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Night-sky orientation with diurnal and nocturnal eyes: dim-light adaptations are critical when the moon is out of sight

Jochen Smolka[a,*], Emily Baird[a], Basil el Jundi[a], Therese Reber[a], Marcus J. Byrne[b], Marie Dacke[a,b]

[a] Department of Biology, Lund University, Sweden

[b] School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, South Africa

[*] Corresponding author:

Jochen Smolka, Department of Biology, Lund University, Biology Building, Sölvegatan 35, 223 62 Lund, Sweden. e-mail: jochen.smolka@biol.lu.se, phone: +46 46 2228097

Abstract

The visual systems of many animals feature energetically costly specialisations to enable them to function in dim light. It is often unclear, however, how large the behavioural benefit of these specialisations is, because a direct comparison in a behaviourally relevant task between closely related day- and night-active species is not usually possible. Here we compare the orientation performance of diurnal and nocturnal species of dung beetles attempting to roll dung balls along straight paths at both day and night. Using video tracking, we quantified the straightness of paths and the repeatability of roll bearings as beetles exited a flat arena in their natural habitat or under controlled conditions indoors. Both species oriented equally well when either the moon or an artificial point light source was available, but when the view of the moon was blocked and only wide-field cues such as the lunar polarisation pattern or the stars were available for orientation, nocturnal beetles were oriented substantially better. We found no evidence that ball-rolling speed changed with light level, which suggests little or no temporal summation in the visual system. Finally, we found that both diurnal and nocturnal beetles tend to choose bearings that lead them towards a bright light source, but away from a dim one. Our results show that even diurnal insects – at least those with superposition eyes – could orient by the light of the moon, but that dim-light adaptations are needed for precise orientation when the moon is not visible.

Keywords: dung beetle, insect, Milky Way, nocturnal adaptation, polarised moonlight, sky compass, straight-line orientation, vision

Introduction

Seeing at night is a challenging task. The skylight on a moonless night can be over one hundred million times dimmer than on a sunny day (Lythgoe, 1979). As light levels drop, fewer photons reach each photoreceptor, and the signal-to-noise ratio in the visual system eventually falls to a level where even objects or light sources that present a large relative contrast to the background can no longer be distinguished from it. Nevertheless, many animals, including small insects, are exclusively active at night and rely on vision to guide them in tasks such as locomotion, foraging, courtship and navigation (Warrant 2008; Warrant and Dacke, 2011). To deal with extremely low light intensities, nocturnal animals have developed visual systems with a wide range of anatomical and physiological adaptations. Insects living in dim light, for example, generally have compound eyes whose ommatidia have larger facet lenses of shorter focal length, as well as longer and wider rhabdoms, in order to increase the photon capture of each photoreceptor (Greiner et al., 2004a, 2007; Meyer-Rochow and Nilsson, 1999; Warrant 2008; Warrant and Dacke, 2011; Warrant and McIntyre, 1991). Many night-active insects also possess superposition compound eyes, where hundreds or thousands of facets contribute light to each photoreceptor instead of just one as in apposition eyes. Even in their sum, however, these optical adaptations rarely boost sensitivity by more than a factor of 1000, and they are therefore not sufficient to explain how some insects can deal with the eight orders of magnitude of light intensity variation between night and day. Various neural mechanisms, including a change of photoreceptor gain, as well as spatial and temporal summation of signals at different stages of the neural processing network, have been suggested as solutions to bridge this sensitivity gap (Frederiksen et al., 2008; Greiner et al., 2004b, 2005; Laughlin, 1981; Theobald et al., 2006; van Hateren 1993; Warrant, 1999). The fact that hornets, for example, can fly and forage at night without any obvious dim-light adaptations at the level of the optics of their compound eyes (Kelber et al., 2011) suggests that neural adaptations alone can provide a large enough sensitivity boost to allow an animal to extend its activity period to much dimmer light intensities.

Considering that large eyes are costly to develop and maintain, and that vision consumes a large proportion of an animal's energy budget (Laughlin et al., 1998; Moran et al., in press), the question arises as to how large an advantage neural and receptor adaptations confer on a nocturnal insect. And how many of the changes in neural processing could also be dynamically engaged in a non-specialised, diurnal eye if it was forced to work at night? Ideally, these questions should be answered by observing an exclusively diurnal species perform its natural behaviour at night. This experiment is possible in ball-rolling dung beetles due to their extremely robust straight-line orientation behaviour, which can be elicited under practically any circumstances – even at times when the species would never naturally be active on the soil surface – allowing us to get a direct comparison of a behaviourally relevant task in the animal's natural habitat.

After landing at a fresh dung pile, ball-rolling dung beetles separate a piece of dung and shape it into a ball. They then select a seemingly random bearing (Baird et al., 2010), and – with their head down, walking backwards – roll the ball away with their hind legs until they have found an expedient spot to bury themselves together with the ball, and consume it in solitude or lay an egg in it. In order to escape from the dung pile as quickly as possible, to avoid competition from other newly arrived beetles keen to steal a ball rather than make one themselves, the ball-rolling beetles move away in straight lines. Simple as this may sound, keeping a straight line is impossible without external "compass" cues (Cheung et al., 2007) – even for humans (Souman et al., 2009). For this compass, dung beetles use celestial cues exclusively. Ignoring even obvious landmarks, beetles lose their way when the sky is overcast or experimentally occluded (Dacke et al., 2013a). Within the sky, however, they use a large range of directional cues, including the azimuthal position of the sun or moon (Byrne et al., 2003; Dacke et al., 2004, 2014), the pattern of polarised light formed around these celestial bodies (Byrne et al., 2003; Dacke et al., 2003a, 2003b; el Jundi et al., 2014, 2015), the gradient of skylight intensity that stretches from the solar to the anti-solar hemisphere (el Jundi et al., 2014) and even the Milky Way (Dacke et al., 2013b). Astonishingly, the precision with which beetles orient to their familiar cues does not change over a very large range of light intensities (Dacke et al., 2011). Like other dim-light active insects, nocturnal dung beetles have a range of visual specialisations, which allow them to be active at night. Their superposition compound eyes (which all dung beetles possess) are enlarged compared to those of their diurnal cousins in all the expected parameters (Fig. 1) (Byrne and Dacke, 2011; Caveney and McIntyre, 1981; Dacke et al., 2003b; Frederiksen and Warrant, 2008; McIntyre and Caveney, 1998; Warrant and McIntyre, 1990), including an enlargement of the dorsal rim area (the region analysing polarised skylight) (Dacke et al., 2003b; Dacke, Smolka and Ribi, unpublished data), and often feature a tracheal tapetum, which reflects light back onto the photoreceptor and effectively doubles the light path (Warrant and McIntyre, 1991). Taken together, these optical specialisations can increase the sensitivity of a nocturnal beetle's eye by up to 85 times compared to that of a diurnal beetle (Frederiksen and Warrant, 2008; McIntyre and Caveney, 1998). Physiologically, some nocturnal dung beetles adapt their photoreceptors to dim light with a slower frequency response and higher gain (Frederiksen, 2008; Warrant and McIntyre, 1990). Taken together, these specialisations should give nocturnal beetles vastly superior light sensitivity compared to diurnal beetles.

Here, we compare the straight-line orientation behaviour of a diurnal and a closely related nocturnal species of South African ball-rolling dung beetle across a large range of light intensities. Our results suggest that orientation to even the smallest crescent moon does not require any dim-light adaptations, but that only nocturnal beetles can reliably orient to dim wide-field cues such as the lunar polarisation pattern or the Milky Way.

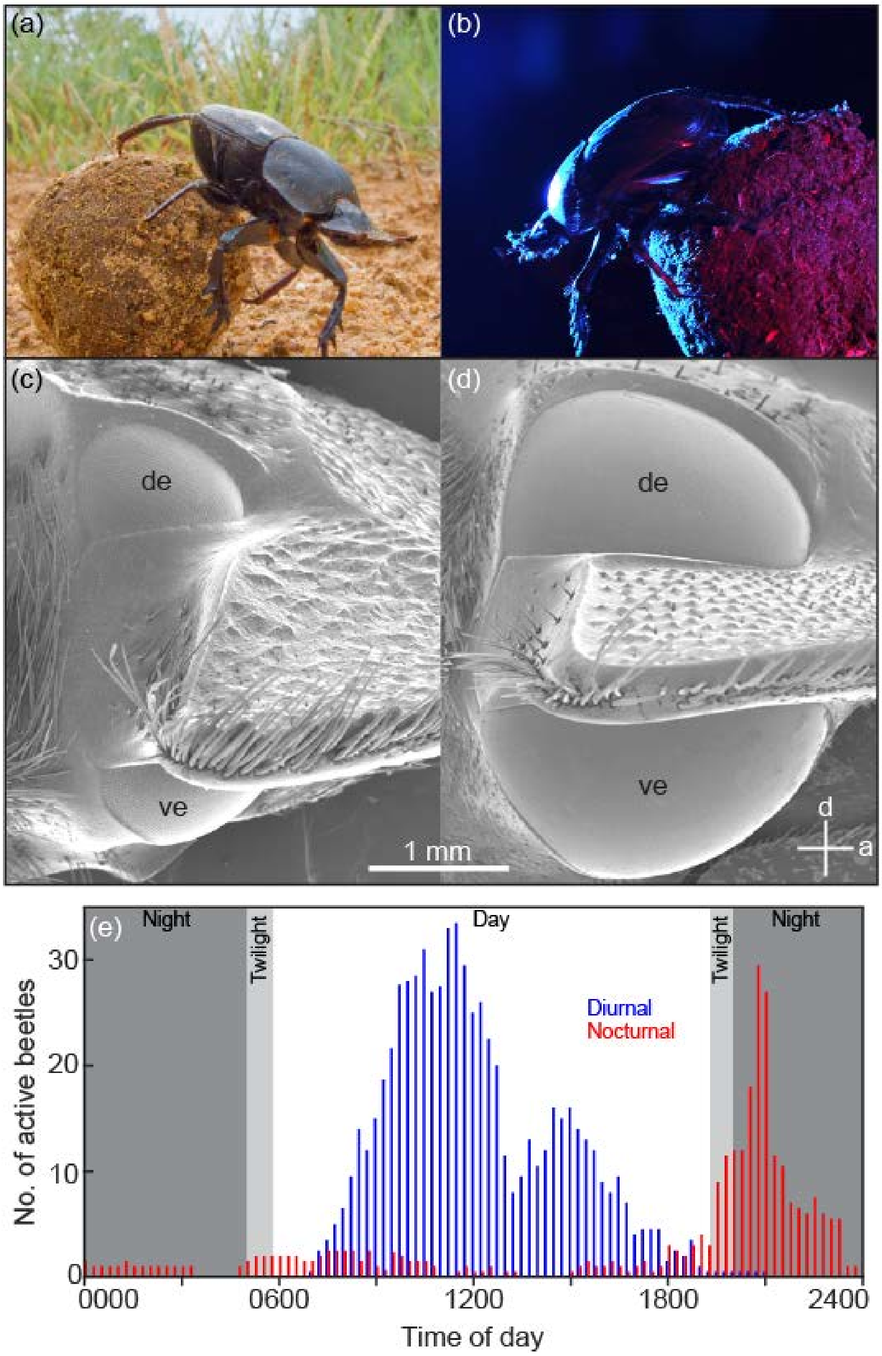

Figure 1: Comparison between study species. **(a, b)** To test what advantage a nocturnal eye design provides in dim light, we compared the orientation performance of the diurnal dung beetle *Scarabaeus lamarcki* (a) and that of the closely related nocturnal species *Scarabaeus satyrus* (b). **(c, d)** Lateral view of the head in scanning electron micrographs of the eyes of the diurnal (c) and the nocturnal species (d), showing that the eyes of the latter are substantially larger. The eyes of these two dung beetle species are split into a dorsal eye (de), which perceives most of the signals relevant for skylight orientation, and a ventral eye (ve), which is most likely involved in general visual processing and flight control. **(e)** Mean activity of 60 diurnal (blue) and 60 nocturnal (red) beetles over two 24-hour periods in sand-filled bins in their natural habitat. Beetles were observed every 15 minutes, and any beetle present at the surface was counted as active.

Materials and methods

Animals

All experiments were performed with the diurnal dung beetle species *Scarabaeus* (*Kheper*) *lamarcki* Macleay, 1821 (Coleoptera, Scarabaeidae) and the nocturnal species *Scarabaeus satyrus* Boheman, 1860. We captured the beetles using pit-fall traps in their natural habitat on the game farm "Stonehenge" in South Africa (24.3°E, 26.4°S). After collection, beetles were kept in plastic boxes (30 x 22 x 22 cm) in the shade, where they were provided with soil and fresh cow dung. Field experiments were performed in January and February 2010 and 2013, and January 2014. Laboratory experiments were performed at Lund University in March 2010, within six weeks of capture of the beetles. These animals were kept under a 12 hour light/dark cycle in a climate-controlled animal room, and fed with fresh cow or horse dung. Before field night-time or laboratory experiments, beetles were placed in a plastic container situated in a heated cool box, where they were provided with a thin layer of sand and some cow dung. For diel activity measurements, six plastic barrels (diameter 50 cm, height 60 cm) were filled with sand to a height of about 50 cm and placed in a shaded location, away from human-made light sources. Ten night-active and ten day-active beetles, as well as ten beetles of a crepuscular species (not reported here) were placed in each barrel and prevented from flying away by a fine mesh placed over the top of the barrel. We then recorded the number of active beetles of each species (i.e. beetles that were at the surface rather than dug down into the soil) every 15 minutes over a period of 48 hours. Beetles were not fed for the full 48 hours of activity measurements.

Behavioural experiments in the field

To test their orientation performance under different light conditions, we observed beetles rolling their balls in their natural habitat under seven different conditions: (1) during the day with a full view of the sky; (2) during a full moon night with a full view of the moon or (3) with the moon blocked by a wooden board; (4) during a crescent moon night with a full view of the moon or (5) with the moon blocked by a wooden board; (6) during a time when the moon was more than 18° below the horizon, and only starlight was available for celestial orientation, and (7) during the same moonless nights, but with a bright LED light (angular size <0.1°, 23 cd m^{-2} at arena centre) as an additional orientation cue. This last control condition was added to test whether the unusual time of day, temperature or other environmental factors were negatively affecting the ability of diurnal beetles to orient and roll their balls. For each condition, we individually placed between 10 and 21 beetles of each species (Table 2) onto a dung ball in the centre of a flat circular experimental arena, from where they rolled the ball towards the edge of the arena (3 m diameter, marked out on a flattened and levelled sandy patch of ground). The beetles' paths out of the arena were filmed from above (height: 3.1 m) with a camcorder (Sony HDR-HC5E or Samsung VP-HMX20C) fitted with a 0.42x wide-angle lens at 25 frames per second. In dim light, we filmed beetles with infrared illumination, which is invisible to the beetles, using the NightShot function of the Sony camcorder, and followed each beetle with an additional infrared LED light to provide sufficient illumination for observation. Indicator lights on the cameras were covered with several layers of black tape to prevent the beetles using them as additional orientation cues. Each individual beetle experienced both conditions on the crescent moon (conditions iv and v) and moonless nights (conditions vi and vii), with half the beetles being tested in the respective brighter condition first, and the other half tested in the dimmer condition first.

