Crucial role of ultraviolet light for desert ants in determining direction from the terrestrial panorama

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Abstract: Ants use the panoramic skyline in part to determine a direction of travel. A theoretically elegant way to define where terrestrial objects meet the sky is to use an opponent-process channel contrasting green wavelengths of light with ultraviolet wavelengths. Compared with the sky, terrestrial objects reflect relatively more green wavelengths. Using such an opponent-process channel gains constancy in the face of changes in overall illumination level. We tested the use of ultraviolet (UV) wavelengths in desert ants by using a plastic that filtered out most of the energy below 400 nm. Ants, Melophorus bagoti, were trained to home with an artificial skyline provided by an arena (Experiment 1) or with the natural panorama (Experiment 2). On a test, a homing ant was captured just before she entered her nest, and then brought back to a replicate arena (Experiment 1) or the starting point (the feeder, Experiment 2) and released. Blocking ultraviolet light led to deteriorations in orientation in both experiments. If the artificial skyline was transformed from opaque to transparent ultraviolet-blocking plastic (Experiment 3) on the other hand, the ants were still oriented. We conclude that UV wavelengths play a crucial role in determining direction based on the terrestrial surround.

Crucial role of ultraviolet light for desert ants in determining direction from the terrestrial panorama

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Running head: Ultraviolet light, panorama, and determining direction

***Detailed Response to Reviewers**

Dear Dr. Jeanson,

Thank you and Reviewer 1 for your comments on our revised manuscript. They have again helped to improve our manuscript. In this revised version, we have added discussion of other insect species when it comes to using UV wavelengths in navigation, a suggestion of yours. We have also done our best to fix up tables and figures in the format for *Animal Behaviour*. Detailed replies follow.

We are happy to make any further changes that you think will improve the manuscript.

On behalf of all authors,

Ken Cheng

Dear Authors,

I am happy to accept your paper "Crucial role of ultraviolet light for desert ants in determining direction from the terrestrial panorama" (ANBEH-D-15-00959) for publication in Animal Behaviour, subject to minor revisions.
I agree with Reviewer 1 that your revision substantially improved the manuscript. However, I am still a bit concerned about the relatively narrow scope of your manuscript. As things stand now, your paper exclusively focuses on ants, with no reference to other taxa. I thus strongly encourage you to broaden the scope of your manuscript by adding some information relative to the use of UV on orientation in other taxa (e.g. beetles).

Reply. We have added a paragraph at the end of the discussion that includes brief mention of dung beetles and desert locusts. Dung beetles use UV wavelengths in their perception of polarised light. We chose them because we deem the work excellent and interesting. Locusts have provided much neurobiology of the celestial compass, and we cited what we think is a great recent review of it (el Jundi et al., 2014). But they also deserve brief mention because green-UV opponent-process neurons have been found in their circuits for the celestial compass. In this way, we have broadened the taxa discussed without roaming far beyond the topic of navigation, which we would deem inappropriate.

Thanks very much for the suggestion.

In addition, I have a few minor queries listed below.

- Table 1: Please made explicit that "ZV UV block combined" is the combination of the results of "ZV UV block inside" and "ZV UV block outside", not an experimental condition combining the UV block inside and outside. Same remark for other tables.
Reply. Done.

- Table 4: Please indicate that Control 1 and Control2 are control trials for two replicates (not two different control trials)

Reply. Done.

- Header of Table 4. Remove "and full-vector (FV)" as the table only report results for ZV ants.
Reply. Done.

- Tables should have a short one-sentence title above the table with other information placed below the table.
Reply. Done.

- Tables. Remove the horizontal lines.
Reply. Done.

- Line 226: Table 4, not Table 2
Reply. Done.

- Line 239: Table 5, not Table 3.
Reply. Done.

- Small P-values should be indicated as P<0.001, e.g. not P<10-22
Reply. Done.

- Table 4: change the header of Table 4 as this table does not include results of FV ants.
Reply. Done.

- Line 281: "confidence" not "confidene"
Reply. Done.

- 4th highlight: "this transparent skyline was sufficient for" instead of "this transparent skyline for sufficient for"
Reply. Done.

- Shorten the 5th highlight (maximum 85 characters including spaces)
Reply. Done.

- Please list keywords alphabetically.
Reply. Done.

- Figures: labels should be in full parentheses (e.g. (a)) and placed inside the axes of the graph.
Reply. Done.

- Note that Animal Behaviour uses APA style for citations and references.
Reply. We have checked over the references for APA style.

- On the title page for each affiliation add the town and country where the university is located.
Reply. Done.

- In statistics, N, P, should be capital letters in italics.
Reply. Done.

- Figures. Remove the horizontal background lines and put the labels in parentheses inside the photo or graph (e.g. (a)). Parts of figures should not have titles as well as labels, e.g. Fig. 2a should just be labelled (a) not (a) Transmission of UV-blocking plastic. The figure legend should say what the graph is about.
Reply. Done.

- Fig. 1. Word labels should start with a capital letter, e.g. Nest to feeder.
Reply. Done.

- Upload only the non-highlighted tables. We do not need highlighted versions.
Reply. We will do this in this round of submission.

- Tables should have a short title above the table with other information placed below the table.
Reply. Done.

As you revise your manuscript, please note that the journal's guidelines require that you address any animal welfare issues arising from your study within the Methods section, preferably in a separate subsection of the Methods headed Ethical Note. Even if your study involves only invertebrates, please address all ethical implications of the experimental design and procedures, including any procedures taken to minimize adverse impacts on the welfare of subjects or to enhance their welfare. For further details on what ethical information to include, please consult the "Animal Welfare" and "Methods" sections of the journal's "Guide for Authors" and "A Guide to Ethical Information Required for Animal Behaviour Papers" (http://www.elsevier.com/framework_products/promis_misc/ethyanbe.doc).

When you revise your paper, you should prepare a detailed explanation of how you have dealt with all of the reviewers' and my own comments. Refer to the Instructions for Authors (on the main menu of the Elsevier Editorial System at http://ees.elsevier.com/anbeh/) for details of our house style and for a list of file types that are acceptable for revised papers. Log in to the Elsevier Editorial System as an Author to submit your response to the comments and your revised paper. Changes in the revised paper should be highlighted in Word or underlined. Please submit both the highlighted version and the non-highlighted version of the revised paper.

We should like to receive the revised paper within 30 days. If you think you will be unable to revise your manuscript in that time please let the Journal Office know (yanbe@elsevier.com). Please do not reply directly to this email.

Best wishes,

Raphael Jeanson
Editor

Reviewer #1: Only trivial comments now, much better.

line 26 perhaps simpler to say 'define the location of the skyline'?

Reply: we changed it to “define where terrestrial objects meet the sky”, taking in part the suggestion from another comment. In this sentence, we wanted to define what a skyline is implicitly.

lines 31, 100, 150 - 152. 'blocked most wavelengths' sounds funny - some blocked some not. Perhaps: 'filtered out most of the energy below 400nm'

Reply: We have adopted the suggested terminology, thanks.

line 58. Don't much like 'the elevations of the tops of the terrestrial panorama' perhaps: where earth meets sky across the 360 deg panorama'

Reply: We changed the phrase to “where terrestrial objects meet the sky across the 360°”. We think that simply using the term “earth” might confuse some readers to interpret it as groud level.

line 306 sentence beginning 'Zero..' would read better if it started In the control condition, zero

Reply: We have changed the sentence as suggested. It indeed reads better, thanks.

Thanks so much for reading and commenting on our manuscript again.

Highlights

Patrick Schultheiss et al.

- A clear plastic was used to reduce ultraviolet (UV) light in the panoramic view
- Ants were much worse at homing using terrestrial cues with UV wavelengths reduced
- The terrestrial panorama was also reproduced with the UV-blocking plastic
- This transparent skyline for sufficient for orientation in homing desert ants
- UV light plays a crucial role in ant navigation based on terrestrial cues

Crucial role of ultraviolet light for desert ants in determining direction from the terrestrial panorama

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Running head: Ultraviolet light, panorama, and determining direction

Abstract

Ants use the panoramic skyline in part to determine a direction of travel. A theoretically elegant way to define where terrestrial objects meet the sky is to use an opponent-process channel contrasting green wavelengths of light with ultraviolet wavelengths. Compared with the sky, terrestrial objects reflect relatively more green wavelengths. Using such an opponent-process channel gains constancy in the face of changes in overall illumination level. We tested the use of ultraviolet (UV) wavelengths in desert ants by using a plastic that filtered out most of the energy below 400 nm. Ants, *Melophorus bagoti*, were trained to home with an artificial skyline provided by an arena (Experiment 1) or with the natural panorama (Experiment 2). On a test, a homing ant was captured just before she entered her nest, and then brought back to a replicate arena (Experiment 1) or the starting point (the feeder, Experiment 2) and released. Blocking ultraviolet light led to deteriorations in orientation in both experiments. If the artificial skyline was transformed from opaque to transparent ultraviolet-blocking plastic (Experiment 3) on the other hand, the ants were still oriented. We conclude that UV wavelengths play a crucial role in determining direction based on the terrestrial surround.

Key words: desert ants, green, orientation, panorama, skyline, ultraviolet,

Navigating ants use a multifaceted toolkit (Wehner, 2009). Along with path integration (Wehner & Srinivasan, 2003), ants are known to use visual terrestrial cues for navigation (*Temnothorax albipennis*: Pratt, Brooks, & Franks, 2001; *Formica rufa*: Graham & Collett, 2002; Lent, Graham, & Collett, 2013; *Cataglyphis fortis*: Wehner, Michel, & Antonsen, 1996; *Melophorus bagoti*: Wystrach, Beugnon, & Cheng, 2011, 2012; Wystrach, Schwarz, Schultheiss, Beugnon, & Cheng, 2011; *Myrmecia croslandi*: Narendra, Gourmaud, & Zeil, 2013; Zeil, Narendra, & Stürzl, 2014). And as a 'back-up', they also engage in systematic searching (Schultheiss, Cheng, & Reynolds, 2015).

Some properties of the panorama have been shown to guide ants travelling on familiar routes, including fractional position of mass, matching of segments of the scene, and the skyline. Fractional position of mass refers to the amount of the visual scene to one's left vs. right as one faces the goal direction. Wood ants (*F. rufa*) use this cue in some conditions in the lab (Lent et al., 2013). In other conditions, *F. rufa* might match a salient segment of the scene (Lent et al., 2013). The skyline is some record of where terrestrial objects meet the sky across the 360° panorama (Dyer, 1987; Graham & Cheng, 2009a, 2009b; Towne, 2008; Towne & Moscrip, 2008; von Frisch & Lindauer, 1954). Its use was demonstrated in Central Australian desert ants (*M. bagoti*) when an artificial skyline in black was created to mimic the natural skyline seen from the start of the journey (Graham & Cheng, 2009a). The ants oriented according to the artificial skyline even when it was rotated so that the celestial cues associated with the panorama did not match in test and training conditions.

Here we investigate further the nature of the sensory input used for view-based matching, focusing on the role of ultraviolet (UV) wavelengths of light in the use of the terrestrial panorama. Ants have been found to have two types of visual receptors in their compound eyes and ocelli (*Cataglyphis bicolor*: Mote & Wehner, 1980), or sometimes three (*Myrmecia croslandi* and *M. vindex*: Ogawa, Falkowski, Narendra, Zeil, & Hemmi, 2015). In these cited cases, one type is most sensitive to light in the green range, with maximum sensitivity at ~510 nm or ~550 nm. One other type has highest sensitivity in the UV range, peaking at ~350 nm or ~370 nm. Ground objects typically do not reflect much in the UV wavelengths, far less so than what is found in the sky (Möller, 2002). Theoretically, UV wavelengths are useful for segregating ground objects from the sky.

Two different ways of using UV wavelengths for delineating the skyline have been proposed. Möller (2002) proposed that UV-green contrast, sensitive to the ratio of UV irradiance to green irradiance, might be used to differentiate sky from ground, and thus delineate the skyline. An opponent-process contrast based on the UV:green ratio buys constancy in the face of fluctuating overall intensity both across time and across space. If a cloud covers the sun temporarily and drops the intensity, both the green reflectance of terrestrial objects and the UV irradiance in the sky diminish. But at the local level, the ratios stay fairly constant, as measured empirically by Möller (2002). While UV-green opponent neurons have been found (in locusts: Kinoshita, Homberg, & Pfeiffer, 2007), a proposed UV-green channel for segregating ground objects from the sky remains hypothetical. But such opponent-process systems are well known in other domains of visual processing in which constancy is important, such as colour vision (in primates: Hurvich & Jameson, 1957; in insects: Backhaus, 1991) and polarisation vision in insects (crickets: Labhart, 1988, 1996). More recently, UV levels alone have been proposed in two separate studies (Differt & Möller, 2015; Stone, Mangan, Ardin, & Webb, 2014). Stone et al. (2014) used UV levels for segregating the skyline for artificial navigation, and found that it worked better than UV-green contrast. Differt and Möller

(2015) also found that UV levels worked well in computational models, with UV-green contrast hardly adding any benefits.

If UV level or UV-green contrast is used by insects in segregating the skyline, light in the UV range should prove important for navigation based on the panoramic scene. Evidence for this claim is till lacking. We tested the importance of the UV wavelengths in the terrestrial scene for the Central Australian *M. bagoti* (Cheng, Narendra, Sommer, & Wehner, 2009; Muser, Sommer, Wolf, & Wehner, 2005; Schultheiss & Nooten, 2013) by using a clear plastic that filtered out most of the energy from UV wavelengths. The material cut out most wavelengths under 400 nm, as spectrometric measurements indicated. This obliterated most, although probably not all of the sensitive range of the ant's UV receptor. It was a serious 'knock-down' manipulation, if not a total 'knock-out' one. Key manipulations consisted of surrounding the scene viewed by homing ants with a tall cylinder of this clear plastic. Overall brightness is reduced a little by this manipulation, and in some cases, for both ground objects and the sky. The greatest change in UV levels or in UV-green contrast, however, would be at the top border of the clear plastic. Because it is at a uniform height, a skyline defined in terms of either parameter would be uninformative. The necessity of the UV wavelengths for orientation was tested both in an impoverished artificial arena defining a skyline, and in the natural panorama. The efficacy of UV wavelengths was tested by replicating the skyline of a training arena with an identical skyline using clear UV-blocking plastic.

METHODS

Location and setting

Field work took place at a private property ~10 km south of the town centre of Alice Springs, Australia, in a region of semi-arid climate with an average annual rainfall of 282.6 mm. The field site is dominated by the invasive buffel grass *Cenchrus ciliaris*, mixed with bushes of *Acacia* and *Hakea* genera, and tall Eucalypts. Low buildings were also scattered around the premises, adding to the panoramic terrestrial cues (Figure 1a). Experiments took place in three southern summers from November to March, from 2012 to 2015.

Insert Figure 1 about here

Test animals

The red honey ant *Melophorus bagoti* is widespread in the area. It occupies the niche of a thermophilic diurnal scavenger (Wehner, 1987), looking for desiccated arthropod remains and plant materials in the heat of the day during the summer (Christian & Morton, 1992; Muser et al., 2005; Schultheiss & Nooten, 2013). Ants from one nest took part in Experiments 1 and 2, while ants from a different nest took part in Experiment 3.

Materials and set ups

In each experiment, ants travelled mostly or completely over natural terrain to a plastic tub (15 × 15 × 9 cm deep) sunk into the ground as a feeder. Feeder-to-nest distance was 12.7 m in Experiment 1, 5 m in Experiment 2 and 10 m in Experiment 3. A circular green plastic arena surrounded the feeder in Experiments 1 and 3 to provide an artificial terrestrial panorama (reflectance characteristics in Figure 2b), while in Experiment 2 the natural scene provided the terrestrial panorama. The arena in Experiments 1 and 3 (diameter 1.4 m) had a uniform green colour but variable height (highest part 0.5 m), providing a panoramic skyline (Figure 1). A bit of dirt was dug out to provide an entrance into the arena, under the part of the wall between the feeder and the nest.

Insert Figure 2 about here

The feeder was stocked with cookie crumbs (Arnott™ brand) and pieces of mealworm for the ants to forage. Slippery tape covered the already slippery feeder walls, so that ants typically cannot climb the walls of the feeder. During training, sticks of natural vegetation and cardboard pieces were placed in the feeder as exit ramps.

Around the route between the feeder and the nest in each experiment, we set up an enclosure of plastic or wooden boards that surrounded the nest and extended to the arena wall (Figure 1). The materials are very hard for ants to climb over, and this increased the number of animals visiting the feeder. This enclosure was wide enough (~1.2 m) so that on the route, the natural scene rose all around above the enclosure for ants travelling away from the walls, which they did most of the time.

