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

Smolka, Jochen; Baird, Emily; el Jundi, Basil; Reber, Therese; Byrne, Marcus J.; Dacke, Marie

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

3

4 Jochen Smolka^{a,*}, Emily Baird^a, Basil el Jundi^a, Therese Reber^a, Marcus J. Byrne^b, Marie
5 Dacke^{a,b}

6

7 ^aDepartment of Biology, Lund University, Sweden

8 ^bSchool of Animal, Plant and Environmental Sciences, University of the Witwatersrand,
9 South Africa

10 * Corresponding author:

11 Jochen Smolka, Department of Biology, Lund University, Biology Building, Sölvegatan 35,
12 223 62 Lund, Sweden. e-mail: jochen.smolka@biol.lu.se, phone: +46 46 2228097

13 **Abstract**

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

31

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

34

35 **Introduction**

36 Seeing at night is a challenging task. The skylight on a moonless night can be over one
37 hundred million times dimmer than on a sunny day (Lythgoe, 1979). As light levels drop,

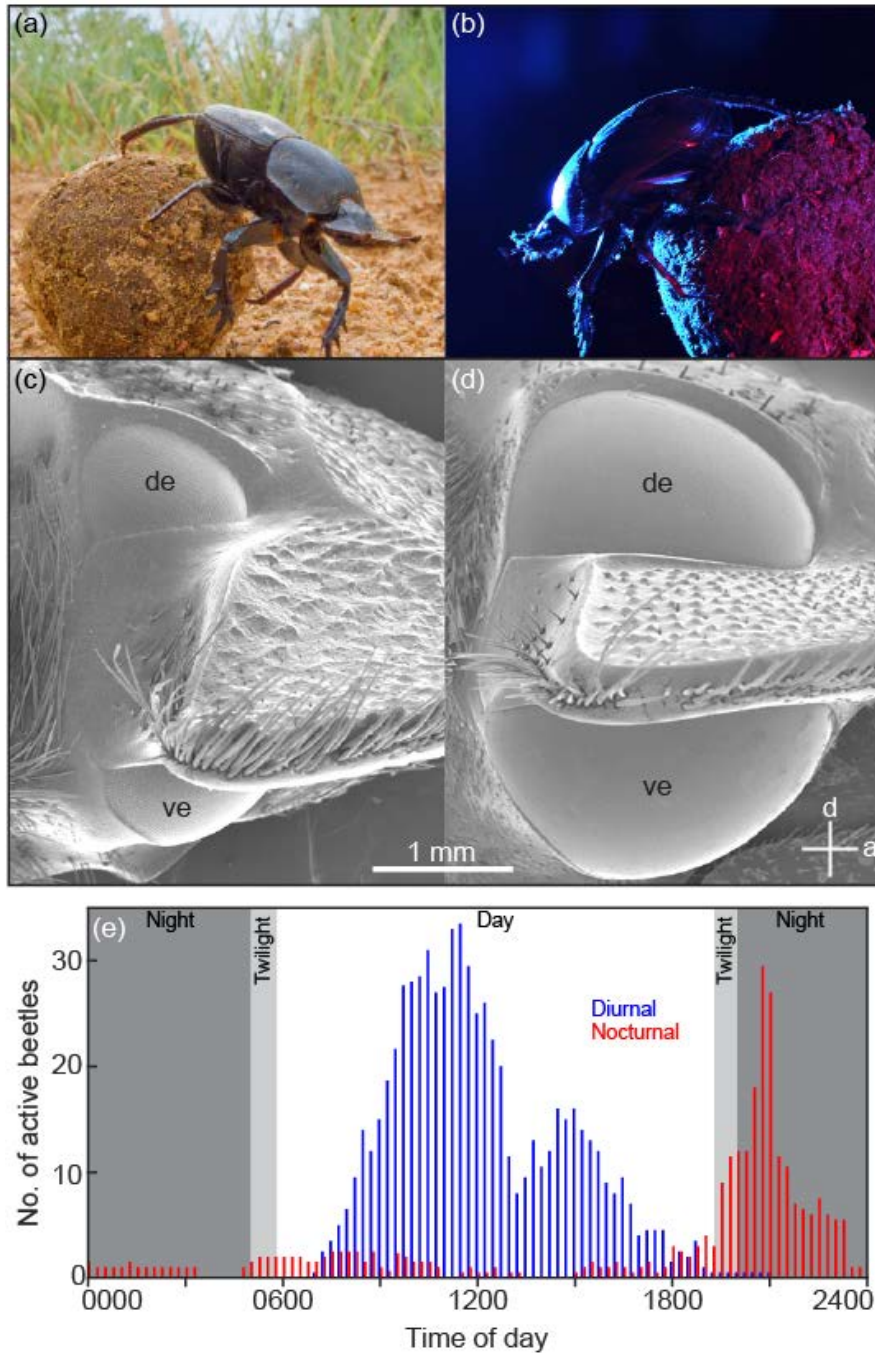
38 fewer photons reach each photoreceptor, and the signal-to-noise ratio in the visual system
39 eventually falls to a level where even objects or light sources that present a large relative
40 contrast to the background can no longer be distinguished from it. Nevertheless, many
41 animals, including small insects, are exclusively active at night and rely on vision to guide
42 them in tasks such as locomotion, foraging, courtship and navigation (Warrant 2008; Warrant
43 and Dacke, 2011). To deal with extremely low light intensities, nocturnal animals have
44 developed visual systems with a wide range of anatomical and physiological adaptations.
45 Insects living in dim light, for example, generally have compound eyes whose ommatidia
46 have larger facet lenses of shorter focal length, as well as longer and wider rhabdoms, in
47 order to increase the photon capture of each photoreceptor (Greiner et al., 2004a, 2007;
48 Meyer-Rochow and Nilsson, 1999; Warrant 2008; Warrant and Dacke, 2011; Warrant and
49 McIntyre, 1991). Many night-active insects also possess superposition compound eyes, where
50 hundreds or thousands of facets contribute light to each photoreceptor instead of just one as
51 in apposition eyes. Even in their sum, however, these optical adaptations rarely boost
52 sensitivity by more than a factor of 1000, and they are therefore not sufficient to explain how
53 some insects can deal with the eight orders of magnitude of light intensity variation between
54 night and day. Various neural mechanisms, including a change of photoreceptor gain, as well
55 as spatial and temporal summation of signals at different stages of the neural processing
56 network, have been suggested as solutions to bridge this sensitivity gap (Frederiksen et al.,
57 2008; Greiner et al., 2004b, 2005; Laughlin, 1981; Theobald et al., 2006; van Hateren 1993;
58 Warrant, 1999). The fact that hornets, for example, can fly and forage at night without any
59 obvious dim-light adaptations at the level of the optics of their compound eyes (Kelber et al.,
60 2011) suggests that neural adaptations alone can provide a large enough sensitivity boost to
61 allow an animal to extend its activity period to much dimmer light intensities.

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

73 After landing at a fresh dung pile, ball-rolling dung beetles separate a piece of dung and
74 shape it into a ball. They then select a seemingly random bearing (Baird et al., 2010), and –
75 with their head down, walking backwards – roll the ball away with their hind legs until they
76 have found an expedient spot to bury themselves together with the ball, and consume it in
77 solitude or lay an egg in it. In order to escape from the dung pile as quickly as possible, to
78 avoid competition from other newly arrived beetles keen to steal a ball rather than make one
79 themselves, the ball-rolling beetles move away in straight lines. Simple as this may sound,
80 keeping a straight line is impossible without external "compass" cues (Cheung et al., 2007) –
81 even for humans (Souman et al., 2009). For this compass, dung beetles use celestial cues
82 exclusively. Ignoring even obvious landmarks, beetles lose their way when the sky is
83 overcast or experimentally occluded (Dacke et al., 2013a). Within the sky, however, they use
84 a large range of directional cues, including the azimuthal position of the sun or moon (Byrne
85 et al., 2003; Dacke et al., 2004, 2014), the pattern of polarised light formed around these
86 celestial bodies (Byrne et al., 2003; Dacke et al., 2003a, 2003b; el Jundi et al., 2014, 2015),

87 the gradient of skylight intensity that stretches from the solar to the anti-solar hemisphere (el
88 Jundi et al., 2014) and even the Milky Way (Dacke et al., 2013b). Astonishingly, the
89 precision with which beetles orient to their familiar cues does not change over a very large
90 range of light intensities (Dacke et al., 2011). Like other dim-light active insects, nocturnal
91 dung beetles have a range of visual specialisations, which allow them to be active at night.
92 Their superposition compound eyes (which all dung beetles possess) are enlarged compared
93 to those of their diurnal cousins in all the expected parameters (Fig. 1) (Byrne and Dacke,
94 2011; Caveney and McIntyre, 1981; Dacke et al., 2003b; Frederiksen and Warrant, 2008;
95 McIntyre and Caveney, 1998; Warrant and McIntyre, 1990), including an enlargement of the
96 dorsal rim area (the region analysing polarised skylight) (Dacke et al., 2003b; Dacke, Smolka
97 and Ribí, unpublished data), and often feature a tracheal tapetum, which reflects light back
98 onto the photoreceptor and effectively doubles the light path (Warrant and McIntyre, 1991).
99 Taken together, these optical specialisations can increase the sensitivity of a nocturnal
100 beetle's eye by up to 85 times compared to that of a diurnal beetle (Frederiksen and Warrant,
101 2008; McIntyre and Caveney, 1998). Physiologically, some nocturnal dung beetles adapt
102 their photoreceptors to dim light with a slower frequency response and higher gain
103 (Frederiksen, 2008; Warrant and McIntyre, 1990). Taken together, these specialisations
104 should give nocturnal beetles vastly superior light sensitivity compared to diurnal beetles.

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



110

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

122

123 **Materials and methods**

124 *Animals*

125 All experiments were performed with the diurnal dung beetle species *Scarabaeus (Kheper)*
126 *lamarcki* Macleay, 1821 (Coleoptera, Scarabaeidae) and the nocturnal species *Scarabaeus*
127 *satyrus* Boheman, 1860. We captured the beetles using pit-fall traps in their natural habitat on
128 the game farm "Stonehenge" in South Africa (24.3°E, 26.4°S). After collection, beetles were
129 kept in plastic boxes (30 x 22 x 22 cm) in the shade, where they were provided with soil and
130 fresh cow dung. Field experiments were performed in January and February 2010 and 2013,
131 and January 2014. Laboratory experiments were performed at Lund University in March
132 2010, within six weeks of capture of the beetles. These animals were kept under a 12 hour
133 light/dark cycle in a climate-controlled animal room, and fed with fresh cow or horse dung.
134 Before field night-time or laboratory experiments, beetles were placed in a plastic container
135 situated in a heated cool box, where they were provided with a thin layer of sand and some
136 cow dung. For diel activity measurements, six plastic barrels (diameter 50 cm, height 60 cm)
137 were filled with sand to a height of about 50 cm and placed in a shaded location, away from
138 human-made light sources. Ten night-active and ten day-active beetles, as well as ten beetles
139 of a crepuscular species (not reported here) were placed in each barrel and prevented from
140 flying away by a fine mesh placed over the top of the barrel. We then recorded the number of
141 active beetles of each species (i.e. beetles that were at the surface rather than dug down into
142 the soil) every 15 minutes over a period of 48 hours. Beetles were not fed for the full 48
143 hours of activity measurements.

