

## RESEARCH ARTICLE

# Diurnal dung beetles use the intensity gradient and the polarization pattern of the sky for orientation

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**ABSTRACT**

To escape competition at the dung pile, a ball-rolling dung beetle forms a piece of dung into a ball and rolls it away. To ensure their efficient escape from the dung pile, beetles rely on a ‘celestial compass’ to move along a straight path. Here, we analyzed the reliability of different skylight cues for this compass and found that dung beetles rely not only on the sun but also on the skylight polarization pattern. Moreover, we show the first evidence of an insect using the celestial light-intensity gradient for orientation. Using a polarizer, we manipulated skylight so that the polarization pattern appeared to turn by 90 deg. The beetles then changed their bearing close to the expected 90 deg. This behavior was abolished if the sun was visible to the beetle, suggesting that polarized light is hierarchically subordinate to the sun. When the sky was depolarized and the sun was invisible, the beetles could still move along straight paths. Therefore, we analyzed the use of the celestial light-intensity gradient for orientation. Artificial rotation of the intensity pattern by 180 deg caused beetles to orient in the opposite direction. This light-intensity cue was also found to be subordinate to the sun and could play a role in disambiguating the polarization signal, especially at low sun elevations.

**KEY WORDS:** Insect, Scarabaeidae, Compass orientation, Polarized light, Intensity difference, Celestial cues

**INTRODUCTION**

Animals use many different cues to determine an optimal route during migration, navigation and orientation. Insects, for example, often rely on celestial signals for orientation (Giurfa and Capaldi, 1999) and, in familiar areas, frequently supplement these ‘celestial compass’ cues with information about local landmarks (Collett, 1992; Kohler and Wehner, 2005). During the day, the sun is usually the brightest spot in the sky, and many insects are known to use it as a reference for orientation (Wehner, 1984). In addition, scattered solar light gives rise to other skylight cues, such as the pattern of polarized light and gradients of light intensity and color across the sky (Strutt, 1871; Coemans et al., 1994). When clouds cover the sun, these signals can still be used for orientation.

The celestial polarization pattern is generated by the scattering of sunlight in the atmosphere and is characterized by electric field ( $E$ -) vectors that oscillate in concentric circles around the sun (Fig. 1A). In 1949, von Frisch was the first to describe the use of polarized skylight for orientation in honey bees (von Frisch, 1949). Since then, compass orientation based on the skylight polarization pattern has been shown

in several diurnal insects, including desert ants, monarch butterflies and fruit flies (Wehner, 1997; Reppert et al., 2004; Weir and Dickinson, 2012). In all these species, a relatively small region of the eye – the dorsal rim area – is specialized for polarization vision (Labhart and Meyer, 1999); painting out this area leads to a loss of perception of polarized skylight (Wehner, 1989).

One problem that arises when using polarized skylight alone as compass cue is that it is not possible to distinguish between the sun and anti-sun direction. However, the skylight intensity gradient (Fig. 1A) could potentially be used by animals for orientation and could aid in solving the ambiguity of polarized light. Curiously, the use of this skylight intensity gradient has never been tested in insects. Among crustaceans, sandhoppers (*Talitrus saltator*) have been shown to orient using only the skylight intensity gradient when the position of the sun is obscured (Ugolini et al., 2009). In insects, indirect evidence of orientation using the intensity gradient is presented by the fact that desert ants are still able to navigate even when the dorsal rim areas of both eyes are painted out and the sun is shielded from view (Wehner, 1997).

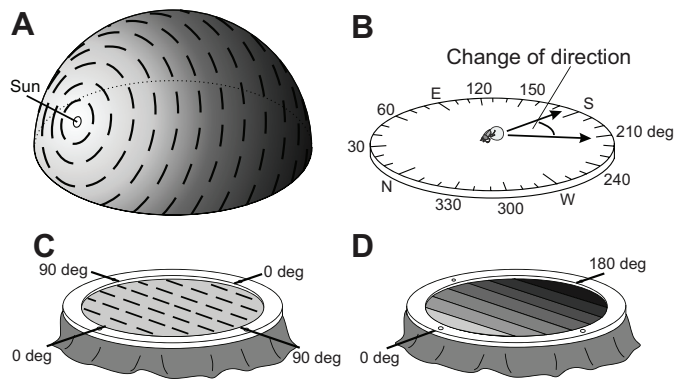
Ball-rolling dung beetles show a unique orientation behavior. After finding a suitable dung pile, they land at this food source, often together with a large number of other beetles. To escape the competition for limited food, they separate a piece of dung from the pile, form it into a ball and roll it away in a randomly chosen direction (Dacke et al., 2003a; Dacke et al., 2003b; Byrne et al., 2003). While rolling, each beetle maintains its chosen rolling direction until a suitable place is found for burial and underground consumption. This straight course prevents a beetle from inadvertently returning its dung ‘treasure’ back to the busy dung pat. To guarantee straight-line orientation, diurnal beetles are known to rely on a sun compass (Byrne et al., 2003; Dacke et al., 2014), and they seem to ignore available landmark cues (Dacke et al., 2013a). After sunset, crepuscular and nocturnal dung beetles use night-sky cues for orientation, such as the moon, polarized light formed around the moon (Dacke et al., 2003a; Dacke et al., 2003b) and light from the Milky Way (Dacke et al., 2013b). Whether diurnal dung beetles, like their nocturnal relatives, can orient to celestial cues other than the sun has, so far, not been tested.

Here, we examined whether diurnal dung beetles can use the celestial polarization pattern and the skylight intensity gradient as sky compass cues. We found that they use both of these celestial signals during orientation. However, these skylight cues are hierarchically subordinate to the sun and are mainly used when the sun is not directly visible to the beetle. We investigated the precision of the beetle’s compass, and found that precision was high when the sun or the polarization pattern were available as a cue; however, the precision decreased as soon as the intensity gradient became the main celestial reference. We therefore conclude that the skylight intensity gradient is primarily used in combination with the polarization pattern for orientation, or as a last resort when other cues are no longer available.

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**Fig. 1. Skylight cues and experimental setups.** (A) Schematic drawing of the skylight polarization pattern and intensity gradient at a solar elevation of 30 deg. The  $E$ -vectors of polarized light (black lines) form a pattern of concentric circles around the sun. The intensity gradient is indicated by increased shading. (B) Orientation precision was defined as the difference between the bearing of the first and second exit of a beetle rolling its ball out of a wooden arena (0 deg oriented towards local magnetic north). (C) To analyze the possible use of the skylight polarization pattern, a polarizer (diameter 42 cm) was placed 10 cm above a 60 cm wooden arena. Possible competing orientation cues were blocked by a skirt of black cloth attached to the filter holder. (D) To analyze whether dung beetles can use the skylight intensity gradient for straight-line orientation, strips of neutral density filters overlaid on each other (42 cm diameter, total change in intensity 2 log units) were placed above the arena.

