The Dung Beetle Dance: The role of visual cues in dung beetle orientation behavior

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Summary

When a dung beetle has arrived at a dung pat, it soon starts to form a ball, which it will later roll away in a straight line. Rolling in a straight line ensures that the beetle will never risk rolling back to the fierce competition at the pat, thus eliminating the chance of getting its ball stolen by others. Before rolling out from the pat, the beetle performs an orientation dance on top of the ball. This dance is believed to aid in establishing and keeping a straight roll bearing. Here, I test the hypothesis that dung beetles take visual snapshots of compass cues, allowing them to both set a roll bearing and to relocate this bearing after a disturbance. If the dance is visually based, it should be affected when visual compass information is removed or modified. Additionally, I predict that the duration of the dance will be longer when visual cues are lacking or are unreliable. My results indicate that the duration of the dance increases when there is a lack, or insufficient amount of visual compass cues. I conclude that the dance is not visually based, but visually regulated, as the beetles also dance in conditions where no visual input is present.

Keywords Dung beetle • Orientation dance • Visual cues • Pause • Total rotation

Introduction

When a foraging dung beetle has found a suitable dung pile for feeding, it is a matter of efficiency that decides whether or not it is able to get a sufficient bite to eat. At the dung pile there is fierce competition with several beetles competing for a piece of dung, many of whom do not even bother making a dung ball themselves, but will try to steal the balls of others (Giller & Doube, 1989). Thus, a dung beetle's best bet to get food is to be as efficient as possible in making the dung ball and to get it away from the pile in the fastest manner possible. To do this, the most effective way is to roll away from the pat in a straight line, which is exactly what the beetle does (Hanski, 1991; Byrne et al., 2003).

In order to roll in a straight line, dung beetles rely on celestial compass cues such as the sun, the moon and the polarization pattern that forms around them, as well as the intensity gradient created by them (Byrne et al., 2003; Dacke et al., 2003; Dacke et al., 2013; Dacke et al., 2014; El Jundi et al., 2014).

The use of different cues to orient is a very common phenomenon amongst animals, not in the least among invertebrates. Bees, wasps, ants and sandhoppers are a few examples of insects known to use multiple compass cues for orientation (Collett, 1996; Ugolini, 2006; Mueller & Wehner, 2010). For navigation between a set position (such as a hive or a nest) and a novel position (such as a food source) using external cues as landmarks or a compass to orient is very effective, as they remain stable throughout the orientation route. Bees and wasps, for instance, use familiar landmarks to navigate between their hive and a food source. While in flight they turn back and make a looping motion, presumably scanning prominent landmarks, before continuing in the forward direction; a so-called turn back and look behavior (Lehrer, 1991, 1993; Fry, 1999). For insects orienting from the ground, such as ants and sandhoppers, a similar behavior to that of bees and wasps can be seen. Ants tend to stop and make small turns while walking. During this characteristic spiral walk, the ant is suggested to visually memorize, or take a "snapshot" of the outside world in order to establish where its nest is in relation to the surroundings (Wehner & Srinivasan, 1981; Lent et al., 2010). Sandhoppers also show similar orientation behavior. To orient themselves in the preferred direction with reference to the sea-land axis, sandhoppers use the magnetic field as a compass cue. When orienting, they make left and right rotations with their body, which is shown to be a form of scanning of the environment and the magnetic field, to establish the correct direction (Ugolini, 2006).

Scanning behavior has also been observed in dung beetles. Just before rolling away from the dung pat, a beetle will climb up on its ball and rotate horizontally around its own axis, occasionally stopping in the midst of the rotation to pause, before continuing the rotation. This so-called "dance" behavior is not only performed at the initial rolling out from the dung pile, but also occurs when the beetle has been disturbed in some way while rolling its ball (Baird et al., 2012). Baird et al. (2012) hypothesize that the dung beetle dance is a visually based orientation behavior that has two functions, (1) to set a roll bearing direction when a new ball is made by allowing the beetles to take a clear snapshot of the celestial compass cues and (2) to recover this bearing by re-matching this snapshot when the beetle experiences a disturbance to its straight-line roll path. Here, I test this hypothesis by presenting the beetles of the species *Scarabaeus (Kheper) lamarcki* with five different conditions, each of which modify the visual information available to the beetle and the different celestial compass cues that beetles are known to use.

If the dance does play a role in the dung beetles' visual orientation behavior, it should be affected when compass cues are modified, become difficult to use or are removed altogether. I predict that the duration of the dance will increase in direct proportion to the unreliability of the available compass cues. With only a few readable cues present, the beetle will lack sufficient amount of information from the surrounding environment to choose a bearing direction or match a previously chosen bearing. This will cause the beetle to search for the information it is lacking by scanning the surrounding environment repeatedly to find any information that will help it to choose or relocate a roll bearing. Thus, when there is a lack of readable cues available, I predict that the total rotation on the ball and the number of pauses the beetle makes will increase.

Methods

General

Males and females of the South African diurnal ball-rolling dung beetle species *Scarabaeus (Kheper) lamarcki* were collected within the game farm "Stonehenge", located 70km north-west of Vryberg, North-West Province, South Africa (24°E, 26°S). All experiments were performed at the field site during the daytime, when *S. lamarcki* are normally active. Experiments 3, 4 and the first condition of Experiment 1 were conducted indoors, while the remaining experiments were performed outdoors. For each experiment, individual beetles were marked with a number on their elytra for individual identification. With the exception of Experiment 2, there were 20 individuals tested in each experiment. For Experiment 2 only 10 individuals were tested.