Crucial to the study was the use of a transparent UV-blocking plastic (Macrolon™ brand) a material that blocks (absorbs) UV light. This material filtered out most of the energy below 400 nm (Figure 2a). It thus blocks much but not all of the wavelengths of light that would excite the UV receptor in *Cataglyphis* ants (Mote & Wehner, 1980). This plastic surrounded the tested ant in some experimental conditions. Its dimensions were 1.6 m (diameter) by 0.61 m (height) in Experiment 1, and 0.7 m by 0.63 m in Experiment 2. The dimensions were chosen to cover the visible terrestrial panorama in both experiments.

Training and testing procedures

During training, ants that arrived at the feeder were painted with non-toxic enamel paint (Tamiya™ brand) on the abdomen, each with a colour that represented the day of arrival. Thereafter, the ants were left to shuttle back and forth between feeder and nest for at least 2 days before testing.

On a test, an ant might be tested as a full-vector (FV) and or a zero-vector (ZV) ant. A full-vector ant is so called because it possesses a vector pointing in the nest direction based on path integration on the outbound trip. Such an ant was taken directly from the feeder in a dark (opaque) vial and placed at the release point for a test. A zero-vector ant is so called because it has run off its vector based on path integration before being tested. We let a ZV ant run home with a bit of food, and captured it just before it entered its nest, using a small plastic enclosure to trap the ant if necessary. Then the ant was taken in the dark to be released for a test.

In testing the use of the terrestrial panorama, tests with zero-vector ants provide the crucial data. Full-vector ants use the celestial compass cues as well as possible terrestrial cues, and the crucial manipulations should not affect their orientation too much. At most, the direction of their orientation might be off slightly compared with unmanipulated conditions because the UV-blocking plastic cuts out a part of the sky. The oriented behaviour of full-vector ants would indicate that ants were still motivated to home under the test conditions. Full-vector test conditions were added in Experiment 1 because zero-vector ants were not oriented in the home direction in the key experimental conditions.

On all tests, an ant was released in the centre of a goniometer consisting of a wooden board with a circle drawn on it divided into 24 sectors of 15° each. Location of testing is described in the following subsection. Only ants that held on to a piece of cookie were tested, to ensure homing motivation. We noted the sector in which the ant crossed at 15 and 30 cm from the release point, these distances being drawn on the goniometer. Each ant was tested individually only once, under one of the conditions to be described next.

Australia does not have ethical regulations concerning ants anywhere, but the manipulations effected in the study are completely non-invasive. From many studies, including this one, we have noted no adverse effects on the ants.

Conditions of testing

Experiment 1. Five test conditions were effected in Experiment 1 using the dark green arena with a skyline shape (Table 1). To minimise interference with ongoing training, ants were tested in a replica of the arena of the same construction placed in the same orientation just behind the training arena from the perspective of the nest. The goniometer was placed at the centre of the test arena. In the ZV-control condition, zero-vector ants were tested in the replica arena, a condition that replicated training conditions. In the ZV-UV-block-inside condition, the transparent UV blocking foil, of a uniform height exceeding the maximum height of the green artificial skyline, was added on the inside of the test arena. In the ZV-UV-block-outside condition, the tall transparent UV blocking foil was added on the outside of the test arena, hugging the walls. Two conditions testing full-vector ants were also effected. In the FV-control conditions, full-vector ants were tested in a replica of the training arena oriented in the same direction. In the FV-UV-block-inside condition, the UV-blocking foil was added inside the walls of the test arena.

Having the UV-blocking plastic both inside and outside the test arena provided more than variations on the theme. The ZV-UV-block-inside was important because it reduces the reflectance of the arena wall more than it does the irradiance of the sky. Being in front of the arena, light had to go through the plastic to reach the wall, and go through the plastic again in bouncing off the wall. This spells a ~16% reduction in transmission according to Figure 2b. Above the wall, the transmission through the plastic is approximately 91% (square root of 84%) in the visible range, a ~9% reduction, but wavelengths < 400 nm were cut out as well. The brightness change of course depends on the sensory system of the ant rather than physical parameters. In this regard, data on *C. bicolor* shows that their ‘green’ receptors (with peak sensitivity at ~510 nm) are more sensitive by almost two orders of magnitude than their ‘UV’ receptors (with peak sensitivity at ~350 nm; Mote and Wehner 1980, Figure 6). Furthermore, in ants’ compound eyes, the majority (~75%) of receptors are ‘green’ receptors (Menzel, 1972). Thus, the ‘green’ channel, whose contrast is at least preserved in the experimental manipulations, probably dominates brightness perception.

In both these conditions, the biggest change in UV levels, and also in UV-green contrast, was found at the upper border of the uniform transparent plastic. We expect both these UV-block conditions to affect the orientation of zero-vector ants adversely, while full-vector ants should not be adversely affected by the UV-blocking plastic.

Experiment 2. Three conditions were effected in Experiment 2, all on zero-vector ants trained with the natural panorama (Table 4). In the ZV-control condition, ants were tested in training conditions. The goniometer was placed on the feeder, so that the location of testing matched the starting point of the homeward journey on training runs. This condition was effected on two replicates from the same nest but at different points in the season, one in mid-November to December, one in February. In the ZV-UV-block condition, ants were again tested at the feeder, but with a UV-blocking foil of uniform height (0.7 m diameter, 0.63 m height) surrounding them. This condition was also effected on two replicates at the same two periods in the season. In the ZV-opaque condition, ants were tested at the feeder with an opaque foil (white colour, 0.7 m diameter, 0.63 m height) surrounding them. The foil effectively cut out terrestrial panoramic information, and forced the ants to use celestial sources for directional information.

Experiment 3. Experiment 3 tested the sufficiency of a clear, UV-blocking cut-out in the shape of the training arena used in Experiment 1 (Table 5). In all conditions, zero-vector ants were tested, with an aim to include at least 100 test individuals in each condition. In the Control condition, ants were tested in a replica of the training arena, an exact repeat of the ZV-control condition of Experiment 1. In the UV-blocking-foil-cut-out condition, ants were tested in the clear cut-out in the shape of the training arena. This cut-out was placed at a distant test site ~143 m away, so that ants would not see a familiar scene through the transparent plastic. In the No-arena condition, ants were tested at the distant test site at which the UV-blocking-foil-cut-out condition took place, but without any arenas, as a test for orientation at that site. Based on suggestive pilot results, we predicted that the control and the UV-blocking-foil-cut-out conditions would produce heading distributions that are significantly oriented, while the No-arena condition would produce an unoriented distribution.

Data analysis

Circular statistics based on Batschelet (1981) and one test of our own invention were used for inferential statistics, calculated using Matlab™. We compared headings at 15 cm and at 30 cm in all conditions, and found that in no condition across the experiments did they differ significantly in orientation or scatter. We thus restricted data analysis to headings at 30 cm. For each condition, we tested whether the distribution was significantly oriented in the feeder-to-nest direction by the V test (Batschelet, 1981). In addition, we examined if the 95% confidence interval contained the predicted direction, and conducted the Rayleigh test (Batschelet, 1981) to test if the distribution was oriented in any direction at all. We set alpha at 0.05 for these tests. Differences in scatter between conditions were tested using the Var test, a test of our own making. The absolute difference of each individual heading from the circular mean of each condition was tabulated. These absolute differences in two conditions were compared using the non-parametric Wilcoxon rank sum test (two-tailed). This test is suitable for any conditions that are oriented, for which a meaningful mean direction can be calculated.

Conditions were compared against appropriate control conditions. We compared directions between a condition and its appropriate control using the Watson-Williams test (Batschelet, 1981). In cases of multiple comparisons with a group in Experiments 1 and 3, we followed Holm's (1979) method for alpha correction. The first alpha was set to 0.05/k (number of comparisons). If the comparison with lowest *P* value is above that value, no null hypothesis is rejected (all deemed non-significant). If the lowest *P* value falls below 0.05/k, the associated null hypothesis is rejected. The next *P* value is set at 0.05/(k-1) to test against the next lowest *P* value, and so on.

Insert Tables 1, 2 and 3 about here

RESULTS

Experiment 1

Ants were trained and tested with artificial panoramas in Experiment 1. Results showed that the UV-blocking foil had a strong effect on the headings of zero-vector ants, but not full-vector ants (Figure 3, Table 1). Full-vector ants oriented well in the nest direction with or without the UV-blocking foil (Figure 3a), although surprisingly, control full-vector ants showed a leftward bias in that the 95% confidence interval did not include the feeder-to-nest direction (Table 1). Zero-vector ants in the control condition oriented well in the nest direction (Figure 3b, Table 1), also with a leftward bias, but zero-vector ants with the UV-blocking foil on either the inside or the outside of the arena were not oriented in the nest direction according to the V test (Figures 3b, c, Table 1). The Rayleigh test showed, however, that these groups were significantly oriented (Table 1). That is because the ants tended to head in the opposite, nest-to-feeder direction (Figures 3b, c). A V test for this direction showed that this tendency was not significant for the ZV-UV-block-inside condition (V = 3.18, P = 0.220, but was significant for the ZV-UV-block-outside condition (V = 11.89, P = 0.001). If the results of these two groups are pooled, the ants were significantly oriented in the nest-to-feeder direction (*V* = 15.07, *P* = 0.004). It should be noted, however, that the 95% confidence interval for either group, or for the two UV-block groups combined, did not include 180°.

Insert Figure 3 about here

In directional scatter, both zero-vector groups with the UV-blocking foil were more scattered than the ZV-control group (Table 2). Comparing the full-vector group with the UV-blocking foil on the inside with the FV-control group, the difference in directional scatter was not significant (Table 2).

Comparing mean directions of headings of zero-vector ants using the Watson-Williams test, both the ZV-UV-block-inside condition and the ZV-UV-block-outside condition differed in mean direction from the ZV-control group (Table 3). For full vector ants, the FV-UV-block-inside group differed significantly in mean direction from the FV-control group (Table 3).

Experiment 2

Ants were trained and tested with a natural panorama in Experiment 2. In the control condition, zero-vector ants were clearly oriented in the nest direction (Figure 4a), but when surrounded with a UV-blocking foil, they appear less well oriented (Figure 4b). The UV-block groups in both replicates, however, were in fact significantly oriented in the nest direction (Table 4). Replicate 1 of the UV-block group, however, erred to the right, with the 95% confidence interval not containing the nest direction. Directional scatter between the ZV-control and ZV-UV-block conditions were compared using the Var test. The scatter did not differ significantly for replicate 1, but did differ significantly for replicate 2 (Table 2). When the two replicates were pooled (Figure 4c), the UV block resulted in more directional scatter in the headings of the ants compared with control conditions (Table 2). Zero-vector ants facing an opaque surround were not significantly oriented (Figure 4d, Table 4), and not significantly oriented in the nest direction (Table 4).

Insert Figure 4 and Table 4 about here

We compared the mean directions of zero-vector control groups against the UV-blocking groups using the Watson-Williams test. The mean direction differed for replicate 1 but not for replicate 2 (Table 3). When the two replicates are combined, ZV-control ants did not differ in mean direction from their counterparts surrounded by the UV-blocking foil (Table 3).

In addition, given the differences in behaviour between the zero-vector ants in Experiments 1 and 2, it is of interest to compare groups across experiments in their mean direction, with the usual cautionary note needed about comparing between experiments. We compared zero-vector control groups (two replicates combined for Experiment 2) using the Watson-Williams test and found that mean direction differed significantly between experiments ($F = 6.35$, $P = 0.013$). We also compared the UV-blocking conditions (ZV-UV-block-inside and ZV-UV-block-outside combined in Experiment 1 vs. two replicates of ZV-UV-block in Experiment 2) and found that as expected, they differed significantly in mean direction ($F = 47.96$, $P < 0.001$).

Experiment 3

Ants in Experiment 3 were trained in the artificial arena. Experimental groups were tested at a distant location from the training site, either with a clear cut-out having the shape and orientation of the training arena (UV-blocking-foil-cut-out), or in the open at the unfamiliar site (No arena). Experiment 3 was high in power, with over 100 individuals tested in each condition. The ants (all zero-vector ants) appear well oriented, somewhere in the vicinity of the feeder-to-nest direction, in the Control and UV-blocking-foil-cut-out conditions, but it is difficult to discern a clear peak in the heading distribution from the No-arena condition (Figure 5a,b). The V test, however, revealed significant orientation in the nest direction in all three groups (Table 5). Both the UV-blocking-foil-cut-out group and the No-arena group erred to the left, in that the 95% confidence interval did not contain the feeder-to-nest direction. The Var test for directional scatter revealed significant differences between all pairs of groups by Holm's (1979) correction method: Control condition vs No-arena condition ($Z = 5.62$, $P < 0.001$), UV-blocking-foil-cut-out condition vs. No-arena condition ($Z = 3.41$, $P < 0.001$), Control condition and UV-blocking-foil-cut-out condition ($Z = 2.29$, $P = 0.022$). These latter two conditions differed significantly in mean direction (Watson-Williams test, $F = 8.54$, $P = 0.004$). The No-arena condition was too scattered in heading distribution to compare with other conditions. The headings in each condition were smoothed by a running average of three bins in Figure 5c,d. That is, the count in each bin consisted of the average of the raw count in that bin and its two immediate neighbours. These figures might show the trend of the data better, but were not used for analyses.

Insert Figure 5 and Table 5 about here

DISCUSSION

To summarise the experimental findings, in Experiment 1, the terrestrial cues consisted of a skyline in a uniformly coloured arena, offering a form of 'pure skyline', while in Experiment 2, ants homed under natural conditions. When wavelengths < 400 nm were greatly reduced at a uniform height surrounding the test ant, ants trained and tested in the arena without directional information from path integration (zero-vector ants) did not orient in the nest direction. Rather, they tended to orient in the opposite nest-to-feeder direction. When zero-vector ants homing in natural conditions had wavelengths < 400 nm knocked down at a uniform height surrounding the test ant, they were still oriented in the nest direction, but the performance was more scattered compared with control zero-vector ants homing under unaltered conditions. These results point to the importance of UV wavelengths in using the terrestrial panorama to orientate. Reducing UV wavelengths up to a uniform height alters the UV:green ratio and the overall UV level found in the skyline. In effect, the test skyline under such conditions would be the uniformly tall top border of the surrounding clear plastic, where the greatest change in either UV:green ratio or UV level was found. Disruption of orientation would show that one of these parameters (or both) plays a major role in defining the skyline.

In Experiment 3, a clear cut-out of the shape of the training arena, made with the UV-blocking plastic foil, was placed at a distant test site. The zero-vector ants used this cut-out readily to home, albeit less precisely and with a distortion in the initial direction compared with controls. This shows a form of sufficiency of the contour of maximum green-UV contrast or maximum change in UV levels in the face of many changes in spectral composition, two theoretically proposed ways of extracting the skyline (Differt & Möller, 2015; Möller, 2002; Stone et al., 2014).

The most serious alternative interpretation to consider is that a slight reduction in brightness contrast, between ground objects (arena wall or the natural scene) and the sky, might have caused the ants' performance to deteriorate in the UV-blocking-foil conditions in Experiments 1 and 2. The UV-blocking foil has the same physical effects on ground objects and sky in Experiment 2 in the natural surround. But physiologically, the sky might show a greater reduction in overall brightness — sum of 'green' and 'UV' receptor stimulation — because it contains more intensity than ground objects in the UV wavelengths, which are knocked down by the UV-blocking foil. In Experiment 1, this is compensated to some extent because the foil reduced the intensity of the wall more (light had to pass through the foil twice in reaching the wall through the foil and then bouncing back out through the foil). It seems, however, that passing clouds covering the sun would have a greater effect in reducing intensity contrast. Such an event might change intensity levels by an order of magnitude (see Möller, 2002). Geophysically, clouds covering the sun blocks transmission of visible (to humans) light more so than transmission of UV wavelengths (Blumenthaler, Ambach, & Salzgeber, 1994), meaning that cloud cover tends to reduce brightness and green contrast of the skyline more so than it does UV contrast and the green:UV ratio. Our observations from working with this species, albeit not formally documented, have suggested that cloud cover does not affect the orientation of zero-vector ants adversely. More formal investigations along these lines, however, would be illuminating and should be carried out.