144

145 *Behavioural experiments in the field*

146 To test their orientation performance under different light conditions, we observed beetles
147 rolling their balls in their natural habitat under seven different conditions: (1) during the day

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

169 All field experiments were performed under a clear sky, with the dominant celestial body
170 at low to medium elevations ($15^\circ - 53^\circ$) to provide an easy-to-read directional cue. All night
171 experiments were performed after the end of astronomical evening twilight and before the
172 beginning of morning twilight to ensure that the sun provided no polarisation pattern that

173 could have been used for orientation (Cronin et al., 2006). Similarly, all experiments under
174 moonless conditions were performed when the moon was more than 18° below the horizon to
175 ensure that no lunar polarisation pattern was available as an orientation cue.

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

185

186 *Behavioural experiments in the laboratory*

187 To test whether diurnal and nocturnal dung beetles differ in their ability to orient to a dim
188 point light source, we also investigated the beetles' orientation behaviour on a wooden arena
189 (1.6 metre diameter) in the laboratory in Sweden. The beetles' paths were recorded in the
190 same manner as in the field, from a height of 1.9 metres. Illumination for the bright light
191 control condition was provided by a halogen spotlight (Dedolight Aspherics 2; Dedotec USA
192 Inc., Ashley Falls, MA, USA, fitted with a 150W halogen lamp; 39,000 cd m⁻² at arena
193 centre) placed with the centre of its aperture at a height of 88 cm above the ground, 140 cm
194 from the centre of the arena. The front aperture measured 70 mm, creating a light spot of 2.4°
195 angular size as seen from the centre of the arena. Illumination for dim light test conditions
196 was provided by a custom-made halogen lamp placed at a distance of 130 cm from the arena
197 centre and a height of 62 cm above the ground. The 16.4 mm aperture created a light spot of

198 0.65° angular size as seen from the centre of the arena, and therefore appeared only slightly
199 larger than the real moon (approximately 0.5°). The aperture was covered by a neutral density
200 filter and a diffuser, which adjusted the maximum light intensity at the centre of the arena to
201 approximately that of a full moon night (cf. Figs 2, 4). By adjusting the voltage to the lamp,
202 we created a total of four light-intensity conditions (12V / 6V / 3V / 1.5V), which covered the
203 range of naturally observed light intensities. As an additional control for non-visual cues, we
204 tested the beetles with the power supply set to 0V, i.e. in complete darkness.

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

222

223 *Video analysis & track filtering*

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

- 228 1) Start: The beetle's initial activity inside a 40 cm diameter circle around the centre was
229 ignored. This removed any activity due to the beetle's initial maintenance of the ball, as
230 well as its first dance and re-orientation on the ball (Baird et al., 2012).
- 231 2) Finish: The track ended when the beetle had either moved across the arena's outer
232 perimeter, or timed out when it had rolled its ball for two minutes after leaving the inner
233 40 cm diameter circle without exiting the arena. A total of 45 out of 535 beetles were
234 timed out in this way (Tables A2, A4). In some cases, beetles were removed from the
235 arena by the experimenter before they crossed the outer perimeter, e.g. to keep them
236 from colliding with an obstacle. In these cases, the tracks were still included in the
237 analysis as long as the beetles were no more than 10 cm away from and clearly moving
238 towards the perimeter at the time of removal.
- 239 3) Gaps: Due to the sometimes less than ideal filming situation at night (under- or
240 overexposed video images, visual obstructions, defocused camera images), a number of
241 tracks had gaps where tracking was not possible. Any gaps that were shorter than four
242 seconds were filled in by linear interpolation. Tracks with longer gaps were discarded for
243 the analysis of tortuosities, but were still included in the analysis of rolling speeds and
244 bearing choice. This was the case for a total of 26 out of 775 recorded tracks.
- 245 4) Pauses: A pause was defined as any segment of the path where a beetle moved no more
246 than 1 cm in 2 s or no more than 2 cm in 4 s. For the calculation of tortuosity, pauses

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

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

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

265 Each track was finally characterised using three characteristics:

266 1) The tortuosity of the track, which is the ratio between the total track length L and
267 the straight distance between start and end point D (Fig. 2a). This measure was
268 calculated from the final, filtered path.

269 2) The mean rolling speed of the beetle. For the calculation of this mean speed, only
270 those segments of the path were included that neither started nor ended with a
271 point that was part of a gap or pause.

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

275

276 *Light source characterisation*

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

286

287 *Statistics*

288 We performed all statistical analyses in Matlab using the in-built statistics toolbox and the
289 CircStat toolbox (Berens, 2009), with the exception of Mardia-Watson-Wheeler tests, which
290 were performed in Oriana (Kovach Computing Services, Anglesey, Wales), and linear model
291 analyses, which were performed in R 3.1.2 (R Core Team, 2013). To compare the
292 distributions of tortuosities or speeds between treatments (Figs 2-4, 6), we applied Wilcoxon
293 rank-sum tests (Mann and Whitney, 1947; Wilcoxon, 1945) to compare medians, and Brown-
294 Forsythe tests (Brown and Forsythe, 1974) to compare the spread. Both tests are robust to
295 non-normally distributed data, which some of the tortuosity data clearly were. Since most of
296 the unexpected results of this study are reflected by statistical tests failing to show a

297 difference between two groups, we performed one-sided comparison tests throughout to
298 increase the power of the tests. To test for an overall difference in orientation performance
299 between species, and to check whether the time of day or the order of conditions had an
300 influence on the beetles' ability to keep to a straight line or on the beetles' rolling speed, we
301 calculated linear models predicting tortuosity and speed from species, condition, order of
302 conditions and time of day. The final model was selected by sequentially fitting parameters of
303 interest and including only those parameters that reached significance at a 5 per cent level
304 when added to the final model.

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

314

315 *Ethical note*

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

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

326

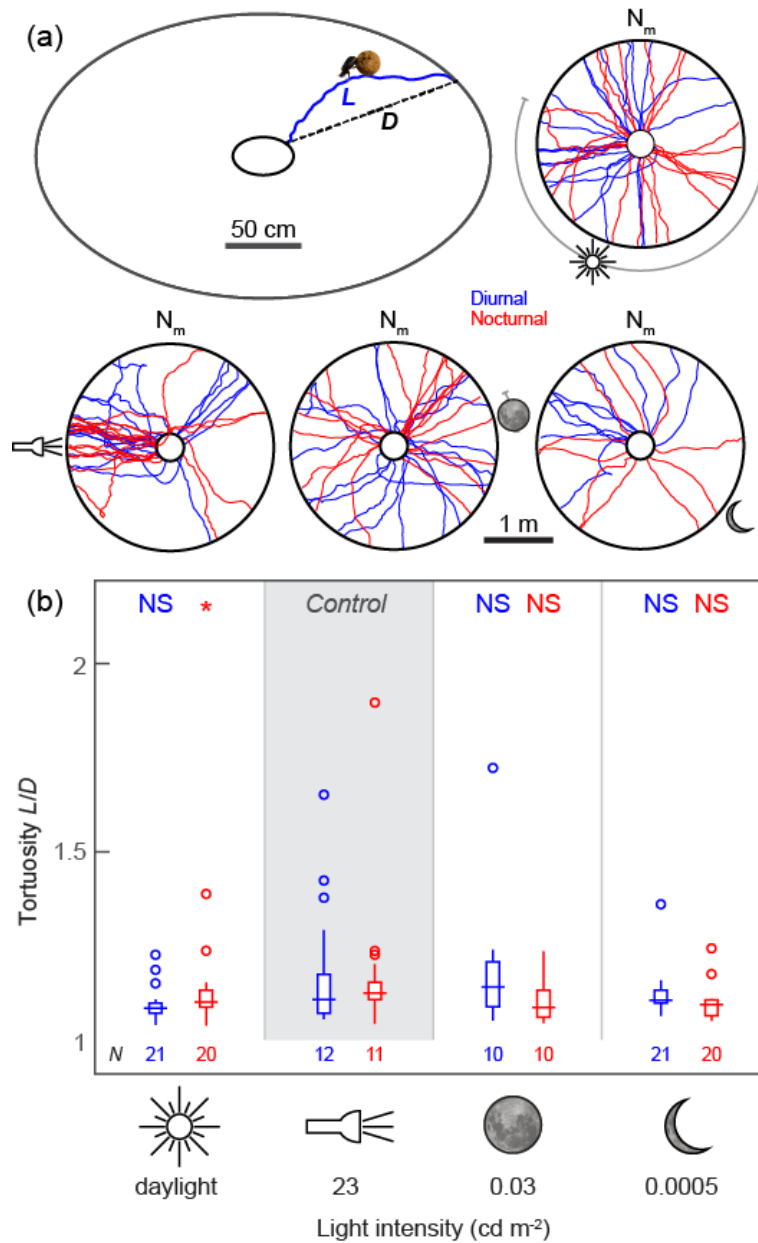
327 **Results**

328 *Orientation with a full view of the sky*

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

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

346 everything else being equal, a doubling of path tortuosity means that a beetle will require
 347 twice the time and expend twice the energy to reach a given distance from the dung pile.
 348



349
 350 **Figure 2:** Nocturnal and diurnal beetles orient equally well to point light sources in their
 351 natural habitat. **(a, top left)** Beetles were placed in the centre of a 3-metre diameter arena in
 352 their natural South African habitat, and their paths recorded as they rolled their dung balls out
 353 of the arena. Initial movements (less than 20 cm away from centre) were ignored. As a
 354 measure of orientation performance, we calculated tortuosity, the ratio between the path
 355 length L and the straight-line distance D between path start and end. **(a)** Bird's eye view of
 356 the rolling paths of diurnal (blue) and nocturnal (red) dung beetles on a clear, sunny day (top
 357 right), with an artificial light at night (bottom left), on a full moon night (bottom centre) and
 358 on a crescent moon night (bottom right). See methods for details of path filtering. Top of each
 359 diagram is local magnetic North (N_m), symbols indicate the position of the light source (with

