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Polarized Light Helps Monarch Butterflies Navigate

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Summary

During their spectacular migratory journey in the fall, North American monarch butterflies (*Danaus plexippus*) use a time-compensated sun compass to help them navigate to their overwintering sites in central Mexico [1–3]. One feature of the sun compass mechanism not fully explored in monarchs is the sunlight-dependent parameters used to navigate. We now provide data suggesting that the angle of polarized skylight (the *e*-vector) is a relevant orientation parameter. By placing butterflies in a flight simulator outdoors and using a linear polarizing filter, we show that manipulating the *e*-vector alters predictably the direction of oriented flight. Butterflies studied in either the morning or afternoon showed similar responses to filter rotation. Monarch butterflies possess the anatomical structure needed for polarized skylight detection, as rhabdoms in the dorsalmost row of photoreceptor cells in monarch eye show the organization characteristic of polarized-light receptors. The existence of polarized-light detection could allow migrants to accurately navigate under a variety of atmospheric conditions and reveals a critical input pathway into the sun compass mechanism.

Results and Discussion

Ultraviolet (UV) light is important for the initiation of flight in monarch butterflies [3]. Once flight is attained, however, the relevant features of skylight used for actual compass orientation are unknown. Based largely on studies in honey bees and desert ants, the recognized compass signals visible to insects in the daytime sky consist of the sun itself and polarization and spectral-intensity gradients, which are generated as sunlight scatters through the atmosphere [4–7]. The skylight pattern of polarized light (the *e*-vector pattern) provides one of the most reliable navigational cues [4–7].

We examined whether monarch butterflies use polarized skylight to orient by placing butterflies in a flight simulator [2] outdoors and manipulating the *e*-vector direction with a linear polarizing filter (Figures 1 and 2A). Flight behavior was monitored using the Mouritsen-Frost flight simulator [2], which was modified by reducing the dimensions of the housing barrel by 50%. With a linear polarizing filter in position, the smaller barrel size ensured that the butterflies could only view the sky through the filter, which restricted the angle of vision to 80° (Figure 1A).

During a control period without the filter, flight orientation of three butterflies was to the southwest with mean direction vectors (α) of 255°, 193°, and 210° (Figure 2B, top), well within the range of orientation values reported for populations of migrating butterflies housed under similar (artificial or natural) lighting conditions [1–3, 8]. When a linear polarizing filter was placed above the butterflies so that the horizontal plane of polarized skylight was parallel with the naturally occurring *e*-vector viewed at the zenith (Figure 2A), the direction of flight was not significantly altered (change of $9.7° \pm 5.2°$ [mean ± SEM, $n = 3$ butterflies] $p > 0.10$, one-sample t-test) (Figure 2B, compare top two panels). Thus, filter placement alone does not substantially alter butterfly orientation. Although these butterflies could choose one of two opposite directions because of the bidirectional *e*-vector imposed by the filter (Figure 2A and see below), the prior orientation period before filter placement likely provides a strong impetus for the butterflies to continue in the same (control) direction with parallel filter placement.

We next examined flight orientation responses to horizontal rotation of the filter by 90°, placing the *e*-vector perpendicular to that at the zenith (Figure 2A). As filter rotation can change horizontal light intensity patterns, which could cause an artifact inside the simulator to which the butterflies might orient [4], we first monitored intensity patterns from 200 to 800 nm in the simulator barrel. Filter rotation did not appreciably change the intensity pattern as measured at the level of the butterfly head in the simulator (Figure 1B). Because the orientation of polarized light-sensing dorsal rim photoreceptors is toward the filter (see below) and the tethered animals flown in the simulator are fixed in the vertical plane (can only rotate horizontally), any potential light reflection off the bottom or sides of the simulator would have a negligible effect on polarized-light detection by the butterflies—the major polarized-light stimulus would be from above, through the filter.

If monarch butterflies use polarized light to orient, then filter rotation to the perpendicular should cause a corresponding change in flight direction (see Figure 2A). In fact, when the filter rotation data from the three butterflies depicted in Figures 2B (compare middle two panels) and eight additional butterflies (studied only with the filter present) were examined, the mean rotation-induced change in flight orientation was not different from the expected 90° ($90.3° \pm 5.3°$, $n = 11$ butterflies, $p > 0.10$).

Not only should the direction of orientation be changed by filter rotation, but also the bidirectional nature of the *e*-vector imposed by the filter should cause

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bimodal orientation patterns (Figure 2A). Bimodal orientation patterns could be manifested in two ways. First, each butterfly might continually switch its orientation from each of two opposite directions, giving rise to bimodal orientation profiles; that is, two equal peaks that are 180° out of phase with each other. Second, individual butterflies could simply orient primarily to one of the two possible positions dictated by the bidirectional *e*-vector [9].

