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Exploration adjustment by ant colonies

Carolina Doran, Martin C. Stumpe, Ana Sendova-Franks and Nigel R. Franks

Article citation details

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Review timeline

Original submission: 6 October 2015
Revised submission: 17 November 2015
Final acceptance: 18 December 2015

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

Review History

RSOS-150533.R0 (Original submission)

Review form: Reviewer 1 (Tomer Czaczkes)

Is the manuscript scientifically sound in its present form?

Yes

Are the interpretations and conclusions justified by the results?

No

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?

Yes

Recommendation?

Major revision is needed (please make suggestions in comments)

Comments to the Author(s)

In this study, the exploration behaviour of the Rock Ant *Temnothorax albigenis* is studied. Colonies are housed in nests of varying quality, and the number of exploration bouts, and their total and individual lengths and durations, are measured. Two conclusions are drawn: Regarding collective exploration, the authors conclude that overall exploration rates increase as current nest value decreases. Regarding individual exploration, the authors conclude that exploration bout length and duration increase with increasing nest quality, while instantaneous speed decreases.

The paper reads well, the motivation behind the experiments is sound, and the experimental methodology is robust. The figures are clear and informative. The statistical analysis is, to my knowledge, generally sound (see some minor comments at the end), although I cannot judge the Python script. Indeed, I applaud the provision of both the preliminary data checking and the scripts use to carry out the analysis (but see some minor comments) – this sort of transparency is what most papers should strive for.

I find the collective results robust, reliable and believable. I will make more detailed suggestions at the end, but have no major comments regarding this aspect of the work.

However, I am very sceptical about the patterns described for the individual level behaviour. While significant differences are found, and I can find no flaw in how the statistics were carried out (I am no expert on the matter). However, the effect size seems from the figures to be very small indeed. If we consider the breadth of the whiskers in all panels of figure 4, we see that variation within groups is much, much greater than variation between groups. If we were to consider non-transformed data, the whiskers would likely be even broader. While there is no agreed upon equivalent for an R-squared value for generalised linear mixed-effect models (GLMM), a rough estimate can be achieved by using an equivalent GLM. Using the raw data supplied, I calculated such R-squared. Log bout duration vs treatment had an adjusted R squared of 0.78% - less than 1% of the variation is explained by the treatment! Similar results are found for speed (2.21%). Very large sample sizes (here they vary from 2205 to 648 samples per treatment) can result in even tiny effects reaching significance. Just because an effect is statistically significant does not mean it is either real or that it has biological meaning.

Even if we were to agree that these patterns represent real and important biological effects (which I do not), I do not necessarily agree with the interpretation assigned to them. The authors present these results as showing a behavioural adaptation by individual scouts to their nest quality. I see a different (and in my opinion more likely) explanation: with higher nest quality all but a few 'super-scouts' stop scouting. This non-random subsample of scouts also happen to walk slower, make longer scouting bouts, and spend longer scouting. Thus, there is no change in the scouting behaviour of individuals – just a removal of all but the most hard working scouts. Had the individual scouts been assigned an identity (perhaps using the software id-Tracker (Pérez-Escudero et al. 2014), although I realise this software is limited to a very few individuals), testing which of these options is true would be possible. Without such individual ID information, both options must be presented as (at least) equally likely.

In light of these concerns, I urge the authors to be much more cautious in both how they present their results regarding individual behaviour, and in the conclusions drawn. This will require a significant rewrite of the title, the abstract, the results, and the discussion. Frankly, I would completely drop any claims being made about adjustment of individual-level behaviour. With that said, as the experimental methods and data collection is solid, I believe that manuscript will be acceptable for publication after a revision.

I hope the authors find my comments constructive and helpful.

Yours sincerely,

Tomer J. Czaczkes
University of Regensburg

I shall now make a series of more minor comments (split by section), aimed at improving the manuscript.

ABSTRACT

- Line 16-18: this (division of labour) does not seem very relevant to the study
- Line 20-21 and elsewhere: you are (perhaps reasonably) assuming the ants are scouting for a new nest, but in fact you do not know what the ants are doing. They may be searching for food, patrolling for enemies, or searching for material with which to modify their nest. Caution is required when assigning motivation to animal behaviour, as the motivational state of the animal will strongly affect how it reacts to stimuli.
- Lines 22-24: some concrete numbers (percentages, ratios, etc) would not go amiss here.
- Line 26-27: looking at the effect strengths, it seems that the collective patterns are driven almost exclusively by the number of bouts made in total.

BACKGROUND

- Line 51: "might be" - this has been concretely shown repeatedly, no?
- Line 53: in the reference list, ref 12 is formatted as a journal article, but it is fact a chapter in a book.
- I feel a lot of the introduction is not strictly relevant, e.g. lines 46-52, and even as far as line 62.
- Lines 55-58: I don't see these as mutually exclusive.
- Lines 67-8: according to ref 21, the ants adapted rather poorly to microgravity. Indeed, I feel this reference is a bit of a stretch.
- Line 72: "ultimately" replace with "finally".