## RESULTS

### The sun compass

In a previous study (Dacke et al., 2014), we found no difference in the accuracy of straight-line orientation in the diurnal dung beetle *Scarabaeus lamarcki* Macleay 1821 under a sunlit sky or in the shade. When given a full view of the sunlit sky, the absolute change of direction between two consecutive rolls was  $16.0 \pm 2.2$  deg (mean  $\pm$  s.e.m.) (Fig. 3A, sun–sun) (see Dacke et al., 2014), and when rolling in the shade, the absolute change of direction was  $11.7 \pm 2.2$  deg (Dacke et al., 2014). Here, we measured the orientation accuracy of 30 beetles rolling once under the sunlit sky and once in the shade (Fig. 1B), and we found that the absolute change of direction was no more than  $14.0 \pm 2.1$  deg from their original bearing (Fig. 3A, sun–shade). The orientation performance under this condition was, again, not significantly different from that observed when the sun was visible during both rolls ( $P=0.522$ ,  $F_{1,58}=0.416$ ). This suggests that *S. lamarcki* does not rely purely on the sun as an orientation cue but also uses additional celestial compass signals for straight-line orientation.

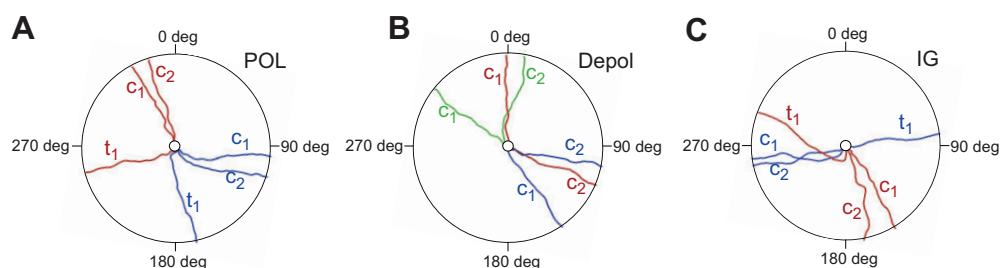
### Polarized light orientation

At low sun elevations, the degree of polarization in the zenith is high and might be used as a reference for orientation. To test whether beetles could orient using polarized light, a UV-transparent polarization filter with a diameter of 42 cm was placed above the arena (Fig. 1C). When released under the polarizer, the beetles selected random exit bearings, irrespective of whether the filter's  $E$ -vector was oriented perpendicular (Fig. 3B, left panel;  $N=16$ ,  $P=0.259$ ) or parallel to the main  $E$ -vector in the sky (Fig. 3B, right panel;  $N=16$ ,  $P=0.169$ ). This suggests that the beetles do not simply roll along the perceived  $E$ -vector but can orient at any angle to it, just as they do towards the sun (Baird et al., 2010).

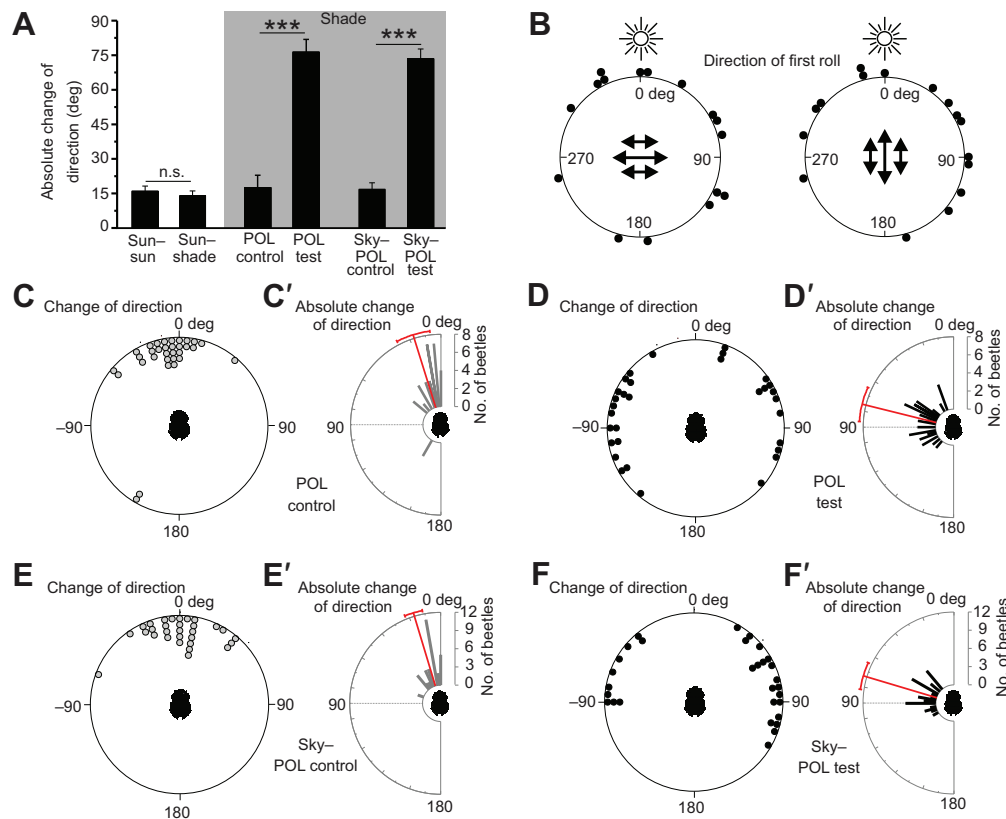
For each beetle, we calculated the angular difference between two consecutive rolls when the filter remained in place (control) (Fig. 2A, Fig. 3C; POL control). Under this control condition, the beetles' change of direction was clustered around 0 deg, i.e. they did not change their bearing (Fig. 3C;  $V$ -test,  $P=4.31 \times 10^{-11}$ ,  $V=24.69$ ). Moreover, the absolute change of direction of  $17.4 \pm 5.4$  deg (Fig. 3C') did not differ from that which we observed when beetles rolled twice under the visible sun ( $P=0.808$ ,  $F_{1,60}=0.059$ ) or twice in the shade ( $11.7 \pm 2.2$  deg,  $P=0.33$ ,  $F_{1,60}=0.966$ ).

