

A sky polarization compass in lizards: the central role of the parietal eye

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SUMMARY

The present study first examined whether ruin lizards *Podarcis sicula* are able to orientate using the e-vector direction of polarized light. Ruin lizards were trained and tested indoors, inside a hexagonal Morris water maze, positioned under an artificial light source producing plane polarized light with a single e-vector, which provided an axial cue. Lizards were subjected to axial training by positioning two identical goals in contact with the centre of two opposite side walls of the Morris water maze. Goals were invisible because they were placed just beneath the water surface, and water was rendered opaque. The results showed that the directional choices of lizards meeting learning criteria were bimodally distributed along the training axis, and that after 90 deg rotation of the e-vector direction of polarized light the lizards directional choices rotated correspondingly, producing a bimodal distribution which was perpendicular to the training axis. The present results confirm in ruin lizards results previously obtained in other lizard species showing that these reptiles can use the e-vector direction of polarized light in the form of a sky polarization compass. The second step of the study aimed at answering the still open question of whether functioning of a sky polarization compass would be mediated by the lizard parietal eye. To test this, ruin lizards meeting learning criteria were tested inside the Morris water maze under polarized light after their parietal eyes were painted black. Lizards with black-painted parietal eyes were completely disoriented. Thus, the present data show for the first time that the parietal eye plays a central role in mediating the functioning of a putative sky polarization compass of lizards.

Key words: lizards, orientation, Morris water maze, sky polarization compass, parietal eye.

INTRODUCTION

A time-compensated celestial compass may function using two different daylight celestial cues. Compass bearings can be calculated using the sun's azimuth (the point where the solar meridian – a line dropped vertically from the sun's position – intersects with the horizon), and for this it is necessary to know the pattern of changes of the sun's azimuth over time. Alternatively, sky polarization patterns can be used, since these patterns also vary predictably with the sun's position (Wehner, 1992). As sunlight passes through the atmosphere it is partially polarized, as a function of the scattering angle. The e-vector of each scattered ray exhibits a prevailing vibration direction, which is perpendicular to the plane in which the ray was deflected [Rayleigh scattering (Brines and Gould, 1982)]. This results in a symmetrical e-vector pattern, which is fixed with respect to the solar and anti-solar meridians. One of these meridians can be used as a reference direction, and compass bearings can be determined by measuring the angle between this reference and the direction of interest. This compass was defined as a sky polarization compass (Frisch, 1949). Like the sun azimuth compass, the sky polarization compass requires time compensation to take into account changes in e-vector direction and pattern rotation as the sun changes elevation and moves horizontally across the sky (Brines and Gould, 1982).

In several lizard species a time-compensated celestial compass has been shown to exist (Freake, 2001), but is still unclear whether these reptiles are actually equipped with a sun azimuth compass, a sky polarization compass, or both. A sky polarization compass may provide an advantage over a sun azimuth compass, because it remains functional even when the sun's position is obscured by vegetation and clouds, as long as some blue sky is visible. Often,

small lizards face situations in which they have to hide themselves under vegetation and cover to avoid predation, and, at the same time, the necessity to orientate within or toward their territories or burrows, or toward known food sources, may render the use of a sky polarization compass adaptive.

Results of experiments carried out by Adler and Phillips (Adler and Phillips, 1985) in the fringe-toed lizard *Uma notata* and by Freake (Freake, 1999) in the sleepy lizard *Tiliqua rugosa* demonstrated that lizards can use the e-vector direction of polarized light in the form of a sky polarization compass. Both groups proposed that such a sky polarization compass sense could be mediated by the lizard parietal eye (Adler and Phillips, 1985; Freake, 1999). This view is supported by anatomical data suggesting that the photoreceptors in the parietal eye are arranged in a pattern that would theoretically allow analysis of the e-vector of linearly polarized light (Hamasaki and Eder, 1977). Up to now, however, there has been no experimental evidence that the lizard parietal eye actually plays a role in the functioning of a sky polarization compass. Field studies, carried out in both the spiny lizard *Sceloporus jarrovi* and the sleepy lizard *T. rugosa*, showed that an intact parietal eye is required to perform goal orientation under the sun, but these data do not indicate whether the lizard's parietal eye mediates the functioning of a sun's azimuth compass or a sky polarization compass (Ellis-Quinn and Simon, 1991; Freake, 2001).

We previously used a Morris water maze to investigate compass orientation mechanisms in the ruin lizard *Podarcis sicula* (Morris, 1984; Foà et al., 2009). In the open field, during sunny days, lizards were individually trained to swim from the centre of the water maze onto a hidden platform (the goal), positioned at the periphery of the maze in a single compass direction. The results (Foà et al., 2009)

showed that: (1) lizards learn to swim directly towards the hidden goal under the sun in the absence of visual feature cues; (2) the learned orientation response is mediated by a time-compensated celestial compass; and (3) an intact parietal eye is required to perform goal orientation under the sun.

