflect light with a high percentage of linear polarization [a correlation known as Umow's law (Umow, 1905)], Meltser et al. suggested that the cue that guides the females' choice is the percentage polarization of the light reflected from the water surface.

Polarization vision (PV) is well known among invertebrates, including insects, as the microvilli in their photoreceptors are often orthogonally arranged (for a review, see Horváth and Varjú, 2004). Insects and other invertebrates use PV for several tasks, including orientation and navigation [bees (von Frisch, 1949); ants (Wehner, 1982); dung beetles (Dacke et al., 2003); spiders (Dacke et al., 1999)] and as a mating signal [butterflies (Sweeney et al., 2003)]. Flying insects are known to use PV to detect water bodies [first demonstrated by Schwind (Schwind, 1991)] and to avoid flying over the sea [*locusts* (Shashar et al., 2005)]. In laboratory experiments, females of the Australian orchard butterfly *Papilio aegeus* showed a clear preference to oviposit on horizontally polarized patches, regardless of the wavelength of light reflected from the substrate (Kelber, 1999; Kelber et al., 2001). Wildermuth (Wildermuth, 1998) showed that dragonflies make more attempts to oviposit on high polarization reflecting substrates, and Kriska et al. (Kriska et al., 1998) also reported that species of mayflies (Ephemeroptera) are attracted to and even occasionally oviposit on horizontally oriented polarization reflecting substrates. Furthermore, Kriska et al. (Kriska et al., 2006) suggested that the large standard deviation from horizontal alignment of the electric vector (e-vector) of the reflected polarized light is the reason for the unattractiveness of burnt stubble fields for aquatic insects, emphasizing the importance of a stable (in their case horizontal) e-vector orientation as a visual cue.

Chironomids are sensitive to light at 370–400 nm (ultraviolet for humans) and at 490–510 nm (green for humans) with no apparent overlap between the two ranges (Kokkinn and Williams, 1989). Chironomids were also found to be attracted to horizontal polarization over vertical polarization (Danthanarayana and Dashper, 1986). Meltser et al. (Meltser et al., 2008) caught more chironomid females in polarized light traps than in unpolarized traps (transmitting equal intensity), whereas male numbers in both traps were the same. This supports the suspicion that the ability of the females to sense polarization is used for a rather feminine activity, such as oviposition. Here, we examine the hypothesis that chironomid females select the water body to oviposit by the percentage polarization of the light reflected from a water surface and not by the light intensity.

MATERIALS AND METHODS

Study site

Two multiple-choice field experiments were conducted in the waste stabilization pond station of Tivon, Israel (32°40′ N 35°07′ E) during September and October 2007, when the local chironomid population is at its peak (Halpern et al., 2006). Experiments were conducted along the banks of the northeastern pond, where the highest number of EB was recorded.

Experiment 1: oviposition preference under controlled illumination

To check whether the females choose a site for oviposition by the reflected intensity or by polarization, their preference for egg traps reflecting linear polarized light *vs* depolarized light was examined at two intensity levels. For this purpose, four traps were placed in a tent (185 cm×305 cm×310 cm). Each trap was constructed of a wooden box (30 cm×30 cm×30 cm) with a 10 W incandescent light bulb placed in the middle of the base (Fig. 1). A 10 cm×10 cm open hatch was placed above the bulb, where a filter including diffusers (commercial wax paper) and a linear plane polarizer (Polaroid HN38S) was placed. The combination of diffusers and polarizers in the four filters was, respectively (Fig. 2): three diffusers + one polarizer (ordered from the light bulb to the viewer), transmitting high intensity polarized light (PHIH); one polarizer + three diffusers, transmitting high intensity unpolarized light (PLIH); 15 diffusers + one polarizer, transmitting low intensity polarized light (PHIL); and one polarizer + 15 diffusers, transmitting low intensity unpolarized light (PLIL). On top of the filter, a glass aquarium of 15 cm×15 cm×10 cm was placed, filled with tap water 2 cm high (450 cm^3). In addition, four fluorescent bulbs were placed inside the tent to attract the chironomids. The experimental procedure included turning on the fluorescent light from 30 min before sunset until 30 min after, then shutting down the fluorescent light and turning on the light bulbs in the boxes overnight. The EB laid in the aquarium of each box were counted on the following morning.

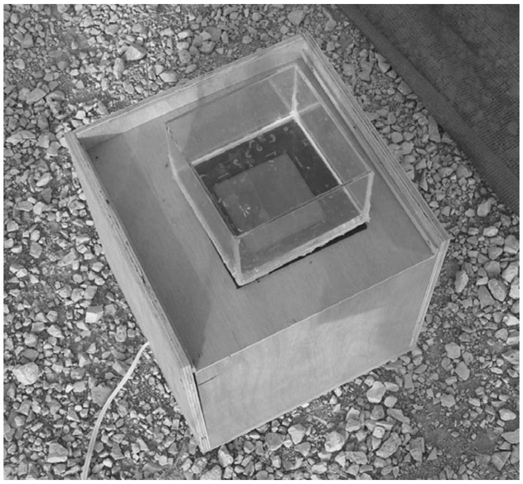

Fig. 1. An egg trap used in experiment 1. The trap consisted of a 30 cm×30 cm×30 cm wooden box, on the bottom of which was a 10 W bulb. Above the bulb there was a 10 cm×10 cm open hatch with a 15 cm×15 cm filter (black rectangle). The filter included diffusers and a plane polarizer that provided polarized/unpolarized illumination at two levels of intensity. A 15 cm×15 cm×10 cm glass aquarium filled with 2 cm (450 cm^3) of tap water was placed on the filter.

