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Experimental evidence for a magnetic sense in Neotropical migrating butterflies (Lepidoptera: Pieridae)

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We tested whether migrating *Aphrissa statira* butterflies orient with a magnetic compass. We captured migrants flying over Lake Gatún, Panama, and exposed experimental butterflies to a strong magnetic field. These and unmanipulated control butterflies were released back over the lake. Experimental butterflies had a more dispersed pattern of orientation than control butterflies. The average direction adopted was northeast, 160° anticlockwise to the natural migratory direction. Unmanipulated control butterflies adopted two diametrically opposed orientations: one shifted 33° clockwise, and another 147° anticlockwise, to the migratory direction. Control and experimental butterflies differed in that some controls oriented towards the migratory direction. These differences in orientation support the hypothesis of a sense for magnetic orientation cues. Unmanipulated butterflies released over the lake when the sky was completely overcast were significantly oriented towards their direction before capture (187° and 203°, respectively), further supporting the magnetic compass hypothesis. In a third experiment, we obstructed sun compass cues and reversed the horizontal component of the local geomagnetic field to position magnetic north towards the geographical south pole within a flight arena into which we released individual butterflies. Experimental butterflies experiencing the reversed magnetic field oriented on average 180° opposite to their natural migratory direction. Control butterflies, for which the position of magnetic north was unaltered, were oriented both towards and 180° opposite to the natural migratory direction. This difference between orientations of control and experimental butterflies also supports the hypothesis of a sense for magnetic orientation cues.

For migrating butterflies, three not mutually exclusive hypotheses have been put forward to explain their abilities to orient and navigate: use of landmarks, orientation with a sun compass, and use of a geomagnetic compass (Brower 1996). To date, we have shown that *Aphrissa statira* butterflies (Pieridae: Coliadinae), which cross the isthmus of Panama in abundance during May–July, use local landmarks to correct for wind drift when crossing the Panama Canal (Srygley et al. 1996). We have also demonstrated use of a time-compensated sun compass by this species, which may be used for orienting over long distances (Oliveira et al. 1998).

As with the sun, the earth's magnetic field may also provide directional information for insects that navigate long distances. When approached with a strong magnet before release, headings of magnetized monarch, *Danaus plexippus*, butterflies were random whereas those of control butterflies were directed to the southwest (Perez et al. 1999). However, these results appear to have been confounded by wind drift (Srygley & Oliveira 2001). Most recently, Mouritsen & Frost (2002) tethered

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monarchs to simulate flight in a boat house under brightly lit Plexiglas to simulate overcast conditions. In this experimental set-up, monarchs oriented randomly and did not adopt an orientation when the polarity of the magnetic field was locally reversed.

Nocturnally active insects may also maintain a preferred orientation without the sun. Baker & Mather (1982) reversed the orientation of caged nocturnal moths *Noctua pronuba* with a magnetic field whose net effect was nearly a mirror image of the geomagnetic intensity and direction, and Baker (1987) reversed the orientation of caged *Agrotis exclamationis* moths. However, these moths are not known to be long-distance migrants and Baker (1987) conceded that the aforementioned experiments lacked the proper controls.

Although there is little evidence that long-distance migrating insects use a magnetic sense for orientation, arthropods that move short distances have the ability to sense the magnetic field (e.g. honeybees, *Apis mellifera*: Lindauer & Martin 1968; Walker & Bitterman 1989; sandhoppers, *Talitrus saltatus*: Ugolini et al. 1999; wood ants, *Formica rufa*: Camlitepe & Stradling 1995; leaf-cutter ants, *Atta colombica*: Banks & Srygley 2003). Our first objective in the present study was to test whether *A. statira* butterflies use a magnetic compass when celestial cues are available. To accomplish this, we exposed migratory butterflies to a strong magnetic field and measured their orientation when released over the lake where they had been captured. Our second objective was to test whether *A. statira* butterflies orient when celestial orientation cues are unavailable. In this case, we released butterflies on the lake when the sky was completely overcast. Our third objective was to test whether the insects obtain polarity information from the horizontal component of the magnetic field. To test this, we experimentally reversed the horizontal component of the earth's magnetic field and measured the butterfly's orientation when flying in an arena in which we obscured celestial cues to the best of our abilities.

Predictions for the Experimental Treatments

Experimental disruption of the magnetic sense

Information that arises from the geomagnetic field may be disrupted by application of a strong magnetic field. This technique has been used successfully with migrating passerine birds (Wiltschko et al. 1994) and monarch butterflies (Perez et al. 1999). We applied a strong magnetic field to experimental butterflies, whereas a control group was handled in the same manner but a strong magnetic field was not applied. We predicted that unmanipulated control butterflies would adopt the natural migratory orientation when released over the lake from which they had been captured. For experimental butterflies, we predicted that they would either orient randomly if celestial cues were not used, or that they would adopt the same orientation as control butterflies if celestial cues were used in the absence of magnetic cues.