In Experiment 1, the ants homed in a uniformly coloured arena that proffered a skyline. The uniform colouration impoverishes spectral cues, but does not eliminate them. While the wall would have the same reflectance characteristics everywhere, the position of the sun would still provide spectral cues (Wehner, 1997). Thus, it was obvious to human observers (without a UV receptor) that one side of the arena looks brighter because the sun was shining on it. The UV-blocking plastic would not alter such a brightness gradient substantially, lowering the brightness on both the sun and anti-sun sides. Polarisation compass cues in the sky would also be left largely intact. The zero-vector ants did not orient in the home direction, but some evidence indicates that they did orient opposite the home direction. This backtracking behaviour may parallel what Wystrach and colleagues (Wystrach, Schwarz, Baniel, & Cheng, 2013) found in this species. In that study, *Melophorus bagoti* backtracked when they were captured near their nest after homing from a familiar site (feeder) and then displaced to a distant, unfamiliar location. Such ants must have been using their celestial compass to head in the nest-to-feeder direction because the distant site had no useful terrestrial information. Evidence that zero-vector ants of this species use the celestial cues for orientation has been found in some circumstances (Legge, Spetch, & Cheng, 2010; Legge, Wystrach, Spetch, & Cheng, 2014; Wystrach & Schwarz, 2013; Wystrach et al., 2013). In our ants homing with the UV-blocking shield in place, we tentatively interpret the manipulation to have rendered the scene unfamiliar to the ants, unfamiliar enough that they too exhibited backtracking behaviour. The interpretation is uncertain because the 95% confidence interval of the mean direction did not include 180°. The distortion, if it is that, could arise because the UV-blocking foil changed the pattern of polarised light visible to the ants. The polarisation compass in ants depends on UV-sensitive receptors in the dorsal rim area (Wehner, 1994). But it remains possible that ants in the key experimental conditions were simply disoriented.

Full-vector ants in Experiment 1 facing the UV-blocking plastic were oriented in the feeder-to-nest direction, albeit with a bias (Table 1). This shows that ants facing the UV-blocking plastic were motivated to home. Their mean direction, however, differed from that of full-vector controls facing the replica of the training environment. Again, changing the amount of UV wavelengths perceptible at different azimuths, compared with training conditions, might have distorted the information based on the polarisation compass.

Full-vector and zero-vector ants facing a replica of the training environment showed a leftward bias. Two explanations, not mutually exclusive, might account for this pattern. The first is that just to the left of the feeder-to-nest direction, the arena presented a distinctive undulating cue, a near-vertical segment (see Figure 1a and 1b), which might provide a more distinct cue for approaching. This explanation assumes that well trained full-vector ants use both the celestial cues and the terrestrial panorama in orientation, and evidence for this claim has been found in this species (Legge et al., 2014). A second, perhaps related reason is that in training, only a small opening allowed exit from the arena. Some of the ants might have erred strategically to one side — and why not the more distinct side — so as to determine the direction to turn when they arrive at the wall. These, however, remain posthoc explanations in need of further confirmation.

Under natural conditions (Experiment 2), obliterating UV wavelengths (< 400 nm) at a uniform height did not knock out homeward orientation. Unlike the arena, the ants were both motivated to and can orient homeward. But their performance was worse, in being more scattered in initial heading. We thus conclude that UV wavelengths provide an important cue for the ants. We can only speculate at this point on what other cues are available. Assuming the UV receptor to be effectively taken out of play by the UV blocking plastic, brightness contrast or contrast in the green channel between ground objects and sky remain possibilities. Of course, the cues linked to the sun, polarised light and spectral patterns, were not blocked, and are in principle available as well.

In Experiment 3, a cut-out made of the UV-blocking plastic mimicking the shape of the green arena was presented on the crucial test at a distant test site. Given that the plastic eliminated most wavelengths of light < 400 nm, we hypothesised that the skyline defined by the cut-out would still be the top border of the arena, matching training conditions. The biggest jump in UV levels or in UV:green contrast would still be found at the top of the clear cut-out. With a sample size >100, the ants were oriented in the nest direction, although less precisely and with a deflection in mean direction compared with controls. With regard to the deflection in mean direction, one possibility is the natural panorama viewed through the clear plastic. We conducted a pixel-by-pixel comparison of natural skyline at the test site and the skyline defined by the training arena found that the best match was at about 85° (results not shown). Perhaps the ants in the clear-cut-out test perceived two skylines, one at the top of the test arena, and one through the cut-out. Combining those two cues would deflect the mean direction to the left relative to controls.

In reducing substantially the UV wavelengths with the plastic, we of course changed the amount of UV light reaching the ants as well as the green:UV ratio. If either parameter is used to segregate out the skyline, similar patterns of results would be found. Navigation based on a skyline defined by measuring the amount of UV light has been demonstrated in autonomously navigating vehicles (Stone et al., 2014). Stone et al.'s vehicles, however, were navigating in environments altered by humans: streets in urban neighbourhoods. Human alterations do not change the UV levels found in the sky, but make the green channel noisier, with some human-made objects reflecting little in the green wavelengths. For biological navigational systems evolving in natural habitats unaltered by humans, some form of green/UV contrast based on opponent-processes may be theoretically more likely (Möller, 2002). Evidence supports such an opponent-process system in the polarisation compass (Labhart, 1988, 1996). Such opponent processes buy constancy in the face of changing overall illumination levels and alleviate the need to adjust the threshold on the basis of overall light levels, a by no means trivial problem. It would be good to effect a similar knock-down manipulation targeting the green wavelengths as well. The green:UV ratio would also be distorted if green wavelengths are substantially reduced, and similar deficits should be found. If the ants use the amount of UV light (or stimulation of the UV receptor) for segregating the skyline, the green knock-down manipulation should have little effect.

Sensitivity to UV wavelengths serves navigation in other ways in insects. Sensory neurons sensitive to UV wavelengths in the dorsal rim of the eyes of desert ants and honeybees serve as receptors for polarised light (Wehner 1994, 1997). Dung beetles, *Scarabaeus zambesianus*, use polarised moon light in order to roll a ball of dung away from the dung pile in a straight line (Dacke, Nilsson, Scholtz, Byrne, & Warrant, 2003). This polarisation channel is also mediated by sensitivity to UV wavelengths (el Jundi et al., 2015). In the desert locust, *Schistocerca gregaria*, the polarisation channel is mediated by blue receptors (el Jundi, Pfeiffer, Heinze, & Homberg, 2014), but intriguingly, UV-green opponent-process neurons have been found in the anterior optic tubercle (Kinoshita et al., 2007). These neurons are excited by unpolarised light in the green wavelengths and inhibited by unpolarised light in the UV wavelengths, or vice versa. They are thought to serve the celestial compass in locusts. Whether such opponent-process neurons can be found in circuits in insects that encode terrestrial cues remains an open question.

In sum, this study has shown that light in the UV range plays an important role in ant navigation based on the terrestrial panorama. Knocking it down by blocking UV wavelengths made zero-vector ants not orient in the nest direction when navigating out of a uniformly coloured arena providing a skyline (Experiment 1), but instead if anything in the opposite nest-to-feeder direction. With UV wavelengths blocked, the ants did not orient as well in the nest direction under natural conditions, although they were still significantly oriented in this direction (Experiment 2). With an opaque artificial arena replaced with a UV-blocking but clear arena of the same shape, the ants managed to orient significantly in the nest direction.

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Figure captions

Figure 1. The set up in Experiments 1 and 2. (a) A photo of the arena used in Experiment 1 with some of the surrounding scenery, which would not be visible to the ants inside the arena. An enclosure (white plastic) surrounding the nest and leading to the arena kept most of the ants foraging in the corridor and increased the number of foragers arriving at the feeder. (b) The panoramic view provided by the arena. The photo was taken with a panoramic lens and rendered into cylindrical form. The photo ‘wraps around’, in that the right side of the photo coincides with the left side. (c) The panoramic view at the feeder in Experiment 2, with again the right side of the photo coinciding with the left side.

Figure 2. (a) Transmission characteristics of the Makrolon UV-blocking plastic. The photospectrometric measurements were taken with an Ocean Optics Jaz™ photospectrometer (Ocean Optics, Dunedin, Florida), with the plastic placed in front of a piece of standard white colour, and compared with the reflectance of standard white alone. Thus, in the measurements of the plastic, the light had to go through the plastic twice, to get to the standard white and then to reflect back from the standard white. Only transmittance in the range of 300-700 nm, a reliable range for the instrument, is shown. (b) Reflectance characteristics of the green wall of the arena used in Experiments 1 and 3, measured with the same instrument. Note that the scale is reduced tenfold, with maximum on graph set at 10%.

Figure 3. Results of Experiment 1. Distributions of heading directions at 30 cm for full-vector ants under control (training) conditions and with the UV-blocking plastic placed inside the arena (a), zero-vector ants under control (training) conditions and with the UV-blocking plastic placed inside or outside the arena, two conditions combined (b), and zero-vector ants with the UV-blocking conditions placed inside or outside the test

arena, two conditions separate (c). Each panel is cylindrical, with +180° and –180° being the same nest-to-feeder direction. Nest direction is at 0°. The line through each distribution is an atheoretical spline that serves only to help readers to visualise the data. *: Two conditions in graph differ significantly in directional scatter. #: Two conditions in the graph differ significantly in mean heading direction.

Figure 4. Results of Experiment 2. Distributions of heading directions at 30 cm for zero-vector ants in control (training) conditions, separately for two replicates (a), zero-vector ants with the UV-blocking foil surrounding them on the test, separately for two replicates (b), zero-vector ants in control (training) conditions and with the UV-blocking foil surrounding them on the test, each with two replicates combined (c), and zero-vector ants with an opaque white foil surrounding them on the test (d). Each panel is cylindrical, with +180° and –180° being the same nest-to-feeder direction. Nest direction is at 0°. The line through each distribution is an atheoretical spline that serves only to help readers to visualise the data. *: Two conditions in graph differ significantly in directional scatter.

Figure 5. Results of Experiment 3. Distributions of heading directions at 30 cm for zero-vector ants in the Control condition and with UV-blocking foil cut out to the shape of the training arena (Clear-cut-out, (a)) and in the No-arena condition (b). Smoothed data for the Control condition and with UV-blocking foil cut out to the shape of the training arena (c), and in the No arena condition (d). Data in (c) and (d) were transformed from those in (a) and (b) by averaging each bin with its two immediate neighbours. Each panel is cylindrical, with +180° and –180° being the same nest-to-feeder direction. Nest direction is at 0°. The line through each distribution is an atheoretical spline that serves only to help readers to visualise the data. *: Two conditions in graph differ significantly

in directional scatter. #: Two conditions in the graph differ significantly in mean heading direction. Inferential statistics was not performed on panels (c) and (d).

Figure 1

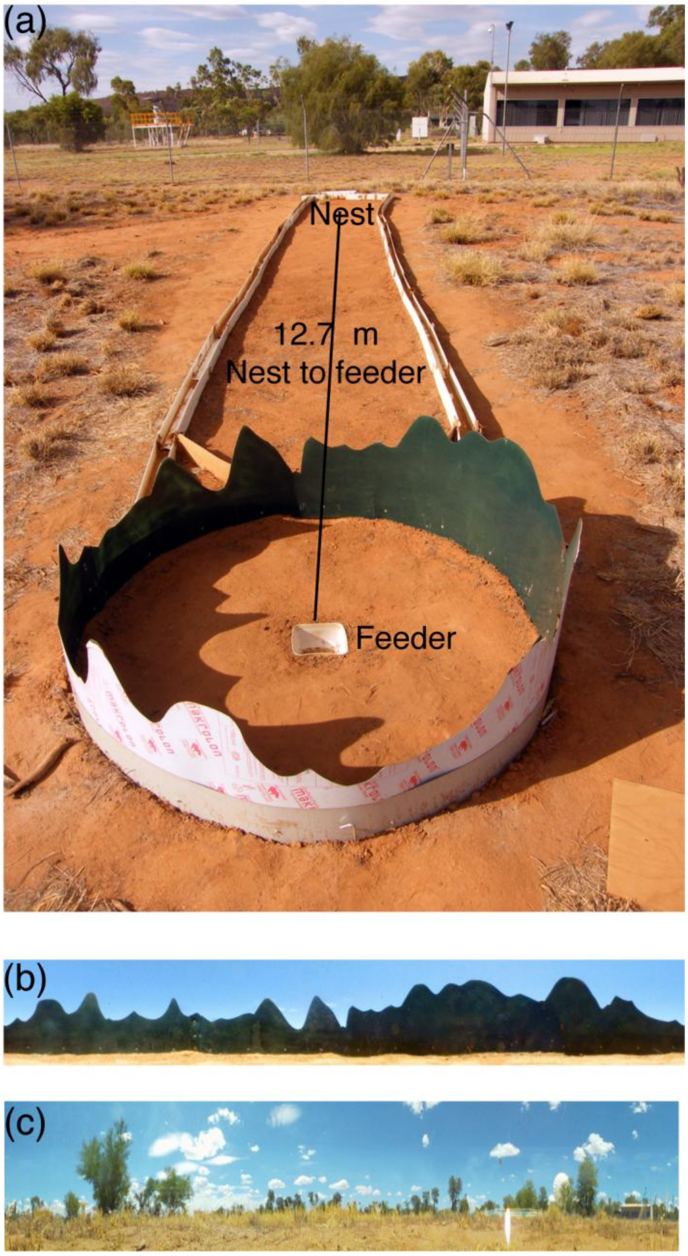


Figure 2

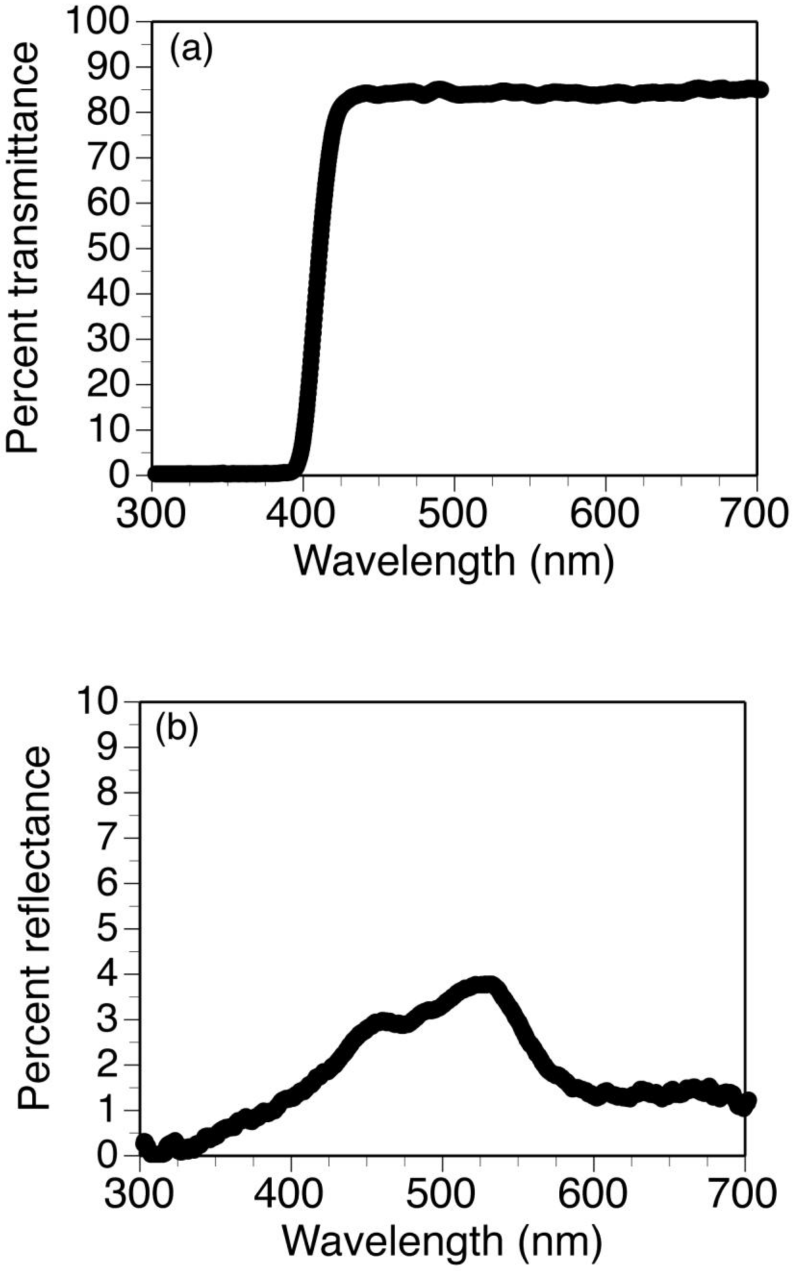

(a)
Percent transmittance
0
10
20
30
40
50
60
70
80
90
100
300
400
500
600
700
Wavelength (nm)
(b)
Percent reflectance
0
1
2
3
4
5
6
7
8
9
10
300
400
500
600
700
Wavelength (nm)