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

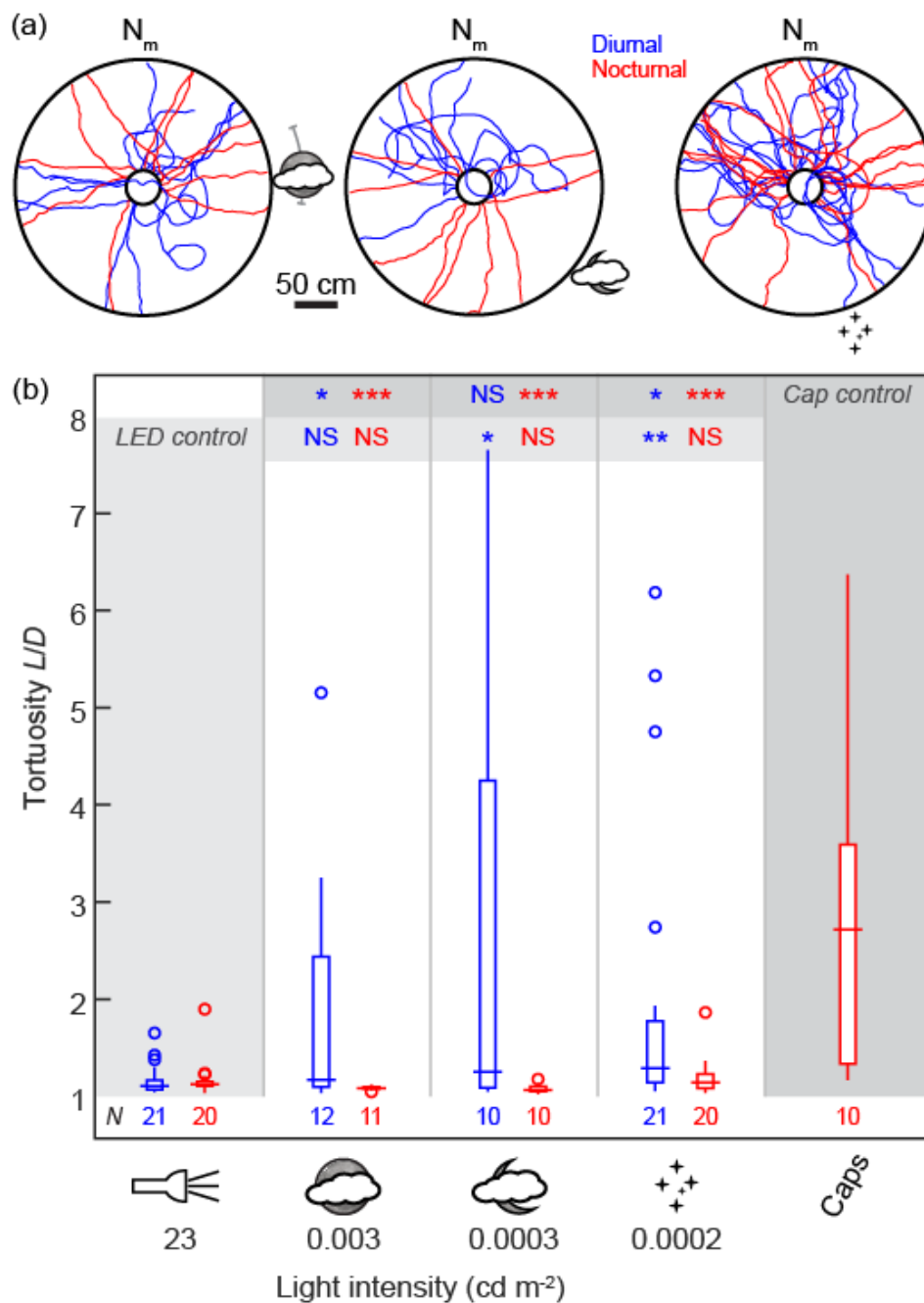
369 Surprisingly, we found that both species performed equally well in all four conditions,
370 and that diurnal beetles oriented as well as nocturnal beetles during day and night (see Fig. 2,
371 results of specific comparisons in Table 1 and linear model analysis in Table 2). While there
372 was a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

384

385 *Orientation to polarised light pattern and starry sky*

386 Even when neither the sun nor the moon itself is visible, dung beetles can still orient using
387 wide-field celestial cues, such as the pattern of polarised skylight surrounding the sun or
388 moon (Dacke et al., 2003; el Jundi et al., 2014), gradients of intensity across the sky (el Jundi

389 et al., 2014) or the Milky Way (Dacke et al., 2013b). To test whether nocturnal beetles are
 390 better than diurnal beetles at keeping a straight line when the moon is hidden from sight, we
 391 compared the orientation performance of both species under three conditions where no major
 392 celestial body was visible: (1) on a full moon night with the moon shaded from view by a
 393 large wooden board, (2) on a crescent moon night with the moon similarly shaded, and (3) on
 394 a moonless night, illuminated only by the light of the stars.



395

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

419
420 Under these conditions, dimmer and arguably more difficult than under even the smallest
421 moon, diurnal and nocturnal beetles no longer oriented equally well (Fig. 3; linear model in
422 Table 2). The paths of nocturnal beetles, on the one hand, were not significantly more
423 tortuous than in the bright light control (Fig. 3b, red bars; Table 1), and the spread of the data
424 did not change (multi-sample Brown-Forsythe test, $F_{3,57} = 1.6$, $P = 0.19$), indicating that
425 these beetles orient equally well to wide-field cues, such as the lunar polarisation pattern or
426 the Milky Way, as they do to when a point light source such as the moon is also available.
427 The paths of diurnal beetles, on the other hand, became substantially less straight once the
428 moon was not visible (Fig. 3b, blue bars; Table 1). While the median tortuosity was only
429 significantly larger than in the control in two of the three wide-field conditions, the spread of
430 tortuosities increased significantly – by up to 30-fold – in all three (Brown-Forsythe tests: full
431 moon shade: $F_{1,31} = 6.4$, $P = 0.017$; crescent moon shade: $F_{1,29} = 9.4$, $P = 0.0047$; stars:
432 $F_{1,40} = 5.1$, $P = 0.029$).

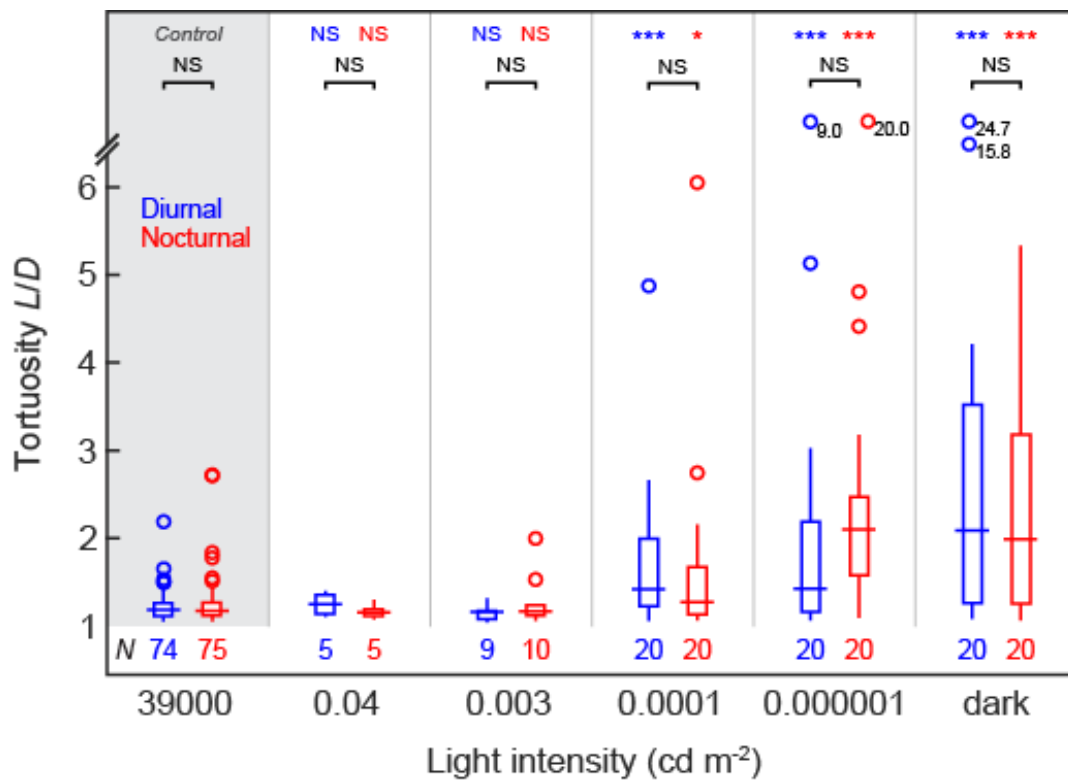
433 Taken together, these results show that, without a dominant point light source as an
434 orientation cue, diurnal dung beetles cannot use the available wide-field cues (polarised light
435 and stars) as effectively as nocturnal beetles can. However, it is interesting to note that even
436 in the dimmest light (under starlight), the paths of diurnal beetles were, on average, only a
437 moderate 17 per cent longer than in their control condition (median tortuosity of 1.29 and
438 1.11, respectively), indicating that even under these extreme conditions a large proportion of
439 diurnal beetles were still able to keep a straight path. This fact becomes even clearer when we
440 compare the diurnal beetles' paths to those of nocturnal beetles whose view of a starlit sky
441 was occluded by a cardboard cap fixed to their head (data from Dacke et al., 2013b, re-
442 analysed to match the two-minute time-out and track filtering used in this study; Fig. 3b,
443 right-most red bar). The paths of these truly lost beetles have a median tortuosity of 2.72,
444 which is significantly larger than that of the diurnal beetles' paths under almost all conditions
445 (Fig. 3b, upper row of significance markers; Table 1). Only under a shaded crescent moon
446 were the diurnal beetles as poorly oriented as the capped nocturnal beetles. In summary, the
447 orientation performance of nocturnal beetles is not affected at night when the moon is not
448 directly visible. Diurnal beetles, on the other hand, are worse at orienting to wide-field
449 orientation cues (polarisation pattern and stars) than nocturnal beetles, but significantly better
450 than could be expected if they were not using these cues at all.

451

452 *Orientation to a simulated celestial body*

453 The fact that diurnal and nocturnal beetles orient equally well under an open sky at night
454 (Fig. 2) but not when the moon is shaded (Fig. 3) suggests that the two species are equally
455 good at measuring the moon's azimuth for orientation. To test this hypothesis, and to
456 investigate what happens to their orientation at intensities lower than that of the crescent

457 moon, we tested both species indoors, with only a dimmable point light source for
 458 orientation.