Statistical analysis

For all five experiments, Mann-Whitney U tests were used in order to establish if an experimental treatment had any significant affect on the beetle's dance behavior regarding the number of pauses and the beetle's total angular rotation on the ball. This non-parametric statistical test was used because it is appropriate for use with the count data (number of pauses) and because some of the data sets were not normally distributed. For this reason, all summary statistics are presented as median ± inter quartile range (IQR). All statistical analyses were performed using Matlab R2013a (Mathworks, USA).

Data analysis

For each experiment, a camera mounted above the arena recorded the dances at 25 frames per second (fps). The films were played back on a computer and a screen protractor was used to calculate the head orientation at different points in the dance. The total angular rotation of the beetle in each dance was measured by finding the head orientation at the start, when the beetle first started climbing onto the ball and at the end, when the beetle started to roll the ball. If the beetle changed its direction of rotation, this was also included in the calculation of total angular rotation. The number of pauses each beetle performed during a dance was also analyzed. A pause was defined as the beetle holding its head stationary for more than two frames, or 80ms.

Experiment 1: The effect of total darkness on dance behavior

In a small arena, surrounded by black cloth to prevent any light from coming through, the beetle was placed by its ball and allowed to dance. To ensure that no light of any sort was present in the arena, the camera was also wrapped in black cloth so as to block any light it produced. To control for the effect of the initial orientation on pause direction, the beetles were initially placed beside the ball facing four different directions (N, S, E, W).

Before dancing in the dark (*Dark* condition), dances of the beetles were first recorded outside in daylight (*Sun* condition), under natural conditions. Thus, each beetle danced under the two different conditions.

Experiment 2: The effect of blocking the dorsal and frontal view on dance behavior

The beetle was provided with a cap made out of black non-transparent paper. The cap was mounted over the head of the beetle, covering its dorsal eyes and its dorsal and frontal field of view (Fig. 1).

On a small sand-covered arena, the beetle, with a cap mounted on its head, was placed alongside its ball and allowed to dance (*With Cap*). Following the *With Cap* condition, the cap was removed from the beetle and the same individual was allowed to dance in the arena again (*Without Cap*). Each beetle danced under the two different conditions.

Fig. 1. Beetle with cap. The image shows a beetle wearing a cap used in the *With Cap* condition of Experiment 2. The cap is made out of black non-transparent paper, blocking the dorsal and frontal view of the beetle.

Experiment 3: The effect of a single light source on dance behavior

The experimental arena was a circular sand-covered board, measuring 0.35m in radius. The arena was surrounded by a black curtain to exclude the presence of any external visual cues (Fig. 2b). On each side of the arena, two light fixtures equipped with white LEDs were placed directly facing each other. The light sources were placed at a height of 0.45m from the arena floor. The angular elevation of the light from the center of the arena was 53°. A third light fixture, with a total of eight white LEDs, creating a circle with a diameter of 25cm, was mounted directly above the arena at a height of 1.5m. A camera was placed in the center of this circle so that the beetle's dance could be recorded from above (Fig. 2a).

The beetles were tested under two different conditions, with a total of seven dances recorded per beetle. In the first condition, the beetles were presented with a light from above (*Above*). To ensure that the properties of the dance in this condition was not only related to it being the first condition the beetle saw, but a result of the light position, the condition was tested two consecutive times. Firstly, two times before presenting the beetle with the side lights (see below), and then again, two consecutive times after the side lights had been presented to the beetle.

For the second condition, only light from the beetle's lateral view was presented (*Side*). Additionally, this condition was divided into two treatments. Either the beetle was presented with a light source emitting from its left side (*Left*), or it was presented with a light source emitting from its right side (*Right*). For each of the two treatments, only one light was presented at a time. To ensure that any change in behavior when changing the direction of light from left to right (or vice versa) depended strictly on the change in light direction, and not on the order of how many times the beetle has seen a lateral light, the side light was shown three times. Either a switch in light position was made