Experimental reversal of the earth's magnetic field

Within an insectary placed in a tent that obscured celestial cues, we predicted that control butterflies would orient in the natural migratory direction (south by southwest). For the experimental butterflies exposed to a reversal of ambient magnetic polarity from geographical north to south, we predicted that they would reverse their orientation 180° to geographical north by northeast.

METHODS

Natural Flight Directions

We captured butterflies flying over the Panama Canal (Lake Gatún) between 0900 and 1200 hours for midday experiments and for experiments the following morning (refer to Srygley et al. 1996 for a map of the flyway and Oliveira et al. 1998 for the phenology of the *Aphrissa* migration). Data were collected during the migratory seasons of 2001, 2002 and 2003 (specifically 24 June–7 July 2001, 13 May–23 July 2002 and 21 May–6 June 2003). Prior to capture of each butterfly as it migrated naturally across the lake, we followed it with an aluminium boat powered by a 40-hp outboard motor and measured the track direction of the butterfly (and the boat) using a Suunto handheld compass. We then captured the butterfly with a handnet and placed it in a glassine envelope containing moistened cotton swabs that maintained humidity and prevented damage from compression. After capture, we measured local wind speed with an Omega anemometer and wind direction with a wind vane and handheld compass.

Strong Magnet Experiment

As we wanted to release butterflies in the morning when wind speeds are typically less than those at midday (Oliveira et al. 1998), we fed them a 20% sugar water solution near midday (1115–1330 hours) and held them overnight in the laboratory at 23 °C in a net bag (diameter: 0.5 m; height: 0.75 m). We fed them again between 0800 and 0830 hours before releasing them on Lake Gatún between 0900 and 1000 hours, a time that we regularly observed *Aphrissa* butterflies migrating naturally across the lake.

We randomly selected butterflies by coin-flip to undergo an experimental or control treatment immediately prior to release over the lake. Butterflies in the experimental treatment were swiped through a strong magnetic field emanating from two rigid 1.22 T block magnets (rectangular dimensions: 5.1 × 2.5 × 1.2 cm) aligned with faces of the same magnetic polarity parallel (i.e. north and south faced one another) and fixed rigidly beside one another at a distance of 7 mm within an aluminium base. For the experimental treatment, the butterflies were held by the wings and swiped quickly (ca. 0.5 s) between the two magnets (the combined field strength measured at the centre point between the two magnets was 0.75 T). The same method was used for control butterflies except that they were swiped between two nonmagnetic aluminium blocks that were set in an aluminium base of equivalent dimensions as used in the magnetic exposure. Each butterfly was carefully handled so that it was never within 1 m of the strong magnets until immediately before the experimental treatment. Control butterflies were always kept away from the magnets.

Before and after each release, we measured the ambient wind speed and direction, temperature and sunlight, and estimated the percentage of sky that was covered by clouds. We classified the sun's visibility into one of four classes: sun's disc fully visible, sun's disc visible behind clouds, sun's disc not visible but sun's rays visible so that the sun's position could be reliably estimated, and sun's position obscured by clouds. This latter category is not the same as an overcast sky, which is quantified by the percentage of cloud cover. An observer followed the butterfly as it flew over the lake until it could no longer be seen. The observer did not set up the experiment and was unaware of the treatment, and so the experiment was conducted blind. The horizon bearing relative to the boat at which the butterfly vanished was measured with a handheld Suunto compass. The vanishing bearing was recorded with the time of release and meteorological data for each uniquely identified butterfly.

From the complete data set, we excluded 19 butterflies that were released in strong winds ($\geq$3.5 m/s). For the control butterflies, wind directions were axially distributed (158° and 338°; Rayleigh test: $r = 0.28$, $N = 42$, $P < 0.05$), as they were for experimental butterflies (154° and 334°, $r = 0.32$, $N = 37$, $P < 0.025$).

Releases on an Overcast Day

To determine whether *Aphrissa* butterflies would orient in the migratory direction without sun or polarized light cues, we captured butterflies migrating across Lake Gatún and released them without further manipulation on the following day (29 June 2001) between 1055 and 1215 hours when the sky was completely overcast. Orientation and meteorological data during capture and release were taken as outlined above.

