Do monarch butterflies use polarized skylight for migratory orientation?

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Summary

To test if migratory monarch butterflies use polarized light patterns as part of their time-compensated sun compass, we recorded their virtual flight paths in a flight simulator while the butterflies were exposed to patches of naturally polarized blue sky, artificial polarizers or a sunny sky. In addition, we tested butterflies with and without the polarized light detectors of their compound eye being occluded. The monarchs' orientation responses suggested that the butterflies did not use the polarized light patterns as a compass cue, nor did they exhibit a specific alignment response towards the axis of polarized light. When given direct view of the sun, migratory monarchs with their polarized light detectors painted out were still able to use their time-compensated compass: non-clockshifted butterflies, with their dorsal rim area occluded, oriented in their typical south–southwesterly migratory direction. Furthermore, they shifted their flight course clockwise by the predicted ~90° after being advance clockshifted 6 h. We conclude that in migratory monarch butterflies, polarized light cues are not necessary for a time-compensated celestial compass to work and that the azimuthal position of the sun disc and/or the associated light-intensity and spectral gradients seem to be the migrants' major compass cue.

Key words: navigation, time-compensated sun compass, polarized skylight, dorsal rim area, *Danaus plexippus*, Lepidoptera.

Introduction

It is now well established that Monarch butterflies (*Danaus plexippus* L.) use a time-compensated sun compass during their autumn migration from eastern North America to their overwintering areas in Central Mexico (Perez et al., 1997; Mouritsen and Frost, 2002; Froy et al., 2003). In previous experiments, migrating monarchs were presented with approximately 120° of clear blue sky including the sun (Mouritsen and Frost, 2002). Therefore, the butterflies could not only see the sun but also the polarized light pattern resulting from the scattering of sunlight by the air molecules of the atmosphere (Strutt, 1871).

Polarization vision is a widespread sensory ability of insects (Waterman, 1981; Wehner, 1982, 1994; Labhart and Meyer, 1999; Horvath and Varju, 2004), and the pattern of polarized light in the sky is involved in several spatial orientation mechanisms (Wehner, 1984, 2001) ranging from course control, as suggested for instance in flies (Wolf et al., 1980; von Philipsborn and Labhart, 1990), to the polarization compass of bees and ants (Wehner, 1984, 1994, 1996). In all insects investigated so far (bees, ants, flies, crickets and locusts), the ability to use polarized skylight for orientation is mediated by a group of specialized ommatidia located at the dorsal margin of the compound eye, termed the dorsal rim area

(DRA) (Wehner, 1982; Wehner and Strasser, 1985; Fent, 1985; von Philipsborn and Labhart, 1990; Brunner and Labhart, 1987; Labhart, 1999; Mappes and Homberg, 2004). Histological studies (Labhart and Baumann, 2003; Reppert et al., 2004) and electrophysiological recordings (J.S. and T.L., unpublished) demonstrated the presence of a specialized DRA in the monarch butterfly eye. Thus, it was suggested that monarchs use polarization vision for spatial orientation (Reppert et al., 2004). The aim of the present study is to investigate the role of skylight polarization in the orientation system of monarch butterflies.

Materials and methods

We caught a total of 331 wild monarch butterflies in fattened migratory condition on the Northern shores of Lake Ontario during autumn 2003 and 2004. The monarchs were individually numbered and housed indoors. We subjected 43 of them to a 6 h-advanced clockshift (lights on 6 h after sunrise, off 6 h after sunset for at least 10 days) and kept the rest in a light/dark cycle matching the local photoperiod. To study the butterflies' behavioral response to linearly polarized light we used four flight simulators (Mouritsen and Frost,

2002). These simulators consist of white, translucent plastic cylinders, which provide a 120° visual field of the sky and prevent the butterflies from seeing landmarks outside the barrel. Butterflies tethered in the center of the simulator are able to orient in any geographical direction they choose while an optical encoder records their instantaneous flight directions. New miniature optical encoders (E4; US Digital, Vancouver, WA, USA) covered less than 7° of the monarchs' visual field in all experiments. The apparatus, butterfly tethering technique and basic experimental procedures are described in detail in Mouritsen and Frost (2002). The undisturbed Earth's magnetic field (measured with a Flux-gate magnetometer; Fluxmaster-X, Mayer Messgeräte, Germany) was available in all experiments.

Experiments were performed outdoors in an open field near Kingston, Canada (76°30′ W, 44°20′ N) during the peak of autumn migration (11 September – 26 October). All procedures were approved by Queen's University Animal Care Committee as compliant with the Canadian Council of Animal Care Guidelines. Only butterflies flying actively for at least 15 min were included in the data analysis. For each individual flight, we calculated the geographical mean flight direction (α_{geo}) of the individual animal and the directedness of its flight, $r_{individual}$ (mean orientation vector length; Batschelet, 1981). Flights with $r_{individual}$ values of <0.1 (40 flights from a total of over 300) were excluded from statistical analysis, since these flight paths were undirected. Within each experimental condition, any given individual monarch was only tested once. To minimize animal use, most butterflies were tested in several experimental conditions.

To investigate whether migratory monarch butterflies can use the natural skylight polarization pattern as an orientation cue, we recorded the flight direction of butterflies under a milky-white, non-UV-transmitting, translucent Plexiglas lid with a circular opening of 15 cm diameter in the zenith. This limited the butterflies' view of the natural sky to a 44° visual angle centered on the zenith. Thus, the butterflies were prevented from seeing the sun, and the availability of other possible sources of directional information such as spectral and light intensity gradients was minimized (Rossel and Wehner, 1984a; Wehner and Strasser, 1985; Wehner, 1997). Wooden sun shades erected outside the butterflies' field of view shadowed the simulators completely, thereby removing potential sun-related directional brightness cues on the barrel walls. Since maximum skylight polarization occurs at a 90° angular distance from the sun, the degree of polarization in the zenith is highest when the sun is near the horizon. Thus, to ensure that the polarization cues presented were above the perception threshold known from other insect species (Wehner, 1991; Labhart, 1996), we restricted the 44° visual angle experiments to the morning and afternoon hours when the sun's elevation was less than 40°. In most of these tests, the sky was completely clear during the entire testing period. Only in a few tests, up to ~20% transient cloud cover was present. As controls, we tested monarchs with a 120° field of view of clear sky with the sun visible, and under simulated complete overcast (milky-white translucent Plexiglas lid without opening) such that neither a view of the sun nor the polarization pattern was available.

