The Journal of Experimental Biology 215, 3577-3586

doi:10.1242/jeb.075028

RESEARCH ARTICLE

Electrophysiological evidence for polarization sensitivity in the camera-type eyes of the aquatic predacious insect larva *Thermonectus marmoratus*

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SUMMARY

Polarization sensitivity has most often been studied in mature insects, yet it is likely that larvae also make use of this visual modality. The aquatic larvae of the predacious diving beetle *Thermonectus marmoratus* are highly successful visually guided predators, with a UV-sensitive proximal retina that, according to its ultrastructure, has three distinct cell types with anatomical attributes that are consistent with polarization sensitivity. In the present study we used electrophysiological methods and single-cell staining to confirm polarization sensitivity in the proximal retinas of both principal eyes of these larvae. As expected from their microvillar orientation, cells of type T1 are most sensitive to vertically polarized light, while cells of type T2 are most sensitive to horizontally polarized light. In addition, T3 cells probably constitute a second population of cells that are most sensitive to light with vertical e-vector orientation, characterized by shallower polarization modulations, and smaller polarization sensitivity (PS) values than are typical for T1 cells. The level of PS values found in this study suggests that polarization sensitivity probably plays an important role in the visual system of these larvae. Based on their natural history and behavior, possible functions are: (1) finding water after hatching, (2) finding the shore before pupation, and (3) making prey more visible, by filtering out horizontally polarized haze, and/or using polarization features for prey detection.

Key words: stemmata, prey capture, eye, predator, e-vector.

Received 14 May 2012; Accepted 20 June 2012

INTRODUCTION

Polarization cues are known to be important for many adult insects. Most commonly they are used for navigation and habitat or ovipositor site detection, as well as for finding mates. In aquatic habitats, animals such as certain fish, lobster, crabs, crayfish, mantis shrimp and cephalopods have been found to use polarization sensitivity for communication, to improve the visual contrast of their surroundings, or to detect prey (Horváth and Varjú, 2004; Shashar et al., 2011; Wehner, 2001). Although it has been suggested that polarization vision for contrast enhancement and prey detection could also play a role in insect visual systems (Schneider and Langer, 1969; Trujillo-Cenóz and Bernard, 1972; Horváth and Varjú, 2004), to the best of our knowledge, this has never been demonstrated. Even less is known about polarization sensitivity in insect larvae. With regard to the latter we only know that some, such as gypsy moth larvae, sawfly larvae, mosquito larvae and tent caterpillar larvae, show polarotaxis (Wellington et al., 1951; Baylor and Smith, 1953; Sullivan and Wellington, 1953; Wellington, 1955; Meyer-Rochow, 1974; Doane and Leonard, 1975; Gilbert, 1994). Previously our group presented ultrastructural data that raised the possibility of the existence of polarization sensitivity in a specialized region of the complex principal eyes of *Thermonectus marmoratus* (Coleoptera: Dytiscidae) larvae (Stecher et al., 2010). These larvae are highly successful visually guided aquatic predators, which could potentially exploit polarization sensitivity to improve contrast and see prey better. We present electrophysiological data that confirms our anatomical predictions, both with regard to the existence of polarization sensitivity, and with regard to the e-vector orientation to which individual cell types are maximally sensitive.

There are two main sources of polarized light in natural environments: (1) the scattering of light in bulk media such as the atmosphere or water, and (2) the light reflected from shiny surfaces (Horváth and Varjú, 2004). In the air, polarized light comes from light scattering in the atmosphere with a predictable polarization pattern that changes slowly over time. It also comes from reflecting surfaces such as leaves or water. The polarization patterns of this light might change rapidly and unpredictably, especially as the orientation of reflecting surfaces changes with waves or wind. Most studies with regard to polarization sensitivity or polarization vision in air show utilization of this ability within three broad categories. First, polarization sensitivity is used to gain insights on compass directions. For example, insects such as bees, ants and locusts exploit the polarization pattern of the sky for orientation and navigation (Fent, 1986; Rossel, 1993; Mappes and Homberg, 2004; Wehner and Müller, 2006). Second, polarization cues are used to recognize specific habitats. For example, water beetles and bugs use the polarization pattern of reflecting surfaces as a visual cue to find habitats (Schwind, 1984; Schwind, 1991), and insects such as mayflies, midges and dragonflies use the pattern to find water surfaces to use as their oviposition sites (Kriska et al., 1998; Wildermuth, 1998; Kriska et al., 2007; Lerner et al., 2008). Finally, polarization sensitivity is used for communication and mate recognition. Some animals have polarization-active body parts. For example, polarization-sensitive butterflies have been shown to use this visual cue for finding mates in the rain forest where there is little interference from other polarized light sources due to the dense vegetation (Sweeney et al., 2003).

Some animals are also known to use underwater polarization cues. Due to its higher refractive index, in water, less polarized light is

reflected from surfaces than in air. Instead, almost all polarization emerges from the scattering of light in bulk media, resulting in polarization patterns that are more predictable but also more complex than those found in air. The complexity arises from factors such as depth, line of view, elevation of the sun, wavelength of the light, visibility of the bottom, proximity of the shore, and water as well as weather conditions (Waterman and Westell, 1956; Ivanoff and Waterman, 1958; Novales Flamarique and Hawryshyn, 1997). However, it is precisely the predictability of polarization patterns that allows for exploitation of polarization sensitivity for orientation, contrast enhancement, and for using the polarization features of animals as reliable visual cues for communication or prey detection (Cronin, 2006; Wehner and Labhart, 2006; Shashar et al., 2011).

Generally there is relatively poor visibility in water compared with air. This is primarily because the contrast of any scenery is drastically decreased due to the scattering of light within the water. However, scattered light is mainly polarized horizontally, so that a vertical polarization filter can increase the overall contrast by filtering out the haze (Lythgoe and Hemmings, 1967; Cronin and Marshall, 2011; Johnsen et al., 2011). Additionally, muscle tissue and other body structures can influence the polarization of light, leading to a visual cue that can be used to detect prey or enhance communication. Specifically, tissue might polarize unpolarized light, or depolarize or change the e-vector orientation of existing polarized light (Shashar et al., 2000; Cronin et al., 2003; Sabbah and Shashar, 2006; Johnsen et al., 2011). Such body parts can increase the visibility of prey to polarization-sensitive predators such as fish and cephalopods (Shashar et al., 1998; Shashar et al., 2000; Johnsen et al., 2011; Kamermans and Hawryshyn, 2011), or might be used for communication as suggested in cephalopods and mantis shrimps (Shashar et al., 1996; Marshall et al., 1999). So far, such use of polarization sensitivity has never been shown for any insect, even though some, such as *T. marmoratus* and other predacious aquatic insects, could clearly benefit from such mechanisms.

Thermonectus marmoratus larvae are aquatic visually guided predators native to the southwest United States (Larson et al., 2000). The larvae are found in shallow ponds and small slow-flowing streams (Velasco and Millan, 1998; Evans and Hogue, 2006) and tend to swim with their principal eyes directed approximately horizontally. Thus the polarization patterns that are formed relatively close to the surface in the horizontal line of view should be most important. In this line of view the polarization of light can be primarily explained by the refractive angle of the incident light. Additionally, it is influenced by weather and water conditions, the wavelength of the light, the albedo of a visible bottom, and the proximity to the shore (Waterman and Westell, 1956; Ivanoff and Waterman, 1958; Novales Flamarique and Hawryshyn, 1997). Overall, the percent polarization during the day might reach up to 40% and the e-vector of the polarized light during the day is approximately horizontal as long as the sun zenith angle is not too large (Novales Flamarique and Hawryshyn, 1997). In the presence of polarized light, zooplankton and many other small transparent organisms that possess polarization-active body parts are potentially more visible to a polarization-sensitive predator (Johnsen et al., 2011). For example, prey of *T. marmoratus* larvae, such as mosquito larvae, show clear polarization features (Stecher et al., 2010) that the larvae could potentially use as visual cues to better detect their prey, if adequate polarization sensitivity exists in the principal eyes of these larvae.