Our butterflies chose predominantly the second response, as six of the 11 butterflies repositioned their orientation to the right (+90°), while the other five changed to the left (−90°) (Figure 3). In addition, there was no direction bias between butterflies flown in the same apparatus at the same position on the same flight arena in either the morning (Figure 3, small open circles) or afternoon (Figure 3, small closed circles). The finding that monarchs show similar magnitudes of orientation response to filter rotation in either the morning or afternoon is consistent with time-compensated use of the *e*-vector. It is noteworthy that some of the butterflies examined with the filter in place showed accentuated asymmetrical bimodal orientation patterns in individual records (e.g., Figure 2B, AM-1, second panel down; data not shown), but these bimodal profiles usually showed a dominant orientation peak.

When the filter was rotated back parallel to the zenith *e*-vector (Figure 2A), two of the three animals in Figure 2B (bottom two panels) oriented back to the control position, whereas the third oriented to the opposite position (PM butterfly, Figure 2B), consistent with the imposed bidirectional *e*-vector. In this case, the prior position of the filter perpendicular to the zenith *e*-vector would eliminate the control position bias noted in the top two panels of Figure 2B.

The dorsalmost row of photoreceptor cells in the compound eye of the monarch butterfly showed the anatomical organization characteristic of polarized-light receptors [10]; they have rectangular-shaped rhabdoms whose rhabdomeres contribute microvilli that are oriented at right angles (Figure 4A). This orthogonal specialization optimizes accurate measurement of incident light *e*-vector direction by the photoreceptor-containing microvilli [10]. This structural specialization in the dorsal margin of the monarch eye is contrasted with the more typical photoreceptor cells found ventrally, in which the microvilli are aligned in different planes to optimize light reception at all angles for more global photoreceptive activities (Figure 4B).

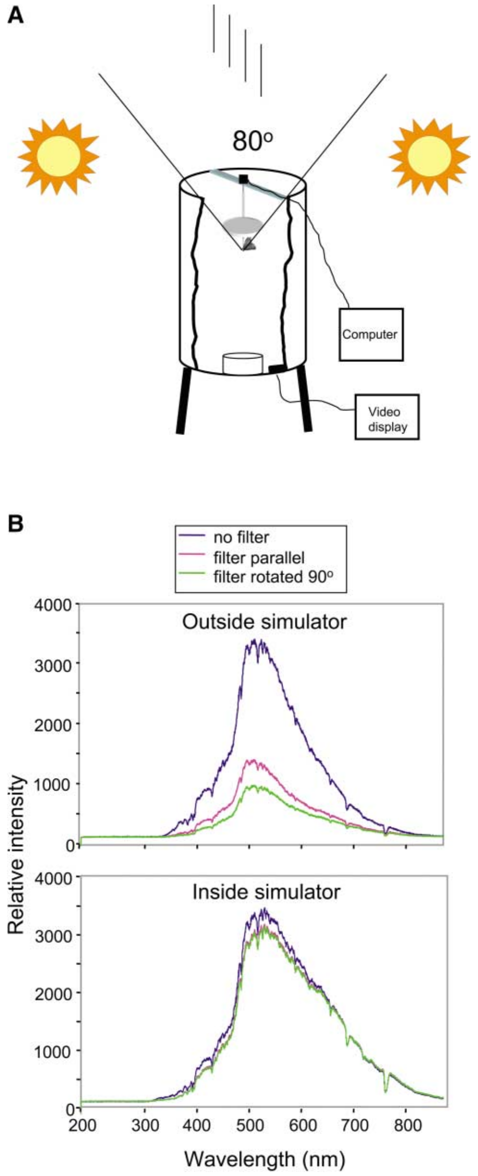

Figure 1. Experimental Setup for Polarized-Light Experiments

(A) Flight simulator with polarizing filter in place. The joining oblique lines denote butterfly angle of vision of open skylight below the filter (gray disc). Vertical lines designate the zenith *e*-vector. Filter studies were performed outdoors in the morning or afternoon when the butterflies could not visualize the sun directly. Modified from [2].

(B) Light spectral-intensity plots of outdoor light with the photocell outside (top) or inside (bottom) the simulator. Light measurements (from a USB2000 spectroradiometer from Ocean Optics; range 200–800 nm) were made inside the simulator at the level of the butterfly head with the filter in place. Measurements outside the barrel were made with the filter directly over the photocell. Peak intensity in each situation was plotted as 3500 units. Skylight penetration from around the outer edge of the filter and through the translucent sides of the barrel itself dampened any change in light intensity in the barrel, with the filter in place and when the filter was rotated. The results were the same when recordings were made in the morning or the afternoon.