Electromagnet and Orientation Cage

Butterflies that were captured migrating over Lake Gatún that same morning were individually released between 1120 and 1330 hours into an octagonal insectary erected within an electromagnet constructed on Barro Colorado Island (BCI), Panama (elevation 40 m, 9°09′N, 79°51′W). The insectary was made of white nylon screen and PVC tubing. It was 2 m high and 2 m across from side to side, and was oriented so that each of its eight sides faced towards a compass point (design adapted from Spieth & Kaschuba-Holtgrave 1996).

The large Merritt four-coil electromagnet (Merritt et al. 1983; Kirschvink 1992) was constructed for the purpose of reversing the local geomagnetic polarity. A wooden frame (2.40 × 2.40 × 2.43 m) supported the 10 Standard Wire Gauge copper wire square coils. The resistance of the coils was 2.4 Ω, which was not different from the value (2.359 Ω) estimated from the length of the wire. The island's AC electric current (115 V) was connected to a Variac and an AC to DC transformer so that the voltage could be set to 6.83 V. When current was flowing through the coil, the voltage and resistance yielded a 2.85-A current that produced a magnetic flux approximately twice the magnitude of the horizontal component of the earth's magnetic field (28 121 nT, data calculated from the World Magnetic Model, wmm-2000: http://geomag.usgs.gov/geomag/geomagAWT.html). The total geomagnetic flux on BCI is 35 278 nT (inclination is 37° and declination −2°). There are no known magnetic anomalies. When the coil was off, the horizontal magnetic flux measured at the coil centre was 25 841 nT. When current flowed through the coil, the horizontal magnetic flux measured at the coil centre was −28 662 nT (negative indicating that its orientation was reversed; inclination of the total flux was 36.5°), weakening to a minimum horizontal flux of −21 800 nT near the coil's frame. The magnet itself was under a translucent, white nylon-reinforced plastic tent (6 m long, 3.4 m wide, height varying from 2 to 4 m, peaking at the centre north–south line; Larin Corp., Ontario, Canada), closed to all sides and above to eliminate gross landmark cues. Although the tent diffused incident sunlight, a brighter spot in the direction of the sun's disc was discernible to the human eye from within the tent on sunlit days.

Prior to release within the Merritt coil, each butterfly was cooled on ice outside of the tent and electromagnet. The treatment was randomly selected by coin-flip and the coil turned on for the experimental treatment or off for the controls. Butterflies were carried to the orientation chamber in the electromagnet and released on to an artificial flower, positioned at the chamber's centre approximately 1.5 m above the ground, from which they fed on the 15% sugar solution. While the butterfly was feeding and warming up, the experimenter left the tent that housed the insectary and electromagnet and closed the door. A video camera set in the earthen floor of the insectary relayed the butterfly's behaviour to a television set in a room 20 m from the tent. An observer who was unaware of the treatment began to record the butterfly's position after the experimenter had left the tent. The observer recorded behaviour for 5 min, and only when the butterfly had not moved from the flower in this first time period were the observations continued for an additional 5 min. The direction of each flight was recorded only if the butterfly approached a cage face and crossed a line drawn on the television monitor, corresponding to approximately 30 cm from each face of the octagonal chamber at the top. A new orientation was not recorded until the butterfly passed back across the imaginary octagon and once again crossed the line heading towards any vertical face of the insectary. Directions were recorded as one of the eight compass points that corresponded to the direction faced by each side of the octagonal insectary. For each butterfly, we calculated its circular mean vector (mean compass orientation with length r) so that observations would be independent in subsequent statistical analyses.

RESULTS

Strong Magnet Experiments

When released, control butterflies were significantly oriented on an axis (68° and 248°; axial analysis, Rayleigh test: $r = 0.36$, $N = 57$, $P < 0.01$; Fig. 1a). Before capture, these same butterflies had a mean orientation of 215° (±9°, 95% confidence interval; Fig. 1b). The 95% confidence interval for the released control butterflies (±28°) overlapped with the 95% confidence interval of the mean orientation before capture, but their axial orientation upon release was obviously different from their univectorial orientation when naturally migrating across the lake.

For the released experimental butterflies, the mean orientation was 50° (Rayleigh test: $r = 0.39$, $N = 59$, $P < 0.01$; 95% confidence interval: ±26°; Fig. 1c). Before capture, these same butterflies flew towards 210° ($r = 0.88$, $P < 0.01$; 95% confidence interval: ±8°; Fig. 1d). Hence after the application of the strong magnet, the butterflies' orientation was shifted 160° anticlockwise to their natural migratory direction.