METHODS

- Line 104: add "sequentially" before "inhabit".
- Figure 1 would be more informative if it was a grab from one of the videos, perhaps showing some ant paths.
 - As there is no id tracking in the video analysis, how can an ant running in and out of the nest repeatedly be distinguished from many ants beginning scouting bouts? Is there a minimum bout length cut-off? Figure S7 suggests mostly very short bouts. Are these, biologically speaking, really scouting bouts?
 - Line 127 - is the median speed a good measure to use here? The ants paths may be characterised by mostly standing still, with bouts of rapid movement. If so, you'll miss changes in these movement bouts if only the median is used. Overall distance per unit time might be more informative.
 - Supplementary code: I applaud the provision of the scripts and code. However, they should be properly annotated in order to make them useful. As they stand, I cannot follow them. For example, in the Rscript model summary modouts1, what are L, Q, C and 4? It took me a while to realise this refers to linear, quadratic, cubic, etc). The same goes for Treat1-4.
- Line 137-8 - was square rooting necessary here?
- Was the experiment order ever tested for an effect? Ants might give up scouting after four weeks of not finding any new nest.

- Line 144 – it is not clear to me why linear, quadratic, cubic or 4th (!) order trends were searched for. Was there any a-priori reason for doing this? In general, higher order trends should only be looked for with good reason.

RESULTS

- Line 150 – I’m not sure “linearly” can be claimed for ordinal data.
- Line 162 – replace ‘work’ with ‘exploration’ or scouting

DISCUSSION

- Lines 224-226 - see main comments above.
- Line 230 – remove one comma

FIGURES

- Figure 1 – a screen grab with scale bars and ant paths would be more informative
- Figure 2 – Y axis should read “total number of EXPLORATION bouts”. For all figures – what do the whiskers represent?
- Figures 3 & 4 – I dislike showing transformed data in figures. It is fine to use them for statistical analysis, but I feel the raw numbers are more useful in a figure.
- Figure 3 – it would be nice to see similar figures to these, but with the path length and exploration time divided by number of bouts. This would allow the reader to assess the relationship between these variables, and would likely show that the number of bouts mostly controls the other two variables.

Review form: Reviewer 2

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)

This is a nice piece of work. It is interesting, it builds on previous work in a productive and useful way and it is generally well-presented. The study is well framed, the Methods and Results are clear and the Discussion is open-handed and does not overreach. It is not my nature to submit short reviews and I know, with my Editor’s hat on, that short reviews are often unhelpful. However, I thought this was a good study and I enjoyed reading the paper.

The only comments I have are:

I think the overall finding of the paper could be expressed more clearly in the Abstract – my issue here was the use of “target nest”, which I think will not be immediately understood in that context by someone not familiar with this body of work, and the use of “nest value”. I think there is room here to be more plain and direct in the Abstract and tell the story in a more intuitive way - “increasing current nest value” might make total sense but it seems to over complicate things. However, this is a matter of taste rather than a “must address” comment

The Introduction is already quite long so it might be difficult to incorporate this, but...it would be nice to have some discussion of nest finding and nest value in other (non-ant) species. Honeybees are obvious of course, but also nesting birds choosing nest boxes etc.

Decision letter (RSOS-150533)

26-Oct-2015

Dear Dr Doran Borges de Sousa,

The editors assigned to your paper ("Ant colonies explore less but individuals search for longer when current housing conditions are better.") has now received comments from reviewers. We would like you to revise your paper in accordance with the referee and Subject Editor suggestions which can be found below (not including confidential reports to the Editor). Please note this decision does not guarantee eventual acceptance.

Please submit a copy of your revised paper within three weeks (i.e. by the 18-Nov-2015). If we do not hear from you within this time then it will be assumed that the paper has been withdrawn. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office in advance. We do not allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Editors, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers.

To revise your manuscript, log into <http://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." Your manuscript number has been appended to denote a revision. Revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you must respond to the comments made by the referees and upload a file "Response to Referees" in "Section 6 - File Upload". Please use this to document how you have responded to the comments, and the adjustments you have made. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response.

In addition to addressing all of the reviewers' and editor's comments please also ensure that your revised manuscript contains the following sections as appropriate before the reference list:

- Ethics statement (if applicable)

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-150533>

- 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.

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.

Yours sincerely,

Emilie Aime

Senior Publishing Editor, Royal Society Open Science

on behalf of Kevin Padian

Subject Editor, Royal Society Open Science

openscience@royalsociety.org

Author's Response to Decision Letter for (RSOS-150533)

See Appendix A.

RSOS-150533.R1 (Revision)

Review form: Reviewer 1 (Tomer Czaczkes)

Is the manuscript scientifically sound in its present form?

No

Are the interpretations and conclusions justified by the results?

No

Is the language acceptable?

Yes

Is it clear how to access all supporting data?

All present, clear, and acceptable

Do you have any ethical concerns with this paper?

No

Have you any concerns about statistical analyses in this paper?

No

Recommendation?

Major revision is needed (please make suggestions in comments)

Comments to the Author(s)

I have been asked to re-review the manuscript "Ant colonies explore less but individuals search for longer when current housing conditions are better" By Doran et al. In my previous review I reported that I found the collective aspect of this manuscript robust and worthy of publication (if not novel) with only minor revisions (which the authors have now carried out). However, I was very sceptical about the results pertaining to the reported modulation of individual-level behaviours of scouting bout length (per bout) and scouting bout path length (per bout). While the results came out "significant" (i.e. lower than $P < 0.05$) the explanatory power of the effect was so low as to be, to my eyes, meaningless. I thus strongly suggested removing all mention of the individual level effects.