Thermonectus marmoratus larvae have 12 eyes, six on each side of the head. Four of these eyes (E1 and E2 on each side) are tubular and look directly forward (Fig. 1A). The larvae scan with these

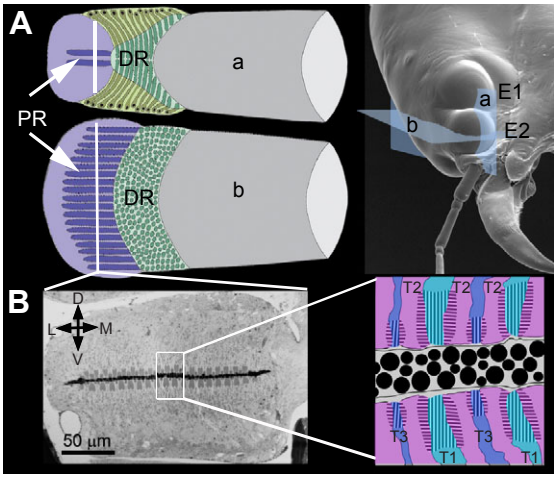

Fig. 1. Schematic of the principal eye's structure of *Thermonectus marmoratus* larvae. (A) Horizontal (a) and sagittal (b) schematic of eye 2 (E2) indicating the position of the distal (DR) and proximal (PR) retinas. The white line marks the approximate position of B. (B) Microstructure of the PR, containing three photoreceptor types: T1, T2 and T3. The insert schematically illustrates the microvillar orientation for each of these cells. D, dorsal; V, ventral; L, lateral; M, medial.

principal eyes by oscillating their heads dorsoventrally as they approach potential prey (Buschbeck et al., 2007). The anatomy of the retinas of these principal eyes is unusual (Mandapaka et al., 2006; Maksimovic et al., 2011). The retinas are divided into distinct distal and proximal portions. The distal retina consists of at least 12 tiers of photoreceptor cells with rhabdomes that are oriented approximately perpendicular to the light path. The microvillar orientation of these cells is irregular (Stecher et al., 2010). The proximal retina lies directly beneath and contains photoreceptor cells, the rhabdomes of which are oriented parallel to the light path, as illustrated in the schematic of Fig. 1A. Based on an ultrastructural study (Stecher et al., 2010), it has been suggested that the proximal retina could be polarization sensitive because it contains cell types that meet common key characteristics that lead to polarization sensitivity in invertebrates. Those include the presence of parallel microvilli within individual photoreceptors, perpendicular orientation of microvilli in neighboring photoreceptors, and the presence of identical spectral sensitivity (Wehner and Labhart, 2006). In *T. marmoratus* the proximal retina is sensitive to UV (Maksimovic et al., 2011) and is composed of three cell types. Two of these types (T1 and T3) have a vertical (dorsoventral) microvillar orientation and one (T2) has a horizontal (mediolateral) microvillar orientation (Fig. 1B). Within the retina, the three types are situated in an alternating pattern so that cells with vertical microvillar orientation are adjacent to cells with horizontal microvillar orientation (Fig. 1B). However, before light reaches the proximal retina, it first travels through the rhabdomeric portion of the distal retina (Fig. 1A). Prior to this study, it was unclear if the polarization sensitivity might diverge from what was expected from the microvillar orientation, since rhabdomes potentially alter polarized light (Chiou et al., 2008).

Based on electrophysiological measurements of third instar larvae, we present data that clearly demonstrate that the proximal retina is indeed polarization sensitive. Our data show that two of the three cell types have relatively high polarization sensitivity (PS), and that the orientation of polarization sensitivity corresponds well with predictions from the anatomical data: T2 cells are most

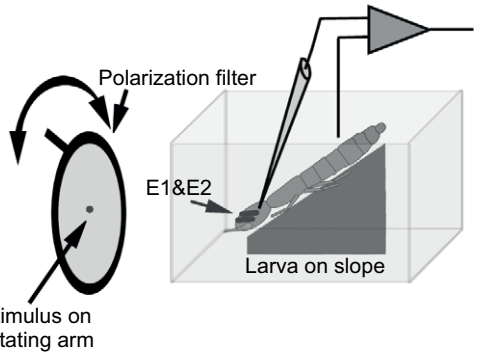

Fig. 2. Schematic of setup, which contains a rotating arm with the light stimulus (that could be moved freely during recordings), a polarization filter that can be rotated, and a sloped specimen holder within a small glass container (filled with saline solution) onto which the larvae was mounted so that the principal eyes were oriented horizontally. During experiments a sharp glass electrode was inserted near the back of each eye tube, and the indifferent electrode was placed in the saline solution.

sensitive to horizontally polarized light, and T1 cells are most sensitive to vertically polarized light.

MATERIALS AND METHODS

Animals

Thermonectus marmoratus (Gray 1831) larvae were offspring of beetles provided by the Insectarium of the Cincinnati Zoo and Botanic Garden, or of beetles collected between 2004 and 2012 near Tucson, AZ, USA. A population of *T. marmoratus* is maintained in our laboratory throughout the year. *Thermonectus marmoratus* larvae were reared in isolation on previously frozen bloodworms and live mosquito larvae. All data were obtained from third instar larvae, 3–5 days after ecdysis.

Animal preparation

The larvae were anesthetized on ice and placed, head downward, onto a 35 deg slope so that the eye tubes of E1 and E2 were oriented approximately horizontally (Fig. 2). Apart from the head and the tip of the abdomen, larvae were immobilized in 2% agar gel. The head and mandibles were immobilized with dental wax (#091-1578, Patterson, St Paul, MN, USA). In some trials, to specifically target photoreceptors of E1 or E2, the excluded eye was occluded with opaque nail polish. The animal was positioned with its eyes 1 cm behind the polarization filter (Fig. 2). Apart from the tip of the abdomen, the animal was submerged in 50% insect Ringer solution (O'Shea and Adams, 1981) containing 0.01% trypsin (Fisher Science Education, Hanover Park, IL, USA) or 0.01% protease from *Streptomyces griseus* (Sigma-Aldrich, St Louis, MO, USA). The protease inhibited the coagulation of the hemolymph, which otherwise formed a gelatinous mass that made it difficult to advance the electrode. To gain access to the photoreceptors of E2, the lens of E6 was removed. To access the photoreceptors of E1, either the lens of E6 or E5 was removed. Immediately thereafter a microelectrode was advanced into the tissue with a motorized manipulator, and from then on manipulations were performed under dim red light, to which the photoreceptors showed no response. In total we recorded from 38 animals (14 E1 and 24 E2). Although we most often only recorded from one cell per eye, in a few instances we recorded from two cells: one most sensitive to vertical e-vector orientation, and one most sensitive to horizontal e-vector orientation.

Intracellular recording and neurobiotin iontophoresis

The electrophysiological setup was composed of standard equipment including an Axoclamp-2A amplifier with a HS-2A gain ×1 headstage (Molecular Devices, Sunnyvale, CA, USA), iWorks AD board 118 (iWorks Systems, Dover, NH, USA), A-M Systems audio monitor 330 (A-M Systems, Sequim, WA, USA), a Tektronix 5103N oscilloscope (Tektronix, Beaverton, OR, USA), a vibration isolation platform (TMC-66-501, Technica Manufacturing, Peabody, MA, USA) and a Faraday cage. A silver wire that was inserted into the insect Ringer solution served as a reference electrode.

The experimental setup also included a UV transmissive polarization filter (BVO UV Polarizer RAW film, Bolder Vision Optics, Boulder, CO, USA) that was mounted onto a rotary optic mount (Edmund Optics, Barrington, NJ, USA). The light stimulus consisted of a UV LED with a peak wavelength of 383 nm and a half-width of 10 nm (30 mW/15, RL5-UV0315-380, Super Bright LEDs, St Louis, MO, USA) that was mounted onto a rotating arm. The peak emission of the LED was close to the peak sensitivity of the photoreceptor cells of the proximal retina, which were previously reported to be 375 nm with a half-width of 75 nm (Maksimovic et al., 2011). The LED was positioned a couple of millimeters behind the polarization filter. Both the polarization filter orientation as well as the stimulus position could be freely adjusted throughout the recording, as they were mechanically uncoupled from the vibration isolation table. The light intensity of the LED was controlled through the AD board with LabScribe2 (v. 2.301, iWorks Systems). The light intensity, measured with a cosine corrector (Ocean Optics, Dunedin, FL, USA), ranged from 7.97×10^{15} to 1.18×10^{19} photons cm^{-2} s^{-1} at the position of the eye. The intensity was measured with a calibrated spectrometer (USB2000+; Ocean Optics).