From the release site (Fig. 2), shorelines to the east and west are equidistant (approximately 1250 m). To the north and south in the direction that the Panama Canal runs, shorelines are further. Released butterflies did not fly disproportionately towards the nearest points of land. Therefore, we conclude that control and experimental butterflies were not affected by the shoreline to a degree that would cause a difference between the treatments. Released butterflies were generally oriented across the axially distributed wind directions rather than downwind (cf. Oliveira et al. 1998).

The control butterflies were oriented significantly axially and the experimental butterflies were oriented unimodally; the distributions of orientations in these two treatments were significantly different (Watson's test: $U^2 = 0.432$, $N_1 = 57$, $N_2 = 59$, $P < 0.001$; conducted with Oriana v. 2.02, Kovach Computing Services, Anglesey, U.K.). The angular dispersion of the experimental butterflies is their displacement from the mean orientation. For the control butterflies, we calculated the displacement around the mean of the doubled angles (152°; Zar 1999, page 608). Experimental butterflies were dispersed significantly more about the mean orientation than controls were dispersed

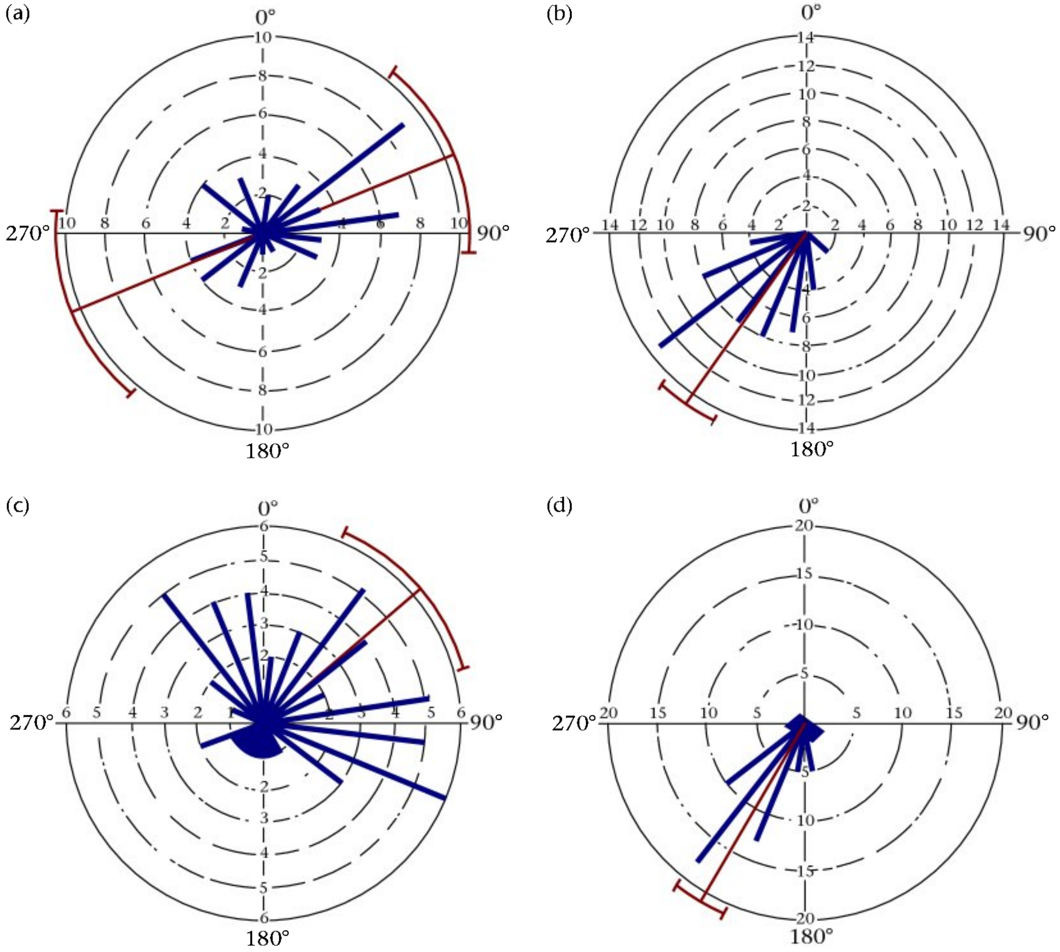

Figure 1. The natural flight directions for *Aphrissa statira* butterflies before capture, and their flight direction after treatment and release over the lake. North is towards the top of the figure. Control butterflies (a) after the sham treatment and (b) before capture. Experimental butterflies (c) after exposure to a strong magnetic field and (d) before capture. Concentric circle: number of butterflies flying in each 10° compass sector; red ray: mean flight direction; red arc: 95% confidence interval for the mean flight direction.