The authors disagreed with my assessment, stating:

"We agree that the effect size is indeed very small. However, these results corroborate the results in Doran et al. 2013 (reference number 25) using another metric. In addition, for social systems such as ant colonies, small effect sizes will have larger effects if there is social amplification such as recruitment."

They thus chose not to reconsider the validity of the conclusions they draw from the data.

I'm afraid I find this response wholly unconvincing.

Firstly, in Doran et al. (2013), which I had studied closely previously, and have now re-read, the authors report changes in colony-level behaviour - the probability of ants scouting depending on current nest quality, as measured by the number of ants scouting. The results we are debating here pertain to changes in mean path length of a scouting bout, mean bout duration, and mean speed of the scouting ants. I simply do not see how these results are a corroboration of the findings of Doran et al. (2013).

I'm afraid the secondary argument, that social amplification will make smaller effects larger, also feels very much like hand-waving. The recruitment system here famously shows linear feedback, and so will not show such large amplification. I remind the authors that I previously estimated the effect strength to be c. 1% - leaving 99% of the variation unexplained. This remaining 99% of random variation will be amplified along with the putative 1% of signal, still resulting in little biological relevance of the factors measured.

I thus cannot accept the response of the authors to my concern, and therefore still cannot recommend that this manuscript be published in its current state. I reiterate my request that "the authors be much more cautious in both how they present their results regarding individual behaviour, and in the conclusions drawn. This will require a significant rewrite of the title, the abstract, the results, and the discussion. Frankly, I would completely drop any claims being made about adjustment of individual-level behaviour."

I also stand by my previous comment, that
 "With that said, as the experimental methods and data collection is solid, I believe that manuscript will be acceptable for publication after a revision."

The authors responded in much more detail to my subsidiary concern about the interpretation of the individual behavioural patterns they report finding. I somewhat regret raising this point, as it perhaps took the emphasis off my main concern. Nonetheless, I appreciate the response of the authors to my comment. I will respond to their response, but emphasise that without handling my main point above, this debate is purely hypothetical.

In brief, I suggested that rather than adjustment of individual level behaviour, the putative findings could be explained by a process of self-selecting subsets of ants. The authors argue that, due to the variation within each groups not changing, one can rule out the hypothesis the alternative hypothesis I raised. I find this to be rather weak evidence to hang the entire message of the paper on. Indeed, why assume that the variance would decrease? The 'super-scouts' (see original comment) might have the same variance, but with a shifted mean in other aspects of their behaviour.

The authors go on to run some new statistical analyses in which the collective effects examined are divided by the total number of bouts. They find opposite effects to the ones reported (good that this is now added to the manuscript!). This is presented as further support for their interpretation. They state:

"If the number of bouts was the cause of the variation in the collective effort we would expect no trend at all after dividing the response variables by the number of bouts."

True, but it is not clear to me how this rules out the super-scout hypothesis? It may just mean that the super-scouts walk further and for longer, and make the same number of bouts. I'm still not fully convinced, and think both hypotheses should have been equal weight. But, as I stated earlier, I am not convinced that there is any real evidence of individual-level behavioural differences between the treatments at all.

I am very sorry to be stubborn on this point - I know that this can be frustrating. I also realise that, without the individual level behaviour, the results presented here are not greatly different to

those shown in Doran (2013). Nonetheless, I cannot in good conscience recommend this manuscript for publication until I am fully convinced, or my suggestions are followed. Perhaps I have misunderstood something key? If discussion via writing is not working, but the authors really are convinced that they are right, I would be happy to talk in person (via skype or similar) to try and work this out.

Yours sincerely,

Tomer Czaczkes

I will now respond to the 'minor comments' bits.

(old comment): Lines 22-24: some concrete numbers (percentages, ratios, etc) would not go amiss here.

We don't usually put values in the abstract as we think the abstract should give a general overview of the paper. However we are happy to do so if the editor sees fit

TC) no, I didn't mean results of statistical tests, so much as descriptive statistics. E.g. "the number of scouting bouts was four times higher in poor compared to excellent nests"

(old comment): Figures 3 & 4 – I dislike showing transformed data in figures. It is fine to use them for statistical

analysis, but I feel the raw numbers are more useful in a figure.

We completely agree with the referee, however, when the non-transformed data is skewed, extreme values

could "squash" the presentation of most of the data and make the figure non informative

TC) Well, you could take out the outliers – the figures are only illustrations of the results, after all, and should serve to help the readers get the main message. But this is all a matter of personal taste.

Line 240 typo – 'wile' instead of 'while'

All other minor responses and changes look good, and are approved.

Decision letter (RSOS-150533.R1)

24-Nov-2015

Dear Dr Doran Borges de Sousa:

Manuscript ID RSOS-150533.R1 entitled "Ant colonies explore less but individuals search for longer when current housing conditions are better." which you submitted to Royal Society Open Science, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.

Please submit a copy of your revised paper within three weeks (i.e. by the 17-Dec-2015). If we do not hear from you within this time then it will be assumed that the paper has been withdrawn. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office in advance. We do not allow multiple rounds of revision so we urge you to make every effort to

fully address all of the comments at this stage. If deemed necessary by the Editors, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers.