To establish the response–stimulus intensity (V–log$I$) relationship, 20 ms light pulses (with 2 s intervals) were presented for 12 light intensities over 3 log units. Driving the LED with the chosen voltages yielded stable and reproducible light intensities and a stable emission spectrum. Our LED stimulus tended to truncate the flatter upper and lower portions of the V–log$I$ curve; however, all critical measurements, as well as the PS value calculations, were performed within its confirmed linear range. A 20 ms stimulus yielded a clean response that did not overlap with the stimulus artifact. Intracellular recordings were performed with high impedance glass microelectrodes (A-M Systems; catalog no. 601000) with a resistance of 70–120 MΩ, which were pulled with a horizontal puller (P97, Sutter Instruments, Novato, CA, USA). The tips of the electrodes were filled with 2% neurobiotin in 3 mol l^{-1} KCl (Vector Laboratories, Burlingame, CA, USA), and the remainder with 3 mol l^{-1} KCl (separated by a small air bubble).

After a photoreceptor cell was impaled, the stimulus was positioned to maximize the response. Measurements were only taken from cells with stable resting potentials and response strengths of at least 20 mV, even when the polarization filter was turned perpendicular to the optimal e-vector orientation. After successful recordings, cells were iontophoretically injected with neurobiotin for ~15 min by either passing a constant or pulsing current (150 ms, 2–3 nA pulses at 3 Hz). Thereafter, intact animals were placed in 50% insect Ringer solution for 10–30 min at room temperature to allow neurobiotin to distribute throughout the cell. The data were recorded and stored, a moving average (10 points; 1 ms) was calculated using LabScribe software (LabScribe2, v. 2.301) and data were analyzed with customized MATLAB (MathWorks, Natick, MA, USA) programs. For each stimulus, the stimulus intensity was calculated from the average resting potential (over 200 µs prior to the stimulus onset) and the maximum response.

Optimal e-vector orientation

Light intensities were chosen that fell in the linear range of the V–log$I$ response curves. Stimulus intensities were slightly adjusted for individual cells. To determine how well each cell responded to polarized light of different orientation, the polarization filter was turned in 5 deg steps over 180 deg. This was repeated up to five times per cell, and the e-vector direction for which a cell showed minimal and maximal responses was determined from these data. To achieve this, for each individual cell, the cycles were normalized to the maximum response magnitude of the cell and fitted to a sinusoidal curve $f(x)=a\sin(bx+c)+d$ using the cftool() function of MATLAB's curve-fitting toolbox. The e-vector direction with respect to the head position (taken from frontal images of the head) that yielded minimum and maximum response was obtained from this fit. To visualize the response magnitude dependency on e-vector direction, for each cell, the response magnitudes were averaged and normalized (maximum=1; minimum=0). After rounding the e-vector directions to the nearest 5 deg, the average of all cells was calculated.

Quantifying polarization sensitivity

V–log$I$ relationships were determined for e-vector orientations that yielded minimum responses (min V–log$I$), as well as to perpendicular e-vector directions (max V–log$I$). For each stimulus intensity the response was measured three to five times. For each cell the response magnitudes of both e-vector orientations were fitted to the hyperbolic Naka–Rushton function (Naka and Rushton, 1966; Menzel et al., 1986; Skorupski et al., 2007): $V=(I^n V_{max})/(I^n+K^n)$, where V is the response magnitude (mV), I is the stimulus intensity, K is the stimulus intensity at $V_{max}/2$ (measured in photons cm^{-2} s^{-1}) and n is the slope of the curve. From this fit, the PS was calculated from the shift of the V–log$I$ response curves at $V_{max}/2$. Specifically, PS is defined as $PS=10^{\Delta i}$, where Δi is the difference in log I units between the two V–log$I$ curves at K (Dacke et al., 2002; Kleinlogel and Marshall, 2006). To visualize the normalized V–log$I$ curves (Fig. 6), we first determined the maximum and minimum responses of the max V–log$I$ of each cell. Subsequently, max V–log$I$ and min V–log$I$ curves were normalized to these values (max=1; min=0). Cells of E1 (Fig. 6A,B) and E2 (Fig. 6C,D) were considered separately.

In order to visualize relative response differences between cell types (Fig. 7), we pooled data from E1 and E2 for cells for which we had V–log$I$ curves and therefore could confirm that measurements were indeed within the linear range of these curves. To normalize measurements without affecting the magnitude of the modulation, for each data point we calculated the difference to the maximum response magnitude of the cell (Δ to max response, in mV).

Histology

After completion of the recordings and injection of neurobiotin, the animal was decapitated and processed as previously described (Maksimovic et al., 2011). In brief, animals were fixed in 4% paraformaldehyde solution (Electron Microscopy Sciences, Hatfield, PA, USA) in 0.2 mol l^{-1} Sorensen's buffer (Electron Microscopy Sciences) for 14–16 h at 4°C. After thorough washing in Sorensen's buffer the tissue was dehydrated, washed in propylene oxide for ~15 min to improve penetration, and rehydrated. Subsequently, the tissue was incubated with streptavidin conjugated with Alexa Fluor 568 (Life Technologies Corporation, Carlsbad, CA, USA) diluted 1:200 (working concentration 0.5 μg ml^{-1}) in Sorensen's buffer with 1% Triton X-100 for 14–16 h at room temperature, washed, dehydrated and embedded in Ultra-Low Viscosity Embedding Medium (Polysciences, Warrington, PA, USA). Finally, the tissue was serially sectioned at 15 μm, mounted and imaged with an Olympus 60806 digital camera (Olympus America, Center Valley, PA, USA) or a Zeiss LSM 510 laser scanning confocal microscope (Carl Zeiss AG, Oberkochen, Germany). For transmission electron microscopy, tissue was processed as described by Wolff (Wolff, 2011), with the following modifications: Sorensen's buffer was used instead of sodium cacodylate, the heads were incubated in the fixative in the refrigerator overnight, and tissue was embedded in Ultra-Low Viscosity Embedding Medium. Ultrathin sections of the proximal retina were taken with an Ultracut E Microtome (Reichert-Jung), visualized with a transmission electron microscope (JOEL JEM-1230) and digital images were taken with a Megaplus ES 4.0 camera. The brightness and contrast of all final images was adjusted with Adobe Photoshop CS3 (Adobe Systems, San Jose, CA, USA).

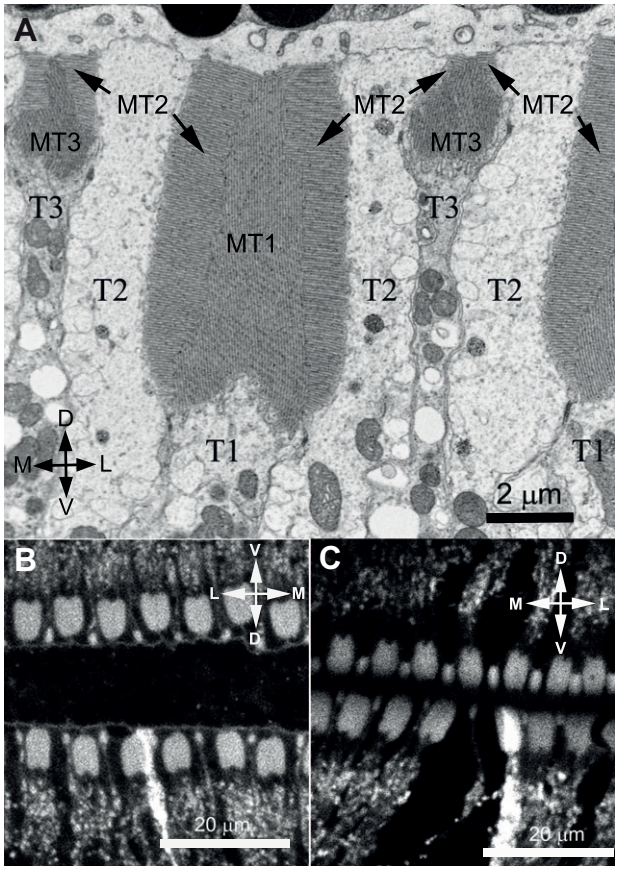

Fig. 3. Histological images. (A) Transmission electron micrograph of a cross section of the proximal retina of E2 of a third instar larva. As has been the case for first instar larvae, three distinct cell types are discernible: T1 and T3 have vertically oriented microvilli; T2 is situated between T1 and T3 and has horizontally oriented microvilli that are immediately adjacent to the microvilli of T1 and T3 (with two sets of microvilli for each cell). MT1, MT2 and MT3 indicate the position of microvilli for each cell. (B) Example of a neurobiotin-stained T2 cell. The bright staining of the cell is visible between the unstained rhabdomeric portions of T1 and T3, which is specific to T2 cells. (C) Example of a T1 cell, which is characterized by bright staining of the center of one of the large rhabdomes. V, ventral; D, dorsal; L, lateral; M, medial.