The present study was aimed at answering the still open question of whether the lizard parietal eye plays a role in the functioning of a sky polarization compass. The first step of the investigation consisted of testing whether ruin lizards can use a linearly polarized light source to orientate inside a Morris water maze positioned indoors. As ruin lizards actually demonstrated this ability, we further examined whether individual lizards whose parietal eyes were painted black could still use the e-vector direction of linearly polarized light for orientation.

MATERIALS AND METHODS

Animals

Ruin lizards *P. sicula* (Rafinesque-Schmaltz 1810; adults only) were collected from the area of Ferrara (Italy; longitude: 12°21'44" E, latitude: 45°03'72" N) under the authority of the Parco Delta del Po-Emilia Romagna (Department of Wildlife and Fisheries). Details of

lizard maintenance have been published previously (see Foà et al., 2009). The captive maintenance procedures and research protocols were approved by the University of Ferrara Institutional Animal Care and Use Committee and by the Italian Ministry of Health.

Experimental apparatus

The Morris water maze was the same maze which was utilized previously (Foà et al., 2009). The maze was placed inside a laboratory with no access to natural light. Water was maintained at a constant temperature of $29 \pm 1.0^\circ\text{C}$ by means of aquarium thermostats (Tetrahat, Tetra, Milan, Italy), and rendered opaque by the addition of fossil flour (Clarcel, Ceca, Honfleur, France). The goals consisted of two identical transparent Plexiglas rectangular platforms ($23.7\text{ cm} \times 16\text{ cm}$, 2.5 cm thick), each mounted on a pedestal (11.5 cm from the maze bottom). The maze was surrounded by a thick black opaque cloth fence to a height of 190 cm (Fig. 1A). This prevented the lizards from seeing laboratory features. Peep holes were built through the cloth fence allowing observers to follow lizard behaviour during releases and for positioning of the lens of a movie camera (GZ-MG21E, JVC, Yokohama, Japan), which recorded all lizard trials. The top of the fence was closed with an