To revise your manuscript, log into <http://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." Your manuscript number has been appended to denote a revision. Revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you must respond to the comments made by the referees and upload a file "Response to Referees" in "Section 6 - File Upload". Please use this to document how you have responded to the comments, and the adjustments you have made. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response.

In addition to addressing all of the reviewers' and editor's comments please also ensure that your revised manuscript contains the following sections before the reference list:

- 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.

- 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.

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.

Sincerely,

Emilie Aime

Senior Publishing Editor, Royal Society Open Science

on behalf of Kevin Padian

Subject Editor, Royal Society Open Science

openscience@royalsociety.org

Author's Response to Decision Letter for (RSOS-150533)

See Appendix B.

RSOS-150533.R2 (Revision)

Review form: Reviewer 1 (Tomer Czaczkes)

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 as is

Comments to the Author(s)

I am pleased with this revision. The authors have thought seriously about my reservations and taken them onboard, while maintaining their own view on the data. The strong conclusions have been emphasised, and the weaker ones stated cautiously without giving the feeling that anything is being hidden. I agree with the authors that the manuscript is now greatly improved. This is how the review process should work.

I now have no reservations about this manuscript, and take great pleasure in recommending it for immediate publication.

Yours sincerely,

Tomer Czaczkes

-typos-

line 232: can not should be one word here

line 236: "...as well as being..."

Decision letter (RSOS-150533.R2)

18-Dec-2015

Dear Dr Doran Borges de Sousa,

I am pleased to inform you that your manuscript entitled "Exploration adjustment by ant colonies" is now accepted for publication in Royal Society Open Science.

You can expect to receive a proof of your article within approximately 10 working days. Please contact the production office (openscience_proofs@royalsociety.org) to let us know if you are likely to be away from e-mail contact during that period. 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,

Dr Matthew Allinson

matthew.allinson@royalsociety.org

<http://rsos.royalsocietypublishing.org/>

Appendix A

Dear Dr Doran Borges de Sousa,

The editors assigned to your paper ("Ant colonies explore less but individuals search for longer when current housing conditions are better.") has now received comments from reviewers. We would like you to revise your paper in accordance with the referee and Subject Editor suggestions which can be found below (not including confidential reports to the Editor). Please note this decision does not guarantee eventual acceptance.

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To revise your manuscript, log into <http://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." Your manuscript number has been appended to denote a revision. Revise your manuscript and upload a new version through your Author Centre.

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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-150533>

- 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.

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- 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.

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.

Yours sincerely,

Emilie Aime

Senior Publishing Editor, Royal Society Open Science

on behalf of Kevin Padian

Subject Editor, Royal Society Open Science

openscience@royalsociety.org

Dear Dr Aime,

We greatly appreciate all the encouraging comments about our manuscript. We have addressed all the comments and have provided justification for the ones we did not agree with below. We feel the manuscript has improved greatly after the suggestions from both referees. The line numbers from the submitted version did not match with our word version so we have highlighted in yellow all the changes we have made.

Comments to Author:

Reviewers' Comments to Author:

Reviewer: 1

Comments to the Author(s)

In this study, the exploration behaviour of the Rock Ant *Temnothorax albipennis* is studied. Colonies are housed in nests of varying quality, and the number of exploration bouts, and their total and individual lengths and durations, are measured. Two conclusions are drawn: Regarding collective exploration, the authors conclude that overall exploration rates increase as current nest value decreases. Regarding individual exploration, the authors conclude that exploration bout length and duration increase with increasing nest quality, while instantaneous speed decreases.

The paper reads well, the motivation behind the experiments is sound, and the experimental methodology is robust. The figures are clear and informative. The statistical analysis is, to my knowledge, generally sound (see some minor comments at the end), although I cannot judge the Python script. Indeed, I applaud the provision of both the preliminary data checking and the scripts use to carry out the analysis (but see some minor comments) – this sort of transparency is what most papers should strive for.

Dear Dr Czaczkes,

We are extremely grateful for all your encouraging words and all your suggestions for our manuscript. We appreciate your very thorough review and will address now all of your comments.

I find the collective results robust, reliable and believable. I will make more detailed suggestions at the end, but have no major comments regarding this aspect of the work.

However, I am very sceptical about the patterns described for the individual level behaviour. While significant differences are found, and I can find no flaw in how the statistics were carried out (I am no expert on the matter). However, the effect size seems from the figures to be very small indeed. If we consider the breadth of the whiskers in all panels of figure 4, we see that variation within groups is much, much greater than variation between groups. If we were to consider non-transformed data, the whiskers would likely be even broader. While there is no agreed upon equivalent for an R-squared value for generalised linear mixed-effect models (GLMM), a rough estimate can be achieved by using an equivalent GLM. Using the raw data supplied, I calculated such R-squared. Log bout duration vs treatment had an adjusted R squared of 0.78% - less than 1% of the variation is explained by the treatment! Similar results are found for speed (2.21%). Very large sample sizes (here they vary from 2205 to 648 samples per treatment) can result in even tiny effects reaching significance. Just because an effect is statistically significant does not mean it is either real or that it has biological meaning.