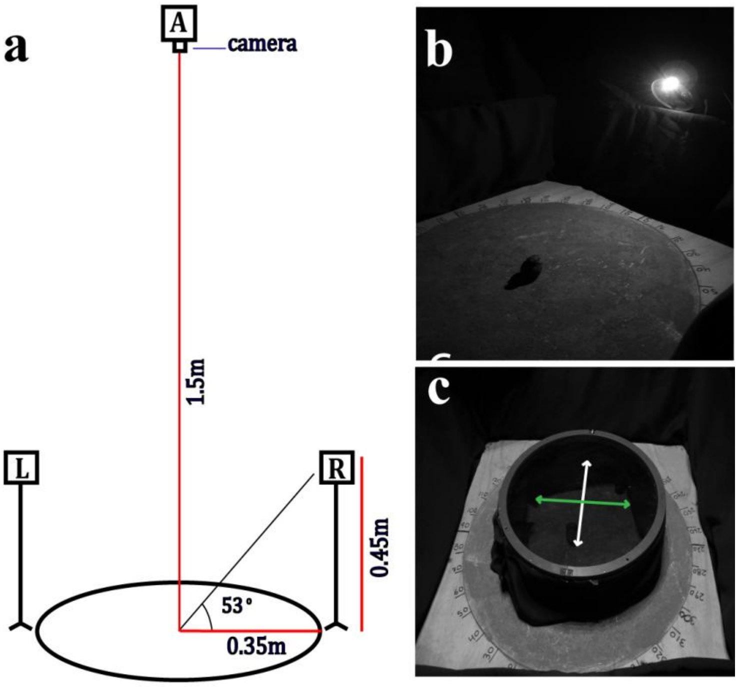

Fig. 2 Experimental set-ups. ***(a)*** Schematic diagram of the experimental set-up in Experiment 3 (and part of Experiment 4). A beetle is placed in the center of a circular sand covered arena, measuring 0.35m in radius. On each side of the arena, directly facing each other are two light fixtures used during the *Side* condition (L and R), each equipped with a LED emitting white light. Both light fixtures are at a height of 0.45m, thus the angular elevation of the light from the center of the arena is counted to 53°. 1.5m above the arena a light fixture with 8 white LEDs, used for the *Above* condition, is positioned (A). In the center of this light fixture, which is arranged in a circle, a camera is mounted allowing recordings of the beetle to be made from above. The light set-up (A), with an addition of 8 LEDs emitting UV-light, also positioned on the same fixture, were used in Experiment 4. ***(b)*** The set-up for Experiment 3. In the center of the arena a beetle with its ball is shown, and the right light (R) is lit. ***(c)*** Experiment 4 has a similar set-up as for the *Above* condition. Aside from the addition of 8 UV-light LEDs, a polarizing filter was placed over the beetle and its ball. The green arrow indicates the direction of the e-vector in the *Pol90°* condition, while the white arrow indicates the direction of the e-vector in the *Pol0°* condition.

when the beetle had been allowed to dance two times with light from the same direction, or a switch was made after the first dance. Furthermore, the order in which the two lateral lights were presented was alternated for each beetle.

Thus, each individual beetle danced under three conditions for a total of seven times (two presentations of the above light, three presentations of the lateral light, and two consecutive presentations of above light). In all conditions, the beetles were placed down facing the ball in the same orientation with respect to the arena.

Experiment 4: The effect of polarized light on dance behavior

The experimental arena and general procedure was the same as that described in Experiment 3.

In this experiment, the light stimulus mounted above the center of the arena consisted of the same 8 white light LEDs as used for the *Above* condition in Experiment 3. In addition to these, an additional 8 LEDs emitting UV-light, arranged in a circle with a diameter of 15cm, were presented together with the white light LEDs. A UV-transparent polarization filter with a diameter of 42cm was placed over the beetle and arena (Fig. 2c) to present the beetles with linearly polarized light with one electronic vector (e-vector) orientation. The beetle was presented with two conditions. For the first condition, the polarizing filter was fixed over the beetle in a manner that resulted in the orientation of the electronic vector (e-vector) being oriented parallel to the head direction of the beetle (*Pol0°*) (white arrow in Fig. 2c).

For the second condition, the filter was turned 90° in relation to the *Pol0°* condition orientation, resulting in the e-vector being oriented perpendicular to the beetle’s head direction (*Pol90°*) (green arrow in Fig. 2c).

To ensure that any change in dance behavior was not a result of the number of times the beetle had danced, but a direct result of the change in orientation of the e-vector, the beetle was allowed to dance and roll out under the polarizing filter two consecutive times before turning the polarizing filter 90°. The beetle was then allowed to dance two additional times under the filter that had now been turned. The first condition was alternated, so that half of the beetles started with the *Pol90°* condition and the other half with the *Pol0°* condition. Each individual beetle danced a total of four times.

Results

For a summary of the number of pauses and total rotation in each condition see Table 1 and Fig. 3.

Experiment 1: The effect of total darkness on dance behavior

The aim of this experiment was to test how complete darkness affects the beetle’s dance behavior.

Comparing the frequency of pauses made by the beetle when it is dancing in an enclosed arena in complete darkness to when the beetle is dancing outside under the sun, it can be shown that the beetles do pause significantly more in the dark (P=<0.001; *Dark*, 5±6; *Sun*, 2±1.25, median ± IQR). A similar trend is seen in the total angular rotation of the beetle. The beetles rotate significantly more during the dance in total darkness, compared to that in natural light (P=0.0012, *Dark*, 327.5±492°; *Sun*, 172.5±100°). This data shows that, when dancing in the dark, beetles perform larger angular rotations and pause more than when dancing in full sunlight.

Experiment 2: The effect of blocking the dorsal and frontal view on dance behavior

The aim of this experiment was to test the effect of occluding celestial compass information in an otherwise bright, natural setting on dance behavior.