The predicted group orientation responses of monarch butterflies given a 44° view of the clear natural sky depend on how the butterflies are expected to utilize polarized light cues for spatial orientation. If polarized light cues are used as a reference for a time-compensated compass system, monarchs tested in the course of the day should all orient along the migratory SW–NE axis. The directional distribution should be bimodal due to the nearly full 180° ambiguity of the polarized stimulus in the zenith. Alternatively, monarchs could line up in a specific preferred direction relative to the polarized skylight pattern (e.g. to e-vectors perpendicular to the body's longitudinal axis, as in the fly *Musca domestica*; see, for example, von Philipsborn and Labhart, 1990). Their predicted bimodal orientation will then shift over the course of the day due to the ~15 deg. h^{-1} rotation of the sun and the associated symmetry axis of the polarization pattern.

To analyze our data for such a line-up response, we corrected each butterfly's mean geographical flight direction (α_{geo}) for the sun-azimuth averaged over the time of the experiment (α_{sun}), thereby revealing the monarch's orientation (α_{rel}) relative to the sun azimuth and thus also relative to the perpendicular sun-derived axis of polarization at the zenith [$\alpha_{rel}=\alpha_{geo}-\alpha_{sun}$ (mod 360°)]. If the monarchs all prefer the same e-vector orientation, α_{rel} is constant. If, however, individual monarch butterflies have different preferred e-vector orientations, as observed for example in the locust *Schistocerca gregaria* (Mappes and Homberg, 2004), the orientation within each experimental group of monarchs will be random.

In a second series of experiments we therefore covered the opening in the Plexiglas lid with a piece of UVA-transmitting linear polarizer (HN42HE; 3M, Norwood, MA, USA), providing each monarch with a zenith-centered polarized light stimulus being 44° wide and having a degree of polarization of 80–100% (between 300 nm and 700 nm). After 15 min of active flight, the lid (including the polarizer) was turned clockwise by 90° in order to test whether a shift in orientation would occur. As a control, we repeated the experiments after removing the polarizer from the Plexiglas lid such that the monarchs could see the natural sky through the opening. The clockwise 90° turn now caused the same lid movement as before, whereas the polarized stimulus (natural skylight polarization) remained the same. Only data from monarchs that kept flying for at least 10 min after the turn had occurred were analyzed for a shift in orientation. We performed the experiments during midday (solar elevation >40°), when the degree of polarization at the zenith was low, and aligned the polarizers ±45° relative to the average sun azimuth during each experimental 30 min flight period. Since the e-vector orientation in the zenith is perpendicular to the azimuth of the sun (Strutt, 1871), this arrangement ensured that the light intensity underneath the filter was the same for the two polarizer orientations.

To test if the monarchs could use the artificial polarized light stimulus for time-compensated orientation, for each flight we calculated (1) the mean geographical flight direction during the first 15 min of flight (α_{geo}), (2) the geographical orientation of one end of the artificial polarizer axis during that particular experiment (α_{pol}) and (3) the sun's azimuth averaged over the time of the experiment (α_{sun}). From these values, we obtained the time-compensated geographical heading of the butterfly, (α_{comp}), as: $\alpha_{comp}=\alpha_{geo}-\alpha_{pol}+\alpha_{sun}\pm90°$ (mod. 360°). The ±-term in this formula is necessary because any orientation of the linear polarizer can indicate two equally likely sun azimuth positions. To allow for this ambiguity, we doubled the angles of the obtained data, transforming the expected bimodal group orientation into a unimodal one (Batschelet, 1981). The value $\alpha_{geo}-\alpha_{pol}$ (mod 360°) indicates the monarch's orientation relative to the polarizer and is therefore used to test whether the monarchs lined up with the polarizer.

While we were in the process of writing this paper, Reppert et al. (2004) reported strong reactions of monarchs towards an artificial polarizer covering ~80° of the animals' visual field of view. These data contradict our findings using a 44°-wide polarized light stimuli. Therefore, we decided to investigate, in a third series of experiments performed in autumn 2004, whether the different sizes of the polarized light stimuli could explain the opposing results. We mounted a UVA-transmitting linear polarizer (3M® Vikuiti® Polarizing Film HNP'B; 3M Canada, London, Ontario, Canada; 10.2 cm diameter) 5.7 cm above the butterflies' heads so as to obtain an 85° highly polarized light stimulus (degree of polarization >99% between 300 nm and 900 nm): a 0.5 cm-diameter hole was punched into the center of each polarizer, which was glued by a small ring of double-sided adhesive tape to the central 1 cm of the bottom side of the clear, UV-transmitting Plexiglas holder (2 cm diameter), so that the hole in the polarizer fitted an equivalent hole in the center of the Plexiglas disc. The disc with the mounted polarizer was fixed on an aluminum tube (0.5 cm diameter) by using a small fastening screw, so that the position of the polarizer could be adjusted in height after the 0.5 cm-wide aluminum tube was slid on the aluminum tube guiding the tungsten rod to which the butterfly was fastened. We also screwed a clear, UV-transmitting Plexiglas ring (1 cm diameter) to the bottom end of the guiding tube to prevent the tightly fitting aluminum tube (and therefore the polarizer) from sliding out of position. Another UV-transmitting Plexiglas disc (3 cm diameter) was fixed to the upper end of the aluminum tube just underneath the Plexiglas bar on which the optical encoder was mounted. This allowed us by turning this disc to shift the polarizer manually without reaching inside the flight simulator. The complete HNP'B polarizer holder is illustrated in Fig. 1. A white, translucent Plexiglas lid with a 30 cm-diameter opening in the center covered the barrel and provided a 75° visual field of clear blue sky in the zenith above the polarizer. Following the experimental procedures described by Reppert et al. (2004), we performed all experiments in the morning and afternoon excluding midday [11:00 h to 13:00 h Eastern Standard Time (EST)]. The polarizers were aligned
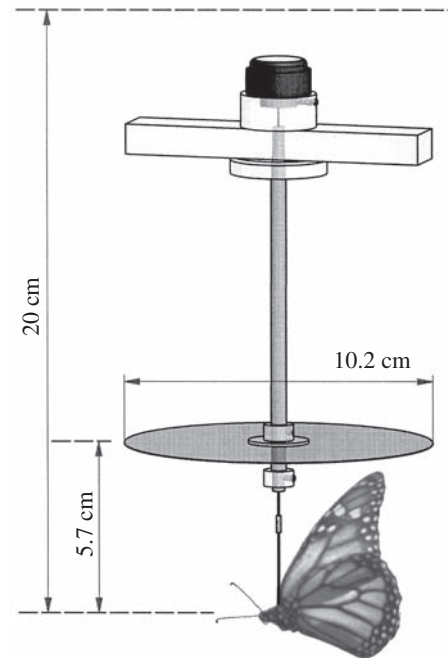