We agree that the effect size is indeed very small. However, these results corroborate the results in Doran et al. 2013 (reference number 25) using another metric. In addition, for social systems such as ant colonies, small effect sizes will have larger effects if there is social amplification such as recruitment.

Even if we were to agree that these patterns represent real and important biological effects (which I do not), I do not necessarily agree with the interpretation assigned to them. The authors present these results as showing a behavioural adaptation by individual scouts to their nest quality. I see a different (and in my opinion more likely) explanation: with higher nest quality all but a few 'super-scouts' stop scouting. This non-random subsample of scouts also happen to walk slower, make longer scouting bouts, and spend longer scouting. Thus, there is no change in the scouting

behaviour of individuals – just a removal of all but the most hard working scouts. Had the individual scouts been assigned an identity (perhaps using the software id-Tracker (Pérez-Escudero et al. 2014), although I realise this software is limited to a very few individuals), testing which of these options is true would be possible. Without such individual ID information, both options must be presented as (at least) equally likely.

We appreciate the alternative interpretation of our results. In fact we had previously considered such a scenario since it would go extremely well in line with the response threshold idea. If colonies are composed of individuals with different response thresholds, we would expect that in better housing conditions only individuals with a really high threshold would not be satisfied and continue searching. However, if we compare the different treatments, the level of variation does not seem to change that much. We have compared this using an F test, which is not the most accurate way since it does not consider colony as a random factor, but it is enough to give us an idea. For the duration, the F-ratio is not significantly different from 1, even with a sample size of 2204 points. For both path length and instantaneous speed it is significant but at 1.25 and 1.47 ratio respectively. Both are closer to 1 than to 2. If indeed in better housing conditions only the 'super scouts' would be scouting we would expect a gradual decrease in variation with increasing nest value. As that is not the case, we think it is more likely the interpretation that the patterns observed are a result of individual flexibility.

Furthermore, if we run the collective effort models (Total path length and Total path duration) but with both response variables divided by the number of bouts, in both cases, we get a significant positive linear trend with increasing nest value, with the addition of treatment to the models having a highly significant effect ($p < 0.001$ in both cases). This trend is opposite to the trend obtained for total path length and total exploration behaviour when not divided by total number of bouts. This is another piece of evidence in support of our interpretation. If the number of bouts was the cause of the variation in the collective effort we would expect no trend at all after dividing the response variables by the number of bouts.

We have added boxplots of both response variables divided by the number of bouts to the supplementary information as well as the R code and output of the models. We have also added some information regarding this analysis both in the results (lines 173 - 175) and in our discussion (lines 211 - 216). We have also added a few lines to our discussion regarding the referee's interpretation since we do think that this alternative interpretation is highly relevant (lines 212 – 219).

We did consider id-Tracker, unfortunately it requires more pixels per ant than in our videos. Something to keep in mind for future experiments.

In light of these concerns, I urge the authors to be much more cautious in both how they present their results regarding individual behaviour, and in the conclusions drawn. This will require a significant rewrite of the title, the abstract, the results, and the discussion. Frankly, I would completely drop any claims being made about adjustment of individual-level behaviour. With that said, as the experimental methods and data collection is solid, I believe that manuscript will be acceptable for publication after a revision.

For the reasons justified above, we decided to keep the title and continue to favour our initial interpretation. However we have now included the alternative interpretation by Dr Czaczkes.

I hope the authors find my comments constructive and helpful.

Yours sincerely,

Tomer J. Czaczkes
University of Regensburg

Dear Dr Gzaczkes

We appreciate greatly the positive comments and encouragement. Please see our detailed point-by-point responses below.

I shall now make a series of more minor comments (split by section), aimed at improving the manuscript.

ABSTRACT

- Line 16-18: this (division of labour) does not seem very relevant to the study

We understand this concern and have made more obvious the relationship between our work and division of labour on lines 58 - 64. This study focusses on how colonies organize their work, within a single task and we believe that it might shed some light on more general models of work organisation such as task allocation.

- Line 20-21 and elsewhere: you are (perhaps reasonably) assuming the ants are scouting for a new nest, but in fact you do not know what the ants are doing. They may be searching for food, patrolling for enemies, or searching for material with which to modify their nest. Caution is required when assigning motivation to animal behaviour, as the motivational state of the animal will strongly affect how it reacts to stimuli.

We agree with the reviewer that caution is required when assigning motivation to animal behaviour. However, as shown both here and in one of our previous papers (reference number 25), with all else being equal, differences in the number of individuals outside have to be attributed to changes in nest value. We have made this clearer in our discussion lines 257 – 258.

- Lines 22-24: some concrete numbers (percentages, ratios, etc) would not go amiss here.

We don't usually put values in the abstract as we think the abstract should give a general overview of the paper. However we are happy to do so if the editor sees fit.

- Line 26-27: looking at the effect strengths, it seems that the collective patterns are driven almost exclusively by the number of bouts made in total.

As mentioned in our first response, the models including total path length and total exploration duration divided by the number of bouts show a linear trend in the opposite direction in comparison to when they are not divided by the number of bouts. Hence the total effort observed is not a result of only variation in total number of bouts as if that was the case we would expect that dividing total path length and total exploration time by the total number of bouts would annihilate the effect of treatment.