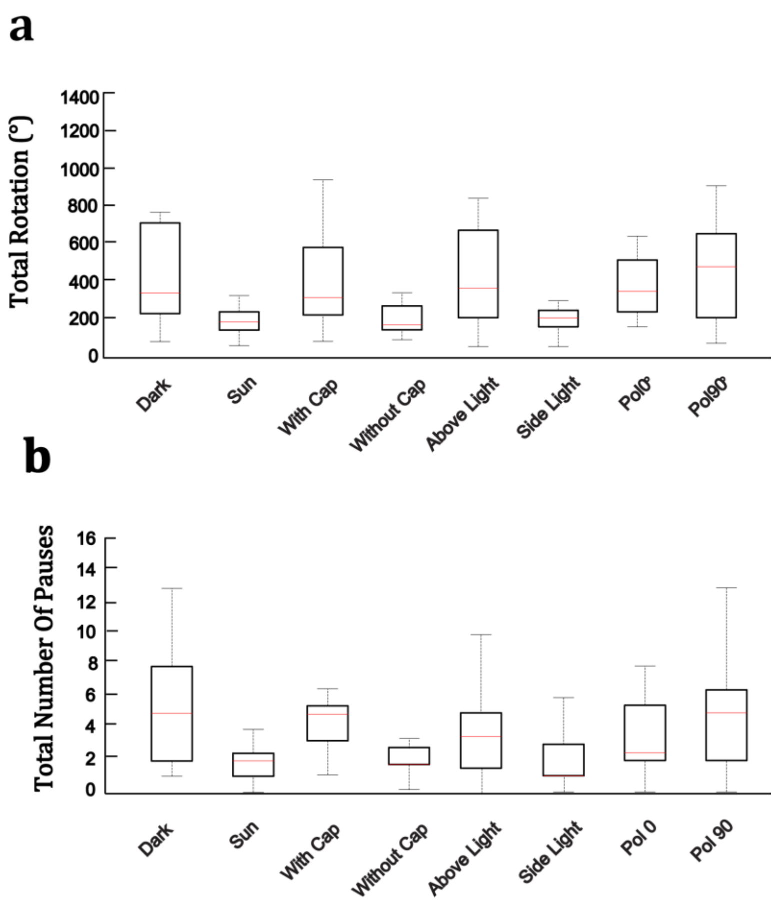

Fig. 3 An overview of the total rotation and the total number of pauses for each condition. For each box plot, the lower and upper part of the box represents the lower and upper quartile values, respectively. The red line of each box represents the median, and the whiskers represent the data values of up to 1.5 times the interquartile range. In all groups but two (*With Cap* and *Without Cap*), the data presented represents a total of 20 individuals. For the remaining two, the data represent 10 individuals. Each individual represents one dance. ***(a)*** The figure shows the total angular rotation of each individual within each group. ***(b)*** The figure represents the total number of pauses of each individual within each group.

Table 1. Median and inter quartile range (IQR) for the frequency of pauses and the total rotation over all conditions. The table below shows the median of the total number of pauses and the total degree of rotation given by each group of beetles during their orientation dance, over all conditions. Included in the table is also the value for the IQR of each data set over each condition. The data for each condition in Experiment 3 and 4 represent the first presentation of them. For a more detailed overview of all experimental conditions see Methods. For a full table of the data from which median and IQR was calculated, see Supplementary Results.

		Experiment 1		Experiment 2		Experiment 3		Experiment 4	
		Dark	*Sun*	*With Cap*	*Without Cap*	*Above*	*Side*	*Pol0°*	*Pol90°*
Frequency of Pauses	**Median:**	**5**	**2**	**4.5**	**1**	**3.5**	**1**	**2.5**	**5**
	Interquartile Range	6	1.25	1.75	0.75	3.25	1.75	3.25	4
Total Rotation (°)	**Median:**	327.5	172.5	270	128.5	355	192.5	335	472.5
	IQR:	473	95	324.25	103.25	433.75	78.75	265	371.25

difference between the number of pauses in the *With Cap* condition and the number of pauses in the *Without Cap* (P=0.05, *With Cap*, 4.5±1.75; *Without Cap*, 1±0.75).

Similarly, there was some indication that the amount of rotation was slightly higher when the beetle was equipped with a cap, compared to when it was not (P=0.0446, *With Cap*, 270±352°; *Without Cap*, 128.5±126°). Thus, when the celestial compass cues are occluded, beetles tend to rotate and pause slightly more than when they have a full view of the sky.

Experiment 3: The effect of a single light source on dance behavior

The aim of this experiment was to observe (1) the effect on dance behavior when the beetle was presented with a single compass cue and (2) if the position of this single cue had an effect on the dance behavior in terms of the total degree of rotation and the total number of pauses the beetle performs during its dance.

In all conditions, the beetles were placed in the same orientation with respect to the arena such that any differences could be related to the change in the position of the light source rather than the orientation of the beetle at the start of the dance.

In terms of the total rotation of the beetle during the three presentations of each treatment, there was no significant difference ($P_{First\ Left\ vs.\ First\ Right}$=0.4270, $P_{Second\ Left\ vs.\ Second\ Right}$=0.9131 and $P_{Third\ Left\ vs\ Third\ Right}$=0.7792, *First Left*, 190±66.25°; *Second Left*, 120±70°; *Third Left*, 135±103.75°, *First Right*, 210±116.25°; *Second Right*, 155±85°; *Third Right*, 130±205°, respectively). As for the total number of pauses performed, no significant difference could be found ($P_{First\ Left\ vs.\ First\ Right}$=0.4158, $P_{Second\ Left\ vs.\ Second\ Right}$=0.5291, $P_{Third\ Left\ vs.\ Third\ Right}$=0.6355, *First Left*, 1±0.75; *Second Left*, 1±2; *Third Left*, 1±1.5; *First Right*, 3±2; *Second Right*, 2±1; *Third Right*, 1±1.75, respectively).