In contrast, when the filter was turned by 90 deg after the first roll (test), the beetles changed their rolling direction by  $76.0 \pm 5.5$  deg (Fig. 2A, Fig. 3D'; POL test) in the second roll. This was significantly larger than the change in direction that we recorded under the control condition (Fig. 3A;  $P<0.0001$ ,  $F_{1,62}=55.31$ ), and the resulting distribution was significantly directed along the expected 90 deg axis (Fig. 3D;  $V$ -test,  $P=6.06 \times 10^{-5}$ ,  $V=7.93$ ). In general, approximately half of the beetles changed their bearing clockwise and half of the beetles turned counterclockwise after a polarizer turn (Fig. 3D). Taken together, these results suggest that diurnal dung beetles can detect polarized light and that they orient along straight paths, at least with respect to artificially produced polarized light.

In the next set of experiments, we compared the beetles' bearings under the natural celestial polarization pattern with those under the artificial polarization pattern. When the  $E$ -vector of the filter was parallel to the main  $E$ -vector in the sky (control), the change of direction was significantly clustered around 0 deg (Fig. 3E;  $V$ -test,  $P=3.04 \times 10^{-12}$ ,  $V=22.13$ ), and the absolute change of direction between these two conditions was only  $16.7 \pm 2.8$  deg (Fig. 3E'). When the  $E$ -vector of the polarizer was oriented perpendicular to the celestial polarization pattern (test), the change of rolling direction was clustered close to the expected 90 deg (Fig. 3F;  $V$ -test,  $P=8.82 \times 10^{-7}$ ,  $V=9.3$ ). The absolute change of direction in this latter condition was  $73.3 \pm 4.3$  deg (Fig. 3F'), which was significantly different from that measured in the control rolls (Fig. 3A,  $P<0.0001$ ;



**Fig. 2. Paths of *S. lamarcki* rolling from the center to the edge of a 60-cm diameter circular arena.** Tracks of the same individual are coded by color. (A) Tracks of two beetles rolling three times from the center of the arena (small, black circles) under a polarizing (POL) filter are shown. Each beetle performed two control rolls under a stationary filter ( $c_1, c_2$ ), and one test roll after the filter had been rotated by 90 deg ( $t_1$ ). (B) Tracks of three beetles rolling twice under a depolarizing (Depol) filter ( $c_1, c_2$ ). (C) The tracks of two beetles rolling three times under an intensity gradient (IG) filter. Each beetle performed two control rolls under a stationary filter ( $c_1, c_2$ ) and one test roll after the filter had been rotated by 180 deg ( $t_1$ ).



**Fig. 3. The dung beetle's sun and polarization compass at low sun elevations (<30 deg).** (A) Summary of the absolute changes of rolling direction between two consecutive rolls under different compass conditions. Under all control conditions (first, third and fifth bar from the left), as well as in the sun–shade condition [second bar,  $14.0 \pm 2.1$  deg (mean  $\pm$  s.e.m.),  $N=30$ ], beetles only changed their bearing by  $\sim 15$  deg (sun–sun:  $16.0 \pm 2.2$  deg,  $N=30$ ; POL control and sky–POL control see C' and E'). However, when the polarization pattern was turned by 90 deg between the two compared rolls (fourth and sixth bar), the beetles turned by a significantly larger angle (POL test and sky–POL test see D' and F', respectively). The gray sector indicates experiments that were conducted in the shade. Raw data for the bars 3–6 are presented in C–F, respectively. The error bars show s.e.m.; \*\*\* $P < 0.001$ ; n.s., not significant. (B) The rolling direction with respect to the sun (=0 deg) of beetles rolling for the first time under the polarization filter. The *E*-vector of the filter (double-headed arrows) was oriented either parallel (left plot,  $N=16$ ) or perpendicular (right plot,  $N=16$ ) to the main *E*-vector in the sky. Directions were randomly distributed under both conditions ( $P=0.259$  and  $P=0.169$ , respectively). (C–F) Change of rolling direction and (C'–F') absolute change of rolling direction of two consecutive rolls under different conditions. Red lines show the circular mean of the absolute change of direction; red sectors indicate 95% confidence intervals of the mean. The data sets are the same as those shown in bars 3–6 of A. (C,C') The results under a stationary polarizing filter (POL control) are significantly clustered around 0 deg ( $P=4.31 \times 10^{-11}$ ) with a mean absolute change of  $17.4 \pm 5.3$  deg ( $N=32$ ). (D,D') After a 90 deg turn (POL test) of the polarizer, beetles changed their rolling direction towards  $\pm 90$  deg ( $P=6.06 \times 10^{-5}$ ,  $N=32$ ) with a mean absolute change of  $76.0 \pm 5.5$  deg. (E,E') When beetles first rolled under the sky and then under the polarizer with the *E*-vector parallel to the main *E*-vector in the sky (sky–POL control), changes of direction were significantly clustered around 0 deg ( $P=3.04 \times 10^{-12}$ ) with an absolute change of  $16.7 \pm 2.8$  deg ( $N=30$ ). (F,F') If the filter was instead perpendicular to the main *E*-vector in the sky (sky–POL test), the beetles changed their rolling direction towards  $\pm 90$  deg ( $P=8.82 \times 10^{-7}$ ) with an absolute change of direction of  $73.4 \pm 4.3$  deg ( $N=30$ ). The sun was hidden from the beetles' view in all polarized light experiments.

$F_{1,58}=116.08$ ). These results show that diurnal dung beetles can use the polarization pattern of the sky for orientation.