We have chosen not to make any changes in the title and abstract but have addressed all the referees concern in other sections.

BACKGROUND

- Line 51: “might be” – this has been concretely shown repeatedly, no?

We have updated line 49.

- Line 53: in the reference list, ref 12 is formatted as a journal article, but it is fact a chapter in a book.

Thank you, we have corrected this reference.

- I feel a lot of the introduction is not strictly relevant, e.g. lines 46-52, and even as far as line 62.

We have made the connection between our work and division of labour clearer. See lines 58 – 64.

- Lines 55-58: I don't see these as mutually exclusive.

We completely agree with this point and have added updated the manuscript, lines 58 - 60

- Lines 67-8: according to ref 21, the ants adapted rather poorly to microgravity. Indeed, I feel this reference is a bit of a stretch.

We reference this paper to show other research where the same tracking software has been used. We have made it clear that colonies indeed adapted very poorly. See line 70.

- Line 72: “ultimately” replace with “finally”.

We have made this correction, now line 74.

METHODS

- Line 104: add “sequentially” before “inhabit”.

We have made this correction, now line 104.

- Figure 1 would be more informative if it was a grab from one of the videos, perhaps showing some ant paths.

We do agree with the referee, however the ants occupy only around 20 pixels and there are several trajectories at the same time. Hence we find it not so informative. We think figure 1, as it stands is quite an elegant diagram, which aims to visualise the experimental set up and how the parameters were calculated.

- As there is no id tracking in the video analysis, how can an ant running in and out of the nest repeatedly be distinguished from many ants beginning scouting bouts? Is there a minimum bout length cut-off? Figure S7 suggests mostly very short bouts. Are these, biologically speaking, really scouting bouts?

We are not able to distinguish if the same ant leaves more than once. We have made this clearer in lines 118 – 119. We considered exploratory bouts as soon an ant's full body entered the scouting arena. This was done exactly the same way for all treatments.

- Line 127 – is the median speed a good measure to use here? The ants paths may be characterised by mostly standing still, with bouts of rapid movement. If so, you'll miss changes in these movement bouts if only the median is used. Overall distance per unit time might be more informative.

This is a really good point. In fact we did look at number of seconds an ant is not moving and this does not change significantly for the 5 nesting conditions. We do not think this information is relevant for the paper but we can certainly add it if necessary.

- Supplementary code: I applaud the provision of the scripts and code. However, they should be properly annotated in order to make them useful. As they stand, I cannot follow them. For example, in the Rscript model summary modouts1, what are L, Q, C and 4? It took me a while to realise this refers to linear, quadratic, cubic, etc). The same goes for Treat1-4.

We completely agree with this point and have added several comments to all the scripts.

- Line 137-8 – was square rooting necessary here?

We have chosen to square-root the data so that a linear mixed model could be used. Such models require that the data is at least approximately normal.

- Was the experiment order ever tested for an effect? Ants might give up scouting after four weeks of not finding any new nest.

We did look at the effect of order for all the response variables. We ran the following models:

Mod0 -> Response variable and colony as a random factor (Null model)

Mod1 -> Response variable, treatment as a fixed factor and colony as a random factor

Mod2 -> Response variable, treatment and order as a fixed factors and colony as a random factor

Mod3 -> Response variable, order as a fixed factor and colony as a random factor

For all 3 response variables (path length, duration and instantaneous speed) the comparison between models Mod0 and Mod3 showed no significant differences. Furthermore comparing models Mod1 with Mod 2 did show a significant difference but with a delta AIC of 2, 4 and 7 for path length, duration and instantaneous speed respectively. According to Burnham and Anderson (2001, 2nd edition) delta AIC > 10 are considered not to be competitive with the best model. Also for all cases Mod2 only showed a significant fit to cubic and 4th order trends which do not have a biological meaning and so we chose the model with only treatment as a fixed factor.

The supplementary information is already quite vast and so we chose to leave all this information out but we are happy to add it if necessary.

- Line 144 – it is not clear to me why linear, quadratic, cubic or 4th (!) order trends were searched for. Was there any a-priori reason for doing this? In general, higher order trends should only be looked for with good reason.

We completely agree with the referee. That is why in our interpretation we focus on the linear trend and overall positive or negative direction as these are of biological interest. By definition the max order of trends in a model is equal to the number of categories minus 1, hence with five nest qualities the software automatically gives from linear to 4th order trend.

RESULTS

- Line 150 – I'm not sure "linearly" can be claimed for ordinal data.

We agree with the referee and have removed the word linearly from here as it causes confusion between linear relationship and a linear contrast.

- Line 162 – replace 'work' with 'exploration' or scouting

We agree and have made this change now in line 159

DISCUSSION

- Lines 224-226 - see main comments above.

Already discussed above

- Line 230 – remove one comma

We agree and have removed one of the commas, lines 232 – 233.

FIGURES

- Figure 1 – a screen grab with scale bars and ant paths would be more informative

Already discussed above

- Figure 2 – Y axis should read "total number of EXPLORATION bouts". For all figures – what do the whiskers represent?

We agree and have made the change to the figure label. The whiskers represent the nearest value within 1.5 of the interquartile range. We find this to be the default for most software's. We have added this information to the figure labels.