459
 460 **Figure 4:** Nocturnal and diurnal beetles orient equally well to point light sources in the
 461 laboratory. Tortuosity of rolling paths of diurnal (blue) and nocturnal (red) dung beetles when
 462 placed in a 1.6-metre diameter arena in the laboratory (paths in Fig. A1). Tests were
 463 performed with a bright spotlight (control) or a dimmable tungsten lamp at a range of
 464 different light intensities covering and exceeding the range of light intensities measured under
 465 natural nocturnal conditions. As light intensity decreased, tortuosities started increasing
 466 significantly in both species (compared to the respective controls). This increase in tortuosity
 467 occurs at 0.0001 cd m⁻², a light intensity slightly lower than the lowest intensity measured in
 468 the field, on a moonless night (blue/red significance indicators reflect the results of one-sided
 469 Wilcoxon rank-sum tests comparing tortuosities to the relevant same-species control).
 470 However, tortuosities were never significantly different between species at the same light
 471 intensity (black significance indicators). Box-plots show median, inter-quartile range and
 472 outliers (any points more than 1.5 inter-quartile ranges above the 75th percentile or below the
 473 25th percentile). Four outliers are presented at a tortuosity of ca. 7 with their actual values
 474 indicated beside them. Numbers at the bottom indicate sample size (N). *: *P* < 0.05; NS: not
 475 significant.

476
 477 The results (Fig. 4; paths in Fig. A1) confirmed what we found in the field: Diurnal beetles
 478 and nocturnal beetles oriented equally well under all conditions (pairwise comparisons in
 479 Table 1; linear model in Table 2). Both species' paths became more tortuous as we decreased
 480 the light intensity, but they did so equally (i.e. there was an effect of light intensity, but no

481 interaction between light intensity and species). At the third test intensity (0.0001 cd m^{-2} ; five
482 times dimmer than the crescent moon, and 8.5 orders of magnitude dimmer than the control;
483 Fig. 4, fourth column), both species performed significantly worse than in the control
484 condition (Table 1). The same was true for the fourth test intensity ($0.000001 \text{ cd m}^{-2}$; 500
485 times dimmer than crescent moon; Fig. 4, fifth column) and in complete darkness (Fig. 5,
486 right-most column). Using linear models, we also explored other factors that could have
487 influenced the beetles' orientation performance (Table 2): We performed approximately half
488 of the experiments at night, and half during the day, but this did not affect orientation
489 performance. Similarly, orientation performance did not depend on whether the bright light
490 control was presented to the animal as the first or second stimulus condition.

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

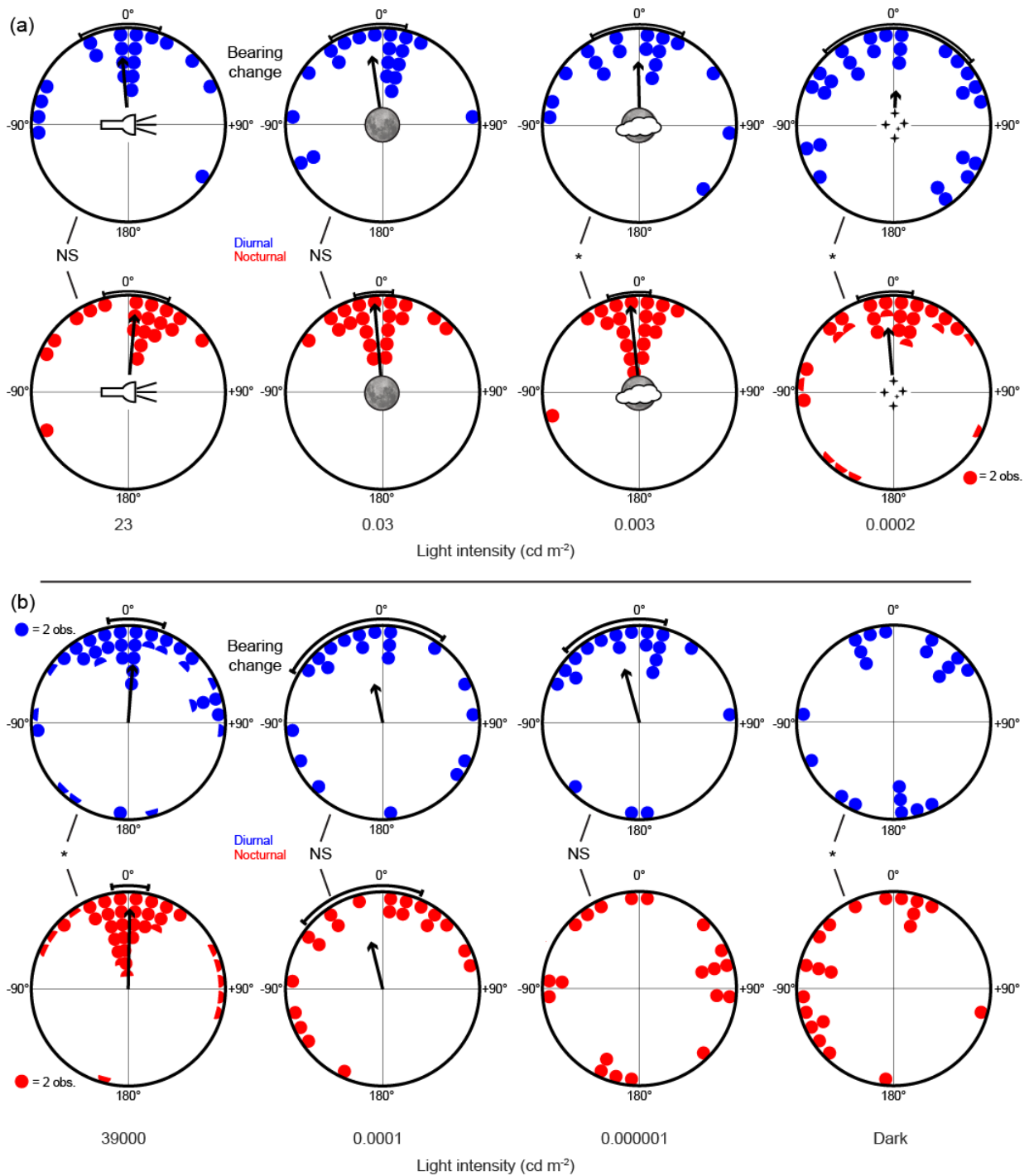
494

495 *Bearing fidelity*

496 Moving in a straight line is impossible without some kind of external compass cue (Cheung
497 et al., 2007; Souman et al., 2009). However, on a small scale, an animal could walk in a
498 reasonably straight line by simply keeping the image on its retinae constant and correcting for
499 any perceived rotations, a behaviour known as the optomotor response (review: Wehner,
500 1981). If such optomotor cues were used exclusively, however, it would be exceedingly
501 difficult to regain a chosen bearing after a major disturbance, e.g. if a beetle falls off its dung
502 ball.

503 To test how well beetles could regain their rolling bearing at night, and whether the
504 orientation mechanisms differ between diurnal and nocturnal beetles in dim light, we allowed
505 a new group of beetles to roll their balls out of the arena three times, and calculated the

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



525

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

536 mean. The spread of bearing changes is equal for both species with an artificial light or a full
537 moon, but is larger for diurnal beetles under a shaded full moon or the starry sky (black
538 significance indicators reflect the results of Mardia-Watson-Wheeler tests comparing
539 between species), reflecting decreased orientation performance for diurnal beetles under these
540 conditions (Fig. 3). For clarity, the bottom right diagram only displays one dot for each two
541 observations; half-dots indicate single observations. *: $P < 0.05$; NS: not significant. **(b)**
542 Bearing changes after being returned to the arena centre for diurnal and nocturnal beetles in
543 the laboratory. The highest light intensity at which beetles did not significantly regain their
544 chosen direction was $0.000001 \text{ cd m}^{-2}$ (500 times dimmer than crescent moon light). For
545 clarity, the two left-most diagrams only display one dot for each two observations; half-dots
546 indicate single observations. Statistical tests, half-dots and significance indicators as in (a).
547

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

566 cues. However, the high tortuosity of their paths (Fig. 4) and the large spread of bearing
567 changes (Fig. 5b, bottom right) suggest that this might have been a chance result.

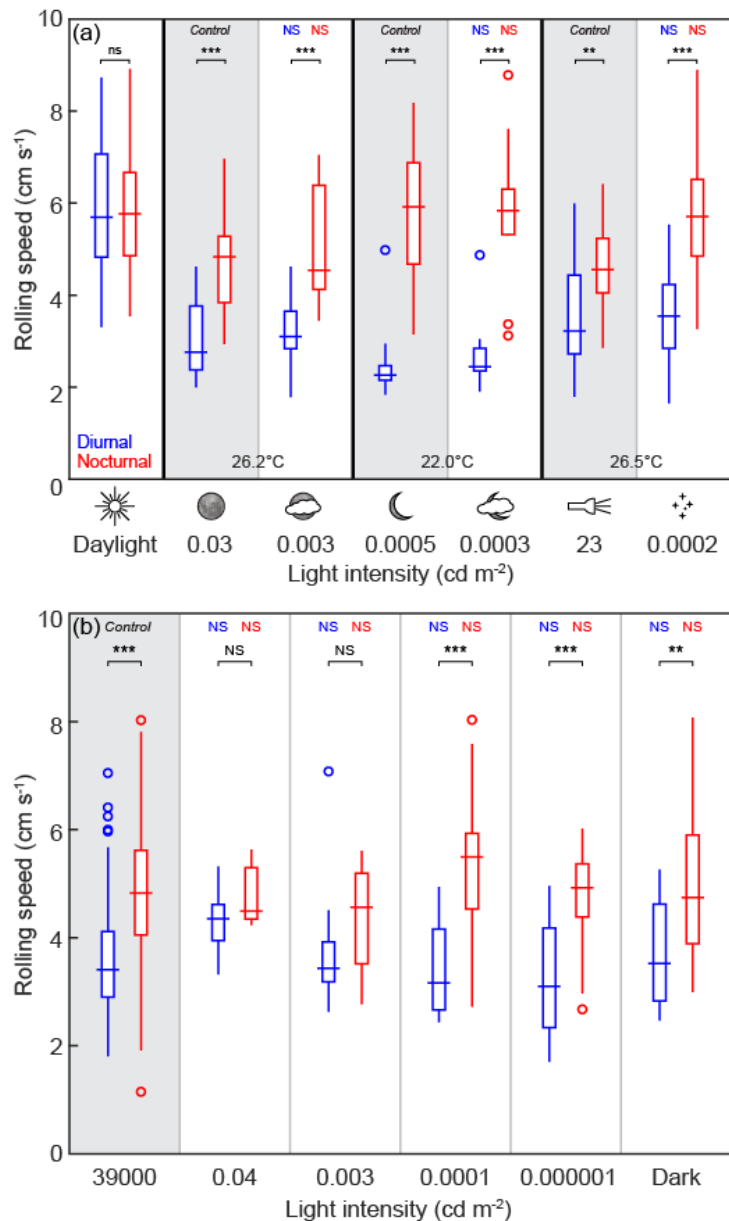
568 The large spread that we observed in both tortuosities (Figs 3-4) and bearing changes
569 (Fig. 5) in groups of poorly oriented beetles could result from two sources: (1) From variation
570 across trials, due to the random nature of cumulative orientation errors (Cheung et al., 2007).
571 An animal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