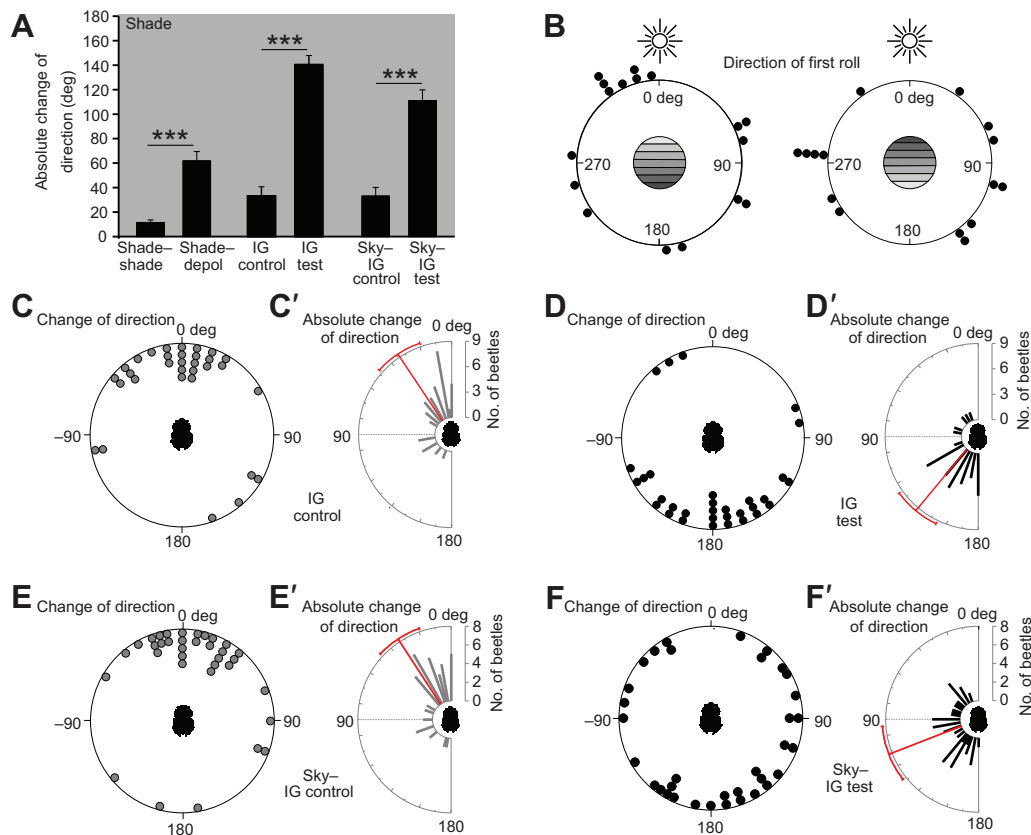
### Orientation without the sun and polarized light

Next, we tested the orientation behavior of beetles that rolled once in the shade (with the celestial polarization pattern available as an orientation cue) and once under a depolarizing filter (shade-depol) or twice under a depolarizing filter (depol-depol). When the sun and the polarization pattern were no longer available as compass cues, the beetles changed their bearing between two consecutive rolls (shade-depol) by  $62.0 \pm 7.5$  deg, which was significantly larger than the  $11.7 \pm 2.2$  deg recorded for beetles that rolled twice in the shade (with the polarization pattern still available as an orientation cue) (Fig. 4A;  $P < 0.0001$ ,  $F_{1,61}=38.32$ ). The beetles still showed weak orientation towards their original direction of travel in both the shade-depol and depol-depol conditions ( $V$ -test,  $P=0.027$ ,  $V=8.62$ ;  $P=0.017$ ,  $V=7.81$ , respectively; supplementary material Fig. S4A,B).

However, further statistical analysis showed that, because the beetles' rolling directions under the filter were not randomly distributed in the first place, this type of test does not provide a reliable indicator of directedness in this condition (supplementary material Fig. S2). The straight tracks that the beetles traveled under the filter (Fig. 2B), however, do suggest that they can move along straight paths even in the absence of the sun and the celestial polarization pattern.

### Orientation to the skylight intensity gradient

A gradient of light is present in the clear sky, with the highest intensity occurring in the solar hemisphere and the lowest occurring in the anti-solar hemisphere (Fig. 1A). To test whether dung beetles use this difference in skylight intensity as a cue for orientation, we created an intensity gradient pattern filter (Fig. 1D) and placed it above the dung beetles in the arena. As with the polarizer and depolarizer experiments, the beetles were able to orient along



**Fig. 4. The effect of depolarized skylight and the skylight intensity gradient on straight-line orientation in beetles.** (A) Summary of absolute changes of rolling direction between two consecutive rolls under different compass conditions. In all control conditions (first, third and fifth bar from the left), beetles changed their bearings by less than 40 deg [shade-shade:  $11.7 \pm 2.2$  deg (mean  $\pm$  s.e.m.,  $N=30$ ); IG control and sky-IG control, see C', E']. However, when the skylight was depolarized (second bar) or the intensity gradient was turned by 180 deg between the two compared rolls (fourth and sixth bar), the beetles turned by a significantly larger angle (shade-depol;  $62.0 \pm 7.5$  deg,  $N=33$ ; IG test and sky-IG test see D' and F', respectively). All experiments were conducted in the shade. Raw data for bars 3–6 are presented in C–F, respectively. Error bars show s.e.m.; \*\*\* $P < 0.001$ . (B) The rolling direction with respect to the sun (=0 deg) of beetles rolling for the first time under the intensity gradient filter. The brighter section of the filter was oriented either towards the sun (left plot,  $N=15$ ) or away from it (right plot,  $N=17$ ). Directions were randomly distributed under both conditions ( $P=0.417$  and  $P=0.912$ , respectively). (C–F) Change of rolling direction and (C'–F') absolute change of rolling direction of two consecutive rolls under different conditions. Red lines show the circular mean of the absolute change of direction, red sectors indicate 95% confidence intervals of the mean. Data sets are the same as those shown in bars 3–6 of A. (C, C') Results under a stationary intensity gradient filter (IG control) are significantly clustered around 0 deg ( $P=5.27 \times 10^{-8}$ ,  $N=32$ ) with a mean absolute change of  $33.4 \pm 7.5$  deg. (D) When the filter was turned by 180 deg between rolls (IG test;  $N=32$ ), beetles changed their rolling direction towards the opposite hemisphere ( $P=3.14 \times 10^{-6}$ ). (D') The absolute change of direction was  $140.4 \pm 7.4$  deg. (E, E') When beetles first rolled under the sky and then under the intensity filter with its bright side turned towards the sun (sky-IG control), the changes in direction were significantly clustered around 0 deg ( $P=1.18 \times 10^{-8}$ ,  $N=35$ ) with an absolute change of  $33.2 \pm 7.0$  deg. (F) If the filter's bright side was instead turned away from the sun (sky-POL test), beetles changed their rolling direction towards 180 deg ( $P=0.021$ ,  $N=35$ ). (F') The absolute change of direction was  $111.1 \pm 8.7$  deg. The sun was hidden from the beetles' view in all experiments.

straight paths under the intensity gradient filter (Fig. 2C). Independently of whether the brighter section of the intensity gradient filter (Fig. 4B, left panel;  $P=0.417$ ) or the darker section of the filter was oriented towards the sun (Fig. 4B, right panel;  $P=0.912$ ), the beetles selected a random distribution of exit directions for their first roll. This suggests that the beetles did not simply orient towards the dark or the bright side of the arena when choosing their initial rolling direction.