Fig. 1. Detailed drawing of the polarizer holder used in the 2004 experiments for testing the monarchs' reactions to a 85° polarized light stimulus. The upper broken line indicates the level of the lid, which limited the butterflies' view of the sky. For detailed description, see Materials and methods.

parallel to the polarization pattern in the zenith during the first 15 min of flight and then turned by 90°, so that they were perpendicular to skylight polarization during the second part of the flight.

The experimental set-up described by Reppert et al. (2004) did not mention any sun shades. The sun, even though not directly visible to the butterfly inside the flight simulator, caused an obvious light intensity pattern on the simulator walls: the simulator side facing the sun was brightly illuminated through the white, translucent barrel walls, while the opposite side showed a very distinct oval-shaped bright spot opposite to the sun's azimuth. In order to eliminate these brightness artefacts, we repeated the experiments while using sun shades positioned outside the butterflies' view. These control experiments were performed late in the season (12–26 October 2004). As the maximal sun elevation was less than 35°, the skylight in the zenith was well polarized throughout the day so that we could skip the midday break. The results obtained under the 85° polarized light stimulus were analyzed as described above for the experiments performed with the 44° polarized light stimulus.

The last experimental series was designed to test whether the perception of polarized skylight is necessary for migratory orientation in monarchs. Therefore, we painted over the polarized light detectors of clockshifted and non-clockshifted monarchs with opaque black paint [1:1 Lascaux Aquacryl (Alois K. Diethelm AG, Brüttisellen, Switzerland) : Marabu Dekorlack (Marabuwerke, Tamm, Germany)], covering the

margin of the entire eye except the caudal-most side. The monarchs were tested under a 120° visual field of clear sky including the direct view of the sun. The results were compared with clockshifted and non-clockshifted controls, which did not have their DRA covered. To confirm that the DRA was completely covered by the paint in the experimentals, in 2003 the butterflies were sacrificed immediately after the test flight. In the lab, each eye was mounted individually and sputtered (BAL-TEC SCD 005 Cool Sputter Coater; Balzers, Liechtenstein) with gold (15 nm layer). Then, the paint was peeled off and the probe was sputtered again (10 nm layer). Due to the difference in gold layer thickness, scanning electron microscopy (SEM; S-3200N; Hitachi, Tokyo, Japan) revealed the exact number of occluded ommatidia. The SEM images confirmed that the DRA was amply covered in all animals. Hence, in 2004, we instead peeled the paint off each eye after the experiment had been performed and verified the number of occluded ommatidia based on the clear negative imprint left by the ommatidia on the inside of the painted mask.

Results

First, we investigated whether the various stimulus conditions had any significant systematic effect on the directedness [mean vector length ($r_{individual}$)] of individual flights. The only significant differences based on pair-wise comparisons occurred between the directedness of animals flying under simulated cloud-cover compared with the flight paths of the clockshifted and non-clockshifted sun control groups of 2004 (one-way ANOVA on ranks followed by Dunn's all pair-wise multiple comparison method, $P<0.05$ for the two mentioned comparisons, $P>0.05$ for all other comparisons). Group-specific values can be found in Table 1.