When comparing the three presentations of the *Side* condition with each other, the total degree of rotation in the first presentation of *Side* is significantly higher than the second and third presentation ($P_{First\ vs\ Second}$=0.0366 and $P_{First\ vs\ Third}$=0.0380, *First Side*, 192.5±79°; *Second Side*, 120±85°; *Third Side*, 135±118°). No significant difference could be seen between second and third *Side* (P=0.0718). In terms of pauses, the frequency does not differ significantly, with the exception of *First Side* compared to *Third Side* ($P_{First\ Side\ vs.\ Second\ Side}$=0.1090, $P_{First\ Side\ vs.\ Third\ Side}$=0.0265, $P_{Second\ Side\ vs.\ Third\ Side}$=0.6326, *First Side*, 1±2; *Second Side*, 1±2; *Third Side*, 1±2).

For the *Above* condition, the total rotation between the first and second presentation show no significant difference (P=0.1895, *First Above*, 355±473°; *Second Above*, 278±365°). However, the second presentation of the *Above* condition shows a significant decrease in total number of pauses (P=0.0135, *First Above*, 3±4; *Second Above*, 2±4).

When comparing the *Side* condition with the first presentation of the *Above* condition, the total degree of rotation is significantly higher for the *Above* condition. This is true for all three presentations of the *Side*, when compared to the presentation of the initial above light ($P_{First\ Above\ vs.\ First\ Side}$=0.0304, $P_{First\ Above\ vs.\ Second\ Side}$=0.0017, $P_{First\ Above\ vs.\ Third\ Side}$=0.0011). In terms of pauses, there is no difference in the frequency between *First Side* and *First Above*. On the contrary, the frequency of pauses decrease significantly for *Second Side* and *Third Side* when compared to the *First Above* condition ($P_{First\ Above\ vs.\ First\ Side}$=0.1741, $P_{First\ Above\ vs.\ Second\ Side}$=0.0048, $P_{First\ Above\ vs.\ Third\ Side}$=0.0015).

The data shows that the direction from which the side light is presented (*Left* or *Right*) has no effect on the dance in terms of total rotation or number of pauses. However, the number of times in which the beetle is presented with the side light, does affect the dance. The beetle will rotate more when presented with the side light for the first time.

The number of times in which the beetle is presented a light also plays a role in the *Above* condition. Here, the beetle will pause less when presented with the above light for a second time.

Because the previous experiments (1 and 2) investigate the dance of the beetle when it first is presented with a condition, only data from the first *Above* and first *Side* are included in the tables and figures (Table1-3, Fig. 3-4).

Experiment 4: The effect of polarized light on dance behavior

The aim of this experiment was to test if a change in the orientation of the e-vector of polarized light had an influence on dance behavior. As in the last experiment, the beetles all started the dance in the same orientation with respect to the arena so that any observed differences in the dance behavior are related to the change in orientation of the compass cue.

There was no significant difference in the frequency of pauses or the total angular rotation between the first and second presentation of the polarizing filter in the *Pol0°* condition (P_{Pause}=0.2412, P_{Total}