The absolute angular deviation between two consecutive rolls that were performed under the same artificial intensity gradient (filter set at 0 deg/0 deg or 180 deg/180 deg) was  $33.4 \pm 7.5$  deg (IG control, Fig. 4C'). The distribution of these changes of direction was clustered around 0 deg (Fig. 4C;  $V$ -test,  $P=5.27 \times 10^{-8}$ ,  $V=20.26$ ), indicating that the beetles still oriented towards their original rolling direction. In contrast, when the filter was turned by 180 deg after the first roll (0 deg/180 deg or 180 deg/0 deg), most beetles rolled in the opposite direction on the second roll (Fig. 4D;  $V$ -test,  $P=3.14 \times 10^{-6}$ ;

$V=18.86$ ). The absolute change of direction under this condition was  $140.4 \pm 7.3$  deg (Fig. 4D'), which was significantly different from the direction of rolling under the control condition (Fig. 4A;  $P < 0.0001$ ;  $F_{1,62}=92.59$ ). This suggests that dung beetles can use an intensity gradient for orientation.

To test whether dung beetles use the skylight intensity gradient for straight-line orientation (rather than an artificial gradient as in the previous experiment), we compared the beetles' rolling directions under the natural intensity gradient with those under the artificial intensity gradient on consecutive rolls. When the filter's intensity gradient was oriented parallel to that of the sky, the beetles did not change their rolling direction (i.e. the change of direction was significantly clustered around 0 deg, Fig. 4E;  $V$ -test,  $P=1.18 \times 10^{-8}$ ,  $V=22.36$ ). The absolute change of direction was only  $33.2 \pm 7.0$  deg (Fig. 4E'). In contrast, when the filter's gradient was anti-parallel to the celestial intensity gradient, the change of direction was clustered around 180 deg (Fig. 4F;  $V$ -test,  $P=0.021$ ,

$V=8.45$ ). The absolute change of direction was  $111.1 \pm 8.7$  deg (Fig. 4F'). Thus, the change in rolling direction was significantly higher when the filter was set in conflict with the skylight intensity gradient (sky-IG test) than when it was parallel to it (sky-IG control) (Fig. 4A;  $P < 0.0001$ ,  $F_{1,68} = 44.89$ ). This indicates that dung beetles can use the skylight intensity gradient for orientation.

### The hierarchy of sky compass cues and their precision during orientation

To identify which cues are most efficient in allowing dung beetles to maintain a constant direction, we determined the precision with which beetles kept their bearing under different conditions. We calculated the length of the mean vector  $r$  of the changes in direction between two consecutive rolls under the following conditions: (1) under the open sky (sun), (2) in the shade (shade), (3) under a stationary polarizer (POL, data from Fig. 2C), (4) under the artificial intensity gradient (IG, data from Fig. 3C) and (5) under a depolarizing filter (depol, data from supplementary material Fig. S4B). The length of the mean vector  $r$  can vary from 0 to 1 and gives information about the directedness of the rolling bearings, where 1 represents a complete agreement between the two rolls in all beetles. As the difference between the bearings increases (i.e. as orientation precision decreases), the value of  $r$  decreases. We found that under all conditions that had clear polarized skylight, the orientation precision was equally high; there was no significant difference between sun and shade ( $0.5 > P > 0.2$ ,  $U^2 = 0.081$ ) or between the sun and POL conditions ( $0.5 > P > 0.2$ ,  $U^2 = 0.078$ ) (Fig. 5A). However, when the beetles rolled under the depolarizing filter (in the shade), the orientation precision was much lower than that under the open sky ( $P < 0.001$ ,  $U^2 = 0.599$ ). This shows that dung beetles can orient with the same precision as long as the polarization pattern is clearly available as a cue. The orientation precision that was recorded under the artificial intensity gradient filter was worse than that recorded under the natural sky ( $P < 0.05$ ,  $U^2 = 0.233$ ) but was not significantly different from that recorded under the polarizing filter ( $0.1 > P > 0.05$ ,  $U^2 = 0.168$ ) or under the depolarizer ( $0.2 > P > 0.1$ ,  $U^2 = 0.142$ ). This may be a consequence of the fact that the artificial intensity gradient does not perfectly represent the celestial intensity

gradient. However, the beetles are still clearly able to use it for orientation.

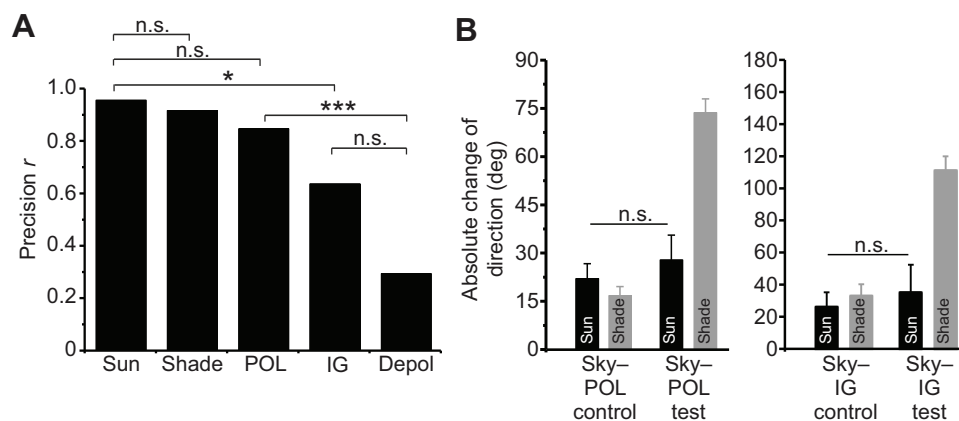
We further examined whether dung beetles follow one cue in preference to another if two cues are set in conflict. We found that, as long as the beetles could see the sun, their orientation was not influenced by a 90 deg change in the  $E$ -vector orientation (Fig. 5B; sky-POL control versus sky-POL test,  $P = 0.546$ ,  $F_{1,18} = 0.379$ ) or a 180 deg change in the direction of the intensity gradient (Fig. 5B; sky-IG control versus sky-IG test,  $P = 0.608$ ,  $F_{1,18} = 0.273$ ). This indicates that the sun is the main cue for orientation in the celestial compass of diurnal dung beetles, supplemented by the polarization pattern and the intensity gradient.