RESULTS

Based on transmission electron microscopy, the proximal retina of the principal eyes of first instar larvae of *T. marmoratus* is composed of three distinct cell types (Stecher et al., 2010). To evaluate if a

Table 1. Overview of recordings and results

	Number of cells stained (type)	e-vector angle (deg)			Polarization sensitivity	
		Min response	Max response	*N*	PS	*N*
Eye 1	4 (T1)	179.6±6.8	268.5±5.7	8	11.1±8.2	7
		271±6.5	182.2±5.2	6	8.8±3.2	5
Eye 2	3 (T1)	178.3±5.4	269.2±4.3	12	12.2±8.0	13
	4 (T2)	268.7±7.4	181.9±6.3	11	9.5±3.4	9

Values are means ± s.d.

similar organization also exists in third instar larvae, we first examined ultrathin sections of both principal eyes. As illustrated for E2 in Fig. 3A, this is indeed the case: three distinct cell types are discernible. T1 and T2 are somewhat larger, and have vertically and horizontally aligned microvilli, respectively. T3 is organized similarly to T1, but its rhabdomeric portion is much smaller. Next, we used intracellular recordings to measure the polarization sensitivity of individual proximal photoreceptors. We found two physiologically distinct cell types in both eyes: one is most sensitive to horizontally polarized light, and the other is most sensitive to vertically polarized light. Comparable data were obtained for E1 and E2. Neurobiotin staining allowed us to link our physiological findings to two (T1 and T2) of the three anatomically distinct cell types (Table 1). In many cases multiple cells were stained, making it impossible to identify the cell that was recorded from. In some cases such staining was used to confirm the eye from which we recorded. If only one cell was stained, without exception, this was cell type T2 for cells most sensitive to horizontally polarized light (see Fig. 3B for example) and T1 for cells that were most sensitive to vertically polarized light (see Fig. 3C for example). Although there is some indication in the physiological data that we may have recorded from two different populations of cells that are most sensitive to vertically polarized light (see below), none of the stained cells was of cell type T3.

Response to changing e-vector orientation

An example of a recording from a cell that was most sensitive to horizontally polarized light is illustrated in Fig. 4. The cell's response is modulated by about 44%, while the e-vector orientation is rotated through 180 deg (Fig. 4A). In addition, the shape of individual voltage responses was slightly different between recordings. Specifically, a cell's maximum response was characterized by a fast initial peak, followed by a slightly slower maximum (Fig. 4B), similar to what has been reported in sawflies (Meyer-Rochow, 1974). Weaker responses did not show the fast initial peak (Fig. 4C). To visualize the response magnitude modulation (Fig. 5), the data were normalized and averaged. Three cells that showed a maximum and minimum response to e-vector orientations that deviated by more than 3s.d. from the average were excluded from this and further analysis. These outliers were probably the result of tissue distortion from excessive gut movement that sometimes occurs during recordings.

On average, cells that were most sensitive to horizontally polarized light had a maximum response to polarized light with an e-vector direction of 182.2±5.2 deg (mean ± s.d., N=6) in E1 (Fig. 5A) and 181.9±6.3 deg (N=11) in E2 (Fig. 5B), and a minimum response to polarized light with an e-vector direction of 271±6.5 deg (N=6) in E1 and 268.7±7.4 deg (N=11) in E2. There was no significant difference between measurements from E1 and E2 (two-tailed Student's t-test, min response P=0.539, max response P=0.957).

Cells most sensitive to vertically polarized light had a maximum response to an e-vector direction of 268.5±5.7 deg (N=8) in E1 (Fig. 5A) and 269.2±4.3 deg (N=12) in E2 (Fig. 5B), and a minimum response to an e-vector direction of 179.6±6.8 deg (N=8) in E1 and 178.3±5.4 deg (N=12) in E2. No significant difference between the two eyes was observed (two-tailed Student's t-test, min response P=0.642, max response P=0.769).

Polarization sensitivity measurements

Trials were excluded when they (a) had an unstable baseline (three recordings), or (b) the response magnitude could not be recovered to within 10% of the initial response (three recordings). Fig. 6 illustrates (separately for E1 and E2) the average of the normalized V–log$I$ curves of cells most sensitive to horizontally and vertically polarized light. Normalized V–log$I$ curves are illustrated for both maximum (max V–log$I$) and minimum (min V–log$I$) response e-vector orientations. To calculate the PS values we first measured the V–log$I$ relationship for each cell at the maximal and minimal

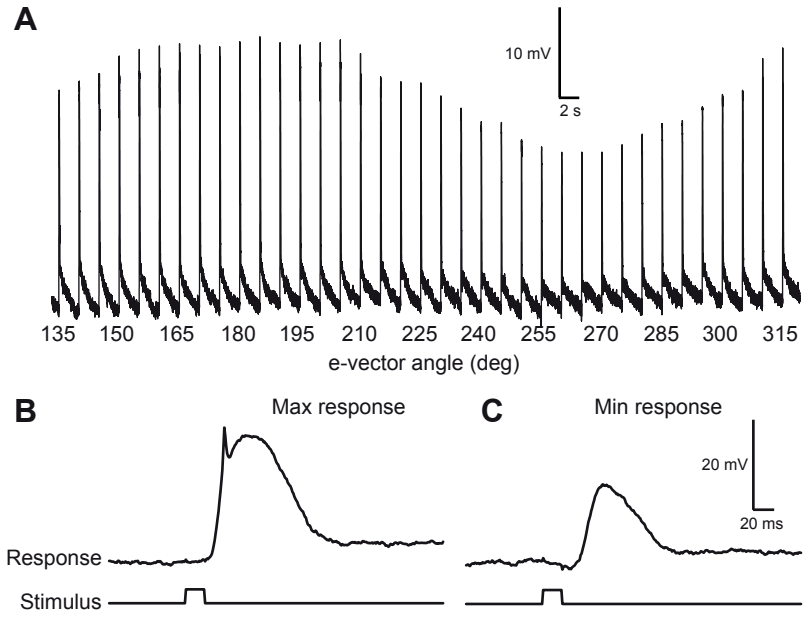

Fig. 4. Example recording of a cell that was most sensitive to horizontally polarized light. (A) During stimulation with light pulses the e-vector orientation was turned through 180 deg in 5 deg steps. (B) Response of the cell to a single 20 ms stimulus at the e-vector orientation (185 deg) that yielded the maximum response. (C) Response of the cell to the e-vector orientation (265 deg) that yielded the minimum response.

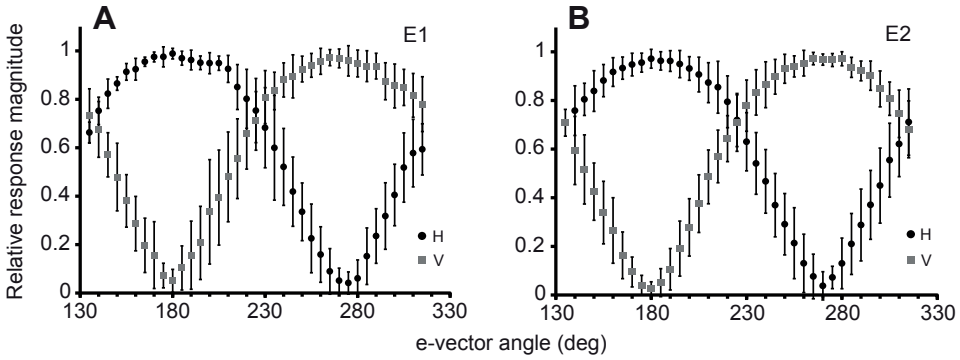

Fig. 5. Average relative response magnitude at different e-vector directions. The response magnitude of each cell was normalized to minimum response=0 and maximum response=1. (A) Average relative response magnitude with s.d. for E1. Cells most sensitive to horizontally (H, *N*=6) and vertically (V, *N*=8) polarized light. (B) Average relative response magnitude with s.d. for E2 (H, *N*=11; V, *N*=12).

sensitive e-vector orientation (Fig. 6A–D). However, at the time of the recordings no exact measurements of these directions were available. Therefore they were estimated by slowly turning the polarization filter while observing the response magnitude. These estimations were on average within 5.2±4.9 deg (mean ± s.d., *N*=30) of the measured value (based on subsequent data analysis). This small diversion from the optimal angle probably leads to a small underestimate of the PS for some of the cells.

