Polarized light use in the nocturnal bull ant, *Myrmecia midas*

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**Article citation details**

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**Review timeline**

Original submission: 2 June 2017  
Revised submission: 1 August 2017  
Final acceptance: 2 August 2017

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Note: This manuscript was transferred from another Royal Society journal without peer review.

**Review History**

RSOS-170598.R0 (Original submission)

Review form: Reviewer 1 (Rachel Muheim)

Is the manuscript scientifically sound in its present form?  
Yes

Are the interpretations and conclusions justified by the results?  
Yes

Is the language acceptable?  
Yes

Is it clear how to access all supporting data?  
Yes

Do you have any ethical concerns with this paper?  
No

Have you any concerns about statistical analyses in this paper?  
No
Recommendation?
Accept with minor revision (please list in comments)

Comments to the Author(s)
Lines 71-74: Light emitted by the sun and moon is unpolarized, but gets polarized when it enters the atmosphere. Your description can be misunderstood that the light emitted by the sun and moon is already polarized, which is not correct.
Lines 75-76: The solar meridian is the position of the sun at midday, but the e-vector is always aligned perpendicular to the direction of the sun, irrespective of time of day.
Line 109 ff: What time of day were these experiments done? Please give range of sun elevation so it is possible to get an idea of how “dark” it was.
Lines 113-117: Please describe how you determined the ambient polarization axis and aligned the filter within the short amount of time it takes an ant to walk a few meters. The natural polarization axis changes over the season and time of day, so it appears to be quite a challenge to determine the exact alignment of the polarizer. Please also make an estimate of the error.
Line 130/131: Please give range of sun elevation here as well. “pre-dawn twilight” is too imprecise.
Lines 157-160: Do you really mean “absolute shift”? Absolut by definitions means disregarding the sign of the shift, but in the results you report positive and negative shifts...
Lines 160-161: In those cases where the mean vectors of both samples are >0.75 (all but Fig. 2B), you should preferentially use the Watson-William-F-test, which is a parametric test and tests for differences in direction between groups. The Mardia-Watson-Wheeler test, on the other hand (and also Watson U2-test), tests for difference in direction or scatter, so to be able to conclude that the difference is in direction, you need to do additional tests (e.g., set the means of the two groups equal and then repeat the Mardia-Watson-Wheeler test). Only then you can say with confidence that the difference is due to direction. So, whenever possible, the F-test is better. Also, before doing any group comparisons, each sample should be tested for randomness with a Rayleigh test, which tests whether or not the samples are significantly directed or not. In addition, you might want to use the confidence interval test to test whether the expected direction (rotation) lies within the 95% CI, i.e., whether the animals fully responded to the shift of the polarization axis.
Results: are very difficult to read, please place basic orientation data in a table, and give mean direction, r-vector length and Rayleigh statistics.

Rachel Muheim
Lund University, Sweden

Decision letter (RSOS-170598)
24-Jul-2017
Dear Dr Freas

On behalf of the Editors, I am pleased to inform you that your Manuscript RSOS-170598 entitled "Polarized light use in the nocturnal bull ant, Myrmecia midas" has been accepted for publication in Royal Society Open Science subject to minor revision in accordance with the referee suggestions. Please find the referees' comments at the end of this email.

The reviewers and handling editors have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the comments and revise your manuscript.
• Ethics statement
If your study uses humans or animals please include details of the ethical approval received, including the name of the committee that granted approval. For human studies please also detail whether informed consent was obtained. For field studies on animals please include details of all permissions, licences and/or approvals granted to carry out the fieldwork.

• Data accessibility
It is a condition of publication that all supporting data are made available either as supplementary information or preferably in a suitable permanent repository. The data accessibility section should state where the article's supporting data can be accessed. This section should also include details, where possible of where to access other relevant research materials such as statistical tools, protocols, software etc can be accessed. If the data has been deposited in an external repository this section should list the database, accession number and link to the DOI for all data from the article that has been made publicly available. Data sets that have been deposited in an external repository and have a DOI should also be appropriately cited in the manuscript and included in the reference list.

If you wish to submit your supporting data or code to Dryad (http://datadryad.org/), or modify your current submission to dryad, please use the following link:
http://datadryad.org/submit?journalID=RSOS&manu=RSOS-170598

• Competing interests
Please declare any financial or non-financial competing interests, or state that you have no competing interests.