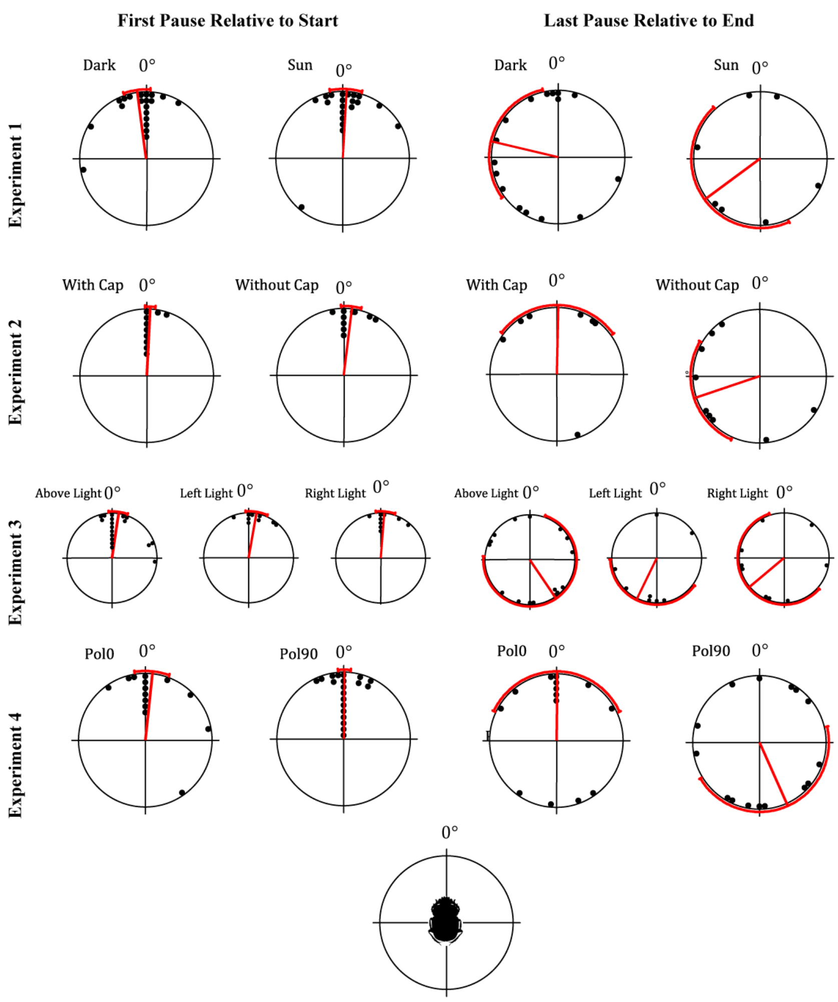

Fig. 4 Angular difference between the beetle's head direction in the first and last pause. The circular plots show the relationship between the beetle's head position at the initiation and completion of the dance compared to the first and last pause performed, respectively. The head direction of the beetle at the start of the dance and at the end of the dance are both represented at 0°, as portrayed by the illustration at the bottom of the figure. The first circular plot of each condition, on each row, represents where the first pause of the beetle is positioned in relation to its head direction at start (when it first climbs on the ball). The second circular plot of each condition, on each row, represents the position of the last pause in relation to the beetle's head direction at the end (immediately before it will roll the ball away). Each point in the plot represents one dance of one individual. The red line of each plot present the mean direction and the curved red line represents the 95% confidence interval, For Experiment 3, the side light condition is further divided in and presented as the two treatments; *Left and Right* condition. All figures shown for Experiments 3 and 4 represent the dance from the first presentation of each condition.

$_{Rotation}$=0.1421, *First Pol0°*, 3±4; *Second Pol0°*, 2±3, *First Pol0°*, 335±280°; *Second Pol0°*, 218±218°, respectively). Likewise, there was no significant difference between the first and second presentation under the *Pol90°* condition regarding the frequency of pauses and the total angular rotation (P_{Pause}=0.1477, $P_{Total\ Rotation}$=0.1574, *First Pol90°*, 5±4.5; *Second Pol90°*, 3±4, *First Pol90°*, 472.5±455°; *Second Pol90°*, 290±297.5°, respectively).

The 90° degree rotation of the e-vector had no effect on the frequency of pauses, nor on the angular rotation of the beetle ($P_{0°\ Condition\ vs.\ 90°\ Condition}$ =0.3116 and $P_{0°\ Condition\ vs.\ 90°\ Condition}$ =0.072, *Pol0°*, 2.5±3.5; *Pol90°*, 5±4.5, *Pol0°*, 335±280°; *Pol90°*, 472.5±455°, respectively). This suggests that a change in e-vector direction has no effect on the dance, despite the beetle being placed in the same start orientation with respect to the arena.

The relationship between pause and dance

To investigate the function of the pauses, the orientation of the beetle in the first pause was compared to the orientation in which it climbs up on the ball (and thus initiates the dance) and the orientation of the last pause was compared to the orientation of the beetle when it begins to roll the ball away. This was compared in all five experiments (Fig. 4).

For all conditions there is a consistent pattern in the relationship between the head direction of the first pause and the initiation of the dance, which is close to 0°. On the contrary, there is no clear pattern in any condition when comparing the head direction in the last pause and the head direction when starting to roll (Fig. 4).

The relationship between conditions

As the first two experimental conditions remove all visual cues from the dorsal visual field, the difference between these dances was investigated to see whether or not they generate similar dances in terms of number of pauses and total rotation. Due to the fact that the *With Cap* condition only had half of the number of individuals as all other conditions (10 individuals compared to 20 individuals), ten individuals were randomly selected from the *Dark* condition group to compare with the *With Cap* condition. Comparison between the two groups presented no significant difference in terms of the number of pauses and total rotation (P=0.7306, P=0.5964, respectively). Further, because the *Above* condition and the *Dark* condition did not provide the beetles with any celestial compass cues, the relationship between these dances was also investigated. No difference could be seen in terms of number of pauses or total rotation between the two conditions (P=0.1677, P=0.9460, respectively).

The *Side* condition from Experiment 3 presents the beetle with a clear, readable light cue. To examine if the *Side* condition influences dance behavior to the same extent as a natural condition outside, it was compared with the *Sun* condition of Experiment 1. No difference regarding the number of pauses or the total rotation could be found between the two conditions (P=0.3167, P=0.9545, respectively).

It should be noted that each experiment showed instances where some beetles did not perform a dance, but instead directly climbed over the top of the ball and started rolling. The frequency of these non-dances was very low in each condition. The highest occurrence of non-dancing under a changed environment was for Experiment 4, where 6.25% of all dances performed were non-dances. Aside from the dances recorded in a modified environment, 20% of individuals in the *Sun* condition, and *Without Cap* condition (were the beetles danced outside in natural conditions) respectively, did not perform a dance.

Moreover, it should also be noted that for the modified conditions, discounting the *With Cap* condition, beetles always danced in the first presentation of an experimental condition.

Discussion

In this paper I investigate the orientation dance of the dung beetle *Scarabaeus (Kheper) lamarcki* and how it is affected by changes in the visual input. These changes can be categorized into two sections; (1) the removal of visual cues, and (2) the modification of different celestial compass cues.