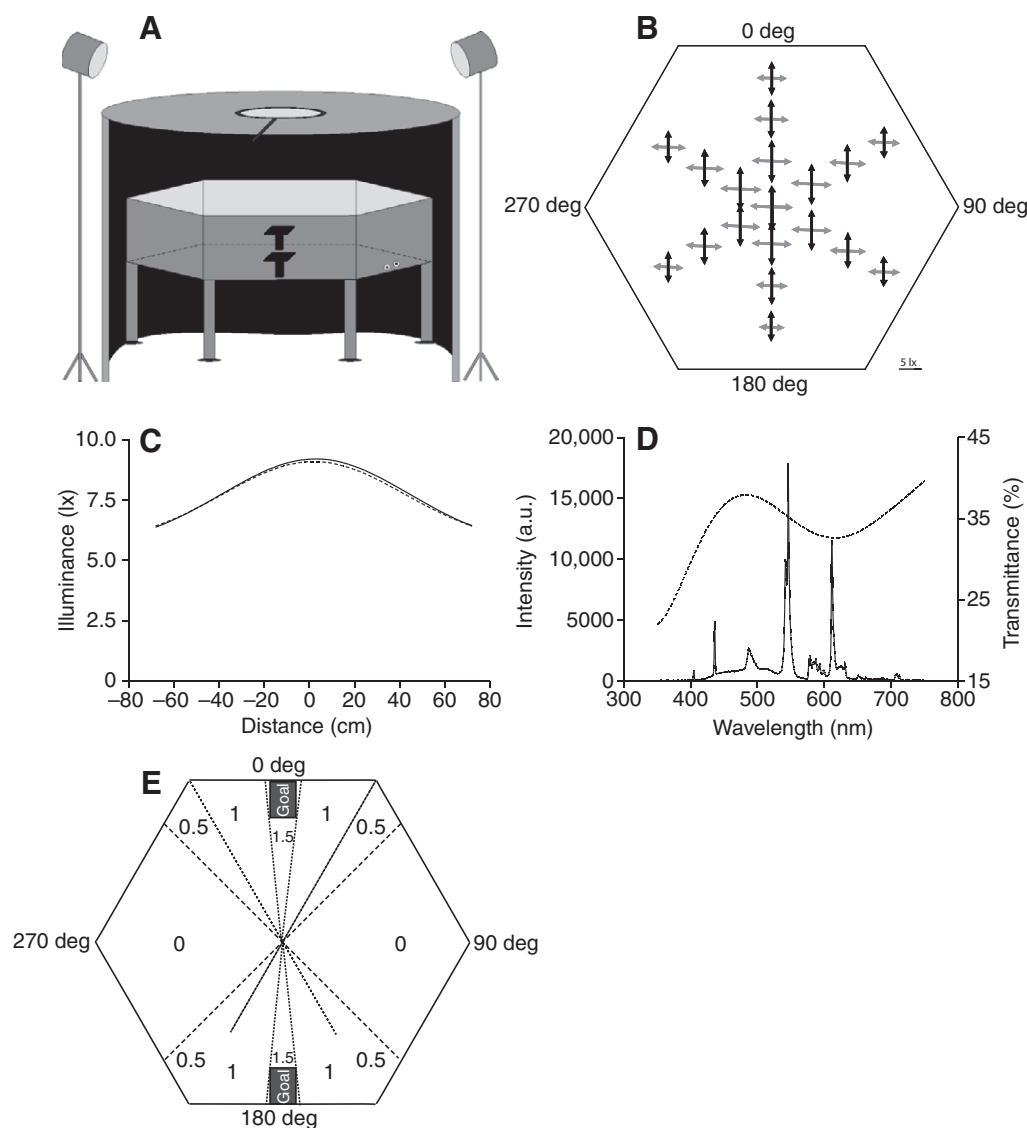


Fig. 1. (A) The hexagonal Morris water maze used in experiments. The figure shows the two goals mounted on their pedestals, located in direct contact with the centre of opposite side walls, and the black wood roof with the linear polarized filter. The black non-transparent fence completely surrounding the water maze is partially shown. Further details are reported in Materials and methods. (B) The intensity and direction of the polarization of light on the horizontal plane at the level of the goal platforms, measured along the three directions connecting opposite side walls of the hexagonal maze. Each arrow indicates the direction of the plane of polarization (e-vector). The length of the arrows is proportional to the intensity of illumination. Arrows were referred to a filter window with the polarization direction parallel (black) or perpendicular (grey) to the 0–180 deg axis. (C) Profile of the illuminance as a function of distance from the centre of the maze for the polarization direction parallel (solid line) or perpendicular (dotted line) to the 0–180 deg axis. (D) Spectral intensity of light transmitted by the polarized window, averaged with respect to both filter orientation (solid line) and spectral transmittance of the polarized filter (dotted line). (E) Schematic drawing of the water maze surface indicating the score system used to establish learning criteria. The top and the bottom of the hexagon identify goals (direction 0 and 180 deg).

opaque black wood roof (diameter 266 cm). Two floor lights (284 cm high; AEF Lighting, Ferrara, Italy), each equipped with four cool electronic energy saving lamps (32 W and 1920 lm each, Atlas Lighting Co., Brescia, Italy) protruded 94 cm above the fence, at opposite positions. A linear polarized filter (diameter 50 cm, HN42, 3M, Norwood, MA, USA) was placed in a hole (diameter 48 cm) cut around the centre of the roof, to illuminate the maze by a plane polarized light with a single e-vector. The polarized filter was sandwiched between a sandblasted glass sheet 5 mm thick on the top side and a planar glass sheet 5 mm thick on the bottom side. The sandblasted glass sheet operates as a light diffuser. Light beams produced by the lamps formed an incidence angle of 45 deg with the horizontal plane containing the polarized filter. The intensity, degree and direction of the polarization of light on the horizontal plane at the level of the goals were measured by an illuminance meter (Fig. 1B,C; Konica Minolta T-10, Konica Minolta Sensing Inc., Tokyo, Japan), equipped with a linear polarized filter (PL-C, Canon Inc., Tokyo, Japan). Because of the spatial arrangement of light beams, asymmetries of illuminance patterns at the level of the surface and vertical walls of the Morris water maze were negligible (Fig. 1B,C). Illuminance changed from 9.3 lx at the centre to 6.3 lx at the periphery of the maze (Fig. 1C). Rotation of the polarized filter by 90 deg did not change the profile and intensity of the illuminance inside the Morris water maze at the level of the goals (Fig. 1B; Student's *t*-test: $t_{36}=1.4$, $P>0.16$). The degree of polarization of light under the filter window was always unitary at all the tested points in the Morris water maze (Fig. 1B). The spectrum of the plane polarized light was measured by a spectrometer operating in the visual–near infrared (Vis-NIR) region (Fig. 1D; Ocean Optics USB 2000, Dunedin, FL, USA). The spectrum is that typical of a fluorescent lamp with colour temperature of 5500–6000 K, the same as that of natural light. The spectral transmittance (Fig. 1D) of the polarized filter was measured by a spectrophotometer (Perkin-Elmer Lambda 900, Waltham, MA, USA). As can be seen, the filter operates well as a polarizer in the full visible spectrum, where its transmittance is around 35%. The reflectance of the linearly polarized light on the water maze walls was measured to verify the existence of variations in brightness on the vertical walls of the Morris water maze [see p381 of Horvath and Varjú (Horvath and Varjú, 2003)]. Variations in brightness were found to be functionally negligible (<0.3 lx).