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

591

592 *Beetles do not reduce their rolling speed in dimmer light*

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



608

609 **Figure 6:** Rolling speed is not related to light intensity. For each roll path (data from Figs 2
 610 & 3), the average movement speed (excluding pauses) was calculated. (a) Rolling speeds
 611 during field experiments. During the day (first column), diurnal (blue) and nocturnal (red)
 612 beetles rolled at the same speed. In all 6 conditions at night, nocturnal beetles rolled
 613 significantly faster than diurnal beetles (black significance markers indicate result of one-
 614 sided Wilcoxon rank-sum tests), but neither species rolled more slowly in dim-light
 615 conditions when compared to appropriate controls (blue/red significance markers; see text for
 616 details), indicating that the slower rolling speed at night was related to body temperature (or
 617 other environmental factors), not to light intensity. This, in turn, indicates that beetles do not
 618 use temporal integration in their visual or nervous system to deal with lower light intensities.
 619 Box-plots show median, inter-quartile range and outliers (any points more than 1.5 inter-
 620 quartile ranges above the 75th percentile or below the 25th percentile). (b) Rolling speeds
 621 during laboratory experiments. Calculation, colours and statistics as in (a). While nocturnal
 622 beetles rolled faster than diurnal ones in most conditions (and overall; see text for details of a
 623 linear model), there was no indication of lower rolling speeds at lower light intensities. ***:
 624 $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$; NS: not significant.

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

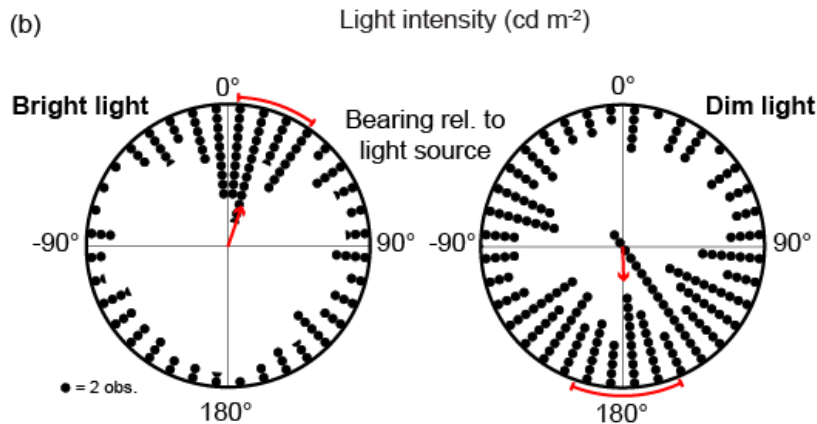
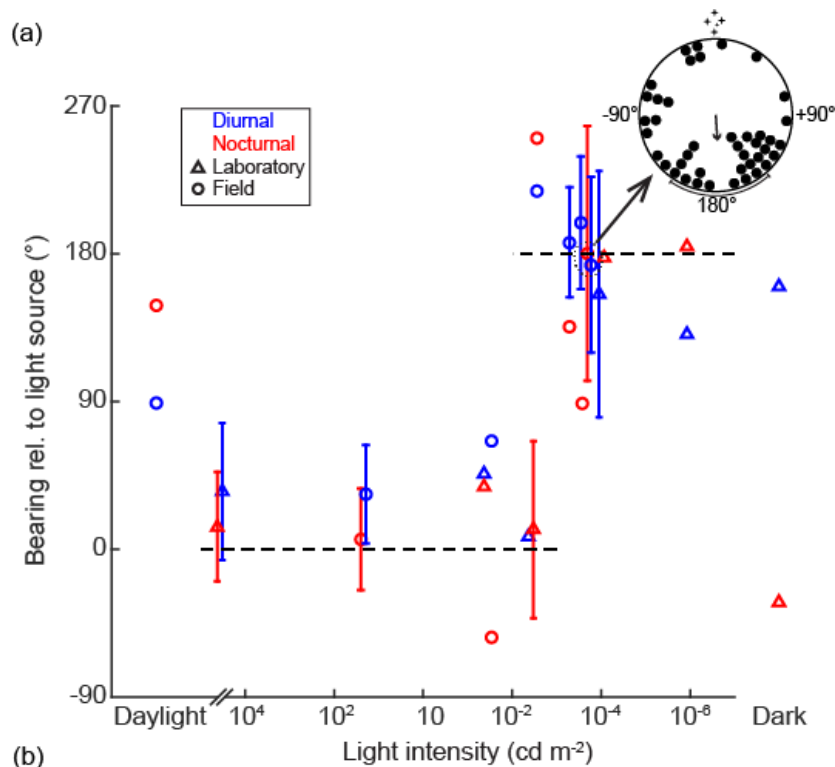
639 In the laboratory, we found once again that nocturnal beetles rolled on average 36 per
640 cent faster than diurnal beetles (4.9 cm/s vs. 3.6 cm/s), but there was no indication that speed
641 depended on light intensity or that the speed difference between species depended on light
642 intensity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

648

649 *Bearing selection is biased and depends on light level*

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

662



663

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

682 When we compared the circular mean of the beetles' chosen bearings across all
683 experiments, a clear pattern emerged (Fig. 7a): At higher light intensities, beetles tended to
684 roll towards the light source, whereas at lower light intensities, they tended to roll away from
685 it. To analyse this bias in bearing selection statistically, we combined the data from all
686 experiments (except those in sunlight and those in complete darkness), and split them into a
687 bright light (light intensities greater than full moon shade; Fig. 7b, left diagram) and a dim
688 light group (light intensities less than or equal to full moon shade; Fig. 7b, right diagram).
689 The results confirmed that the bearings chosen in bright light were significantly clustered
690 with a mean of 19.0° (Rayleigh-test, $Z = 22.78$, $N = 257$, $P < 0.0001$), which would take the
691 beetle towards the light source. The bearings chosen in dim light were also significantly
692 clustered (Rayleigh-test, $Z = 12.01$, $N = 196$, $P < 0.0001$), but with a mean of 178.2° , which
693 is almost directly away from the light source. A comparison between distributions confirmed
694 that they are significantly different at different light levels (Circular two-way ANOVA,
695 Harrison and Kanji, 1988; $\chi^2_2 = 64.4$, $P < 0.0001$), but there is no evidence that they are
696 different between the two species ($\chi^2_2 = 5.8$, $P = 0.055$) or that different light levels affected
697 the bearing choice of diurnal and nocturnal beetles differently (interaction: $\chi^2_1 = 1.4$,
698 $P = 0.83$). It should be noted that both distributions are comparatively broad (circular
699 standard deviations of 89° and 96° for bright and dim light, respectively), suggesting that
700 even under dim light conditions, beetles do not simply perform a phototactic behaviour.
701 Instead, they choose from the full range of possible bearings, but do so with a bias at the
702 population level.

703 One reason for beetles to choose a particular bearing in a visually challenging orientation
704 environment could be that successful orientation is more likely when the most salient visual
705 cues are kept in a particularly sensitive part of the visual field. If that was the case, we would
706 expect to see a correlation between an animal's first chosen bearing (relative to the light

707 source) and the error it makes in regaining that bearing after a disturbance. When we
708 analysed this for all bearing fidelity data (data from Fig. 5), we only found two relevant
709 significant correlations (Fig. A4): (1) Under a shaded full moon, diurnal beetles made smaller
710 errors when rolling towards the moon, a bearing which placed the area of highest skylight
711 polarisation in their fronto-dorsal visual field. (2) Under the starry sky, diurnal beetles made
712 smaller errors when rolling away from the brightest spot of the Milky Way, a bearing which
713 placed this bright spot in their fronto-dorsal visual field. While we found no overall trend
714 when combining all data for the "bright" and "dim" conditions (as defined above; Fig. A4c),
715 these two examples indicate that there might, indeed, be an orientation advantage of certain
716 bearings under particularly challenging environmental conditions.

717

718 **Discussion**

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

729

730 *Celestial cues for orientation in bright and dim light*

731 Before discussing the broader implications of our results, it may be useful to consider which
732 parts of the celestial light field the two different beetle species used as their main orientation
733 cues in our different experiments. The diurnal species we investigated seems to follow a
734 comparatively simple strategy. The fact that these beetles were well-oriented under a crescent
735 moon (Fig. 2b), but that at least some beetles were disoriented under a shaded full moon (Fig.
736 3b) – even though the ambient light intensity was six times higher in the latter situation –
737 suggests that they rely heavily on measuring the position of the moon itself, rather than
738 reading wide-field cues such as the polarisation pattern or gradients of intensity and colour.
739 This result is consistent with previous studies, which show that these diurnal beetles follow a
740 180° shift of the sun's or moon's apparent azimuth, but will follow a 90° shift in the
741 polarisation pattern only when their view of the celestial body itself is blocked (Byrne et al.,
742 2003; Dacke et al., 2014; el Jundi et al., 2014, 2015). The diurnal species we investigated
743 therefore seems to always weight the available cues in the same way – using the azimuth of a
744 celestial body over wide-field cues – independent of light intensity. Nocturnal beetles, on the
745 other hand, follow different strategies in different light intensities. While they too use the
746 position of a celestial body as a primary cue in very bright light, they prioritise the lunar
747 polarisation pattern over the moon itself at night (el Jundi et al., 2015). This difference in
748 strategy between day- and night-active beetles can even be found in compass neurons in the
749 beetle brain (el Jundi et al., 2015), and is most likely based on the different relative size of the
750 dorsal rim area in each species, the part of the eye specialised for polarised light processing.
751 In the diurnal species, this area is only one or a few ommatidial rows wide, while in
752 crepuscular and nocturnal beetles, it can take up half of the dorsal eye (Dacke et al., 2003b;
753 Dacke, Smolka and Ribi, unpublished data). It is therefore possible and likely that in any
754 experiments where beetles had a full view of the clear night sky (Fig. 2), different parts of the

755 sky were used as primary orientation cues by the two species: diurnal beetles seem to have
756 relied more on the moon itself, while nocturnal beetles probably weighted the lunar
757 polarisation pattern more strongly.