Experiment 2: oviposition preference under natural illumination

To investigate the females' preference under natural illumination, sets of four black plastic tubs of 40 cm×50 cm×20 cm (40 l) were placed around the pond's banks. Each tub was filled with 20 L of tap water (clear, no organic matter) or pond water (turbid; total organic carbon (TOC) concentration, 23.1 mg l^{-1}). The tap and pond water turbidities were measured using a HACH portable turbidimeter 2100P at 0.9±0.2 and 46.0±11.1 NTU, respectively (*N*=20). During a preliminary check, we observed that tubs that were white above water level were not attractive to the females, as no EB were laid in them although they were filled with pond water and reflected high polarization. Therefore, two of the tubs in each set were painted white up to water level (WT/WP for white painted tubs filled with tap/pond water), and the other two tubs were not painted and were left black (BT/BP for black tap/pond water tubs). The distance between the tubs was 1 m to allow the females to choose between several available oviposition habitats located nearby. The reflected intensity, spectra, percentage polarization, and e-vector orientation were measured at sunset within an hour before complete darkness, when the sun was obscured by the Carmel Mountains. EB laid in the tubs were counted in the following morning.

Light measurements

Intensity, spectra, percentage linear polarization, and e-vector orientation were measured using a custom made polarimeter as described by Cronin and Shashar (Cronin and Shashar, 2001; Sabbah and Shashar, 2007). Briefly, an optic fiber (Ocean Optics, Dunedin, Florida, USA; UV/VIS 600 μm) was attached to one side of the tub to a spectrophotometer calibrated for intensity and spectral measurements (Ocean Optics ADC-1000-USB), recording readings at 1 nm practical resolution (0.33 nm nominal resolution) in the range 300–800 nm. The other end of the optic fiber was connected to a 5 deg. acceptance angle restrictor, to which a rotatable plane polarizer (Polaroid HN38S) was attached. Each measurement included a set of three readings with the polarizer set at orientations

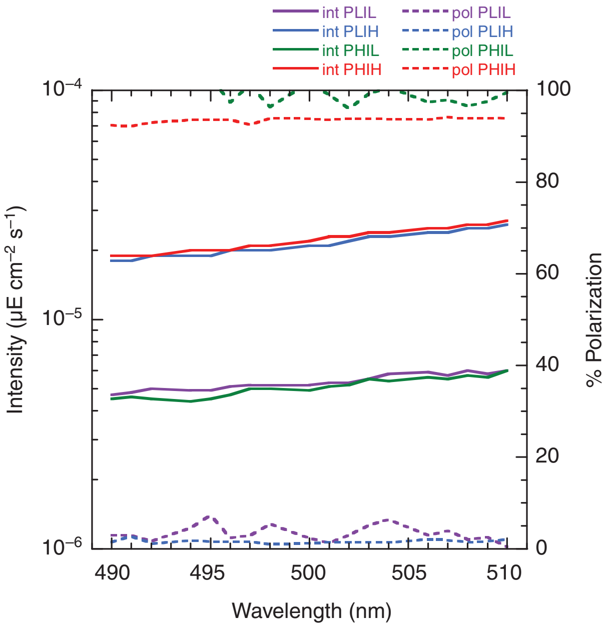

Fig. 2. Intensity (solid lines) and percentage polarization (dashed lines) of radiation measured in the range 490–510 nm reflected from the egg traps in experiment 1. The light source was a 10 W bulb. PLIL, polarization low (unpolarized) intensity low [1 polarizing sheet (1p)+15 diffusing sheets (15d)] ordered from light source to viewer; PLIH, polarization low intensity high (1p+3d); PHIL, polarization high (polarized) intensity low (15d+1p); PHIH, polarization high intensity high (3d+1p).