Figure 3

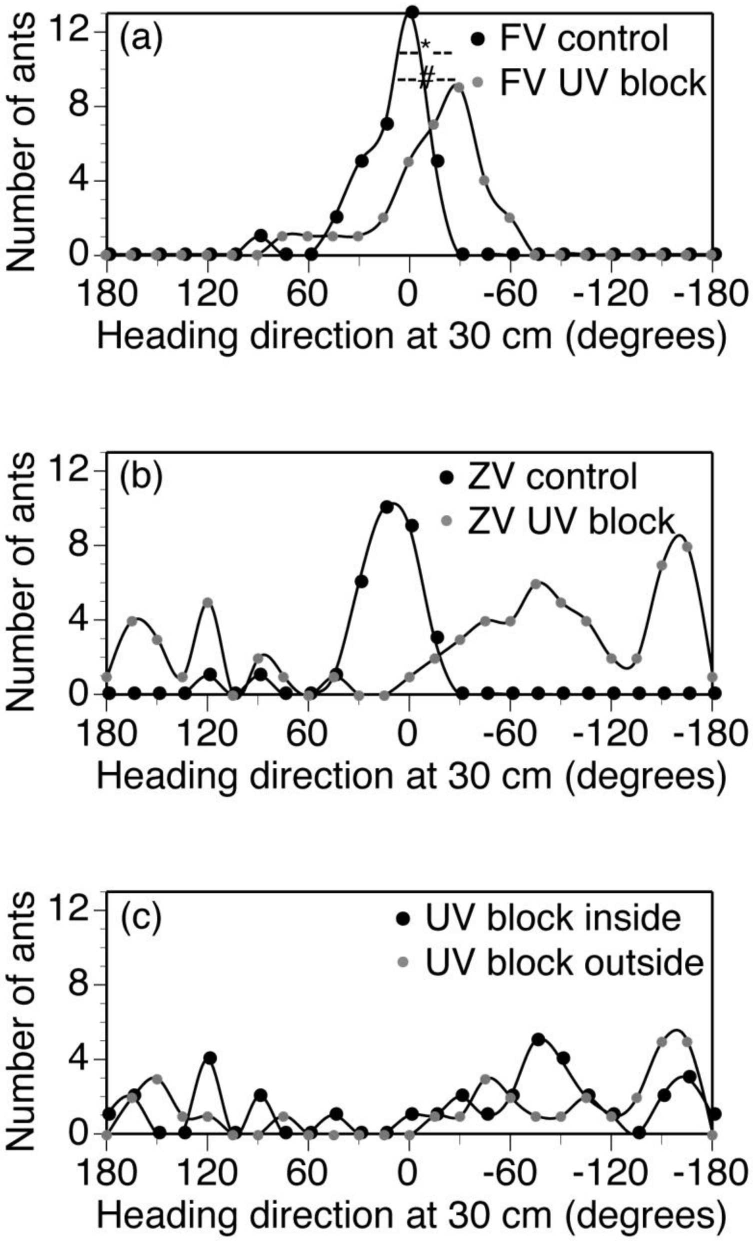

(a)
● FV control
● FV UV block
*
#
(b)
● ZV control
● ZV UV block
(c)
● UV block inside
● UV block outside
Number of ants
0
4
8
12
180
120
60
0
-60
-120
-180
Heading direction at 30 cm (degrees)

Figure 4

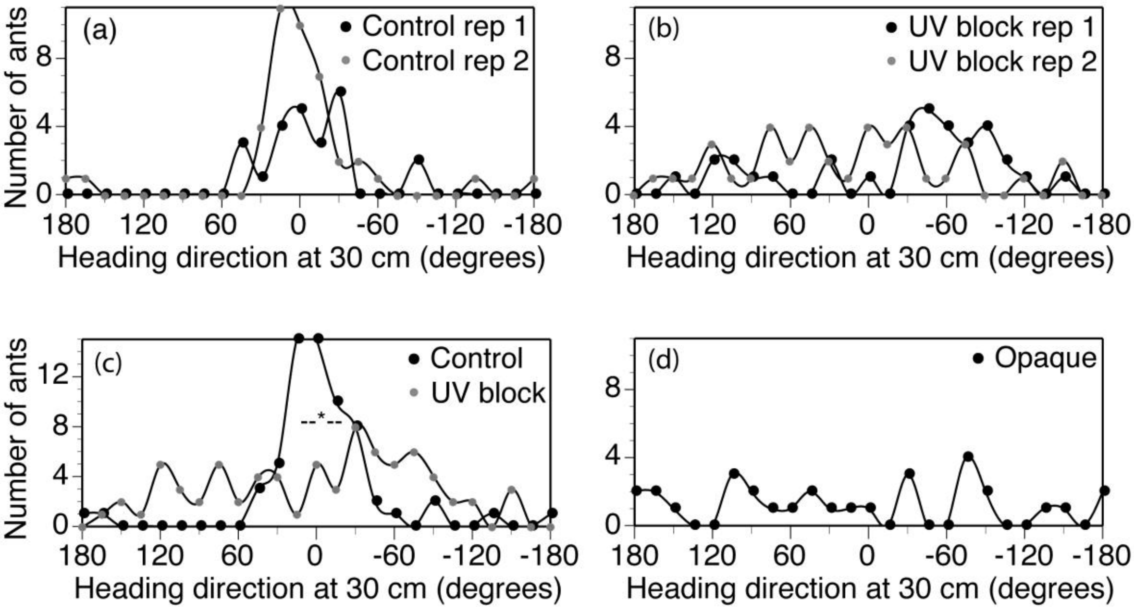
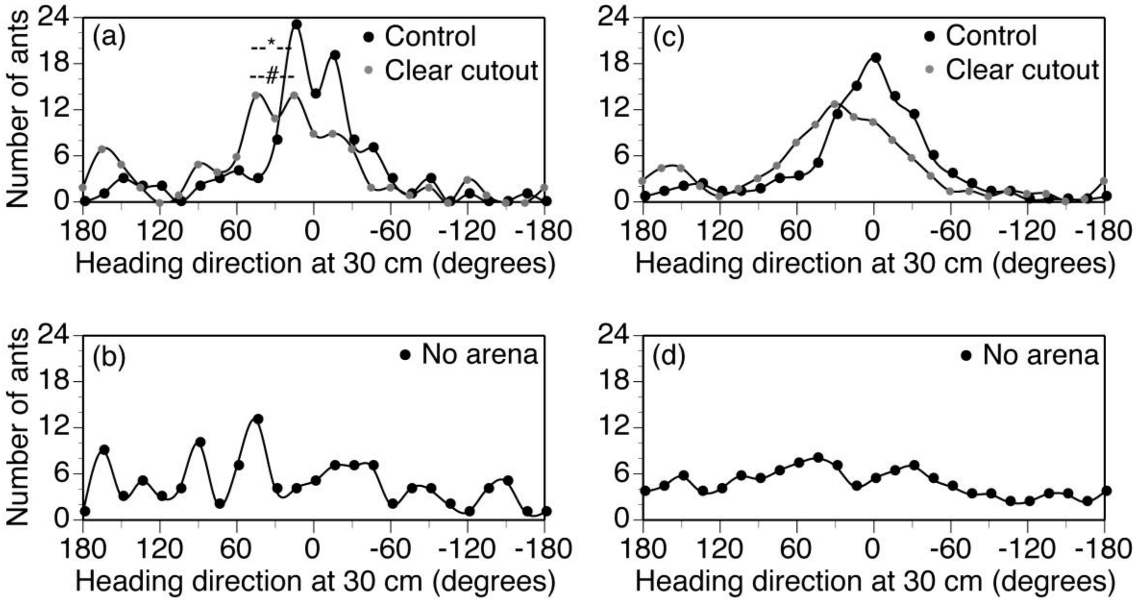

(a)
Control rep 1
Control rep 2
(b)
UV block rep 1
UV block rep 2
(c)
Control
UV block
--*--
(d)
Opaque
Number of ants
0
4
8
12
180
120
60
0
-60
-120
-180
Heading direction at 30 cm (degrees)

Figure 5


(a)
● Control
● Clear cutout
--*--
--#--
(b)
● No arena
(c)
● Control
● Clear cutout
(d)
● No arena
Number of ants
24
18
12
6
0
180
120
60
0
-60
-120
-180
Heading direction at 30 cm (degrees)

Crucial role of ultraviolet light for desert ants in determining direction from the terrestrial panorama

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Running head: Ultraviolet light, panorama, and determining direction

Abstract

Ants use the panoramic skyline in part to determine a direction of travel. A theoretically elegant way to define where terrestrial objects meet the sky is to use an opponent-process channel contrasting green wavelengths of light with ultraviolet wavelengths. Compared with the sky, terrestrial objects reflect relatively more green wavelengths. Using such an opponent-process channel gains constancy in the face of changes in overall illumination level. We tested the use of ultraviolet (UV) wavelengths in desert ants by using a plastic that filtered out most of the energy below 400 nm. Ants, *Melophorus bagoti*, were trained to home with an artificial skyline provided by an arena (Experiment 1) or with the natural panorama (Experiment 2). On a test, a homing ant was captured just before she entered her nest, and then brought back to a replicate arena (Experiment 1) or the starting point (the feeder, Experiment 2) and released. Blocking ultraviolet light led to deteriorations in orientation in both experiments. If the artificial skyline was transformed from opaque to transparent ultraviolet-blocking plastic (Experiment 3) on the other hand, the ants were still oriented. We conclude that UV wavelengths play a crucial role in determining direction based on the terrestrial surround.

Key words: desert ants, green, orientation, panorama, skyline, ultraviolet,

Navigating ants use a multifaceted toolkit (Wehner, 2009). Along with path integration (Wehner & Srinivasan, 2003), ants are known to use visual terrestrial cues for navigation (*Temnothorax albipennis*: Pratt, Brooks, & Franks, 2001; *Formica rufa*: Graham & Collett, 2002; Lent, Graham, & Collett, 2013; *Cataglyphis fortis*: Wehner, Michel, & Antonsen, 1996; *Melophorus bagoti*: Wystrach, Beugnon, & Cheng, 2011, 2012; Wystrach, Schwarz, Schultheiss, Beugnon, & Cheng, 2011; *Myrmecia croslandi*: Narendra, Gourmaud, & Zeil, 2013; Zeil, Narendra, & Stürzl, 2014). And as a 'back-up', they also engage in systematic searching (Schultheiss, Cheng, & Reynolds, 2015).

Some properties of the panorama have been shown to guide ants travelling on familiar routes, including fractional position of mass, matching of segments of the scene, and the skyline. Fractional position of mass refers to the amount of the visual scene to one's left vs. right as one faces the goal direction. Wood ants (*F. rufa*) use this cue in some conditions in the lab (Lent et al., 2013). In other conditions, *F. rufa* might match a salient segment of the scene (Lent et al., 2013). The skyline is some record of where terrestrial objects meet the sky across the 360° panorama (Dyer, 1987; Graham & Cheng, 2009a, 2009b; Towne, 2008; Towne & Moscrip, 2008; von Frisch & Lindauer, 1954). Its use was demonstrated in Central Australian desert ants (*M. bagoti*) when an artificial skyline in black was created to mimic the natural skyline seen from the start of the journey (Graham & Cheng, 2009a). The ants oriented according to the artificial skyline even when it was rotated so that the celestial cues associated with the panorama did not match in test and training conditions.

Here we investigate further the nature of the sensory input used for view-based matching, focusing on the role of ultraviolet (UV) wavelengths of light in the use of the terrestrial panorama. Ants have been found to have two types of visual receptors in their compound eyes and ocelli (*Cataglyphis bicolor*: Mote & Wehner, 1980), or sometimes three (*Myrmecia croslandi* and *M. vindex*: Ogawa, Falkowski, Narendra, Zeil, & Hemmi, 2015). In these cited cases, one type is most sensitive to light in the green range, with maximum sensitivity at ~510 nm or ~550 nm. One other type has highest sensitivity in the UV range, peaking at ~350 nm or ~370 nm. Ground objects typically do not reflect much in the UV wavelengths, far less so than what is found in the sky (Möller, 2002). Theoretically, UV wavelengths are useful for segregating ground objects from the sky.

Two different ways of using UV wavelengths for delineating the skyline have been proposed. Möller (2002) proposed that UV-green contrast, sensitive to the ratio of UV irradiance to green irradiance, might be used to differentiate sky from ground, and thus delineate the skyline. An opponent-process contrast based on the UV:green ratio buys constancy in the face of fluctuating overall intensity both across time and across space. If a cloud covers the sun temporarily and drops the intensity, both the green reflectance of terrestrial objects and the UV irradiance in the sky diminish. But at the local level, the ratios stay fairly constant, as measured empirically by Möller (2002). While UV-green opponent neurons have been found (in locusts: Kinoshita, Homberg, & Pfeiffer, 2007), a proposed UV-green channel for segregating ground objects from the sky remains hypothetical. But such opponent-process systems are well known in other domains of visual processing in which constancy is important, such as colour vision (in primates: Hurvich & Jameson, 1957; in insects: Backhaus, 1991) and polarisation vision in insects (crickets: Labhart, 1988, 1996). More recently, UV levels alone have been proposed in two separate studies (Differt & Möller, 2015; Stone, Mangan, Ardin, & Webb, 2014). Stone et al. (2014) used UV levels for segregating the skyline for artificial navigation, and found that it worked better than UV-green contrast. Differt and Möller

(2015) also found that UV levels worked well in computational models, with UV-green contrast hardly adding any benefits.

If UV level or UV-green contrast is used by insects in segregating the skyline, light in the UV range should prove important for navigation based on the panoramic scene. Evidence for this claim is till lacking. We tested the importance of the UV wavelengths in the terrestrial scene for the Central Australian *M. bagoti* (Cheng, Narendra, Sommer, & Wehner, 2009; Muser, Sommer, Wolf, & Wehner, 2005; Schultheiss & Nooten, 2013) by using a clear plastic that filtered out most of the energy from UV wavelengths. The material cut out most wavelengths under 400 nm, as spectrometric measurements indicated. This obliterated most, although probably not all of the sensitive range of the ant's UV receptor. It was a serious 'knock-down' manipulation, if not a total 'knock-out' one. Key manipulations consisted of surrounding the scene viewed by homing ants with a tall cylinder of this clear plastic. Overall brightness is reduced a little by this manipulation, and in some cases, for both ground objects and the sky. The greatest change in UV levels or in UV-green contrast, however, would be at the top border of the clear plastic. Because it is at a uniform height, a skyline defined in terms of either parameter would be uninformative. The necessity of the UV wavelengths for orientation was tested both in an impoverished artificial arena defining a skyline, and in the natural panorama. The efficacy of UV wavelengths was tested by replicating the skyline of a training arena with an identical skyline using clear UV-blocking plastic.

METHODS

Location and setting

Field work took place at a private property ~10 km south of the town centre of Alice Springs, Australia, in a region of semi-arid climate with an average annual rainfall of 282.6 mm. The field site is dominated by the invasive buffel grass *Cenchrus ciliaris*, mixed with bushes of *Acacia* and *Hakea* genera, and tall Eucalypts. Low buildings were also scattered around the premises, adding to the panoramic terrestrial cues (Figure 1a). Experiments took place in three southern summers from November to March, from 2012 to 2015.

Insert Figure 1 about here

Test animals

The red honey ant *Melophorus bagoti* is widespread in the area. It occupies the niche of a thermophilic diurnal scavenger (Wehner, 1987), looking for desiccated arthropod remains and plant materials in the heat of the day during the summer (Christian & Morton, 1992; Muser et al., 2005; Schultheiss & Nooten, 2013). Ants from one nest took part in Experiments 1 and 2, while ants from a different nest took part in Experiment 3.

Materials and set ups

In each experiment, ants travelled mostly or completely over natural terrain to a plastic tub (15 × 15 × 9 cm deep) sunk into the ground as a feeder. Feeder-to-nest distance was 12.7 m in Experiment 1, 5 m in Experiment 2 and 10 m in Experiment 3. A circular green plastic arena surrounded the feeder in Experiments 1 and 3 to provide an artificial terrestrial panorama (reflectance characteristics in Figure 2b), while in Experiment 2 the natural scene provided the terrestrial panorama. The arena in Experiments 1 and 3 (diameter 1.4 m) had a uniform green colour but variable height (highest part 0.5 m), providing a panoramic skyline (Figure 1). A bit of dirt was dug out to provide an entrance into the arena, under the part of the wall between the feeder and the nest.

Insert Figure 2 about here

The feeder was stocked with cookie crumbs (Arnott™ brand) and pieces of mealworm for the ants to forage. Slippery tape covered the already slippery feeder walls, so that ants typically cannot climb the walls of the feeder. During training, sticks of natural vegetation and cardboard pieces were placed in the feeder as exit ramps.

Around the route between the feeder and the nest in each experiment, we set up an enclosure of plastic or wooden boards that surrounded the nest and extended to the arena wall (Figure 1). The materials are very hard for ants to climb over, and this increased the number of animals visiting the feeder. This enclosure was wide enough (~1.2 m) so that on the route, the natural scene rose all around above the enclosure for ants travelling away from the walls, which they did most of the time.

Crucial to the study was the use of a transparent UV-blocking plastic (Macrolon™ brand) a material that blocks (absorbs) UV light. This material filtered out most of the energy below 400 nm (Figure 2a). It thus blocks much but not all of the wavelengths of light that would excite the UV receptor in *Cataglyphis* ants (Mote & Wehner, 1980). This plastic surrounded the tested ant in some experimental conditions. Its dimensions were 1.6 m (diameter) by 0.61 m (height) in Experiment 1, and 0.7 m by 0.63 m in Experiment 2. The dimensions were chosen to cover the visible terrestrial panorama in both experiments.