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

777 The lower extreme of natural nocturnal light intensities can be found during moonless
778 nights, when neither the moon itself nor the polarisation pattern is available to guide the
779 beetles' paths. The stars and, in particular, the Milky Way provide the only celestial light

780 available to the beetles. Although diurnal beetles rolled along more tortuous paths than
781 nocturnal beetles in this condition, the difference in median tortuosities was small (1.29 vs
782 1.14) and, more importantly, the diurnal beetles' orientation performance was still
783 substantially better than that of cap-wearing, truly lost beetles (Fig. 3). This, surprisingly,
784 indicates that some diurnal beetles were still able to orient using the dim starlight. It will be
785 interesting to see in future experiments which particular cues they were using, and whether
786 these are the same cues that nocturnal beetles use under these circumstances.

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

803

804 *Anatomical and neuronal specialisations for higher sensitivity*

805 What are the visual adaptations that allow nocturnal dung beetles to orient in almost perfectly
806 straight lines under practically all nocturnal conditions? Two main modifications have been
807 suggested to increase the sensitivity of the visual system in nocturnal animals. The first of
808 these is anatomical: by creating larger apertures and shorter focal lengths, and by widening
809 and elongating the photoreceptive structures (i.e. the rhabdoms in insect eyes), a brighter
810 image is created on the retina. Optical modelling and intracellular recordings from
811 photoreceptors in the eyes of onitine dung beetles show that a nocturnal beetle's eye can be
812 made up to 85 times more sensitive in this way compared to that of a diurnal beetle
813 (Frederiksen and Warrant, 2008; McIntyre and Caveney, 1998). Preliminary histological data
814 suggest that the difference in optical sensitivity between the diurnal and nocturnal species
815 investigated in the present study might be of a similar magnitude (Dacke, Smolka and Ribí,
816 unpublished data). The second set of adaptations that increase the sensitivity of nocturnal
817 visual systems can be found at the retinal and neuronal level: by increasing photoreceptor
818 gain and integration times, the signal that is sent to the brain can be boosted (Frederiksen,
819 2008). Higher neural mechanisms, such as spatial and temporal summation of photoreceptor
820 signals in the optic lobes, can then further increase the signal-to-noise ratio and potentially
821 enhance the sensitivity of an insect visual system by as much as 100 000 times again
822 (Warrant 1999).

823 Why then did we not find nocturnal beetles to be more sensitive than diurnal beetles
824 when orienting to a point light source indoors (Fig. 4)? Three reasons come to mind. Firstly,
825 due to the specific optics of a refracting superposition compound eye, the sensitivity
826 advantage of the nocturnal optics can only be fully utilised for extended light sources, and not
827 for point light sources (for a detailed explanation, see Warrant and McIntyre, 1990;
828 Frederiksen and Warrant, 2008). This explanation is consistent with the result that nocturnal

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

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

866

867 *Bearing selection at night*

868 When they first leave the dung pile, dung beetles select a roll bearing apparently at random
869 (Baird et al., 2010), with environmental factors, such as the position of the sun, introducing
870 only small population biases under certain circumstances (Baird et al., 2010; Byrne et al.,
871 2003; Reber, 2012). In our experiments, we found a weak but reliable population bias of
872 beetles moving towards a bright dominant light source, and away from a dim one (Fig. 7).

873 The fact that the beetles' bearing choices were biased in both of these circumstances
874 suggests that there are distinct visual advantages in both situations. In comparatively bright
875 light, it might be advantageous to roll a ball towards a light source because this places it
876 between the beetle and the light, thereby reducing the risk that large parts of the retina will
877 become light-adapted. For an insect with superposition eyes, this is even more important at

878 night than during the day, because the enlarged superposition pupil will transmit the direct
879 light of the bright source onto a greater number of photoreceptors. While it might seem
880 problematic, at first, for the beetle to move in a way that obscures one of its main orientation
881 cues, it should be kept in mind that, under natural conditions, there are always wide-field cues
882 available in addition to point sources. In fact, since the area of strongest polarisation in the
883 sky is found at a 90° angle to the sun or moon direction, the backwards-rolling beetles are
884 placing that area in their fronto-dorsal visual field, and probably optimising the signal
885 generated in the dorsal rim area when rolling into the light.

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

902 when carefully tested in controlled experiments, e.g. by shading different parts of the sky or
903 by rotating a simulated "Milky Way" in the laboratory.

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

913

914 *Conclusion*

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

923

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931

932 **Competing interests**

933 The authors declare no competing interests.

934

935 **Author contributions**

936 Study concept and design of behavioural experiments: J.S., M.B., E.B., M.D. Data

937 acquisition of behavioural data: J.S., B.E.J., M.B., T.R., E.B., M.D. Data analysis: J.S.

938 Interpretation of data: J.S., B.E.J., M.B., E.B., M.D. Drafting of the manuscript: J.S. Critical

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944

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- 1088
- 1089

1090 **Table 1**

1091 Pairwise comparisons (one-tailed Wilcoxon rank-sum tests) of path tortuosities under
 1092 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		

1093 With a **full view of the sky**, beetles are always at least as well oriented as in the LED control
 1094 condition. When the moon is shaded and **wide-field cues only** are available, however, diurnal
 1095 beetles are less well oriented in two out of three conditions, while nocturnal beetles still
 1096 perform as well as in the control. However, in every condition but one (under a shaded
 1097 crescent moon), diurnal beetles still perform better than truly lost beetles that had their view
 1098 of the sky blocked by a cardboard cap glued to their head. With an artificial **point-light**
 1099 **source** in the laboratory, beetles first become less well-oriented than in the control at a light

1100 intensity of 0.0001 cd m⁻² (five times dimmer than under a crescent moon). However, diurnal
 1101 and nocturnal beetles perform equally well under all conditions.

1102

1103 **Table 2**

1104 Results of linear models exploring factors influencing tortuosity in the field and laboratory

Experiment	Independent variables	<i>F</i>	<i>df</i>	<i>P</i>
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

1105 With a **full view of the sky**, no difference was found in orientation performance under
 1106 different moon phases, or between species. Once the moon was shaded and **wide-field cues**
 1107 **only** were available, diurnal beetles performed significantly worse than nocturnal beetles.
 1108 With an artificial **point-light source** in the laboratory, orientation performance decreased at
 1109 lower light levels, but it did so equally in both species. Other factors (time of day or the order
 1110 of presented conditions) were not found to influence orientation performance.

1111

1112 **Table 3**

1113 Tests for directed distributions of bearing changes (*V*-tests)

	Species	Light condition	<i>V</i>	<i>N</i>	<i>P</i>
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

1114 We calculated the difference in bearing before and after a beetle was removed from its ball
 1115 and returned to the arena centre. In the **field**, this bearing difference was always significantly
 1116 clustered around 0°, i.e. the beetles returned to their previously chosen bearing after a
 1117 disturbance. In the **laboratory**, this was also the case except in total darkness and under the
 1118 lowest illumination condition for the nocturnal species.

1119

1120 **Table 4**

1121 Pairwise comparisons of circular distributions of bearing changes (Mardia-Watson-Wheeler
 1122 tests)

	Light condition	<i>W</i>	<i>N</i> ₁	<i>N</i> ₂	<i>P</i>
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

1123 We calculated the difference in bearing before and after a beetle was removed from its ball
 1124 and returned to the arena centre, and compared the distribution of these changes in bearing
 1125 between diurnal and nocturnal beetles. In the **field**, we found no significant difference in the
 1126 re-orientation accuracy between species in the control condition and under a full moon, but
 1127 found a significant difference under a shaded full moon and starlight, most likely due to the
 1128 fact that the spread of the distribution is substantially larger for the diurnal species under
 1129 these conditions, indicating a poorer re-orientation performance. In the **laboratory**, we found
 1130 no difference between species except in complete darkness.

1131

1132 **Table 5**

1133 Pairwise comparisons (one-tailed Wilcoxon rank-sum tests) of rolling speeds under different
 1134 light conditions

Experiment	Species	Light condition	<i>W</i>	<i>N</i> ₁	<i>N</i> ₂	<i>P</i>	
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		

1135 Both in the **field** and in the **laboratory**, we found no indication that beetles rolled more
 1136 slowly in dimmer light, as might have been expected if the beetles use temporal integration to
 1137 create a more reliable visual signal.

1138

1139 **Table 6**

1140 Results of linear models exploring factors that influence rolling speed in the laboratory

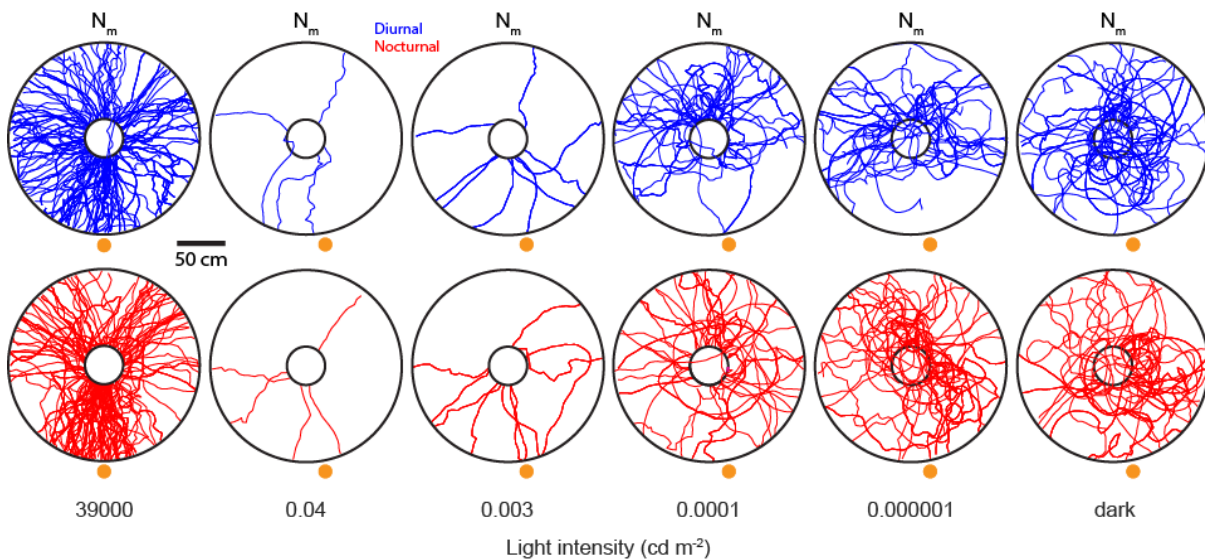
Experiment	Independent variables	<i>F</i>	<i>df</i>	<i>P</i>
Rolling speed	Species	94.8	1, 296	<0.0001
	Light condition	0.89	5, 291	0.49
	Species * Light condition	1.19	5, 286	0.29

1141 While diurnal beetles rolled their balls on average significantly slower than nocturnal beetles,
 1142 there was no effect of light intensity on rolling speed, as might have been expected if the
 1143 beetles use temporal integration to create a more reliable visual signal.