All field experiments were performed under a clear sky, with the dominant celestial body at low to medium elevations (15° – 53°) to provide an easy-to-read directional cue. All night experiments were performed after the end of astronomical evening twilight and before the beginning of morning twilight to ensure that the sun provided no polarisation pattern that could have been used for orientation (Cronin et al., 2006). Similarly, all experiments under moonless conditions were performed when the moon was more than 18° below the horizon to ensure that no lunar polarisation pattern was available as an orientation cue.

To test whether beetles were able to keep a constant bearing after a disturbance, we performed additional experiments in 2013 and 2014. We tested a total of 114 beetles repeatedly under full moon, full moon shade, starlight and artificial light conditions (defined as in previous experiments). We recorded their bearings to the nearest 5° as they exited the three-metre arena, and measured the bearing difference as the circular distance between the bearings taken in consecutive rolls. The exact timing of all experiments, the position of the dominant light source or celestial body, as well as the ambient light levels and temperatures can be found in Table A1; the relevant sample sizes for all field experiments are presented in Table A2.

Behavioural experiments in the laboratory

To test whether diurnal and nocturnal dung beetles differ in their ability to orient to a dim point light source, we also investigated the beetles' orientation behaviour on a wooden arena (1.6 metre diameter) in the laboratory in Sweden. The beetles’ paths were recorded in the same manner as in the field, from a height of 1.9 metres. Illumination for the bright light control condition was provided by a halogen spotlight (Dedolight Aspherics 2; Dedotec USA Inc., Ashley Falls, MA, USA, fitted with a 150W halogen lamp; 39,000 cd m^{-2} at arena centre) placed with the centre of its aperture at a height of 88 cm above the ground, 140 cm from the centre of the arena. The front aperture measured 70 mm, creating a light spot of 2.4° angular size as seen from the centre of the arena. Illumination for dim light test conditions was provided by a custom-made halogen lamp placed at a distance of 130 cm from the arena centre and a height of 62 cm above the ground. The 16.4 mm aperture created a light spot of

0.65° angular size as seen from the centre of the arena, and therefore appeared only slightly larger than the real moon (approximately 0.5°). The aperture was covered by a neutral density filter and a diffuser, which adjusted the maximum light intensity at the centre of the arena to approximately that of a full moon night (cf. Figs 2, 4). By adjusting the voltage to the lamp, we created a total of four light-intensity conditions (12V / 6V / 3V / 1.5V), which covered the range of naturally observed light intensities. As an additional control for non-visual cues, we tested the beetles with the power supply set to 0V, i.e. in complete darkness.

Experiments were performed during four experimental sessions – two days and two nights. On the first day, we tested five beetles of each species in each dim light condition. Each individual beetle was also tested with the bright control light, with approximately half the beetles experiencing this control condition directly before, and the other half directly after being tested in dim light. The same number of beetles were tested in the other three experimental sessions with the exception of the 12V and 6V conditions: As it became clear early on that the beetles' orientation performance under these relatively bright conditions was indistinguishable from the control, we dropped these conditions after the first and second session, respectively, leading to a total sample size (Table A4) of five and ten beetles per species for these conditions (with one additional sample being removed from the diurnal species in 6V due to technical problems). To test whether beetles were able to keep a constant bearing after a disturbance, we tested all beetles in the third and fourth experimental session three times in succession, and calculated the bearing differences between the first and second, as well as the second and third roll from the videos. The exact timing of all laboratory experiments, the position of the dominant light source, as well as the ambient light levels measured at the centre of the arena can be found in Table A3; the relevant sample sizes for all laboratory experiments are presented in Table A4.

Video analysis & track filtering

Videos were digitised, calibrated for perspective and optical distortion (Bouguet, 2010), and analysed at a sampling interval of 400 ms using custom-made tracking software (Smolka et al., 2012) in Matlab 2014b (The Mathworks Inc., Natwick MA, USA). The raw tracks were processed in five steps:

1) Start: The beetle’s initial activity inside a 40 cm diameter circle around the centre was ignored. This removed any activity due to the beetle’s initial maintenance of the ball, as well as its first dance and re-orientation on the ball (Baird et al., 2012).
2) Finish: The track ended when the beetle had either moved across the arena’s outer perimeter, or timed out when it had rolled its ball for two minutes after leaving the inner 40 cm diameter circle without exiting the arena. A total of 45 out of 535 beetles were timed out in this way (Tables A2, A4). In some cases, beetles were removed from the arena by the experimenter before they crossed the outer perimeter, e.g. to keep them from colliding with an obstacle. In these cases, the tracks were still included in the analysis as long as the beetles were no more than 10 cm away from and clearly moving towards the perimeter at the time of removal.
3) Gaps: Due to the sometimes less than ideal filming situation at night (under- or overexposed video images, visual obstructions, defocused camera images), a number of tracks had gaps where tracking was not possible. Any gaps that were shorter than four seconds were filled in by linear interpolation. Tracks with longer gaps were discarded for the analysis of tortuosities, but were still included in the analysis of rolling speeds and bearing choice. This was the case for a total of 26 out of 775 recorded tracks.
4) Pauses: A pause was defined as any segment of the path where a beetle moved no more than 1 cm in 2 s or no more than 2 cm in 4 s. For the calculation of tortuosity, pauses

were removed and replaced by a single point. All automatically detected pauses and gaps were thoroughly checked manually prior to further analysis.

5) Due to tracking noise and small sideways deviation of a beetle induced by an irregular ball shape, an unfiltered estimate of track length – or any related measures such as tortuosity, straightness or sinuosity – is dependent on the video frame rate and a beetle's rolling speed (Benhamou, 2004). To make the track length independent of how often the track was sampled, and therefore make the tracks comparable across species and across different studies, we developed a filtering algorithm designed to resample all tracks to a minimum segment length of 40 mm. In a first step to reduce the tracking error in segments of the path where the beetles moved slowly, tracks were smoothed by replacing each point with the mean of all points in a 20 mm circle around that point. In a second step, we resampled each track to a minimum segment length of 40 mm. The first point of this resampled track was the first tracked point outside the inner 40 cm circle. The algorithm then moved along the smoothed track point-by-point, and added a new point to the resampled track whenever the distance of the current point to the last added point reached or exceeded 40 mm.

Small changes in any of the above parameters do not have any major effects on our results or conclusions.

Each track was finally characterised using three characteristics:

1) The tortuosity of the track, which is the ratio between the total track length *L* and the straight distance between start and end point *D* (Fig. 2a). This measure was calculated from the final, filtered path.
2) The mean rolling speed of the beetle. For the calculation of this mean speed, only those segments of the path were included that neither started nor ended with a point that was part of a gap or pause.

3) The bearing of the recorded endpoint, which was calculated as the compass bearing of a straight line between the arena centre and the last point of the filtered track.

Light source characterisation

We determined luminances using an IL1700 photometer (International Light Technologies Inc., Peabody, MA, USA) by measuring the intensity of light reflected from a horizontally placed white sheet of paper. The sensor was placed at a vertical angle of 45° to the piece of paper, and a horizontal angle of 90° to the dominant light source. To calculate the beetles' bearings relative to the position of the dominant light source, the position of sun and moon were calculated in Matlab (Koblick, 2009a; Koblick, 2009b), and the azimuth corrected for the local magnetic declination. For experiments on moonless nights, the azimuth of the brightest part of the Milky Way was determined in Stellarium 0.12.4 (Stellarium Developers, www.stellarium.org).

Statistics

We performed all statistical analyses in Matlab using the in-built statistics toolbox and the CircStat toolbox (Berens, 2009), with the exception of Mardia-Watson-Wheeler tests, which were performed in Oriana (Kovach Computing Services, Anglesey, Wales), and linear model analyses, which were performed in R 3.1.2 (R Core Team, 2013). To compare the distributions of tortuosities or speeds between treatments (Figs 2-4, 6), we applied Wilcoxon rank-sum tests (Mann and Whitney, 1947; Wilcoxon, 1945) to compare medians, and Brown-Forsythe tests (Brown and Forsythe, 1974) to compare the spread. Both tests are robust to non-normally distributed data, which some of the tortuosity data clearly were. Since most of the unexpected results of this study are reflected by statistical tests failing to show a difference between two groups, we performed one-sided comparison tests throughout to increase the power of the tests. To test for an overall difference in orientation performance between species, and to check whether the time of day or the order of conditions had an influence on the beetles' ability to keep to a straight line or on the beetles' rolling speed, we calculated linear models predicting tortuosity and speed from species, condition, order of conditions and time of day. The final model was selected by sequentially fitting parameters of interest and including only those parameters that reached significance at a 5 per cent level when added to the final model.

To test whether directional data (changes of bearing, Fig. 5; initial bearing choices, Fig. 7) were distributed in a non-random fashion, we employed the *V*-test (Batschelet, 1981) with an expected direction of 0° or 180°, depending on the experiment. When comparing two such circular distributions, we used the Mardia-Watson-Wheeler test (Batschelet, 1981). To test whether re-orientation errors after a disturbance were dependent on the individual (Table A1), we calculated Pearson correlations between each individual's first and second error measurement. Similarly, we calculated Pearson correlations between an individual's first chosen bearing (relative to the light source) and its error angle to test whether orientation errors depends on the chosen bearing (Fig. A4).

Ethical note

In the field, we kept the beetles in boxes filled with soil from their natural habitat – deep enough to allow all beetles to bury themselves together with their dung balls – and regularly fed them with fresh cow dung. We stored the boxes in the shade, and changed the soil every few days. After experiments, we released all beetles that were not transported back to Sweden. In Sweden, boxes were filled with sand of a similar consistency to the animals' natural soil, and the beetles were fed with dung collected from Swedish dairy cows.

No animals were harmed during any of the behavioural experiments. Before laboratory experiments, we warmed up the room to approximately 30°C to reduce temperature-related stress. After the experiments, we returned the beetles to their holding boxes with fresh dung, and allowed them to rest for at least one full day before further experiments.

Results

Orientation with a full view of the sky

To test how much of an advantage a nocturnal eye design provides in dim light, we compared the orientation performance of the diurnal dung beetle *Scarabaeus lamarcki* (Fig. 1a) to that of the closely related nocturnal species *Scarabaeus satyrus* (Fig. 1b). The two species are of similar body size, but the eyes of the nocturnal species are substantially larger (Fig. 1c-d), probably an adaptation to the distinctly different activity times of the species (Fig. 1e).

In a first set of experiments, we tested the orientation performance of nocturnal and diurnal beetles under three conditions where they had a full view of the sky: (1) during a clear day, (2) on a full moon night, and (3) on a crescent moon night, 4-5 days before new moon (Fig. 2). These three conditions were compared against a control condition, in which we provided the beetles, at night, with a single point-source of bright artificial LED light of controlled intensity and elevation. Under each of these conditions, we let 10-20 beetles of each species individually roll a ball of dung out of a 3-metre diameter arena. As a measure of orientation performance, we calculated the tortuosity of each beetle's path. Tortuosity is defined as the ratio of the actual path length L to the straight-line distance between start and end point D (Fig. 2a); a tortuosity of 1.0 therefore indicates a perfectly straight path, whereas higher tortuosity values describe more and more curved and twisted paths. This rather straight-forward measure of orientation performance is also ecologically meaningful:

everything else being equal, a doubling of path tortuosity means that a beetle will require twice the time and expend twice the energy to reach a given distance from the dung pile.

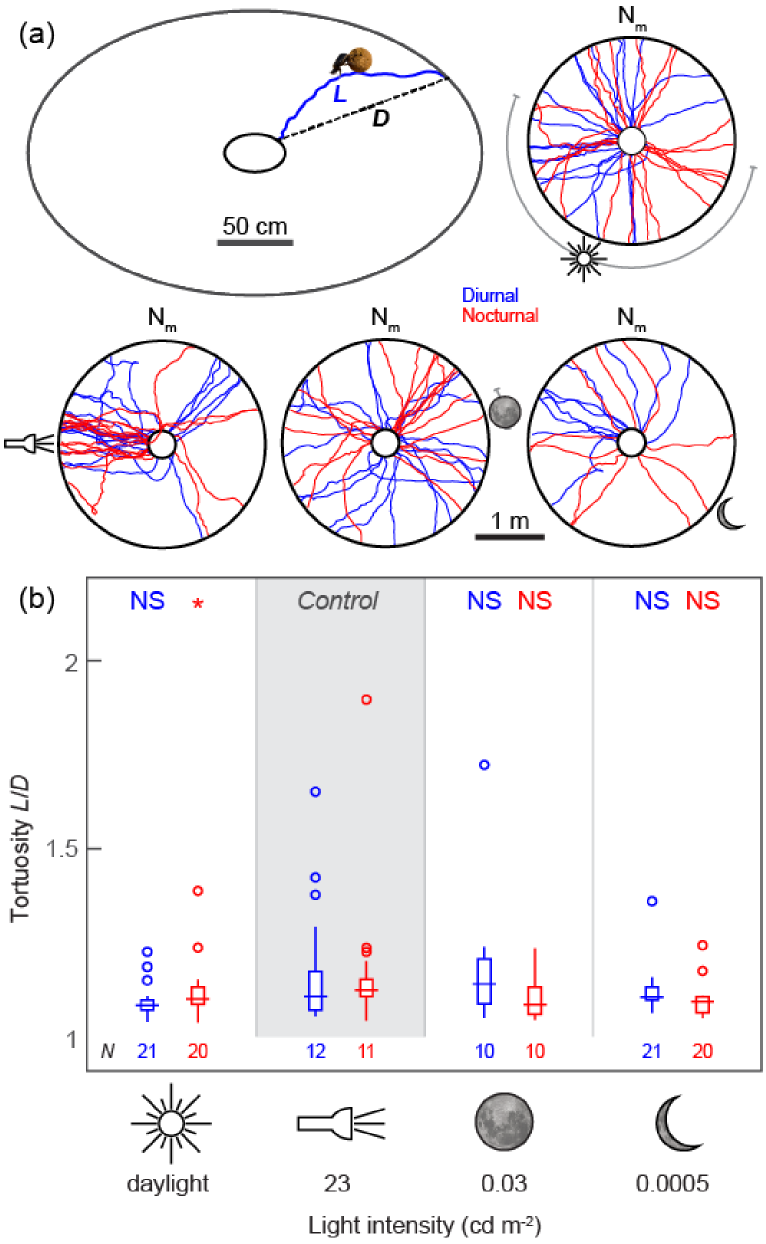

Figure 2: Nocturnal and diurnal beetles orient equally well to point light sources in their natural habitat. **(a, top left)** Beetles were placed in the centre of a 3-metre diameter arena in their natural South African habitat, and their paths recorded as they rolled their dung balls out of the arena. Initial movements (less than 20 cm away from centre) were ignored. As a measure of orientation performance, we calculated tortuosity, the ratio between the path length *L* and the straight-line distance *D* between path start and end. **(a)** Bird's eye view of the rolling paths of diurnal (blue) and nocturnal (red) dung beetles on a clear, sunny day (top right), with an artificial light at night (bottom left), on a full moon night (bottom centre) and on a crescent moon night (bottom right). See methods for details of path filtering. Top of each diagram is local magnetic North (N_m), symbols indicate the position of the light source (with

grey line marking the full range of light source positions during the experiments). **(b)** Tortuosity of rolling paths is not significantly different from the control (except for nocturnal beetles during the day, which are slightly better oriented than in the control). Colours and symbols as in (a). Box-plots show median, inter-quartile range and outliers (any points more than 1.5 inter-quartile ranges above the 75th percentile or below the 25th percentile). Text at the top indicates the results of one-sided Wilcoxon rank-sum tests comparing the tortuosities to the same-species control, numbers at the bottom indicate sample size (N). *: $P < 0.05$; NS: not significant.