Removal of visual cues

When all visual cues were removed (*Dark* condition, Experiment 1) beetles perform an orientation dance that is longer, both in terms of the number of pauses and in total angular rotation, compared to when they dance in full sunlight (*Sun* condition, Experiment 1). Because the beetle is lacking visual cues, it cannot gather the compass information that is needed. However, the beetle will attempt to gather compass information despite the lack of visual information, resulting in a longer dance. In the *Sun* condition, the visual scene provided sufficient information for the beetle, so the beetle would not need a long time to acquire the information it needs to keep a straight roll bearing direction. With a cap on, the visual input from the celestial cues was removed, as the dorsal field of view was occluded. However, the ventral view was not obstructed, so the beetle could get visual input from the ventral side. But as the visual input given is not of any readable celestial cues, the features of the dance are similar to that of the *Dark* condition. In addition, the beetles rotated significantly more when wearing a cap than when the cap was removed. This suggests that the beetle had a greater difficulty in finding a readable compass cue when wearing a cap. The need to search more for a cue results in a higher total rotation. Because there was a lack of dorsal visual input during the *With Cap* condition, I would have also expected to see a higher frequency of pauses compared to *Without Cap* condition. I did not. A possible explanation could be that as there were only ten beetles tested in this experiment, and not twenty as for the other four, the low n-number might have had an effect on the statistical outcome. Would I have had more individuals for this experiment, I might have obtained a different p-value.

From these results we can conclude that the orientation dance of the beetle is visually regulated, as removal of all or some of the visual celestial cues affects the dance.

Modification of visual cues

After investigating the effect on the orientation dance by removal of visual cues, I next explored the role of different celestial compass cues and their importance for the dance.

A single light source in what is otherwise a dark, featureless room can act as an orientation cue for a beetle if it is placed at an offset from the zenith.

A light presented from above does not provide sufficient information for orientation. There is no contrast to any other point in the visual scene, so no clear reference point is conveyed to aid in deciding a bearing. This situation is similar to that of the *Dark* condition. In this condition the visual scene provided no useful visual compass information. Comparing the two conditions, no significant difference can be seen. This suggests that a change in the orientation dance is not caused by the lack of light, but by the lack of readable cues.

When light is presented from the side, the cue is reliable as it gives the beetle a clear reference point in the visual scene. The basis of a clear, readable cue in the visual scene is reminiscent of a natural condition, where there is also a clear, readable cue – the sun. If we compare the dances of the beetles in the *Side* condition to that of the *Sun* condition, we see no significant difference between them. This indicates that the *Side* condition provides similar sufficient information for the beetle, as a natural condition outside. With this information, it can be assumed that the dance will give rise to a higher number of pauses and a higher total rotation when the light is presented from above than from the side. This has been found to be true in all cases but one. The only instance that stands out is between the first *Above* condition and the first *Side* condition. As these conditions are the first of their treatments, the beetles are naïve in both of these conditions. The beetle will thus pause more to grow accustomed to the new compass cues. In the remaining conditions (second and third *Side* and second *Above*) the beetles are already familiar with the compass cues now and do not need to pause as frequently to gather information.

Changing the direction of a reliable cue by 180° does not affect the orientation dance. When the position of the light changes, the beetle will already have a visual memory of the scene, the only difference now is that this stored view is mirrored to the current view. This is not a drastic change for the beetle. It still only needs to match the information it has gathered via the snapshots taken in the previous dance, to the current view. The same principle can explain why a change in the direction of the e-vector with 90° has no effect on the orientation dance of the beetle. The beetle already has a visual memory of the scene, and changing the direction of the e-vector does not have a large enough impact to confuse the beetle and affect its dance.

From these results we can conclude that modifying the location of a visual cue will not affect the number of pauses and the total rotation of the dance as long as it has similar properties to the cues that it has seen in the previous dance

Is *the dance an innate behavior?*

We see that the orientation dance is affected by visual cues. In addition to this, I suggest that the dance is not purely visually based. This can be seen from the *Dark* condition. Although the dance is affected by the lack of visual input when compared to the *Sun* condition, dances are still performed when no visual information is present. Of course, because the *Dark* condition is performed inside while the *Sun* condition is performed outside, one can argue that there are many non-visual factors that could potentially influence the dance. Scent, humidity or wind cues are a few examples of information that the beetles may be reading while dancing. To show that the dance performed by the beetle when visual cues are lacking is not because of changes in non-visual cues we can look at Experiment 3. Here, both conditions are performed inside, in the exact same set-up. We have previously showed that the *Above* condition does not provide any readable cues, while the *Side* condition does. If the dance were purely visually based, the beetle would not dance when it is not given any visual input of any kind. However, the beetle will still dance in the *Dark* and the *Above* conditions. I therefore suggest that while the dance is visually regulated, it is not visually based and thus seem to be an innate behavior that is triggered when the beetle comes into contact with the ball.