The PS was calculated from the shift of the *V*–log*I* curves along the intensity axis (Fig. 6A). The range of PS values, especially for the cells that were most sensitive to vertically polarized light (of both eyes), was very large (as illustrated in Fig. 6E,F). Cells of E1, which were most sensitive to vertically polarized light, had a PS of 11.1±8.2 (*N*=7) and cells that were most sensitive to horizontally polarized light had a PS of 8.8±3.2 (*N*=5). For E2 the PS of cells most sensitive to vertically polarized light was 12.2±8.0 (*N*=13), and of those most sensitive to horizontally polarized light the PS was 9.5±3.4 (*N*=9). From these data we could detect neither a significant difference in PS values between eyes, nor between cells that were most sensitive to vertically or horizontally polarized light within each eye (Student's *t*-test, $P>0.05$).

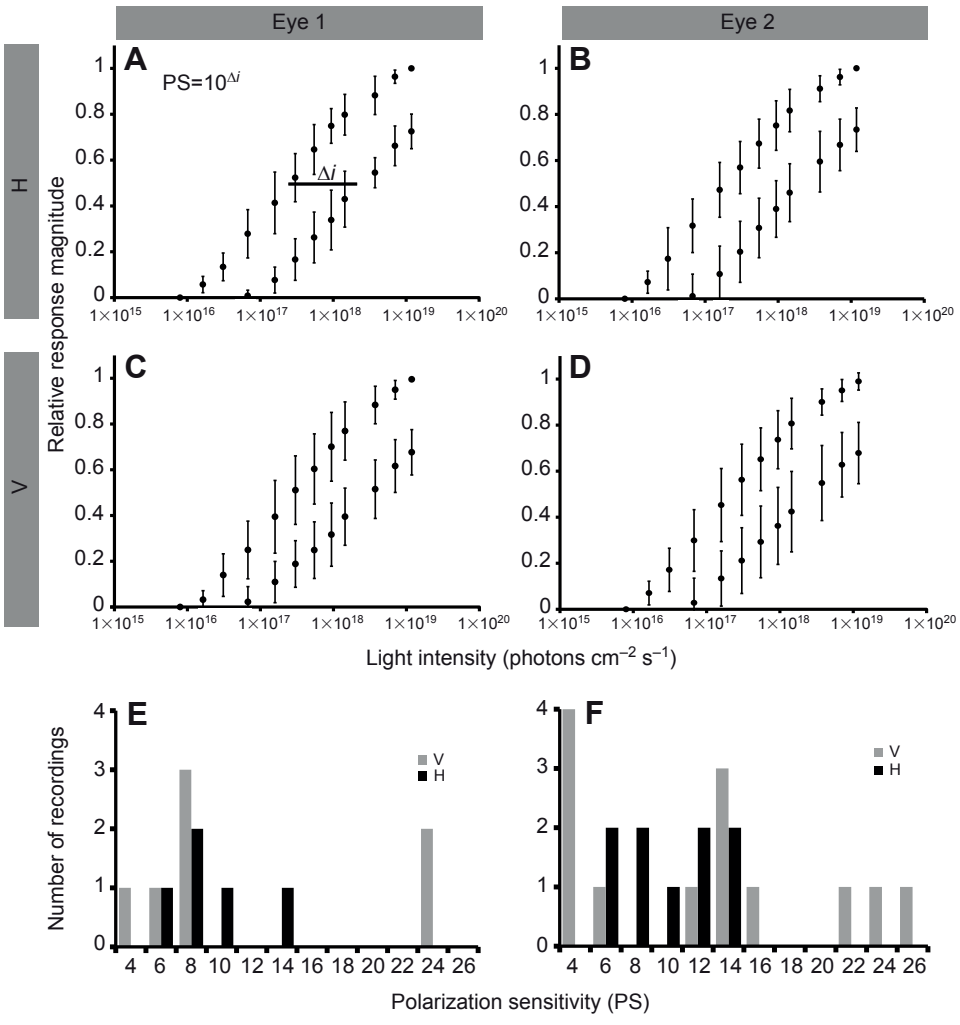

Fig. 6. Average normalized *V*–log*I* curves with s.d. and PS values of cells most sensitive to vertically (V) and horizontally (H) polarized light. The PS was calculated from the shift of the *V*–log*I* curves. (A) Cells of eye 1 most sensitive to horizontally polarized light (*N*=5). (B) Cells of eye 2 most sensitive to horizontally polarized light cells (*N*=9). (C) Cells of eye 1 most sensitive to vertically polarized light (*N*=7). (D) Cells of eye 2 most sensitive to vertically polarized light (*N*=13). E and F, PS values of eye 1 and eye 2, respectively.

Table 2. Comparison of polarization sensitivity (PS) values in different arthropods, from dorsal rim area (DR) or other areas of the eye, and with spectral sensitivity as indicated

Organism	Species	PS values	Reference
Insects			
Beetle	*Pachysoma striatum*	12.8±1.2 (DR, UV) 6.5±1.1 (DR, UV/green)	Dacke et al., 2002*
	Scarabacus zambesianus	7.7 (DR, UV) 12.9 (DR, UV/green) 1.5–1.6	Dacke et al., 2004*
	T. marmoratus larvae	10.8±6.4 (UV)	Present study
Bee	*Megalopta genalis*	21.2±7.5 (DR, UV) 1.4 (green)	Greiner et al., 2007*
	Apis mellifera mellifera	5.6±2.1 (DR, UV/green) <2 (UV) 3.8±2.4 (DR, UV/green) 13±4.5 (DR, UV)	Labhart, 1980
Ant	*Cataglyphis bicolor*	6.3±2.4 (DR, UV) 2.9±1.6	Labhart, 1986
Fly	*Musca domestica*, *Calliphora erythrocephala*	6–19 (DR, UV)	Hardie, 1984*
	Pega affinis larvae	Larval eye 6.1±2.1	Meyer-Rochow, 1974
Bug	*Gerris lacustris*	6.7±1.7 (blue) 6.9±0.63 (green)	Bartsch, 1995
Cricket	*Gryllus campestris*	6.2	Blum and Labhart, 2000
	Gallus campestris	2.6±0.8 (DR, green) 8.3±5.9 (DR, blue)	Labhart et al., 1984
Crustacea			
Stomatopod	*Odontodactylus scyllarus*	2.75±0.42 (rows 5 and 6, UV)	Kleinlogel and Marshall, 2009*
	Gonodactylus chiragra	3.8±1.6 6.2±1.3	Kleinlogel and Marshall, 2006*
Crab	*Leptograpsus variegatus*	5	Doujak, 1984
	Leptograpsus variegatus	10	Stowe, 1980
	Carcinus maenas, *Callinectes sapidus*	4.5	Mote, 1974*
Crayfish	*Procambarus clarkii*	1.3–12.5	Glantz, 2007

*These studies derived PS values from the shift of maximum and minimum *V*–log*I* curves.

DISCUSSION

Although polarization sensitivity has been studied fairly well in adult insects, little is known about it in larvae. Nevertheless, it is likely that at least some larvae, such as those of *T. marmoratus*, could substantially benefit from it. In previous work, the possibility of polarization sensitivity in these larvae has been raised based on the ultrastructure of their eyes (Stecher et al., 2010). Here we used electrophysiological methods to confirm that the proximal retinas of the principal eyes E1 and E2 are indeed polarization sensitive. As expected from the ultrastructure, cells of the type T1 are most sensitive to vertically polarized light, and cells of the type T2 are most sensitive to horizontally polarized light.