Surprisingly, we found that both species performed equally well in all four conditions, and that diurnal beetles oriented as well as nocturnal beetles during day and night (see Fig. 2, results of specific comparisons in Table 1 and linear model analysis in Table 2). While there was a trend towards lower tortuosities during daytime, the difference in medians was only 2 percentage points (diurnal: 1.09 in sunlight, 1.11 in night-time control; nocturnal: 1.10 in sunlight, 1.12 in night-time control). This slight difference in orientation performance could either be related to less favourable environmental conditions at night, or (less likely) to the presence of two conflicting visual cues in the control condition – the bright LED light, and the much dimmer pattern of stars. We also tested whether the *spread* of tortuosities changed with different lighting conditions, which (in the absence of a change in the median) might indicate a minority of beetles getting more and more disoriented. We found no such difference in spread between the four light conditions for either the diurnal (multi-sample Brown-Forsythe test, $F_{3,67} = 1.6$, $P = 0.21$) or the nocturnal beetles ($F_{3,66} = 0.44$, $P = 0.72$). This further confirms that diurnal and nocturnal dung beetles orient equally well as long as they have a full view of the sky and at least a crescent moon to orient by.

Orientation to polarised light pattern and starry sky

Even when neither the sun nor the moon itself is visible, dung beetles can still orient using wide-field celestial cues, such as the pattern of polarised skylight surrounding the sun or moon (Dacke et al., 2003; el Jundi et al., 2014), gradients of intensity across the sky (el Jundi et al., 2014) or the Milky Way (Dacke et al., 2013b). To test whether nocturnal beetles are better than diurnal beetles at keeping a straight line when the moon is hidden from sight, we compared the orientation performance of both species under three conditions where no major celestial body was visible: (1) on a full moon night with the moon shaded from view by a large wooden board, (2) on a crescent moon night with the moon similarly shaded, and (3) on a moonless night, illuminated only by the light of the stars.

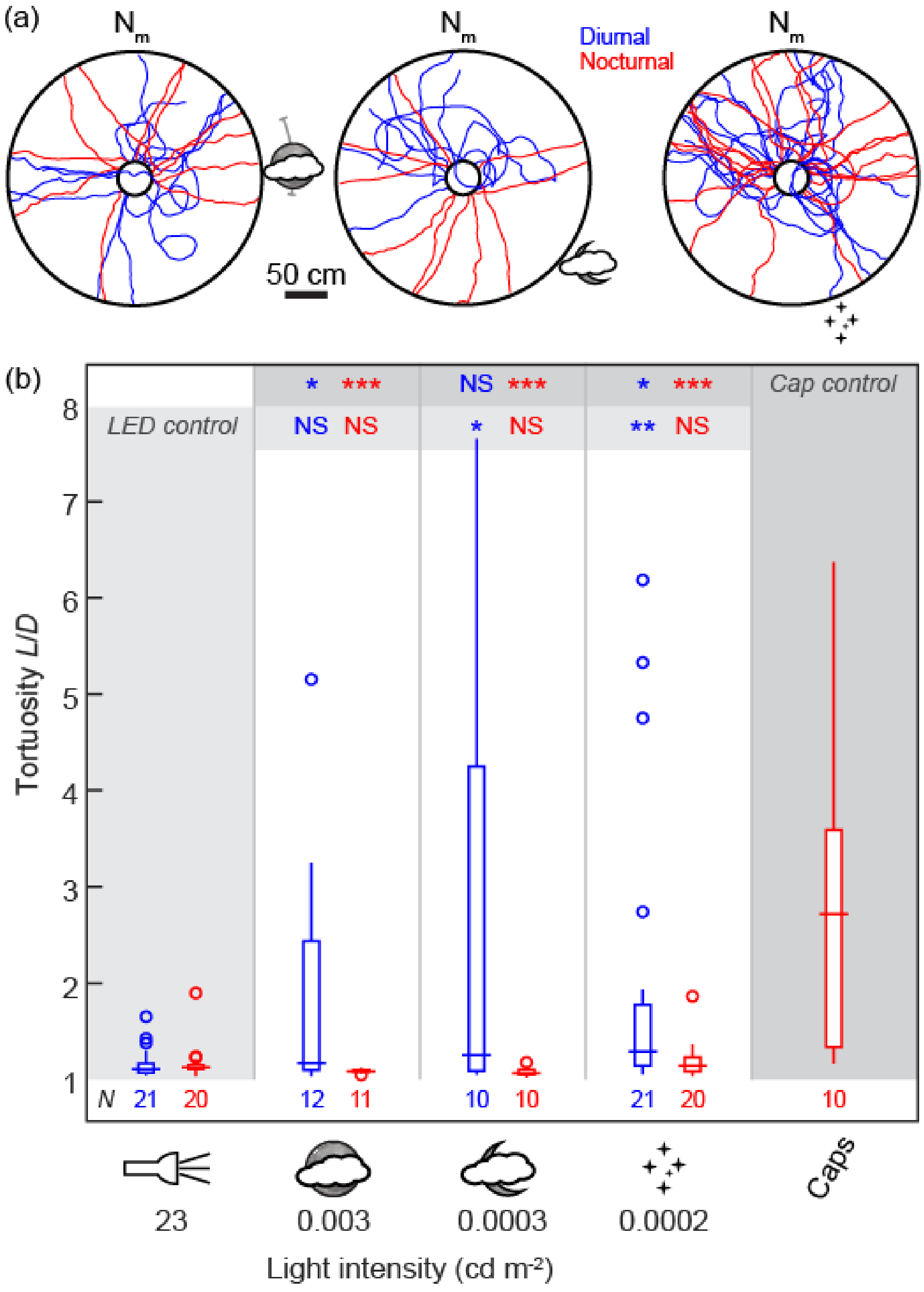

Figure 3: Nocturnal beetles orient equally well to wide-field cues as to point light sources, but diurnal beetles do not. **(a)** Bird's eye view of the rolling paths of diurnal (blue) and nocturnal (red) dung beetles when placed in a 3-metre diameter arena in their natural habitat. Tests were performed under three conditions where only wide-field celestial cues (polarised light pattern / starlight) were available as orientation cues: on a full moon night with the moon shaded by a wooden board (left), on a crescent moon night with the moon similarly shaded (centre), and on a night with only the light of the stars to guide the beetles (right). See methods for details of path filtering. Top of each diagram is local magnetic North (N_m), symbols indicate the position of the dominant light source (grey line: range of light source positions). **(b)** Tortuosity of rolling paths. For nocturnal beetles, the tortuosity was not significantly different from the LED control (paths in Fig. 2a) in any of the three wide-field conditions. For diurnal beetles, paths in two of the three conditions were significantly less well-oriented than the control (results of one-sided Wilcoxon rank-sum tests in lightly shaded area) and the spread of tortuosities in all three conditions was significantly larger than in the control (see text for statistics). However, path tortuosities under almost all conditions for both species were significantly lower than in truly lost beetles – nocturnal beetles with their view of the sky occluded by a cardboard cap (data from Dacke et al., 2013b, re-analysed; results of one-sided Wilcoxon rank-sum tests comparing with this cap-control can be found in the dark shaded area), indicating that the majority of diurnal beetles were still well-oriented. Box-plots show median, inter-quartile range and outliers (any points more than 1.5 inter-quartile ranges above the 75th percentile or below the 25th percentile). Numbers at the bottom indicate sample size (*N*), colours and symbols as in (a). ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; NS: not significant.

Under these conditions, dimmer and arguably more difficult than under even the smallest moon, diurnal and nocturnal beetles no longer oriented equally well (Fig. 3; linear model in Table 2). The paths of nocturnal beetles, on the one hand, were not significantly more tortuous than in the bright light control (Fig. 3b, red bars; Table 1), and the spread of the data did not change (multi-sample Brown-Forsythe test, $F_{3,57} = 1.6$, $P = 0.19$), indicating that these beetles orient equally well to wide-field cues, such as the lunar polarisation pattern or the Milky Way, as they do to when a point light source such as the moon is also available. The paths of diurnal beetles, on the other hand, became substantially less straight once the moon was not visible (Fig. 3b, blue bars; Table 1). While the median tortuosity was only significantly larger than in the control in two of the three wide-field conditions, the spread of tortuosities increased significantly – by up to 30-fold – in all three (Brown-Forsythe tests: full moon shade: $F_{1,31} = 6.4$, $P = 0.017$; crescent moon shade: $F_{1,29} = 9.4$, $P = 0.0047$; stars: $F_{1,40} = 5.1$, $P = 0.029$).

Taken together, these results show that, without a dominant point light source as an orientation cue, diurnal dung beetles cannot use the available wide-field cues (polarised light and stars) as effectively as nocturnal beetles can. However, it is interesting to note that even in the dimmest light (under starlight), the paths of diurnal beetles were, on average, only a moderate 17 per cent longer than in their control condition (median tortuosity of 1.29 and 1.11, respectively), indicating that even under these extreme conditions a large proportion of diurnal beetles were still able to keep a straight path. This fact becomes even clearer when we compare the diurnal beetles' paths to those of nocturnal beetles whose view of a starlit sky was occluded by a cardboard cap fixed to their head (data from Dacke et al., 2013b, re-analysed to match the two-minute time-out and track filtering used in this study; Fig. 3b, right-most red bar). The paths of these truly lost beetles have a median tortuosity of 2.72, which is significantly larger than that of the diurnal beetles' paths under almost all conditions (Fig. 3b, upper row of significance markers; Table 1). Only under a shaded crescent moon were the diurnal beetles as poorly oriented as the capped nocturnal beetles. In summary, the orientation performance of nocturnal beetles is not affected at night when the moon is not directly visible. Diurnal beetles, on the other hand, are worse at orienting to wide-field orientation cues (polarisation pattern and stars) than nocturnal beetles, but significantly better than could be expected if they were not using these cues at all.

Orientation to a simulated celestial body

The fact that diurnal and nocturnal beetles orient equally well under an open sky at night (Fig. 2) but not when the moon is shaded (Fig. 3) suggests that the two species are equally good at measuring the moon's azimuth for orientation. To test this hypothesis, and to investigate what happens to their orientation at intensities lower than that of the crescent moon, we tested both species indoors, with only a dimmable point light source for orientation.

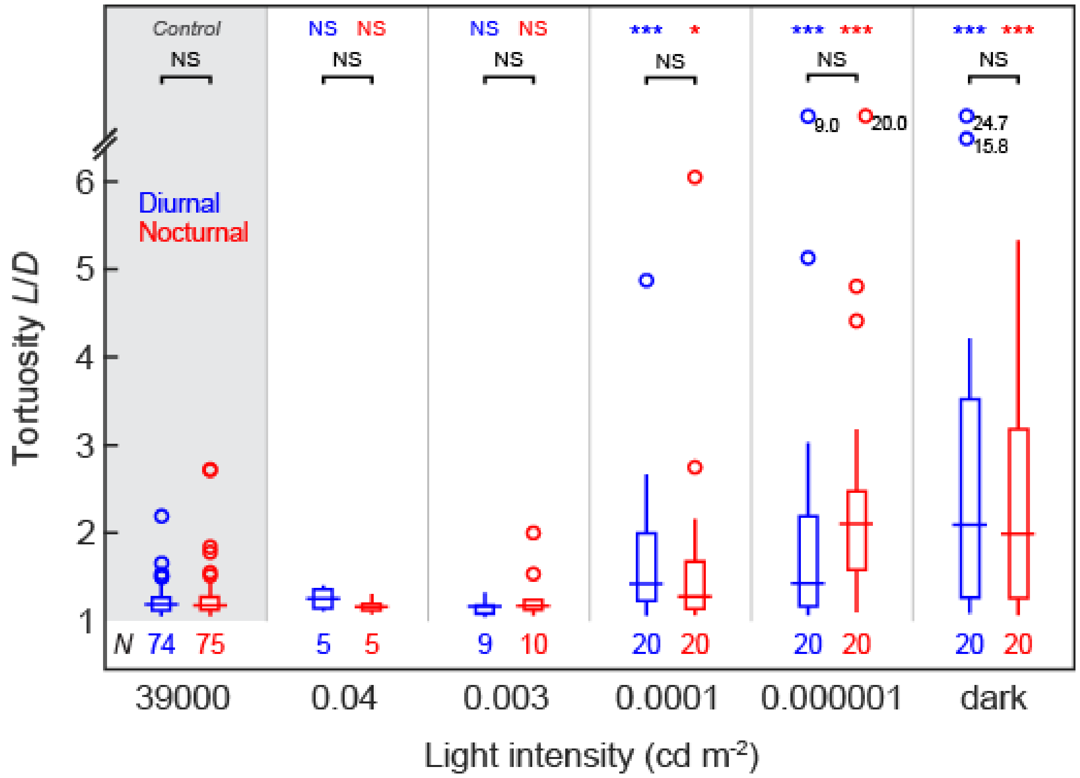

Figure 4: Nocturnal and diurnal beetles orient equally well to point light sources in the laboratory. Tortuosity of rolling paths of diurnal (blue) and nocturnal (red) dung beetles when placed in a 1.6-metre diameter arena in the laboratory (paths in Fig. A1). Tests were performed with a bright spotlight (control) or a dimmable tungsten lamp at a range of different light intensities covering and exceeding the range of light intensities measured under natural nocturnal conditions. As light intensity decreased, tortuosities started increasing significantly in both species (compared to the respective controls). This increase in tortuosity occurs at 0.0001 cd m^{-2}, a light intensity slightly lower than the lowest intensity measured in the field, on a moonless night (blue/red significance indicators reflect the results of one-sided Wilcoxon rank-sum tests comparing tortuosities to the relevant same-species control). However, tortuosities were never significantly different between secies at the same light intensity (black significance indicators). Box-plots show median, inter-quartile range and outliers (any points more than 1.5 inter-quartile ranges above the 75th percentile or below the 25th percentile). Four outliers are presented at a tortuosity of ca. 7 with their actual values indicated beside them. Numbers at the bottom indicate sample size (*N*). *: $P < 0.05$; NS: not significant.