My hypothesis is further confirmed when we study the head direction of the beetles in each condition. Throughout all conditions, the common denominator is the initial pause. The majority of individuals that paused in their dance, paused within the vicinity of 90° from where they mounted the ball (Fig. 3), irrespective of which direction they were initially placed in (see the *Dark* condition). No such relationship can be seen between the last pause of the beetle and the head direction when the beetle dismounts the ball and begins to roll. This indicates that the first pause and its direction is hard-wired and a definite part of the dance. As the first pause is present in all dances, I hypothesize that this first pause is where beetles decide their roll bearing. However, this hypothesis has not been tested in the present study and further detailed investigations are required to determine whether this is indeed the case

Conclusion

In order to navigate to and from a specific goal, most insects use vision as their primary sensory modality (Collett et al., 2006; Zeil, 2012). These animals take a "snapshot" of a specific goal, creating a visual memory of its location with respect to different directional cues. To navigate back to this goal, the stored view is matched and compared to their current view. This "snapshot model" is a very plausible model for many insects seen to perform a scanning behavior, such like the turn back and look of bees, or the scanning pirouettes of ants (Wehner & Raber, 1979; Cartwright & Collett, 1983; Collett et al., 2006; Zeil et al., 2014).

Similar snapshot behavior is suggested to be used here by ball-rolling dung beetles. The main difference, however, is that the dung beetles snapshot includes only information about the celestial compass cues, rather than landmark cues. I propose that dung beetles take snapshots of the current view of the celestial compass cues during their dance. These snapshots are then later stored and matched to the current view given whilst rolling, allowing the beetle to roll in a constant direction. When clear directional cues are present, few snapshots are sufficient to give the information needed in order to keep a straight roll bearing. On the contrary, when cues are lacking, or unreliable, a few snapshots are not sufficient, but the beetle needs to take several, at different angles to keep a straight bearing.

It is still unclear, however, how the beetle chooses its initial roll bearing. The snapshot model helps us understand the reasoning behind the dance, but it cannot answer how and when the beetle chooses its roll bearing. One can speculate that because the first pause is present in all conditions, regardless of visual input, this could be where the beetle decides its roll bearing. This however is mere speculation. Further investigations into how and when dung beetles set their preferred bearing are required. Overall, the results presented here provide a better understanding of the behavioral mechanisms that orienting, but non-goal oriented, animals use to set a bearing, to maintain it while moving and to find it again when the path is disturbed.

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Supplementary Results

The data for the number of pauses and the total angular rotation performed by each individual beetle, for all conditions, is presented in the tables below (Table 2 and 3, respectively). It is important to note that each experiment had an independent group of individuals that did not perform conditions of any other experiments but of their own.

Table 2.Total number of pauses for all conditions. The table below shows the total number of pauses (including median and inter quartile range, IQR) given by each beetle during its orientation dance, over all conditions. For a more detailed overview of all experimental conditions see Methods.

	Experiment 1		**Experiment 2**		**Experiment 3**		**Experiment 4**	
Beetle	*Dark*	*Sun*	*With Cap*	*Without Cap*	*Above*	*Side*	*Pol0°*	*Pol90°*
1	6	2	1	0	2	2	8	3
2	2	4	2	0	5	5	2	5
3	2	1	3	1	1	1	2	1
4	1	1	4	1	4	1	4	8
5	3	2	4	1	5	6	4	0
6	8	5	5	1	1	1	2	7
7	2	2	5	1	6	1	2	2
8	13	0	5	2	2	1	8	5
9	8	4	6	7	11	1	1	2
10	9	2	6	9	2	1	1	6
11	4	1			10	3	6	1
12	5	2			2	1	6	13
13	5	1			5	1	13	2
14	9	3			3	3	5	16
15	2	2			6	7	3	3
16	4	2			5	7	2	5
17	5	4			1	1	2	2
18	5	1			4	3	1	6
19	1	1			0	3	4	6
20	11	1			0	1	0	7
Median	**5**	**2**	**4.5**	**1**	**3.5**	**1**	**2.5**	**5**
IQR	**6**	**1.25**	**1.75**	**0.75**	**3.25**	**1.75**	**3.25**	**4**

Table 3. Total rotation angle for all conditions. The table below shows the total angular rotation (including median and the inter quartile range, IQR) for each beetle during its orientation dance, over all conditions. For a more detailed overview of all experimental conditions see Methods.

	Experiment 1		**Experiment 2**		**Experiment 3**		**Experiment 4**	
Beetle	*Dark*	*Sun*	*With Cap*	*Without Cap*	*Above*	*Side*	*Pol0°*	*Pol90°*
1	240	145	300	293	240	190	580	650
2	190	285	884	227	535	215	145	435
3	547	170	96	157	140	190	180	170
4	694	140	530	444	350	195	445	540
5	240	185	178	123	730	570	475	125
6	620	45	40	101	180	245	300	750
7	65	143	240	48	840	125	230	145
8	660	75	460	65	100	170	635	570
9	766	120	219	134	755	40	370	220
10	720	120	540	122	225	135	155	360
11	290	180			800	420	540	115
12	165	260			360	220	610	885
13	340	175			520	140	535	230
14	720	135			280	100	460	1355
15	105	225			605	220	270	615
16	270	195			370	540	410	650
17	720	485			210	150	245	55
18	315	60			780	200	225	510
19	150	230			75	290	205	365
20	720	315			40	175	225	905
Median	**327.5**	**172.5**	**270**	**128.5**	**355**	**192.5**	**335**	**472.5**
IQR	**473**	**95**	**324.25**	**103.25**	**433.75**	**78.75**	**265**	**371.25**