Release conditions

One hour before starting the individual releases, lizards were transferred from the vivaria to glass containers in which they were kept wet, at the same temperature as the Morris water maze, as previously described (Foà et al., 2009).

Experimental protocols

Experiments were carried out from June to October between 11:00 and 14:00 h. Lizards were trained and tested under an artificial light source producing plane polarized light with a single e-vector that provided an axial cue. Lizards were subjected to axial training by positioning two identical goals in contact with the centre of two opposite side walls along the axis 0–180 deg (the training axis) of the maze. If lizards were able to use a single e-vector of plane polarized light as a reference for orientation, their directional choices should show a symmetrical, bimodal distribution along the training axis 0–180 deg. Before starting pre-training and training, lizards were subdivided in two groups. The Parallel group was trained along an axis which was parallel to the e-vector direction. The Perpendicular group was trained along an axis which was

perpendicular to the e-vector direction, an experimental condition which was achieved by rotating the polarized filter by 90 deg. For each lizard the release order changed in a random sequence from one trial series to another, during both pre-training and training. Each lizard was subjected to two trials per day.

Pre-training

Goal platforms were above the opaque water surface. Twelve trials per lizard were performed, for which the release box containing the lizard was faced alternately toward one or the other goal. In the first four trials the release box was placed in direct contact with the goal, in the subsequent four trials the box was moved 10 cm away from the goal, while in the remaining four trials the box was positioned in the centre of the water maze.

Training

For training, the surface of the goal platform was placed just beneath the opaque water surface, and was thus invisible to the lizards. Each lizard was released from the centre of the maze by orienting the release box each time in a different direction, randomly chosen. The straight course from the centre of the maze to the centre of each side wall containing the goal measures 77 cm. The first point of a side wall touched by a lizard was recorded as the directional choice in that trial. After each trial the side walls of the maze were cleaned to avoid the appearance of unevenness in the visual field of the lizards which might be used as orienting feature cues. Lizards reaching one of the two goals (± 5 deg from platforms) were rewarded, and their trials were given a score of 1.5 (Fig. 1E). The reward consisted of immediately lowering the water level in the maze, so that the goal and the lizard placed on it could emerge completely from the water within 5–6 s. The lizard was kept there for 30 s before recapture. Lizards reaching the correct side walls, but not the goal platforms, were not rewarded and their trials were given a score of 1 (Fig. 1E). Lizards reaching one of the two side walls contiguous with the goal side wall were not rewarded, and their trials were scored from 0.5 to 0, with the score decreasing with distance from the goal (Fig. 1E). In all cases, lizards that reached one of the four side walls not including the goal platforms were left in the water for 30 s before recapture. Sometimes lizards sank immediately at release, and after 30–65 s spent underwater re-emerged and swam around in circles or, more often, escaped towards the nearest wall of the maze. In all these situations the lizard's directional choice was discarded, and the trial of the unsuccessful lizard was repeated. Methods to measure the compass bearings of lizards have been reported previously (Foà et al., 2009).

Learning criteria

To meet the learning criteria each lizard had to obtain a score of 6 or higher within six consecutive trials, with a maximum of one trial scoring ≤ 0.5 , and with the last trial scoring ≥ 1 (Fig. 1E). Lizards failing to meet these learning criteria were excluded from experiments.

E-vector rotation test

Once they had met the learning criteria, we tested whether lizards had learned to orientate along the training axis by using the e-vector direction of plane polarized light. To do this, the orientation of either Parallel or Perpendicular lizards was tested after 90 deg rotation of the e-vector direction with respect to the e-vector direction during training. Either Parallel or Perpendicular lizards were expected to orientate along a parallel axis with respect to training axis. For each lizard of either group the orientation test consisted of a single trial carried out in the absence of goal platforms.

Refreshing training trials

At the end of the e-vector rotation test, lizards were subjected to a second training session consisting of two trials in the presence of both goal platforms, placed as previously along the 0–180 deg axis. Again, Parallel lizards were trained with the e-vector parallel to the training axis while Perpendicular lizards were trained with the e-vector perpendicular to the training axis. Only lizards scoring ≥ 1.0 in both trials were admitted to the subsequent orientation test, which was aimed at establishing the role of the parietal eye in perceiving the e-vector direction of polarized light.

Black painted parietal eye test (Par-P)

Twelve Parallel and 17 Perpendicular lizards were admitted to the test. For either group the e-vector direction was the same as during training. One hour prior to testing the parietal eye of all lizards was painted black (Par-P) with non-dangerous colour (Craft color, Bologna, Italy) by means of a fine paintbrush. For each Par-P lizard the orientation test consisted of a single trial in the absence of goal platforms.