Polarization sensitivity in arthropods

To the best of our knowledge, there has only been one other physiological study (Meyer-Rochow, 1974) of polarization sensitivity within holometabolous insect larvae. In that study the PS values of the sawfly larval eye had a mean of 6.1, with a maximum of 10. Much more is known about polarization sensitivity in adult insects and crustaceans. For the former, the highest PS values are generally found in the dorsal rim area, an area of the compound eye that is known to be specialized for polarization vision.

The PS values of *T. marmoratus* larvae are comparable to values commonly found in the dorsal rim area (for example, those of crickets, locusts and ants; Table 2). Moreover, they are clearly higher than the typically low PS values found in other areas of insect eyes. Specifically, our values are most similar to those of bees, scarab beetles and some flies (*Musca*, *Calliphora*). Similarly, when compared with crustaceans, our values are similar to the higher PS values in the literature. In some of these species behavioral relevance has been demonstrated (Chiou et al., 2008). Taken together, these comparisons make it clear that PS values in the visual system of *T. marmoratus* larval eyes are fairly high, making it likely that polarization sensitivity plays an important role for them.

PS values are often quite variable in invertebrates (Stowe, 1983). Correspondingly, the range of the measured PS values in *T. marmoratus* was large, ranging from 4.5 to 14.2 for cells most sensitive to horizontal e-vector orientation, and from 2.7 to 24.9 for cells most sensitive to vertical e-vector orientation. Some, but probably not all, of the variability might be due to measurement inaccuracies (Stowe, 1983). Another previously discussed source of the typically large range in PS values is natural variability in microvillar orientation, as well as distortions that might be caused by the microelectrode penetration. Nilsson et al. (Nilsson et al., 1987) modeled the effects of microvillar misalignment on PS values and found that relatively minor misalignments can strongly affect PS values. In addition, in fused rhabdomes, neighboring cells can act as lateral filters for one another, adding further variability (Shaw, 1969; Nilsson et al., 1987; Stowe, 1983).

In addition to sensitivity to linearly polarized light, animals can be sensitive to circularly polarized light. In the mantis shrimp compound eye distally situated photoreceptors act as a retarder that converts circularly polarized light into linearly polarized light (and *vice versa*), allowing them to be sensitive to circularly polarized light instead of linearly polarized light (Chiou et al., 2008). In E1 and E2 of *T. marmoratus*, light that enters the polarization-sensitive proximal retina also first has to cross the microvilli of distally situated photoreceptor cells (Fig. 1A), an organization that could potentially alter the incoming light. However, in contrast to the mantis shrimp organization, the microvilli of the distally located

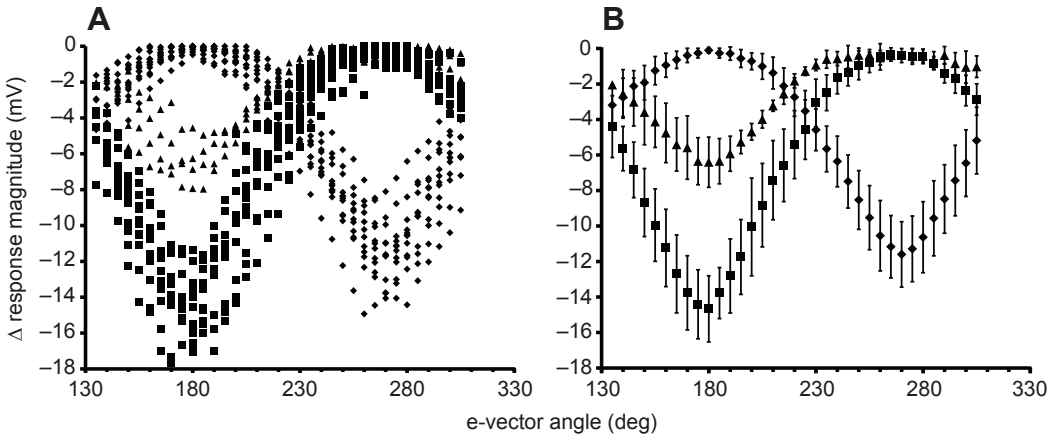

Fig. 7. Change in response magnitude of E1 and E2 cells most sensitive to vertically and horizontally polarized light. (A) Data of all cells. Triangles indicate a population of cells that is most sensitive to vertically polarized light with a relatively low modulation, when compared with other cells with equivalent e-vector orientation sensitivity (squares). Diamonds indicate cells that are most sensitive to horizontally polarized light. (B) Average of all cells (with s.d.) after separating vertical sensitive cells into shallow and large modulation groups.

photoreceptor cells of *T. marmoratus* are relatively irregular (Stecher et al., 2010). Moreover, cells are typically most sensitive to either linearly or circularly polarized light (Chiou et al., 2008). Therefore it is unlikely that T1–T3 cells are sensitive to circularly polarized light, though we did not directly test for this possibility.

Although PS values are generally highly variable, the range of values for those cells that were most sensitive to vertical e-vector orientations was particularly large. In the next section we discuss evidence that this may be due to the presence of two distinct groups of cells.

Evidence for two cell types that are sensitive to vertically polarized light

The proximal retina is composed of three cell types (T1, T2 and T3) that are arranged in an alternating pattern (Fig. 1B). All three cell types have the same spectral sensitivity in the UV range (Maksimovic et al., 2011), there is no obvious optical barrier between cells, and the microvilli are directly adjacent. Based on our transmission electron micrographs (Fig. 3A), in third instar larvae two of these cells (T1 and T3) have microvilli that are oriented vertically, whereas only one cell type (T2) has microvilli that are oriented horizontally. From post-recording staining of cells we could confirm that, as expected from their microvillar orientation, T2 cells are indeed most sensitive to horizontally polarized light, and that T1 cells are most sensitive to vertically polarized light. However, we were not successful in staining any of the much smaller T3 cells. Considering that post-recording injection of neurobiotin only succeeded in single cell staining in less than a third of the experiments, it is conceivable that some of our physiology data are nevertheless from T3 cells. Based on the confirmed directional sensitivity of the T1 cells and the more or less identical microvillar orientation of T3, it is highly likely that these cells too are most sensitive to vertically polarized light. However, the large structural difference between these cells (including the sizes of adjacent rhabdomeres) could result in differences in PS values. As modeled by Nilsson et al. (Nilsson et al., 1987), an unequal light absorbance ratio between neighboring cells (that act as lateral filters for one another) leads to different modulation strengths and hence unequal PS values for these cells. Specifically, the model shows that a cell with a relatively large rhabdomere, next to a cell with a smaller, orthogonal rhabdomere, would result in less modulation and lower PS values. Conversely, the cell with the smaller rhabdomere is expected to have increased modulation and a higher PS value. As is apparent in Fig. 3A, the rhabdomere of T3 cells (labeled MT3) might indeed be surrounded by very small T2 cell rhabdomeres (MT2). Accordingly, from the anatomy it might be expected that T3 cells have relatively low PS values.

Based on our combined physiological data, cells most sensitive to vertical e-vectors appear to fall into two distinct populations (Fig. 7): one showing shallower modulation (lower Δ response magnitude) than the other group of cells, some of which have been identified as T1 cells. In addition, when we recalculated the average PS values according to these groupings, we found that the PS values of the cells most sensitive to horizontal e-vector orientations (9.3±3.2, mean±s.d., N=14) tend to fall in between the values of the low (3.1± 0.4, N=4) and high (13.9±7.5, N=16) modulated cells most sensitive to vertical e-vector orientations. The shallower population could potentially represent T3 cells. No separation into two groups could be observed for cells most sensitive to horizontally polarized light, neither anatomically nor based on physiology. Interestingly though, the shape of the polarization modulation for all cells, with a broadened range around the peak and a narrow range around the trough, corresponded well with theoretical curves (Nilsson et al., 1987).

Despite the relatively large amount of literature on polarization sensitivity, few studies have evaluated the modulation strength of neighboring cells in the light of rhabdomere anatomy. The unequal rhabdomere organization of *T. marmoratus* makes it well suited to test existing theoretical models, and we are excited that our data are conceptually consistent with theoretical considerations (Nilsson et al., 1987). It would be interesting to empirically investigate the reciprocal influence of neighboring cells in greater depth by examining other comparable systems.