## DISCUSSION

### Orientation to polarized skylight

In this study, we analyzed the skylight-dependent orientation behavior of dung beetles at low sun elevations. We found that, when shaded, diurnal dung beetles changed their bearing to follow the rotation of a polarization filter. Thus, in addition to the sun (Byrne et al., 2003; Dacke et al., 2014), diurnal beetles can also use the polarization pattern of the daytime sky for orientation. This finding is consistent with previous studies that have shown that the crepuscular dung beetle *Scarabaeus zambesianus* uses the polarization pattern formed around the moon for orientation (Dacke et al., 2003a; Dacke et al., 2003b). In contrast to honey bees, desert ants and monarch butterflies (von Frisch, 1949; Wehner, 1997; Reppert et al., 2004) – all of which are known to use the polarization pattern of the sky for orientation – most ball-rolling dung beetles do not have to navigate back to a nest, or to travel thousands of kilometers to find a suitable breeding site. It is, therefore, likely that dung beetles use the pattern of polarized light as a direct compass reference, independent of the time of day or the position of the sun.

How dung beetles analyze the skylight polarization pattern via their dorsal rim area is still poorly understood, but before rolling its ball away from the dung pile, *S. lamarcki* performs a series of horizontal rotations on top of its ball, referred to as the dung beetle dance (Baird et al., 2012). These scanning movements could aid the insects in analyzing the celestial polarization pattern (Wehner, 1989;



**Fig. 5. Hierarchy and relative precision of sky compass cues.** (A) The length of the mean vector  $r$  of the change in direction between two consecutive rolls was used to analyze the precision of straight-line orientation under five different conditions: under the open sky (Sun;  $r = 0.95$ ,  $N = 30$ ), in the shade (Shade;  $r = 0.91$ ;  $N = 30$ ), in the shade with a stationary polarizer on top (POL;  $r = 0.84$ ,  $N = 32$ ), with a stationary intensity gradient filter on top (IG;  $r = 0.63$ ,  $N = 32$ ) and with a depolarizer on top (Depol;  $r = 0.29$ ,  $N = 33$ ). Precision was significantly reduced under the intensity filter and under a depolarizer. (B) Absolute change of rolling direction after skylight cue manipulation (polarized light or intensity gradient) when the sun was visible (black bars,  $N = 10$ ) or when the sun was shaded (gray bars; the same data is shown as that in Fig. 3A,E,F and Fig. 4A,E,F). Although beetles changed their direction under a rotated polarization or intensity gradient filter in the shade, this change was abolished when the sun was visible [left: sky-POL control, black bar,  $22.0 \pm 5.4$  deg (mean  $\pm$  s.e.m.); sky-POL test, black bar,  $27.8 \pm 9.1$  deg. Right: sky-IG control, black bar,  $26.4 \pm 8.9$  deg; sky-IG test, black bar,  $35.3 \pm 17.1$  deg]. Error bars indicate the s.e.m.; \*\*\* $P < 0.001$ ; \* $P < 0.05$ ; n.s., not significant.

Wehner and Labhart, 2006). On a neuronal level, we know from experiments in locusts that the signals from the dorsal rim areas in each eye converge to a single neural network for compass orientation (Homberg et al., 2011). When a beetle rotates around its vertical body axis, such a network could potentially balance the input from both eyes, informing the beetle when the body axis is in line with the symmetry plane of the skylight polarization pattern, and therefore with the solar meridian (Wehner, 1989). However, whether these dances are indeed used to read skylight polarization information remains to be investigated.

### Orientation to the skylight intensity gradient

When an intensity gradient filter was positioned anti-parallel to the gradient of the sky, the beetles changed their bearing by an absolute mean of 111 deg to the opposite celestial hemisphere. This response is similar to that which we observe when the sun is reflected by 180 deg at the same time of day (Dacke et al., 2014) and provides the first direct evidence that an insect can use the skylight intensity gradient for orientation. However, when the sun was shaded and the celestial polarization pattern artificially depolarized, the beetles' orientation behavior was strongly impaired. This suggests that the intensity gradient cannot be used as an individual cue for orientation, rather it is used in combination with the skylight polarization pattern. An alternative explanation for this result is that it might be an artifact of our filter design. To guarantee full depolarization, we had to filter the skylight through a linear polarizer before it entered the depolarizer. This linear polarizer slightly reduces light intensity across the whole visual field but does so more strongly in parts of the sky where the degree of polarization is low. Depending on how exactly the beetles measure the skylight intensity gradient, this intensity change might have interfered with the beetles' detection of it.

*Talitrus saltator* (Crustacea: Amphipoda) are the only other species known to use the intensity gradient for orientation. When shown only the skylight intensity gradient through a translucent white-glaze Plexiglas dome, these beach-dwelling amphipods are still able to orient seawards (Ugolini et al., 2009), but when the intensity gradient is also eliminated, they become disoriented. In addition, a study on the desert ant *Cataglyphis* showed that these navigating insects are still able to maintain their orientation towards their nest when the sun is shaded and the polarization pattern is no longer available (Wehner, 1997). This suggests that other insects could also rely on the intensity gradient for their navigation, but this remains to be confirmed.

A potential confounding factor of our intensity gradient filter is that we introduced an intensity-based stripe pattern (Fig. 1D), which might enable the beetles to use an edge-detection or contrast-based mechanism. This contrast-based strategy could be used by the beetles to orient in the same direction, with respect to the artificial pattern, when rolling twice under the intensity gradient filter. However, when we tested beetles first under a natural sky and then under the intensity gradient filter (oriented anti-parallel to the skylight intensity gradient), they turned to follow the artificial intensity gradient. This result indicates that the beetles use the same orientation strategy in both conditions, a gradient detection mechanism. How this gradient detection is implemented – i.e. which points in their visual field beetles compare – is still unknown.