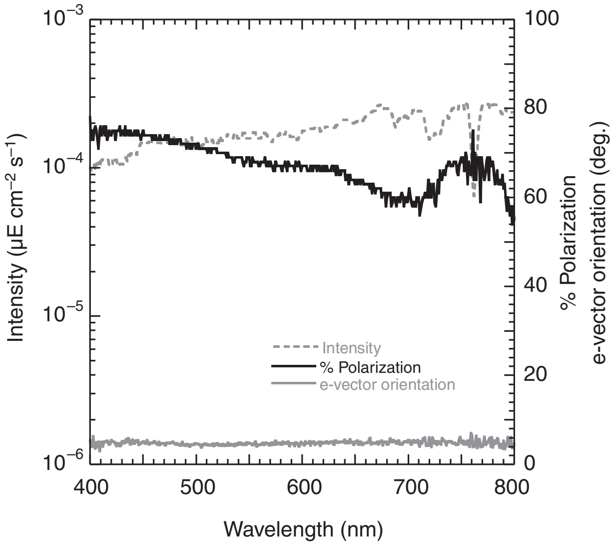

Fig. 3. The spectrum of intensity (dashed grey line, measured without a polarizing filter), percentage polarization (black solid line) and e-vector orientation (grey solid line) reflected from the pond's surface, measured on 17 October 2007 at 16.23 h local time (UTC+2 h). The sun was obscured by the Carmel Ridge from 16.00 h and sunset time was 17.05 h. The sensor was directed 45 deg. from the nadir and 230 deg. from the north. The intensity, percentage polarization and e-vector orientation calculated from the readings integrated over the range 490–510 nm were 2.8×10^{-3} µE cm^{-2} s^{-1}, 71.5%, and 4.4 deg., respectively (where 0 deg. represents horizontal alignment).

of 0 deg., 45 deg. and 90 deg. with respect to the horizon (I_0, I_{45}, I_{90}). Since the polarizer we used absorbs UV radiation (below 400 nm), the three light components, intensity, percentage polarization, and e-vector orientation were calculated from the three readings for each nanometer in the range 490–510 nm in which chironomids are known to be sensitive (Kokkinn and Williams, 1989). This range coincides well with the spectral range of light reflected from the pond before sunset, the starting time of chironomid activity (Fig. 3). The sets of three readings were analyzed using the equations developed by Wolff and Andreou (Wolff and Andreou, 1995), modified by Shashar et al. (Shashar et al., 2004), and described in detail by Sabbah and Shashar (Sabbah and Shashar, 2007). In experiment 1, the distance between the fiber and water surface was 5 cm, oriented to the nadir, and in experiment 2, the fiber was mounted on a tripod, 40 cm above water level, 30 deg. from the nadir, at varying azimuthal angles. In the measurements from the 'natural' chironomid pond, the sensor was placed on the bank edge, 40 cm above water level, 45 deg. from the nadir, and 230 deg. from the north. All light measurements from the tubs in the second experiment and from the pond were performed under clear sky, after the sun was obscured by the Carmel Mountains before sunset. Therefore only diffuse skylight was available as a light source.

Polarization as a predictor to the amount of food in the water

To examine the correlation of polarization with the amount of food in the water, the intensity, percentage polarization, and e-vector orientation of radiation reflected from different pond water concentrations were measured in the waste stabilization station on January 1, 2008; between 7.00 h and 10.00 h local time (UTC+2 h). Reflected light was measured from a 70 l white barrel filled with 60 l (water depth, 45 cm) of pond water diluted with tap water. The amount of food in the water was estimated as the total organic carbon (TOC) concentration, measured for a concentration of 100% of pond water using a Tekmar Dohrmann Apollo 9000 TOC Combustion Analyzer and calculated for each dilution according to the partial volume of the pond water (TOC was assumed to be zero in tap water and to be homogenously distributed in the pond water). The sensor was mounted on a tripod at 40 cm above water level, oriented 30 deg. from the nadir and 230 deg. from the north. The measurements were conducted in a location shaded from direct sunlight.

Statistical analyses

EB counts were analyzed using repeated measures analysis of variance [RM-ANOVA (Sokal and Rohlf, 1995)]. The RM-ANOVA treats the EB values of the four patches within each sampling day as a repeat. In experiment 1, we examined whether intensity and polarization affected the EB number laid, and therefore we applied RM-ANOVA with two within-subject factors: intensity (low/high) and polarization (low/high). We used planned comparisons in experiment 1 to test whether specific pairs of egg traps differ statistically. In experiment 2, we examined whether tub type affected the EB number laid, and therefore we applied RM-ANOVA with a single within-subject factor, consisting of four levels. Pairwise comparisons of the four artificial patches in experiment 2 were examined using Bonferroni *post-hoc* tests. The mean and standard deviation of the e-vector orientations reflected from the tubs in experiment 2 were calculated using the equations for circular statistics (Batschelet, 1981). The statistical analyses of the first experiment were conducted using STATISTICA® 8 (StatSoft, 2007) and those of the second experiment using SPSS® 13.0 (SPSS, 2004).