Control test

Another group of Parallel lizards was subjected to pre-training and training until they met the learning criteria. Before testing, the plane polarized filter was taken off the roof. For each Parallel lizard the orientation test under diffused, not polarized, light consisted of a single trial in the absence of goal platforms.

Data analysis and statistics

Our results showed that in most training and orientation tests lizards' directional choices were distributed in an approximately bimodal fashion. In all these situations mean vector length would be zero (or would approach zero) and no mean angle (mean direction) could be determined [see p.17 of Batschelet (Batschelet, 1981) and p.607 of Zar (Zar, 1999)]. One can get meaningful results from such bimodal bearing distributions only by doubling the angles, so that unimodal distributions are obtained on which statistical tests can be finally applied (Batschelet, 1981). In previous studies the method of doubling the angles was invariably applied when bearings distributions were found to be bimodal (e.g. Adler and Phillips, 1985; Freake, 1999; Wiltshko and Wiltshko, 2001; Muheim et al., 2007). In the present study we doubled all angles (directions) chosen by lizards during the last training trial and used the data obtained to calculate the training mean vector. We also doubled all angles chosen by the same lizards during the single trial orientation test and used the data obtained to calculate the test mean vector. The Rayleigh test was used to test whether the directions chosen by the lizards deviated from uniform (Batschelet, 1981). When bearing distributions were not bimodal angles chosen by lizards were not doubled. This happened, for instance, when directions chosen by lizards did not deviate from uniform. For each treatment, the Hotelling test for paired data and the Watson U^2 -test were applied to test for differences between the directions chosen by lizards in the last training trial and the directions chosen by the same lizards in the respective single trial orientation test (Batschelet, 1981). The Watson U^2 -test was applied to test for differences in orientation between Parallel lizards and Perpendicular lizards.

RESULTS

Training trials

Fifteen of 26 lizards belonging to the Parallel group, and 17 of 29 lizards of the Perpendicular group met the learning criteria

(Fig. 2A,E). There were no significant differences between Parallel and Perpendicular lizards in the number of training trials necessary to reach the learning criteria (Parallel: 19.04 ± 1.55 ; Perpendicular: 18.94 ± 1.50 , means \pm s.e.m.; Student's t -test: $t_{39}=0.04$, $P>0.95$). As expected, the directional choices of both groups showed a symmetrical, bimodal distribution along the training axis (0–180 deg). After doubling the angles, the directional choices of either the Parallel group or the Perpendicular group in the last training trial were found to deviate from uniform (Rayleigh test: $Z=12.46$, $P<0.001$, and $Z=15.18$, $P<0.001$, respectively). In the last training trial the directional choices of Parallel lizards were not significantly different from those of Perpendicular lizards (Watson U^2 -test: $U^2_{15,17}=0.038$, $P>0.50$).

E-vector rotation tests

When tested after 90 deg rotation of the e-vector axis, the directional choices of lizards had a symmetrical, bimodal distribution along the rotated axis (90–270 deg) (Fig. 2B,F). After doubling the angles, the directional choices of the Parallel group in the e-vector rotation test were found to deviate from uniform ($N=15$; Rayleigh test: $Z=4.48$, $P<0.01$) and the 95% confidence limits (34–146 deg) included the expected direction (90 deg); the same was true for the Perpendicular group ($N=17$; Rayleigh test: $Z=7.16$, $P<0.001$) and the 95% confidence limits (35–107 deg) included the expected direction (90 deg). The directions chosen by the Parallel group in the e-vector rotation test were significantly different from those that the same lizards chose in the last training trial before rotation (Hotelling test for paired data: $F_{2,13}=15.93$, $P<0.0005$); the same was true for the Perpendicular group (Hotelling test for paired data: $F_{2,15}=17.66$, $P<0.0005$). After rotation, the directional choices of Parallel lizards were not significantly different from those of Perpendicular lizards (Watson U^2 -test: $U^2_{15,17}=0.029$, $P>0.50$).

Refreshing training trials

Twelve of 15 lizards of the Parallel group and all 17 Perpendicular lizards scored ≥ 1 in both refreshing training trials (Fig. 2C,G). Their directional choices in the second refreshing training trial were found to deviate from uniform (Rayleigh test: $Z=10.0$, $P<0.001$, and $Z=10.53$, $P<0.001$, respectively). The directions chosen by the Parallel group in the refreshing trials were significantly different from those the same group chose in the previous e-vector rotation test (Hotelling test for paired data: $F_{2,10}=16.16$, $P<0.001$) and the same was true for the Perpendicular group (Hotelling test for paired data: $F_{2,15}=13.25$, $P<0.0005$). In the refreshing trials the directional choices of Parallel lizards were not significantly different from those of Perpendicular lizards (Watson U^2 -test: $U^2_{12,17}=0.025$, $P>0.50$).