Training and testing procedures

During training, ants that arrived at the feeder were painted with non-toxic enamel paint (Tamiya™ brand) on the abdomen, each with a colour that represented the day of arrival. Thereafter, the ants were left to shuttle back and forth between feeder and nest for at least 2 days before testing.

On a test, an ant might be tested as a full-vector (FV) and or a zero-vector (ZV) ant. A full-vector ant is so called because it possesses a vector pointing in the nest direction based on path integration on the outbound trip. Such an ant was taken directly from the feeder in a dark (opaque) vial and placed at the release point for a test. A zero-vector ant is so called because it has run off its vector based on path integration before being tested. We let a ZV ant run home with a bit of food, and captured it just before it entered its nest, using a small plastic enclosure to trap the ant if necessary. Then the ant was taken in the dark to be released for a test.

In testing the use of the terrestrial panorama, tests with zero-vector ants provide the crucial data. Full-vector ants use the celestial compass cues as well as possible terrestrial cues, and the crucial manipulations should not affect their orientation too much. At most, the direction of their orientation might be off slightly compared with unmanipulated conditions because the UV-blocking plastic cuts out a part of the sky. The oriented behaviour of full-vector ants would indicate that ants were still motivated to home under the test conditions. Full-vector test conditions were added in Experiment 1 because zero-vector ants were not oriented in the home direction in the key experimental conditions.

On all tests, an ant was released in the centre of a goniometer consisting of a wooden board with a circle drawn on it divided into 24 sectors of 15° each. Location of testing is described in the following subsection. Only ants that held on to a piece of cookie were tested, to ensure homing motivation. We noted the sector in which the ant crossed at 15 and 30 cm from the release point, these distances being drawn on the goniometer. Each ant was tested individually only once, under one of the conditions to be described next.

Australia does not have ethical regulations concerning ants anywhere, but the manipulations effected in the study are completely non-invasive. From many studies, including this one, we have noted no adverse effects on the ants.

Conditions of testing

Experiment 1. Five test conditions were effected in Experiment 1 using the dark green arena with a skyline shape (Table 1). To minimise interference with ongoing training, ants were tested in a replica of the arena of the same construction placed in the same orientation just behind the training arena from the perspective of the nest. The goniometer was placed at the centre of the test arena. In the ZV-control condition, zero-vector ants were tested in the replica arena, a condition that replicated training conditions. In the ZV-UV-block-inside condition, the transparent UV blocking foil, of a uniform height exceeding the maximum height of the green artificial skyline, was added on the inside of the test arena. In the ZV-UV-block-outside condition, the tall transparent UV blocking foil was added on the outside of the test arena, hugging the walls. Two conditions testing full-vector ants were also effected. In the FV-control conditions, full-vector ants were tested in a replica of the training arena oriented in the same direction. In the FV-UV-block-inside condition, the UV-blocking foil was added inside the walls of the test arena.

Having the UV-blocking plastic both inside and outside the test arena provided more than variations on the theme. The ZV-UV-block-inside was important because it reduces the reflectance of the arena wall more than it does the irradiance of the sky. Being in front of the arena, light had to go through the plastic to reach the wall, and go through the plastic again in bouncing off the wall. This spells a ~16% reduction in transmission according to Figure 2b. Above the wall, the transmission through the plastic is approximately 91% (square root of 84%) in the visible range, a ~9% reduction, but wavelengths < 400 nm were cut out as well. The brightness change of course depends on the sensory system of the ant rather than physical parameters. In this regard, data on *C. bicolor* shows that their ‘green’ receptors (with peak sensitivity at ~510 nm) are more sensitive by almost two orders of magnitude than their ‘UV’ receptors (with peak sensitivity at ~350 nm; Mote and Wehner 1980, Figure 6). Furthermore, in ants’ compound eyes, the majority (~75%) of receptors are ‘green’ receptors (Menzel, 1972). Thus, the ‘green’ channel, whose contrast is at least preserved in the experimental manipulations, probably dominates brightness perception.

In both these conditions, the biggest change in UV levels, and also in UV-green contrast, was found at the upper border of the uniform transparent plastic. We expect both these UV-block conditions to affect the orientation of zero-vector ants adversely, while full-vector ants should not be adversely affected by the UV-blocking plastic.

Experiment 2. Three conditions were effected in Experiment 2, all on zero-vector ants trained with the natural panorama (Table 4). In the ZV-control condition, ants were tested in training conditions. The goniometer was placed on the feeder, so that the location of testing matched the starting point of the homeward journey on training runs. This condition was effected on two replicates from the same nest but at different points in the season, one in mid-November to December, one in February. In the ZV-UV-block condition, ants were again tested at the feeder, but with a UV-blocking foil of uniform height (0.7 m diameter, 0.63 m height) surrounding them. This condition was also effected on two replicates at the same two periods in the season. In the ZV-opaque condition, ants were tested at the feeder with an opaque foil (white colour, 0.7 m diameter, 0.63 m height) surrounding them. The foil effectively cut out terrestrial panoramic information, and forced the ants to use celestial sources for directional information.

Experiment 3. Experiment 3 tested the sufficiency of a clear, UV-blocking cut-out in the shape of the training arena used in Experiment 1 (Table 5). In all conditions, zero-vector ants were tested, with an aim to include at least 100 test individuals in each condition. In the Control condition, ants were tested in a replica of the training arena, an exact repeat of the ZV-control condition of Experiment 1. In the UV-blocking-foil-cut-out condition, ants were tested in the clear cut-out in the shape of the training arena. This cut-out was placed at a distant test site ~143 m away, so that ants would not see a familiar scene through the transparent plastic. In the No-arena condition, ants were tested at the distant test site at which the UV-blocking-foil-cut-out condition took place, but without any arenas, as a test for orientation at that site. Based on suggestive pilot results, we predicted that the control and the UV-blocking-foil-cut-out conditions would produce heading distributions that are significantly oriented, while the No-arena condition would produce an unoriented distribution.

Data analysis

Circular statistics based on Batschelet (1981) and one test of our own invention were used for inferential statistics, calculated using Matlab™. We compared headings at 15 cm and at 30 cm in all conditions, and found that in no condition across the experiments did they differ significantly in orientation or scatter. We thus restricted data analysis to headings at 30 cm. For each condition, we tested whether the distribution was significantly oriented in the feeder-to-nest direction by the V test (Batschelet, 1981). In addition, we examined if the 95% confidence interval contained the predicted direction, and conducted the Rayleigh test (Batschelet, 1981) to test if the distribution was oriented in any direction at all. We set alpha at 0.05 for these tests. Differences in scatter between conditions were tested using the Var test, a test of our own making. The absolute difference of each individual heading from the circular mean of each condition was tabulated. These absolute differences in two conditions were compared using the non-parametric Wilcoxon rank sum test (two-tailed). This test is suitable for any conditions that are oriented, for which a meaningful mean direction can be calculated.

Conditions were compared against appropriate control conditions. We compared directions between a condition and its appropriate control using the Watson-Williams test (Batschelet, 1981). In cases of multiple comparisons with a group in Experiments 1 and 3, we followed Holm's (1979) method for alpha correction. The first alpha was set to 0.05/k (number of comparisons). If the comparison with lowest *P* value is above that value, no null hypothesis is rejected (all deemed non-significant). If the lowest *P* value falls below 0.05/k, the associated null hypothesis is rejected. The next *P* value is set at 0.05/(k-1) to test against the next lowest *P* value, and so on.

Insert Tables 1, 2 and 3 about here

RESULTS

Experiment 1

Ants were trained and tested with artificial panoramas in Experiment 1. Results showed that the UV-blocking foil had a strong effect on the headings of zero-vector ants, but not full-vector ants (Figure 3, Table 1). Full-vector ants oriented well in the nest direction with or without the UV-blocking foil (Figure 3a), although surprisingly, control full-vector ants showed a leftward bias in that the 95% confidence interval did not include the feeder-to-nest direction (Table 1). Zero-vector ants in the control condition oriented well in the nest direction (Figure 3b, Table 1), also with a leftward bias, but zero-vector ants with the UV-blocking foil on either the inside or the outside of the arena were not oriented in the nest direction according to the V test (Figures 3b, c, Table 1). The Rayleigh test showed, however, that these groups were significantly oriented (Table 1). That is because the ants tended to head in the opposite, nest-to-feeder direction (Figures 3b, c). A V test for this direction showed that this tendency was not significant for the ZV-UV-block-inside condition (*V* = 3.18, *P* = 0.220, but was significant for the ZV-UV-block-outside condition (*V* = 11.89, *P* = 0.001). If the results of these two groups are pooled, the ants were significantly oriented in the nest-to-feeder direction ($V = 15.07$, $P = 0.004$). It should be noted, however, that the 95% confidence interval for either group, or for the two UV-block groups combined, did not include 180°.

Insert Figure 3 about here

In directional scatter, both zero-vector groups with the UV-blocking foil were more scattered than the ZV-control group (Table 2). Comparing the full-vector group with the UV-blocking foil on the inside with the FV-control group, the difference in directional scatter was not significant (Table 2).

Comparing mean directions of headings of zero-vector ants using the Watson-Williams test, both the ZV-UV-block-inside condition and the ZV-UV-block-outside condition differed in mean direction from the ZV-control group (Table 3). For full vector ants, the FV-UV-block-inside group differed significantly in mean direction from the FV-control group (Table 3).

Experiment 2

Ants were trained and tested with a natural panorama in Experiment 2. In the control condition, zero-vector ants were clearly oriented in the nest direction (Figure 4a), but when surrounded with a UV-blocking foil, they appear less well oriented (Figure 4b). The UV-block groups in both replicates, however, were in fact significantly oriented in the nest direction (Table 4). Replicate 1 of the UV-block group, however, erred to the right, with the 95% confidence interval not containing the nest direction. Directional scatter between the ZV-control and ZV-UV-block conditions were compared using the Var test. The scatter did not differ significantly for replicate 1, but did differ significantly for replicate 2 (Table 2). When the two replicates were pooled (Figure 4c), the UV block resulted in more directional scatter in the headings of the ants compared with control conditions (Table 2). Zero-vector ants facing an opaque surround were not significantly oriented (Figure 4d, Table 4), and not significantly oriented in the nest direction (Table 4).

Insert Figure 4 and Table 4 about here

We compared the mean directions of zero-vector control groups against the UV-blocking groups using the Watson-Williams test. The mean direction differed for replicate 1 but not for replicate 2 (Table 3). When the two replicates are combined, ZV-control ants did not differ in mean direction from their counterparts surrounded by the UV-blocking foil (Table 3).

In addition, given the differences in behaviour between the zero-vector ants in Experiments 1 and 2, it is of interest to compare groups across experiments in their mean direction, with the usual cautionary note needed about comparing between experiments. We compared zero-vector control groups (two replicates combined for Experiment 2) using the Watson-Williams test and found that mean direction differed significantly between experiments ($F = 6.35$, $P = 0.013$). We also compared the UV-blocking conditions (ZV-UV-block-inside and ZV-UV-block-outside combined in Experiment 1 vs. two replicates of ZV-UV-block in Experiment 2) and found that as expected, they differed significantly in mean direction ($F = 47.96$, $P < 0.001$).

Experiment 3

Ants in Experiment 3 were trained in the artificial arena. Experimental groups were tested at a distant location from the training site, either with a clear cut-out having the shape and orientation of the training arena (UV-blocking-foil-cut-out), or in the open at the unfamiliar site (No arena). Experiment 3 was high in power, with over 100 individuals tested in each condition. The ants (all zero-vector ants) appear well oriented, somewhere in the vicinity of the feeder-to-nest direction, in the Control and UV-blocking-foil-cut-out conditions, but it is difficult to discern a clear peak in the heading distribution from the No-arena condition (Figure 5a,b). The V test, however, revealed significant orientation in the nest direction in all three groups (Table 5). Both the UV-blocking-foil-cut-out group and the No-arena group erred to the left, in that the 95% confidence interval did not contain the feeder-to-nest direction. The Var test for directional scatter revealed significant differences between all pairs of groups by Holm's (1979) correction method: Control condition vs No-arena condition ($Z = 5.62$, $P < 0.001$), UV-blocking-foil-cut-out condition vs. No-arena condition ($Z = 3.41$, $P < 0.001$), Control condition and UV-blocking-foil-cut-out condition ($Z = 2.29$, $P = 0.022$). These latter two conditions differed significantly in mean direction (Watson-Williams test, $F = 8.54$, $P = 0.004$). The No-arena condition was too scattered in heading distribution to compare with other conditions. The headings in each condition were smoothed by a running average of three bins in Figure 5c,d. That is, the count in each bin consisted of the average of the raw count in that bin and its two immediate neighbours. These figures might show the trend of the data better, but were not used for analyses.

Insert Figure 5 and Table 5 about here

DISCUSSION

To summarise the experimental findings, in Experiment 1, the terrestrial cues consisted of a skyline in a uniformly coloured arena, offering a form of 'pure skyline', while in Experiment 2, ants homed under natural conditions. When wavelengths < 400 nm were greatly reduced at a uniform height surrounding the test ant, ants trained and tested in the arena without directional information from path integration (zero-vector ants) did not orient in the nest direction. Rather, they tended to orient in the opposite nest-to-feeder direction. When zero-vector ants homing in natural conditions had wavelengths < 400 nm knocked down at a uniform height surrounding the test ant, they were still oriented in the nest direction, but the performance was more scattered compared with control zero-vector ants homing under unaltered conditions. These results point to the importance of UV wavelengths in using the terrestrial panorama to orientate. Reducing UV wavelengths up to a uniform height alters the UV:green ratio and the overall UV level found in the skyline. In effect, the test skyline under such conditions would be the uniformly tall top border of the surrounding clear plastic, where the greatest change in either UV:green ratio or UV level was found. Disruption of orientation would show that one of these parameters (or both) plays a major role in defining the skyline.

In Experiment 3, a clear cut-out of the shape of the training arena, made with the UV-blocking plastic foil, was placed at a distant test site. The zero-vector ants used this cut-out readily to home, albeit less precisely and with a distortion in the initial direction compared with controls. This shows a form of sufficiency of the contour of maximum green-UV contrast or maximum change in UV levels in the face of many changes in spectral composition, two theoretically proposed ways of extracting the skyline (Differt & Möller, 2015; Möller, 2002; Stone et al., 2014).

The most serious alternative interpretation to consider is that a slight reduction in brightness contrast, between ground objects (arena wall or the natural scene) and the sky, might have caused the ants’ performance to deteriorate in the UV-blocking-foil conditions in Experiments 1 and 2. The UV-blocking foil has the same physical effects on ground objects and sky in Experiment 2 in the natural surround. But physiologically, the sky might show a greater reduction in overall brightness — sum of ‘green’ and ‘UV’ receptor stimulation — because it contains more intensity than ground objects in the UV wavelengths, which are knocked down by the UV-blocking foil. In Experiment 1, this is compensated to some extent because the foil reduced the intensity of the wall more (light had to pass through the foil twice in reaching the wall through the foil and then bouncing back out through the foil). It seems, however, that passing clouds covering the sun would have a greater effect in reducing intensity contrast. Such an event might change intensity levels by an order of magnitude (see Möller, 2002). Geophysically, clouds covering the sun blocks transmission of visible (to humans) light more so than transmission of UV wavelengths (Blumenthaler, Ambach, & Salzgeber, 1994), meaning that cloud cover tends to reduce brightness and green contrast of the skyline more so than it does UV contrast and the green:UV ratio. Our observations from working with this species, albeit not formally documented, have suggested that cloud cover does not affect the orientation of zero-vector ants adversely. More formal investigations along these lines, however, would be illuminating and should be carried out.

In Experiment 1, the ants homed in a uniformly coloured arena that proffered a skyline. The uniform colouration impoverishes spectral cues, but does not eliminate them. While the wall would have the same reflectance characteristics everywhere, the position of the sun would still provide spectral cues (Wehner, 1997). Thus, it was obvious to human observers (without a UV receptor) that one side of the arena looks brighter because the sun was shining on it. The UV-blocking plastic would not alter such a brightness gradient substantially, lowering the brightness on both the sun and anti-sun sides. Polarisation compass cues in the sky would also be left largely intact. The zero-vector ants did not orient in the home direction, but some evidence indicates that they did orient opposite the home direction. This backtracking behaviour may parallel what Wystrach and colleagues (Wystrach, Schwarz, Baniel, & Cheng, 2013) found in this species. In that study, *Melophorus bagoti* backtracked when they were captured near their nest after homing from a familiar site (feeder) and then displaced to a distant, unfamiliar location. Such ants must have been using their celestial compass to head in the nest-to-feeder direction because the distant site had no useful terrestrial information. Evidence that zero-vector ants of this species use the celestial cues for orientation has been found in some circumstances (Legge, Spetch, & Cheng, 2010; Legge, Wystrach, Spetch, & Cheng, 2014; Wystrach & Schwarz, 2013; Wystrach et al., 2013). In our ants homing with the UV-blocking shield in place, we tentatively interpret the manipulation to have rendered the scene unfamiliar to the ants, unfamiliar enough that they too exhibited backtracking behaviour. The interpretation is uncertain because the 95% confidence interval of the mean direction did not include 180°. The distortion, if it is that, could arise because the UV-blocking foil changed the pattern of polarised light visible to the ants. The polarisation compass in ants depends on UV-sensitive receptors in the dorsal rim area (Wehner, 1994). But it remains possible that ants in the key experimental conditions were simply disoriented.