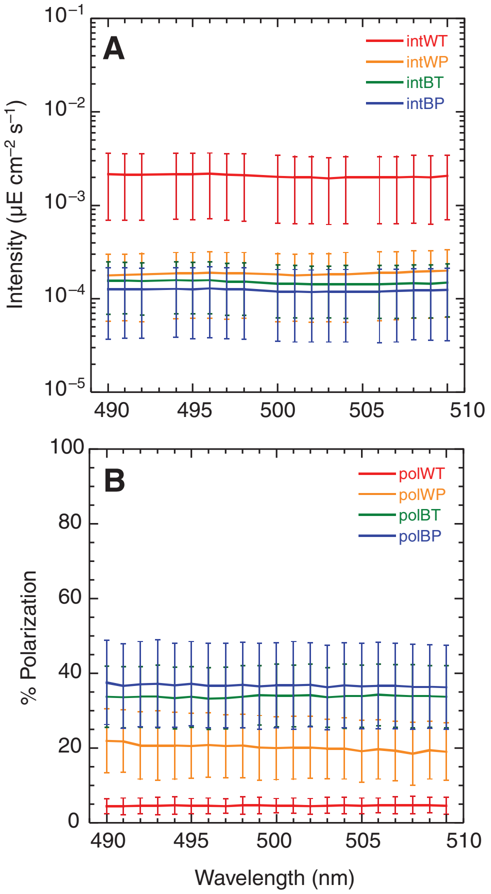

Fig. 4. Mean ± s.d. of the intensity (A) and percentage polarization (B), reflected from the four tubs in experiment 2, as a function of wavelength in the range 490–510 nm. WT, black tub painted white up to water level filled with tap water (N=10; 10 tubs, one measurement from each tub); WP, black tub painted white up to water level filled with pond water (N=10); BT, black tub filled with tap water (N=18); and BP, black tub filled with pond water (N=18). For the reflected intensity, only WT differed significantly from the other tubs (Kruskal–Wallis non-parametric ANOVA; P<0.05). For the reflected percentage polarization, only BT and BP did not differ significantly from each other (pair-wise comparisons, one-way ANOVA, P<0.001). None of the e-vector orientations reflected from the tubs differed significantly from 0 deg. (horizontal).

Electron microscopy

The eyes of *Chironomus transvaalensis* females were sectioned from the ventral part of the eye to check the alignment of the microvilli within the ommatidia. Adult heads were fixed and processed following the protocol described in Zelhof et al. (Zelhof et al., 2006) for *Drosophila*. Whole heads were embedded in an Araldite resin and sectioned using a Leica UltraCut UCT ultramicrotome. The sections were stained with uranyl acetate and lead citrate, and viewed and photographed with a JEOL JEM-1230 transmission electron microscope (TEM) operated at 120 kV.

RESULTS

Intensity and polarization reflected from the egg traps of experiment 2

The intensity and polarization reflected from the tubs in experiment 2 were measured within an hour before sunset each sampling day (Fig. 4A,B, respectively). The mean ± standard deviation (s.d.) of the reflected percentage polarization measured from the tubs in the spectral range 490–510 nm for the tub treatments WT, WP, BT and BP was 4.6±2.1% (N=10), 20.4±8.3% (N=10), 35.4±7.0% (N=18) and 38.5±12.0% (N=18), respectively. Pair-wise comparisons of the means of all combinations showed that the percentage polarization differed between all four patches significantly (one-way ANOVA, $P\leq 0.001$), except for the pair BT and BP, which did not differ significantly from one another ($P\sim 1$). The mean ± s.d. of the mean reflected intensity measured from the tubs at each nanometer over the spectral range of 490–510 nm was 2.06±0.07×10^{-3}, 1.86±0.06×10^{-4}, 1.53±0.06×10^{-4} and 1.37±0.04×10^{-4} (µE cm^{-2} s^{-1} nm^{-1}), for the tub treatments WT, WP, BT and BP, respectively. Pair-wise comparisons following Kruskal–Wallis non-parametric ANOVA (Siegel and Castellan, 1988) applied for wavelengths 400, 450, 490, 500, 510, 550, 600, 650 and 700 nm revealed that in wavelengths <600 nm (the range relevant to chironomid vision), only WT intensity differed significantly from each of the other three patches (P<0.05). None of the e-vector orientations reflected from the tubs differed significantly from 0 deg. (horizontal).

Intensity and polarization reflections from the chironomids' native pond during twilight

The intensity and polarization reflected from the chironomids' 'natural' pond surface were recorded from an hour before sunset until darkness to document the visual cues available for the chironomid females in their natural habitat (Fig. 5). While the intensity decreased by 96% during this period, the percentage polarization remained high and stable at around 60%. The e-vector orientation was also stable around the horizontal (0±4 deg.; mean ± s.d.).

Species identification by laid eggs

During the course of experiment 1, in which EB were collected from egg traps under controlled illumination, EB of four species were found: (1) *Kiefferulus* sp. (6.3% of the total EB; Fig. 6B), which lays green EB that were found on the bottom of the aquarium; (2) *Chironomus transvaalensis* Kieffer 1923 (83.5% of the total EB number; Fig. 6C), which lays brown EB that were found on the air–glass–water interface; (3) *Polypedilum nubifer* Skuse 1889 (10.1% of the total EB number; Fig. 6D), which lays brown curled EB that were found on the bottom of the aquarium; and (4) an unidentified genus of chironomid (0.1% of the total EB number; Fig. 6A), which lays string-shaped EB that were also found on the bottom of the aquarium. During the course of experiment 2, in which EB were collected from egg traps under natural illumination, only *C. transvaalensis* eggs were found.

Egg number counted under controlled illumination in experiment 1

The mean ± s.d. of EB percentages per repeat laid in each trap were 9.5±2.5% in PLIL, 26.4±6.9% in PLIH, 22.8±8.2% in PHIL and 41.3±6.8% in PHIH, respectively (Fig. 7; see Table 1 for the actual number of eggs found). Both intensity and polarization were significant factors affecting EB numbers (RM-ANOVA; $F_{1,9}$=30.2, P<0.001 and $F_{1,9}$=18.5, P=0.002, for intensity and percentage polarization, respectively), whereas the interaction term between them was not ($F_{1,9}$=0.4, P=0.55). Planned comparisons of the means for PLIL *vs* PLIH, PLIL *vs* PHIL, PHIL *vs* PHIH, and PLIH *vs* PHIH were found significant in all four pairs (P<0.005).