Second, for each experimental condition, we investigated the group mean orientation of the butterflies. Detailed numerical data on all orientation experiments can be found in Table 1. Monarch butterflies flying under clear skies with an unobstructed view of the sun in 2003 oriented in the typical south–southwesterly migratory direction ($\alpha_{geo}=202°$, $r=0.80$, $P<0.001$; Fig. 2A,B) at all times of the day [time range 10:00 h to 15:00 h EST in this study; even wider time range in Mouritsen and Frost (2002)]. Butterflies tested under complete overcast simulation showed random group orientation ($\alpha_{geo}=134°$, $r=0.22$, $P=0.35$; Fig. 2C,D). The experimental groups provided with a 44°-wide visual field of clear, natural sky in the zenith but without direct view of the sun oriented
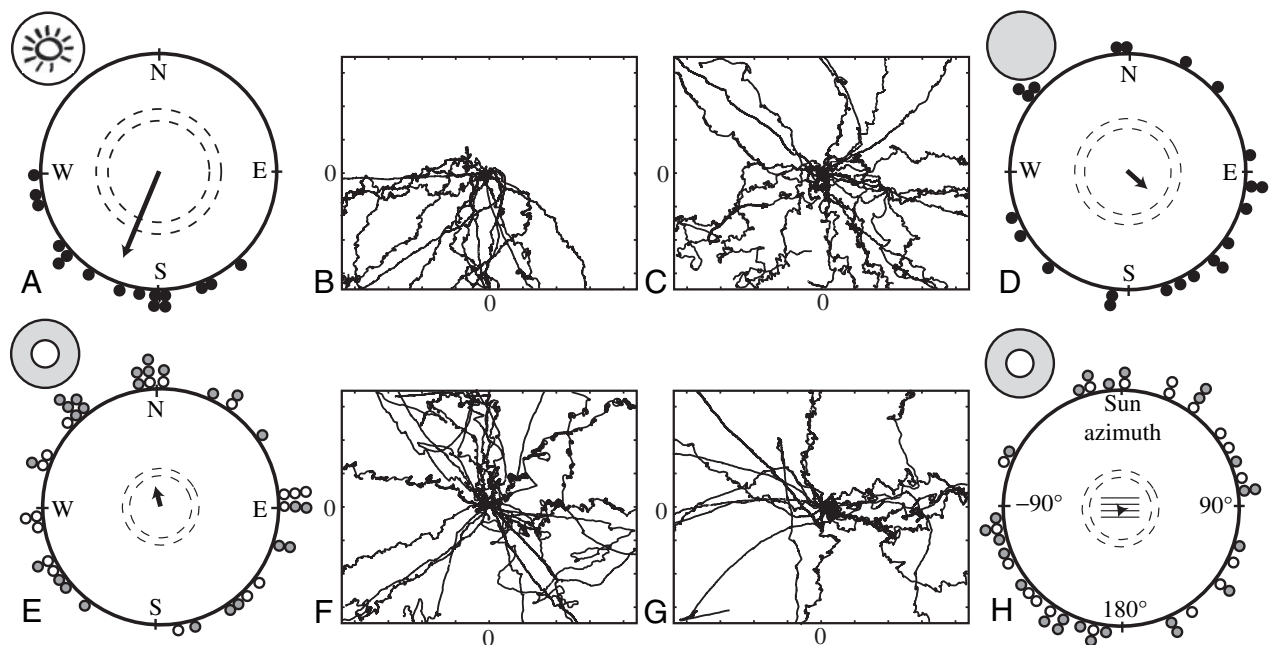

Fig. 2. Orientation of monarch butterflies exposed to different celestial cues. Each dot at the circle periphery (A,D,E,H) indicates the mean orientation of one butterfly flight. The arrows indicate group mean vectors. The broken circles indicate the radius of the mean vector required for significance at the $P<0.05$ and $P<0.01$ levels according to the Rayleigh test (Batschelet, 1981). B,C and F,G show the virtual flight paths flown by individual butterflies assuming a constant flight speed. They start in the center of the diagram and travel towards the periphery. Distances have been normalized. (A,B) With a 120° view of the clear blue sky including the sun, monarchs orient in their south–southwesterly migratory direction. (C,D) Under simulated overcast with only the geomagnetic field available, but no sun or polarized light cues, the butterflies were randomly oriented. (E–G) A 44° visual field of clear blue sky in the zenith but no direct view of the sun also led to random orientation (E) both for monarchs tested in the morning (grey dots, tracks in F) and in the afternoon (open dots, tracks in G). Since a few flights are bimodal and only the prominent peak of each bimodal distribution is indicated on the circular diagrams, a few points in the circular diagrams do not seem to coincide with the corresponding tracks. (H) The monarchs also did not show any preference to line up with the axis of skylight polarization in the zenith (indicated by four parallel lines).

randomly ($\alpha_{geo}=345°$, $r=0.16$, $P=0.39$; Fig. 2E–G), and doubling of the orientation angles did not reveal significant bimodal orientation either ($\alpha_{geo}=116°/296°$, $r=0.14$, $P=0.42$). No preference to line up in a specific direction relative to the sky's polarization pattern was observed ($\alpha_{rel}=198°$, $r=0.06$, $P>0.90$; Fig. 2H), and no significant bimodal orientation could be revealed by doubling the angles ($\alpha_{rel}=39°/219°$, $r=0.23$, $P=0.10$).

Table 1. *Overview of experimental conditions*

	Individual orientation behavior				Group orientation behavior					Group orientation behavior doubled angles			
	n	$r_{individual}$*	S.D.	Random[†]	n	α	r_{group}[‡]	P	95% conf. interval	n	α	r_{group}[‡]	P
Sun visible (2003)	16	0.44	0.23	0	16	202°	0.80	<0.001	182–222°	–	–	–	–
Sun visible, overpainted DRA (2003)	20	0.47	0.28	0	20	208°	0.58	<0.001	178–238°	–	–	–	–
Sun visible (2004)	33	0.49	0.26	4	29	166°	0.61	<0.001	142–190°	–	–	–	–
Sun visible, clock-shifted +6 h (2004)	21	0.51	0.28	2	19	262°	0.66	<0.001	235–289°				
Sun visible, clock-shifted +6 h, overpainted DRA (2004)	28	0.46	0.32	4	24	252°	0.48	<0.01	217–287°				
Complete overcast simulation	27	0.26	0.17	5	22	134°	0.22	0.35	–	22	140°/320°	0.20	0.42
44° visual field of sky													
Geographical orientation													
Morning	30	0.35	0.22	5	25	354°	0.23	0.27	–	25	151°/331°	0.18	0.45
Afternoon	21	0.35	0.25	3	18	309°	0.09	0.86	–	18	94°/274°	0.36	0.10
Combined data	51	0.35	0.23	8	43	345°	0.16	0.39	–	43	116°/296°	0.14	0.42
Orientation towards e-vector axis (E–W)													
Morning		Same as for geographical orientation			25	210°	0.15	0.57	–	25	35°/215°	0.19	0.41
Afternoon					18	59°	0.07	>0.90	–	18	43°/223°	0.30	0.20
Combined data					43	198°	0.06	>0.90	–	43	39°/219°	0.23	0.10
90° turn of lid[§]	–	–	–	–	10	4°	0.72	<0.01	329–39°	–	–	–	–
44° polarized light stimulus (sun shades up)													
Time-compensated orientation	48	0.37	0.24	5	–	–	–	–	–	43	89°/269°	0.23	0.13
Orientation towards e-vector axis (N–S)					43	231°	0.14	0.43	–	43	174°/354°	0.20	0.20
Flight direction change after 90° turn of polarizer	–	–	–	–	16	348°	0.61	<0.01	316–20°	–	–	–	–
85° polarized light stimulus (no sun shades)													
Time-compensated orientation	49	0.41	0.25	6	–	–	–	–	–	43	71°/251°	0.26	0.06
Orientation towards e-vector axis (N–S)					43	115°	0.23	0.19	–	43	42°/222°	0.25	0.11
Flight direction change after 90° turn of polarizer	–	–	–	–	29	5°	0.57	<0.001	342–28°	–	–	–	–
85° polarized light stimulus (sun shades up)													
Time-compensated orientation	38	0.32	0.21	6	–	–	–	–	–	32	89°/269°	0.14	0.56
Orientation towards e-vector axis (N–S)					32	288°	0.25	0.16	–	32	100°/280°	0.16	0.47
Flight direction change after 90° turn of polarizer	–	–	–	–	5	14°	0.66	0.11	321–67°	–	–	–	–