• Authors’ contributions
All submissions, other than those with a single author, must include an Authors’ Contributions section which individually lists the specific contribution of each author. The list of Authors should meet all of the following criteria; 1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published.

All contributors who do not meet all of these criteria should be included in the acknowledgements.

We suggest the following format:
AB carried out the molecular lab work, participated in data analysis, carried out sequence alignments, participated in the design of the study and drafted the manuscript; CD carried out the statistical analyses; EF collected field data; GH conceived of the study, designed the study, coordinated the study and helped draft the manuscript. All authors gave final approval for publication.

• Acknowledgements
Please acknowledge anyone who contributed to the study but did not meet the authorship criteria.

• Funding statement
Please list the source of funding for each author.

Please note that we cannot publish your manuscript without these end statements included. We have included a screenshot example of the end statements for reference. If you feel that a given heading is not relevant to your paper, please nevertheless include the heading and explicitly state that it is not relevant to your work.
Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript within 7 days (i.e. by the 02-Aug-2017). If you do not think you will be able to meet this date please let me know immediately.

To revise your manuscript, log into https://mc.manuscriptcentral.com/rsos and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions". Under "Actions," click on "Create a Revision." You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you will be able to respond to the comments made by the referees and upload a file "Response to Referees" in "Section 6 - File Upload". You can use this to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response to the referees.

When uploading your revised files please make sure that you have:

1) A text file of the manuscript (tex, txt, rtf, docx or doc), references, tables (including captions) and figure captions. Do not upload a PDF as your "Main Document".
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4) Included the raw data to support the claims made in your paper. You can either include your data as electronic supplementary material or upload to a repository and include the relevant doi within your manuscript
5) All supplementary materials accompanying an accepted article will be treated as in their final form. Note that the Royal Society will neither edit nor typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details where possible (authors, article title, journal name).

Supplementary files will be published alongside the paper on the journal website and posted on the online figshare repository (https://figshare.com). The heading and legend provided for each supplementary file during the submission process will be used to create the figshare page, so please ensure these are accurate and informative so that your files can be found in searches. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

Once again, thank you for submitting your manuscript to Royal Society Open Science and I look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Kind regards,
Andrew Dunn
Senior Publishing Editor
Royal Society Open Science
openscience@royalsociety.org

on behalf of Kevin Padian
Subject Editor, Royal Society Open Science
openscience@royalsociety.org
Associate Editor Comments to Author:
As you can see, you have received a review from a single referee. We had assigned a second, but they were very late with the review. Given the enthusiasm of this review, we decided to move forward with acceptance of the manuscript. Please address the minor concerns of the reviewer.

Reviewer comments to Author:
Reviewer: 1

Comments to the Author(s)
Lines 71-74: Light emitted by the sun and moon is unpolarized, but gets polarized when it enters the atmosphere. Your description can be misunderstood that the light emitted by the sun and moon is already polarized, which is not correct.
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Rachel Muheim
Lund University, Sweden

Author's Response to Decision Letter for (RSOS-170598)

See Appendix A.
Dear Dr Freas,

I am pleased to inform you that your manuscript entitled "Polarized light use in the nocturnal bull ant, Myrmecia midas" is now accepted for publication in Royal Society Open Science.

You can expect to receive a proof of your article in the near future. Please contact the editorial office (openscience_proofs@royalsociety.org and openscience@royalsociety.org) to let us know if you are likely to be away from e-mail contact. Due to rapid publication and an extremely tight schedule, if comments are not received, your paper may experience a delay in publication.

Royal Society Open Science operates under a continuous publication model (http://bit.ly/cpFAQ). Your article will be published straight into the next open issue and this will be the final version of the paper. As such, it can be cited immediately by other researchers. As the issue version of your paper will be the only version to be published I would advise you to check your proofs thoroughly as changes cannot be made once the paper is published.

In order to raise the profile of your paper once it is published, we can send through a PDF of your paper to selected colleagues. If you wish to take advantage of this, please reply to this email with the name and email addresses of up to 10 people who you feel would wish to read your article.

On behalf of the Editors of Royal Society Open Science, we look forward to your continued contributions to the Journal.

Best wishes,

Alice Power
Editorial Coordinator
Royal Society Open Science
openscience@royalsociety.org
Reviewer comments to Author:
Reviewer: 1
Comments to the Author(s)
Lines 71-74: Light emitted by the sun and moon is unpolarized, but gets polarized when it enters the atmosphere. Your description can be misunderstood that the light emitted by the sun and moon is already polarized, which is not correct.