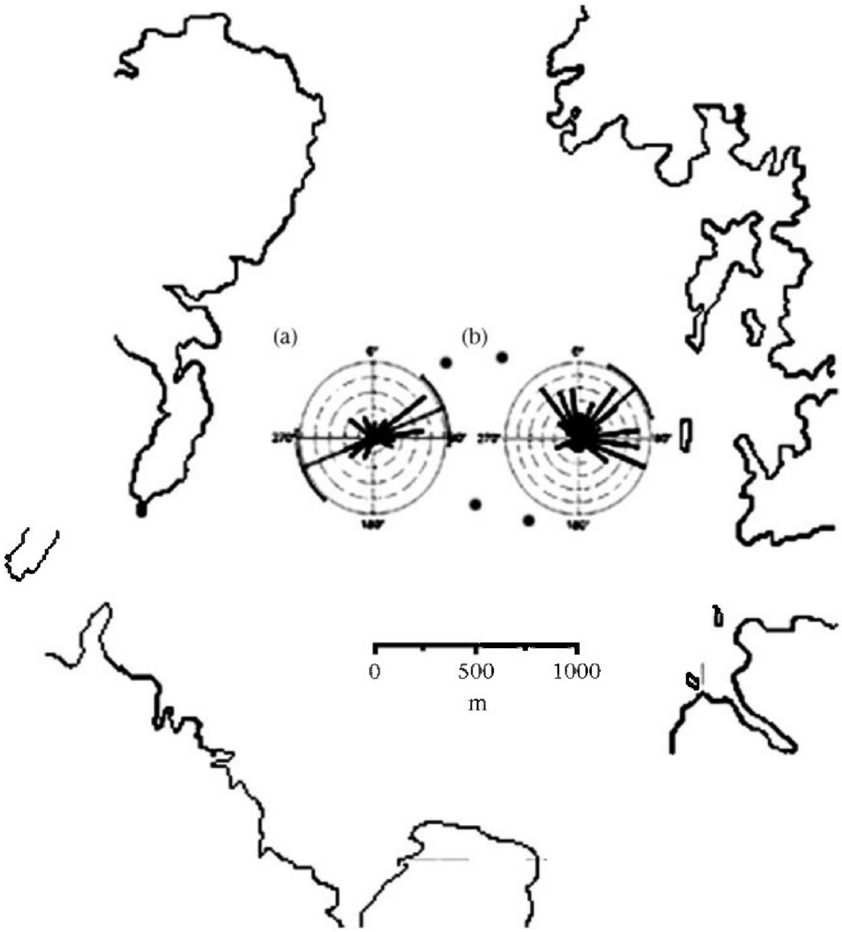

Figure 2. The release site for *Aphrissa statira* butterflies on Lake Gatún, Panama. North is towards the top of the figure. The four black dots in the lake mark Panama Canal buoys between which we conducted the releases. The lake shore and islands in the vicinity of the release site are outlined. The circular diagrams indicate orientations for (a) released control butterflies, and (b) released experimental butterflies (corresponding to Fig. 1a and c).

from the mean of the doubled angles (Mann–Whitney U test: $U = 2462$, $Z = -4.31$ by normal approximation, $N_1 = 57$, $N_2 = 59$, $P < 0.001$). Although the treatment did not cause the uniform orientation that was predicted, experimental butterflies were more dispersed in their orientations relative to controls, as predicted.

Orientation Under Overcast Skies

The mean orientation of the released butterflies was 187° (Rayleigh test: $r = 0.64$, $N = 19$, $P < 0.01$) and did not differ significantly from their natural migratory orientation before capture (203°; $r = 0.98$, $N = 18$, $P < 0.01$; Watson's F test: $F_{35} = 1.35$, $P = 0.25$). Winds were 1.23 m/s on average (range 0.5–1.9 m/s) from 328° (range 304°–358°). The range of incident light was 21 000–41 500 lx and of ambient temperature 27–28 °C.

Experimental Reversal of the Magnetic Field

In the locally reversed magnetic field, magnetic north was positioned towards the geographical south pole (declination in Panama is less than 2°, Peddie 1993). Because the orientation chamber had eight faces, the mean vector of the orientation of all of the butterflies was tested with bivariate second-order statistics (Batschelet 1981). The control butterflies were not significantly oriented with this test (Moore's statistic: $D^* = 0.43$, $N = 61$ butterflies, $P > 0.10$), but an axial orientation would not be significant with this univectorial test (Zar 1999, see below). The experimental group was significantly oriented with a mean vector towards 7° relative to geographical north ($D^* = 1.31$, $N = 64$, $P < 0.01$).