- Figures 3 & 4 – I dislike showing transformed data in figures. It is fine to use them for statistical analysis, but I feel the raw numbers are more useful in a figure.

We completely agree with the referee, however, when the non-transformed data is skewed, extreme values could "squash" the presentation of most of the data and make the figure non informative.

- Figure 3 – it would be nice to see similar figures to these, but with the path length and exploration time divided by number of bouts. This would allow the reader to assess the relationship between these variables, and would likely show that the number of bouts mostly controls the other two variables.

We have done this and have added to the supplementary figure document (figure S33). We have added a sentence referring to these to the main document. Lines 174 – 176. If the number of bouts controlled most of the other variables we would expect no trend, but instead we found a positive trend (as expected based on the individual bout analysis).

Reviewer: 2

Comments to the Author(s)

This is a nice piece of work. It is interesting, it builds on previous work in a productive and useful way and it is generally well-presented. The study is well framed, the Methods and Results are clear and the Discussion is open-handed and does not overreach. It is not my nature to submit short reviews and I know, with my Editor's hat on, that short reviews are often unhelpful. However, I thought this was a good study and I enjoyed reading the paper.

[We thank the referee for the encouraging comments on our manuscript.](#)

The only comments I have are:

I think the overall finding of the paper could be expressed more clearly in the Abstract – my issue here was the use of “target nest”, which I think will not be immediately understood in that context by someone not familiar with this body of work, and the use of “nest value”. I think there is room here to be more plain and direct in the Abstract and tell the story in a more intuitive way - “increasing current nest value” might make total sense but it seems to over complicate things. However, this is a matter of taste rather than a “must address” comment

[We agree with the referee that even though this terms are very obvious to us they might not be to some readers and hence we have changed the abstract Lines 20, 21 and 24\).](#)

The Introduction is already quite long so it might be difficult to incorporate this, but...it would be nice to have some discussion of nest finding and nest value in other (non-ant) species. Honeybees are obvious of course, but also nesting birds choosing nest boxes etc.

[We agree with this point and since our introduction was indeed already long we have included a paragraph in the discussion regarding house hunting in other animal species, lines \(238 - 244\).](#)

Appendix B

24-Nov-2015

Dear Dr Doran Borges de Sousa:

Manuscript ID RSOS-150533.R1 entitled "Ant colonies explore less but individuals search for longer when current housing conditions are better." which you submitted to Royal Society Open Science, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.

Please submit a copy of your revised paper within three weeks (i.e. by the 17-Dec-2015). If we do not hear from you within this time then it will be assumed that the paper has been withdrawn. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office in advance. We do not allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Editors, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers.

To revise your manuscript, log into <http://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." Your manuscript number has been appended to denote a revision. Revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you must respond to the comments made by the referees and upload a file "Response to Referees" in "Section 6 - File Upload". Please use this to document how you have responded to the comments, and the adjustments you have made. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response.

In addition to addressing all of the reviewers' and editor's comments please also ensure that your revised manuscript contains the following sections before the reference list:

- 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.

- 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.

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.

Sincerely,
Emilie Aime
Senior Publishing Editor, Royal Society Open Science

Dear Emilie Aime,

We are very grateful for the opportunity of a second round of revision. We really enjoyed the fruitful discussions and once again think the manuscript is more solid after the suggestions from the referee.

on behalf of Kevin Padian
Subject Editor, Royal Society Open Science
openscience@royalsociety.org

Comments to Author:

Reviewer: 1

Comments to the Author(s)

I have been asked to re-review the manuscript "Ant colonies explore less but individuals search for longer when current housing conditions are better" By Doran et al. In my previous review I reported that I found the collective aspect of this manuscript robust and worthy of publication (if not novel) with only minor revisions (which the authors have now carried out). However, I was very sceptical about the results pertaining to the reported modulation of individual-level behaviours of scouting bout length (per bout) and scouting bout path length (per bout). While the results came out "significant" (i.e. lower than $P < 0.05$) the explanatory power of the effect was so

low as to be, to my eyes, meaningless. I thus strongly suggested removing all mention of the individual level effects.

Dear Tomer Czaczkes,

We are extremely grateful for all the time and effort you have invested in our manuscript. After careful consideration we have decided to take your advice. We have now made changes to the title, abstract, results and discussion. The manuscript now focuses mainly on the collective responses. However we still discuss the idea of individual adjustment and quote 2 papers where small effect size actually showed a significant effect on the collective.

We hope you are now happy with the manuscript. We will now respond to every point separately.

The authors disagreed with my assessment, stating:

“We agree that the effect size is indeed very small. However, these results corroborate the results in Doran et al. 2013 (reference number 25) using another metric. In addition, for social systems such as ant colonies, small effect sizes will have larger effects if there is social amplification such as recruitment.”

They thus chose not to reconsider the validity of the conclusions they draw from the data.

I'm afraid I find this response wholly unconvincing.

Firstly, in Doran et al. (2013), which I had studied closely previously, and have now re-read, the authors report changes in colony-level behaviour - the probability of ants scouting depending on current nest quality, as measured by the number of ants scouting. The results we are debating here pertain to changes in mean path length of a scouting bout, mean bout duration, and mean speed of the scouting ants. I simply do not see how these results are a corroboration of the findings of Doran et al. (2013).