Egg number laid under natural illumination in experiment 2

The mean ± s.d. of EB percentages laid in the tubs were 0.6±1.2% in WT, 6.1±5.0% in WP, 38.3±12.7% in BT and 55.1±10.6% in

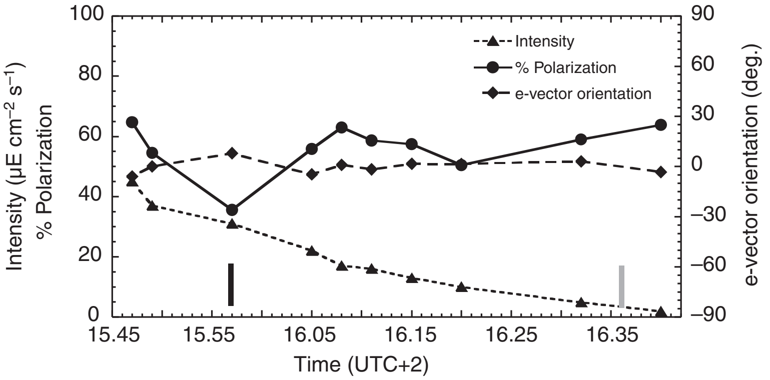

Fig. 5. Intensity (triangles), percentage polarization (circles), and e-vector orientation (diamonds) of the radiance reflected from the pond water surface during sunset, measured on December 10, 2007. Values were measured for the wavelength range 490–510 nm. The sun was obscured by the Carmel Ridge at 15.57 h local time (black vertical bar) and sunset time was 16.36 h (grey vertical bar). Complete darkness occurred at 17.01 h. The e-vector orientation did not differ from horizontal (0±4 deg.). Note that the light intensity decreased by 96%, while the percentage polarization remained stable at around 60%.

BP, respectively (Fig. 8; see Table 2 for the actual number of eggs found). The percentage of EB laid in WT did not differ significantly from 0% [95% confidence interval (CI_{95})=–0.1% to 1.2%]. The RM-ANOVA with a single within-subject factor revealed a significant difference between the number of EB laid in the four tubs ($F_{3,13}=6.5$, $P=0.006$). *Post-hoc* Bonferroni pair-wise comparisons of EB numbers showed a significant difference between all treatments ($P<0.05$).

Intensity and polarization correlation with organic carbon concentrations in the water

The correlation of the reflected intensity and polarization with the TOC concentrations in the water is shown in Fig. 9. Although the intensity decreased with increasing TOC concentration, the percentage polarization increased, reaching a maximum of 13%. The e-vector orientation did not change with increasing TOC concentration and was nearly horizontal at –6°±17°.

Alignment of the microvilli in the ventral part of the female eye

Transverse sections of the ommatidia from the ventral part of the female eye reveal tight alignment of the microvilli in the rhabdomeres (Fig. 10). Two pairs of rhabdomeres (R4, R7 and R1, R2) were found to be aligned orthogonal to one another, whereas R5, R6 and R3 were found to be aligned at an angle of around 45° with respect to the other pairs.

DISCUSSION

The choice of oviposition habitat by flying insects has been demonstrated in the past to be dependent on the reflected polarization of light in the habitat (Schwind, 1991; Kriska et al., 1998; Kelber, 1999; Kelber et al., 2001). Here we quantitatively show that the choice of chironomid females as to where to oviposit is guided by the percentage polarization, independent of the intensity reflected from water bodies, and that the preference to oviposit (the number of EB laid) increases as the percentage polarization of the light reflected from the water surface increases.

In experiment 1, chironomid females held within the tent had to oviposit in one of the treatments presented to them. The EB numbers revealed the animals' preference for both high light intensity and high percentage polarization, independently (Fig. 7). In experiment 2, conducted under open, natural conditions, the females could oviposit in the experimental patches as well as in the nearby pond. The results of this experiment demonstrate that percentage polarization is the signal attracting females to water bodies, as the number of EB laid in the unpolarized patches was not significantly different from zero (Fig. 8), whereas it was positively related to the percentage polarization in polarized patches. This finding agrees with the observations in previous studies that flying insects tend to oviposit on high polarization reflecting substrates (Schwind, 1991; Kriska et al., 1998; Kelber, 1999; Kelber et al., 2001; Bernath et al., 2002).