With the caveat that a principal assumption was violated because the mean vectors for each butterfly differed in length, we also applied parametric statistics because there is no nonparametric equivalent to an axial analysis. Control butterflies were oriented in two directions (27° and 207°; Rayleigh test: $r = 0.31$, $N = 61$, $P < 0.01$; Fig. 3a). Hence as in the releases on Lake Gatún, a proportion of the butterflies were oriented in their natural migratory direction before capture (209°; $r = 0.87$, $P < 0.01$; Fig. 3b), but a larger proportion were oriented 180° opposite to the natural migratory direction. Butterflies in the experimental treatment were oriented towards geographical north by northeast (9°; $r = 0.29$, $N = 64$, $P < 0.01$; Fig. 3c). As predicted, this direction was reversed relative to their mean natural migratory direction before

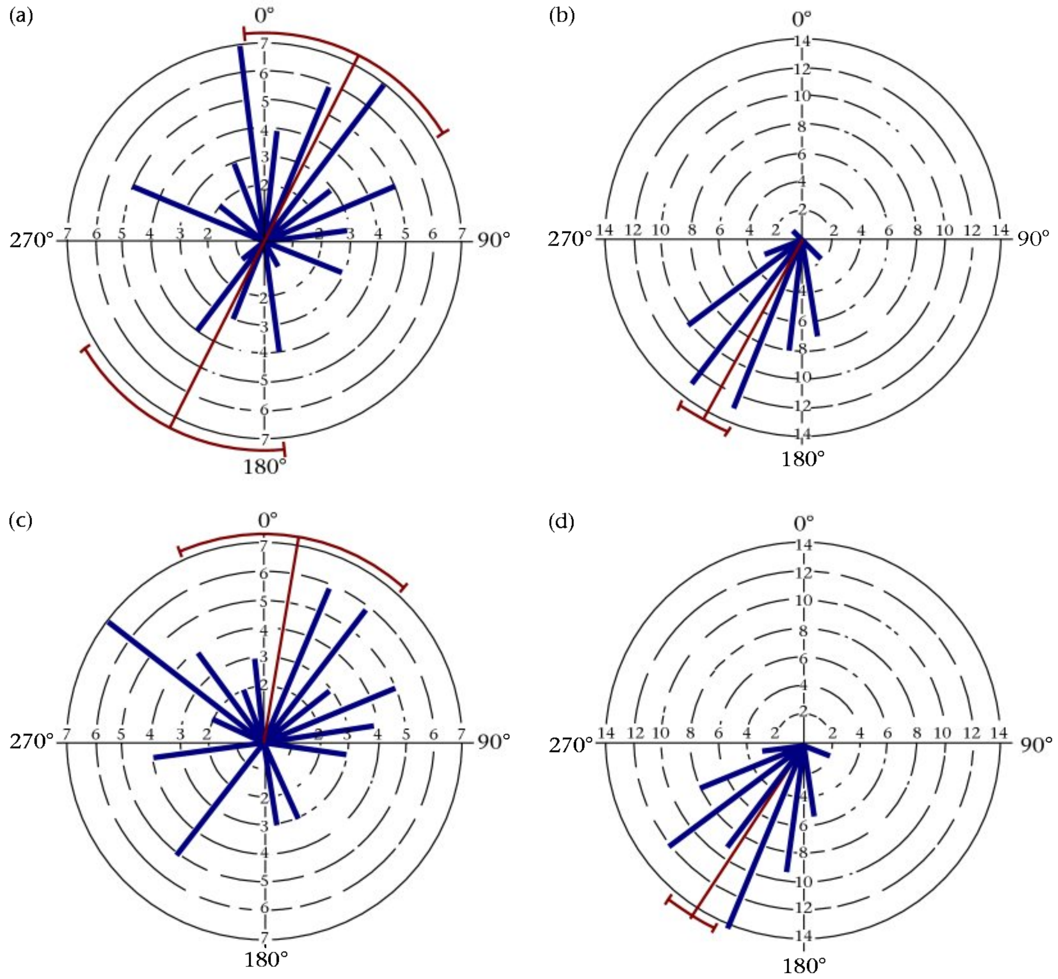

Figure 3. The natural flight directions for *Aphrissa statira* butterflies before capture, and their mean flight direction after release in the orientation chamber. Geographical north and, except in (c), magnetic north are towards the top of the figure. Control butterflies (a) in the chamber with geomagnetic north unchanged and (b) before capture. Experimental butterflies (c) in the chamber with magnetic north repositioned toward geographical south and (d) before capture. Concentric circle: number of butterflies flying in each 10° direction class; red ray: mean flight direction; red arc: 95% confidence interval for the mean flight direction.

capture (213°; $r = 0.87$, $P < 0.01$; Fig. 3d). An axial model applied to the experimental butterflies was not significant ($P = 0.66$). The distributions of orientations in the treatments were significantly different (Watson's test: $U^2 = 0.413$, $N_1 = 61$, $N_2 = 64$, $P < 0.001$).