### The hierarchy of compass cues

When the polarization pattern or the intensity gradient was set in conflict with the sun position (at solar elevations of <30 deg), the ball-rolling beetles relied exclusively on their sun compass when

exiting the arena. This agrees with conclusions drawn from other experiments (Byrne et al., 2003; Dacke et al., 2014) and further supports the idea that the sun is the most important cue for the orientation system of diurnal dung beetles. This conclusion also holds true for monarch butterflies (Reppert et al., 2004), where polarized skylight seems to play only a minor role as a compass cue (Stalleicken et al., 2005). In contrast to this, ants and crepuscular beetles, primarily, rely on polarized light for orientation (Dacke et al., 2004; Wehner and Müller, 2006). Experiments on honey bees using conflicting cues suggest that they use the sun and polarization compass equally (von Frisch, 1967). Why different skylight cues have different significance in different orientation systems is still a matter of speculation. It might, in part, depend on the required precision of the orientation system. Vector navigation (path integration), as performed by honey bees and ants, requires high orientation precision, whereas ball-rolling dung beetles and migrating monarch butterflies can afford to deviate somewhat from their intended course. Crepuscular dung beetles might have shifted their reliance in favor of the sky-wide polarization pattern (Dacke et al., 2004) simply because integration over the entire sky provides the visual system with a much more reliable cue in dim light conditions compared with a single light source, the moon, which can be one million times dimmer than the sun (Gál et al., 2001). Furthermore, at night and during twilight, the sky is often cloudy in the natural habitat of the dung beetles. Therefore, the polarization pattern might be more reliable than the moon position as the *E*-vectors are still detectable underneath clouds (Pomozi et al., 2001).

Even though the sun is the main orientation cue for diurnal dung beetles, their orientation precision was not significantly affected when made to roll their balls in the shade, as long as the celestial polarization and intensity patterns were available as compass cues. When the intensity gradient was the only available cue, however, the beetles' orientation precision was significantly impaired. The same holds true for sandhoppers, which are less accurate in their orientation in the absence of the sun or the moon (Ugolini et al., 2003; Ugolini et al., 2009). This suggests that dung beetles and sandhoppers use the intensity gradient of the sky primarily in combination with other skylight cues, or as a last resort when other, more precise cues, are no longer available. In dung beetles, the intensity gradient might also help to disambiguate the polarization signal and to distinguish between the solar and anti-solar sky hemispheres.

One remaining sky compass cue that has not been described in the dung beetle sky compass system is the chromatic skylight gradient (Coemans et al., 1994). Bees interpret artificial light sources of longer wavelengths as the sun direction, whereas UV light is taken as a direction opposite to the sun (Brines and Gould, 1979; Edrich et al., 1979; Rossel and Wehner, 1984). In desert ants, the removal of the spectral gradient (in the absence of the sun and polarized light) induces a change from a navigational behavior to a phototactic orientation towards the brighter part of the sky (Wehner, 1997). Whether dung beetles are able to use the spectral contrast of the sky for straight-line orientation remains to be tested.

### Conclusions

Dung beetles use an impressive variety of celestial cues, including the sun (Byrne et al., 2003; Dacke et al., 2014), the moon (Dacke et al., 2004; Dacke et al., 2011), the solar and lunar polarization patterns (present study; Dacke et al., 2003a; Dacke et al., 2003b), the intensity gradient (present study) and even the Milky Way (Dacke et al., 2013b) for straight-line orientation. The flexibility offered by the ability to use more than one sky compass cue

generates a robust orientation system in an animal that seems to rely solely on the sky as a reference for orientation.

## MATERIALS AND METHODS

### General

All experiments were performed using the diurnal dung beetle *Scarabaeus (Kheper) lamarcki* (Coleoptera: Scarabaeidae) in its natural habitat on the game farm 'Stonehenge' in North West Province, South Africa (24.32°E, 26.39°S). Experiments were conducted in the afternoon, at solar elevations of <30 deg in January–February and November 2013. Beetles were collected using pit-fall traps, kept in soil-filled plastic bins (30×22×22 cm) and fed fresh cow dung.

### Orientation performance under the natural sky

To investigate the accuracy of straight-line orientation in the absence or presence of the sun, 30 beetles were individually released to the south of their dung ball in the center of a 1-m diameter flat wooden arena that was coated with a thin layer of sand. As each beetle rolled its ball, the bearing was recorded with an angular resolution of 5 deg as it crossed the perimeter of the arena. The beetle was then returned with its ball to the center of the arena, and the experiment was repeated. Half of the beetles rolled with the sun visible in the first run, and with the sun shaded by an opaque board (100×75 cm) in the second run. The other half of the beetles rolled first in the shade and then in the sun. The angular difference between the two consecutive rolls was used to define the orientation precision under each condition. The effect of shading the sun was quantified (sun–shade) by calculating the angular difference between each beetle's second and third roll (one with the sun present and one with it shaded).

### The use of polarized light for orientation

#### Use of artificial polarized light

To test whether beetles could orient using polarized light, a UV-transparent polarization filter (Polaroid HN-22) with a diameter of 42 cm was placed above an arena with a diameter of 60 cm. The filter was mounted on 10-cm legs with a black cloth 'skirt' attached to the edge of the holder to prevent the use of any cues in the beetle's lateral or frontal visual field (Fig. 1C). During all trials, the sun was shaded. In the first set of experiments, 32 beetles were individually allowed to roll their ball three times from the center to the perimeter of the arena, where the bearings were recorded with an angular resolution of 5 deg. All beetles were released to the south of their dung ball in the center of the arena, and as a beetle reached the edge of the filter holder, the 'skirt' was lifted to allow it to roll to the perimeter of the arena. The orientation of the filter's electric field (*E*-) vector was alternated for the first roll of each beetle such that half of the beetles started with the filter's *E*-vector parallel (0 deg) to the main *E*-vector in the sky, and the other half started with the *E*-vectors perpendicular (90 deg) to each other. Once the beetle reached the edge of the arena, the bearing was recorded and the beetle was returned to the center of the arena. Before the second roll, the filter was alternately placed with its *E*-vectors in the same orientation as the first roll (POL control) or rotated by 90 deg (POL test). Each beetle thus experienced one of the following combinations of filter orientations: 0 deg/0 deg/90 deg; 0 deg/90 deg/90 deg; 90 deg/90 deg/0 deg, or 90 deg/0 deg/0 deg giving both a control and a test condition for each beetle. The influence of polarized light on the beetle's bearing was then evaluated by comparing the angular difference between two consecutive rolls without turning the filter (POL control) with that of two consecutive rolls where the filter had been turned by 90 deg between rolls (POL test).

#### Use of polarized skylight

In the next set of experiments, we tested whether beetles could use the natural polarization pattern in the sky as a compass cue during orientation. The impact of polarized skylight was evaluated using the same experimental procedure as that used for artificial polarized light, except that beetles first rolled under the open sky and then under the filter. The filter's *E*-vector was either parallel (sky–POL control) or perpendicular (sky–POL test) to the main *E*-vector in the sky. In the first set of tests (30 beetles), the sun was

shaded by a board. In the second set of tests (10 beetles), the sun was visible from within the arena.