Par-P tests

The directional choices of Par-P lizards of either the Parallel ($N=12$) or the Perpendicular group ($N=17$) did not deviate from uniform (Rayleigh test: $Z=0.96$, $P>0.20$, and $Z=0.81$, $P>0.20$, respectively) (Fig. 2D,H).

Control test

A new group of 18 lizards was attributed to the Parallel group and subjected to the usual training procedure (Fig. 3). Nine lizards met the learning criteria, and their directional choices showed a symmetrical, bimodal distribution along the training axis (0–180 deg). After doubling the angles the directional choices in the last training trial deviated from uniform (Rayleigh test:

$Z=6.85$, $P<0.001$). After the plane polarized filter was taken off the roof, the directional choices of the group in the orientation test did not deviate from uniform (Rayleigh test: $Z=0.69$, $P>0.50$) and were significantly different from those expressed in the last trial of the last training session (Watson U^2 -test: $U^2_{9,9}=0.37$, $P<0.002$).

DISCUSSION

The present study first examined whether ruin lizards *P. sicula* are able to orientate using the e-vector direction of polarized light. For this purpose, lizard orientation was tested indoors, under an artificial light source producing plane polarized light with a single e-vector that provided an axial cue. Ruin lizards were therefore subjected to

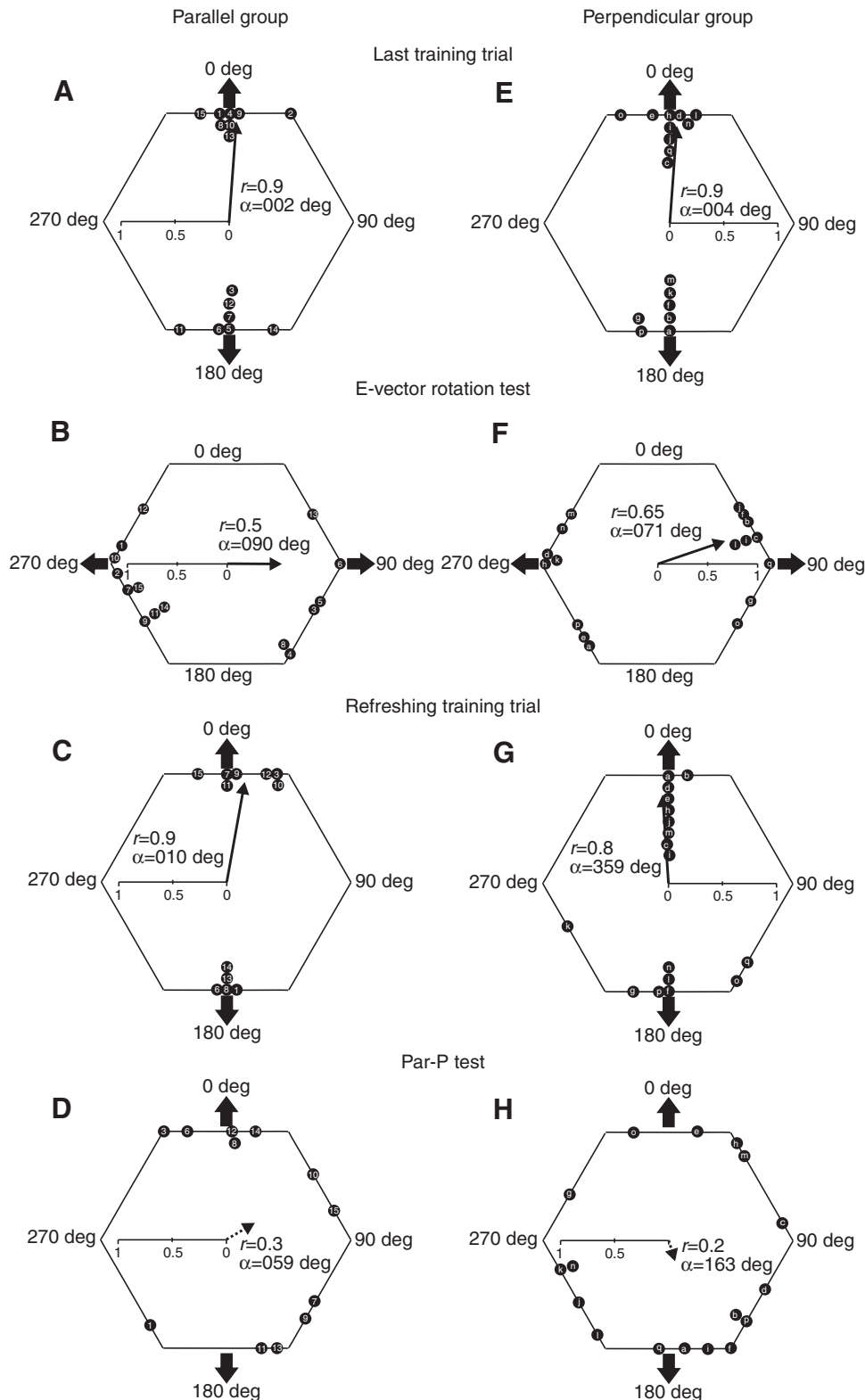


Fig. 2. Orientation behaviour of intact and Par-P lizards trained and tested under an artificial light source producing plane polarized light with a single e-vector. Each symbol indicates the directional choice of a single lizard identified by its number (Parallel) or letter (Perpendicular). In each hexagon the inner arrow represents the mean vector of the group calculated after doubling the angles. In each hexagon the mean vector length (r) and the mean direction (α) of the group are reported. Solid line mean vector: the bearings distribution deviated from uniform; dotted line mean vector: the bearings distribution did not deviate from uniform. For each hexagon, the two outer solid arrows in A, C, E and G mark the expected axis of orientation of intact lizards in training trials (0–180 deg), and in D and H the expected axis of orientation of Par-P lizards in test trials (0–180 deg). The two outer solid arrows in B and F mark the expected axis of orientation of intact lizards in test trials after 90 deg rotation of the e-vector (90–270 deg).

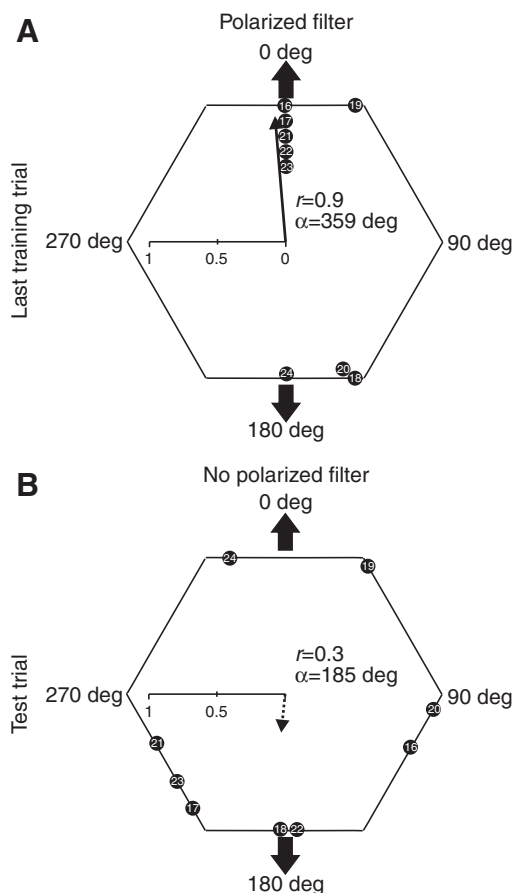