Conclusions

The *e*-vector pattern is an important navigational cue for some foraging hymenopterans, such as honey bees and desert ants [4–7]. We now suggest that lepidopterans can also use the polarized skylight pattern to orient. We were unable to clearly establish the importance of polarized light in the UV range, because butterflies do not fly well in the absence of UV light [3]. Besides using polarized light to orient, monarch butterflies may use the sun itself [3] and/or spectral gradients, which are used by honey bees and desert ants [11–13]. The relative importance of these cues as compass signals in mon-

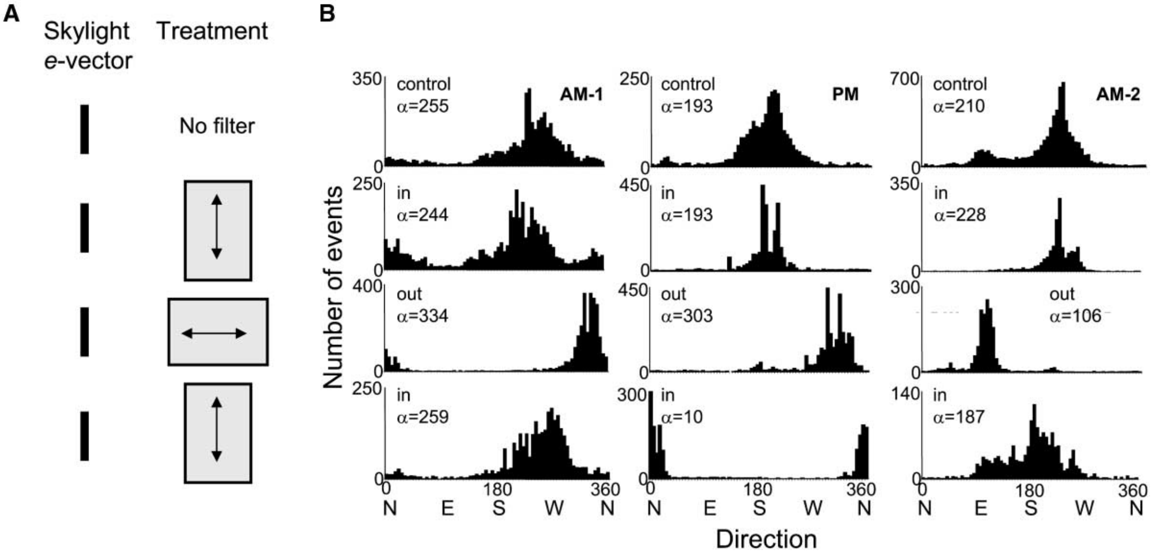

Figure 2. Flight Orientation of Individual Butterflies in Which the Plane of Polarized Skylight Was Varied

(A) Experimental paradigm. Each thick vertical bar in the left-hand column represents the major *e*-vector viewed at the zenith. Treatments (right-hand column) were sequential from top to bottom (described in [B]). Shaded rectangles represent the linear polarizing filter, with the double-arrowed lines depicting the bidirectional *e*-vector imposed by the filter.

(B) A sequential record of flight orientation histograms is shown for each of three butterflies; each histogram is a record of continuous flight lasting at least 5 min and with sampling at 200 ms intervals. After initiating oriented flight (control), we applied a linear polarizing filter with the *e*-vector parallel to that in the zenith (in). The filter was then rotated clockwise by 90° (out), and finally, the filter was rotated clockwise by another 90° to put the *e*-vector back parallel with the zenith (in). The individual on the left (AM-1) was studied from 08:56 to 09:41 hr EST, in the middle (PM) from 13:39 to 14:25 hr, and on the right (AM-2) from 09:55 to 10:34 hr. α, mean vector.

archs remains to be determined. The existence of polarized skylight detection in monarch butterflies would help ensure accurate celestial navigation under a variety of atmospheric conditions encountered during migration, including cloudy skies with some blue sky visible [4–7, 14]. In addition, polarized-light reception reveals a critical input pathway into the time-compensated sun compass mechanism.

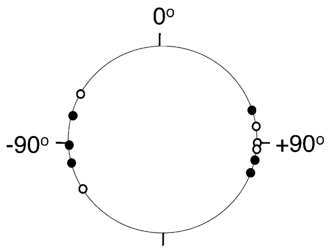

Figure 3. Monarch Butterflies Orient to Polarized Skylight

Flight orientation of 11 butterflies in which the direction of polarized skylight was varied. When the polarizing filter was rotated horizontally by 90°, each of 11 butterflies (small circles) changed its orientation. The data include the three animals depicted in Figure 2 (middle two panels). 0°, denotes the starting position for each butterfly with the polarizer parallel with the *e*-vector at the zenith; +90°, change in orientation to the right; −90°, change in orientation to the left. Small open circles, orientation of butterflies studied between 08:00–11:00 hr; small closed circles, butterflies studied between 13:00–17:00 hr. Two additional animals studied did not significantly change their orientation to filter rotation (<10° change). Both appeared to orient to light patterns inside the barrel, ignoring the polarized skylight pattern through the filter.