The escape behaviours of the experimental and a proportion of the control butterflies were consistent with earlier observations. Our preliminary experiments from the orientation cage had indicated that the butterflies oriented towards the sun's azimuth when sufficient information was available for them to discern its position (both treatments flew towards the east between 0900 and 1100 hours, and the west between 1400 and 1600 hours). The experiment was not possible by any other means because the butterflies would not fly from the artificial flower when clouds covered the sun.

DISCUSSION

We have presented experimental evidence for a magnetic sense in migratory *A. statira* butterflies. Orientations of butterflies experimentally exposed to a strong magnetic field were significantly more dispersed than those of control butterflies. Significant differences between experimental and control groups indicate behavioural sensitivity to magnetic perturbation, although additional factors pertain in the interpretation of the orientational distributions. In addition, butterflies released into a magnetic field of locally reversed polarity were oriented opposite to the natural migratory direction. However, both treatment groups showed an escape response towards the solar azimuth.

In particular, control butterflies in both experiments showed an axial distribution such that only some individuals flew near to the prevailing migratory direction, whereas orientation of the remainder was nearly 180° opposite. This opposing direction may have derived from a behavioural tendency of the butterflies to fly towards the sun's azimuth. Polarity-reversal experiments were conducted from 1120 to 1330 hours, during which time the sun's azimuth swept across the north, ranging from 44° (northeast) to 312° (northwest) and crossing three faces of the octagonal insectary. In these experiments, those control butterflies not oriented in the prevailing southwesterly migratory direction were instead oriented towards 27°, on average. This value lies within the range of the sun's azimuthal position during the time of the experiments. Similarly, high-intensity strong magnet experiments were conducted when the sun's azimuth ranged from 63° to 67° (representative data from 15 June 2002). On average, the mean orientation for those control butterflies not flying southwesterly was 68°. We suggest that the most parsimonious explanation for these orientations towards the solar azimuth is the combined handling and escape effect for some control butterflies (Froy et al. 2003).

For experimental butterflies released into the orientation chamber with a reversed magnetic field, the observed flight direction (7° relative to geographical north) was nearly 180° opposite to the natural migratory direction (213°, i.e. the predicted flight direction was 33°). If the butterflies had flown towards the sun's azimuth during the experiments (northeast 44° to northwest 312°), then the predicted orientation based on a magnetic compass and this postulated escape direction would have been indistinguishable. The axial orientation of the control butterflies suggests that some experimental ones were oriented towards the natural migratory direction (rather than simply adopting an escape response towards the sun), but further experiments where the polarity of the ambient magnetic field is shifted less than 180° should be conducted.

Similar to our control butterflies, some migratory birds within orientation chambers also tend to adopt axial orientations (e.g. Able & Able 1993, 1999). Birds use the inclination of the magnetic field relative to gravity to distinguish equatorial from polar directions (Wiltschko & Wiltschko 1972, 1995). Reversal of both the vertical and horizontal components, that is, reversing the polarity while holding the field lines constant, did not alter the birds' orientations, and both garden warblers, *Sylvia borin*, and European robins, *Erithacus rubecula*, were disoriented in horizontal fields. In contrast, bees, ants (Hymenoptera, e.g. Schmitt & Esch 1993; Banks & Srygley 2003), mealworm beetles (Coleoptera, e.g. Arendse 1978; Vácha & Soukopová 2004) and spiny lobsters, *Panuliris argus* (Crustacea, Lohmann et al. 1995) obtain polarity information from the horizontal component of the magnetic field. Animals may use only one component of the earth's magnetic field for compass orientation, and a combination of components to position themselves in a cognitive map, such as that observed in spiny lobsters (Boles & Lohmann 2003). The potential ability of butterflies to assay both vertical and horizontal components of the geomagnetic field has not been determined.