Full-vector ants in Experiment 1 facing the UV-blocking plastic were oriented in the feeder-to-nest direction, albeit with a bias (Table 1). This shows that ants facing the UV-blocking plastic were motivated to home. Their mean direction, however, differed from that of full-vector controls facing the replica of the training environment. Again, changing the amount of UV wavelengths perceptible at different azimuths, compared with training conditions, might have distorted the information based on the polarisation compass.

Full-vector and zero-vector ants facing a replica of the training environment showed a leftward bias. Two explanations, not mutually exclusive, might account for this pattern. The first is that just to the left of the feeder-to-nest direction, the arena presented a distinctive undulating cue, a near-vertical segment (see Figure 1a and 1b), which might provide a more distinct cue for approaching. This explanation assumes that well trained full-vector ants use both the celestial cues and the terrestrial panorama in orientation, and evidence for this claim has been found in this species (Legge et al., 2014). A second, perhaps related reason is that in training, only a small opening allowed exit from the arena. Some of the ants might have erred strategically to one side — and why not the more distinct side — so as to determine the direction to turn when they arrive at the wall. These, however, remain posthoc explanations in need of further confirmation.

Under natural conditions (Experiment 2), obliterating UV wavelengths (< 400 nm) at a uniform height did not knock out homeward orientation. Unlike the arena, the ants were both motivated to and can orient homeward. But their performance was worse, in being more scattered in initial heading. We thus conclude that UV wavelengths provide an important cue for the ants. We can only speculate at this point on what other cues are available. Assuming the UV receptor to be effectively taken out of play by the UV blocking plastic, brightness contrast or contrast in the green channel between ground objects and sky remain possibilities. Of course, the cues linked to the sun, polarised light and spectral patterns, were not blocked, and are in principle available as well.

In Experiment 3, a cut-out made of the UV-blocking plastic mimicking the shape of the green arena was presented on the crucial test at a distant test site. Given that the plastic eliminated most wavelengths of light < 400 nm, we hypothesised that the skyline defined by the cut-out would still be the top border of the arena, matching training conditions. The biggest jump in UV levels or in UV:green contrast would still be found at the top of the clear cut-out. With a sample size >100, the ants were oriented in the nest direction, although less precisely and with a deflection in mean direction compared with controls. With regard to the deflection in mean direction, one possibility is the natural panorama viewed through the clear plastic. We conducted a pixel-by-pixel comparison of natural skyline at the test site and the skyline defined by the training arena found that the best match was at about 85° (results not shown). Perhaps the ants in the clear-cut-out test perceived two skylines, one at the top of the test arena, and one through the cut-out. Combining those two cues would deflect the mean direction to the left relative to controls.

In reducing substantially the UV wavelengths with the plastic, we of course changed the amount of UV light reaching the ants as well as the green:UV ratio. If either parameter is used to segregate out the skyline, similar patterns of results would be found. Navigation based on a skyline defined by measuring the amount of UV light has been demonstrated in autonomously navigating vehicles (Stone et al., 2014). Stone et al.'s vehicles, however, were navigating in environments altered by humans: streets in urban neighbourhoods. Human alterations do not change the UV levels found in the sky, but make the green channel noisier, with some human-made objects reflecting little in the green wavelengths. For biological navigational systems evolving in natural habitats unaltered by humans, some form of green/UV contrast based on opponent-processes may be theoretically more likely (Möller, 2002). Evidence supports such an opponent-process system in the polarisation compass (Labhart, 1988, 1996). Such opponent processes buy constancy in the face of changing overall illumination levels and alleviate the need to adjust the threshold on the basis of overall light levels, a by no means trivial problem. It would be good to effect a similar knock-down manipulation targeting the green wavelengths as well. The green:UV ratio would also be distorted if green wavelengths are substantially reduced, and similar deficits should be found. If the ants use the amount of UV light (or stimulation of the UV receptor) for segregating the skyline, the green knock-down manipulation should have little effect.

Sensitivity to UV wavelengths serves navigation in other ways in insects. Sensory neurons sensitive to UV wavelengths in the dorsal rim of the eyes of desert ants and honeybees serve as receptors for polarised light (Wehner 1994, 1997). Dung beetles, *Scarabaeus zambesianus*, use polarised moon light in order to roll a ball of dung away from the dung pile in a straight line (Dacke, Nilsson, Scholtz, Byrne, & Warrant, 2003). This polarisation channel is also mediated by sensitivity to UV wavelengths (el Jundi et al., 2015). In the desert locust, *Schistocerca gregaria*, the polarisation channel is mediated by blue receptors (el Jundi, Pfeiffer, Heinze, & Homberg, 2014), but intriguingly, UV-green opponent-process neurons have been found in the anterior optic tubercle (Kinoshita et al., 2007). These neurons are excited by unpolarised light in the green wavelengths and inhibited by unpolarised light in the UV wavelengths, or vice versa. They are thought to serve the celestial compass in locusts. Whether such opponent-process neurons can be found in circuits in insects that encode terrestrial cues remains an open question.

In sum, this study has shown that light in the UV range plays an important role in ant navigation based on the terrestrial panorama. Knocking it down by blocking UV wavelengths made zero-vector ants not orient in the nest direction when navigating out of a uniformly coloured arena providing a skyline (Experiment 1), but instead if anything in the opposite nest-to-feeder direction. With UV wavelengths blocked, the ants did not orient as well in the nest direction under natural conditions, although they were still significantly oriented in this direction (Experiment 2). With an opaque artificial arena replaced with a UV-blocking but clear arena of the same shape, the ants managed to orient significantly in the nest direction.

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Figure captions

Figure 1. The set up in Experiments 1 and 2. (a) A photo of the arena used in Experiment 1 with some of the surrounding scenery, which would not be visible to the ants inside the arena. An enclosure (white plastic) surrounding the nest and leading to the arena kept most of the ants foraging in the corridor and increased the number of foragers arriving at the feeder. (b) The panoramic view provided by the arena. The photo was taken with a panoramic lens and rendered into cylindrical form. The photo ‘wraps around’, in that the right side of the photo coincides with the left side. (c) The panoramic view at the feeder in Experiment 2, with again the right side of the photo coinciding with the left side.

Figure 2. (a) Transmission characteristics of the Makrolon UV-blocking plastic. The photospectrometric measurements were taken with an Ocean Optics Jaz™ photospectrometer (Ocean Optics, Dunedin, Florida), with the plastic placed in front of a piece of standard white colour, and compared with the reflectance of standard white alone. Thus, in the measurements of the plastic, the light had to go through the plastic twice, to get to the standard white and then to reflect back from the standard white. Only transmittance in the range of 300-700 nm, a reliable range for the instrument, is shown. (b) Reflectance characteristics of the green wall of the arena used in Experiments 1 and 3, measured with the same instrument. Note that the scale is reduced tenfold, with maximum on graph set at 10%.

Figure 3. Results of Experiment 1. Distributions of heading directions at 30 cm for full-vector ants under control (training) conditions and with the UV-blocking plastic placed inside the arena (a), zero-vector ants under control (training) conditions and with the UV-blocking plastic placed inside or outside the arena, two conditions combined (b), and zero-vector ants with the UV-blocking conditions placed inside or outside the test

arena, two conditions separate (c). Each panel is cylindrical, with +180° and –180° being the same nest-to-feeder direction. Nest direction is at 0°. The line through each distribution is an atheoretical spline that serves only to help readers to visualise the data. *: Two conditions in graph differ significantly in directional scatter. #: Two conditions in the graph differ significantly in mean heading direction.

Figure 4. Results of Experiment 2. Distributions of heading directions at 30 cm for zero-vector ants in control (training) conditions, separately for two replicates (a), zero-vector ants with the UV-blocking foil surrounding them on the test, separately for two replicates (b), zero-vector ants in control (training) conditions and with the UV-blocking foil surrounding them on the test, each with two replicates combined (c), and zero-vector ants with an opaque white foil surrounding them on the test (d). Each panel is cylindrical, with +180° and –180° being the same nest-to-feeder direction. Nest direction is at 0°. The line through each distribution is an atheoretical spline that serves only to help readers to visualise the data. *: Two conditions in graph differ significantly in directional scatter.

Figure 5. Results of Experiment 3. Distributions of heading directions at 30 cm for zero-vector ants in the Control condition and with UV-blocking foil cut out to the shape of the training arena (Clear-cut-out, (a)) and in the No-arena condition (b). Smoothed data for the Control condition and with UV-blocking foil cut out to the shape of the training arena (c), and in the No arena condition (d). Data in (c) and (d) were transformed from those in (a) and (b) by averaging each bin with its two immediate neighbours. Each panel is cylindrical, with +180° and –180° being the same nest-to-feeder direction. Nest direction is at 0°. The line through each distribution is an atheoretical spline that serves only to help readers to visualise the data. *: Two conditions in graph differ significantly

in directional scatter. #: Two conditions in the graph differ significantly in mean heading direction. Inferential statistics was not performed on panels (c) and (d).

Figure 1

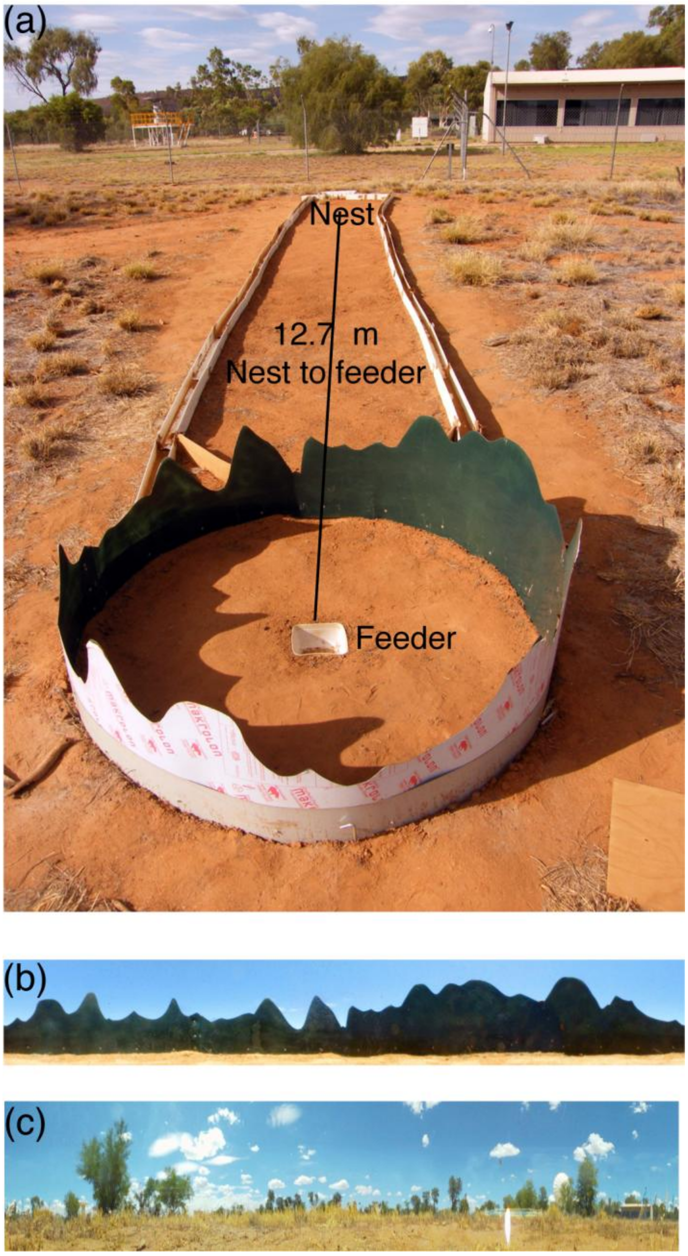


Figure 2

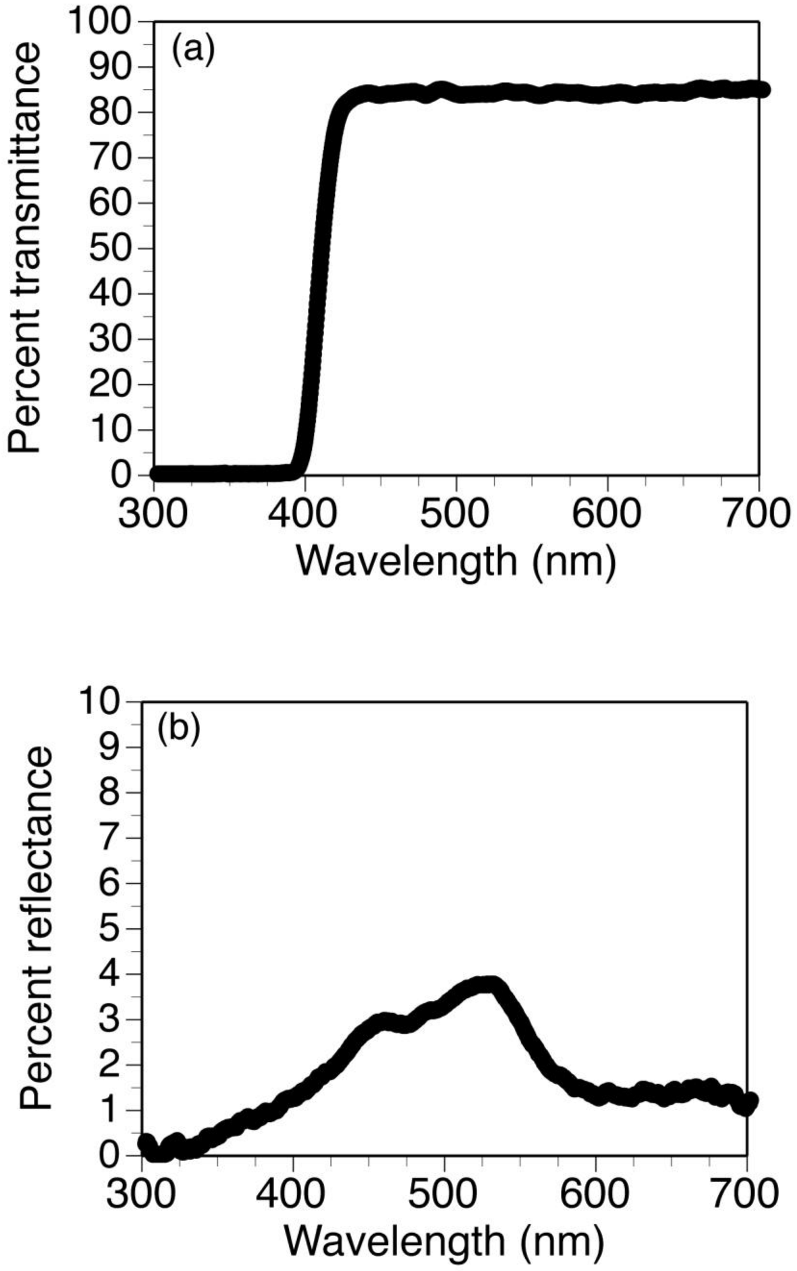

(a)
Percent transmittance
0
10
20
30
40
50
60
70
80
90
100
300
400
500
600
700
Wavelength (nm)
(b)
Percent reflectance
0
1
2
3
4
5
6
7
8
9
10
300
400
500
600
700
Wavelength (nm)