The results (Fig. 4; paths in Fig. A1) confirmed what we found in the field: Diurnal beetles and nocturnal beetles oriented equally well under all conditions (pairwise comparisons in Table 1; linear model in Table 2). Both species' paths became more tortuous as we decreased the light intensity, but they did so equally (i.e. there was an effect of light intensity, but no interaction between light intensity and species). At the third test intensity (0.0001 cd m^{-2}; five times dimmer than the crescent moon, and 8.5 orders of magnitude dimmer than the control; Fig. 4, fourth column), both species performed significantly worse than in the control condition (Table 1). The same was true for the fourth test intensity (0.000001 cd m^{-2}; 500 times dimmer than crescent moon; Fig. 4, fifth column) and in complete darkness (Fig. 5, right-most column). Using linear models, we also explored other factors that could have influenced the beetles' orientation performance (Table 2): We performed approximately half of the experiments at night, and half during the day, but this did not affect orientation performance. Similarly, orientation performance did not depend on whether the bright light control was presented to the animal as the first or second stimulus condition.

Together, the results of our laboratory experiments confirm that diurnal and nocturnal dung beetles – despite their differences in diel activity and visual adaptations – orient equally well in the presence of a point light source over a large range of light intensities.

Bearing fidelity

Moving in a straight line is impossible without some kind of external compass cue (Cheung et al., 2007; Souman et al., 2009). However, on a small scale, an animal could walk in a reasonably straight line by simply keeping the image on its retinae constant and correcting for any perceived rotations, a behaviour known as the optomotor response (review: Wehner, 1981). If such optomotor cues were used exclusively, however, it would be exceedingly difficult to regain a chosen bearing after a major disturbance, e.g. if a beetle falls off its dung ball.

To test how well beetles could regain their rolling bearing at night, and whether the orientation mechanisms differ between diurnal and nocturnal beetles in dim light, we allowed a new group of beetles to roll their balls out of the arena three times, and calculated the change in bearing between consecutive pairs of rolls as a measure of bearing fidelity. We performed this experiment at night (1) with an artificial light, (2) under a full moon, (3) under a shaded full moon and (4) under a moonless, starry sky. In all four conditions, the change in bearing for both diurnal and nocturnal beetles was significantly clustered around 0°, indicating that beetles re-oriented towards their initial bearing even after the disturbance of being removed from their ball and re-positioned at the arena centre (Fig. 5a; see Table 3 for *V*-test results). The distribution of bearing changes was not significantly different between diurnal and nocturnal beetles in the presence of an artificial light or under a full moon (see Mardia-Watson-Wheeler tests in Table 4), which is consistent with the finding that path tortuosities under these conditions are similar (Fig. 2b). Under a shaded full moon and a starry sky, however, the distributions of bearing fidelity were significantly different. A significant difference in the Mardia-Watson-Wheeler test can be due to a difference in means or in spread. Since the mean vectors of all compared groups are very similar, these significant differences probably reflect the larger spread for diurnal beetles (circular standard deviation is 108 per cent and 60 per cent larger in diurnal beetles, respectively), which indicates that diurnal beetles were regaining their original direction with lower precision than nocturnal beetles. To account for the use of repeated measures (each beetle contributed two bearing changes), we performed a permutation analysis, which confirmed the results of all *V*- tests (Fig. A2a).

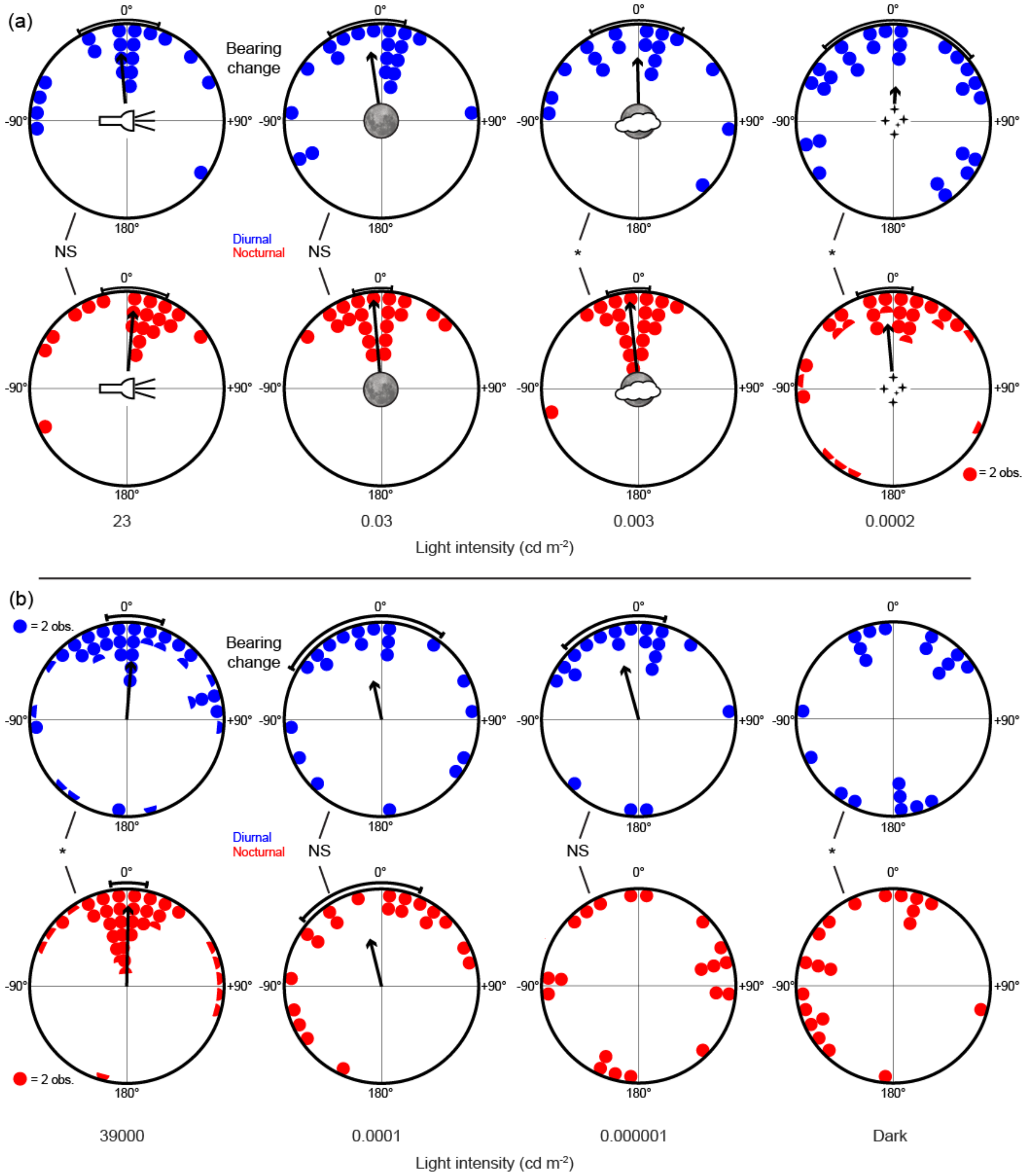

Figure 5: Both diurnal and nocturnal beetles can re-acquire their chosen bearing after a disturbance. **(a)** Beetles were placed in the centre of a 3-metre outdoor arena and allowed to roll their balls to the perimeter three times. Experiments were performed with diurnal (blue) and nocturnal (red) beetles at night, with illumination from (from left to right) (1) an artificial light, (2) the full moon, (3) the shaded full moon or (4) only starlight. The difference between consecutive bearing choices was calculated, and was significantly clustered around 0° in all cases (*V*-test with an expected mean of 0°), indicating that beetles did not just use optomotor cues to keep to a straight line, but could re-acquire their former bearing even after a significant disturbance. Black arrows show the direction and length (from the centre) of the circular mean vector, black lines indicate the 95% confidence interval around the circular

mean. The spread of bearing changes is equal for both species with an artificial light or a full moon, but is larger for diurnal beetles under a shaded full moon or the starry sky (black significance indicators reflect the results of Mardia-Watson-Wheeler tests comparing between species), reflecting decreased orientation performance for diurnal beetles under these conditions (Fig. 3). For clarity, the bottom right diagram only displays one dot for each two observations; half-dots indicate single observations. *: $P < 0.05$; NS: not significant. **(b)** Bearing changes after being returned to the arena centre for diurnal and nocturnal beetles in the laboratory. The highest light intensity at which beetles did not significantly regain their chosen direction was 0.000001 cd m^{-2} (500 times dimmer than crescent moon light). For clarity, the two left-most diagrams only display one dot for each two observations; half-dots indicate single observations. Statistical tests, half-dots and significance indicators as in (a).

In their natural habitat, neither diurnal nor nocturnal beetles therefore appear to exclusively use optomotor cues for orientation. To test whether this was also true in the indoor arena, we performed an analogous analysis using a subset of beetles that had been allowed to roll three times in succession during the original experiments (Fig. 5b; see Table 3 for *V*-test results). The results show that both species were able to regain their original bearing in the bright light control (Fig. 5b, 1st column) as well as with a point light source that was five times dimmer than the crescent moon (Fig. 5b, 2nd column). The same was true for diurnal, but not for nocturnal beetles in the dimmest condition (Fig. 5b, 3rd column). Not unexpectedly, the bearing changes were not significantly clustered around 0° for either species in the dark (Fig. 5b, 4th column). A comparison between species (Table 4) showed a significant difference between the distributions of bearing changes in the bright light control, reflecting the fact that in the bright light, nocturnal beetles were better at regaining their original bearing than diurnal beetles (circular standard deviation of 35° and 58°, respectively). In the two test conditions, there was no difference between species, while in complete darkness, a significant difference was found. Furthermore, while a permutation analysis (Fig. A2b) confirmed almost all *V*-test results, it indicated a significant orientation for nocturnal beetles in the dark ($P = 0.048$), hinting at the possibility that some nocturnal beetles might still have been able to regain their original bearing using non-visual orientation cues. However, the high tortuosity of their paths (Fig. 4) and the large spread of bearing changes (Fig. 5b, bottom right) suggest that this might have been a chance result.

The large spread that we observed in both tortuosities (Figs 3-4) and bearing changes (Fig. 5) in groups of poorly oriented beetles could result from two sources: (1) From variation across trials, due to the random nature of cumulative orientation errors (Cheung et al., 2007). An animal without a functioning compass will accumulate small angular errors with each step, which might lead to a very curvy path (high tortuosity), but due to the random nature of the error the same animal might also, by chance, walk in an almost straight line (low tortuosity). (2) The large spread could stem from differences between individual beetles, i.e. some beetles might be consistently well-oriented, while others are consistently lost. To investigate this, we analysed the correlation between the first and second measurement of bearing change (=orientation error) of each individual beetle for all conditions shown in Fig. 5. A significant correlation indicates a condition under which some beetles were reproducibly lost while others were reproducibly well-oriented. The analysis (Table A5) shows a significant correlation in only one condition (diurnal beetles under shaded full moon), which interestingly is also the only condition where we found a significantly increased spread of tortuosities, but not an increased median tortuosity. Together, these results indicate that in this condition there were some beetles that were truly lost, while others were still perfectly oriented.

In summary, whenever beetles rolled in straight paths, they were also able to regain their bearing after a disturbance, indicating that optomotor cues do not play a dominant role in their orientation behaviour. The overall pattern of the errors made in this re-orientation matches the pattern of path tortuosities: diurnal beetles perform as well as nocturnal beetles as long as a point light source is available for orientation, but fare worse when only wide-field celestial cues such as the polarisation pattern or the stars are available.

Beetles do not reduce their rolling speed in dimmer light

As light levels drop, fewer photons arrive at each photoreceptor in an animal's eye, leading to a less reliable visual signal. One way for the nervous system to deal with this problem dynamically is to integrate photons over a longer period, as a photographer would increase their camera's shutter time to allow more time for light to enter. But, to avoid excessive blurring of the retinal image, the animal consequently has to move more slowly. This strategy, known as temporal summation or integration, can be observed, for example, in hornets (Spiewok and Schmolz, 2006), honeybees (Rose and Menzel, 1981), bumblebees (Reber et al., 2015) and spiders (Nørgaard et al., 2008), but interestingly not in tropical sweat bees, which fly at extremely low light intensities under the canopy of tropical rain forests (Theobald et al., 2007). In the context of navigation, a similar behaviour can be observed in Australian bull ants, which walk more slowly and pause for longer periods during twilight and night than they do during the day (Narendra et al., 2013). To find out whether dung beetles, too, might improve the reliability of their visual system by moving more slowly in dimmer light, we analysed rolling speed as a function of light intensity in our field and laboratory experiments.