We have corrected this portion of the manuscript to make clear that polarized light is a product of light passing through the atmosphere and does not originate from the sun.

Lines 75-76: The solar meridian is the position of the sun at midday, but the e-vector is always aligned perpendicular to the direction of the sun, irrespective of time of day.

We have corrected this portion of the manuscript to make clear that the e-vector aligns (perpendicular) with the direction of the sun regardless of the sun position at different periods of the day. We have removed the portion referencing the solar meridian to reduce confusion.

Line 109 ff: What time of day were these experiments done? Please give range of sun elevation so it is possible to get an idea of how “dark” it was.

All experiments were conducted during the evening or morning twilight when the sun’s position was between -18º and 0º below the horizon. Experiments never started before sunset and always halted before the end of twilight or sunrise. We have added this information to the manuscript.

Lines 113-117: Please describe how you determined the ambient polarization axis and aligned the filter within the short amount of time it takes an ant to walk a few meters. The natural polarization axis changes over the season and time of day, so it appears to be quite a challenge to determine the exact alignment of the polarizer. Please also make an estimate of the error.

The reviewer is absolutely right that natural polarisation axis changes over season and time of day. Each night we checked the position of the sun at sunset or sunrise and set the ambient e-vector 180º from this point. Because the ants maintain predictable nest-foraging tree route patterns in a well-defined corridor, we could easily pinpoint the orientation of the overhead e-vector and rotate the polariser relative to that. We relied on a compass for these measurements by resting the compass on the polar filter along the filter's polarisation pattern during placement. Only after the placement was deemed correct did we remove the compass. We have added this explanation to the manuscript.

As the rotation of the polariser was carried out relying on a hand held compass. We unfortunately cannot estimate the errors involved in this, but we will definitely consider this in our future experiments.
Line 130/131: Please give range of sun elevation here as well. “pre-dawn twilight” is too imprecise.

The sun’s elevation position during testing is now described at the beginning of the methods section. We thought it best to describe the evening and morning twilight positions together.

Lines 157-160: Do you really mean “absolute shift”? Absolute by definitions means disregarding the sign of the shift, but in the results you report positive and negative shifts…

In the results we report the shift data with positive and negative shifts corresponding with the changes in the e-vector.

Data comparisons between conditions use absolute shifts in order to make direct comparisons of shift magnitude between conditions. Absolute shift is really an incorrect term at we actually mirror the -45º data across the 0º mark. Thus an overall shift of -20º is now +20º but a forager that shifts away from the predicted shift (i.e. +15º under a -45º shift) does become negative (-15º). This was done to be able to compare plus and minus data and to combine these conditions for comparisons of the separate nest distance conditions.

We now call this shift magnitude and hope that it is clearer.

Lines 160-161: In those cases where the mean vectors of both samples are >0.75 (all but Fig. 2B), you should preferentially use the Watson-William-F-test, which is a parametric test and tests for differences in direction between groups. The Mardia-Watson-Wheeler test, on the other hand (and also Watson U2-test), tests for difference in direction or scatter, so to be able to conclude that the difference is in direction, you need to do additional tests (e.g., set the means of the two groups equal and then repeat the Mardia-Watson-Wheeler test). Only then you can say with confidence that the difference is due to direction. So, whenever possible, the F-test is better. Also, before doing any group comparisons, each sample should be tested for randomness with a Rayleigh test, which tests whether or not the samples are significantly directed or not. In addition, you might want to use the confidence interval test to test whether the expected direction (rotation) lies within the 95% CI, i.e., whether the animals fully responded to the shift of the polarization axis.

We have changed all of the stats to align with the reviewer’s request. All tests are now Watson-William-F-tests and there is no difference in the pattern of statistical significances in our findings.

We have added the 95% CI to Table 1 as requested. Only the Inbound 4-6m +45º shift does not quite have a 95% CI that represents a full 45º shift with the CI ending at 42º. In our manuscript we do not define these shift as full shifts except in the last sentence, which we have changed to address this issue.

Results: are very difficult to read, please place basic orientation data in a table, and give mean direction, r-vector length and Rayleigh statistics.

We have added a Table 1 which contains these basic orientation statistics.