Spectral information as an orientation cue in dung beetles

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During the day, a non-uniform distribution of long and short wavelength light generates a colour gradient across the sky. This gradient could be used as a compass cue, particularly by animals such as dung beetles that rely primarily on celestial cues for orientation. Here, we tested if dung beetles can use spectral cues for orientation by presenting them with monochromatic (green and UV) light spots in an indoor arena. Beetles kept their original bearing when presented with a single light cue, green or UV, or when presented with both light cues set 180° apart. When either the UV or the green light was turned off after the beetles had set their bearing in the presence of both cues, they were still able to maintain their original bearing to the remaining light. However, if the beetles were presented with two identical green light spots set 180° apart, their ability to maintain their original bearing was impaired. In summary, our data show that ball-rolling beetles could potentially use the celestial chromatic gradient as a reference for orientation.

1. Introduction

In contrast to the vector-based navigation displayed by some insects [1], the straight-line orientation behaviour of South African ball-rolling dung beetles is more simplistic in its nature. On arrival at a dung pile, they shape a piece of dung into a ball, perform a brief ‘orientation dance’ on top of it [2], and then roll the ball away along a straight-line path to minimize the chance of it being stolen by nearby beetles. The beetle maintains its chosen direction until a suitable place is found to bury and consume the ball. To maintain their straight paths, ball-rolling dung beetles use a broad repertoire of celestial cues such as the sun, moon, the skylight polarization and intensity pattern and even the Milky Way [3–6].

Another sky compass cue, generated by the wavelength-dependent scattering of sunlight, is the chromatic contrast between long (460–700 nm) and short-wavelength light (293–460 nm) along the solar meridian [7]. While the relative intensity of green light is much higher in the direction of the sun, the intensity of ultraviolet (UV) light is relatively higher in the opposite sky hemisphere. Some insects, such as bees [8–10] and dung beetles [11], interpret an artificial green light spot as the sun direction. In addition, an artificial UV light stimulus is interpreted by bees during their waggle dances to be in the antisolar hemisphere, indicating that they can use the celestial chromatic gradient for navigation, as has also been suggested for ants [12]. Here, we test whether ball-rolling dung beetles similarly interpret a UV light spot as the ‘anti-sun’ direction, and whether they can use the celestial chromatic gradient for orientation.

2. Material and methods

(a) Animals

Diurnal dung beetles (Scarabaeus lamarcki) were kept in soil-filled plastic bins and fed fresh cow dung. Experiments were performed at a field station in South Africa.
(24.32° E, 26.39° S) and at the Department of Biology (Lund University). Beetles transported to Sweden were kept under a 12 h light/dark cycle (lights on at 10.00), at a room temperature of 26 °C.

(b) Experimental set-up
Experimental light spots were produced by single green (530 nm, LedEngin Inc.) and UV LEDs (365 nm, LedEngin Inc.) attached to two metal plates mounted 180° apart at about 45° elevation, at the perimeter of a wooden circular experimental arena (1 m diameter). All LEDs were adjusted to an equal photon flux of approximately 3.89 × 10^12 photons cm^-2 s^-1 (measured from the arena centre using a spectrometer (QE65000; Ocean Optics)). In one experiment, the light intensity of the LEDs was reduced using neutral density filters (LEE filters). The beetles were recorded using a video camera (Samsung VP-HMX20C) mounted in the centre of a metal plate suspended 160 cm above the arena centre. A thick black curtain hanging down from the edge of the plate blocked any visual cues from outside the arena.

(c) Experimental procedure
To test the beetles’ orientation behaviour to spectral cues, we released beetles individually, with their balls, in the centre of the arena. As soon as each beetle reached the perimeter of the arena, it was returned to the centre, and the experiment was repeated in the presence of the same or a different light stimulus combination (single green or UV light, UV/green light or green/green light, set 180° apart; figure 1a). Note that after each roll, the stimulus position was turned by 180° to exclude other cues that could have been used by the beetles during rolling (for ease of comparison, the data in the figures have been normalized to the orientation of the stimulus rather than to its geographical position). The beetles’ paths and the endpoints of the paths at the perimeter of the arena of the two rolls were recorded. A more detailed description of the experimental procedure can be found in the electronic supplementary material.

(d) Data analysis
All data were analysed in Matlab (MathWorks). Changes of direction were calculated by measuring the angular difference between two rolls. The distribution of the changes of direction was tested using a V-test with an expected mean of 0° or 180°. Similar to previous studies [3,11,13], the reliability of the V-test was analysed using permutation tests (electronic supplementary material, figure S1). Mardia–Watson–Wheeler tests were used to test for differences between circular data. The exit tracks were digitized using customized code (for details, see [13,14]) and compared using an ANOVA Games–Howell post hoc test. Further
details about the track analysis can be found in the electronic supplementary material.