*Mean directedness of individual flights; [†]number of flights where $r_{sing}<0.1$; [‡]mean directedness of experimental group; [§]control experiment for 90° turn of polarizers.

Replacing the natural polarized light stimulus with an artificial polarized light stimulus led to similar results: the
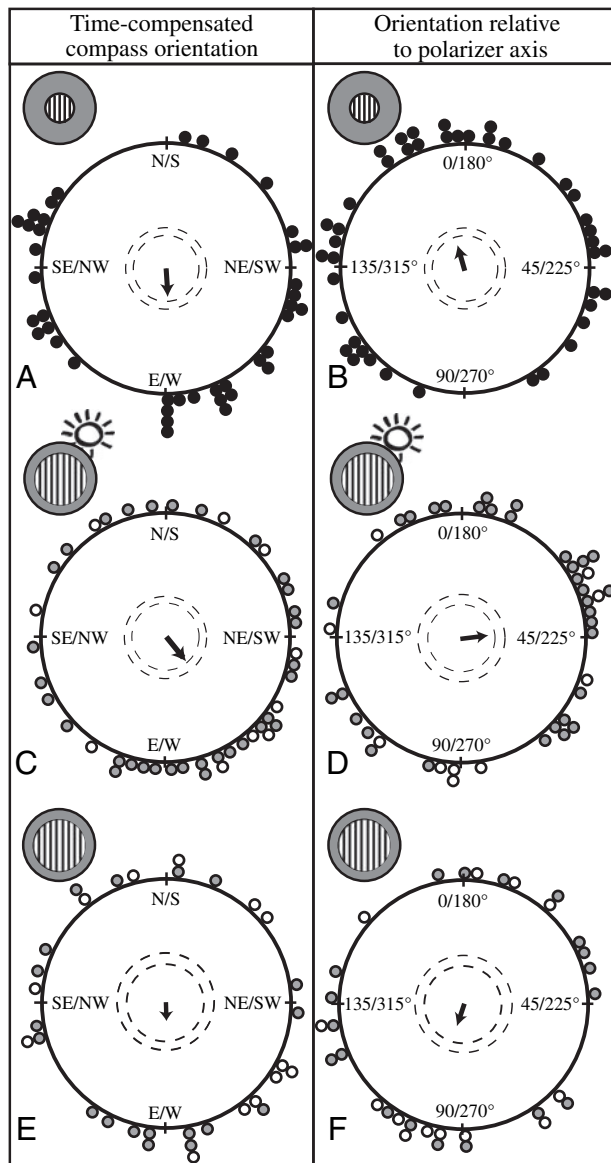

Fig. 3. Orientation of monarch butterflies tested under different artificial polarized stimuli. (A,B) 44° UVA-containing stimulus; (C,D) 85° UVA-containing stimulus without sun shades; (E,F) 85° UVA-containing stimulus with sun shades. None of the polarized light stimuli led to time-compensated compass orientation in monarchs based on the orientation of the polarizer (A,C,E; a point in NE/SW indicates orientation towards northeast or southwest) and the butterflies did not line up in any specific direction relative to the axis of polarization (B,D,F; a point in 0°/180° indicates orientation along the polarizer's axis whereas a point in 90°/270° indicates orientation perpendicular to the polarizer axis). All angles are doubled because of the bimodal ambiguity of all linear polarizers (see Materials and methods). The lack of orientation towards the large polarizer was observed at all times of day (grey dots and open dots indicate flights before and after 12:00 h EST, respectively; total time range 08:15 h to 16:30 h EST). For further explanation of symbols, see legend to Fig. 2.

monarchs' group orientation towards the expected e-vector direction at the time of the experiment (time-compensated orientation) was random under a 44° UVA-containing polarized light stimulus (doubled angles: α_{comp}=89°/269°, r=0.23, P=0.13; Fig. 3A). Seen as a group, the monarchs also did not show any line-up reaction relative to the e-vector axis of the 44° polarized light stimulus (doubled angles: α_{geo}–α_{pol}=174°/354°, r=0.20, P=0.17; Fig. 3B). Although a shift in orientation after a 90° clockwise turn of the polarizer was occasionally observed (see Discussion), no significant group response to a 90° clockwise turn of the polarizer occurred, and the mean shift in orientation following the turn did not differ significantly from 0° (α=348°, r=0.61, P<0.01, 95% confidence interval=316–20°; Fig. 4A). The changes in mean direction after the turn compared with those before the turn did not differ significantly from those observed in control experiments where lids with an opening exposing clear skies but without polarizer were turned [Fig. 4C; 95% confidence interval of the mean direction (α) and directedness of the experimental group (r_{group}) overlap with those of the control group].