Figure 3

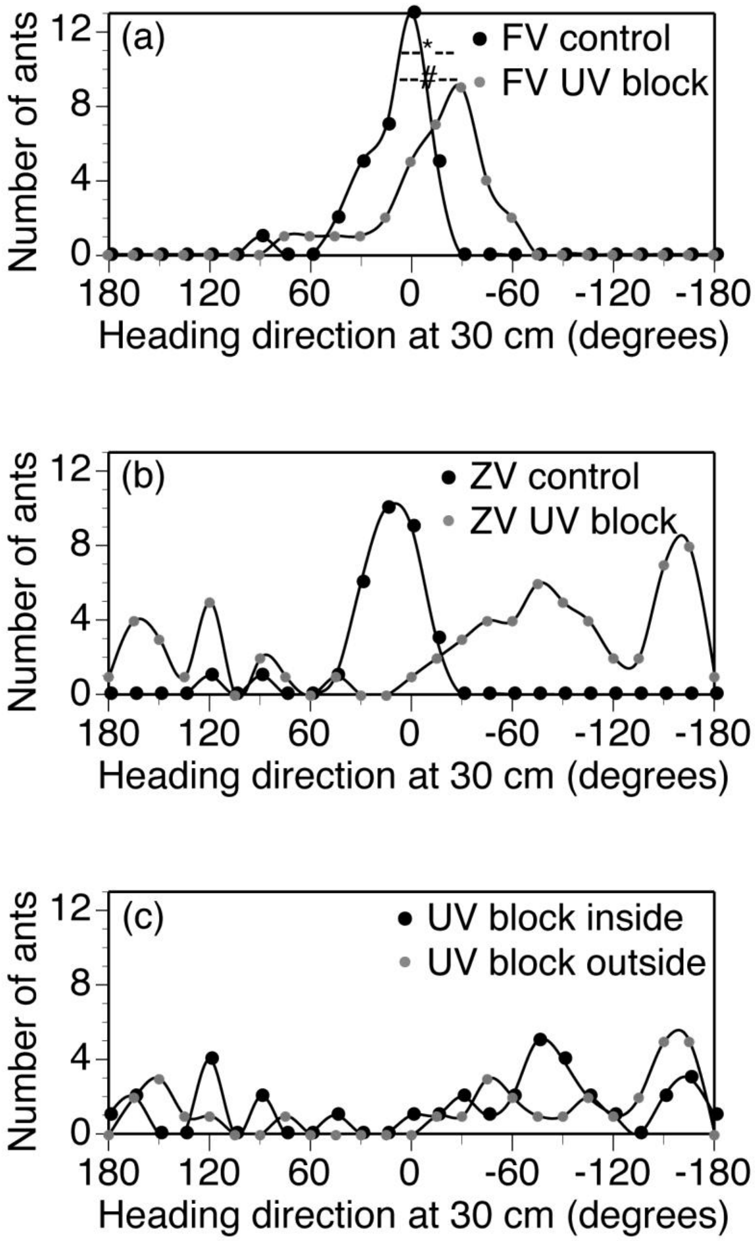

(a)
● FV control
● FV UV block
*
#
Number of ants
12
8
4
0
180
120
60
0
-60
-120
-180
Heading direction at 30 cm (degrees)
(b)
● ZV control
● ZV UV block
Number of ants
Heading direction at 30 cm (degrees)
(c)
● UV block inside
● UV block outside
Number of ants
Heading direction at 30 cm (degrees)

Figure 4

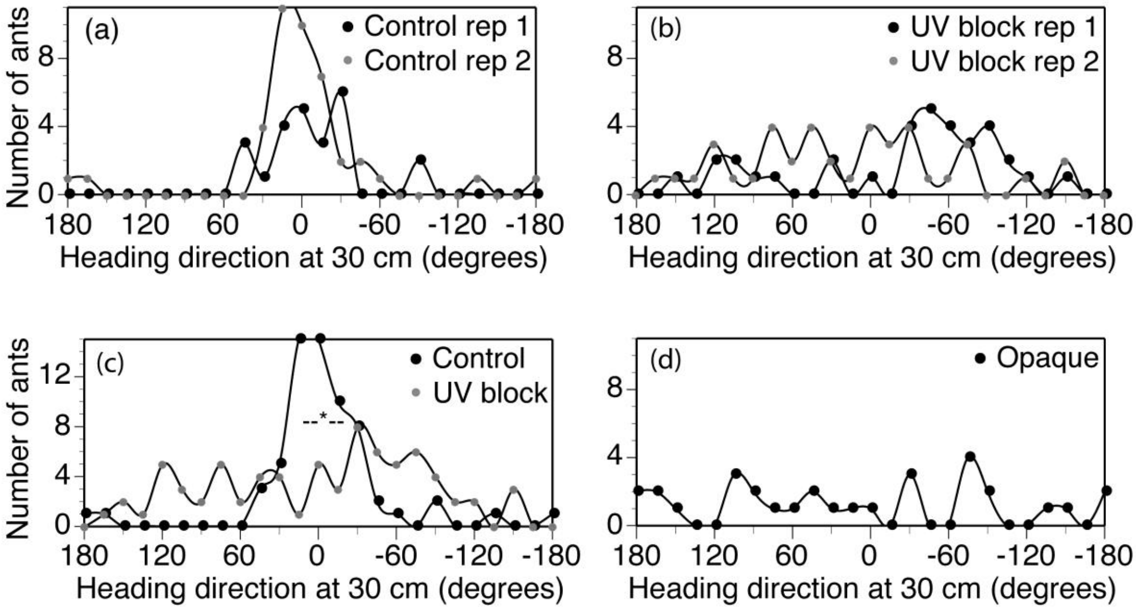
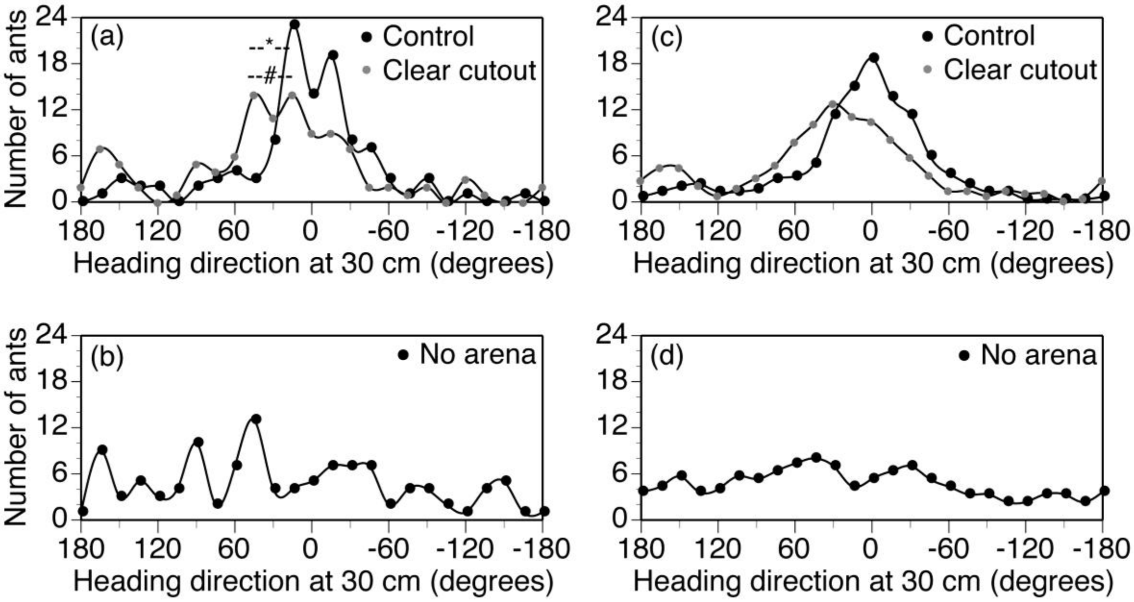

(a)
Control rep 1
Control rep 2
Number of ants
0
4
8
180
120
60
0
-60
-120
-180
Heading direction at 30 cm (degrees)
(b)
UV block rep 1
UV block rep 2
0
4
8
180
120
60
0
-60
-120
-180
Heading direction at 30 cm (degrees)
(c)
Control
UV block
--*--
Number of ants
0
4
8
12
180
120
60
0
-60
-120
-180
Heading direction at 30 cm (degrees)
(d)
Opaque
0
4
8
180
120
60
0
-60
-120
-180
Heading direction at 30 cm (degrees)

Figure 5


(a)
● Control
● Clear cutout
--*--
--#--
(b)
● No arena
(c)
● Control
● Clear cutout
(d)
● No arena
Number of ants
24
18
12
6
0
Heading direction at 30 cm (degrees)
180
120
60
0
-60
-120
-180

Acknowledgements

This research was supported by a grant from the Australian Research Council (DP110100608). We thank the Centre for Appropriate Technology for letting us work on their site, and for providing space for storage. We thank the CSIRO, Alice Springs, for providing some administrative support and letting us rent a house, Martin Whiting for help with photospectrometric measurement, and three anonymous reviewers for helpful comments. The research conducted complied with the laws of the Northern Territory, Australia. The authors declare no conflict of interest.

Figure 1

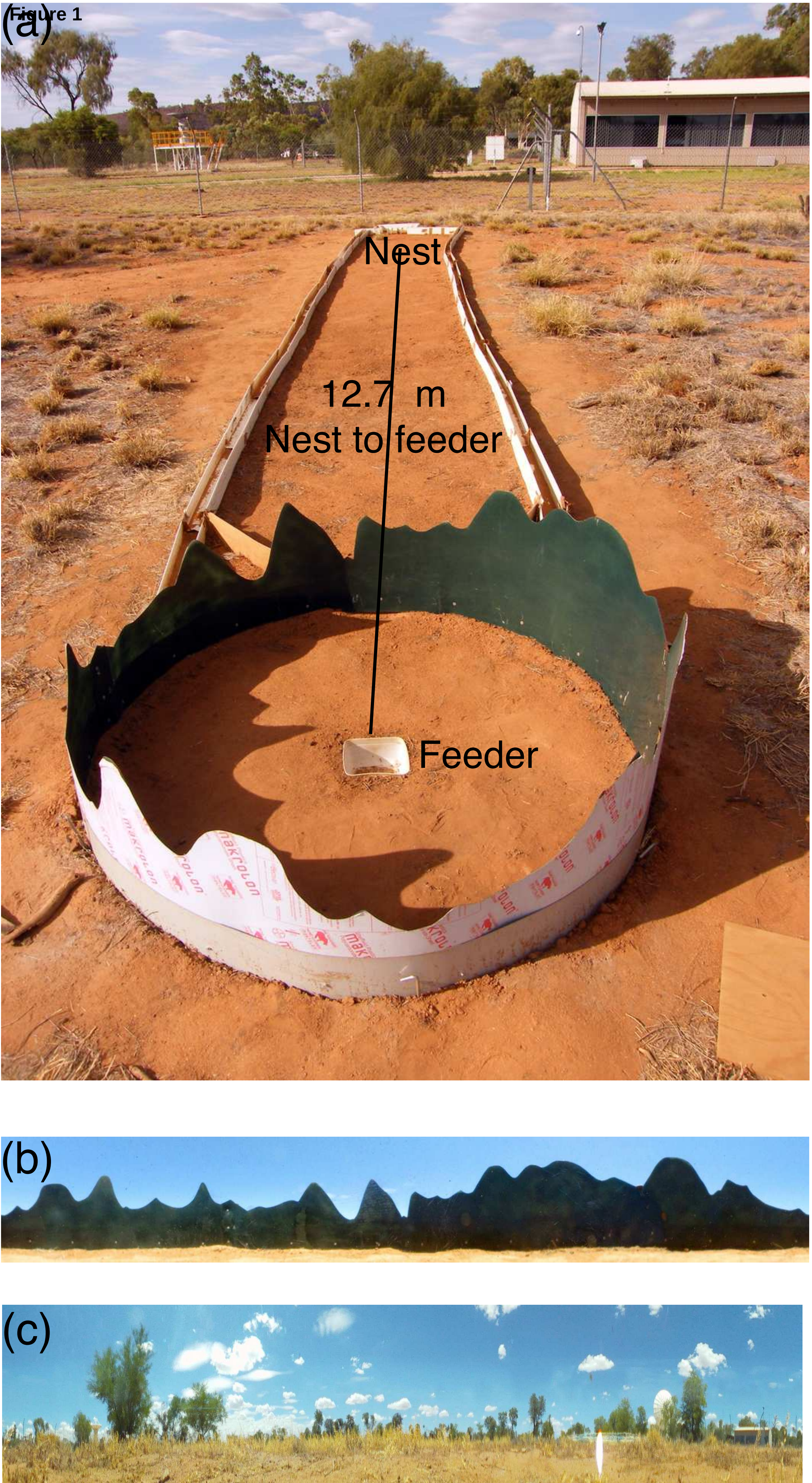
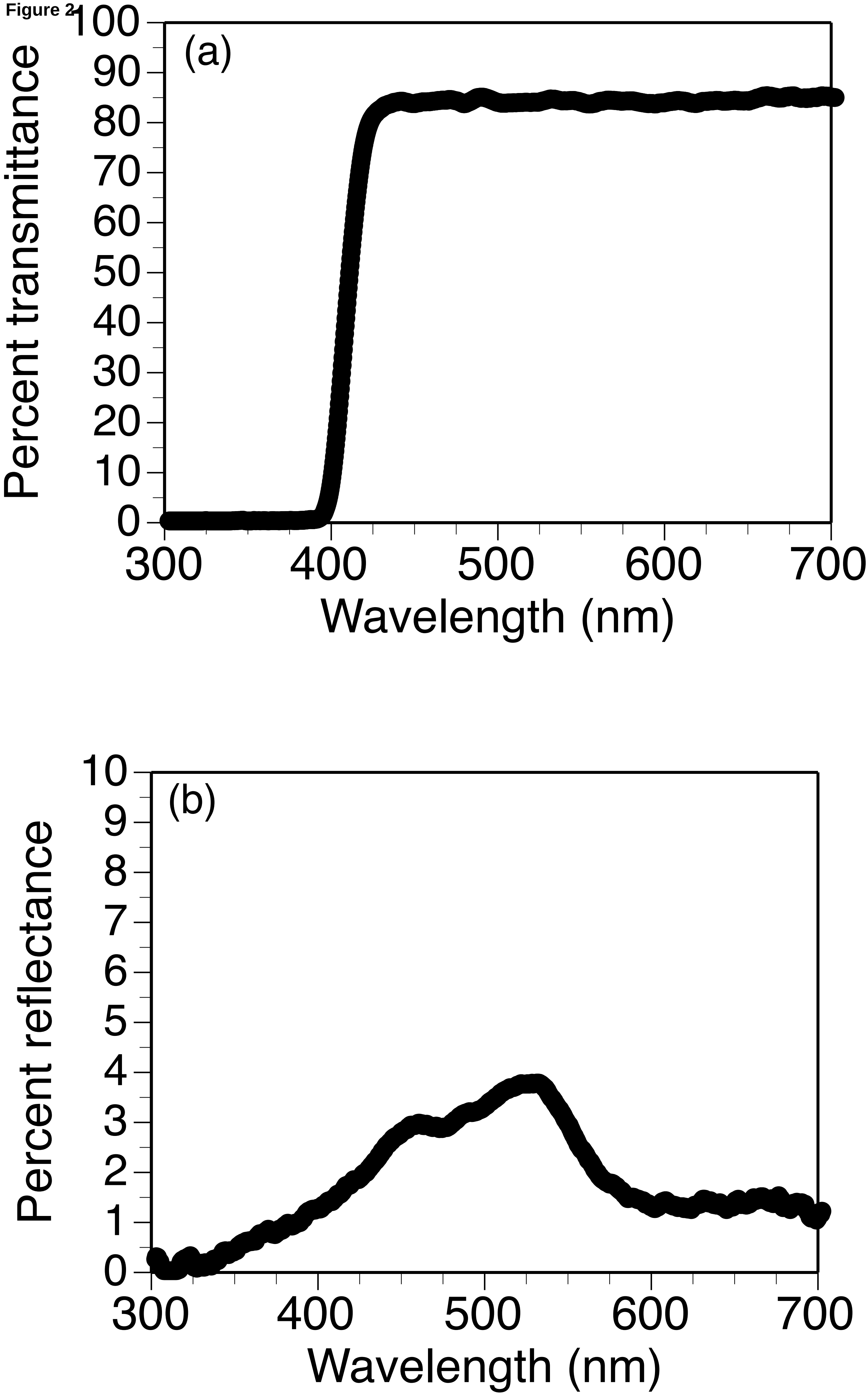


Figure 2


(a)
Percent transmittance
0
10
20
30
40
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100
300
400
500
600
700
Wavelength (nm)
(b)
Percent reflectance
0
1
2
3
4
5
6
7
8
9
10
300
400
500
600
700
Wavelength (nm)