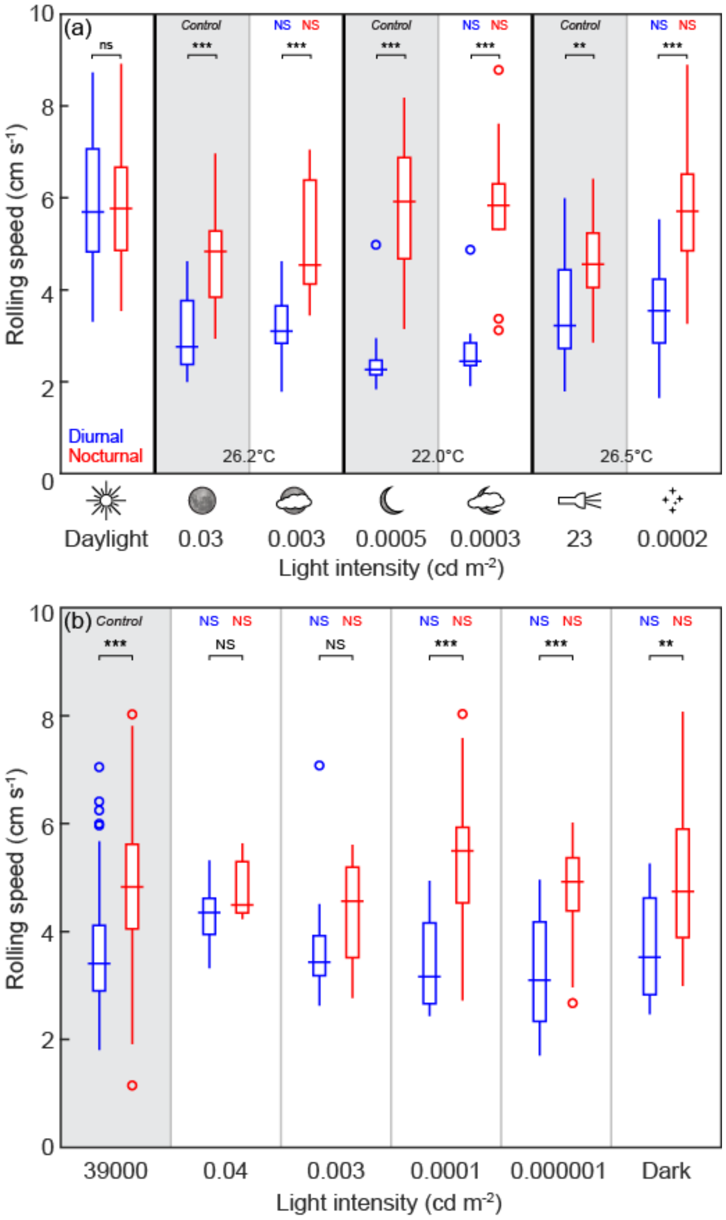

Figure 6: Rolling speed is not related to light intensity. For each roll path (data from Figs 2 & 3), the average movement speed (excluding pauses) was calculated. **(a)** Rolling speeds during field experiments. During the day (first column), diurnal (blue) and nocturnal (red) beetles rolled at the same speed. In all 6 conditions at night, nocturnal beetles rolled significantly faster than diurnal beetles (black significance markers indicate result of one-sided Wilcoxon rank-sum tests), but neither species rolled more slowly in dim-light conditions when compared to appropriate controls (blue/red significance markers; see text for details), indicating that the slower rolling speed at night was related to body temperature (or other environmental factors), not to light intensity. This, in turn, indicates that beetles do not use temporal integration in their visual or nervous system to deal with lower light intensities. Box-plots show median, inter-quartile range and outliers (any points more than 1.5 inter-quartile ranges above the 75th percentile or below the 25th percentile). **(b)** Rolling speeds during laboratory experiments. Calculation, colours and statistics as in (a). While nocturnal beetles rolled faster than diurnal ones in most conditions (and overall; see text for details of a linear model), there was no indication of lower rolling speeds at lower light intensities. ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; NS: not significant.

To rule out an influence of temperature (Heatwole, 1996), we only compared field experiments that were conducted at the same time and therefore under similar environmental conditions. We found no evidence that beetles decrease their rolling speed when light levels decrease or when fewer celestial cues are available (Fig. 6; Table 5). Specifically, neither species rolled more slowly under a shaded compared to an unshaded full moon, under a shaded compared to an unshaded crescent moon, or even under the dim starry sky compared to a bright LED light (Table 5). In each of these night-time experiments, however, nocturnal beetles rolled significantly faster than diurnal beetles, whereas we found no speed difference between diurnal and nocturnal beetles rolling their balls during the day. This suggests that diurnal beetles roll more slowly at night not because of lower light levels, but due to other factors such as lower night-time temperatures, which these beetles have not evolved physiological mechanisms to cope with. While our experiments were not designed to test this hypothesis, the fact that diurnal beetles rolled more slowly on experimental days with lower ground temperatures (Fig. 6a), supports this conclusion.

In the laboratory, we found once again that nocturnal beetles rolled on average 36 per cent faster than diurnal beetles (4.9 cm/s vs. 3.6 cm/s), but there was no indication that speed depended on light intensity or that the speed difference between species depended on light intensity (see linear model analysis in Table 6 and pairwise comparisons in Table 5). This speed difference between species, like that found outdoors, is probably due to different optimal body temperature between species, because the nocturnal beetles' physiology appears to be more suited to the comparatively cool laboratory temperatures. Taken together, our results show no evidence that dung beetles use temporal integration, in the same way as navigating ants do, to provide a more reliable visual signal in dim light.

Bearing selection is biased and depends on light level

When they start rolling a newly made ball away from the dung pile, diurnal dung beetles select an apparently random bearing (Baird et al., 2010), but environmental factors, such as the position of the sun, seem to introduce a small population bias under some circumstances (Baird et al., 2010; Byrne et al., 2003; Reber, 2012). To test whether the bearing choice of dung beetles at night is similarly biased, whether this bias differs between diurnal and nocturnal beetles and whether this effect is influenced by light levels, we analysed the bearing choices relative to the azimuth of the dominant light source in all our field and laboratory experiments. For experiments where the moon was shaded, we still counted the moon as the dominant light source, because its position in the sky could still be inferred from the celestial gradients of colour and intensity or the lunar polarization pattern. For moonless nights, we defined the dominant light source as the position of the brightest spot of the Milky Way (near the constellation Crux and the Southern Pleiades: Fig. A3).

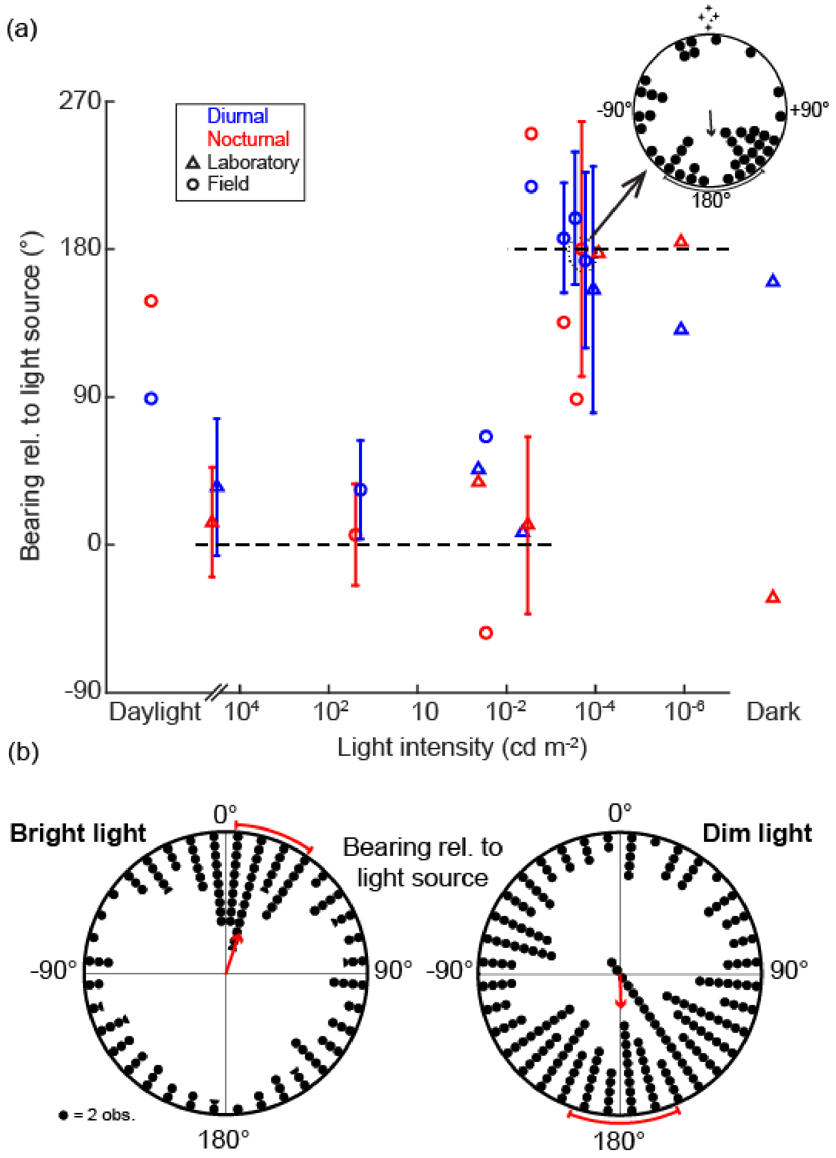

Figure 7: Beetles tend to roll towards a bright light source, but away from a dim one. **(a)** Mean bearing relative to the light source for all experiments (Figs 2-4) as a function of light intensity. Markers are circular means for diurnal (blue) and nocturnal (red) beetles, in laboratory (triangles) and field (circles) experiments. Error bars show the 95% confidence interval around the mean for those data sets that are significantly directed (*V*-test with an expected direction of 0° or 180°). As indicated by the dashed lines, beetles tend to choose a bearing of 0° (i.e. towards the light source) at high light intensities, and of 180° (i.e. away from the light source) at lower light intensities. Inset shows the combined data from diurnal and nocturnal beetles under the starry sky, where 0° indicates the brightest visible part of the Milky Way (near the Southern Pleiades and the constellation of Crux). The fact that beetles were significantly oriented towards 180° (*V*-test, $P < 0.001$, V = 14.24) indicates that, rather than using the whole band of the Milky Way, they might be using its brightest part like a broad point light source to orient by. **(b)** Circular histograms of all bearings relative to the light source in bright light (left) and dim light (right). Range of data included in each diagram is indicated by the dashed lines in (a). Red arrows show the direction and length of the mean vector, red lines indicate the 95% confidence interval around the circular mean. For clarity, the left diagram only displays one dot for each two observations; half-dots indicate single observations.

When we compared the circular mean of the beetles' chosen bearings across all experiments, a clear pattern emerged (Fig. 7a): At higher light intensities, beetles tended to roll towards the light source, whereas at lower light intensities, they tended to roll away from it. To analyse this bias in bearing selection statistically, we combined the data from all experiments (except those in sunlight and those in complete darkness), and split them into a bright light (light intensities greater than full moon shade; Fig. 7b, left diagram) and a dim light group (light intensities less than or equal to full moon shade; Fig. 7b, right diagram). The results confirmed that the bearings chosen in bright light were significantly clustered with a mean of 19.0° (Rayleigh-test, $Z = 22.78$, $N = 257$, $P < 0.0001$), which would take the beetle towards the light source. The bearings chosen in dim light were also significantly clustered (Rayleigh-test, $Z = 12.01$, $N = 196$, $P < 0.0001$), but with a mean of 178.2°, which is almost directly away from the light source. A comparison between distributions confirmed that they are significantly different at different light levels (Circular two-way ANOVA, Harrison and Kanji, 1988; $\chi^2_2 = 64.4$, $P < 0.0001$), but there is no evidence that they are different between the two species ($\chi^2_2 = 5.8$, $P = 0.055$) or that different light levels affected the bearing choice of diurnal and nocturnal beetles differently (interaction:$\chi^2_1 = 1.4$, $P = 0.83$). It should be noted that both distributions are comparatively broad (circular standard deviations of 89° and 96° for bright and dim light, respectively), suggesting that even under dim light conditions, beetles do not simply perform a phototactic behaviour. Instead, they choose from the full range of possible bearings, but do so with a bias at the population level.

One reason for beetles to choose a particular bearing in a visually challenging orientation environment could be that successful orientation is more likely when the most salient visual cues are kept in a particularly sensitive part of the visual field. If that was the case, we would expect to see a correlation between an animal's first chosen bearing (relative to the light source) and the error it makes in regaining that bearing after a disturbance. When we analysed this for all bearing fidelity data (data from Fig. 5), we only found two relevant significant correlations (Fig. A4): (1) Under a shaded full moon, diurnal beetles made smaller errors when rolling towards the moon, a bearing which placed the area of highest skylight polarisation in their fronto-dorsal visual field. (2) Under the starry sky, diurnal beetles made smaller errors when rolling away from the brightest spot of the Milky Way, a bearing which placed this bright spot in their fronto-dorsal visual field. While we found no overall trend when combining all data for the "bright" and "dim" conditions (as defined above; Fig. A4c), these two examples indicate that there might, indeed, be an orientation advantage of certain bearings under particularly challenging environmental conditions.

Discussion

Our findings show that diurnal and nocturnal dung beetles orient equally well over a large range of light intensities – even well below those commonly found in their natural habitat – as long as a full view of the moonlit sky or a single point light source is available. However, nocturnal beetles are markedly better oriented than diurnal beetles once the moon itself is not visible and only wide-field cues such as the lunar polarisation pattern or the Milky Way are available for orientation. We found no evidence that beetles roll their dung balls more slowly at lower light intensities, which would have indicated substantially increased temporal summation, or that their sensitivity to a point light source changes between day and night. The initial choice of the direction in which the balls are rolled, on the other hand, depends on light intensity: beetles tend to roll towards a bright light source, but away from a dim one.

Celestial cues for orientation in bright and dim light

Before discussing the broader implications of our results, it may be useful to consider which parts of the celestial light field the two different beetle species used as their main orientation cues in our different experiments. The diurnal species we investigated seems to follow a comparatively simple strategy. The fact that these beetles were well-oriented under a crescent moon (Fig. 2b), but that at least some beetles were disoriented under a shaded full moon (Fig. 3b) – even though the ambient light intensity was six times higher in the latter situation – suggests that they rely heavily on measuring the position of the moon itself, rather than reading wide-field cues such as the polarisation pattern or gradients of intensity and colour. This result is consistent with previous studies, which show that these diurnal beetles follow a 180° shift of the sun's or moon's apparent azimuth, but will follow a 90° shift in the polarisation pattern only when their view of the celestial body itself is blocked (Byrne et al., 2003; Dacke et al., 2014; el Jundi et al., 2014, 2015). The diurnal species we investigated therefore seems to always weight the available cues in the same way – using the azimuth of a celestial body over wide-field cues – independent of light intensity. Nocturnal beetles, on the other hand, follow different strategies in different light intensities. While they too use the position of a celestial body as a primary cue in very bright light, they prioritise the lunar polarisation pattern over the moon itself at night (el Jundi et al., 2015). This difference in strategy between day- and night-active beetles can even be found in compass neurons in the beetle brain (el Jundi et al., 2015), and is most likely based on the different relative size of the dorsal rim area in each species, the part of the eye specialised for polarised light processing. In the diurnal species, this area is only one or a few ommatidial rows wide, while in crepuscular and nocturnal beetles, it can take up half of the dorsal eye (Dacke et al., 2003b; Dacke, Smolka and Ribi, unpublished data). It is therefore possible and likely that in any experiments where beetles had a full view of the clear night sky (Fig. 2), different parts of the sky were used as primary orientation cues by the two species: diurnal beetles seem to have relied more on the moon itself, while nocturnal beetles probably weighted the lunar polarisation pattern more strongly.