Interactions between solar and magnetic cues may also come into play in the experimental manipulations used here. *Aphrissa statira* uses a time-compensated sun compass to maintain migratory orientation (Oliveira et al. 1998), but at equatorial latitudes the variation in solar azimuthal speed potentially renders a sun compass inaccurate (e.g. Wehner 1984). In equatorial sandhoppers, for example, a magnetic compass overrides the sun compass when the height of the sun is too low for accurate zenithal determination (Ugolini 2001). The relative importance of solar versus magnetic cues in multiple compass systems within butterflies is likely to change with the quality of solar directional information (e.g. solar cues obscured by the tent used here in the polarity-reversal experiments) as well as with motivational condition (e.g. extent of holding time prior to experimentation). Butterflies released on the lake under an overcast sky oriented in the migratory direction. Magnetic perturbations under conditions of variable cloud cover would elucidate the relative importance of the sun's azimuth, polarized light and geomagnetism as directional cues.

When exposed to a strong magnetic field, experimental butterflies were disoriented relative to controls. Scatter in vanishing bearings of the experimental group was much higher than that of controls, but experimental butterflies also tended to orient away from the natural migratory direction and towards the sun's easterly azimuthal location. In the strong magnet experiments, the sun's azimuth ranged from 63° to 67°, and the mean orientation for experimental butterflies was 50°. We interpret flight towards the sun's azimuth as an escape behaviour adopted by experimental butterflies that were disoriented because of disruption of the magnetic sense. In any event, significant differences between orientations of control and experimental individuals indicate magnetic sensitivity, whereas the disoriented nature of experimental butterflies exposed to a strong magnetic field suggests that orientation with the geomagnetic field is a behaviourally relevant directional cue even when the sun is visible.

Strong magnetic fields may alter the dipole moment of particles within ferromagnetic materials and thus change the alignment of the magnetic moment (Beason et al. 1995). However, treatment by a strong magnet could also cause the magnetic material as a whole to move and inadvertently disrupt surrounding tissue. The magnetic material may also simply rotate and return to its origin when the magnet is removed which may make any behavioural outcome of the perturbation less consistent. In contrast to strong magnets, a magnetic pulse of sufficient strength and short duration has a singular effect of overcoming the internal magnetization of a magnetic material. No one has applied a pulse magnet to migratory butterflies to observe its effect on orientation. Our results indicate that further experiments are warranted. Because of the large power demand of the capacitors (e.g. 250 V), it would be necessary to apply the pulse treatment onshore, where electricity is available, before conducting the release experiment on a lake. A strong magnetic field may have other generalized effects on physiological function unrelated to orientation that may have made the experimental insects lethargic or otherwise less inclined to orient in the migratory direction. This caveat applies to pulse magnets as well (Beason et al. 1995).

Magnetic cues are used by a diversity of animal taxa for purposes of spatial orientation (Wiltschko & Wiltschko 1995), and in many cases these responses are also light dependent and wavelength sensitive (Deutschlander et al. 1999). With the exception of the monarch butterfly, magnetic sensitivities of migrant butterflies are essentially unstudied. The presence of magnetic material within the bodies of monarch butterflies is consistent with such sensory capacity (Jones & MacFadden 1982; Jungreis 1987), but the inability to induce magnetic remanence within four other migrant butterfly species is puzzling (Jungreis 1987). In preliminary results (O. Alves, E. Wajnberg & D. Esquivel, unpublished data), electron paramagnetic resonance measurements indicated the presence of ferromagnetic tissue in *A. statira* (see Esquivel et al. 1999 for methods). Well-established behavioural responses to magnetic fields by honeybees (e.g. Gould et al. 1980; Walker & Bitterman 1989) demonstrate the feasibility for insects to evolve and use such sensory capacities. Identification and physiological elucidation of geomagnetic transduction pathways within butterfly migrants, including the danaid monarch butterfly and the pierid *A. statira* studied here, would clearly be desirable goals.

Using tethered monarchs in a flight simulator, Mouritsen & Frost (2002) found no effect on flight orientation when externally imposed magnetic fields were used to rotate the geomagnetic field. However, the butterflies may have lost motivation to fly towards their preferred migratory direction when tethered in an indoor enclosure. Tethered honeybees fail to respond to an externally imposed magnetic field in a feeding protocol, whereas free-flying honeybees show consistent responses to the same stimulus (Walker et al. 1989). Particularly with migrant insects that fly continuously for many hours, behavioural experiments using tethered individuals should be interpreted conservatively given potentially large differences in motivation. In the present set of manipulations, orientation of untethered butterflies within a large cage as well as flight directionality within the natural migratory setting were evaluated and shown to be sensitive to magnetic perturbation in their immediate surroundings. We accordingly suggest that the migratory pierid *A. statira* is sensitive to geomagnetic cues, and propose that free-flying monarch butterflies should be similarly evaluated.

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