One concern in examining the response to polarization in insects is that specific orientations of polarization may be perceived by the

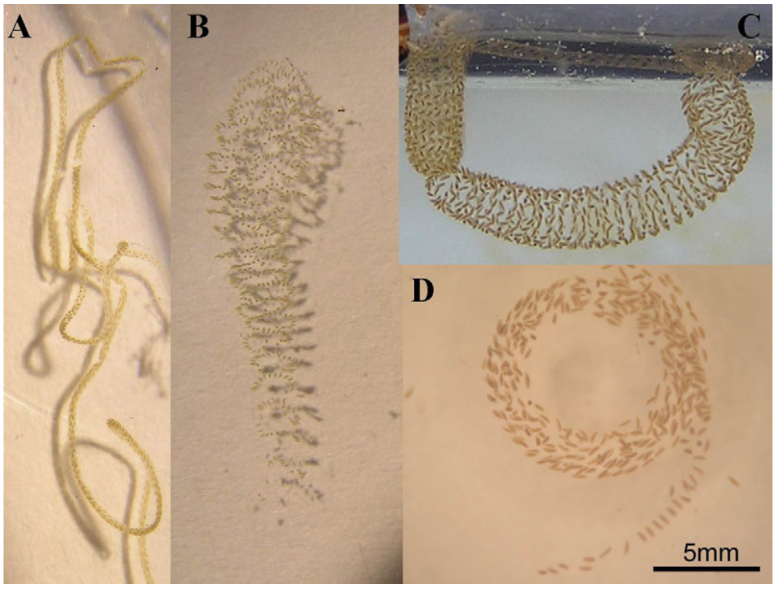

Fig. 6. Chironomid egg batch (EB) types and their relative contribution (%) to the total eggs laid in experiment 1. (A) An unidentified genus of chironomids (0.1%), (B) *Kiefferulus* sp. (6.3%), (C) *Chironomus transvaalensis* (83.5%), and (D) *Polypedilum nubifer* (10.1%). Note that in experiment 2 conducted outdoors, only *C. transvaalensis* eggs were found.

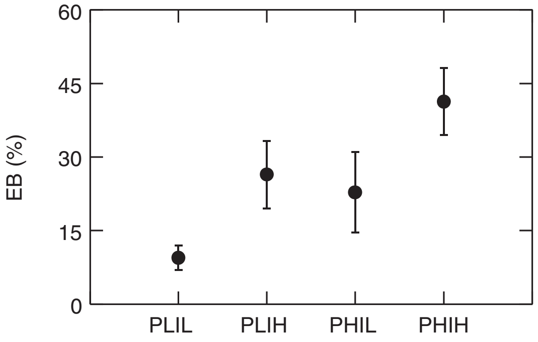

Fig. 7. Mean ± s.d. of percentages of egg batches (EB) laid in artificial egg traps in experiment 1 (total EB=10592; *N*=10 repeats). Planned comparison of means of PLIL *vs* PLIH, PLIL *vs* PHIL, PHIL *vs* PHIH, and PLIH *vs* PHIH were found significant in all four pairs ($P<0.005$). PLIL, polarization low (unpolarized) intensity low; PLIH, polarization low intensity high; PHIL, polarization high (polarized) intensity low; PHIH, polarization high intensity high.

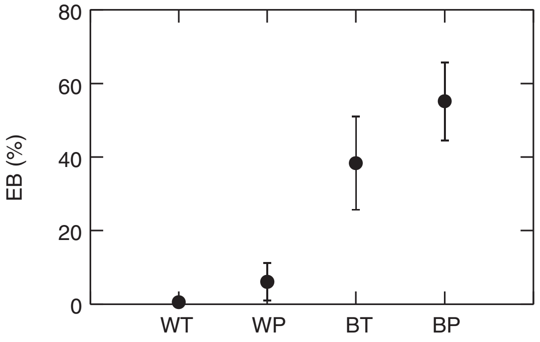

Fig. 8. Mean ± s.d. of percentages of egg batches (EB) laid in the tubs in experiment 2 (total EB=3543; *N*=16 repeats). A Bonferroni *post-hoc* test revealed a significant difference between all treatments ($P<0.05$). Note that EB laid in WT (black tub painted white up to water level and filled with tap water) was not significantly different from 0%. WP, black tub painted white up to water level filled with pond water; BT, black tub filled with tap water; BP, black tub filled with pond water.

animals as exceptionally bright (Kentaro Arikawa, personal communication), and if the insects are attracted to bright areas, they might show pseudo-polarotaxis. However, in our tent experiment, the light passed through a linearly polarizing filter, and the animals' flight and orientation of approach was not restricted; on average the filter could have been seen from any direction and could have presented a range of polarization orientations. Furthermore, the position of the polarizing filters was shuffled randomly between the days of the experiments. Therefore, such a polarization-based intensity gain is unlikely in our setting.

Another concern in most behavioral choice studies is that other unexpected visual cues may guide the studied behavior, producing false positive results. In our case, we tried to avoid, or at least critically examined, such undesired cues in both experiments. In the tent experiment (experiment 1), we used the very same filters in differing order to maintain the spectra of reflected radiation and intensity (when relevant) of the targets. In the outdoor experiment (experiment 2), at wavelengths <600 nm, all treatments had nearly the same spectra, and with the exception of the white painted tubs, all treatments had similar intensities (Fig. 4A). The low numbers of EB laid in the white painted tubs in experiment 2, despite the attraction to high intensity reflection exhibited in experiment 1, strengthen our reasoning that the polarization of reflection is the most important cue directing oviposition by chironomids in the wild. Having said that, one should note that in experiment 2, pond water was always favored over tap water (Fig. 4). Although there is no question that chironomid females readily lay eggs in tap water, and that polarization has a greater impact than the origin/quality of the water, it may possibly be that pond water carries an additional, yet undetermined, quality adding to its attractiveness.