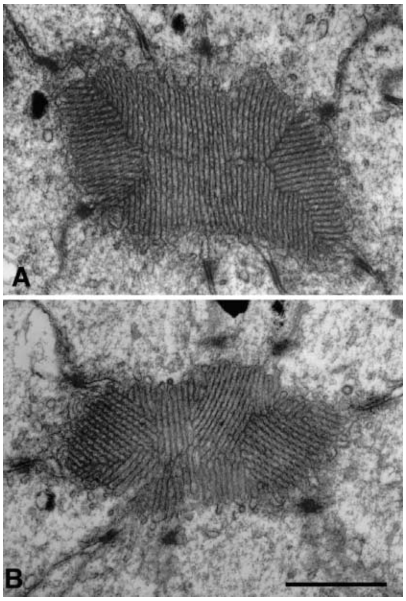

Figure 4. Monarch Butterflies Have the Anatomical Substrate for Polarized-Light Detection

Electron micrographs of cross-sectioned retinulae at the dorsal margin of the monarch butterfly eye. (A) Rhabdom from the dorsalmost row of photoreceptor cells showing the rectangular profile and orthogonal microvilli characteristic of dorsal rim polarized light detectors. (B) Rhabdom from a nearby more ventral row, showing the more typical organization in butterfly eye in which the microvilli are twisted. Scale bar = 1 μm.

Experimental Procedures

Flight Behavior

Monarch butterflies migrating through Western Massachusetts in the fall of 2002 and 2003 were captured and most were housed outside in plastic mesh cages with free access to 10% sucrose in water. Some butterflies were placed in glassine envelopes and housed in the laboratory in environmental compartments (at 18°C and 70% humidity) in lighting conditions approximating the outdoor light-dark cycle [3].

Butterflies were tethered as previously described [2], and flight behavior was monitored using a modified Mouritsen-Frost flight simulator [2]. For filter experiments, an HNP′B linear polarizing filter (3M Company; 10.2 cm in diameter) was placed directly above the butterfly (6.1 cm from filter to head). The filter was rotated manually. In all but one instance, flight studies were performed when the butterfly could not see the sun directly from its position in the simulator.

Flight direction was recorded by a computer running USB1 Explorer (US Digital) configured to record the direction of flight every 200 ms. Flight was monitored visually through a small hole in the bottom of the simulator by an externally mounted surveillance camera.

All butterflies flew for at least 10 min and flew for at least 5 min under each treatment condition. The individuals depicted in Figure 2B flew for 39 to 46 min. Filter experiments were performed on sunny days between 08:00–11:00 and 13:00–17:00 hr, Eastern Standard Time. Midday (11:00–13:00 hr) was avoided because of the reduced intensity of the overhead polarization pattern at that time [4–7].

Data in each histogram were analyzed for significance of orientation and to determine mean direction of orientation using circular statistics. All animals had highly significant orientation during each treatment condition ($p < 0.001$). Reanalysis of several histograms using 2 s sampling periods revealed the same patterns as with 200 ms sampling intervals that are highly significant in terms of the calculated mean vector. Throughout each record, animals rotate several times fully around 360° with pauses around the major orientation position. The butterflies usually accomplish a full rotation within a 2 s period.

Electron Microscopy

Butterfly heads were bisected into cacodylate buffered glutaraldehyde-formaldehyde fixative [15]. After aldehyde fixation for 1 to 2 hr, the tissue was washed in buffer and postfixed in 0.5% OsO_4 for 1 hr, rinsed in water, and stained in 2% uranyl acetate in darkness for 1–2 hr. The tissue was dehydrated in ethanol and propylene oxide and embedded in Spurr's resin (Polysciences, Warrington. PA). Cured blocks were oriented so that thin sections could be cut perpendicular to retinulae along the dorsal margin of the eye. Sections were stained with lead citrate and photographed in a Philips 300 electron microscope. Selected micrographs were processed in Adobe Photoshop, with only contrast and density adjusted for the preparation of Figure 4.

Acknowledgments

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Note Added in Proof

After acceptance of this manuscript, we became aware of a dissertation containing experiments that suggest monarch butterflies can orient to polarized light.

Hyatt, M.B. (1993). The use of sky polarization for migratory orientation by monarch butterflies. PhD thesis, University of Pittsburgh, Pittsburgh, Pennsylvania.