1144

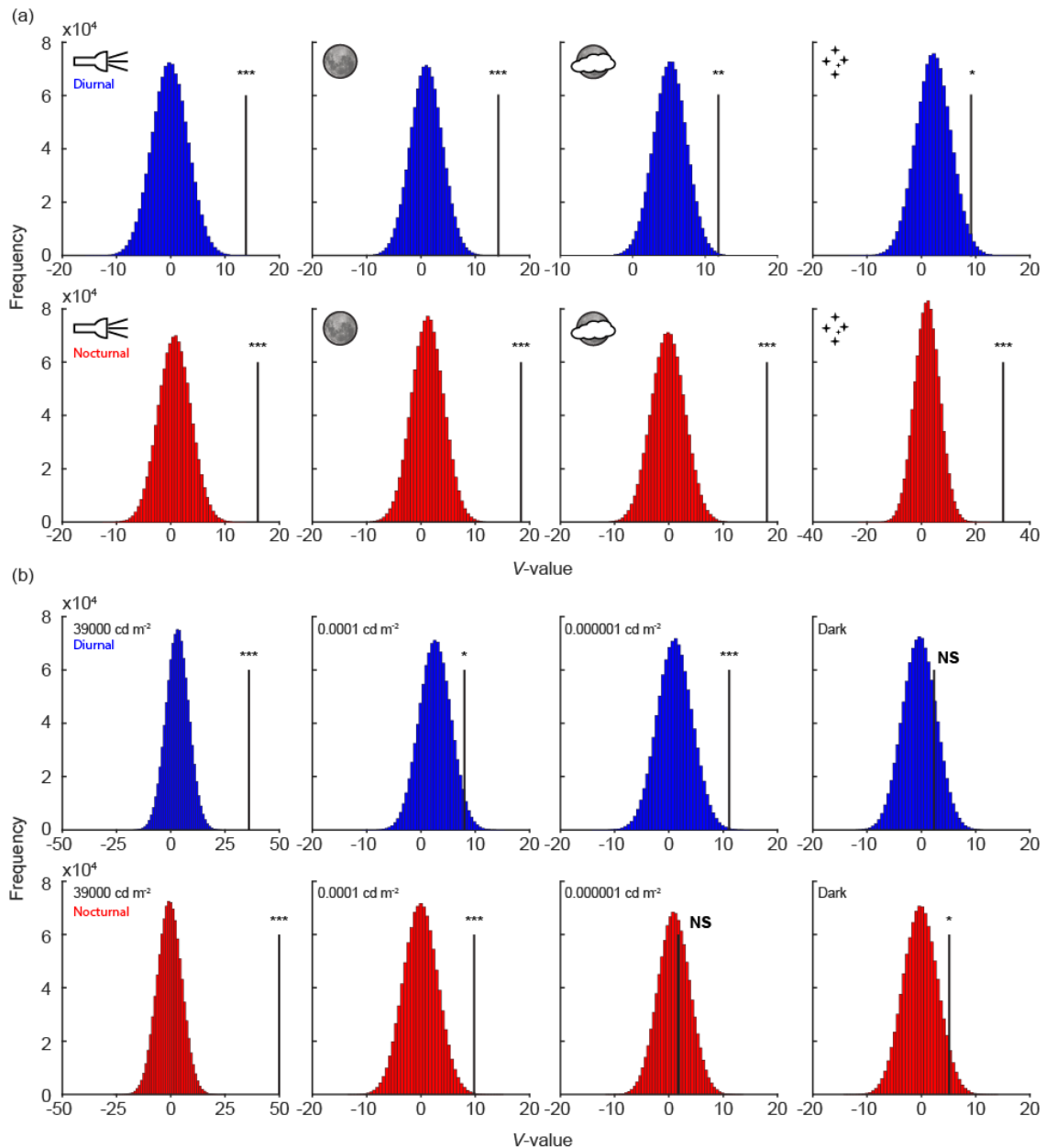
1145 **Appendix**

1146



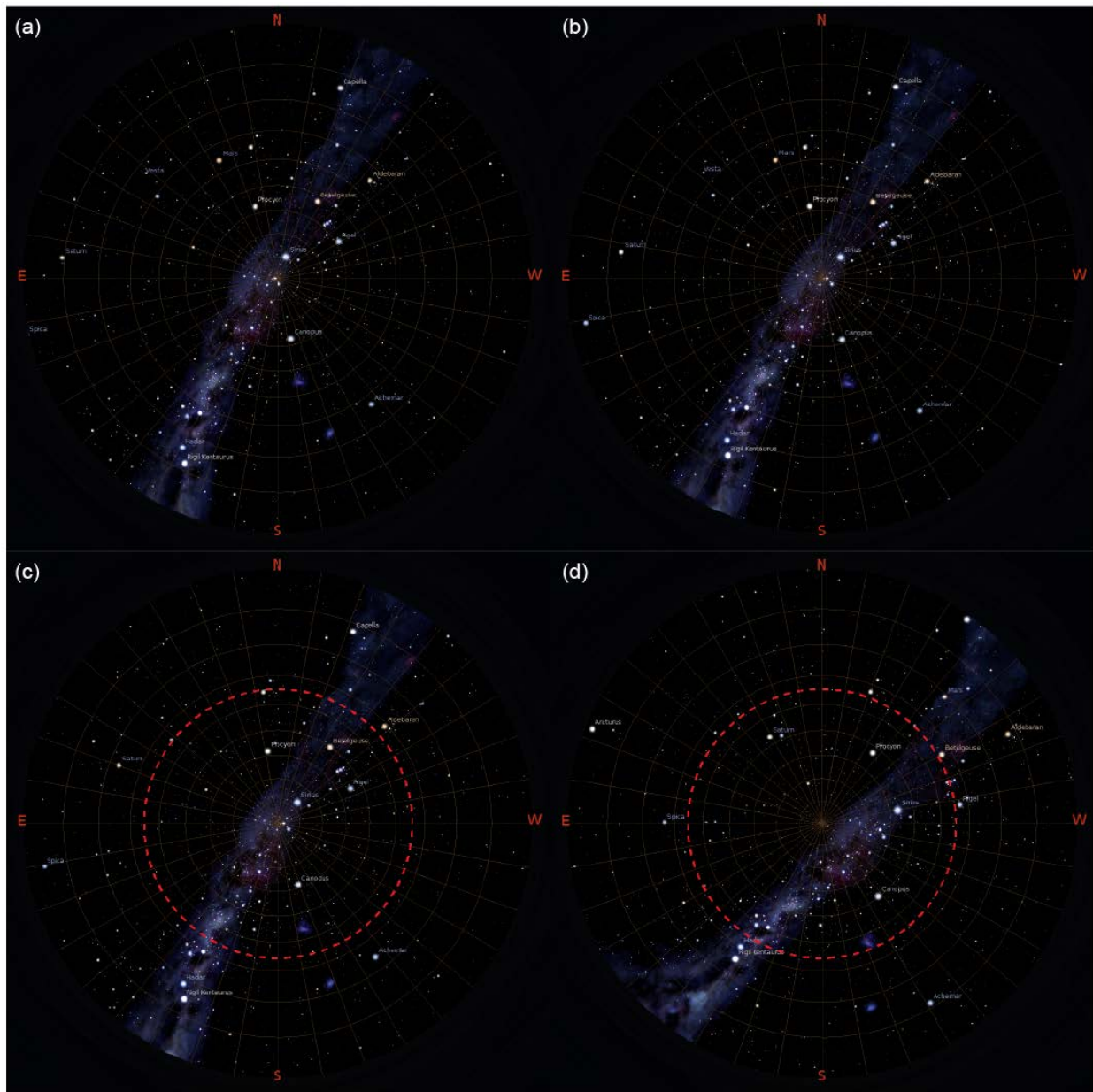
1147

1148 **Figure A1:** Paths of beetles leaving a 1.6-metre indoor arena. Bird's eye view of the rolling
 1149 paths of diurnal (blue) and nocturnal (red) dung beetles when placed in a 1.6-metre diameter
 1150 indoor arena (see Fig. 4). Tests were performed with a bright spotlight (control, 39000 cd m⁻²,
 1151 1st condition) or a dimmable tungsten lamp at a range of different light intensities covering
 1152 and exceeding the range of light intensities measured under natural nocturnal conditions (2nd
 1153 to 6th condition). As light intensity decreases, paths become more tortuous. See methods for
 1154 details of path filtering. Top of each diagram is local magnetic North (N_m), orange circles
 1155 indicate the position of the light source.