One should note that the microvilli arrangement found in an ommatidium from the ventral part of a female eye (Fig. 10) support the behavioral findings. The microvilli in each rhabdom are seen to be parallel to one another and are generally aligned along three axes within the ommatidium, providing a potential anatomical basis for polarization sensitivity. Further detailed examination of the microvilli in different regions of the eye and a comparison with

Table 1. Number of egg batches in the four eggs traps in experiment 1

	Type of light in the trap			
Repeat	PLIL	PLIH	PHIL	PHIH
1	38	49	82	149
2	18	104	31	149
3	71	191	94	353
4	126	257	336	440
5	243	489	660	857
6	96	417	209	405
7	81	345	321	372
8	59	272	164	415
9	95	297	210	490
10	225	297	581	504
Average	105.2	271.8	268.8	413.4

PLIL, polarization low (unpolarized) intensity low; PLIH, polarization low intensity high; PHIL, polarization high (polarized) intensity low ; PHIH, polarization high intensity high.

Table 2. Number of egg batches in the four eggs traps in experiment 2

	Type of trap			
Repeat	WT	WP	BT	BP
1	0	3	12	26
2	0	14	29	58
3	0	3	5	42
4	1	28	37	91
5	0	2	23	37
6	1	7	16	29
7	2	4	17	21
8	0	0	23	12
9	0	5	50	73
10	0	5	94	80
11	0	2	47	78
12	0	2	68	83
13	3	13	216	290
14	0	36	349	323
15	4	28	175	228
16	2	25	342	379
Average	0.8	11.1	93.9	115.6

WT, black tub painted white up to the water level, filled with tap water; WP, black tub painted white up to water level, filled with pond water; BT, black tub filled with tap water; BP, black tub filled with pond water.

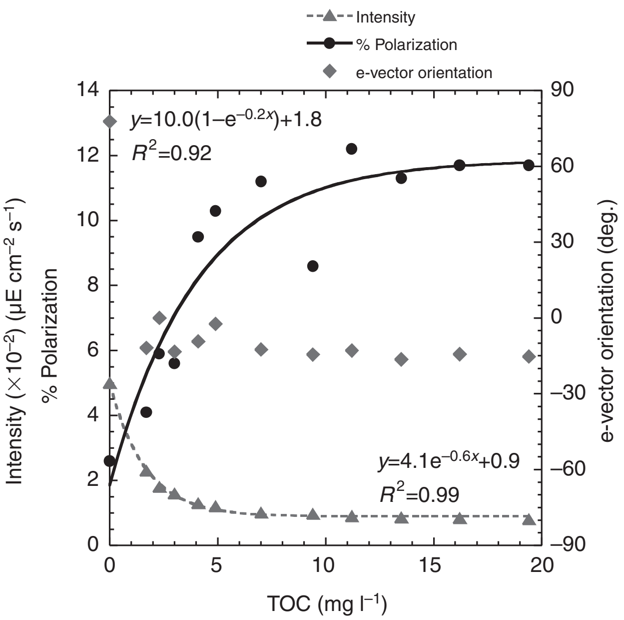

Fig. 9. Intensity (triangles), percentage polarization (circles), and e-vector orientation (diamonds) of the radiance reflected from a 70 l white barrel filled with 60 l of pond water *versus* the total organic carbon (TOC) concentration measured in the water. Values were measured for the wavelength range 490–510 nm. The e-vector orientation did not differ significantly from horizontal (–6±17 deg.). A value of TOC=0 mg l^{-1} was excluded, since the corresponding reflected radiance was almost unpolarized (percentage polarization=2.6%).

males, as well as a neurological study of the response of chironomids to polarized light, are needed to fully understand the properties of their polarization sensitivity.

Advantages of using polarization over intensity as an environmental cue during sunset

Short-lived animals, such as chironomids, need to have rapid and stable environmental cues in their search for ovipositing sites. As mentioned earlier, Meltser et al. (Meltser et al., 2008) showed that, unlike mosquitoes, the preference of chironomid females for an oviposition site is not guided by odor. Our light measurements confirmed that whereas intensity decreases rapidly during sunset, percentage polarization and e-vector orientation, which are determined by scattering and absorption of light by suspended matter in the water, remain high and stable throughout this period (Fig. 5). Furthermore, by using polarization as a cue, polarization-sensitive animals can prolong their time of activity into the twilight period [e.g. by using polarized moonlight, as do dung-beetles (Dacke et al., 2003)].

Polarization as a predictor of the amount of food in water

In Fig. 9, it is demonstrated that polarization reflected from the water surface is positively correlated with the total organic carbon (TOC) concentration in the water. We therefore believe that polarization can serve as a visual proxy for the amount of food in the water available to the larvae of chironomid females. It is important to note, however, that water depth and the darkness of the pond floor, as well as non-organic light absorbing particles, which reflect low intensity and high percentage polarization even in clear water, can easily deceive ovipositing chironomid females, as exemplified in the results from the black tub filled with tap water (BT) in experiment 2.