Using a larger polarized light stimulus equivalent to that used by Reppert et al. (2004) resulted in similar findings: under a 85° UVA-containing polarized light stimulus, the orientation of the time-compensated group showed a non-significant tendency to orient on the northeast/southwest directional axis (α_{comp}=71°/251°, r=0.26, P=0.06; Fig. 3C). This tendency, however, disappeared when sun shades shaded the barrel (α_{comp}=89°/269°, r=0.14, P=0.56; Fig. 3E). No specific group alignment towards the e-vector axis of the 85° polarized light stimulus was observed, either with (doubled angles: α_{geo}–α_{pol}=100°/280°, r=0.16, P=0.47; Fig. 3F) or without sun shades (doubled angles: α_{geo}–α_{pol}=42°/222°, r=0.25, P=0.11; Fig. 3D). The mean orientation shift in the tracks of the monarchs tested in both shaded or non-shaded barrels was not different from 0° (Fig. 4B; without sun shades: α=5°, r=0.57, P<0.001, 95% confidence interval=324–28°; with sun shades: α=14°, r=0.7, P=0.11, 95% confidence interval=321–67°).

Migratory, non-clockshifted monarchs with occluded DRAs showed the typical south–southwesterly group mean orientation under a clear sunny sky with view of the sun (α_{geo}=208°, r=0.58, P<0.001; Fig. 5B). Neither the group mean orientation (95% confidence intervals overlap) nor the group directedness (non-parametric bootstrap with 5×10 000 replications: 0.39<r_{group}<0.78 and 0.72<r_{group}<0.90, respectively) differed significantly from that observed in untreated control butterflies that could see the sun and the pattern of polarized skylight (Fig. 5A).

In 2004, a year of abnormal monarch migratory patterns in eastern North America (http://learner.org/jnorth/fall2004/monarch/index.html), monarchs headed slightly east of south (α=166°, r=0.61, P<0.001; Fig. 5D). Monarchs clockshifted by +6 h in 2004 and tested under identical conditions showed a highly significant shift (P<0.01, 99% confidence intervals do not overlap) in their orientation towards west (α=262°, r=0.66, P<0.001; Fig. 5E). Clockshifted monarchs with overpainted
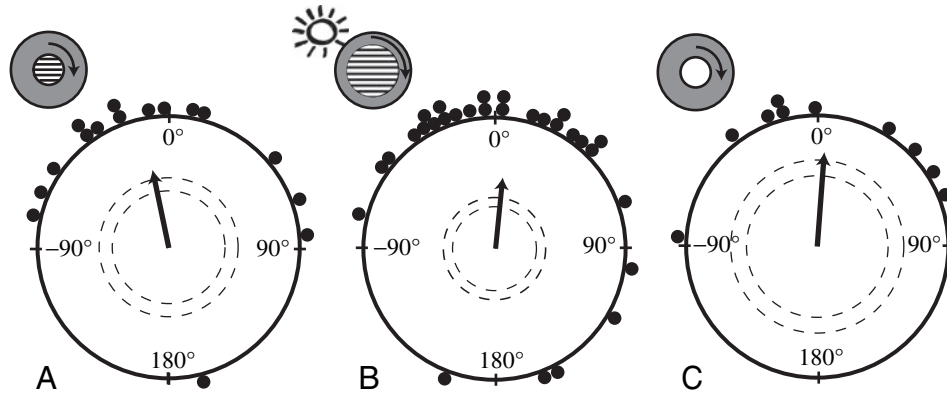
Fig. 4. Responses of monarch butterflies to 90° turns of polarizers or lids. Even though some monarchs changed their mean heading following a 90° clockwise turn of the 44° polarizer (A) or the 85° polarizer (without sun shades; B), their mean change was 0°. Furthermore, the animals' reactions in response to a turn of the polarizers were no different from their reactions when a lid with an opening exposing the blue sky was turned 90° (C). 0° indicates the mean direction chosen by each individual butterfly prior to the turn of the polarizer. Thus, a data point at 0° means that the orientation before and after the turn was identical. For explanation of symbols, see legend to Fig. 2.

DRA also oriented in the shifted westerly direction (α=252°, r=0.48, P<0.01; Fig. 5F), and their group mean orientation did not differ significantly from the clockshifted monarchs that did not have their DRA occluded (95% confidence intervals overlap; see Table 1). As in the non-clockshifted monarchs, a slight but non-significantly increased scatter in the group mean was observed when the DRA was painted out (non-parametric bootstrap with 5×10 000 replications: 0.25<r_{group}<0.72 and 0.47<r_{group}<0.86, respectively), but the directedness of individual flight paths was virtually identical (0.44<mean $r_{individual}$<0.51 in all five groups). Thus, migratory monarchs with overpainted DRA were well able to perform time-compensated compass orientation.