Functional considerations

The high PS makes it likely that polarized light plays an important role in the vision of *T. marmoratus*. The larval eyes nearly completely degenerate during pupation while the adult compound eye develops *de novo* (Sbita et al., 2007). Thus the polarization sensitivity of the larval eyes can only benefit their vision in the larval phase. In order to discuss possible functions, we need to first

consider the natural history and behavior of these beetle larvae. They are highly successful visual predators: once a prey item is detected, they stalk and follow it using their principal eyes E1 and E2. While slowly approaching the prey, larvae scan their visual field with dorsoventral head movements and finally strike to catch the prey (Buschbeck et al., 2007). It has been shown in other aquatic animals that polarization sensitivity can be used to either enhance visual contrast by filtering out horizontally polarized haze, or to use its prey's polarization features for detection (Shashar et al., 1998). It is conceivable that polarization sensitivity in *T. marmoratus* has similar functions. However, there are other ways in which polarization sensitivity could be beneficial. For example, *T. marmoratus* embryos develop on land, near water. After hatching, young larvae need to find the nearby water, a behavior for which the use of polarization cues has been demonstrated in a variety of insects (Schwind, 1991; Schwind, 1999). Moreover, late instar *T. marmoratus* larvae need to return to land to pupate and therefore need to find the shore. Within a pond, horizontal background polarization is expected to be highest away from the shore, and it has been shown that such cues can be used to find open water (Schwind, 1999). It is conceivable that *T. marmoratus* uses similar visual cues for the opposite purpose, namely to find shore when it is time to pupate. Behavioral experiments will be necessary to determine for which of these behaviors polarization sensitivity might be important.

ACKNOWLEDGEMENTS

We thank Dr Ilya Vilinsky and the Buschbeck and Layne laboratory groups for helpful discussion, Dr John E. Layne for lending us his rotating arm, and Marisano J. James for help in editing the manuscript. Shannon Werner assisted in beetle care, and Randy Morgan and the Cincinnati Zoo and Botanical Garden provided sunburst diving beetles.

FUNDING

This work was supported by the National Science Foundation under grants IOS0545978 and IOS1050754 to E.K.B., and a University Research Council summer graduate fellowship to A.S.

REFERENCES

Bartsch, K. (1995). Polarization-sensitive photoreceptors of different spectral types in the compound eye of waterstriders. *Naturwissenschaften* **82**, 292-293.
Baylor, E. R. and Smith, F. E. (1953). The orientation of *Cladocera* to polarized light. *Am. Nat.* **87**, 97-101.
Blum, M. and Labhart, T. (2000). Photoreceptor visual fields, ommatidial array, and receptor axon projections in the polarisation-sensitive dorsal rim area of the cricket compound eye. *J. Comp. Physiol. A* **186**, 119-128.
Buschbeck, E. K., Sbita, S. J. and Morgan, R. C. (2007). Scanning behavior by larvae of the predacious diving beetle, *Thermonectus marmoratus* (Coleoptera: Dytiscidae) enlarges visual field prior to prey capture. *J. Comp. Physiol. A* **193**, 973-982.
Chiou, T.-H., Kleinlogel, S., Cronin, T., Caldwell, R., Loeffler, B., Siddiqi, A., Goldizen, A. and Marshall, J. (2008). Circular polarization vision in a stomatopod crustacean. *Curr. Biol.* **18**, 429-434.
Cronin, T. W. (2006). Invertebrate vision in water. In *Invertebrate Vision* (ed. E. J. Warrant and D.-E. Nilsson), pp. 291-348. Cambridge: Cambridge University Press
Cronin, T. W. and Marshall, J. (2011). Patterns and properties of polarized light in air and water. *Philos. Trans. R. Soc. Lond. B* **366**, 619-626.
Cronin, T. W., Shashar, N., Caldwell, R. L., Marshall, J., Cheroske, A. G. and Chiou, T.-H. (2003). Polarization vision and its role in biological signaling. *Integr. Comp. Biol.* **43**, 549-558.
Dacke, M., Nordström, P., Scholtz, C. H. and Warrant, E. J. (2002). A specialized dorsal rim area for polarized light detection in the compound eye of the scarab beetle *Pachysoma striatum*. *J. Comp. Physiol. A* **188**, 211-216.
Dacke, M., Byrne, M. J., Scholtz, C. H. and Warrant, E. J. (2004). Lunar orientation in a beetle. *Proc. Biol. Sci.* **271**, 361-365.
Doane, C. C. and Leonard, D. E. (1975). Orientation and dispersal of late-stage larvae of *Porthetria dispar* (Lepidoptera: Lymantriidae). *Can. Entomol.* **107**, 1333-1338.
Doujak, F. E. (1984). Electrophysiological measurement of photoreceptor membrane dichroism and polarization sensitivity in a Grapsid crab. *J. Comp. Physiol. A* **154**, 597-605.
Evans, A. V. and Hogue, J. N. (2006). *Field Guide to Beetles of California*. Berkeley, CA: University of California Press.
Fent, K. (1986). Polarized skylight orientation in the desert ant *Cataglyphis*. *J. Comp. Physiol. A* **158**, 145-150.
Gilbert, C. (1994). Form and function of stemmata in larvae of holometabolous insects. *Annu. Rev. Entomol.* **39**, 323-349.
Glantz, R. M. (2007). The distribution of polarization sensitivity in the crayfish retinula. *J. Comp. Physiol. A* **193**, 893-901.
Greiner, B., Cronin, T. W., Ribi, W. A., Wcislo, W. T. and Warrant, E. J. (2007). Anatomical and physiological evidence for polarisation vision in the nocturnal bee *Megalopta genalis*. *J. Comp. Physiol. A* **193**, 591-600.
Hardie, R. C. (1984). Properties of photoreceptors R7 and R8 in dorsal marginal ommatidia in the compound eyes *Musca* and *Calliphora*. *J. Comp. Physiol. A* **154**, 157-165.
Horváth, G. and Varjú, D. (2004). *Polarized Light in Animal Vision: Polarization Patterns in Nature*. Berlin: Springer.
Ivanoff, A. and Waterman, T. H. (1958). Factors, mainly depth and wavelength, affecting the degree of underwater light polarization. *J. Mar. Res.* **16**, 283-307.
Johnsen, S., Marshall, N. J. and Widder, E. A. (2011). Polarization sensitivity as a contrast enhancer in pelagic predators: lessons from *in situ* polarization imaging of transparent zooplankton. *Philos. Trans. R. Soc. Lond. B* **366**, 655-670.
Kamermans, M. and Hawryshyn, C. (2011). Teleost polarization vision: how it might work and what it might be good for. *Philos. Trans. R. Soc. Lond. B* **366**, 742-756.
Kleinlogel, S. and Marshall, N. J. (2006). Electrophysiological evidence for linear polarization sensitivity in the compound eyes of the stomatopod crustacean *Gonodactylus chiragra*. *J. Exp. Biol.* **209**, 4262-4272.
Kleinlogel, S. and Marshall, N. J. (2009). Ultraviolet polarisation sensitivity in the stomatopod crustacean *Odontodactylus scyllarus*. *J. Comp. Physiol. A* **195**, 1153-1162.
Kriska, G., Horváth, G. and Andrikovics, S. (1998). Why do mayflies lay their eggs en masse on dry asphalt roads? Water-imitating polarized light reflected from asphalt attracts Ephemeroptera. *J. Exp. Biol.* **201**, 2273-2286.
Kriska, G., Bernáth, B. and Horváth, G. (2007). Positive polarotaxis in a mayfly that never leaves the water surface: polarotactic water detection in *Palingenia longicauda* (Ephemeroptera). *Naturwissenschaften* **94**, 148-154.
Labhart, T. (1980). Specialized photoreceptors at the dorsal rim of the honeybee's compound eye: Polarizational and angular sensitivity. *J. Comp. Physiol. A* **141**, 19-30.
Labhart, T. (1986). The electrophysiology of photoreceptors in different eye regions of the desert ant *Cataglyphis bicolor*. *J. Comp. Physiol. A* **158**, 1-7.
Labhart, T., Hodel, B. and Valenzuela, I. (1984). The physiology of the cricket's compound eye with particular reference to the anatomically specialized dorsal rim area. *J. Comp. Physiol. A* **155**, 289-296.
Larson, D. J., Alarie, Y. and Roughley, R. E. (2000). *Predaceous Diving Beetles (Coleoptera: Dytiscidae) of the Nearctic Region, with Emphasis on the Fauna of Canada and Alaska*. Ottawa: NRC Research Press.
Lerner, A., Meltser, N., Sapir, N., Erlick, C., Shashar, N. and Broza, M. (2008). Reflected polarization guides chironomid females to oviposition sites. *J. Exp. Biol.* **211**, 3536-3543.
Lythgoe, J. N. and Hemmings, C. C. (1967). Polarized light and underwater vision. *Nature* **213**, 893-894.
Maksimovic, S., Layne, J. E. and Buschbeck, E. K. (2011). Spectral sensitivity of the principal eyes of sunburst diving beetle, *Thermonectus marmoratus* (Coleoptera: Dytiscidae), larvae. *J. Exp. Biol.* **214**, 3524-3531.
Mandapaka, K., Morgan, R. C. and Buschbeck, E. K. (2006). Twenty-eight retinas but only twelve eyes: an anatomical analysis of the larval visual system of the diving beetle *Thermonectus marmoratus* (Coleoptera: Dytiscidae). *J. Comp. Neurol.* **497**, 166-181.
Mappes, M. and Homberg, U. (2004). Behavioral analysis of polarization vision in tethered flying locusts. *J. Comp. Physiol. A* **190**, 61-68.
Marshall, J., Cronin, T. W., Shashar, N. and Land, M. (1999). Behavioural evidence for polarisation vision in stomatopods reveals a potential channel for communication. *Curr. Biol.* **9**, 755-758.
Menzel, R., Ventura, D. F., Hertel, H., de Souza, J. M. and Greggers, U. (1986). Spectral sensitivity of photoreceptors in insect compound eyes: Comparison of species and methods. *J. Comp. Physiol. A* **158**, 165-177.
Meyer-Rochow, V. B. (1974). Structure and function of the larval eye of the sawfly, *Perga*. *J. Insect Physiol.* **20**, 1565-1591.
Mote, M. I. (1974). Polarization sensitivity. A phenomenon independent of stimulus intensity or state of adaptation in retinular cells of the crabs *Carcinus* and *Callinectes*. *J. Comp. Physiol. A* **90**, 389-403.
Naka, K. I. and Rushton, W. A. H. (1966). An attempt to analyse colour reception by electrophysiology. *J. Physiol.* **185**, 556-586.
Nilsson, D. E., Labhart, T. and Meyer, E. (1987). Photoreceptor design and optical properties affecting polarization sensitivity in ants and crickets. *J. Comp. Physiol. A* **161**, 645-658.
Novales Flamarique, I. and Hawryshyn, C. W. (1997). Is the use of underwater polarized light by fish restricted to crepuscular time periods? *Vision Res.* **37**, 975-989.
O'Shea, M. and Adams, M. E. (1981). Pentapeptide (proctolin) associated with an identified neuron. *Science* **213**, 567-569.
Rossel, S. (1993). Navigation by bees using polarized skylight. *Comp. Biochem. Physiol.* **104A**, 695-708.
Sabbah, S. and Shashar, N. (2006). Polarization contrast of zooplankton: a model for polarization-based sighting distance. *Vision Res.* **46**, 444-456.
Sbita, S. J., Morgan, R. C. and Buschbeck, E. K. (2007). Eye and optic lobe metamorphosis in the sunburst diving beetle, *Thermonectus marmoratus* (Coleoptera: Dytiscidae). *Arthropod Struct. Dev.* **36**, 449-462.
Schneider, L. and Langer, H. (1969). Die Struktur des Rhabdoms im 'Doppelauge' des Wasserläufers *Gerris lacustris*. *Cell Tissue Res.* **99**, 538-559.
Schwind, R. (1984). The plunge reaction of the backswimmer *Notonecta glauca*. *J. Comp. Physiol. A* **155**, 319-321.
Schwind, R. (1991). Polarization vision in water insects and insects living on a moist substrate. *J. Comp. Physiol. A* **169**, 531-540.