Fig. 3. Orientation behaviour of intact Parallel lizards trained under the plane polarized filter (A), and tested after removal of the filter (B). For each hexagon, the two outer black arrows mark the expected axis of orientation of lizards in training trials and the test trial (0–180 deg). Further details are given in Fig. 2.

axial training by positioning two identical goals in contact with the centre of two opposite side walls of our hexagonal Morris water maze (Fig. 1A,E). The directional choices of ruin lizards meeting the learning criteria showed a symmetrical, bimodal distribution along the 0–180 deg direction, thus indicating that lizards actually learned to orientate along the training axis (Fig. 2A,E). Several lines of evidence support the contention that lizards use the e-vector direction of polarized light for orientation: (1) following 90 deg rotation of the e-vector direction, lizard orientation rotated correspondingly, thus producing a symmetrical, bimodal distribution which was perpendicular to the training axis (Fig. 2B,F); (2) after the e-vector direction was rotated back 90 deg for the refreshing training, lizard orientation counter-rotated, thus lining up again with the training axis (Fig. 2C,G); (3) the 90 deg-rotated orientation was due to the 90 deg rotation of the e-vector, and not to changes in the pattern and intensity of illumination following filter rotation, since changes in illumination intensity with filter rotation were found to be negligible (Fig. 1B,C); (4) elimination of the polarizing filter produced complete disorientation in the lizards, showing that in the absence of plane polarized light no further cues remained available to them for orientation in the Morris water maze (Fig. 3B). Overall, the present results in *P. sicula* confirm those gathered in other species, such as *U. notata* and *T. rugosa*, showing that lizards are able to orientate using the e-vector direction of polarized light (Adler

and Phillips, 1985; Freake, 1999). Although these studies did not show specifically that lizards use a time-compensated sky polarization compass during natural orientation tasks, they do demonstrate unequivocally the existence in several lizard species of the sensory and cognitive mechanisms required for the functioning of a sky polarization compass. It is important to recall here that a celestial compass has been shown to be time compensated in several species of lizards, including *U. notata*, *S. jarrovi* and *P. sicula*, as their orientation under the sun was found to deviate as expected on the basis of the imposed (fast or slow) clock shift (Adler and Phillips, 1985; Ellis-Quinn and Simon, 1991; Foà et al., 2009).