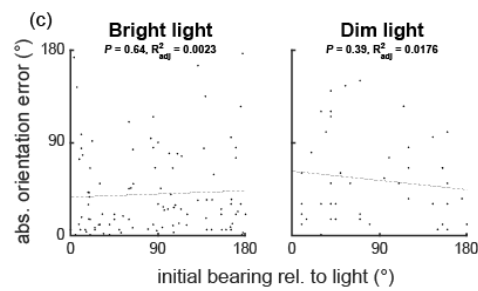
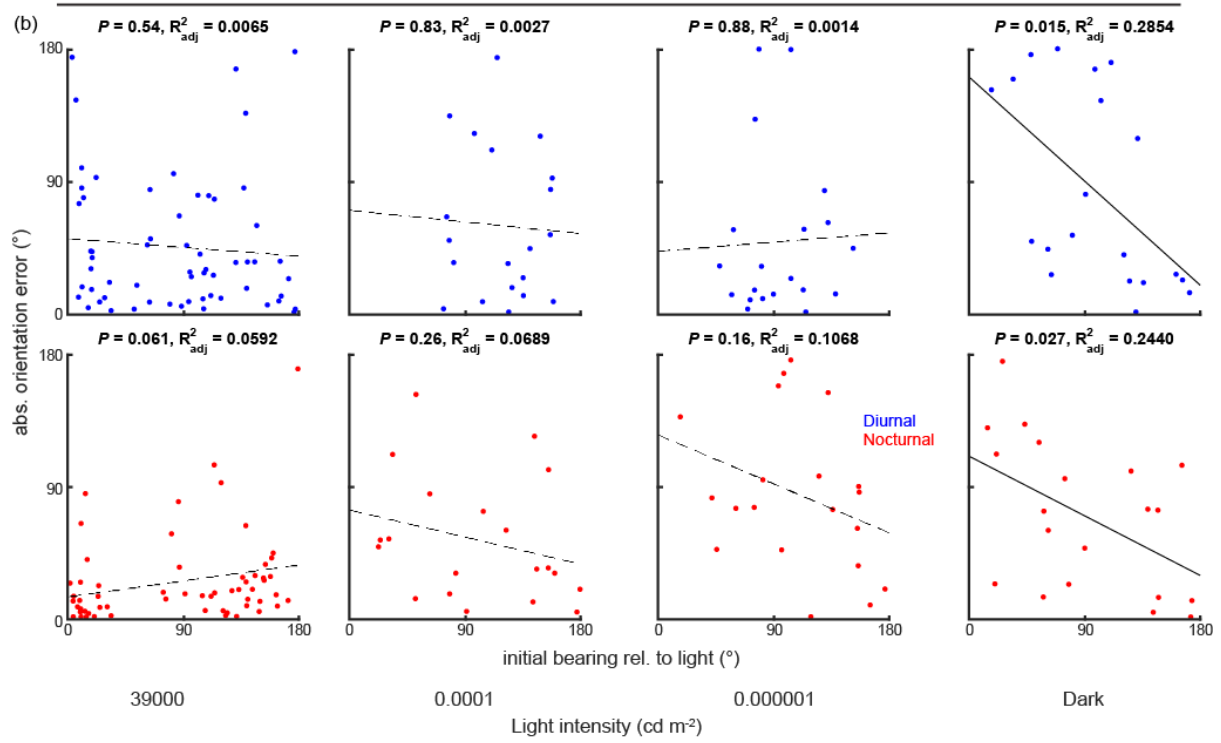
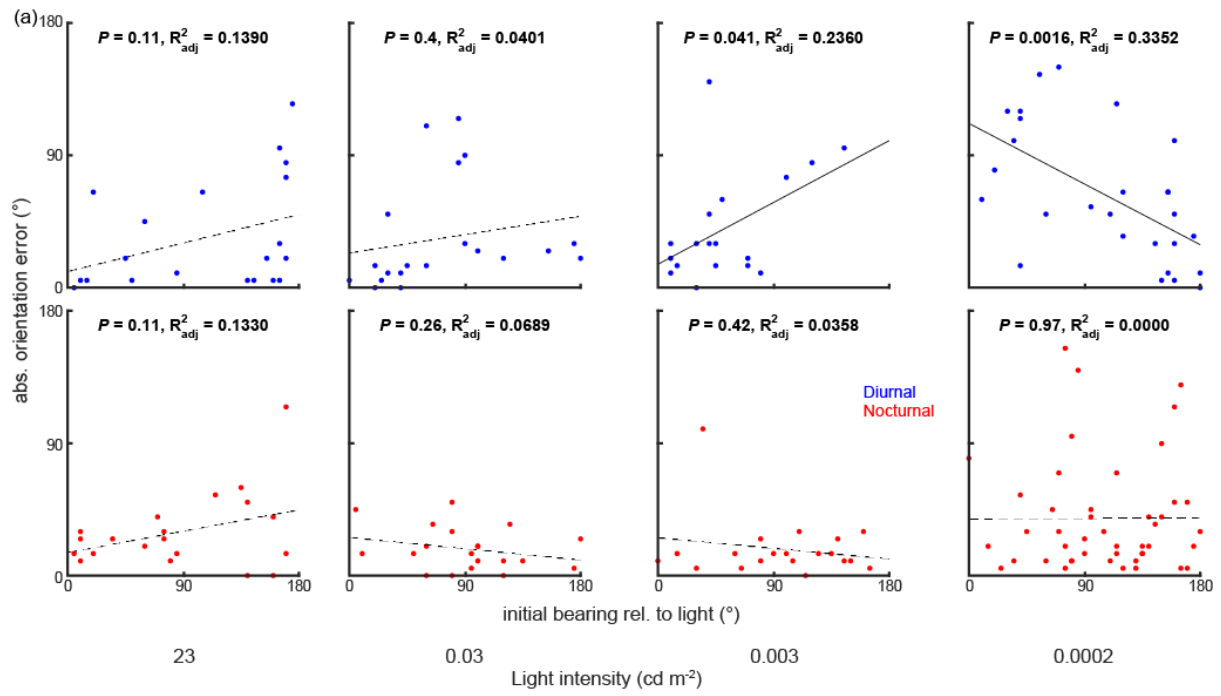
1156

1157 **Figure A2:** Permutation tests for all bearing fidelity experiments. Data and order of
 1158 subpanels as in Fig. 5. Measured bearings were randomly permuted across individuals and
 1159 experimental conditions. The plots show the V -value obtained from 1,000,000 random
 1160 permutations. Black lines indicate the unpermuted V -values. Probabilities were calculated as
 1161 the proportion of permutations that resulted in a higher V -value than that obtained from the
 1162 unpermuted data (equivalent to a one-sided test). ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$;
 1163 NS: not significant. **(a)** Experiments were performed in the beetles' natural habitat with
 1164 diurnal (blue) and nocturnal (red) beetles at night, with illumination from (from left to right)
 1165 (1) an artificial light, (2) the full moon, (3) the shaded full moon or (4) only starlight. The
 1166 difference between consecutive bearing choices was calculated, and was significantly
 1167 clustered around 0° in all cases, indicating that beetles did not just use optomotor cues to
 1168 keep to a straight line, but could re-acquire their bearing even after a significant disturbance.
 1169 **(b)** Bearing changes after replacement to the arena centre for diurnal and nocturnal beetles
 1170 indoors. The highest light intensity at which beetles did not significantly regain their chosen
 1171 direction was $0.000001 \text{ cd m}^{-2}$ (500 times dimmer than crescent moon light).



1172

1173 **Figure A3:** Simulated view of the starry sky. (a, b) Night sky on the two moonless
 1174 experimental days of the present study showing the position of the Milky Way and the
 1175 brightest visible stars and planets. (c, d) Night sky during previous studies examining the
 1176 Milky Way orientation behaviour of *S. satyrus*. (c: Dacke et al., 2011; d: Dacke et al., 2013b).
 1177 In both of these studies, the test arena was surrounded by a 1-metre high wall to block the
 1178 beetles' view of the landmark panorama. From the centre of the arena, this wall would have
 1179 blocked any skylight cues below 34°, i.e. any light outside the red dashed circle. If the
 1180 beetles, as we hypothesise, use the brightest Southern part of the Milky Way for orientation,
 1181 parts of this pattern would have been blocked (to a smaller or larger extent, depending on a
 1182 beetle's position in the arena). Images were created in and exported from Stellarium 0.12.4
 1183 (Stellarium Developers, www.stellarium.org; full-sky Milky Way panorama by Axel
 1184 Mellinger, University of Potsdam, Germany).



1185

1186

1187 **Figure A4:** Re-orientation error after a disturbance does not generally depend on chosen
 1188 bearing. (a, b) We calculated the correlation between the initial bearing relative to the light
 1189 source (i.e. the absolute difference between bearing and light source azimuth) and the
 1190 absolute orientation error, i.e. the difference between this bearing and the bearing chosen
 1191 after the beetle was removed from its ball and returned to the arena centre. Conditions in the
 1192 field (a) and laboratory (b) are in the same order as in Fig. 5. After this disturbance, beetles
 1193 re-orient to their former bearing with a random error that depends on the lighting condition
 1194 (Fig. 5). A significant correlation was only found in two conditions (diurnal beetles under a
 1195 full moon and under the starry sky), indicating that in these visually difficult conditions,
 1196 beetles were more precise when they kept the main orientation cue in their fronto-dorsal
 1197 visual field. We also found a significant correlation for both species in the laboratory in
 1198 complete darkness, again indicating that there might have been a very weak non-visual cue
 1199 for the beetles to orient by (see Result for Fig. 5). (c) When data was pooled for all "bright"
 1200 and "dim" conditions (defined as in Fig. 7), no overall correlation was found.
 1201

1202 **Table A1**

1203 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		Shade: 2.8 x 10 ⁻³
	01/02/2010, 2215 – 0037	75 – 99	15 – 48	0.79		
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		---

1204 Time is local South African time (GMT + 2). Azimuth (0° represents local magnetic North;
 1205 90° East; 180° South; 270° West) and elevation (not corrected for atmospheric refraction)
 1206 ranges are given for the main light source available to the beetles at the time: sun, moon,
 1207 artificial light or the brightest (Southern) part of the Milky Way. Fullness of the moon is
 1208 reported as -cos of the three-dimensional angle between sun and moon (1 is full moon, 0
 1209 half-moon, -1 new moon).

1210

1211 **Table A2**

1212 Numbers of subjects included in field experiments

	Day		Art. light		Full moon		Full moon shade		Crescent moon		Crescent moon shade		Stars		Total
	D	N	D	N	D	N	D	N	D	N	D	N	D	N	
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
<i>n</i> tortuosities	21	21	21	20	19	19	12	11	10	10	10	10	21	20	225
<i>n</i> bearings/speeds	21	21	21	20	19	19	16	18	10	10	10	10	21	21	237
<i>n</i> repeated rolls	–	–	10	10	10	10	10	10	–	–	–	–	18	36	114

1213 For each experimental condition, the number of *beetles* of the diurnal (D) and nocturnal (N)
 1214 species that performed this condition is given. Each beetle only performed each condition
 1215 once, resulting in an equal number of *tracks* as beetles. The results for a total of twelve
 1216 beetles had to be *discarded* because of long gaps in their tracks due to insufficient lighting,
 1217 poor camera focus or obstructions. A total of 19 beetles did not reach the arena perimeter
 1218 within the two-minute time limit (*time-outs*). The final rows indicate the final number of
 1219 animals included in analyses of *tortuosities* (Figs 2-4), *bearings and speeds* (Figs 6-7) and
 1220 *bearing reacquisition* (Fig. 5).

1221

1222 **Table A3**

1223 Light conditions during laboratory experiments

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

1224 Time is local Swedish time (GMT + 1). Azimuth (0° represents local magnetic North; 90°
 1225 East; 180° South; 270° West) and elevation are given for the artificial light sources as seen
 1226 from the arena centre.