Wavelength sensitivity to polarization by chironomids

Schwind (Schwind, 1995) showed that different species of aquatic insects respond differently to surfaces reflecting polarization at different wavelengths. He divided species into short wavelength users (UV; λ<400 nm) and long wavelength users (green-yellow; λ=500–550 nm). Chironomids are known to be sensitive to light in both spectral regions (Kokkinn and Williams, 1989). In this study, UV radiance could not be correlated with oviposition response due to system (polarizer) limitations. Since the radiance of green light could be correlated with oviposition response, we can say that it is likely that at least the 'green' photoreceptors are polarization sensitive. The use of spectral polarization by chironomids warrants further examination.

Percentage polarization or e-vector orientation – which is the guiding cue?

Over the years, most studies investigating the use of light polarization by polarization-sensitive animals surmised that the cue

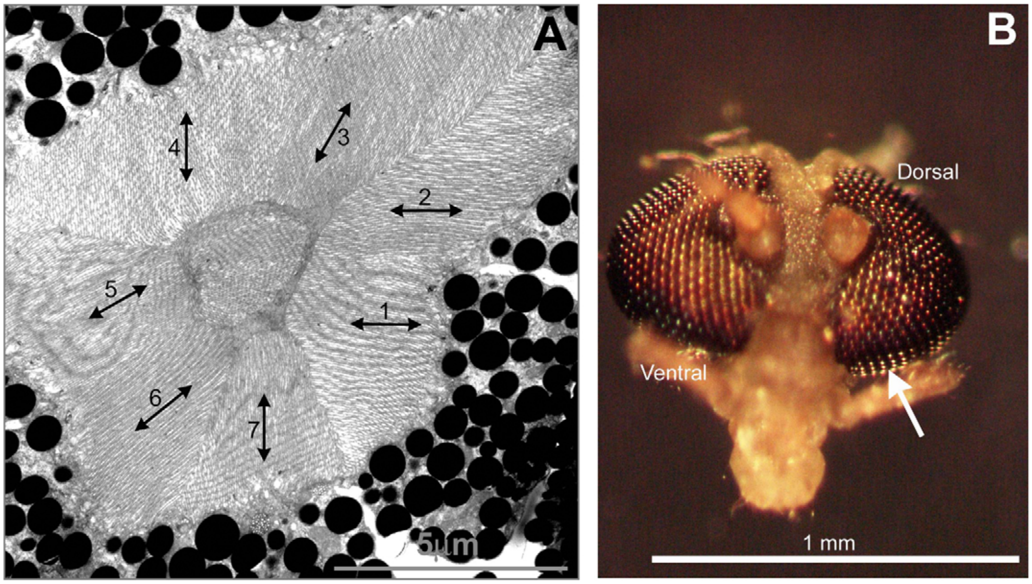

Fig. 10. (A) Transmission electron micrograph (TEM) of a transverse section of an ommatidium from the ventral part (white arrow in B) of the *Chironomus transvaalensis* female eye. The double-headed black arrows in A indicate the general alignment of the microvilli of rhabdomeres R1–R7. Note that the microvilli of R4 and R7 are orthogonal to the microvilli of R1 and R2, and both are aligned at 45 deg. with respect to R5,R6 and R3, providing the anatomical basis for linear polarization detection.

actually used by the animals is the e-vector orientation rather than the percentage polarization. This is the case for navigating insects such as bees, ants, crickets and for flying insects that use polarization to detect water bodies. Our results indicate that at least in the case of chironomids, the cue is the percentage polarization (Fig. 8). In our experiments, the orientation of polarization was close to horizontal in all cases because of strong attenuation (scattering and absorption) by suspended matter in the water. As mentioned earlier, percentage polarization, which is determined by the concentration of the suspended matter, can serve as a proxy for the amount of particles containing organic carbon.

It is possible, however, that the low oviposition rates that we found at low percentage polarizations (<10%) are the result of high noise in the visual system of the animals that makes them insensitive to this low polarization signal. Such high neurological noise could also lower the response to polarizations of less than 20% (Figs 4 and 8). Further neurological measurements are required to assess and characterize the signal within the chironomid's brain.

Chironomid populations and cholera control

Chironomids are the carriers of the *Vibrio cholerae* bacterium, a human pathogen responsible for the fatal cholera disease. The bacterium, which feeds on the eggs, can be dispersed between water bodies by the adults (Broza and Halpern, 2001; Halpern et al., 2004; Broza et al., 2005; Halpern et al., 2006; Paz and Broza, 2007). This study suggests two ways to control chironomid abundance and dispersal and consequently to limit the persistence and spread of cholera: (1) decrease the number of preferred habitats for oviposition, and (2) direct the females to alternative, more attractive artificial habitats and then collect or destroy the eggs. The first strategy can be achieved by decreasing the percentage polarization of light reflected from a water body by increasing the reflectivity of the water. The second strategy can be achieved by creating artificial trapping ponds near natural ponds that will be more attractive to the females because of their high polarization reflection. This may be expanded to polarized light traps placed alongside specific water bodies (Kentaro Arikawa, personal communication). It is worth mentioning again our preliminary observation that tubs with white edges above water level are completely unattractive to chironomid females.

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