Schwind, R. (1999). *Daphnia pulex* swims towards the most strongly polarized light – a response that leads to 'shore flight'. *J. Exp. Biol.* **202**, 3631-3635.
Shashar, N., Rutledge, P. and Cronin, T. (1996). Polarization vision in cuttlefish in a concealed communication channel? *J. Exp. Biol.* **199**, 2077-2084.
Shashar, N., Hanlon, R. T. and Petz, A. deM. (1998). Polarization vision helps detect transparent prey. *Nature* **393**, 222-223.
Shashar, N., Hagan, R., Boal, J. G. and Hanlon, R. T. (2000). Cuttlefish use polarization sensitivity in predation on silvery fish. *Vision Res.* **40**, 71-75.
Shashar, N., Johnsen, S., Lerner, A., Sabbah, S., Chiao, C.-C., Mäthger, L. M. and Hanlon, R. T. (2011). Underwater linear polarization: physical limitations to biological functions. *Philos. Trans. R. Soc. Lond. B* **366**, 649-654.
Shaw, S. R. (1969). Sense-cell structure and interspecies comparisons of polarized-light absorption in arthropod compound eyes. *Vision Res.* **9**, 1031-1040.
Skorupski, P., Döring, T. F. and Chittka, L. (2007). Photoreceptor spectral sensitivity in island and mainland populations of the bumblebee, *Bombus terrestris*. *J. Comp. Physiol. A* **193**, 485-494.
Stecher, N., Morgan, R. and Buschbeck, E. (2010). Retinal ultrastructure may mediate polarization sensitivity in larvae of the sunburst diving beetle, *Thermonectus marmoratus* (Coleoptera: Dytiscidae). *Zoomorphology* **129**, 141-152.
Stowe, S. (1980). Spectral sensitivity and retinal pigment movement in the crab *Leptograpsus variegatus* (Fabricius). *J. Exp. Biol.* **87**, 73-98.
Stowe, S. (1983). A theoretical explanation of intensity-independent variation of polarisation sensitivity in crustacean retinula cells. *J. Comp. Physiol. A* **153**, 435-441.
Sullivan, C. R. and Wellington, W. G. (1953). The light reactions of larvae of the tent caterpillars, *Malacosoma disstria* (Hbn.), *M. americanum* (Fab.), and *M. pluviale* (Dyar). (Lepidoptera: Lasiocampidae). *Can. Entomol.* **85**, 297-310.
Sweeney, A., Jiggins, C. and Johnsen, S. (2003). Insect communication: polarized light as a butterfly mating signal. *Nature* **423**, 31-32.
Trujillo-Cenóz, O. and Bernard, G. D. (1972). Some aspects of the retinal organization of *Sympycnus linetaus* Loew (Diptera, Dolichopodidae). *J. Ultrastruct. Res.* **38**, 149-160.
Velasco, J. and Millan, A. (1998). Feeding habits of two large insects from a desert stream: *Abedus herberti* (Hemiptera: Belostomatidae) and *Thermonectus marmoratus* (Coleoptera: Dytiscidae). *Aquat. Insects* **20**, 85-96.
Waterman, T. H. and Westell, W. E. (1956). Quantitative effect of the sun's position on submarine light polarization. *J. Mar. Res.* **15**, 149-169.
Wehner, R. (2001). Polarization vision – a uniform sensory capacity? *J. Exp. Biol.* **204**, 2589-2596.
Wehner, R. and Labhart, T. (2006). Polarisation vision. In *Invertebrate Vision* (ed. E. J. Warrant and D.-E. Nilsson), pp. 291-348. Cambridge: Cambridge University Press.
Wehner, R. and Müller, M. (2006). The significance of direct sunlight and polarized skylight in the ant's celestial system of navigation. *Proc. Natl. Acad. Sci. USA* **103**, 12575-12579.
Wellington, W. G. (1955). Solar heat and plane polarized light versus the light compass reaction in the orientation of insects on the ground. *Ann. Entomol. Soc. Am.* **48**, 67-76.
Wellington, W. G., Sullivan, C. R. and Green, G. W. (1951). Polarized light and body temperature level as orientation factors in the light reactions of some hymenopterous and lepidopterous larvae. *Can. J. Zool.* **29**, 339-351.
Wildermuth, H. (1998). Dragonflies recognize the water of rendezvous and oviposition sites by horizontally polarized light: a behavioural field test. *Naturwissenschaften* **85**, 297-302.
Wolff, T. (2011). Preparation of *Drosophila* eye specimens for transmission electron microscopy. *Cold Spring Harb. Protoc.* **2011**, 1386-1388.