### Orientation without sun and polarized light

To test whether beetles could orient in the absence of the sun and the polarization pattern, we analyzed the rolling bearing of beetles in the shade under a UV-impermeable polarizer on top of a quarter wave retarder (American Polarizers, quarter wave centered at 560 nm). By orienting the axis of the polarizer at an angle of 45 deg to the axis of the quarter wave retarder, we created a depolarizing filter that blocked UV light and transformed green light into circularly polarized light [the dorsal rim area receptors of dung beetles are maximally sensitive to UV or green light (Dacke et al., 2004)]. The depolarizing filter had a diameter of 42 cm and was mounted on the skirted holder (the same holder as for the polarizer). Sixteen beetles rolled three times each, first under the natural sky and then twice under the filter. The sun was shaded during all experiments. Seventeen beetles were tested using a reversed order of conditions. In all runs, the beetles were always released to the south of the ball in the center of the arena. To test whether beetles could orient when the sun and the polarization pattern were removed, the angular difference between two rolls under the depolarizing filter was calculated (depol–depol) and compared with the angular difference between the bearings of a roll in the shade and a roll under the depolarizer (shade–depol).

### The use of the intensity gradient for orientation

#### Filter design

To investigate whether dung beetles could use the skylight intensity gradient for orientation, we created an intensity gradient filter that was 42 cm in diameter from overlapping gray filters (Fig. 1D). This filter comprised seven 6-cm-wide strips, each of which transmitted a particular amount of light that depended on the number of overlapped gray filters (0.3 neutral density, LEE filters) it comprised. Together, the seven strips of neutral density filters created a filter where the transmitted light decreased successively from one strip to the next, over a total of approximately two logarithmic steps. According to Ugolini and colleagues (Ugolini et al., 2012), the skylight intensity gradient changes over 0.8 logarithmic steps along the solar meridian. We performed similar measurements in the beetles' natural habitat [using a Gossen Mavolux 5032C/B illuminance meter, with a cylinder (height of 1.5 cm) attached around the light sensor (acceptance angle of 20 deg)] and found a difference in skylight intensity of approximately 1.5 logarithmic steps at a sun elevation of approximately 12 deg. Thus, when orienting the bright side of the filter in the anti-solar direction, the intensity gradient was artificially turned by 180 deg.

#### Use of an artificial intensity gradient

The use of an intensity gradient for orientation was tested in 32 beetles in a procedure analogous to that used to investigate the use of polarized skylight but modified so that the intensity gradient filter was either oriented with its bright side towards the sun [i.e. the filter gradient was parallel (0 deg) to the skylight gradient, IG control] or with its dark side towards the sun [i.e. the filter gradient was anti-parallel (180 deg) to the skylight gradient, IG test]. Each beetle thus experienced one of the following sequences of filter orientations: 0 deg/0 deg/180 deg; 0 deg/180 deg/180 deg; 180 deg/180 deg/0 deg; 180 deg/0 deg/0 deg.

#### Use of the skylight intensity gradient

To test the dung beetles' use of the natural celestial intensity gradient for orientation (rather than the intensity gradient of the filter as in the previous experiment), the experiments were conducted analogously, except that the intensity gradient filter was excluded during the second roll. Each beetle thus experienced one of the following sequences of conditions: 0 deg/sky/180 deg, or 180 deg/sky/0 deg. This allowed for a comparison between the rolling bearing under the natural and the artificial intensity gradient with the brighter part oriented towards the sun (0 deg, sky–IG control) or away from the sun (180 deg, sky–IG test) in each beetle. In a first set of tests (35 beetles) the sun was shaded using a board, and in a second set of tests (10 beetles) the sun was clearly visible to the beetle.

## Data analysis

Circular statistics were calculated using Oriana 4.0 (Kovach Computing Services, Anglesey, UK) or (for permutation tests) the CircStat toolbox for MATLAB (Berens, 2009). All reported mean values are circular means. The bearing of the first roll of each beetle was calculated with respect to the azimuthal position of the sun at the time of the experiment. The distributions of these angles were analyzed using Rayleigh's uniformity test for circular data (Batschelet, 1981). Changes of direction were calculated by measuring the angular difference between two rolls. The distribution of the changes of direction was tested using the *V*-test with an expected mean of 0 deg for the control experiments, 90 deg for the POL test and sky-POL test, and 180 deg for the IG test and sky-IG test. If the distribution of the changes of direction was directed around the expected mean, this *V*-test was significant. However, as the *V*-test only tests against a null hypothesis of uniformity (Aneshansley and Larkin, 1981), we additionally performed a Watson test (Batschelet, 1965) to determine whether a confidence interval around the sample mean included the predicted direction. This was the case in all our experimental data (data not shown). To further test the reliability of the *V*-test, we analyzed all data in MATLAB (MathWorks) using permutation tests. For each experiment, the measured bearings were randomly permuted 1,000,000 times across individuals and experimental conditions, and the corresponding *V*-value was calculated. The significance of the experiment was judged by calculating the percentage of permutations that resulted in a *V*-value greater or equal to that calculated from the unpermuted data (supplementary material Figs S1–S3). We performed similar permutation tests for the depolarized sky and intensity gradient experiments, using the absolute changes of direction (see below) as the test statistic. The results were qualitatively the same as for the *V*-based permutations (data not shown).

As a measure of response strength, we also calculated the absolute changes of direction between two rolls. These absolute changes of direction under different conditions were then compared using a Watson–Williams *F*-test.

To test the precision of dung beetles rolling under different conditions, we calculated the length of the mean vector *r*. This value describes the concentration and, thus, the precision of a circular distribution and ranges from 0 (random distribution) to 1 (angles are all exactly the same). To test whether the precision of orientation changed between different conditions, the distributions of the changes of direction were shifted to a mean direction of 0 deg. Subsequently, the data were compared using Watson's *U*<sup>2</sup>-test.

Dung beetle tracks shown in Fig. 2 were reconstructed from images of the tracks that the beetles left as they rolled across the sand-covered arena.

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## Competing interests

The authors declare no competing financial interests.

## Author contributions

All authors had full access to all data of the study and approved the final version of the article. B.E.J., J.S., E.B., M.B. and M.D. contributed to conception of the study and design. B.E.J., J.S., E.B., M.B. and M.D. acquired data. B.E.J. and J.S. analyzed data. B.E.J., J.S., E.B. and M.D. interpreted data. B.E.J. drafted the manuscript. J.S., E.B., M.B. and M.D. critically revised the manuscript.

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## Supplementary material

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