Discussion

Skylight polarization is known to provide insects with a reliable compass cue used during path integration in the context of foraging and homing (reviewed by Wehner and Srinivasan, 2003). This polarization compass works even if the insect is presented with only small isolated patches of polarized skylight (10°-wide apertures: honey bees – Edrich and Helversen, 1976; Rossel and Wehner, 1984b; desert ants – Fent, 1985; for measurements with large-field integrators and full-sky polarimeters, see Labhart, 1999; Pomozi et al., 2001, respectively). Since the discovery of the monarch's DRA
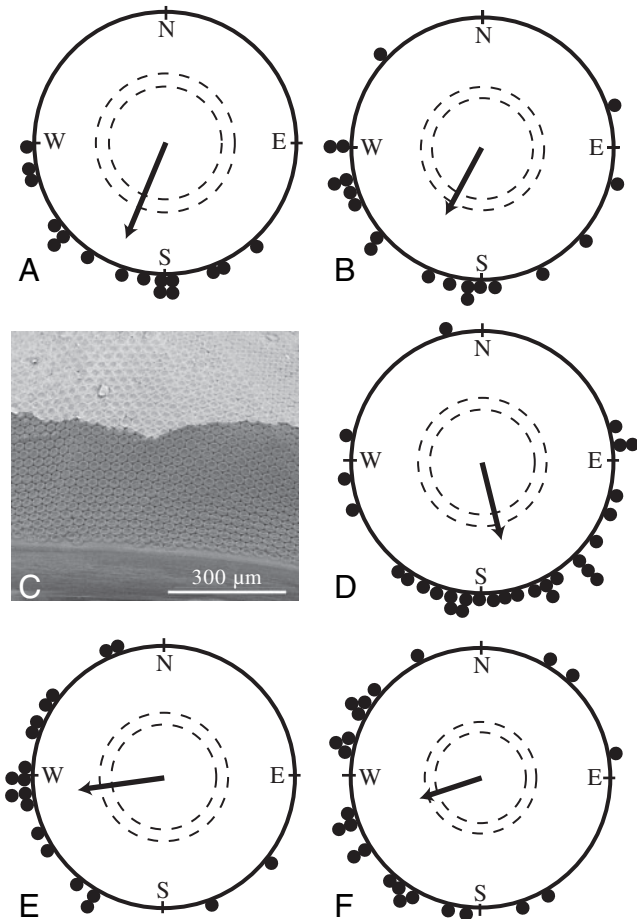

Fig. 5. Time-compensated orientation of monarch butterflies having a 120° view of the sky including the sun with and without occluded dorsal rim area (DRA). (A) In 2003, control monarchs oriented in their typical south–southwesterly autumn migratory direction. (B) Overpainting of the DRA did not affect their ability to orient in the migratory direction. (C) SEM image of the dorsal-most part of a monarch eye showing the extent of the paint mask covering the DRA. Such images revealed that the 14±4 (mean ± s.d.; range 4–37) most marginal rows of ommatidia were occluded in the dorsal half of the eye in the DRA-animals. Thus, the DRA, which has a maximal width of three rows of ommatidia (Labhart and Baumann, 2003), was amply covered in all overpainted eyes. (D) In 2004, non-clockshifted control monarchs showed an unusual mean migratory direction just east of south. (E) Clockshifted monarchs shifted their orientation +96°. (F) Clockshifted monarchs with their DRA occluded also shifted their orientation clockwise (+86°) as predicted when they use a time-compensated sun compass. For explanation of symbols, see legend to Fig. 2.

(Labhart and Baumann, 2003; Reppert et al., 2004), it has been hypothesized that these long-distance migrants may use a time-compensated polarization compass (Reppert et al., 2004). Monarchs would have to use polarized light information in a different way compared with foraging bees and ants: it would have to be time-compensated in order to work during the whole day, and the consistent migratory direction relative to a geographical frame of reference must be fixed genetically. The existence of such a compass based exclusively on polarized light rather than the sun has yet to be demonstrated in any insect.

The results of our experiments do not provide any evidence that polarized light cues are used by migratory monarchs in one way or another: monarchs showed well-oriented migratory flight behavior when given a 120° view of blue sky including the sun. But if their visual field was restricted to 44° of blue sky without the sun, centered in the zenith, their group orientation vanished, suggesting that the butterflies were unable to extract directional information for their time-compensated compass system from the natural polarized light pattern. The monarchs also did not show any specific group alignment relative to the skylight polarization axis, as for instance found in flies (von Philipsborn and Labhart, 1990). This is in line with the observation that our monarchs also were oriented at random in relation to the e-vector axis of UVA-transmitting polarizers providing 44°- or 85°-wide strongly polarized light stimuli in the zenith. Only in one situation (85° polarized light stimulus, no sun shades) did the butterflies show a slight tendency to orient bimodally in a northeast/southwest

direction in a time-compensated manner. However, this effect disappeared after sun shades excluded distinct light patterns on the flight simulator walls originating from the sun and which therefore also moved their position with the sun azimuth.

In summary, the evaluation of more than 160 15–30 min flights of migratory monarch butterflies tested under polarizers suggests that they do not use the polarization patterns for compass orientation during autumn migration. This finding is in agreement with the results of the first orientation experiments performed on monarch butterflies by Kanz (1977), whereas Hyatt's (1993) experiments suggested a time-compensated polarization compass. However, this study was based on very short flights (<1 min) during which the animals could see the observer, who scored the flights *via* direct visual observation. Reppert et al. (2004) reported that 11 monarchs changed their orientation by ±90° as a result of a 90° turn of a UV-transmitting polarizer, also suggesting polarization sensitivity in monarch butterflies. The mean change of the 50 animals that we tested for this reaction did not differ from 0°. However, three out of the 50 butterflies showed what appeared as a perfect 90° shift immediately or shortly after the polarizer turn and kept the new direction during the entire second part of the flight (Fig. 6A,B). Occasionally, we also observed monarchs that seemed to shift orientation for a short period of time after the turn of the polarizer, but they soon returned to their old flight direction (Fig. 6C,D). These observations might indicate that some individuals do refer to the axis of the polarizer as a reference cue rather than as a compass cue. However, these occasional shifts in orientation might not be
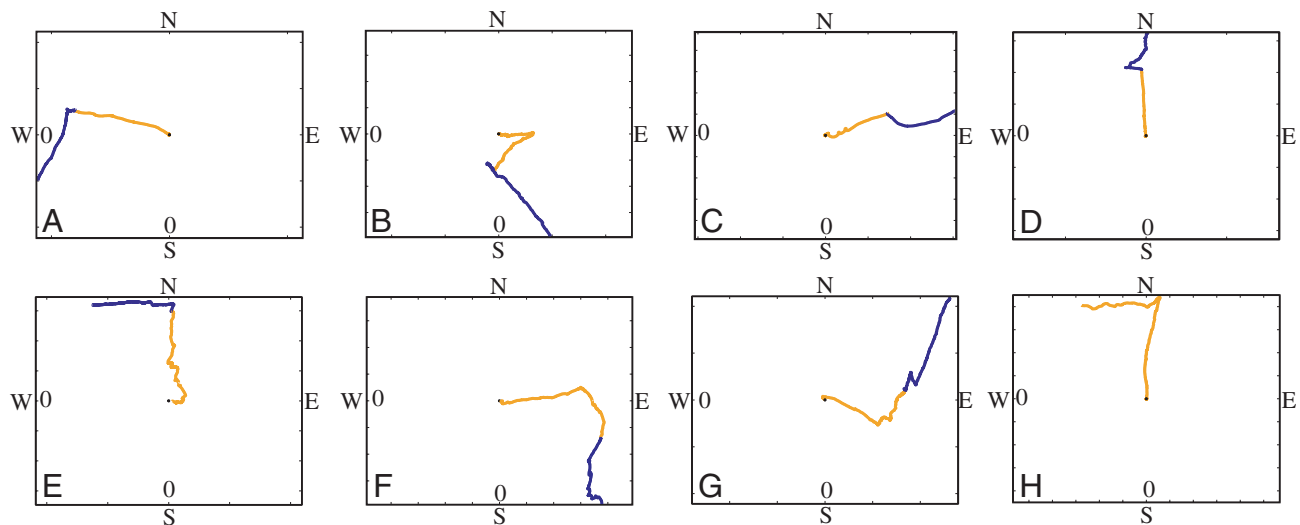