Possibly the most difficult skylight pattern for dung beetles to orient by is the one observed when the crescent moon is shaded by clouds (or experimental wooden boards). Here, the light of individual stars and the Milky Way is superimposed on the dim lunar polarisation pattern. Our light measurements suggest that the scattered lunar skylight (which provides the polarisation pattern and an intensity gradient) makes up only about a third of the light available under this condition. To make matters worse, only a small proportion of the remaining two-thirds is direct starlight, while the rest is due to nocturnal airglow (ambient excitation of upper-atmosphere molecules) and Zodiacal light (sunlight scattered by interplanetary dust) (Roach and Gordon, 1973), which might not provide useful directional information for the purposes of orientation. In their sum, these celestial light sources fill the sky with arguably one of the lowest-contrast, lowest-degree-of-polarization patterns that dung beetles could ever encounter under natural conditions. Remarkably, nocturnal dung beetles still orient using this pattern with the same precision as under brighter conditions (Fig. 3b; Dacke et al., 2011), while diurnal beetles roll their balls in circuitous paths that are indistinguishable in tortuosity from those of a completely disoriented beetle (Fig. 3b). It is not clear from our data, or from previous studies, which cues nocturnal beetles prioritise under these circumstances – polarised light or the Milky Way – but our results here demonstrate that it is in these conditions that their visual specialisations provide them with the greatest advantage over diurnal beetles.

The lower extreme of natural nocturnal light intensities can be found during moonless nights, when neither the moon itself nor the polarisation pattern is available to guide the beetles' paths. The stars and, in particular, the Milky Way provide the only celestial light available to the beetles. Although diurnal beetles rolled along more tortuous paths than nocturnal beetles in this condition, the difference in median tortuosities was small (1.29 vs 1.14) and, more importantly, the diurnal beetles' orientation performance was still substantially better than that of cap-wearing, truly lost beetles (Fig. 3). This, surprisingly, indicates that some diurnal beetles were still able to orient using the dim starlight. It will be interesting to see in future experiments which particular cues they were using, and whether these are the same cues that nocturnal beetles use under these circumstances.

In the present study, we found nocturnal beetles to be equally well-oriented under all nocturnal conditions, including a moonless night with only starlight to orient by. In two previous studies, however, beetles of the same nocturnal species exited a starlit arena of the same size with mean path lengths of 207.9 cm (Dacke et al., 2013b) and 185.1 cm (Dacke et al., 2011), measured over a radial distance of 120 cm. This corresponds to mean tortuosities of 1.73 and 1.54, respectively, whereas the median tortuosity of our beetles was only 1.14 (Fig. 3). It is not immediately obvious why beetles of the same species should perform so differently when tested in the same habitat and at the same time of the year, when astronomical conditions are similar. One reason for this discrepancy could be that the arena used in both previous studies was surrounded by a 1 metre-high wall to block out potential landmark cues. From the centre of this arena, the wall blocked the beetles' view of any celestial objects below an elevation of 34° (Fig. A3). The brightest part of the Milky Way near the Southern Pleiades and the constellation of Crux, for example, was positioned at elevations of approximately 30-50° during the previous studies, and was therefore partially obscured. The diminished orientation performance on moonless nights in previous studies might thus have simply been due to a partial obstruction of skylight cues.

Anatomical and neuronal specialisations for higher sensitivity

What are the visual adaptations that allow nocturnal dung beetles to orient in almost perfectly straight lines under practically all nocturnal conditions? Two main modifications have been suggested to increase the sensitivity of the visual system in nocturnal animals. The first of these is anatomical: by creating larger apertures and shorter focal lengths, and by widening and elongating the photoreceptive structures (i.e. the rhabdoms in insect eyes), a brighter image is created on the retina. Optical modelling and intracellular recordings from photoreceptors in the eyes of onitine dung beetles show that a nocturnal beetle's eye can be made up to 85 times more sensitive in this way compared to that of a diurnal beetle (Frederiksen and Warrant, 2008; McIntyre and Caveney, 1998). Preliminary histological data suggest that the difference in optical sensitivity between the diurnal and nocturnal species investigated in the present study might be of a similar magnitude (Dacke, Smolka and Ribi, unpublished data). The second set of adaptations that increase the sensitivity of nocturnal visual systems can be found at the retinal and neuronal level: by increasing photoreceptor gain and integration times, the signal that is sent to the brain can be boosted (Frederiksen, 2008). Higher neural mechanisms, such as spatial and temporal summation of photoreceptor signals in the optic lobes, can then further increase the signal-to-noise ratio and potentially enhance the sensitivity of an insect visual system by as much as 100 000 times again (Warrant 1999).

Why then did we not find nocturnal beetles to be more sensitive than diurnal beetles when orienting to a point light source indoors (Fig. 4)? Three reasons come to mind. Firstly, due to the specific optics of a refracting superposition compound eye, the sensitivity advantage of the nocturnal optics can only be fully utilised for extended light sources, and not for point light sources (for a detailed explanation, see Warrant and McIntyre, 1990; Frederiksen and Warrant, 2008). This explanation is consistent with the result that nocturnal beetles were perfectly oriented whenever wide-field cues were available, but performed no better than diurnal beetles when the main illumination was provided by a dim point light source. Secondly, it is possible that diurnal beetles, as well as nocturnal beetles, may be able to dynamically engage neural mechanisms, in particular temporal integration, in order to increase sensitivity. We cannot rule this out because we currently do not have a good understanding of how and where these processes are implemented in the insect brain. Even though we found no evidence that beetles were moving more slowly in dimmer light (Fig. 5), it is entirely possible that temporal integration is employed, but with integration times that are too short to affect the sky compass. A comparison of flight speeds at different light intensities might still reveal a similarly strong relationship as it has been shown in some hymenopterans (Reber et al., 2015; Spiewok and Schmolz, 2006). Thirdly, it is likely that we did not find nocturnal beetles to be more sensitive than diurnal beetles when orienting to a point light source indoors simply because these beetles ignore single point light sources that are too dim to be of ecological relevance. A *single* point light source without an associated polarisation pattern never occurs under natural conditions, and nocturnal beetles might therefore simply not possess the neural hardware or the motivation to orient to it. This might also explain why we found no sensitivity difference between night and day, even though the retinae of many insects, including other dung beetles, are known to undergo a circadian shift in sensitivity. By moving screening pigments down along the rhabdom, the amount of off-axis light entering any one photoreceptor is increased at night, and the absolute sensitivity of the eye can thereby be increased by as much as two orders of magnitude (Warrant and McIntyre, 1990). The fact that no such sensitivity difference was observed in our indoor experiments suggests once again that beetles might be effectively ignoring single small stimuli that are too dim to be of ecological relevance.

Rejection of single dim point light sources might be necessary for a celestial orientation system to work at night, because of their abundance in the night sky – even with an apposition compound eye, many animals are theoretically able to see the brightest stars in the night sky (Doujak, 1985), as well as the even brighter inner planets of our solar system (Venus, Jupiter, Mars and Mercury). Dung beetles with their much more sensitive superposition eyes should be able to see an even larger number of individual stars. (Coincidentally, the dimmest light intensity we tested indoors was about 30,000 times dimmer than the full moon, which roughly equals the intensity of Sirius, the brightest star in the night sky (Fig. A3)). If dung beetles, however, used individual stars (rather than a wide-field *pattern* of stars) as orientation cues at night, it is hard to imagine how they could avoid reorienting to a different star as soon as the primary one was blocked, e.g. by a tree or cloud. In fact, this ambiguity might be one of the main reasons why nocturnal beetles prioritise wide-field cues at night: they are simply more reliable than point cues.

Bearing selection at night

When they first leave the dung pile, dung beetles select a roll bearing apparently at random (Baird et al., 2010), with environmental factors, such as the position of the sun, introducing only small population biases under certain circumstances (Baird et al., 2010; Byrne et al., 2003; Reber, 2012). In our experiments, we found a weak but reliable population bias of beetles moving towards a bright dominant light source, and away from a dim one (Fig. 7).

The fact that the beetles' bearing choices were biased in both of these circumstances suggests that there are distinct visual advantages in both situations. In comparatively bright light, it might be advantageous to roll a ball towards a light source because this places it between the beetle and the light, thereby reducing the risk that large parts of the retina will become light-adapted. For an insect with superposition eyes, this is even more important at night than during the day, because the enlarged superposition pupil will transmit the direct light of the bright source onto a greater number of photoreceptors. While it might seem problematic, at first, for the beetle to move in a way that obscures one of its main orientation cues, it should be kept in mind that, under natural conditions, there are always wide-field cues available in addition to point sources. In fact, since the area of strongest polarisation in the sky is found at a 90° angle to the sun or moon direction, the backwards-rolling beetles are placing that area in their fronto-dorsal visual field, and probably optimising the signal generated in the dorsal rim area when rolling into the light.

In dim light, beetles show the opposite tendency – choosing bearings which lead them away from the light source – but this population bias is much weaker than in bright light. In other words, the beetles avoid rolling towards the light source, and instead preferentially choose a bearing that allows them to see the dim light source itself. The fact that we found diurnal beetles that kept the main orientation cue in their fronto-visual field to be more precise in their orientation under a shaded full moon and a starry sky (Fig. A4) suggests that this strategy might improve orientation performance under difficult conditions. This tendency to roll away from a dim source also has interesting implications for the interpretation of the beetles' orientation under starlight, where they similarly tended to roll away from the southern part of the Milky Way. While previous studies have proposed that nocturnal beetles might use the entire strip of the Milky Way as an orientation cue (Dacke et al., 2013b), this result instead suggests that they use this comparatively bright group of deep-sky objects as an extended light source to orient by. Clearly, this result should be interpreted with caution since we selected the cut-off in light intensity separating "bright" from "dim" conditions based on the available data, and other environmental factors might have biased the beetles' bearing choices. It will be interesting to see, therefore, whether either of these hypotheses hold up when carefully tested in controlled experiments, e.g. by shading different parts of the sky or by rotating a simulated "Milky Way" in the laboratory.

Overall, we found no statistical difference in bearing choice between nocturnal and diurnal beetles, as might have been expected if the switch between the two bearing preferences is related to light sensitivity within each species. However, the experimental groups that were pooled into the "dim" and "bright" categories were extremely diverse, and this (together with the above-mentioned data-driven cut-off of light intensity) obviously limits the scope of our conclusion. Whether differences in sensitivity or cue priority in diurnal and nocturnal beetles influence their bearing choice in different light levels will need to be tested in controlled experiments with a much larger individual sample size per condition.

Conclusion

In summary, our work reveals that the ability to orient to the moon is more readily accessible than previously expected, as even diurnal beetles were found to orient in a perfectly straight line under even the smallest of moons. The large eyes and specialised visual systems of nocturnal dung beetles, however, allow them to continue to orient equally well when the moon is obstructed or absent from the sky. Our study shows how a detailed comparison of the natural behaviour of closely related species can serve to give us insights, and open up new questions, about the function, constraints and evolution of sensory and navigation systems under relevant natural conditions.

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Competing interests

The authors declare no competing interests.

Author contributions

Study concept and design of behavioural experiments: J.S., M.B., E.B., M.D. Data acquisition of behavioural data: J.S., B.E.J., M.B., T.R., E.B., M.D. Data analysis: J.S. Interpretation of data: J.S., B.E.J., M.B., E.B., M.D. Drafting of the manuscript: J.S. Critical revision of the manuscript: J.S., T.R., M.B., E.B., B.E.J., M.D.

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Table 1

Pairwise comparisons (one-tailed Wilcoxon rank-sum tests) of path tortuosities under different light conditions in the field (A&B) and laboratory (C)

Experiment	Species	Light condition	*W*	N_1	N_2	*P*
Full view of the sky (Fig. 2)	Diurnal	Day	387	21	21	0.054
		Full moon	399	21	19	0.20
		Crescent moon	333	21	10	0.46
	Nocturnal	Day	369	20	21	**0.031**
		Full moon	475	20	19	0.98
		Crescent moon	351	20	10	0.97
Wide-field cues only (Fig. 3)	Diurnal compared to LED control	Full moon shade	316	21	16	0.065
		Crescent moon shade	294	21	10	**0.040**
		Starlight	332	21	21	**0.001**
	Nocturnal compared to LED control	Full moon shade	396	20	18	0.999
		Crescent moon shade	371	20	10	0.997
		Starlight	393	20	21	0.33
	Diurnal compared to cap control	Full moon shade	103	10	16	**0.012**
		Crescent moon shade	88	10	10	0.11
		Starlight	287	10	21	**0.020**
	Nocturnal compared to cap control	Full moon shade	66	10	18	**0.0001**
		Crescent moon shade	55	10	10	**0.0001**
		Starlight	224	10	21	**0.0001**
Point-light source (Fig. 4)	Diurnal compared to bright control	0.04	2766	74	5	0.20
		0.003	3071	74	9	0.96
		0.0001	2976	74	20	**0.0002**
		0.000001	3001	74	20	**0.0005**
		dark	2872	74	20	**<0.0001**
	Nocturnal compared to bright control	0.04	2932	75	5	0.84
		0.003	3092	75	10	0.64
		0.0001	3226	75	20	**0.028**
		0.000001	2850	75	20	**<0.0001**
		dark	2997	75	20	**<0.0001**
	Diurnal compared to nocturnal	39000	5164	74	75	0.36
		0.04	33	5	5	0.89
		0.003	76	9	10	0.14
		0.0001	435	20	20	0.75
		0.000001	350	20	20	0.054
		dark	376	20	20	0.57

With a **full view of the sky**, beetles are always at least as well oriented as in the LED control condition. When the moon is shaded and **wide-field cues only** are available, however, diurnal beetles are less well oriented in two out of three conditions, while nocturnal beetles still perform as well as in the control. However, in every condition but one (under a shaded crescent moon), diurnal beetles still perform better than truly lost beetles that had their view of the sky blocked by a cardboard cap glued to their head. With an artificial **point-light source** in the laboratory, beetles first become less well-oriented than in the control at a light intensity of 0.0001 cd m^{-2} (five times dimmer than under a crescent moon). However, diurnal and nocturnal beetles perform equally well under all conditions.