Figure 3

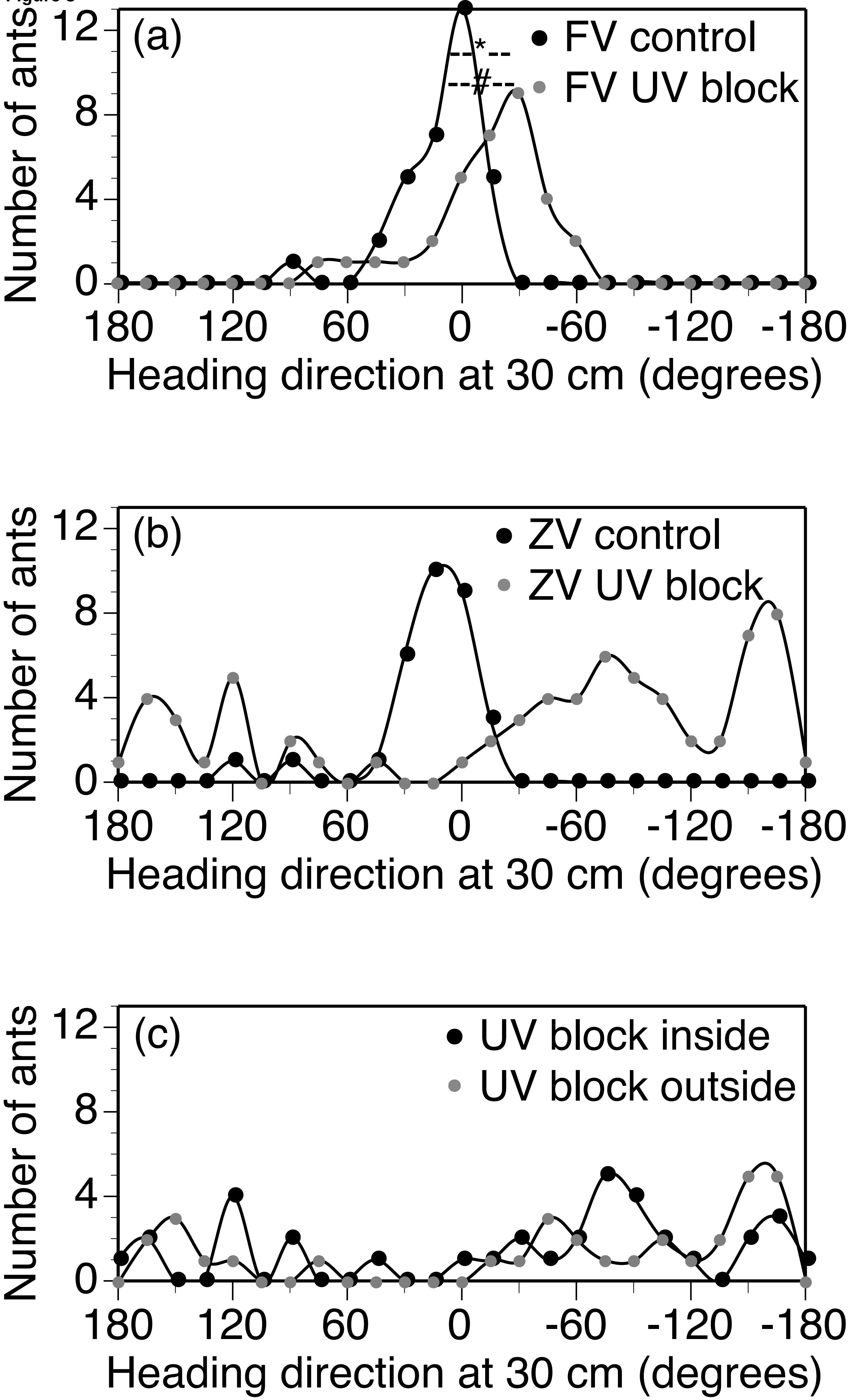

(a)
● FV control
● FV UV block
--*--
--#--
Number of ants
0
4
8
12
180
120
60
0
-60
-120
-180
Heading direction at 30 cm (degrees)
(b)
● ZV control
● ZV UV block
Number of ants
0
4
8
12
180
120
60
0
-60
-120
-180
Heading direction at 30 cm (degrees)
(c)
● UV block inside
● UV block outside
Number of ants
0
4
8
12
180
120
60
0
-60
-120
-180
Heading direction at 30 cm (degrees)

Figure 4

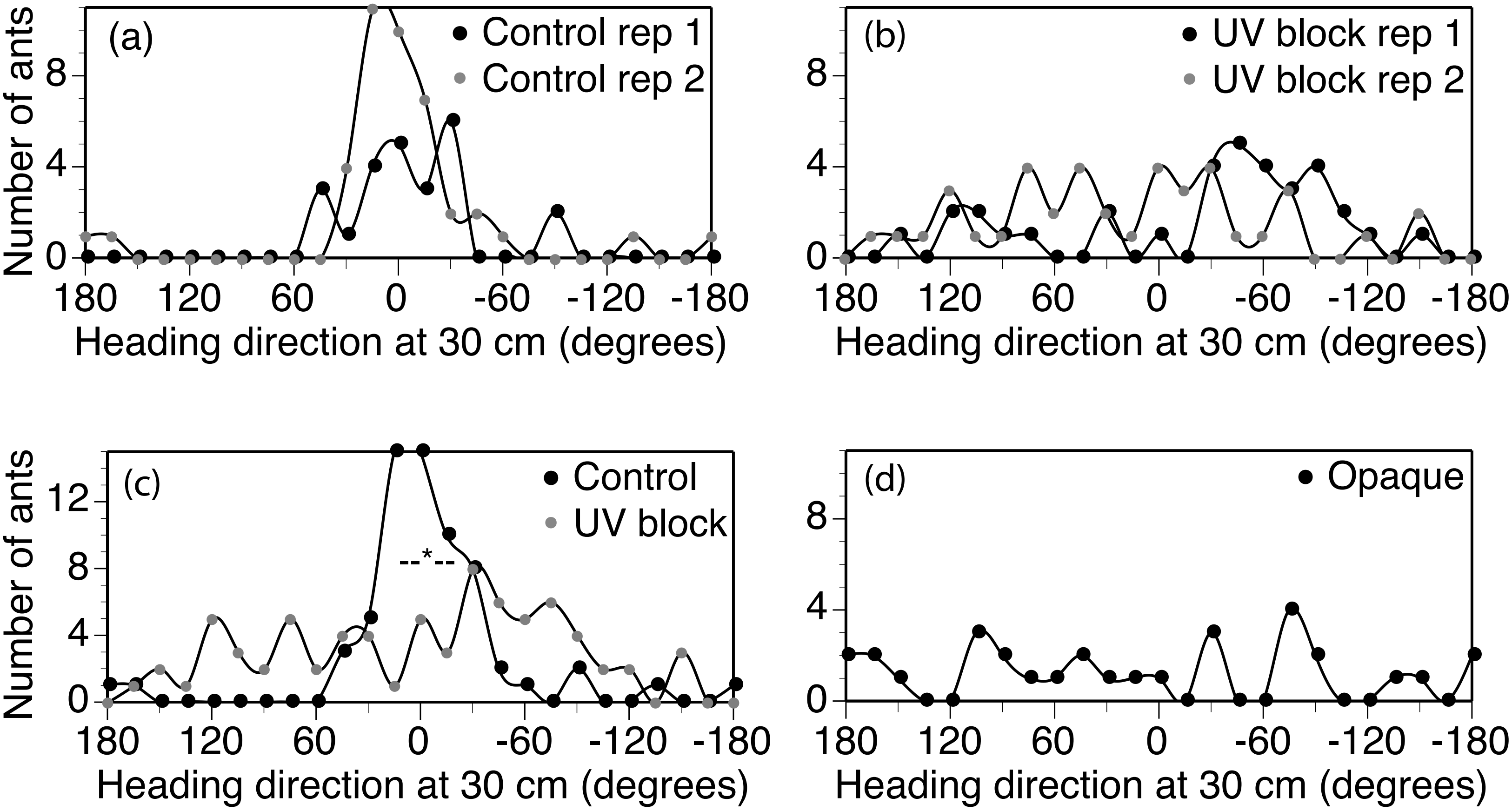
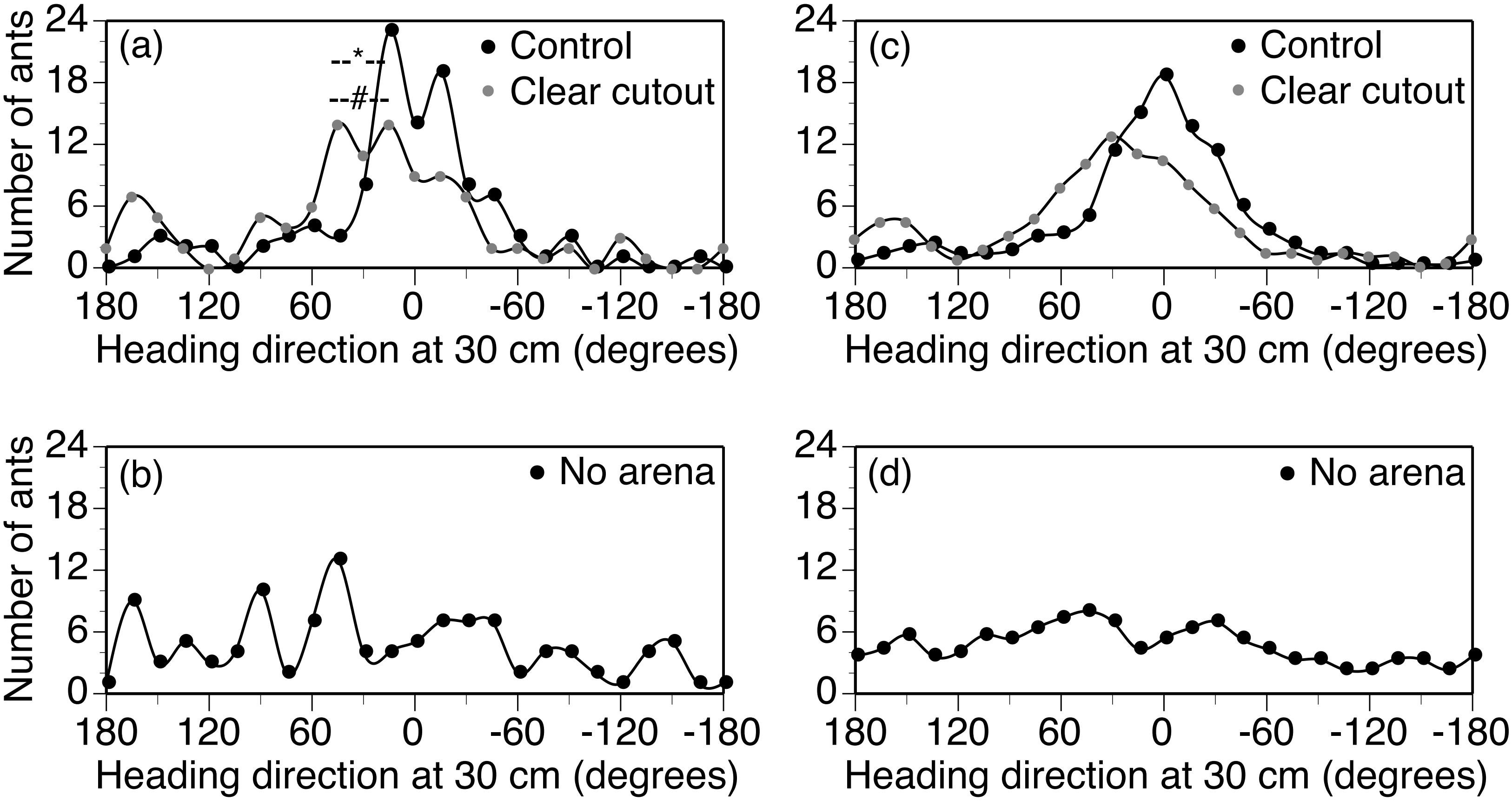

(a)
Control rep 1
Control rep 2
(b)
UV block rep 1
UV block rep 2
(c)
Control
UV block
--*--
(d)
Opaque
Number of ants
0
4
8
12
180
120
60
0
-60
-120
-180
Heading direction at 30 cm (degrees)

Figure 5


(a)
(b)
(c)
(d)
Control
Clear cutout
No arena
--*--
--#--
Number of ants
24
18
12
6
0
180
120
60
0
-60
-120
-180
Heading direction at 30 cm (degrees)

Tables

Table 1

Descriptive and inferential statistics for Experiment 1

		95%CI L	**M**	**95%CI R**		**Rayleigh test**		**V test**	
Condition	***N***	**(deg)**	**(deg)**	**(deg)**	**R**	**z**	***P***	**V**	***P***
ZV control	31	25.2	15.3	5.4	0.90	25.21	<0.001	27.04	<0.001
ZV UV block inside	34	–60.0	–106.9	–153.9	0.32	3.49	0.029	–3.18	0.780
ZV UV block outside	32	–111.1	–139.8	–168.5	0.49	7.54	<0.001	–11.89	0.999
ZV UV block, combining ‘inside’ and ‘outside conditions	66	–100.9	–126.3	–151.7	0.39	9.75	<0.001	–15.07	0.996
FV control	33	17.7	10.2	2.6	0.94	28.78	<0.001	30.42	<0.001
FV UV block inside	33	–2.0	–14.8	–27.7	0.87	24.79	<0.001	27.73	<0.001

Shown are results for zero-vector (ZV) and full-vector (FV) conditions, including the number of ants tested (*N*), mean vector direction (M), 95% confidence intervals to the left (95%CI L) and right (95%CI R), mean vector length (R), Rayleigh test results, and V test results testing for significant orientation in the fictive nest direction, or exit direction according to the arena.

Table 2

Inferential statistics comparing the directional scatter of conditions in Experiments 1 and 2

Experiment	**Comparison**	**Z**	***P***
1	ZV UV block inside vs. ZV control	5.36	<0.001
1	ZV UV block outside vs. ZV control	3.97	<0.001
1	FV UV block inside vs. FV control	1.39	0.163
2	ZV UV block vs. ZV control replicate 1	1.92	0.055
2	ZV UV block vs. ZV control replicate 2	4.92	<0.001
2	ZV UV block vs. ZV control, combining replicate 1 and replicate 2	5.70	<0.001

Comparisons were based on the Var test. Absolute differences of individual headings from the mean circular heading of each of two conditions are computed. The scores for each group are then compared with the Wilcoxon rank sum test, two-tailed. Different zero-vector (ZV) and full-vector (FV) conditions were compared against appropriate control groups.

Table 3

Inferential statistics comparing mean directions of conditions in Experiments 1 and 2

Experiment	**Comparison**	**F**	***P***
1	ZV UV block inside vs. ZV control	44.74	<0.001
1	ZV UV block outside vs. ZV control	104.93	<0.001
1	FV UV block inside vs. FV control	14.61	<0.001
2	ZV UV block vs. ZV control replicate 1	9.14	0.004
2	ZV UV block vs. ZV control replicate 2	3.43	0.068
2	ZV UV block vs. ZV control, combining replicate 1 and replicate 2	<1	0.376

Comparisons were based on the Watson-Williams test. Mean directions of different zero-vector (ZV) and full-vector (FV) conditions were compared against appropriate control groups.

Table 4

Descriptive and inferential statistics for Experiment 2

Condition	***N***	**95%CI L (deg)**	**M (deg)**	**95%CI R (deg)**	**R**	**Rayleigh test z**	**Rayleigh test *P***	**V test V**	**V test *P***
ZV control replicate 1	24	10.0	–6.1	–22.2	0.84	16.76	<0.001	20.00	<0.001
ZV control replicate 2	40	12.0	–1.2	–14.5	0.80	25.33	<0.001	31.92	<0.001
ZV control, combining replicate 1 and replicate 2	64	7.0	–3.1	–13.2	0.81	42.00	<0.001	51.92	<0.001
ZV UV block replicate 1	34	–23.0	–54.8	–86.6	0.44	6.41	0.001	8.52	0.019
ZV UV block replicate 2	40	61.1	26.3	–8.6	0.37	5.56	0.003	13.42	0.001
ZV UV block, combining replicate 1 and replicate 2	74	17.2	–14.0	–45.2	0.31	6.87	<0.001	21.94	<0.001
ZV opaque	28	---	42.2	---	0.07	0.14	0.868	1.50	0.345

Shown are results for zero-vector (ZV) conditions, including the number of ants tested (N), mean vector direction (M), 95% confidence intervals to the left (95%CI L) and right (95%CI R), mean vector length (R), Rayleigh test results, and V test results testing for significant orientation in the fictive nest direction, or exit direction according to the arena.

Table 5

Descriptive and inferential statistics for Experiment 3

Condition	*N*	95% CI L (deg)	M (deg)	95% CI R (deg)	R	Rayleigh test z	Rayleigh test *P*	V: nest direction V	V: nest direction *P*
Control	108	13.0	3.0	–7.1	0.67	48.9	$<10^{-24}$	72.80	<0.001
UV blocking foil cut-out	107	42.7	27.8	13.0	0.49	25.8	$<10^{-11}$	15.51	<0.001
No arena	114	79.5	41.5	3.6	0.21	5.0	0.007	6.52	0.009

Shown for each conditions are the number of zero-vector ants tested (N), mean vector direction (M), 95% confidence intervals to the left (95%CI L) and right (95%CI R), mean vector length (R), Rayleigh test results, and V test results testing for significant orientation in the fictive nest direction.