The second step of the present investigation aimed at answering the still open question of whether the ability of lizards to orientate using the e-vector direction of polarized light is mediated by the parietal eye. For this purpose, ruin lizards which had learned to orientate along the training axis under polarized light were tested inside the Morris water maze under the same polarized light after their parietal eyes had been painted black. These experimental lizards were completely disoriented (Fig. 2D,H), and their behaviour was indistinguishable from the (disoriented) behaviour of lizards with their parietal eyes intact tested in the absence of plane polarized light (Fig. 3B). In other words, impairing the sensory organ (the parietal eye) had the same behavioural effects as eliminating the orientating cue (polarized light). The possibility that the disorientation observed after covering the parietal eye is due not to disruption of an orientation mechanism but to unspecific effects, such as reduced motivation or altered thermoregulation, seems unlikely, as has been pointed out previously (Ellis-Quinn and Simon, 1991; Freake, 2001). In our study, all lizards were kept at a constant temperature in each phase of the experiment, so that they could not thermoregulate at all. Taking all this into consideration, it is important to emphasize that the present results demonstrate for the first time that the parietal eye of lizards plays a central role in mediating functioning of a putative sky polarization compass.

Previous investigations showed a central role of the parietal eye in lizard orientation in the field under the sun (Ellis-Quinn and Simon, 1991; Freake, 2001). In both experiments lizards released on sunny days outside their home ranges were orientated at random when their parietal eyes were covered with a patch (*T. rugosa*) or painted black (*S. jarrovi*). A previous study in which we used the Morris water maze outdoors confirmed the central role of the parietal eye in lizard orientation under the sun (Foà et al., 2009). Since painting the parietal eye might prevent lizards from perceiving light not only by the parietal eye itself but also by the pineal and deep brain photoreceptors (Pasqualetti et al., 2003; Bertolucci and Foà, 2004), further experiments outdoors in the Morris water maze were done at that time to compare the orientation of ruin lizards whose parietal eyes were painted black with that of ruin lizards whose parietal eyes were completely ablated. As both experimental groups were randomly orientated in the Morris water maze, these results demonstrated for the first time that only the parietal eye, and not other brain photoreceptors, is involved in lizard orientation (Foà et al., 2009).

In the light of the present results, one is led to attribute disorientation of lizards tested under the sun with covered or ablated parietal eyes to the consequent loss of function of their sky polarization compass. However, this might not be the whole story. In fact there is some evidence that the lizard parietal eye might also mediate functioning of a sun azimuth compass. First of all, in all the experiments cited above lizards with covered or ablated parietal eyes were disoriented in spite of the fact that the sun disc was fully visible and their lateral eyes were completely

unobstructed. Furthermore, in our previous experiments outdoors, ruin lizards were tested at midday in summer in a Morris water maze surrounded by high fences (Foà et al., 2009). In such a situation ruin lizards were allowed to see a very limited patch of sky around the zenith, at a time of day (midday) when the sunlight directly above the parietal eye is mostly unpolarized (maximum polarization occurs at an angular distance of 90 deg from the sun, and thus near the horizon during midday). All this in our opinion strongly supports the contention that the parietal eye plays a central role in mediating not only a sky polarization compass but also an azimuth sun compass.

As stated by Wehner, there is no particular region of the spectrum predestined to be used preferentially by animals for detection of the e-vector of polarized light under all possible sky conditions [see p.110 of Wehner (Wehner, 1994)]. Several insects, such as the honeybee *Apis mellifera*, the desert ant *Cataglyphis bicolor* and scarab beetles *Lethrus* spp., perceive skylight polarization in the ultraviolet (UV) range, and the same is true in the damselfish *Chromis viridis* (Labhart, 1980; Duelli and Wehner, 1973; Frantsevich et al., 1977; Mussi et al., 2005). In the present experiments, ruin lizards were able to orientate by using the e-vector direction of polarized light, though such light did not include wavelengths in the UV range (Fig. 1D). Thus, the UV range is not necessary for perceiving polarized light in ruin lizards. This seems to be consistent with the results of electrophysiological studies carried out in the desert night lizard *Xantusia vigilis* and the common side-blotched lizard *Uta stansburiana*, showing maximal spectral sensitivity of their parietal eyes for green and blue lights (Solessio and Engbretson, 1993; Solessio and Engbretson, 1999; Su et al., 2006). The next step of the investigation in ruin lizards will be aimed at testing whether there is a preferential region of the spectrum for perception of the e-vector direction of polarized light used by the parietal eye of these lizards for compass orientation.

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