Table 2

Results of linear models exploring factors influencing tortuosity in the field and laboratory

Experiment	Independent variables	*F*	*df*	*P*
Full view of the sky (Fig. 2)	Light condition	1.98	3, 137	0.12
	Species	0.50	1, 139	0.48
	Species * Light condition	1.10	3, 133	0.35
Wide-field cues only (Fig. 3)	Light condition	2.59	3, 121	0.056
	Species	13.3	1, 123	**0.0004**
	Species * Light condition	3.07	3, 117	**0.031**
Point-light source (Fig. 4)	Light condition	7.77	5, 286	**<0.0001**
	Species	0.10	1, 285	0.75
	Species * Light condition	1.46	6, 280	0.19
	Time of day (day vs night)	1.29	1, 285	0.26
	Order of conditions	0.0075	1, 285	0.93
	Order * Species	0.83	3, 283	0.48

With a **full view of the sky**, no difference was found in orientation performance under different moon phases, or between species. Once the moon was shaded and **wide-field cues only** were available, diurnal beetles performed significantly worse than nocturnal beetles. With an artificial **point-light source** in the laboratory, orientation performance decreased at lower light levels, but it did so equally in both species. Other factors (time of day or the order of presented conditions) were not found to influence orientation performance.

Table 3

Tests for directed distributions of bearing changes (*V*-tests)

	Species	Light condition	*V*	*N*	*P*
Field (Fig. 5A)	Diurnal	LED control	13.9	20	**<0.0001**
		Full moon	14.2	20	**<0.0001**
		Full moon shade	11.8	18	**<0.0001**
		Starlight	9.7	27	**0.0041**
	Nocturnal	LED control	16.0	20	**<0.0001**
		Full moon	18.4	20	**<0.0001**
		Full moon shade	18.1	20	**<0.0001**
		Starlight	30.2	45	**<0.0001**
Laboratory (Fig. 5B)	Diurnal	39000	36.0	60	**<0.0001**
		0.0001	8.0	20	**0.0058**
		0.000001	11.1	20	**0.00022**
		dark	2.4	20	0.23
	Nocturnal	39000	49.9	60	**<0.0001**
		0.0001	9.8	20	**0.00096**
		0.000001	1.7	20	0.29
		dark	5.1	20	0.053

We calculated the difference in bearing before and after a beetle was removed from its ball and returned to the arena centre. In the **field**, this bearing difference was always significantly clustered around 0°, i.e. the beetles returned to their previously chosen bearing after a disturbance. In the **laboratory**, this was also the case except in total darkness and under the lowest illumination condition for the nocturnal species.

Table 4

Pairwise comparisons of circular distributions of bearing changes (Mardia-Watson-Wheeler tests)

	Light condition	*W*	N_1	N_2	*P*
Field (Fig. 5A)	LED control	3.4	20	20	0.19
	Full moon	3.3	20	20	0.19
	Full moon shade	8.9	18	20	**0.012**
	Starlight	6.1	27	45	**0.048**
Laboratory (Fig. 5B)	39000	8.7	60	60	0.01
	0.0001	0.68	20	20	0.71
	0.000001	6.0	20	20	0.050
	dark	6.15	20	20	**0.046**

We calculated the difference in bearing before and after a beetle was removed from its ball and returned to the arena centre, and compared the distribution of these changes in bearing between diurnal and nocturnal beetles. In the **field**, we found no significant difference in the re-orientation accuracy between species in the control condition and under a full moon, but found a significant difference under a shaded full moon and starlight, most likely due to the fact that the spread of the distribution is substantially larger for the diurnal species under these conditions, indicating a poorer re-orientation performance. In the **laboratory**, we found no difference between species except in complete darkness.

Table 5

Pairwise comparisons (one-tailed Wilcoxon rank-sum tests) of rolling speeds under different light conditions

Experiment	Species	Light condition	*W*	N_1	N_2	*P*
Field	Diurnal	Shaded vs. unshaded full moon	318	19	16	0.79
		Shaded vs. unshaded crescent moon	91	10	10	0.86
		Stars vs. bright LED	445	21	21	0.57
	Nocturnal	Shaded vs. unshaded full moon	356	19	18	0.57
		Shaded vs. unshaded crescent moon	107	10	10	0.45
		Stars vs. bright LED	296	20	21	1.00
	Diurnal compared to nocturnal	Daylight	457	21	21	0.56
		Full moon	331	21	20	**0.0021**
		Full moon shade	232	19	19	**<0.0001**
		Crescent moon	158	16	18	**<0.0001**
		Crescent moon shade	58	10	10	**0.0002**
		Bright LED	27	10	10	**0.0002**
		Stars	269	21	21	**<0.0001**
Laboratory	Diurnal compared to bright control	0.04	2867	74	5	0.97
		0.003	3084	74	9	0.64
		0.0001	3603	74	20	0.21
		0.000001	3677	74	20	0.68
		dark	3471	74	20	0.66
	Nocturnal compared to bright control	0.04	3042	75	5	0.47
		0.003	3312	75	10	0.12
		0.0001	3428	75	20	0.94
		0.000001	3613	75	20	0.45
		dark	3595	75	20	0.52
	Diurnal compared to nocturnal	39000	4015	74	75	**<0.0001**
		0.04	22	5	5	0.15
		0.003	71	9	10	0.067
		0.0001	251	20	20	**<0.0001**
		0.000001	256	20	20	**<0.0001**
		dark	301	20	20	**0.0017**

Both in the **field** and in the **laboratory**, we found no indication that beetles rolled more slowly in dimmer light, as might have been expected if the beetles use temporal integration to create a more reliable visual signal.

Table 6

Results of linear models exploring factors that influence rolling speed in the laboratory

Experiment	Independent variables	*F*	*df*	*P*
Rolling speed	Species	94.8	1, 296	**<0.0001**
	Light condition	0.89	5, 291	0.49
	Species * Light condition	1.19	5, 286	0.29

While diurnal beetles rolled their balls on average significantly slower than nocturnal beetles, there was no effect of light intensity on rolling speed, as might have been expected if the beetles use temporal integration to create a more reliable visual signal.

Appendix

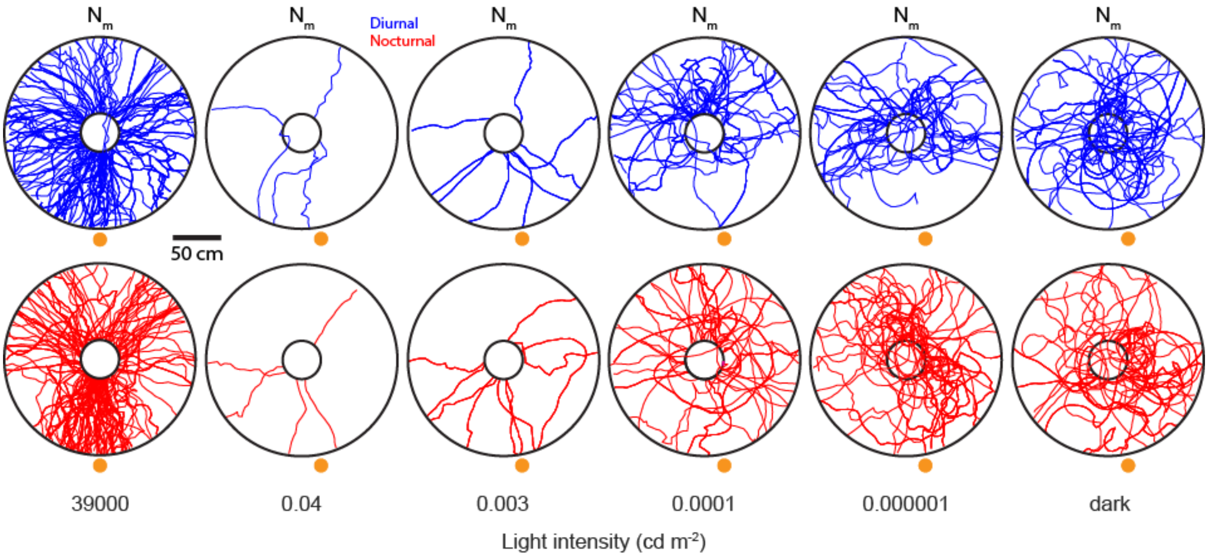

Figure A1: Paths of beetles leaving a 1.6-metre indoor arena. Bird's eye view of the rolling paths of diurnal (blue) and nocturnal (red) dung beetles when placed in a 1.6-metre diameter indoor arena (see Fig. 4). Tests were performed with a bright spotlight (control, 39000 cd m^{-2}, 1st condition) or a dimmable tungsten lamp at a range of different light intensities covering and exceeding the range of light intensities measured under natural nocturnal conditions (2nd to 6th condition). As light intensity decreases, paths become more tortuous. See methods for details of path filtering. Top of each diagram is local magnetic North (N_m), orange circles indicate the position of the light source.

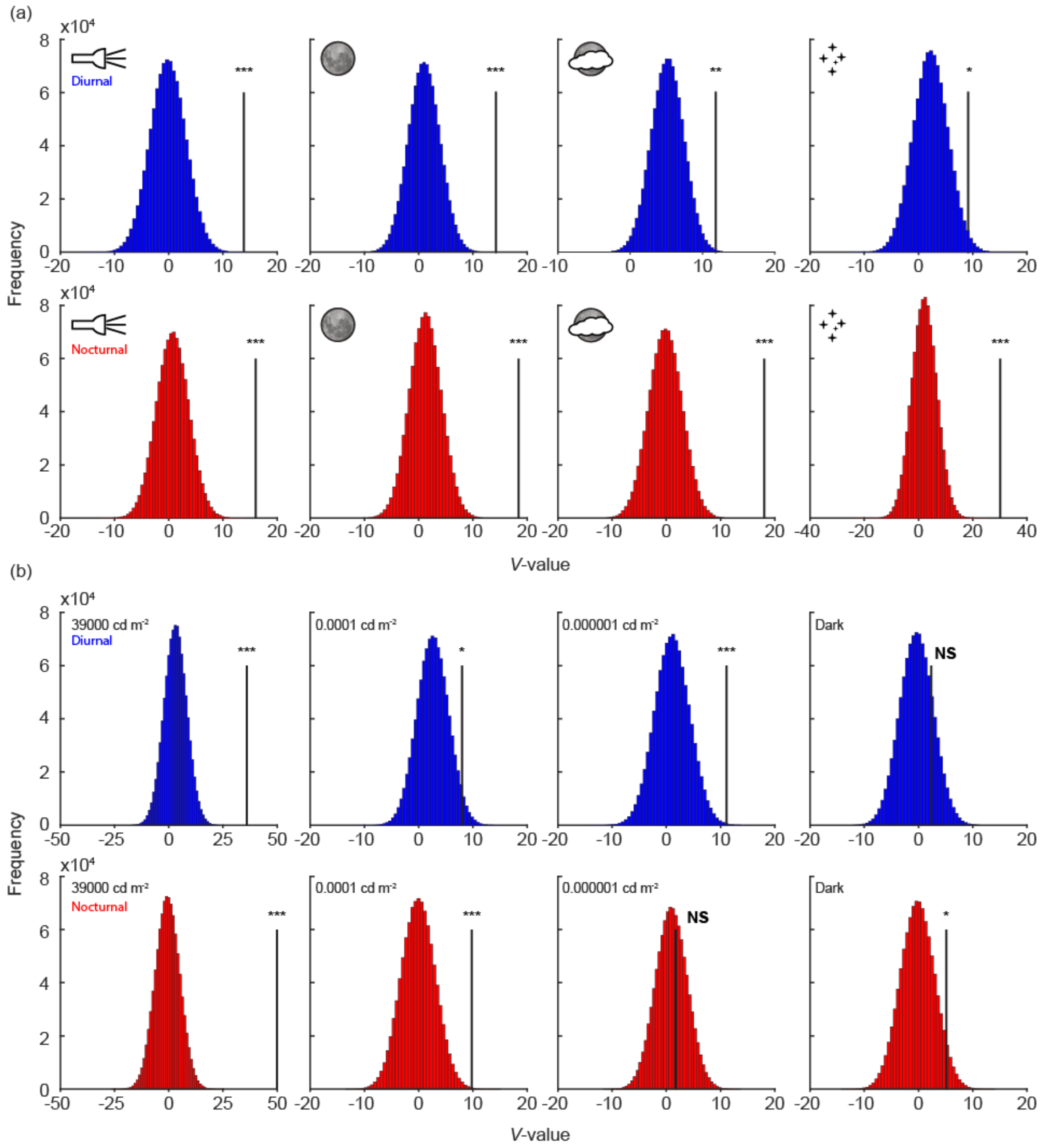

Figure A2: Permutation tests for all bearing fidelity experiments. Data and order of subpanels as in Fig. 5. Measured bearings were randomly permuted across individuals and experimental conditions. The plots show the *V*-value obtained from 1,000,000 random permutations. Black lines indicate the unpermuted *V*-values. Probabilities were calculated as the proportion of permutations that resulted in a higher V-value than that obtained from the unpermuted data (equivalent to a one-sided test). ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; NS: not significant. **(a)** Experiments were performed in the beetles' natural habitat with diurnal (blue) and nocturnal (red) beetles at night, with illumination from (from left to right) (1) an artificial light, (2) the full moon, (3) the shaded full moon or (4) only starlight. The difference between consecutive bearing choices was calculated, and was significantly clustered around 0° in all cases, indicating that beetles did not just use optomotor cues to keep to a straight line, but could re-acquire their bearing even after a significant disturbance. **(b)** Bearing changes after replacement to the arena centre for diurnal and nocturnal beetles indoors. The highest light intensity at which beetles did not significantly regain their chosen direction was 0.000001 cd m^{-2} (500 times dimmer than crescent moon light).