I'm afraid the secondary argument, that social amplification will make smaller effects larger, also feels very much like hand-waving. The recruitment system here famously shows linear feedback, and so will not show such large amplification. I remind the authors that I previously estimated the effect strength to be c. 1% - leaving 99% of the variation unexplained. This remaining 99% of random variation will be amplified along with the putative 1% of signal, still resulting in little biological relevance of the factors measured.

I thus cannot accept the response of the authors to my concern, and therefore still cannot recommend that this manuscript be published in its current state. I reiterate my request that “the authors be much more cautious in both how they present their results regarding individual behaviour, and in the conclusions drawn. This will require a significant rewrite of the title, the abstract, the results, and the discussion. Frankly, I would completely drop any claims being made about adjustment of individual-level behaviour.”

We have now made changes to the title, abstract (lines 22 – 26), results (lines 185 – 186) and discussion (lines 211 – 212, 219 – 224 and 271 – 272). We have maintained a few lines in the discussion arguing how the individual effect might still influence the collective pattern observed, even though very small. The same had been previously shown in reference 34 and 35. However

we have taken on board the suggestion of the referee and have changed the manuscript such that equal weight is given to both interpretations.

I also stand by my previous comment, that

“With that said, as the experimental methods and data collection is solid, I believe that manuscript will be acceptable for publication after a revision.”

The authors responded in much more detail to my subsidiary concern about the interpretation of the individual behavioural patterns they report finding. I somewhat regret raising this point, as it perhaps took the emphasis off my main concern. Nonetheless, I appreciate the response of the authors to my comment. I will respond to their response, but emphasise that without handling my main point above, this debate is purely hypothetical.

In brief, I suggested that rather than adjustment of individual level behaviour, the putative findings could be explained by a process of self-selecting subsets of ants. The authors argue that, due to the variation within each groups not changing, one can rule out the hypothesis the alternative hypothesis I raised. I find this to be rather weak evidence to hang the entire message of the paper on. Indeed, why assume that the variance would decrease? The 'super-scouts' (see original comment) might have the same variance, but with a shifted mean in other aspects of their behaviour.

We very much agree with this point. In fact these data support the suggestion to combine both interpretations: There could be a process of self-selecting ants, and such ants might be adjusting their behaviour and that might be why the effect size is rather small. We have added this idea both in the abstract (lines 22 – 26) and discussion (221 – 224).

The authors go on to run some new statistical analyses in which the collective effects examined are divided by the total number of bouts. They find opposite effects to the ones reported (good that this is now added to the manuscript!). This is presented as further support for their interpretation. They state:

“If the number of bouts was the cause of the variation in the collective effort we would expect no trend at all after dividing the response variables by the number of bouts.”

True, but it is not clear to me how this rules out the super-scout hypothesis? It may just mean that the super-scouts walk further and for longer, and make the same number of bouts. I'm still not fully convinced, and think both hypotheses should have been equal weight. But, as I stated earlier, I am not convinced that there is any real evidence of individual-level behavioural differences between the treatments at all.

I am very sorry to be stubborn on this point – I know that this can be frustrating. I also realise that, without the individual level behaviour, the results presented here are not greatly different to those shown in Doran (2013). Nonetheless, I cannot in good conscience recommend this manuscript for publication until I am fully convinced, or my suggestions are followed. Perhaps I have misunderstood something key? If discussion via writing is not working, but the authors really are convinced that they are right, I would be happy to talk in person (via skype or similar) to try and work this out.

Yours sincerely,

Tomer Czaczkes

Again, we are very grateful for all the investment of time and effort by the referee in our manuscript. We have taken on board your suggestion and have acknowledged that for the individual level the effect size is very small and argue that we might have a combination of both processes. A self-selection of the most hard-working ants who might be adjusting their behaviour to their current housing conditions. We are very happy with this improved version and hope it is now suitable for publication.

I will now respond to the 'minor comments' bits.

(old comment): Lines 22-24: some concrete numbers (percentages, ratios, etc) would not go amiss here.

We don't usually put values in the abstract as we think the abstract should give a general overview of the

paper. However we are happy to do so if the editor sees fit

TC) no, I didn't mean results of statistical tests, so much as descriptive statistics. E.g. "the number of scouting bouts was four times higher in poor compared to excellent nests"

The number of bouts is 5 times smaller in excellent nests in comparison with Poor nest sites, however, due to the word limit of the abstract we have chosen not to add this. However, if the reviewer and editor advise us otherwise, we are happy to add this information.

(old comment): Figures 3 & 4 – I dislike showing transformed data in figures. It is fine to use them for statistical

analysis, but I feel the raw numbers are more useful in a figure.

We completely agree with the referee, however, when the non-transformed data is skewed, extreme values

could "squash" the presentation of most of the data and make the figure non informative

TC) Well, you could take out the outliers – the figures are only illustrations of the results, after all, and should serve to help the readers get the main message. But this is all a matter of personal taste.

We have discussed this at length but we don't like removing outliers and have chosen to maintain the transformed data.

Line 240 typo – 'wile' instead of 'while'

We have corrected this, line 227.

All other minor responses and changes look good, and are approved.