3. Results and discussion

(a) Orientation with respect to a single light source
First, we presented the beetles with a single green (n = 15) or UV (n = 15) light spot as an orientation cue during two consecutive rolls (figure 1b). In both conditions, the beetles kept to their original direction of travel between the two rolls (p_{green} < 0.001, p_{UV} < 0.001; V-test; figure 1b). In addition, the beetles oriented along equally straight paths (green: 64.7 ± 16.6 cm (mean ± s.d. path length); UV: 63.2 ± 10.9 cm; p = 0.97; figure 1e) and with the same precision (as measured from the change in bearing between rolls) in the presence of either of these cues (p = 0.89, Mardia–Watson–Wheeler test). Even when the green light spot was replaced by a UV light spot during the second roll (or vice versa), the beetles (n = 15) kept their original rolling direction (p < 0.01; V-test; figure 1b). These results suggest that dung beetles can orient with the same precision to a single UV or green light spot. In contrast to bees [8–10], however, they do not inherently interpret a UV light spot as the ‘anti-sun’ direction but rather use the brightness information of a single light spot as the orientation cue.

(b) Orientation to two light cues of the same colour
Next, the beetles were presented with an ambiguous stimulus created by placing two green light spots of the same brightness 180° apart. The beetles (n = 21) did not keep their original bearing under this condition (p = 0.96; V-test; figure 1c), and the data distribution was significantly different from that with single light cues (p_{green} < 0.001, p_{UV} < 0.001; Mardia–Watson–Wheeler test). In addition, many of the beetles did not exit the arena in straight lines but rolled in circles (figure 1d). Consequently, the average path length (92.8 ± 45.9 cm) was significantly longer than under the conditions with only one light cue as reference (p_{green} < 0.01, p_{UV} < 0.01; figure 1c). This suggests that the beetles’ straight-line orientation is strongly compromised by the presence of two identical light sources.

(c) Orientation to spectral cues
When presented with a green and a UV light spot (set 180° apart with the same brightness) the beetles’ changes of direction were again tightly clustered around 0° (p < 0.001; V-test, n = 30; figure 1c), and the average path length (61 ± 12.5 cm) was not significantly different from those recorded in the presence of a single light (p_{green} = 0.71, p_{UV} = 0.83; figure 1c). Thus, the beetles were again well oriented along straight paths (figure 1d). In this experiment, beetles might use only one light spot as a reference (while ignoring the other) or use an intensity-based, rather than a spectrally based strategy, because green receptors also absorb UV light via a second band [15]. To exclude these possibilities, we turned one of the lights off (green or UV) prior to the second roll (figure 2a), and found that the beetles were still able to maintain their original rolling direction in the presence of either the green (p < 0.01) or the UV light (p < 0.001; V-test; figure 2a). If the beetles had used a strategy based on relative intensities, mediated by the green receptors (or simply ignored the presence of the UV light), they should have changed their bearing by 180° (or been disoriented) in response to the removal of the green or UV light.

Finally, the beetles might have used the light spots’ absolute intensity as cues. To rule out this possibility, we presented the beetles with a bright and a dim green light spot (figure 2b) and turned off the bright light prior to the second roll. In response to this, the beetles turned by 180° (p < 0.01; V-test, n = 15; figure 2b), suggesting that, when the two light sources no longer differ spectrally, beetles use relative, not absolute, brightness information for orientation. Taken together, our data not only demonstrate that beetles register the position of both spectral cues when presented together, but also indicate that they may use the celestial chromatic contrast for orientation as bees do [8–10]. However, the beetles seem to lack the bees’ ‘inbuilt’ knowledge of the celestial chromatic contrast (where green represents the sun and UV the anti-sun direction) and therefore need to experience and memorize the spatial distribution of the celestial chromatic signals before rolling (figure 2a). The difference in the utilization of spectral cues in these two groups of insects is likely a consequence of the different orientation demands between a once-off straight-line journey away from a food source and repeated navigation to and from a nest.

4. Conclusion
Dung beetles can use colour contrast information to maintain their rolling direction. These spectral cues might be combined...
with intensity and polarization signals to generate an orientation compass that is robust even if the sun is hidden. This robustness is crucial for an animal that apparently uses the sky as its only source of visual orientation cues [16].

**Ethics.** The experiments were performed in accordance with the South African and Swedish guidelines for animal experiments.

**Data accessibility.** Tables of all exit angles and paths are available as electronic supplementary material.

**Authors’ contributions.** B.e.J., J.J.F., E.B., M.J.B. and M.D. conducted experiments; B.e.J., J.J.F., E.B., M.B. and M.D. designed experiments; B.e.J. analysed data and drafted the manuscript; J.J.F., E.B., M.B., M.D. revised the manuscript. All authors are accountable for the presented work and approved the final version of the manuscript for publication.

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**References**