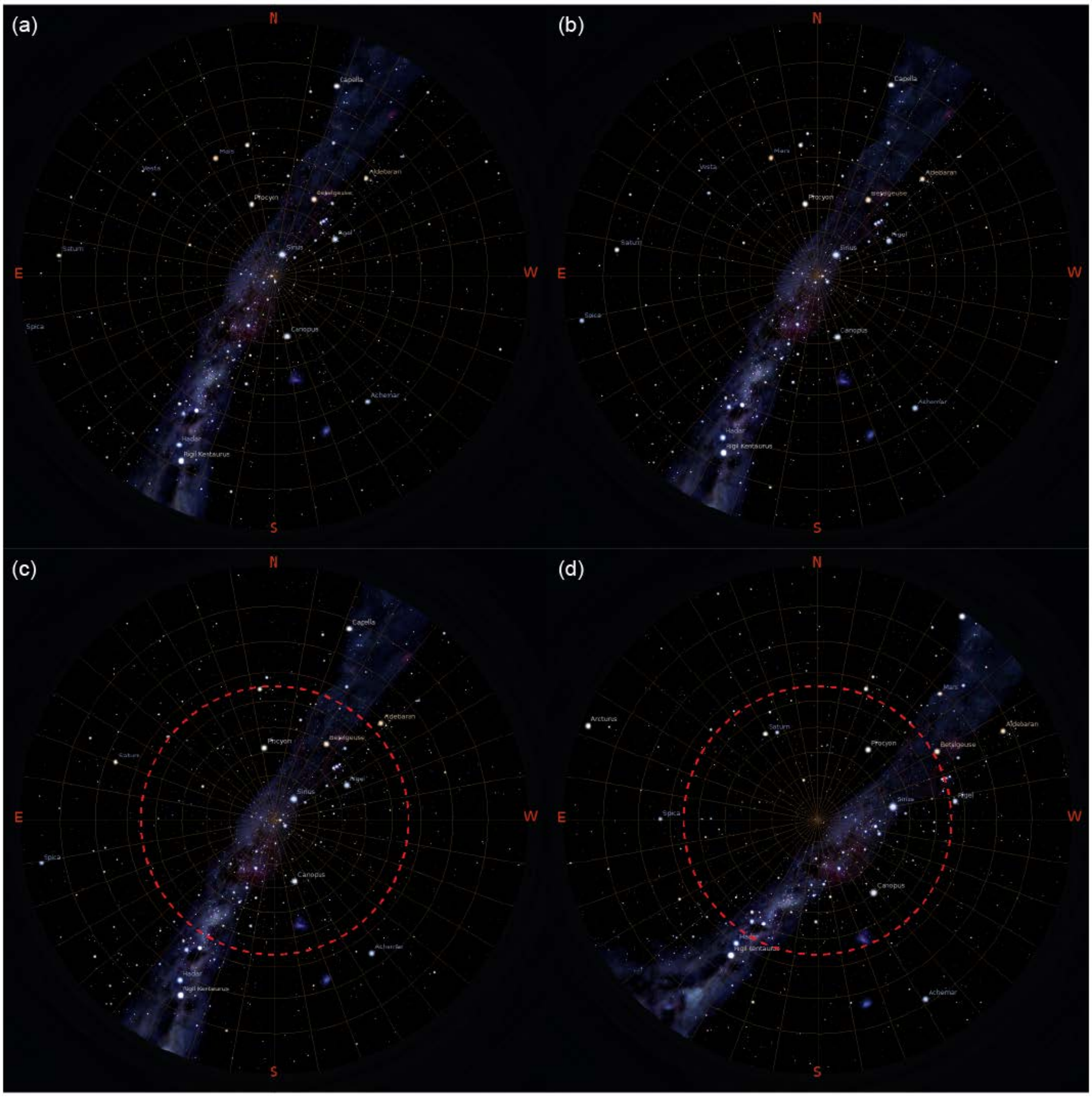

Figure A3: Simulated view of the starry sky. **(a, b)** Night sky on the two moonless experimental days of the present study showing the position of the Milky Way and the brightest visible stars and planets. **(c, d)** Night sky during previous studies examining the Milky Way orientation behaviour of *S. satyrus*. (c: Dacke et al., 2011; d: Dacke et al., 2013b). In both of these studies, the test arena was surrounded by a 1-metre high wall to block the beetles' view of the landmark panorama. From the centre of the arena, this wall would have blocked any skylight cues below 34°, i.e. any light outside the red dashed circle. If the beetles, as we hypothesise, use the brightest Southern part of the Milky Way for orientation, parts of this pattern would have been blocked (to a smaller or larger extent, depending on a beetle's position in the arena). Images were created in and exported from Stellarium 0.12.4 (Stellarium Developers, www.stellarium.org; full-sky Milky Way panorama by Axel Mellinger, University of Potsdam, Germany).

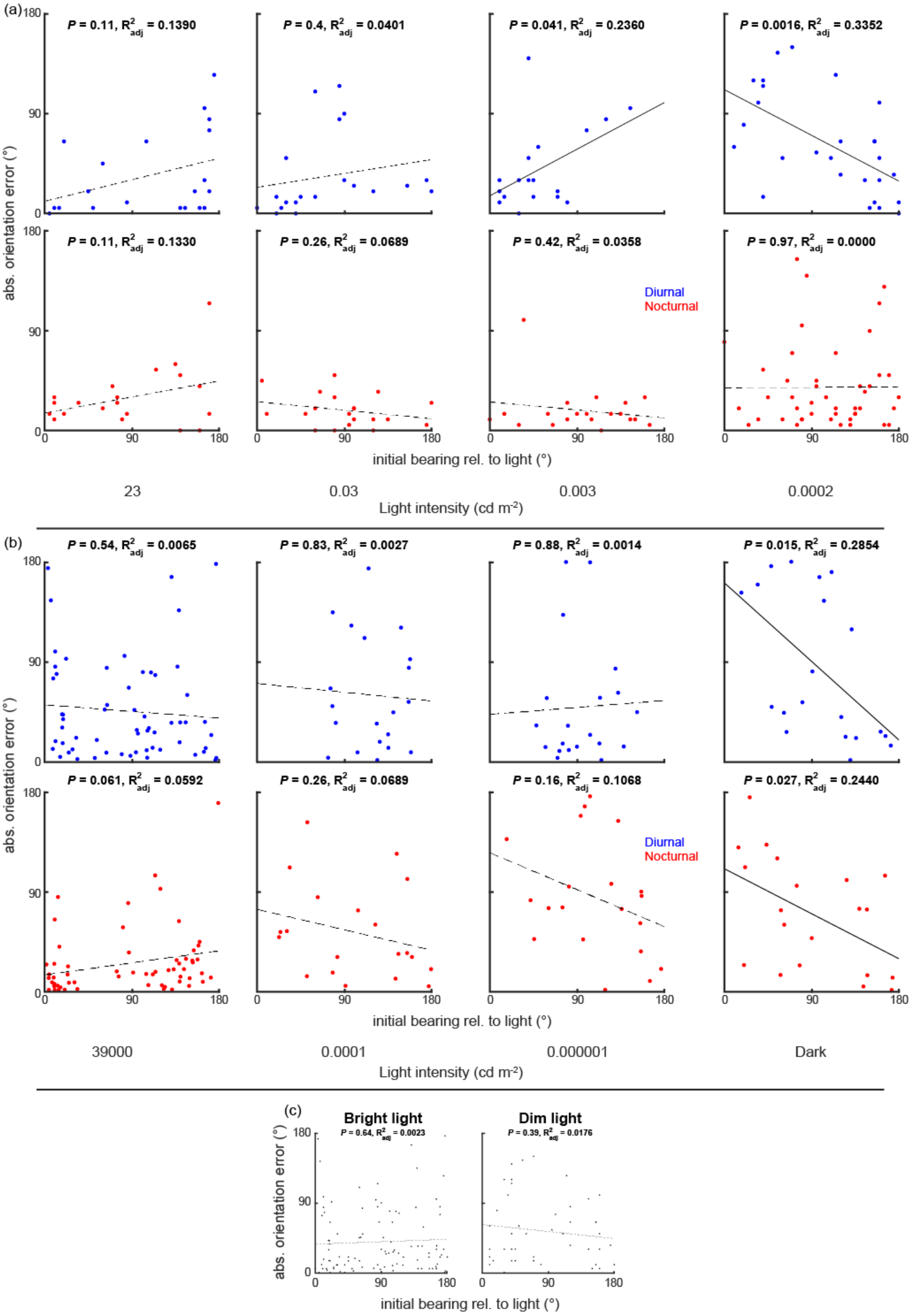
(a)
P = 0.11, R2adj = 0.1390
P = 0.4, R2adj = 0.0401
P = 0.041, R2adj = 0.2360
P = 0.0016, R2adj = 0.3352
P = 0.11, R2adj = 0.1330
P = 0.26, R2adj = 0.0689
P = 0.42, R2adj = 0.0358
P = 0.97, R2adj = 0.0000
Diurnal
Nocturnal
abs. orientation error (°)
0
90
180
initial bearing rel. to light (°)
23
0.03
0.003
0.0002
Light intensity (cd m-2)
(b)
P = 0.54, R2adj = 0.0065
P = 0.83, R2adj = 0.0027
P = 0.88, R2adj = 0.0014
P = 0.015, R2adj = 0.2854
P = 0.061, R2adj = 0.0592
P = 0.26, R2adj = 0.0689
P = 0.16, R2adj = 0.1068
P = 0.027, R2adj = 0.2440
Diurnal
Nocturnal
abs. orientation error (°)
initial bearing rel. to light (°)
39000
0.0001
0.000001
Dark
Light intensity (cd m-2)
(c)
Bright light
Dim light
P = 0.64, R2adj = 0.0023
P = 0.39, R2adj = 0.0176
abs. orientation error (°)
initial bearing rel. to light (°)

Figure A4: Re-orientation error after a disturbance does not generally depend on chosen bearing. **(a, b)** We calculated the correlation between the initial bearing relative to the light source (i.e. the absolute difference between bearing and light source azimuth) and the absolute orientation error, i.e. the difference between this bearing and the bearing chosen after the beetle was removed from its ball and returned to the arena centre. Conditions in the field (a) and laboratory (b) are in the same order as in Fig. 5. After this disturbance, beetles re-orient to their former bearing with a random error that depends on the lighting condition (Fig. 5). A significant correlation was only found in two conditions (diurnal beetles under a full moon and under the starry sky), indicating that in these visually difficult conditions, beetles were more precise when they kept the main orientation cue in their fronto-dorsal visual field. We also found a significant correlation for both species in the laboratory in complete darkness, again indicating that there might have been a very weak non-visual cue for the beetles to orient by (see Result for Fig. 5). **(c)** When data was pooled for all "bright" and "dim" conditions (defined as in Fig. 7), no overall correlation was found.

Table A1

Light and temperature conditions during field experiments

Condition	Date & time (GMT+2)	Azimuth (°)	Elevation (°)	Fullness	Air / ground temp. (°C)	Light levels (cd m^{-2})
Daylight	28/01/2010, 1511 – 1546	288 – 292	45 – 53	---	---/---	---
	01/02/2010, 0926 – 0959	101 – 105	45 – 52			
Full moon	30/01/2010, 2124 – 2243	64 – 78	23 – 37	0.99	19.5 / 26.2	No shade: 2.9 x 10^{-2}
	31/01/2010, 2135 – 2312	75 – 90	18 – 37	0.92		
	01/02/2010, 2215 – 0037	75 – 99	15 – 48	0.79		Shade: 2.8 x 10^{-3}
Crescent moon	09/02/2010, 0325 – 0404	124 – 127	18 – 26	-0.59	19.1 / 22.0	No shade: 5.2 x 10^{-4}
	10/02/2010, 0400 – 0500	122 – 127	15 – 27	-0.74		Shade: 2.6 x 10^{-4}
Stars / art. light	10/02/2010, 2108 – 2245	160 / 270	--- / 5	-0.81	19.5 / 26.5	Stars: 2.0 x 10^{-4}
	11/02/2010, 2142 – 2248	160 / 270	--- / 5	-0.91		Art. light: 23.0
Repeated rolls moon	29/01/2013, 2200 – 0025	69 – 94	18 – 47	0.85	---	4.1 x 10^{-3} – 1.3 x 10^{-2}
Repeated rolls stars	22/01/2013, 0330 – 0425	160 – 210	---	0.54	---	1.2 x 10^{-4}
	01/02/2013, 2030 – 2100	160		0.38		9.0 x 10^{-5}
	02/02/2013, 2030 – 2107	160		0.17		8.6 x 10^{-5}
	24/01/2014, ~2045	160		-0.12		---
	25/01/2014, ~2115	160		-0.34		---

Time is local South African time (GMT + 2). Azimuth (0° represents local magnetic North; 90° East; 180° South; 270° West) and elevation (not corrected for atmospheric refraction) ranges are given for the main light source available to the beetles at the time: sun, moon, artificial light or the brightest (Southern) part of the Milky Way. Fullness of the moon is reported as –cos of the three-dimensional angle between sun and moon (1 is full moon, 0 half-moon, -1 new moon).

Table A2

Numbers of subjects included in field experiments

	Day		Art. light		Full moon		Full moon shade		Crescent moon		Crescent moon shade		Stars		
	D	N	D	N	D	N	D	N	D	N	D	N	D	N	**Total**
beetles	21	21	21	20	19	19	16	18	10	10	10	10	21	21	237
tracks	21	21	21	20	19	19	16	18	10	10	10	10	21	21	237
discarded	0	0	0	0	0	0	4	7	0	0	0	0	0	1	12
time-outs	0	0	3	0	1	0	3	0	0	0	6	0	6	0	19
n tortuosities	21	21	21	20	19	19	12	11	10	10	10	10	21	20	225
n bearings/speeds	21	21	21	20	19	19	16	18	10	10	10	10	21	21	237
n repeated rolls	–	–	10	10	10	10	10	10	–	–	–	–	18	36	114

For each experimental condition, the number of *beetles* of the diurnal (D) and nocturnal (N) species that performed this condition is given. Each beetle only performed each condition once, resulting in an equal number of *tracks* as beetles. The results for a total of twelve beetles had to be *discarded* because of long gaps in their tracks due to insufficient lighting, poor camera focus or obstructions. A total of 19 beetles did not reach the arena perimeter within the two-minute time limit (*time-outs*). The final rows indicate the final number of animals included in analyses of *tortuosities* (Figs 2-4), *bearings and speeds* (Figs 6-7) and *bearing reacquisition* (Fig. 5).

Table A3

Light conditions during laboratory experiments

Date & time (GMT+1)	Condition	Azimuth (°)	Elevation (°)	Light levels (cd m^{-2})
07/03/2010, 1107 – 1722 08/03/2010, 2241 – 0320 10/03/2010, 1140 – 1812 11/03/2010, 2151 – 0324	0V	170	25	1.3×10^{-9}
	1.5V			1.2×10^{-6}
	3V			1.1×10^{-4}
	6V			3.3×10^{-3}
	12V			4.4×10^{-2}
	Bright light	180	32	39000

Time is local Swedish time (GMT + 1). Azimuth (0° represents local magnetic North; 90° East; 180° South; 270° West) and elevation are given for the artificial light sources as seen from the arena centre.