Fig. 6. Abrupt orientation changes following a turn of the polarizer were observed in a few butterflies, although this behavior also occurred in other situations. (A,B) Tracks of two of the three individual butterflies (out of a total of 50 monarchs tested) that changed their orientation by ~90° after the polarizer was turned by 90°. (A) 44° polarizer. (B) 85° polarizer. (C,D) Examples of what might be transient orientation responses towards the polarizer being turned by 90°. (E) However, one out of 10 monarchs showed a distinct ~90° shift in orientation when the lid with an opening exposing the blue sky instead of a polarizer was turned. (F) A ~90° turn observed in the same experimental condition (see E) but occurring before the turn of the lid. (G,H) Spontaneous ~90° changes in orientation of monarchs flying under the artificial polarizer. Note that the animal in G altered its mean flight direction before the polarizer was turned and not in response to the turn. In H, the butterfly abruptly changed its orientation by ~90° although the polarizer was never turned. In two-colored tracks, the orange parts indicate the virtual flight paths of the monarchs before the polarizer and/or the lid was turned. The blue parts indicate the tracks flown after the turn.

responses to the turn of the polarizer, since a 90° shift in orientation also occurred in one out of 10 flights where only the lid with an opening but without polarizer was turned (Fig. 6E). Furthermore, monarchs also occasionally performed abrupt 90° turns in experiments where nothing at all was changed during the course of the flight (Fig. 6F–H).

Our results show that monarch butterflies are able to use a time-compensated compass to orient in their migratory direction without perceiving polarized skylight *via* the DRA, as long as they are given direct view of the sun. Furthermore, after time-shifting their internal clock by +6 h, the monarchs' group mean orientation was shifted by the predicted ~90° independently of whether or not the DRA was occluded. As there was no significant difference in individual or group directedness of monarchs with and without occluded DRAs, polarization vision does not seem to add significantly to the accuracy of the butterflies' compass system. The slight tendency to reduced group directedness when the DRA was occluded could be due to the fact that the overpainted area included several hundred ommatidia outside the DRA. Hence, we conclude that polarized light input is not necessary for time-compensated sun compass orientation in migratory monarch butterflies.

The present study also strongly supports our former suggestion that monarchs do not use the Earth's magnetic field for orientation (Etheredge et al., 1999; Taylor et al., 2000; Mouritsen and Frost, 2002): a total of 140 butterflies was tested without direct view of the sun but with access to the undisturbed geomagnetic field (Fig. 7), and their group orientation was random (α=19°, r=0.09, P=0.33). The fact that as many as 140 butterflies not seeing the sun still show random orientation further strengthens our confidence in our flight simulator results, since even the slightest systematic artifact would have emerged after testing such a large number of individuals.

The presence of a specialized DRA in monarch butterflies (Labhart and Baumann, 2003; Reppert et al., 2004) strongly suggests that monarch butterflies are able to perceive polarized light. However, all butterfly and moth species (representing five families of Lepidoptera) tested so far,

including the non-migratory ones, show an anatomically specialized DRA (Labhart and Meyer, 1999). Thus, presence of a DRA alone does not necessarily mean that the butterflies use it for migratory orientation. It could serve several other functions.

In conclusion, our study shows that monarchs can use their time-compensated sun compass to orient in their normal south–southwesterly migratory direction without relying on polarized light information. In other words, polarized light input is not necessary for a time-compensated celestial compass orientation in migratory monarch butterflies. Our data further suggest that monarch butterflies are unable to make navigational use, during their autumn migration, of either a natural or an artificial polarized light stimulus covering a large (up to 85° wide) zenith-centered part of their visual field. Thus, it seems to be the sun and/or the associated light intensity and spectral gradients rather than the pattern of polarized light in the sky that plays the key role in the monarch's time-compensated sun compass guiding the butterflies on their way to Mexico.
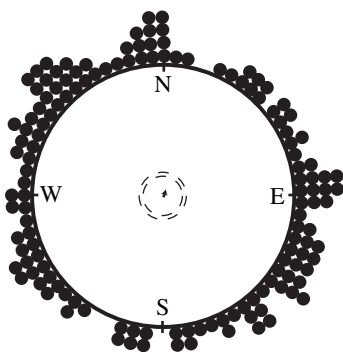
Fig. 7. The geographical orientation of all butterflies that did not see the sun directly was random even with 140 tested individuals. The undisturbed geomagnetic field was available during all flights. For explanation of symbols, see legend to Fig. 2.

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